Determining Sources of Fecal Pollution in Water for a Rural Virginia Community

By

Alexandria Kristen Graves

Thesis submitted to the Faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

IN

CROP AND SOIL ENVIRONMENTAL SCIENCES

APPROVED:

_____

Charles Hagedorn III, Chair

_____ _____

Raymond B. Reneau, Jr. Matthew Eick

August 1, 2000

Blacksburg, Virginia

Keywords: bacterial source tracking, fecal coliforms, fecal streptococci, watersheds

DETERMINING SOURCESOF FECAL POLLUTION IN WATER FOR A RURAL VIRGINIA COMMUNITY

By

Alexandria Kristen Graves

Dr. Charles Hagedorn III, Chairman

Crop and Soil Environmental Sciences

(ABSTRACT)

This project involves developing and applying bacterial source tracking (BST) methodology to determine sources of fecal pollution in water for a rural community (Millwood, VA). Antibiotic resistance analysis (ARA) is the primary BST method for fecal source identification, followed by randomly amplified polymorphic DNA (RAPD) analysis for confirmation. Millwood consists of 66 homes, all served by individual septic systems, and a stream (Spout Run) passes through the center of the community. Spout Run drains a 5,800 ha karst topography watershed that includes large populations of livestock and wildlife. Stream and well samples were collected monthly and analyzed for fecal coliforms and fecal streptococci, starting in 5/99 and ending in 5/00. Twelve percent of the well samples and 92% of the stream samples were positive for fecal coliforms, and 26% of the stream samples exceeded the recreational water standard (1,000 fecal coliforms/100 ml). ARA of fecal streptococci recovered from the stream samples indicated that isolates of human origin appeared throughout the stream as the stream passed through Millwood with a yearly average of (≈10% human, 30% wildlife, and 63% livestock), and the percent human origin isolates declined downstream from Millwood. These results were obtained by comparing the antibiotic resistance profiles of stream isolates against a library of 1,174 known source

isolates with correct classification rates of 94.6% for human isolates, 93.7% for livestock isolates, and 87.8% for wildlife isolates.

There is a human signature in Spout Run, but it is small compared to the proportion of isolates from livestock and wildlife. The sporadic instances where well water samples were positive appeared primarily during very dry periods. Restricting livestock access to streams can dramatically lower fecal coliform counts during the unusually hot and dry periods. Reducing FC counts to below recreational water standards for Virginia (1000 per 100ml for any one sample) may be achievable, however to maintain streams below standards may prove to be difficult, as Spout Run is in an area where there are large populations of Canada geese, deer, and other wildlife, and will be hard to restrict these animals.

I dedicate this thesis

In honor of my parents

ALEXANDER AND CLARETTA GRAVES

and my brother

VINCENT L. GRAVES

ACKNOWLEDGEMENTS

The author began her Master of Science in Crop and Soil Environmental Sciences at Virginia Tech in 1998 under the guidance of Dr. Charles Hagedorn III. Many people were instrumental in helping the author with her research project and the collection of the information for this thesis. She wishes to acknowledge these individuals.

The author's research project was funded by the Virginia Department of Conservation and Recreation (VADCR) in cooperation with Ms. Alison Teeter, the Natural Resource Planner for Clarke County, Virginia. The VADCR staff and Alison Teeter are thanked for their assistance with devotion to this project and its successful completion. The monthly sampling and monitoring would not have been impossible without the support of Ms. Alison Teeter.

The author graciously appreciates the continued assistance and devotion of her committee chairman, Dr. Charles Hagedorn III, and her committee members, Dr. Raymond B. Reneau, Jr., and Dr. Matthew Eick. As the leader of the VADCR project, Dr, Charles Hagedorn taught her scores of indispensable lessons regarding bacterial source tracking, which aided her appreciably in the completion of her project. She appreciates the enthusiasm and support from her committee.

The laboratory technicians in Dr. Reneau's lab performed many of the chemical tests on the routine monitoring samples. Their assistance permitted monthly results to be generated and analyzed in a timely fashion. Norma Nelson, Mechelle Mahal, Amy Bowman and Karen Hix were all very instrumental in preparing media and setting up samples for routine monitoring analyses. Their assistance helped the author tremendously and the author is very

appreciative to them for all their hard work. Countless thanks to Ms. Sybil Phoenix who many times answered questions and gave assistance to the author.

The author could not have completed this document without the constant support of her parents Alexander and Claretta Graves. She is indebted to her parents for their extraordinary assistance, support, and encouragement throughout the completion of this Master of Science degree. Many times she was reminded by her father to "stay focused". This was the advice that most encouraged her to not lose sight of her goals. The author also appreciates the humorous support that her brother Vincent L. Graves gave to her.

Finally, the author thanks God for his love, protection, and the desire he placed in her heart to persevere until the end.

TABLE OF CONTENTS

ABSTRACT	**i**
ACKNOWLEDGEMENTS	**iv**
LIST OF TABLES	**ix**
LIST OF FIGURES	**xii**

Chapter	**Page**
I. INTRODUCTION	**1**
II. REVIEW OF LITERATURE	**4**
A. 1. Health Hazards Associated with Water	6
A. 2. Risk Assessment	7
A. 3. Sources of Water in the United States	8
A. 4. Waste Disposal in the United States	9
B. Exposure Pathways for Fecal Contamination	10
B. 1. Livestock	11
B. 2. Wildlife	11
B. 3. Human	12
C. Bacterial Indicators for Drinking Water Quality	13
C. 1. Criteria for Indicator Organisms	14
C. 2. MPN and Membrane Filtration	15
C. 3. Fecal Coliforms as Indicators of Pollution	16
C. 4. Fecal Streptococci as Indicators of Pollution	17
D. Watershed Studies	18
D. 1 The Regulatory Process and TMDLS	20
D. 2 Fecal Sourcing in Watersheds	22
E. Non-Genomic Techniques in Fecal Typing	25
E. 1. Antibiotic Resistance Patterns	28
F. Genomic Techniques in Fecal Typing	28
F. 1. Isolation of Genomic DNA from Fecal Coliforms and Streptococci	30
F. 2. Quantification an preparation of Genomic DNA Stocks	31
F. 3. Gel electrophoresis of Genomic DNA Samples	31
F. 4. Polymerase Chain Reaction	32
F. 5. RAPD Analysis	33
G. Summary	33
III. MATERIALS AND METHODS	**36**
A. Research and Site Description	36
B. Experimental Setup	37
C. Sample Collection	37
D. Water Monitoring	39

D. 1. Flow Rates 39
D. 2. Turbidity 39
D. 3. Hydrogen Ion Activity 40
D. 4. Ammonium 40
D. 5. Nitrate 40
D. 6. Phosphate 41
D. 7. Biological Oxygen Demand 41
E. Microbial Detection and Recovery Methods 41
E. 1. Presence-Absence Test 42
E. 2. Membrane Filtration for Fecal Coliforms 42
E. 3. Membrane Filtration for Fecal Streptococci 43
E. 4. Filtration for Solids 43
F. Antibiotic Resistance Analysis 44
G. Statistical Methods 48
G. 1. Discriminate Analysis 48

IV. RESULTS AND DISCUSSION 50

A. Studies of Fecal Contamination in Water 50
B. Flow Rate Observations 51
B. 1. Flow Rates for Summer 51
B. 2. Flow Rates for Fall 51
B. 3. Flow Rates for Winter 51
B. 4. Flow Rates for Spring 52
B. 5. Summary 52
C. Water Quality in the Spout Run Stream 54
C. 1. Background Water Quality 54
C. 2. Stream Quality for Summer 54
C. 3. Stream Quality for Fall 58
C. 4. Stream Quality for Winter 60
C. 5. Stream Quality for Spring 63
C. 6. Relationship of FC counts to Turbidity 66
D. Stream Water Quality Comparisons for the Year 68
E. Well Water Quality in Spout Run 71
E. 1. Well Water Quality for Summer 71
E. 2. Well Water Quality for Fall 71
E. 3. Well Water Quality for Winter 73
E. 4. Well Water Quality for Spring 73
E. 5. Summary 74
F. Well Water Quality Comparisons for the Year 76
F. 1. Seasonal Comparisons 76
F. 2. Overall Comparisons 76
F. 3. Summary 76
G. Antibiotic Resistance Analysis (ARA) 76
G. 2. ARA Database 76
G. 1. ARA for Summer 79

G. 2. ARA for Fall 81
G. 3. ARA for Winter 85
G. 4. ARA for Spring 87
G. 5. Summary 89

V. CONCLUSIONS 90
VI. LITERATURE CITED 91

APPENDICES A AND B 94

VITA 120

LIST OF TABLES

	Page
1. Antibiotics and their concentrations used in antibiotic resistance analysis for Spout Run.	47
2. Stream flow measurements (liters/minute) for Spout Run, Clarke County, VA.	53
3. Separation of fecal coliform counts into low, moderate, and high categories for Spout Run, Clarke County, VA.	56
4. Fecal coliform (FC) and fecal streptococci (FS) counts for summer stream samples, averaged over July, August, and September 1999, for each site.	57
5. Average fecal coliform (FC) and fecal streptococci (FS) counts for fall stream samples, averaged over October, November, and December 1999, for each site.	61
6. Fecal coliform (FC) and fecal streptococci (FS) counts for winter stream samples averaged over January, February, and March 2000, for each site.	62
7. Fecal coliform (FC) and fecal streptococci (FS) counts for winter stream samples averaged over May and June 1999 and April and May 2000, for each site.	65
8. Fecal coliform counts versus turbidity.	67
9. Separation of fecal coliform counts into low, moderate and high categories for well water samples by well and season, Millwood, Clarke County, VA.	72
10. Comparison of positive well water samples by well and season, Millwood, Clarke County, VA.	75
11. The antibiotic resistance analysis (ARA) database of 1,174 fecal streptococcal isolates from known sources in the Spout Run watershed, Clarke County, VA.	78
12. Antibiotic resistance analysis of fecal streptococci isolates from Spout Run (July, 1999), Clarke County, VA.	80
13. Antibiotic resistance analysis of fecal streptococci isolates from	82

Spout Run (October, 1999), Clarke County, VA.

14. Antibiotic resistance analysis of fecal streptococci isolates from Spout Run (December, 1999), Clarke County, VA. 84

15. Antibiotic resistance analysis of fecal streptococci isolates from Spout Run (March, 2000), Clarke County, VA. 86

16. Antibiotic resistance analysis of fecal streptococci isolates from Spout Run (May, 1999), Clarke County, VA. 88

17. Appendix A. Fecal coliform and fecal streptococcus counts for May 1999, Spout Run, Clarke County, VA. 95

18. Appendix A. Fecal coliform and fecal streptococcus counts for June 1999, Spout Run, Clarke County, VA. 96

19. Appendix A. Fecal coliform and fecal streptococcus counts for July 1999, Spout Run, Clarke County, VA. 97

20. Appendix A. Fecal coliform and fecal streptococcus counts for August 1999, Spout Run, Clarke County, VA. 98

21. Appendix A. Fecal coliform and fecal streptococcus counts for September 1999, Spout Run, Clarke County, VA. 99

22. Appendix A. Fecal coliform and fecal streptococcus counts for October 1999, Spout Run, Clarke County, VA. 100

23. Appendix A. Fecal coliform and fecal streptococcus counts for November 1999, Spout Run, Clarke County, VA. 101

24. Appendix A. Fecal coliform and fecal streptococcus counts for December 1999, Spout Run, Clarke County, VA. 102

25. Appendix A. Fecal coliform and fecal streptococcus counts for January 2000, Spout Run, Clarke County, VA. 103

26. Appendix A. Fecal coliform and fecal streptococcus counts for February 2000, Spout Run, Clarke County, VA. 104

27. Appendix A. Fecal coliform and fecal streptococcus counts for March 2000, Spout Run, Clarke County, VA. 105

28. Appendix A. Fecal coliform and fecal streptococcus counts 106

for April 2000, Spout Run, Clarke County, VA.

29. Appendix A. Fecal coliform and fecal streptococcus counts for May 2000, Spout Run, Clarke County, VA. 107

30. Appendix B. Chemical analyses for June, 1999, Spout Run, Clarke County, VA. 109

31. Appendix B. Chemical analyses for July, 1999, Spout Run, Clarke County, VA. 110

32. Appendix B. Chemical analyses for August, 1999, Spout Run, Clarke County, VA. 111

33. Appendix B. Chemical analyses for September, 1999, Spout Run, Clarke County, VA. 112

34. Appendix B. Chemical analyses for October, 1999, Spout Run, Clarke County, VA. 113

35. Appendix B. Chemical analyses for November, 1999, Spout Run, Clarke County, VA. 114

36. Appendix B. Chemical analyses for December,1999, Spout Run, Clarke County, VA. 115

37. Appendix B. Chemical analyses for January, 2000, Spout Run, Clarke County, VA. 116

38. Appendix B. Chemical analyses for February, 2000, Spout Run, Clarke County, VA. 117

39. Appendix B. Chemical analyses for March, 2000, Spout Run, Clarke County, VA. 118

40. Appendix B. Chemical analyses for April, 2000, Spout Run, Clarke County, VA. 119

LIST OF FIGURES

		Page
1.	Spout Run Watershed, Clarke County, VA.	3
2.	Average of fecal coliform (FC) counts overall. FC-61, upper Millwood, FC-56, middle Millwood, FC-54, lower Millwood.	69
3.	Average of fecal streptococci (FS) counts overall. FC-61, upper Millwood, FC-56, middle Millwood, FC-54, lower Millwood.	70

I. INTRODUCTION

Many disadvantaged communities suffer from health-related problems attributed to poor water quality and sanitation. Contaminated water has many possible sources that include lack of adequate waste treatment and disposal facilities, rapid-paced rural development, and agricultural (livestock and poultry) activities. Numerous surface and ground waters in Virginia (and all states) are contaminated by fecal pollution. This pollution results in increased health risks to persons exposed to the water, degradation of recreational and drinking water quality, and nutrient loss from the watersheds to larger water bodies such as Chesapeake Bay. Unfortunately, the source(s) of this pollution often cannot be readily determined. Knowledge of the origins of pollution sources will aid in the restoration of water quality, reduce the amounts of nutrients leaving the watershed, and reduce the danger of infectious disease from exposure to these waters. The National Watershed Database, maintained by the U.S. Environmental Protection Agency (EPA), contains the following quote: (http://www.epa.gov/surf3/states/VA) "Virginia: Fecal coliform bacteria are the most widespread problem in rivers and streams."

The Spout Run watershed is a Virginia Department of Conservation and Recreation (DCR)-funded (Section 319) project in Clarke County, Virginia, in cooperation with Ms. Alison Teetor, the Natural Resource Planner for Clarke County. The Spout Run watershed contains the community of Millwood, and Spout Run is the stream that flows through the community. Spout Run is 10.2 km long and the area for the Spout Run water basin is 2,665 ha. Of the 2,665 ha, 396 ha is wooded land, 1,182 ha is pasture/crop, 150 ha is residential and 66 ha is urban.

This project has two distinct objectives: the first objective is to develop a library of fecal bacterial profiles from known sources in the watershed around Millwood. The second objective is to compare fecal bacterial isolates from stream samples (unknown origin) to determine the sources of fecal pollution in the water. Water samples were taken monthly at points in upper,

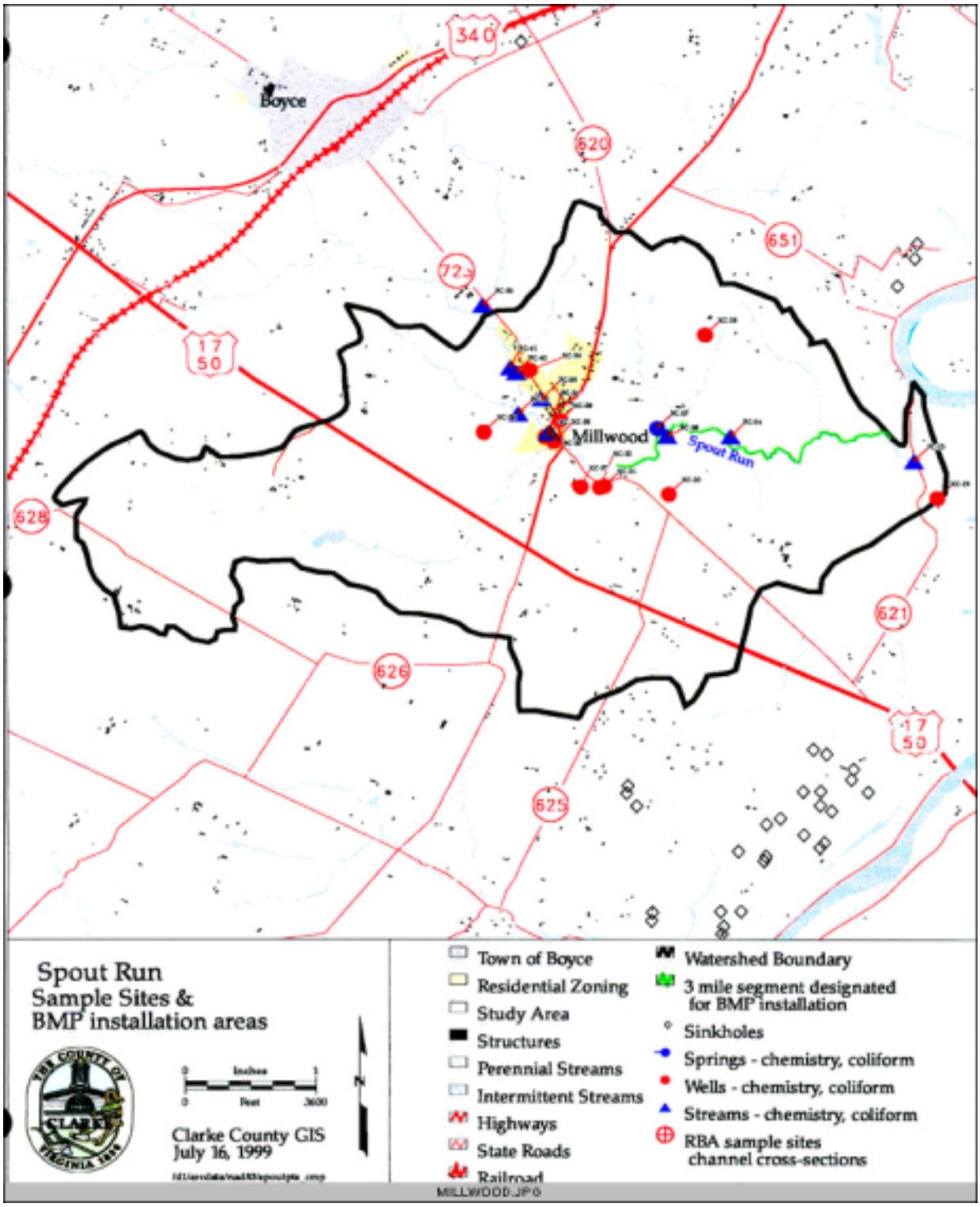
middle, and lower Millwood (Figure 1). The fecal bacteria in the water samples were isolated, characterized, and run against the isolate profile library to determine the fecal load that Millwood adds to Spout Run and the proportion of the isolates from human sources.

A novel methodology, bacterial source tracking, (BST) will be used to meet the research objectives. BST methods can be subdivided into three basic groups: molecular, biochemical and chemical. BST methodology is based on biochemical characteristics (antibiotic resistance profiling) and randomly amplified polymorphic DNA (RAPD) profiles in fecal bacteria. Development of source tracking methodology is new, and is being driven by the total maximum daily loading (TMDL) concept that is now being implemented in Virginia by DCR and EPA.

Millwood is a disadvantaged community of approximately 130 people in 82 homes on either lots or small farms. Most of the homes are over 50 years old, most obtain water from individual wells, and a few homes do not have indoor plumbing. A septic tank and drainfield, outhouse, or cesspool provides waste disposal. The Virginia Department of Environmental Quality (DEQ) and Virginia Department of Health (VDH) have designated Millwood as an "at risk" community due to fecal bacteria in well water samples and in Spout Run.

Preliminary sampling by county and state officials identified spout run as contaminated by fecal pollution and resulted in Spout Run being added to DEQ's impaired stream list. No research to source isolates from Spout Run has been performed. Until sources of pollution can be identified, risk to communities cannot be accurately assessed, and water quality improvement will remain a hit-or-miss affair that is not efficient. My research results can provide regulatory agencies with a critical tool to identify sources of fecal pollution in water. Information obtained from my project can be an example for future research and education projects that will provide both water supply and waste treatment improvement options to target communities.

Figure 1. Map of Spout Run Watershed, Clarke County, VA.



REVIEW OF LITERATURE

A. Sanitary Significance of Fecal Coliforms in the Environment

The presence of coliform bacteria in potable water indicates unsuitable sanitation practices. Such occurrences may be a result of poor water treatment, plant design problems, improper operating procedures, inadequate hygienic practices in plant operation, or aftergrowths in the distribution system (Geldteich, 1996). Nearly all-natural waters are habitats for coliform bacteria (Kott, 1977). The populations of coliforms should be noticeably curtailed by treatment, together with disinfection. Nonetheless, fecal coliform bacteria are not innate inhabitants of water, but are also automatically attributable to fecal pollution. Only a small number of the many fecal microorganisms found in wastewater and capable of contaminating drinking water could be considered as pollution indicators (Kott, 1977). These organisms include C*lostridium perfringens, Streptococcus faecalis,* the fecal coliforms, and *Escherichia coli* (Kott, 1977).

Water is necessary to sustain life and therefore every attempt should be made to achieve a drinking water quality as high as feasibly possible (Gleeson and Gray, 1997). Failure to maintain high water quality exposes the population to the peril of disease, principally the very young, elderly, the sick and those who live in sub-standard sanitary surroundings. Microbial contamination is yet considered to be the most serious risk factor in drinking water quality because of the possible consequences of waterborne disease (Gleeson and Gray, 1997).

Great value is placed on the quality of recreational waters and their appropriateness for activities such as swimming, surfing, boating and fishing. Vigilant management of rivers,

lakes and coastal waters is essential to protect them for recreational use and to avoid further damage (EPA, 1997). Parameters to be used for determining recreational water quality include:

- an assessment of bacterial water quality using fecal coliforms and enterococci as indicator organisms
- an assessment of the presence of nuisance organisms, such as algae
- as assessment of visual clarity and color
- a measurement of pH
- a visual assessment of surface films
- a measurement of temperature.

Guidelines both make a distinction between primary and secondary contact recreation. Primary contact recreation includes sports such as swimming and surfing, where the user comes into frequent direct contact with water. Secondary contact recreation includes activities such as boating and fishing, in which there is less frequent body contact with the water and there is little chance of swallowing water.

A particular issue for recreational use is algal blooms. These decrease the aesthetic value of recreational waters by reducing water clarity and accumulating along shorelines. They may also pose a health risk to humans who have direct contact with affected waters. Some species, blue-green algae for example, are potentially toxic and may cause skin irritations, gastro-intestinal disorders and influenza-like symptoms. Although monitoring of recreational water quality does not address the problem of water contamination directly, it is vital for protecting public health by identifying areas of particular concern for correction and educating the community (EPA, 1997).

A.1 Health Hazards Associated with Contaminated Water

Existing approaches for controlling health risks posed by microbes in drinking waters are centered on a barrier methodology involving treatment of wastewater as well as the treatment of raw waters (Gleeson and Gray, 1997). This methodology consists of disinfection and the creation of acceptable limits for indicators of water quality. In 1997, roughly two-thirds of the world's population lacked access to potable water supplies and ample sanitation (Gleeson and Gray, 1997). The World Health Organization estimates that 80% of all illness in the world was attributable to insufficient water supplies or sanitation. Over 250 million new cases of waterborne diarrhea are reported worldwide each year, resultant in more than 10 million deaths. Today there are many recognized waterborne pathogens. All are present in large numbers in human or animal waste; sporadically both are commonly resistant to environmental decomposition. Many of these pathogens are proficient in causing infections even when ingested in extremely small numbers (Gleeson and Gray, 1997).

Viruses, bacteria and protozoa are the three principal groups of microorganisms that can be transmitted via drinking water (Gleeson and Gray, 1997). They are all transmitted by the fecal-oral route, and so largely arise either directly or indirectly by contamination of water resources by sewage or possibly animal wastes (Gleeson and Gray, 1997).

Infections related to water are classified into four main groups. Waterborne disease refers to transmission of a pathogen followed by ingestion of contaminated water (Gleeson and Gray, 1997). Cholera and typhoid fever are classical examples of waterborne diseases. Secondly, in disease outbreaks pathogens can also be spread from one person to another, facilitated by a lack of an adequate supply of water for washing. Many diarrhea diseases as

well as disease of the eyes and skin are transmitted this way. Thirdly, water-based infections such as schistosomiasis are diseases caused by pathogenic organisms that spend part of their life cycle in aquatic organisms. Lastly, insect vectors that breed in water cause water-related diseases, such as example the spread of malaria by mosquitoes (Gleeson and Gray, 1997).

A. 2 Risk Assessment

Any dialogue on drinking water quality standards should include some consideration of risk assessment. Risk assessment is an effort to compute the potential health consequences of human exposure to specific conditions (Gleeson and Gray, 1997). Risk to human health is defined as the probability or chance that a given exposure to a microbial pathogen may possibly bring about illness or damage to health of the exposed persons. In setting any standard with regard to public safety, a definite balance must be attained between the profits of safety and the expenditures of accomplishing it (Gleeson and Gray, 1997). When considering the risks presented by consumption of drinking water several factors must be carefully evaluated. Some of these factors include determining the current health risk posed by modern day water supplies to public health and whether it is an acceptable risk. Another issue of concern involves the cost of upholding stringent standards in economic and social terms.

When considering the risks to human health from consumption of water supplies a number of other factors must be evaluated. Microorganisms causing illness by the water route must be identified and characterized, and the occurrence and distribution of the organism must also be assessed (Gleeson and Gray, 1997). The limits of analytical tools for pathogen and indicator detection must also be evaluated. The risks involved in terms of

human health and social costs can be determined using this type of information (Gleeson and Gray, 1997).

A. 3 Sources of Water in the United States

Raw source waters, surface waters and ground water are the three major sources of water in the United States (Geldreich, 1996). A number of issues have entered into the options of best obtainable raw source water for potable supply. These considerations include adequate quantity during any seasonal variation in flow, water quality that is modifiable to cost-effective treatment, and some degree of watershed protection from domestic, industrial, and agricultural pollution. Water supply utilities have a greater burden placed on them due to increasing urban populations (Geldreich, 1996). This burden is in terms of the quantity demanded and in terms of contamination potential through the use of less sought-after raw water, resulting from polluted rivers, lakes and groundwater. Wastewater treatment plants, urban and rural runoff, agricultural activities and industrial chemical spills are repeatedly discharged to watercourses that may well be the source of a public water supply (Geldreich, 1996). Many of these wastes contain pathogens and innumerable of other waterborne organisms. There are a few essential ingredients to protecting downstream use of water supplies such as waste minimization management, waste treatment of point source discharges, and controlled treatment of urban stormwater (Geldreich, 1996). There must be a concentrated attempt to make the most of public health protection through a multiple treatment barrier concept and not force the water purveyor to either demonstrate a public health threat to make upstream treatment of waste mandatory or have utilities assume the full burden of treatment for poor-quality water resources (Geldreich, 1996).

Raw surface water is subject to an assortment of bacterial contaminants introduced in stormwater runoff over its supply watershed and the upstream discharges of domestic and industrial wastes. These sources of pollution introduce a wide range of infectious agents to waters that may ultimately be used in water supply (Geldreich, 1996).

Receiving waters between the point of discharge of sewage effluents and water intakes may not just serve as a buffer to accidental spills and treatment bypasses but can add to water quality improvements by way of natural self-purification (Geldreich, 1996). Every stream, lake, and groundwater aquifer has some limited capacity to assimilate waste effluents and storm water runoff entering the drainage basin. This self-purification process is a multifaceted and ill-defined mechanism that includes bacterial adsorption with sedimentation, predation, dilution, hydrologic tributary effects, water temperature, and solar radiation (Geldreich, 1996).

Rivers are subject to considerable fluctuations in water quality as a result of stormwater runoff and spills of municipal and industrial wastes. While these sources assure that quantity will be available to meet the needs of the growing community, these waters are not necessarily of the best quality (Geldreich, 1996).

A. 4 Waste Disposal in the United States

There is a rising consciousness of the high amenity value of clean water in the U.S. today. Agriculture is regularly held responsible for increasing the eutrophication status of water bodies, and landspreading of animal manure in a haphazard fashion is one of the practices that causes the biggest outcry (DiPaola, 1998).

When publicly owned treatment works (POTW) are not obtainable, discarding of human waste transpires through the use of a septic system, cesspool, privy or other analogous types

of on-site wastewater treatment and disposal systems (OSWTDS). Septic tanks and privies account for the highest total volume of water discharged directly into groundwater and are frequently implicated as sources of groundwater contamination (DiPaola, 1998).

Septic effluent entering aquifers used for drinking water are the most common ground water contamination problems reported from individual home sites (Geraghty and Miller, 1978). Contaminated groundwater used for human, domestic, or recreational purposes can be the cause of occurrences of waterborne disease (Craun, 1985). Contamination of surface or groundwater from the overflow or seepage of sewage from septic systems and cesspools has been known to cause high percentages of outbreaks of illnesses reported from contaminated, untreated groundwater supplies (Craun, 1981). Researchers have suggested preventing contamination of drinking water sources, appropriate treatment of contaminated sources, and monitoring to identify contamination as areas of emphasis in the quest to safeguard drinking water sources (DiPaola, 1998).

B. Exposure Pathways for Fecal Contamination

Water quality is most directly impacted by how nutrient management practices affect the environment (Brady and Weil, 1999). The principal water pollutants to be considered are nitrogen, phosphorus, and sediments well as fecal indicator bacteria that can be found amongst these substances. The primary means to avoid damaging surface waters with excess nitrogen, phosphorous, and fecal contamination is to manage the rate at which these nutrients are applied (Brady and Weil, 1999). Fecal contamination in water occurs from three main sources, livestock, wildlife and humans.

B.1 Livestock

Where animal and crop production are integrated on a farm, manure handling is not too much of a problem (Brady and Weil, 1999). The use of pasture can be maximized so that the animals themselves spread much of the manure while grazing. The manure from confined animals is produced in small enough quantities to be hauled daily to the fields or be stored under cover during periods when soil conditions are not favorable for spreading manure (Brady and Weil, 1999).

The problem of manure disposal takes precedence over its utilization where animals are concentrated in large confinement systems (Brady and Weil, 1999). Manure from such systems is handled by four general management procedures. The manure can be collected and spread daily or the manure may be stored packed in a pile where it is allowed to partially decompose before spreading. Also, the manure can be stored in aerated ponds that are sufficiently shallow to permit fairly ready oxidation of the organic materials. Lastly, manure is stored in deep anaerobic lagoons where it is allowed to ferment in the absence of elemental oxygen (Brady and Weil, 1999).

Water and excess nutrients can leach through soils to the groundwater and into streams and lakes, giving rise to contamination, algal blooms and eutrophication. Methods of manure handling that both prevent pollution and preserve nutrients would make a major contribution to ameliorating the manure problem for concentrated animal production enterprises (Brady and Weil, 1999).

B.2. Wildlife

Animals such as deer, geese, beavers, etc. contribute to fecal contamination in streams. Often it is hard to eliminate contamination from wildlife sources because of the inability to

control the movement of the animals. Contamination from these sources is likely to be more severe in the summer months as the animals seek a means of cooling themselves and require greater amounts of water.

Most fecal contamination in watercourses occurs as a result of an animal defecating directly into the flowing water (Miner, 1990). Fecal material deposited on the stream banks will reach the water only under conditions of overland flow (Miner, 1990).

B.3. Human

Effluent form septic tanks that pollute natural soils and water by contaminating organisms is a serious concern (DiPaola, 1998). The number of families not serviced by sanitary sewage systems continues to increase with the development of suburban and rural areas. Septic tanks and subsurface absorption fields are usually employed for the disposal of domestic wastes in the absence of centralized facilities (DiPaola, 1998). Over 32 million people in the United States utilize septic tanks and subsurface absorption fields for disposal of wastewater. Effluents from septic tanks should be disposed of in such a manner as not to result in bacteriological contamination of surface or ground waters (DiPaola, 1998). The Federal Water Pollution Control Administration (1968) has suggested that safe, clear, potable, aesthetically pleasing, and acceptable public water supplies can be obtained from raw surface water containing up to 10,000 coliform/100 ml or 2000 fecal coliforms /100 ml. The desired levels, however, are less than 100 coliforms or 20 fecal coliforms/100 ml (DiPaola, 1998).

Fecal coliforms characteristically inhabit the intestines of warm-blooded animals such as humans and are, therefore, more indicative of recent and potentially dangerous sources of

pollution. Members of the total coliform count may be routinely detected in soil, on plants and insects, in old sewage, and in water polluted sometime in the past (DiPaola, 1998).

C. Bacterial Indicators for Drinking Water Quality

The absence of coliforms when a minimum of 100 ml is tested by the membrane filter (MF) technique or 50ml by the most probable number (MPN) method indicates drinking water of good bacteriological quality in the United States. Due to limitations such as media selection, temperature and incubation time, some ambiguity exists regarding the coliform group (Ptak and Ginsburg, 1977). Consequently, pathogens and secondary invaders are not recognized even though their numbers sometimes appear to exceed those of the coliform group (Ptak and Ginsburg, 1977).

The enterococci or fecal streptococci have been proposed as an indicator to the quality of drinking water, but data has shown that coliforms are better suited since the fecal streptococci die off very rapidly in water of good quality and are not as numerous (Ptak and Ginsburg, 1977). However in studies involving natural or fecally polluted water or soils, fecal streptococci appear to survive longer than bacterial pathogens (Clausen et al., 1977). The enterococci might well be better indicators of pollution in wells (animal contamination), swimming pools, and sewage treatment plants that are high in nutrient and low in oxygen. Similarly the fecal coliforms might be used in areas other than drinking water, since they are already included in the total coliform count and also die off rapidly in water of good quality (Ptak and Ginsburg, 1977).

The modern interpretation of the presence of the coliform group as a pollution indicator depends on the objectives of the pollution investigation (Geldreich, 1996). In some studies, the total coliform group will be the indicator of choice, whereas in another investigation with

a different objective a portion of the group or even a single species may be utilized (Geldteich, 1996).

C.1. Criteria for Indicator Organisms

For an organism to serve as an indicator organism, it must meet several criteria (Gleeson and Gray, 1997). The organism must be of value as an assessor of fecal contamination and should be a member of the normal intestinal flora of healthy people and should be exclusively intestinal in habitat and therefore exclusively fecal in origin if found outside the intestine. Ideally they should only be found in humans and should be present when fecal pathogens are present. These organisms should be present in greater numbers than the pathogen they are intended to indicate (Gleeson and Gray, 1997). They should be unable to grow outside the intestine with a die-off rate slightly less than the pathogenic organism and be easy to isolate, identify and enumerate. Indicator organisms should also be non-pathogenic (Gleeson and Gray, 1997).

The concentration of indicator organisms should be related to the extent of fecal contamination and by implication to the concentration of pathogens and the incidence of waterborne disease. There is no absolute correlation between the numbers of the indicator organism present and the actual presence or numbers of enteric pathogens. The finding of an indicator organism in properly treated water indicates the presence of material of fecal origin and thus has been the principal means by which the sanitary quality of water has been determined (Gleeson and Gray, 1997).

C.2. MPN and Membrane Filtration

Assessment of microbiological drinking water quality by the detection and enumeration of coliform bacteria has existed in some manner since 1912 (Bancroft et al., 1989). The original test for coliforms involved the presumptive, confirmed and completed tests. The presumptive step is performed by a process of tubes inoculated with three different sample volumes to give an estimate of the most probable number (MPN) of coliforms in the water. The complete process, including the confirmed and completed tests entail at least 4 days of incubations and transfers (Prescott et al., 1996).

Regrettably the coliforms comprise a wide range of bacteria whose initial source may not be the intestinal tract. Specific tests have been developed to test for coliforms and fecal coliforms. These tests include the membrane filtration technique, the presence-absence (P-A) test for coliforms and the related Colilert defined substrate test for detecting both coliforms and *E. coli* (Prescott et al., 1996)

The membrane filtration technique has become a widespread and often favored method of assessing the microbiological quality of water. The water sample is passed through a membrane filter. The filter can have trapped absorptive pad containing the desired liquid medium or the filter may be transferred with sterile forceps to solid media. The use of the proper medium allows the rapid detection of total coliforms based on identification of characteristic colonies. There are several advantages and disadvantages to the use of the membrane filter technique for evaluation of the microbial quality of water. Some advantages include 1) good reproducibility 2) single-step results often possible 3) filters can be transferred between different media 4) large volumes can be processed to increase assay sensitivity 5) time savings are considerable 6) ability to

complete filtrations on site 7) lower total cost in comparison with MPN procedure. Some disadvantages include 1) high-turbidity waters limit volumes sampled ,2) high populations of background bacteria cause overgrowth and 3) metals and phenols can adsorb to filters and inhibit growth (Prescott et al., 1996).

The presence-absence test (P-A test) can be used for coliforms. This is a modification of the MPN procedure, in which a larger water sample (100 ml) is incubated in a single culture bottle with a triple-strength broth containing lactose broth, lauryl tryptose broth and bromcresol purple indicator. The P-A test is based on the theory that no coliforms should be present in 100 ml of drinking water. A positive test results in the production of acid (a yellow color) and represents a positive presumptive test requiring affirmation (Prescott et al., 1996)

To test for both coliforms and *E. coli* the related Colilert defined substrate test can be used. A water sample of 100 ml is added to a specialized medium containing O-nitrophenyl-β-D-galactopyranoside (ONPG) and 4-methylumbelliferyl- β-D-glucuronide (MUG) as the only nutrients. The medium will turn yellow within 24 hours at 35˚C due to cleavage of the ONPG in the presence of coliforms. To test for *E. coli* the medium is viewed under long-wavelength UV light for fluorescence. The MUG is modified to yield a fluorescent product when *E. coli* is present. If the test is negative for the presence of coliforms, the water is considered acceptable for human consumption (Prescott et al., 1996).

C.3. Fecal Coliforms as Indicators of Pollution

The single most important indicator of public health hazard from infectious agents is the occurrence of fecal coliform bacteria in water (McFeters and Stuart, 1972). The

original concept of using coliforms and fecal coliforms as indicator organisms revolutionized the whole approach to public health microbiology. The considerable decline in the number of reported waterborne outbreaks of classical communicable disease such as cholera and typhoid fever can be attributed to the effectiveness of the coliform index. While numerous studies have shown that the presence of thermotolerant coliforms generally indicates that fecal contamination has occurred, their presence in water does not always imply a health hazard. Also, the absence of coliforms does not necessarily indicate that the water is safe for consumption, in particular from enteric viruses and pathogenic protozoans (Gleeson and Gray, 1997).

The presence of fecal coliform bacteria is a reliable indicator of fecal contamination from warm-blooded animals (USDA, 1997). Fecal material in water where humans swim or where shellfish are harvested presents a significant risk of infection from pathogenic organisms. The major sources are untreated sewage are effluent from sewage treatment plants, and runoff from pastures, feedlots, and urban areas (USDA, 1997).

C.4. Fecal Streptococci as Indicators of Pollution

Fecal streptococci are present in the feces of humans and warm-blooded animals (Clausen et al., 1977). Although consistently recovered from waters known to receive fecal contamination, there is no indication that they multiply in natural or fecally polluted water or soils. They appear to survive longer than bacterial pathogens and are not considered to be of pathogenic significance themselves. For these reasons, fecal streptococci have been long regarded as potential indicators of fecal pollution (Clausen et. al, 1977). Although fecal streptococci may not prove to be an ideal indicator in all

circumstances, the use of streptococci may be advised in several situations. For instance, when assessing the quality of reservoirs, drinking water, and other waters in which viral contamination is particularly undesirable. Also, when determining the quality of organically rich water, especially those waters receiving chlorinated sewage effluent. Finally, when attempting to locate the sources of fecal pollution (Clausen et. al, 1977).

D. Watershed Studies

Watersheds are areas of land that drain to a stream or other water body. Most pollution control projects concentrate their activities around watersheds, because watersheds incorporate the impacts that land use, climate, hydrology, drainage, and vegetation have on water quality. By concentrating pollution control project activities around a watershed, individuals living in that area are allowed to learn about the water resource they affect, and how to participate in its protection (EPA, 1995).

Monitoring the water resource in a watershed is necessary to identify and record pollution. Monitoring is also essential to constantly evaluate water quality and the health of the water resource. The most dependable way to ascertain if changes in land-based activities have affected water quality is to monitor the land and the water resource before, during, and after a change in land management or restoration occurs (EPA, 1995).

At a watershed scale, the relationship between changes inland management and water quality can only be established by following a precise experimental plan, or monitoring protocol. Detailed pursuits of both land management and water quality is important to supply information to decision makers about the efficiency of pollution control efforts (EPA, 1995).

In non-urban and suburban areas, rainwater often flows directly over farms, roads, golf courses and lawns into waterways (Testing the Watershed, 1998). Farm runoff may contain high concentrations of pathogenic animal waste, fertilizers, and pesticides. Significant amounts of fertilizer, animal waste and other chemicals are often found in suburban runoff. This uncontrolled runoff can foul streams in a watershed. Animal waste can also contain pathogens usually not found in human waste, such as *Cryptosporidium*. Animal waste from large feedlots, when spread too heavily on fields, run off and has been linked to outbreaks of a toxic microorganism, *Pfisteria piscicida*, in the Chesapeake Bay region and North Carolina, causing numerous waterway closings (Testing the Watershed, 1998).

In watersheds where even low levels of fecal contamination are of concern it is logical to look for economical ways to limit livestock defecation directly into live streams (Miner, 1990). One promising way to reduce the water quality impact of grazing cattle, without exceeding the current water quality constraints, is to reduce the amount of time the animals spend in the stream, so the opportunity for direct fecal deposition into the water is diminished (Miner, 1990).

Studies showed that the amount of time cattle spend drinking or loafing in the area of the spring was dramatically reduced around 90% by the presence of a water tank (Miner, 1990). Even when the feed source was placed equal distance between the water tank and the stream, the water tank was effective in reducing the amount of time the cattle spent in the stream (Miner, 1990). In terms of water quality, the relationship between time spent in the stream and fecal pollution is evident since it was possible to eliminate 90% of the animal's use of the stream through the use of a watering tank. Economic and

environmental implications suggest that this may be a viable alternative to the total exclusion of livestock along sensitive stream systems (Miner, 1990).

D. 1 The Regulatory Process and TMDLs

The publishing of the Public Health Service Act of 1914 was the beginning of legislation to protect bacteriological quality of drinking water in the USA (Gleeson and Gray, 1997). Initially these regulations were not mandatory for all public water supplies. However regulations did become mandatory in 1974 with the passing of the Safe Drinking Water Act (SDWA). This particular act required the US-EPA to establish national standards for drinking water quality. All public water systems serving 25 or more persons or from which 15 service connections were now federal responsibility. "EPA first regulated chemicals in drinking water by establishing maximum contaminant levels (MCLs) and sampling requirements for nine inorganic chemicals (IOCs), and six synthetic organic chemicals (SOCs) in the Interim Primary Drinking Water Regulations of 1975" (EPA, 1997). "In accordance with the Safe Drinking Water Act Amendments of 1986, EPA began adding to its list of regulated chemicals. In 1987, EPA adopted standards for eight volatile organic chemicals (VOCs) in the Phase I Rule. From that point on, regulations for contaminants in drinking water have been referred to as National Primary Drinking Water Regulations (NPDWRs)" (EPA, 1997). Interim regulations were published in 1975 that provided for a minimum number of samples to be examined each month and established the maximum number of coliforms (Maximum Contaminant Level or MCL) allowable per 100 ml of finished water (Gleeson and Gray, 1997). MCLs are based on the presence or absence of coliforms in a sample. For systems where at least 40 samples per month are analyzed more than 5% of the monthly samples may be

coliform positive. The MCLs for those systems collecting less than 40 samples per month is no more that one coliform positive sample per month. If the system is found to be in violation of the MCL for total coliforms then, the public must be notified (Gleeson and Gray, 1997).

The SWDA was amended in 1986, significantly expanding the original act. Under the 1986 amendment disinfection was required for all water systems (Gleeson and Gray, 1997). Also the use of lead in any pipe, solder, flux or fittings in any public water system was prohibited with the amendment as well (Gleeson and Gray, 1997). Enforcement procedures were streamlined, which included raising penalties to $25,000 per day for infringements (Gleeson and Gray, 1997).

In July 1989, USDA began the Water Quality Initiative (WQI) to promote both traditional and new land treatment and agrochemical management practices that would reduce pollutant loading to ground and surface water (USDA, 1997). The Rural Clean Water Program (RCWP), initiated in 1980 and ended in 1995, was an experimental program that addressed agricultural nonpoint source pollution in 21 U.S. watersheds. The RCWP was administered by the Agricultural Stabilization and Conservation Service in consultation with EPA (USDA, 1997).

Setting Total Maximum Daily Loads (TMDLs) has been required for years. It was mandated in 1972 by Section 303(d) of the Clean Water Act (CTIC, 200). At the time TMDLs were mandated people had a more limited idea of what constituted pollution than we have now (US EPA, 1999). In 1972 people usually imagined a big pipe spewing stuff into the river (CTIC, 2000). Today we know a whole variety of sources and activities that can degrade water. In addition to setting a TMDL the responsibility for reducing

pollution among both point sources (pipes) and non-point sources such as run-off, septic systems and damage to a riparian area is still a priority (CTIC, 2000). TMDLs are critical for achieving water quality standards and are used to determine impaired waters. Implementing TMDLs is key to success in restoring impaired waters (US EPA, 1999).

D.2. Fecal Sourcing in Watersheds

While most emphasis on bacterial indicators of water quality has been placed on fecal coliforms, there are several good reasons to consider the fecal streptococci as well (Wiggins et al., 1999). There has been little success in determining the source of fecal coliforms using natural antibiotic resistance patterns as identifying markers (Wiggins et al., 1999). Conversely, these patterns do seem to have potential with the fecal streptococci. There is an observance of a higher level of antibiotic resistance in fecal streptococci that are isolated form sewage than those found in soil or on vegetation, when using fecal streptococci as indicators (Wiggins et al., 1999).

There are standards for recreational water quality with the enterococci (33 cells per 100 ml for fresh water), so these organisms are still useful as indicators (Hagedorn et al., 1999). The fecal streptococci tend to persist longer in the environment than fecal coliforms and this may be useful as indicator for less recent fecal pollution. However for determining the sources of fecal pollution, an indicator with a longer survival time can be an advantage (Hagedorn et al., 1999). Lastly, there are some potential sources of fecal contamination (e.g. composted animal and poultry litters, and advanced-treatment Class B biosolids) where it is difficult to isolate fecal coliforms while there is no difficulty in isolating fecal streptococci. Fecal coliforms would not be suitable for sourcing contamination from these types of materials (Hagedorn et al., 1999).

The Virginia Department of Environmental Quality's (VADEQ) most recent water-quality assessment (1998) states the following about bacterial contamination in Virginia: 'In general fecal coliform bacteria exceedances are the leading cause of non or partial support of designated uses in rivers and streams. Agricultural practices appear to be one of the primary sources causing the loss of designated use support. Indications are present that uncontrolled agricultural and pasture land use result in much of the fecal coliform bacteria and nutrient contamination in Virginia's waters' (Keeling, 2000). The presence of fecal coliforms in water supplies indicates that the waste of humans, another mammal, or birds has contaminated the water. This contamination can cause health risks to people exposed to the water. Drinking and recreational water quality is diminished by fecal coliform contamination and can lead to closure and bans on recreational water uses (Hagedorn, 2000). In the past, sources of fecal bacterial pollution in water could not be accurately or reliably determined.

Fecal coliform bacteria are the most widespread problem in Virginia's rivers and streams (EPA, 1995). Fecal coliform populations that exceed water quality standards have caused over one-half of the stream segments in Virginia that have been evaluated to date to be listed as impaired waters (Hagedorn et. al, 1999). The ability to develop realistic TMDLs for fecal bacteria, and implementation of cost-effective best management practices (BMPs) for watershed restoration will largely depend on using the new BST methodology to accurately identify sources of fecal pollution in water (Hagedorn et al., 1999).

Bacterial Source Tracking or BST is an innovative technology used to identify fecal coliforms in water polluted sources. Reliable identification of fecal contamination

sources will help direct efforts and expenditures to the correct source(s) of the impairment (Hagedorn, 2000).

Bacterial Source Tracking or BST is new methodology that is being used on fecal bacteria from environmental samples such as from human, livestock, or wildlife origins to determine the sources of those bacteria (Hagedorn et. al, 1999). A methodology having the ability to turn nonpoint sources into point sources is how BST has been described (Hagedorn, 2000). Fecal sourcing, fecal typing and molecular source tracking are other aliases for BST that are found in scientific literature (Hagedorn et al., 1999). The recent implementation of the TMDL concept by US-EPA is the main driving force behind BST development in Virginia (Hagedorn et al., 1999).

There are three basic groups of BST methods: molecular, biochemical, and chemical. Molecular (genotype) are all referred to as DNA fingerprinting and are based on the unique genetic makeup of different strains, or subspecies, of fecal bacteria (Hagedorn, 2000). Biochemical (phenotype) methods are based on an effect of an organism's genes that actively produce a biochemical substance. The type and quantity of these substances produced is what is actually measured. Chemical methods are based on finding chemical compounds such as detergents and optical brighteners that are associated with human wastewaters (Hagedorn, 2000).

Molecular methods or DNA fingerprinting have received the greatest publicity, but to date there are at least ten or so different methods described in the scientific literature that show potential (Hagedorn, 2000). BST development is so new that no research comparing BST methods or identifying their relative strengths and weaknesses has yet been completed. It is most reasonable to assume that some combination of BST

methods will be needed to provide the most accurate and reliable source identification answers (Wiggins et al., 1999). It is doubtful that any one BST method will emerge as the "best" method for all situations. Molecular methods offer very precise source identification, however they are limited by cost, detailed and time-consuming procedures, and are not yet suitable for assaying large numbers of samples in a reasonable time frame (Hagedorn, 2000). Biochemical methods may or may not be as precise, but are simpler, quicker, less costly, and allow large numbers of samples to be assayed in a short period of time. Chemical methods are based on finding chemical compounds that are associated with human wastewaters, and are very experimental at present. Perhaps the best approach is to use a biochemical and/or chemical method(s) to run large numbers of samples, and then confirm both the method(s) and the results by assaying some subset of samples by a molecular procedure (Hagedorn et. al, 1999).

Presently, BST can very reliably determine if fecal bacteria are from human or animal sources (Hagedorn, 2000). If the bacteria are from animal sources, BST can also tell if the animals are livestock or wildlife, but less reliably than the human vs. animal separation. The key to correct existing fecal bacterial pollution and to prevent further problems is by reliably and accurately finding the contamination source(s). Therefore, BST should be heavily used in the future as a tool much like watershed computer models (Hagedorn, 2000).

E. Non-Genomic Techniques in Fecal Typing

Several attempts have been made to develop methods to determine the sources of fecal pollution (Wiggins et al., 1999). Initially, the ratio of fecal coliforms to fecal streptococci was used as an indicator of the source: a high ratio (>4) was considered to

indicate an animal source, while a low ratio (<0.7) suggested a human source. This ratio has since proven unreliable, and the method has been abandoned (APHA, 1995).

There are several biochemical methods for determining fecal contamination. Antibiotic Resistance Analysis (ARA) is a method used to differentiate between bacterial sources (Wiggins et al., 1999). This method uses fecal streptococcus or *E. coli* and patterns of antibiotic resistance for separation of source animals. The premise is that human fecal bacteria will have the greatest resistance to antibiotics and that domestic and wildlife animal fecal bacteria will have significantly less resistance to the battery of antibiotics used in this method. Since this is not a molecular method there is no genetic linkage to source animals. Additionally, the antibiotics used on livestock can change by region, which may require unique isolate libraries for each region of the state or country (Wiggins et al., 1999). ARA was chosen for use in this research. Details regarding ARA is located in the materials and methods section.

F-specific (F+ or FRNA) coliphage is a method that is used to source track *E. coli* (Miesfeld, 1999). Considered more indicative of viral contamination FRNA coliphage is a pathogen of *E. coli* infecting the pilus of the male *E. coli.* These coliphages can be differentiated using serology (Miesfeld, 1999). There are four antigenically distinct serogroups of FRNA coliphages, and those predominating in humans (groups II and III) differ from those predominating in animals (groups I and IV). Consequently, it may be possible to distinguish between human and animal wastes by serotyping FRNA coliphage isolates. However, there is a problem with separation between human serotypes and serotypes associated with pigs, which also contain group II. Additionally not all animals have FRNA coliphage associated with their respective *E. coli* (Miesfeld, 1999). The

coliphage is persistent in the environment for less than a week and survival is a function of sunlight and water temperature. Ultraviolet light denatures the virus and below 25˚C F-pilus synthesis ceases. The coliphage does not replicate in the environment but only in the presence of F-pilus *E. coli*, and is not found in sediments, but rather just in the water column (Miesfeld, 1999).

Sterols or fatty acid analysis involves the constituents fatty acids in cell walls and membranes. This method is currently under development, however there are hopes to differentiate between the types and quantities of sterols in human *E. coli* cell walls and membranes verses those in other animals (Keeling, 2000).

A nutritional pattern is a technique is based on differences among bacteria in their use of a wide range of carbon and nitrogen sources for growth. This method works well in the laboratory. However, there are many environmental factors in a watershed that can affect bacterial nutrient requirements that may make this method impractical for field determination (Hagedorn, 2000).

Fecal-bacteria ratios are based on the ratios of many different types of stomach and intestinal bacteria, not just fecal coliform bacteria and enterococci. This method is not considered reliable for accurate source identification, but can be used as a general indicator of human verses non-human fecal bacterial contamination (Hagedorn, 2000).

Bacteriophages have also been proposed as indicators of the source of fecal pollution. However the usefulness of bacteriophages as an indicator is limited because only a small percentage of fecal samples contain phages (Wiggins et al., 1999). Other studies have shown that there are differences in the species composition of fecal streptococci among

various types of animal as seen in different percentages of various fecal streptococci in the feces of poultry, cattle, and other animals.

E.1. Antibiotic Resistance Patterns

The latest trend in non-genomic techniques in fecal typing has been the introduction of antibiotic resistance patterns (Wiggins et al., 1999). The use of antibiotics in animals can result in the occurrence of antibiotic-resistant bacteria in those animals (Wiggins et al., 1999). Several attempts have been made to compare the pattern of antibiotic resistance in fecal coliform with the sources of the isolates. The multiple-antibiotic-resistance (MAR) index of *E. coli* shows that resistance in wild animals was generally low, while human and poultry isolates had higher MAR indices. There were also fewer MAR strains of *E.coli* isolated from rural that from urban sources (Wiggins et al., 1999).

Fecal streptococci have also been used to try to identify sources of pollution (Wiggins et al., 1999). Antibiotic resistance in fecal streptococci where higher when isolated from sewage than those that were found in soil or on vegetation. Although there have been many studies that have measured antibiotic resistance of fecal isolates from various sources, it has been difficult to use that information to identify the sources of fecal pollution (Parveen et al., 1997).

F. Genomic Techniques for Fecal Typing

The fecal bacteria in any two animals (including humans) are very much genetically the same (Hagedorn, 2000). There are unique differences, but the differences are only in a small percentage of an organism's total DNA. The key to molecular BST is finding these differences amongst a large amount of similarity (Hagedorn, 2000). The distinctions among fecal bacteria of different animal species occur because of the

different milieu found in the animal's intestines. These strains of microorganisms generally *E. coli,* are isolated using standardized laboratory procedures before fingerprinting the organisms DNA. Typically 20 to 50 isolates are obtained from each water sample (Hagedorn, 2000).

All molecular techniques require highly trained personnel and there are many places in the procedures where mistakes could happen that would skew the results and produce different band patterns. Quality assurance/quality control (QA/QC) plans should be documented and must be adhered to meticulously or results will be misleading or at worst useless (Hagedorn, 2000).

Pulse Field Gel Electrophoresis (PFGE) is one method that has been used to differentiate sources of *E. coli*. This method is considered very accurate in differentiating between strains of the same microorganism and for DNA analysis or fingerprinting. Reports show a good average rate of correct classification (ARCC >80%) however, about 88% has been the best ARCC result to date for PFGE (Simmons, 1994). All genomic methods for source tracking depend on number of mutations in the DNA, number of strains of E. coli any source animal may have, and the amount or degree of crossover from one strain to another (Hagedorn, 2000).

PFGE method is relatively slow and expensive, costing about $30 to $75 per isolate (Hagedorn, 2000). Time for analysis depends on staffing levels and generally takes days to weeks to process samples. Randomly Amplified Polymorphic DNA (RAPD) is another method that is accurate and quick for conducting DNA analysis and bacterial differentiation. This method involves a total genomic digestion that focuses on the unique polymorphisms within the DNA of an organism (Hagedorn, 2000). It is

suspected that these polymorphisms are responsible for things such as antibiotic resistance. Newer analytical devices are creating very high-resolution digital photos of the separation gels and there are software packages that will automatically add and compare results to existing databases or libraries (Hagedorn, 2000). This method is currently faster than either PFGE or ribotyping although it is comparable in expense. This method is still under development but is showing very promising results (Hagedorn, 2000).

Ribotyping (RT) is a method that has non-PCR and PCR (polymerase chain reaction) variations that can be used with it (Parveen et. al, 1999). RT is being used in the USA by other investigators with ARCC's between 68 and 82%. This method looks at the genetic differences in ribosomal RNA and is considered a very useful method of bacterial strain identification. RT is slow and expensive taking several weeks to months to get results and costs about $75 per isolate (Bernhard et al., 2000).

F. 1. Isolation of Genomic DNA from Fecal Coliforms and Fecal Streptococci

When preparing to isolate genomic DNA for fingerprinting a method that will provide high molecular weight DNA that is free of contaminants that interfere with the amplification process must be used (Graves and Swaminathon, 1993). There are affinity-based methods that result in highly pure genomic DNA but may not provide adequate yields of high molecular weight DNA. Some solution-based methods exist that provide an inexpensive and viable alternative (Graves and Swaminathan, 1993).

The solution based genomic DNA isolation method consists of five general steps: 1) addition of a detergent lysis to solubilize cellular components; 2) Rnase treatment to eliminate contaminating RNA; 3) protein precipitation that removes cytoplasmic and

nuclear proteins in the presence of salt; 4) DNA precipitation; and 5) DNA Hydration (Graves and Swaminathan, 1993).

F. 2. Quantification and Preparation of Genomic DNA

Quantitating the DNA provides the ability to accurately determine the concentration and purity of the sample. A UV-range spectrophotometer that is equipped with an ultramicrovolume quartz cuvette is needed to measure small volumes of a sample (Graves and Swaminathan, 1993).

The amount of DNA in a solution can be measured directly by determining its absorbance at 260 nm, the wavelength at which it shows a strong absorption maximum (Youston, 1999). At this wavelength the extinction coefficient of DNA is 20. This indicates that DNA at a concentration of 1mg/ml will have an absorbance of 20. The relationship between DNA concentration and A_{260} is linear up to an A_{260} of about 2.0. If $A_{260} = 20$, it means that the DNA concentration is 1000µg/ml. This method of determining DNA concentration is usually applied to purified DNA. The presence of RNA, proteins, detergents, etc., also contribute to absorbance at 260 nm and will give erroneous results for the DNA determination (Youston, 1999).

F. 3. Gel Electrophoresis of Genomic DNA Samples

DNA fragments can be electrophoretically separated from each other based upon the size of the molecules (usually expressed as the number of base pairs [bp] or kilobase pairs [kb]) (Youston, 1999). When electrophoresed through agarose (a polymer derived from seaweed), the larger fragments of DNA move more slowly through the pores in the agarose than do the smaller fragments of DNA (Youston, 1999). The population of DNA fragments generated by restriction enzymes will move through an agarose gel under the

influence of an electric field, where negatively charged DNA molecules will be repelled by the anodes and drawn to the cathodes (Becker et. al, 1996). The concentration of agarose in the gel determines the pore size, and a DNA fragment having a particular size will migrate at different rates through gels of different concentrations (Becker et. al, 1996).

There is a linear relationship between the mobility and gel concentration over a certain range of fragment sizes, so a gel concentration must be chosen that will effectively separate the molecules in the DNA population (Becker et al., 1996). Gels of 0.8% (w/v) agarose are suitable for separating linear DNA molecules 0.5-10 kb in size (Becker et al., 1996). The log molecular weights of the know marker fragments, such as λ phage cut with HindIII, can be plotted against mobility. The resulting calibration curve can be used to determine the molecular weights of unknown fragment can be estimated by direct comparison by eye to the position of λ fragments on the gel (Becker et al., 1996). The bands are visualized by ultraviolet (UV) light illumination after staining with the fluorescent dye, ethidium bromide (Becker et. al, 1996).

F. 4. Polymerase Chain Reaction

One of the most important developments in applied molecular genetics in the last decade was the invention of an amazingly simple DNA amplification strategy called the polymerase chain reaction (PCR) (Miesfeld, 1999). The logic of the PCR protocol follows directly from well-understood principles of nucleic acid biochemistry. The basic components of a PCR reaction are one or more molecules of target DNA, oligonucleotide primers, thremostable DNA ploymerase and deoxynucleotide triphosphates (dNTPs). This reaction mix is repeatedly heated and cooled to 95, 55, and 72°C, in that order, a

total of 25-35 times to produce a $>10^6$ – fold amplification of the target DNA (Miesfeld, 1999). Although the PCR reaction is relatively simple, the biochemical and kinetic parameters that affect PCR specificity and sensitivity are actually quite complex and it can sometimes be troublesome to define a reaction condition that generates reproducible results. In fact, during the first few years after PCR was described in the literature, researchers, primarily with regard to reaction specificity and sample contamination encountered numerous problems. At the time, these drawbacks seem to indicate that PCR applications may not be well suited for routine diagnostic procedures (Miesfeld, 1999). This dire prediction did not hold up as continued refinement of the technique solved the most serious problems. Nevertheless, it is important to keep in mind that each time a new DNA target or PCR primer pair is designed for a specific research objective, a number of key parameters will have to be optimized (Miesfeld, 1999).

F. 5. RAPD Analysis

The approach of random amplified polymorphic DNA (RAPD) is based on use of the PCR to amplify (make many copies) of chromosomal DNA segments that are unique to each bacterial strain. These amplified segments are separated by agarose gel electrophoresis to create a pattern or "fingerprint" that is unique to each strain or group of closely related strains. RAPD analysis has the capability to distinguish between strains of the same species (Youston, 1999).

G. Summary

Monitoring and detection of indicator and disease-causing microorganisms are a key element of microbiological studies of water quality. By chlorinating drinking water supplies, most of the principal disease-causing microbes can be eliminated. Additional

problems caused by contaminated surface run-off at recreational bodies of water and areas where shellfish are harvested pose an issue as well. At these sites water treatment barriers against contamination may not be present, and the risk of transmission of microbial diseases is higher (Prescott et al., 1996).

Watershed conditions are affected by sometimes indeterminate and always multifaceted environmental trends. The watershed approach to long-term research and monitoring of natural and remote areas within the national parks and related reserves offers significant data on ecosystem processes and relations for identifying both spatial and temporal changes in water environments.

Watershed ecosystem studies are designed for the compilation of long-term baseline data on the ecosystem health of park and equivalent reserves. Watershed level research and monitoring procedures have been a factor in the accumulation of important baseline information on deposition, hydrology, ecosystem performance, and biology. The data collections permit the separation of cause and effect relationships of ecological change within watersheds. They also function to meet both reference and early warning objectives in correlation to natural ecosystems transformation (Prescott et al., 1996).

Differentiation of sources of fecal contamination of ground and surface waters is an important problem, especially for waters receiving mixed agricultural and human waste (Wiggins et al., 1999). In most contaminated water the presence of fecal indicator organisms can be demonstrated even though the source of the pollution is unknown (Wiggins et al., 1999). The contamination of natural water with untreated fecal material may result in an increased risk of transmission of diseases to the humans who use those waters. Because risk to humans may be greater from human than from animal waste, the

knowledge of the source of the pollution is an important factor in determining the degree of risk (Wiggins et al., 1999). It would thus be desirable to be able to determine the source of the fecal material, both to assess the risk to the people who are exposed to the waters and to assist in the location of the sources of pollution (Wiggins et al., 1999). BST will help with the development and implementation of realistic TMDLs and will be applied widely by regulatory agencies to investigate violation of fecal bacteria stands in the nation's waters (Hagedorn et al., 1999).

III. MATERIALS AND METHODS

A. Research Site Description

The Spout Run watershed is located in Clarke County, VA, at the northern end of the Shenandoah Valley. The watershed is a typical karst-based landscape and agriculture is the principal land use.

The Spout Run watershed contains the community of Millwood and has a stream, Spout Run, that flows through it (Figure 1). Spout Run is 10.2 km long and the area for the Spout Run drainage basin is 2,665 ha. Of the 2,665 ha, 396 ha is wooded land, 1,182 ha is pasture/crop, 150 ha is residential and 66 ha is urban. Millwood is an unsewered community of approximately 130 people in 82 homes on either lots or small farms. Most of the homes are over 50 years old, all obtain water from individual wells, and a few homes do not have indoor plumbing. A septic tank and drainfield, outhouse, or cesspool provides waste disposal.

Fecal samples from known sources were collected chiefly during the first phase of the project to build the library of known source profiles. Additional fecal samples (for known sources) were collected periodically during the project to both amplify and verify the accuracy of the profile library. Stream samples were collected monthly (May 1999 thru May 2000) and analyzed for fecal coliforms, fecal streptococci, and water chemistry. To determine sources of fecal pollution antibiotic resistance analysis (ARA) was performed on fecal streptococci stream isolates on a seasonal basis including the months of May, August, October, December 1999, and March 2000 to cover seasonal flow levels and water conditions. Drinking water samples (from wells) were also collected periodically from homes where the owners elected to participate in a voluntary water-testing program.

B. Experimental Setup

Stream water samples were collected and analyzed on a monthly schedule from the same sample collection sites (Section C). Flow rates and water chemistry analyses were conducted as described in Section D. Fecal bacteria were detected, enumerated, and recovered from the water samples as described in Section E, and ARA was determined as described in Section F. Computations were carried out on a per-sample basis (48 isolates per sample), using analysis of variance (ANOVA) to conduct tests of significance. The data was then evaluated using discriminate analysis (Section G).

C. Sample Collection

Sample collection began in May 1999 and ended in May 2000. Items prepared before leaving the laboratory included a cooler filled with ice, one set of latex gloves per sample to be collected, flame-sterilized spatulas (one for each known sample, in a whirl-pac bag), a sterile Whirl-Pac bag for each sample, and boots, waders, or galoshes (if necessary). The Whirl-Pac bags were labeled prior to taking any samples so samples and labels did not become mixed up. For livestock, mixtures of at least 3-4 fresh "patties" were placed in Whirl-Pac bags using a flame-sterilized spatula. A fresh sample is defined as one that is very wet without a dry top layer or one that is witnessed to be fresh. For human samples, a 250ml sample was obtained from septic tank pump-out trucks, and stored in a sterile bottle. For wildlife samples, mixtures of several identified droppings were placed in Whirl-Pac bags using a flame-sterilized spatula. For stream water samples a sterile bottle was placed in the water with the opening upstream to prevent any contact of the sampler's hand and the water. This ensured that any bacteria that may be on the sampler's glove did not enter the bottle. For all sampling, latex gloves were used to avoid direct contact with the sample. The filled Whirl-Pac bags and bottles were placed on ice in

a cooler and taken back to the laboratory for analysis. Samples were filtered within 6 hours of collection when possible, but no later than 24 hours after collection. Refrigeration of the samples maintained the viability of fecal bacteria and samples were routinely kept at 4°C until enough isolated colonies had been obtained to perform ARA. The samples were then discarded.

Samples were collected from sites in upper Millwood, middle Millwood and lower Millwood (Figure 1). Sites located upper Millwood included FC-61 and FC-62. FC-61 is the section of the stream that flows over an old colonial-era dam. The stream at FC-61 is approximately 4.88 meters wide, 0.152-0.203 meters deep, with a velocity of 0.24 meters per second at high flow. FC-62 is where the millrace starts. Sampling sites FC-60, FC-59, FC-55, FC-56, FC-57, and FC-58 are all located within the Millwood community. FC-60 is the section of the stream that flows through a cattle farm. FC-59 is the millrace midway to the Mill, and FC-55 is the millrace below the Mill. FC-56 is the stream just above where the millrace enters the stream. At point FC-56 the stream is approximated 7.62 meters wide, and 0.203-0.254 meters deep with an average velocity of 0.27 meters per second at high flow. The Mill and the millrace are original construction from the 1780s. The millrace is 0.610-0.914 meters wide and 0.305-0.610 meters deep. FC-57 is the spring at Carter Hall and FC-58 is the section of the stream at Carter Hall. Carter Hall, a colonial plantation is now the location for the headquarters for Project Hope. Sampling sites FC-53 and FC-54 are downstream from Millwood. FC-54 is the section of the stream that flows through a horse farm that contains roughly 120 horses. FC-53 is a section of the stream located in a wooded area near the Shenandoah River. There is a large cattle Farm between sites FC-54 and FC-53. At site FC-54 the stream is approximately 8.53 meters wide and 0.304 meters deep with an average velocity of 0.73 meters per second at seasonal high flow.

D. Water Monitoring

D.1 Flow Rates

Flow rates in Spout Run were determined by a Global Water flow meter model FP201 (Global Water, Inc., Gold River, CA). "The Flow Probe is a highly accurate water velocity instrument for measuring flows in open channels and partially filled pipes. The Flow Probe consists of the protected Turbo-Prop positive displacement sensor coupled by the expandable probe handle to the digital readout display. The Flow Probe incorporates true velocity averaging for the most accurate flow measurements. The Flow Probe is ideal for storm water runoff studies, measuring flows in rivers and streams as well as ditches and sewers" (www.globalw.com/flow.html). Results from the flow meter were recorded as feet/sec and then converted to liters/min.

D.2. Turbidity

Turbidity measurements were made by the nephelometric method using a Hanna HI93703 Microprocessor Turbidity Meter (Hanna Instruments Inc., Woonsocket, RI), with a range from 0.00 to 1,000 formazin turbidity units (FTUs). The method is based on a comparison of the intensity of light scattered by the sample under defined conditions as compared to the intensity of light scattered by standard reference suspensions under the same conditions. The standards were used to reference and calibrate the turbidity meter. The higher the intensity of scattered light, the higher the turbidity. Results were reported as nephelometer turbidity units, or NTUs.

D. 3. Hydrogen Ion Activity (measurements for D3 through D7 were conducted in Dr. Reneau's lab)

Hydrogen ion activity (pH) measurement are normally made by means of a glass H^+ sensing electrode paired with a reference electrode, usually a calomel electrode, or a combination electrode, which contains a glass sensing and a reference electrode in a single probe body (APHA, 1995). The electrode is attached to an appropriate meter that indicates the pH from the millivolts of a potential generated when the electrode is placed in the solution.

In the actual measurement of pH the water sample is in equilibrium with the CO_2 in the air. The CO_2 concentration of the air can have an appreciable effect on measured pH in waters with a pH considerably above 7, but in waters below pH 7 measured pH is practically unaffected. Standard buffer solutions, pH 7.0 and 4.0 are used to calibrate the electrode system. An Orion PerpHecT® Meter Model 370 (ATI Orion Inc., Boston, MA) was used with a PerpHecT® Ag/AgCl Sure-flow Electrode, Model 9272, to determine pH (APHA, 1995).

D.4. Ammonium

When ammonium is heated with sodium saliclyate and hypochlorite in an alkaline phosphate buffer, an emerald green color is produced which is proportional to the ammonia concentration. The color is intensified by the addition of sodium nitroprusside. Ammonium was determined on a dual channel autoanalyzer. The autoanalyzer used was a continuous flow Quick Chem 8000 made by Lachat Instruments (Milwaukee, WI).

D.5. Nitrate

Nitrate is quantitatively reduced to nitrite by passage of the sample through a copperized cadmium column (USEPA, 1983). The nitrite (reduced nitrate plus original nitrite) is then determined by diazotizing with sulfanilamide followed by coupling with N- (1-naphthyl)

ethylenediamine dihydrochloride. The resulting soluble dye has a magenta color that is read at 520 nm. Nitrite alone can be determined by removing the cadmium column. Nitrate was determined on a dual channel autoanalyzer. The autoanalyzer is a continuous flow Quick Chem 8000 made by Lachat Instruments (Milwaukee, WI).

D.6. Phosphate

Orthophosphate (OP) was determined with a Hitachi Model 100-20 spectrophotometer (Hitachi Instruments Ltd., Tokyo, Japan). Ammonia molybdate and antimony potassium tartrate react in an acid medium with dilute solutions of phosphorus to form an antimony phospho-molybdate complex. This complex is reduced to an intensely blue colored dye by ascorbic acid. Orthophosphate is the only form of phosphorus to form a blue dye in this method (APHA, 1995).

D.7. Biological Oxygen Demand (BOD)

BOD determination is an empirical test to determine the relative oxygen requirements of wastewaters, effluents, and polluted waters. The test measures the molecular oxygen utilized during a specified incubation period for the biochemical degradation of organic material (carbonaceous demand) and the oxygen used to oxidize inorganic material such as sulfides and ferrous iron. The oxygen consumed in a 5 day test period (BOD_5) was measured with a YSI Model 57 Oxygen meter (YSI Inc., Yellow Springs, OH) with a YSI 5905 BOD probe (APHA, 1995).

E. Microbial Detection and Recovery

For membrane filtration, a known volume of a water sample is passed through a membrane filter that is then placed on M-Endo, M-FC or LES-Endo agar (Becton Dickinson,

Cockeysville, MD) for 24 h incubation at 37˚C or 44.5˚C, depending on the medium used (APHA, 1995). All organisms that produce red colonies with a metallic sheen within 24 h of incubation are considered members of the coliform group on M-Endo agar at 37˚C, or dark blue colonies on m-FC agar at 44. 5˚C to detect and enumerate fecal coliforms. A benefit of the membrane filter method over the Most Probable Number (MPN) technique is that results are available within 24 h instead of the 3 to 4 days required for confirming MPN results, although confirmation of colonies observed after membrane filtration is advisable. The membrane filter technique is as effective as the MPN test for detecting bacteria of the coliform group (APHA, 1995).

E.1. Presence-Absence Test

The Colilert system (IDEXX Laboratories Inc., Westbrook, ME) was developed to enumerate total coliforms and *E. coli* from drinking and source waters without the need for confirmatory tests. No equipment other than an incubator and ultraviolet light (366 nm) is necessary. All that is necessary is to add water from the sampling sites to the powdered formula in a test tube or vessel. A colorless solution results. After incubation at 35°C for 24 hours, yellow color indicates the presence of total coliforms, and fluorescence under ultraviolet light indicates the presence of *E. coli* (Prescott et al., 1999). It is a primary water test (i.e., a test directly from a water sample) that requires no confirmation. (Prescott et al., 1999). The Colilert system was used to determine if samples were positive or negative for coliforms. Only positive samples were membrane filtered.

E.2. Membrane Filtration for Fecal Coliforms

Water samples (100 ml) were filtered through a 0.45 um pore-size membrane filter. The filters were then transferred to a 50-mm petri dish containing 5 ml of m-FC agar. The filters

were incubated for 24 h at 44.5°C. Dark blue colonies were counted and the fecal coliform density was reported as the membrane filter count per 100 ml (APHA, 1995).

E.3. Membrane Filtration Method for Fecal Streptococci

Water samples (100 ml) were filtered through a 0.45 um pore-size membrane filter (APHA. 1995). The filters were then transferred to a 50-mm petri dish containing 5 ml of m-Enterococcus agar (Becton Dickinson, Cockeysville, MD). The filters were incubated for 24-48 h at 37°C. After 48 h of incubation, red to burgundy colored colonies were counted and the fecal streptococci density was reported as the membrane filter count per 100 ml.

E.4. Filtration for Solids

Fecal bacteria from manure, septage, and scat samples were isolated after they were collected, and then mixed in a Whirl-Pac bag by massaging the bag. After sample collection, 0.1 -0.2 g of sample was added to 50 ml of sterile saline buffer (SB) in a 125 ml screw-cap bottle and then shaken for 30 sec. Heavy solids were allowed to settle before pipetting. For all materials, 0.1, 1.0, and 3.0 ml of buffered sample were normally filtered, although sometimes other volumes were necessary. Since fecal and septage samples can be difficult to filter, prefiltration using a very fast flowing filter paper (e.g., grade 363) was useful. Petri dishes with 5 ml of Enterococcus agar were labeled with the sample number and volume filtered in triplicate. The autoclaved filter towers were placed in the filtration manifold, and sterile membrane filters were added by using sterile filter forceps (tips are dipped in 95% ethanol and flamed). Five milliliters of 95% ethanol was added to each tower to check for leaks in the filters. An aliquot of each volume of sample was pipetted into each of the three towers (for a total of 3 filters/volume tested). After the vacuum pump was turned on and the sample was filtered, 5-10 milliliters of saline buffer were then added to wash the filter and the sides of the tower. Sterilized forceps

were used to transfer the filter to the 50-mm petri dishes containing the agar. After all three volumes are filtered (9 plates total), the petri dishes were placed in plastic bags, labeled with the sample number and date, and incubated at 37°C for 48 hours.

F. Antibiotic Resistance Analysis

Samples were obtained from several types of known sources: human cattle, geese, deer, sheep, chicken, raccoon, and horse for the development of a known database. After fecal streptococci had been enumerated and colonies were available on membrane filter plates, sterile toothpicks were used to transfer 48 individual colonies per sample to 96-well microtiter plates. No set number of colonies was taken from any one plate, but rather colonies were selected randomly and equally (where possible) from the plates that constituted one sample. No more than 48 colonies were taken from any one composite known sample. If 48 colonies were not available, then less than 48 were used. The 96-well plates (pre-sterilized) were first filled with Enterococcosel broth (Becton Dickinson, Cockeysville, MD) using an 8-channel multi-well pipetter to add 0.2 ml of broth to each of the wells. Care was taken to ensure that each colony picked was separate and distinct. Each isolate was scraped from the filter with the toothpick and thoroughly inoculated into one well of the 96-well plate. The 96-well plate was placed in a plastic container to prevent the microwells from drying up, and then incubated at 37˚C for 24 to 48 h.

The antibiotic-containing plates were prepared by adding filter-sterilized stock solutions (10 mg/ml) in sterile water (ampicillin, neomycin, oxytetracycline, streptomycin), 1:1 water: ethanol (chlorotetracycline, erythromycin, tetracycline, vancomycin), and 1:1 water: methanol (amoxicillin,), to autoclaved and cooled Trypticase Soy Agar (TSA; (Becton Dickinson, Cockeysville, MD) at initial concentrations of 2.5, 5, 10, 20, 40, 60, 80, and 100 μg/ml. The

isolates were transferred with a stainless steel 48-prong replica-plater (Sigma Chemical Company, St. Louis, MO) from the Enterococcosel-containing microwells to a set of 30 Tryptocase Soy Agar (TSA) plates containing the various concentrations of each antibiotic to be tested, and to a control plate containing no antibiotic (Table 1). The plates were incubated at 37^{o}C for 48 h and growth of each isolate on each concentration of every antibiotic was determined. An isolate was considered to be resistant to a given concentration of antibiotic if growth comparable to the controls occurred on that plate. Any isolate that did not grow on the control plates (containing no antibiotic) was not used in the analysis. A data set for one sample was 48 isolates x 30 antibiotics/concentrations (Hagedorn et al., 1999).

Data on the ability of each of the known isolates to grow in the presence of each concentration of each antibiotic were analyzed with SAS-JMP statistical software (version 3.2.2, SAS Institute Inc.) (Wiggins et al., 1999). Several variations of discriminant analysis were performed by varying the combination of antibiotics and the level of pooling of source types. The classification table produced by the DISCRIM procedure was used to calculate the percentages of misclassified isolates and determine the average rate of correct classification (ARCC). The table is a source-by-source matrix in which the numbers and percentages of correctly classified isolates are found on the diagonal. The ARCC for a give combination of antibiotics was computed by averaging the percentages along the diagonal. The percentage of misclassified isolates for a given source was determined by adding the percentages of misclassified isolates in the appropriate row of the table (excluding the value in the diagonal). The percentage of isolates from other sources that the average of the percentages in the appropriate column (excluding the value in the diagonal)(Wiggins et al., 1999).

Results are recorded in laboratory notebooks and in computer files prior to evaluation by discriminate analysis (Hagedorn et al., 1999).

Table 1. Antibiotics and their concentrations used in the Antibiotic Resistance Analysis.

Antibiotic	Concentrations (μg/ml)
Amoxicillin, AMX	2.5
Cephalothin, CEP	10, 15, 30, 50
Chloratetracycline, CTC	60, 80, 100
Erythromycin, ETC	10, 15, 30, 50
Oxytetracycline, OTC	20, 40, 60, 80, 100
Streptomycin, STR	40, 60, 80, 100
Tetracycline, TET	10, 15, 30, 50, 100
Neomycin, NEO	40, 60, 80
Vancomycin, VAN	2.5

G. Statistical Methods

Analysis of variance (ANOVA) performs comparisons like the t-Test, but for an arbitrary number of factors. Each factor can also have an arbitrary number of levels. Furthermore each factor combination can have any number of replicates. ANOVA works on a single dependent variable while, the factors must be discrete. The ANOVA can be thought of in a practical sense as an extension of the t-Test to an arbitrary number of factors and levels. It can also be thought of as a linear regression model whose independent variables are restricted to a discrete set (Sall and Lehman, 1996).

When performing discriminate analysis (SAS-JMP statistical software, version 3.2.6 SAS Institute, Inc.), the software program first performs ANOVA on the data set and tests for significance by subjecting the data to chi-square, the Likelihood Ratio test, the Pearson test and generates a kappa value to measure the degree of agreement within the data set. If the data set fails the chi-square test, discriminate analysis is not performed.

G.1. Discriminate Analysis

Data for all fecal bacterial isolates ability to grow in the presence of each concentration of each antibiotic was analyzed with SAS-JMP statistical software (version 3.2.6, SAS Institute Inc., Cary, NC) Variables for the analyses include the number of drugs used and the degree of pooling of sources. Each analysis produces a classification set for every known source isolate. The average rate of correct classification (ARCC) for each analysis is determined by averaging the percentages of correctly classified isolates for each source. A database is built for each known source (e.g. human, beef cattle, etc.). The discriminant analysis procedure compares each

set of isolates from an unknown source against the database of known sources and then classifies each isolate into one of the possible sources.

IV. RESULTS AND DISCUSSION

A. Studies of Fecal Contamination in Water

The ability to perform accurate and reliable fecal source tracking has been lacking in watershed monitoring. As the Environmental Protection Agency (EPA) initiates the Total Maximum Daily Loading (TMDL) process, bacterial source tracking (BST) will be essential to effectively use the TMDL process at the local level. The purpose of BST is to use biochemical and molecular characteristics of bacteria to determine the sources of the fecal pollution. The knowledge of the type of pollution and sources will aid in the restoration of water quality. The site chosen for this experiment was designated as an at-risk community by the Virginia Department of Health (VDH) and the Virginia Department of Environmental Quality (DEQ) due to fecal bacteria in well water samples and in Spout Run.

During the 12 month monitoring period antibiotic resistance analysis (ARA) was used to determine sources of contamination. Ultimately, surface waters eventually reach ground water and may recharge waters that could be used for drinking. It is very important that sources of contamination be identified to assist in preserving the quality of well and stream waters (Moberg and Rice, 1999).

Flow rate measurements were taken on a seasonal basis at locations in upper Millwood (FC-61), middle Millwood (FC-56) and lower Millwood (FC-54) (Section IV.B.). Water quality in the Spout Run stream was determined with monthly monitoring and included the membrane filtration technique and chemical analyses (Section IV.C.). Overall comparisons for the seasons and the year are described in Section IV.D. Well water quality is described in section IV.E., and

the comparisons for the seasons and year are located in Section IV. F. Antibiotic resistance analysis (ARA) was used to determine the source of fecal contamination (Section IV. G). Water Chemistry from both Spout Run and Millwood community well water was determined with regular monitoring over the 12 month research period. (Sections IV. C and IV. E). A description of each site is located in Materials and Methods Section III. C.

B. Flow Rate Observations

B.1. Flow Rates for Summer

Summer monitoring included the months of July, August, and September, 1999, and flows were recorded in July. During the summer of 1999 the northern Shenandoah Valley experienced a severe drought. Water tables throughout the area reached record lows and the Spout Run drainage basin was no exception. The summer drought had a negative effect on stream flow and flow rates for July were nearly 50 percent less than for the fall, winter, and spring seasons (Table 2).

B.2. Flow Rates for Fall

Fall monitoring included the months of October, November, and December, 1999, and flows were recorded in December. Remnants of the drought (low rainfall) were still in effect during the month of October. However, there was a recharge to streams during November and December, 1999, as a result of rain and snowmelt, and flows had returned to levels near those recorded in May 1999, prior to the drought (Table 2).

B.3. Flow Rates for Winter

Winter monitoring included the months January, February and March, 2000, and flows were recorded in March. During this period there were adequate amounts of precipitation and these produced seasonal high stream flow rates (Table 2).

B.4. Flow Rates for Spring

Spring monitoring included the months of April 2000, May 2000, and May and June 1999. Flows were recorded in May 1999. During the spring there were near seasonal high flow rates (Table 2).

B.5. Summary

The data in Table 2 indicated that flow rates were fairly constant throughout the year with the exception of the summer season and part of the fall. The summer drought had an adverse effect on stream flow and was worse than usual due to prolonged elevated temperatures and low precipitation. The high temperatures attract wildlife and livestock to the streams for longer periods of time, so they remain in the stagnate waters, resulting in overloading the streams with fecal bacteria (Sections IV. C2 and IV. C3).

Stream flow data are used by regulatory agencies, such as the DEQ; to establish permits for the discharge of treated wastewater and water use. This information is also essential to water managers, policy makers, scientists, and other members of the general public for characterizing and comprehending the water-quality conditions of our nation's streams, lakes, reservoirs, and estuaries. Stream flow data aids in preserving, managing, and improving the use of our nation's water resources (Moberg and Rice, 1999).

Table 2. Stream flow measurements (liters/minute) for Spout Run, Clarke County VA.[a]

Location	May 1999	July 1999	December 1999	March 2000
Upper	20,100	8,070	20,236	26,967
Millwood (FC-61)				
Middle	27,847	11,710	25,651	29,825
Millwood (FC-56)				
Lower	47,256	20,955	50,406	54,021
Millwood (FC-54)				

[a]Spout Run is formed where Pagebrook and Roseville Run flow together (Figure 1). Refer to Materials and Methods, Section III. C. for sampling site descriptions.

C. Water Quality in the Spout Run Stream

C.1 Background Water Quality

The EPA designates three levels of fecal coliform contamination for recreational waters. These levels are termed as low, moderate or high based on the values of the fecal coliform (FC) counts. Low is designated as FC counts less than 100, moderate is characteristic of FC values of 100-1000 and high levels of contamination are defined as FC values greater than 1000 (the regulatory standard for recreational waters based on monthly grab samples).

C.2 Stream Water Quality for Summer

Summer observations included the months of July, August and September 1999(Table 3). For July, 10 of 10 stream samples were positive. For the 10 positive stream samples one was low (<100 FCs per 100 ml, FC-57), seven were moderate and ranged from 200 (FC-59) to 930 FCs/100 ml (FC-62), and two were high and ranged from 1,430 (FC-55) to 3,300 (FC-53). All 10 positive stream samples were positive for fecal streps, and counts ranged from <100 (FC-57) to 1,852 (FC-35) fecal streps/1000 ml (Appendix A, Table 19).

For the month of August, 9 of 10 stream samples were positive. For the 9 positive stream samples, none were low (<100 FCs per 100ml). All were moderate and ranged form 140 (FC-58) to 800 FCs/100ml (FC-53). All ten positive stream samples were also positive for fecal streps and counts ranged form 410 (FC-61) to 1,050 (FC-56) fecal streps per 100ml (Table 3 and Appendix A, Table 20).

For the month of September, 9 of 10 stream samples were positive. For the 9 positive stream samples three were low (<100 FC/100ml, FC-53, FC-55, and FC-59); and the remaining six were moderate and ranged from 160 (FC-61) to 780 FCs/100ml (FC-60). All nine positive stream samples were also positive for fecal streptococci and counts ranged from 430 (FC-54) to 1,204 (FC-35) fecal streps per 100ml (Table 3 and Appendix A).

In July 1999 stream flow levels were reduced by at least 50 to 60% at all locations and cattle were evident wherever there was unrestricted access (Table 4). Approximately 30 cattle were observed in an around the stream at FC-53 in July and August. Higher fecal bacterial counts in the stream, with subsequent degradation of recreational water uses are the inevitable result.

Table 3. Separation of fecal coliform counts into low, moderate, and high categories for Spout Run, Clarke County, VA.[a]

Season	No. of Samples	Fecal Coliforms		
		Low	Moderate	High
Summer (1999)				
July	10	1	7	2
August	10	0	9	0
September	10	3	6	0
Fall (1999)				
October	10	0	0	10
November	10	0	1	9
December	10	6	3	0
Winter (2000)				
January	10	4	5	0
February	10	8	0	0
March	10	0	9	0
Spring				
May 1999	10	5	4	0
June 1999	10	1	4	4
April 2000	10	5	4	0
May 2000	10	0	2	7
Totals	130	14	74	31

[a]Low equals <100 colony forming units (CFU)/100ml, moderate equals CFUs from 100 to 1,000/100 ml, and high equals CFUs >1000ml. Of 130 samples, 119 or 91.5% were FC positive. Of the 119, 14 or 11.8% were low, 74 or 62.2% were moderate, and 31 or 26.1% were high.

Table 4. Fecal coliform (FC) and fecal streptococci (FS)counts for summer stream samples, average over July, August, and September, 1999, for each site.

Location	Site No.	FC/(CFU/100ml)[a]	FS/(CFU/100ml)
Upper Millwood	FC-60	600	520
	FC-61	470	430
	FC-62	545	420
Middle Millwood	FC-55	615	545
	FC-56	635	690
	FC-59	195	390
Lower Millwood	FC-53	1,370	600
	FC-54	370	400
	FC-57[b]	0	0
	FC-58	445	395

[a]Recorded as colony forming units (CFU) per 100ml.

[b]FC-57 is Carter Hall Spring.

Chemical analyses for summer indicated that none of the measured parameters were problematic (Appendix B, Tables 32 and 33). There was a pH range of 7.38-8.20, PO_4 – P had a range of 0.01-0.25 mg/L, NO_3 -N had a range of 0.15-9.39 mg/L, NH_4 –N had a range of 0.04-0.34 mg/L, BOD_5 had a range of 0.2-3.0 mg/L, and turbidity had a range of 0-11.21 FTU. Of these six parameters none are regulated for recreational water use. According to EPA, NO_3 should not reach a measurement higher than 10mg/L for drinking water, and the NO_3 standard for groundwater in Virginia is 5mg/L.

C.3. Stream Quality for Fall

Fall observation included the months October, November, and December, 1999 (Table 3). For the month of October, 10 of 10 stream samples were positive. For the 10 positive stream samples all were all high (>1000 FCs per 100ml) and ranged form 1,190 (FC-35 and FC-53) to 3,070 (FC-56). All 10 positive stream samples were also positive for fecal streptococci and counts ranged form 50 (FC-57) to 1,228 (FC-56) fecal streps/100 ml. sampling was done shortly after the remnants of Hurricane Dennis. This storm activity provided some 5 cm of rain in the area. This was the first sampling where every stream sample was positive and high (Appendix A, Table 22).

For the month of November, 10 of 10 stream samples were positive. For the 10 positive stream samples 9 were high and ranged from 2,080 (FC-61) to 3,450 (FC-58). Sample FC-57 (Carter Hall Spring) was low (<100 FCs per 100 ml). All 10 positive stream samples were also positive for fecal streps and counts ranged from 0 (FC-57) to 632 (FC-61) fecal streptococci per 100ml. This was the second sampling where every stream sample was

positive and high, except for FC-57, the Carter Hall spring sample (Table 3 and Appendix A, Table 23).

For the month of December, 9 of 10 stream samples were positive. For the 9 positive stream samples none were high, six were low (<100 FCs per 100ml) and three were moderate and ranged from 140 (FC-56) to 160 (FC-62). Sample FC-57 (Carter Hall Spring) was negative. All nine positive steam samples were also positive for fecal streptococci, and counts ranged from 1 (FC-54) to 6 (FC-61) fecal streptococci per 100ml (Table 3 and Appendix A, Table 24).

October, 1999 microbiological monitoring indicated a substantial rise in FC and FS counts in comparison to July, 1999's microbiological monitoring (Tables 4 and 5). Each of the samples FC counts were higher than the recreational standard of 1000 FCs/100ml, except for FC-57. However, FC-57 had a moderate value and this was significant increase from zero for July, 1999 (Tables 4 and 5).

Chemical analysis for fall was not problematic (Appendix B, Tables 34-36). There was a pH range of 7.50-8.18. Phosphate had a range of 0.05-0.15 mg/L, NO_3-N had a range of 1.69-3.41 mg/L, NH_4-N had a range of 0.09-0.28 mg/L, BOD_5 had a range of 0.0-9.6 mg/L, and turbidity had a range of 0.31-22.41 FTU.

The month of October was the first sampling where every stream sample was positive and high. It would seem that some time was necessary after the onset of several rain events before higher fecal counts were observed in the stream samples. November's sampling was characteristic of October. In December the counts were low compared to previous months and reflected colder weather where livestock and wildlife did not seek water as often.

C.4. Stream Quality for Winter

Observations for winter included the months of January, February, and March 2000 (Table 3). For the month of January, 9 of 10 stream samples were positive. For the 9 positive stream samples, none were high, 4 were low <100 FC per 100ml, FC-58) and 5 were moderate ranging from (100, FC-62 to 180 at FC-56). All 9 positive stream samples were also positive for fecal streptococci and counts ranged from 20 (FC-53 and FC-54) to 70 (FC-61) fecal streptococci/100ml (Appendix A, Table 25), Sample FC-57 (Carter Hall Spring) was negative.

For the Month of February, 8 of 10 stream samples were positive. For the 8 positive stream samples seven were low and ranged from 30 (FC-54 and FC-55) to 80 (FC-60). One stream sample was moderate with a value of 120 (FC-62). Sample FC-57 (Carter Hall Spring) was negative (Table 3 and Appendix A, Table 26).

For the month of March, 9 of 10 stream samples were positive. For the 9 positive stream samples, none were high and 1 was low, 8 were moderate and ranged from 120 (FC-58) to 280 (FC-60) fecal streptococci per 100 ml. Sample FC-57 (Carter Hall Spring) was negative (Table 3 and Appendix A, Table 27).

There was a significant drop in the FC and FS counts for March 2000 as compared to October 1999 (Tables 5 and 6). The results for March 2000, were similar to those of May 1999 as the results were lower in value(Tables 4 and 6). FC-57 returned to a value of zero.

Chemical analysis for the winter was not problematic (Appendix B, Tables 37-39). There was a pH range of 7.71-8.12. Phosphate had a range of 0.02-0.06 mg/L, NO_3-N had a range of 1.86-3.43 mg/L, NH_4-N had a range of 0.02-0.20 mg/L, BOD_5 had a range of 0.0-2.7 mg/L, and turbidity had a range of 0.0-16.04 FTU.

Table 5. Average fecal coliform (FC) and fecal streptococci (FS) counts for fall stream samples, averaged over October, November, and December, 1999, for each site.

Location	Sample No.	FC (CFU/100ml)[a]	FS(CFU/100ml)
Upper Millwood	FC-60	1,640	450
	FC-61	1,665	520
	FC-62	1,150	450
Middle Millwood	FC-55	1,255	620
	FC-56	2,040	400
	FC-59	1,520	450
Lower Millwood	FC-53	1,265	430
	FC-54	1,405	460
	FC-57 [b]	675	15
	FC-58	2,035	505

[a]Recorded as colony forming units (CFU) per 100 ml.
[b]FC-57 is Carter Hall spring.

Table 6. Fecal coliform (FC) and fecal streptococci (FS)counts for winter stream samples, averaged over January, February, and March, 2000. For each site.

Location	Sample No.	FC(CFUs/100ml)[a]	FS(CFUs/100ml)
Upper Millwood	FC-60	140	70
	FC-61	130	70
	FC-62	125	65
Middle Millwood	FC-55	100	40
	FC-56	150	40
	FC-59	130	50
Lower Millwood	FC-53	40	30
	FC-54	90	35
	FC-57[b]	0	0
	FC-58	55	25

[a]Recorded as colony forming units (CFU) per 100ml.

[b]FC-57 is Carter Hall spring.

C.5. Stream Quality for Spring

Spring observations included the months of April and May 2000, May and June, 1999 (Table 3). For the month May 1999, 9 of 10 stream samples were positive. For the 9 positive stream samples, five were low (<100 FCs per 100 ml) and four were moderate and ranged from 100 (FC-55 and FC-61) to 630 (FC-58 and FC-60) FCs/100ml. Sample FC-57 (Carter Hall Spring) was negative. All ten positive stream samples were also positive for fecal streps and counts ranged from 84 (FC-54) to 300 (FC-56) fecal streps/100ml (Appendix A, Table 17).

For June 1999, 9 of 10 stream samples were positive. For the 9 positive stream samples, one was low (<10 FCs per 100ml, FC-60), four were moderate and ranged from 260 (FC-54) to 960 FCs/100 ml (FC-58). Four were high and ranged from 1,020 (FC-62) to 1,850 FCs/100ml (FC-56). Sample FC-57 (Carter Hall Spring) was negative. All nine were also positive for fecal streps and counts ranged form 242 (FC-53) to 8000 (FC-56) fecal streps/100ml (Table 3 and Appendix A, Table 18).

In April 2000, 9 of 10 stream samples were positive. None were high and five were low (<100 FCs per 100ml, FC-53, FC-54, FC-55, FC-56, and FC-62), and four were moderate and ranged from 100 (FC-58) to 290 (FC-61). Sample FC-57 (Carter Hall Spring) was negative. All 9 positive stream samples were also positive for fecal streptococci and counts ranged from <10 (FC-53) to 504 (FC-60) fecal streptococci/100ml (Table 3 and Appendix A, Table 28).

For May 2000, 9 of 10 stream samples were positive. For the 9 positive stream samples none were low, two were moderate and ranged form 530 (FC-53) to 630 (FC-54) FCs/100ml. The remaining 7 were high and ranged from 1,030 (FC-58) to 1,570 (FC-62) FCs/1000ml. All

ten positive stream samples were also positive for fecal streps and counts range from 298 (FC-54) to 896 (FC-55) fecal streps /100 ml (Table 3 and Appendix A. Table 29).

FC and FS counts for May 1999 were similar to those July 1999, with samples predominately in the moderate range (Tables 4 and 7). FC-57 remained negative.

Chemical analysis for the spring was not problematic (Appendix B, Tables 30 and 40). There was a pH range of 7.62-8.05, PO_4–P had a range of 0.15-0.22 mg/L, NO_3-N had a range of 2.08-2.90 mg/L, NH_4-N had a range of 0.17-0.34 mg/L, BOD_5 had a range of 0.0-4.2 mg/L, and turbidity had a range of 0.0-82.0 FTU.

Sampling in Spout Run began in May 1999 and ended in May 2000 (Table 3). For the first sampling in May 1999 the downstream sections were quite clean (<10 for FC-53 and FC-54), where some stream fencing had already occurred. FC-58 was higher (630), however the sample was taken below a beaver dam. There were some fecal coliforms in the millrace (FC-55, FC-59, and FC-62) and in Spout Run (FC-56, FC-60, and FC-61). For June 1999, observations were characteristic of May 1999 observations.

January through April 2000 were characteristic of December 1999 and reflect colder weather coupled with livestock and wildlife not seeking water as often as well as dilution from snowmelt, precipitation, and high stream flow levels. May 2000 sampling results were elevated as compared to results from the winter and probably a result of rising livestock use of the stream.

Table 7. Fecal coliform (FC) and fecal streptococci (FS) counts for spring stream samples, averaged over May and June1999, and April and May 2000, for each site.

Location	Sample No.	FC(CFUs/100ml)[a]	FS/(CFUs/100ml)
Upper Millwood	FC-60	570	370
	FC-61	600	340
	FC-62	790	290
Middle Millwood	FC-55	740	405
	FC-56	795	2,240
	FC-59	670	1,575
Lower Millwood	FC-53	235	185
	FC-54	240	175
	FC-57[b]	0	0
	FC-58	680	2,090

[a]Recorded as colony forming units (CFU) per 100ml.

[b]FC-57 is Carter Hall spring.

C.6. Relationship of FC counts to Turbidity.

According to the literature it is difficult to find good correlations between any chemical parameters and FC counts. Sometimes a good correlation is found between FC counts and turbidity, however this was not the case with this research. A correlation of turbidity and fecal coliform counts were preformed using (SAS-JMP statistical software, version 3.2.6 SAS Institute, Inc.) (Table 8).

Table 8. Fecal coliform counts versus Turbidity for the year.

Section	Site Number	Correlation[a]
Upper Millwood	FC-61	-0.3273
Middle Millwood	FC-56	0.7840
Lower Millwood	FC-54	0.1987

[a]If there is an exact linear relationship between two variables, then the correlation is 1 or -1, depending on whether the variables are positively or negatively related. If there is no linear relationship the correlation tends towards zero (Sall and Lehman, 1996).

D. Stream Water Quality Comparisons for the Year

On a seasonal basis fecal coliforms were problematic regarding recreational water quality standards during the summer, fall and spring seasons (Table 3). Effects such as reduced stream flow, higher temperatures, and lower precipitation all influenced the levels of FC found in the streams, especially during the summer and fall.

Overall, there was a serious problem with fecal contamination as 88.3% of 130 water samples were found to fall within a moderate to high range. This data supports EPA's findings that identify FC bacteria as the most widespread problem in Virginia's rivers and streams. The TMDL program only allows streams to exceed recreational standards by no more than 10% of monthly samples. Based on those samples that were high (<1,000) Spout Run exceeded stands 26.1% of the time (Table 3).

Nearly 92% of the 130 water samples were FC positive (Table 3). This is an issue of obvious concern as the presence of FC bacteria is a reliable indicator of fecal contamination from warm-blooded animals. As a result of such high fecal bacterial counts the spout run waters are not fit for recreational uses. It is also imperative that best management practices (BMPs) be implemented such as fencing and riparian buffers to prevent access of livestock and some wildlife into the streams, and to evaluate options for improving waste disposal and treatment in middle Millwood.

On a seasonal basis, both FC and FS counts increased at FC-56 (middle Millwood), for nearly every season (Figure 2 and 3). This indicates that there is a substantial addition of waste to the Spout Run stream as the stream flows through middle Millwood.

Figure 2. Average of fecal coliform (FC) counts overall. FC-61, upper Millwood; FC-56, middle Millwood; FC-54, lower Millwood.

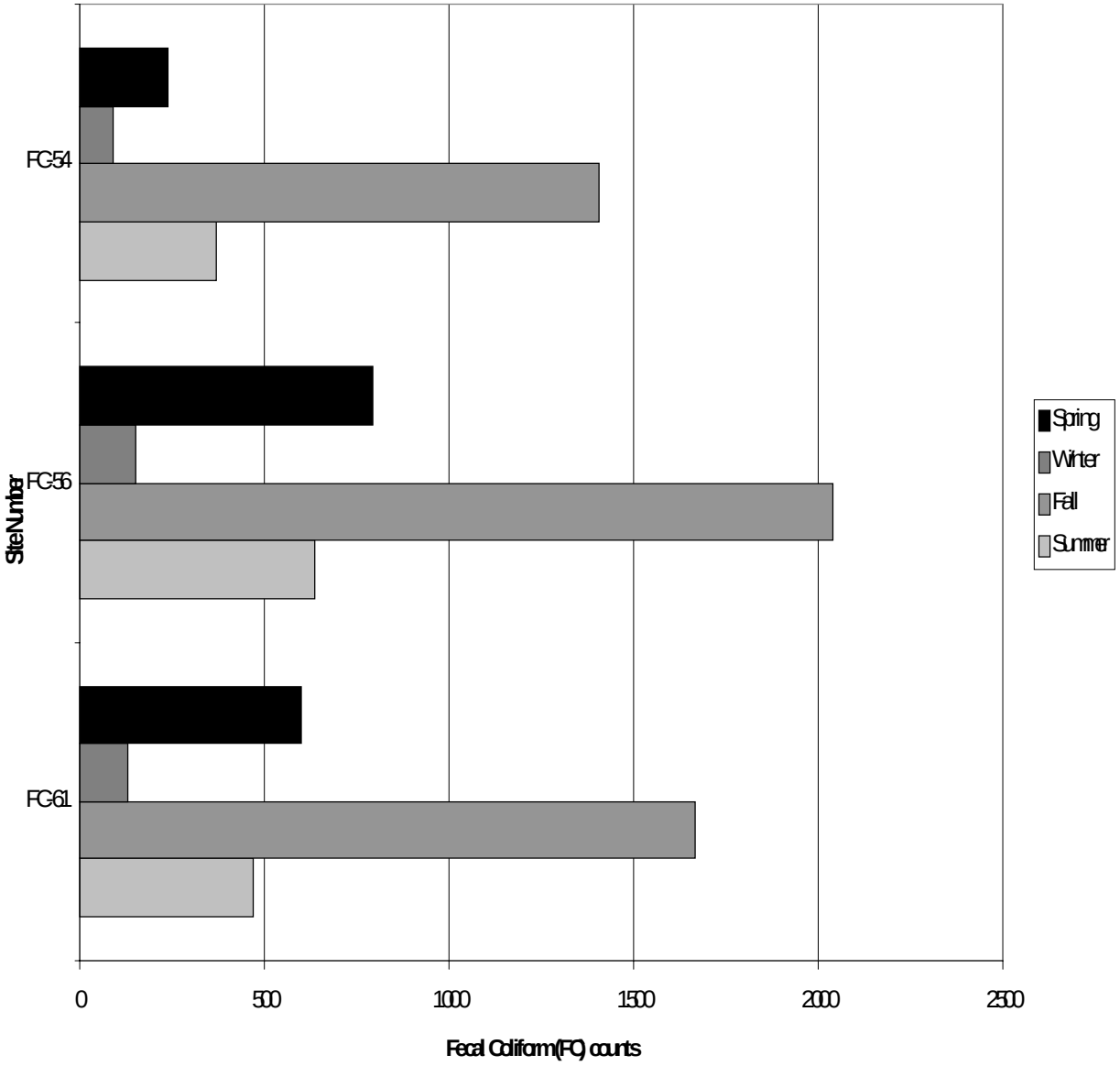
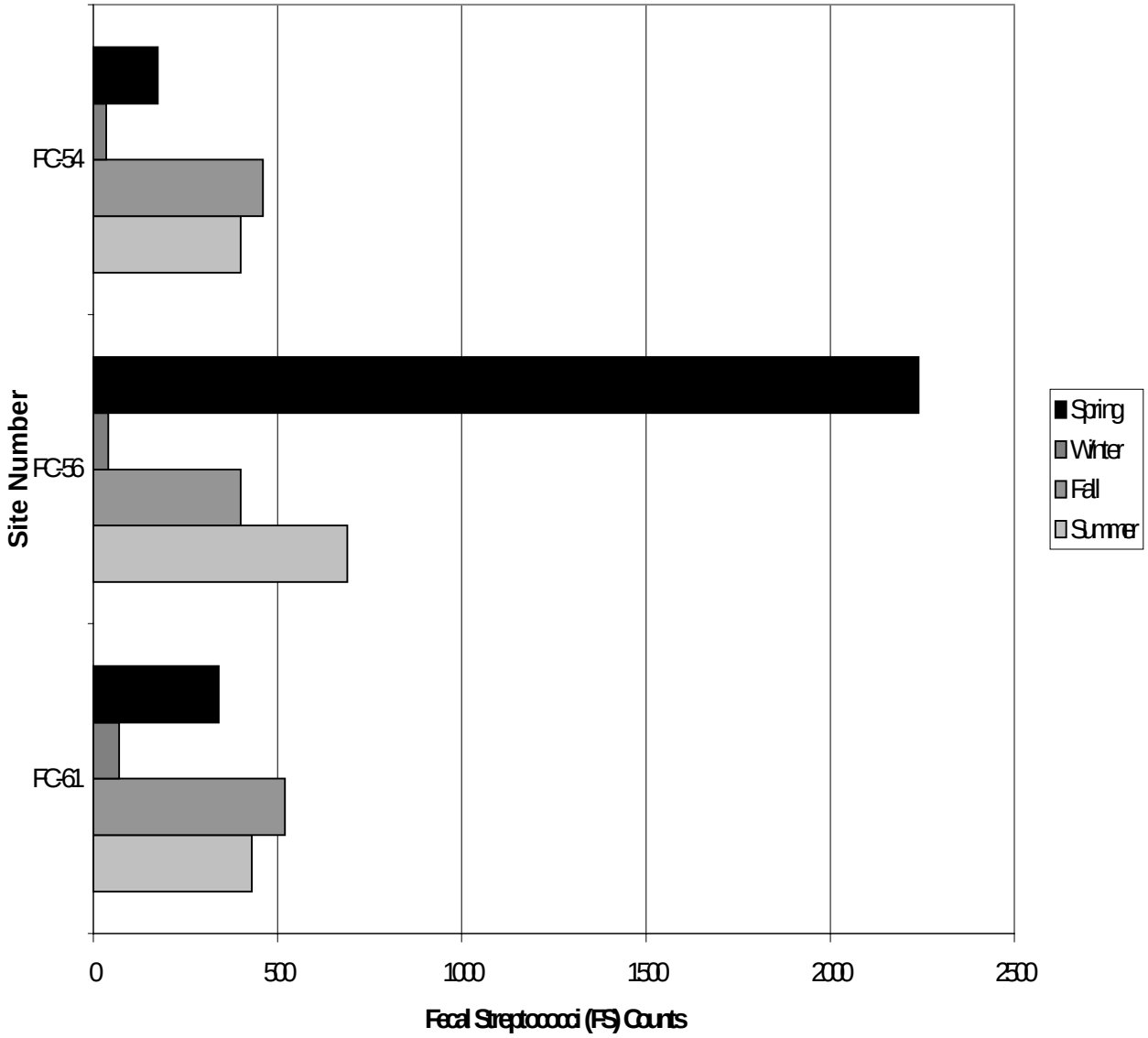


Figure 3. Average of fecal streptococci (FS) counts overall. FC-61, upper Millwood, FC-56, middle Millwood, FC-54 lower Millwood.



E. Well Water Quality in Spout Run

All wells were not sampled every month. The well water monitoring was based on those in the community who volunteered to participate in this project. Access to homes for sample collection changed from month to month.

E.1. Well Water Quality for Summer

Summer observations included the months July, August, and September 1999 (Table 9). For the month of July 1 of 6 well samples was positive (XC-37) with a low value <10 FCs per 100ml. For the months August and September 0 of 9 well water samples were positive each month (Table 9 and Appendix A, Tables 19-21).

Chemical analysis for the summer was not problematic (Appendix B). There was a pH range of 7.14-8.17, PO_4-P had a range of 0.13-0.21 mg/L, and NO_3-N had a range of 0.17-6.09 mg/L, NH_4-N had a range of 0.13-0.33 mg/L, BOD_5 had a range of 0.0-3.2 mg/L, and turbidity had a range of 0.0-179 FTU (Appendix B, Tables 31-33).

E.2. Well Water Quality for Fall

Fall observations included the months October, November, and December, 1999 (Table 9). For the month of October, 6 of the 9 well water samples were positive. For the six positive well samples, 5 were low (<10 FCs per 100ml) (XC-31, XC-33, XC-35, XC-37, XC-38) and 1 was moderate (XC-28, 140 FCs per 100ml). This was the first sampling where so many of the well samples were positive, and was the same month where 10 of 10 stream samples were high (Table 3). For the month of November, 2 of 8 well samples were positive (XC-30, XC-38). For the 2 positive well samples both were low (<10 FCs per 100ml). Zero of 8 well samples were positive for the month of December (Appendix A, Tables 22-24).

Table 9. Separation of fecal coliform counts into low, moderate and high categories for well water samples, Millwood, Clarke Count, VA.[a]

Season	No of Samples	Fecal Coliforms		
		Low	Moderate	High
Summer (1999)				
July	6	1	0	0
August	9	0	0	0
September	9	0	0	0
Fall (1999)				
October	9	5	1	0
November	8	2	0	0
December	8	0	0	0
Winter (2000)				
January	7	0	0	0
February	7	1	0	0
March	7	0	0	0
Spring				
May 1999	8	0	0	0
June 1999	8	1	0	0
April 2000	10	0	1	0
May 2000	10	0	0	0
Total	109	10	2	0

[a]Low equals <100 colony forming units (CFU)/100ml, moderate equals CFUs from 100 to 1,000/100 ml, and high equals CFUs >1000ml. Of 109 samples, 12 or 11% were FC positive. Of the 12, 10 or 9% were low, and 2 or 2% were moderate.

Chemical analysis was not problematic for the fall. There was a pH range of 7.13-7.62, PO_4–P had a range of 0.01-0.13 mg/L, NO_3-N had a range of 0.13-7.48 mg/L, NH_4–N had a range of 0.10-0.28 mg/L, BOD_5 had a range of 0.0-2.3 mg/L, and turbidity had a range of 0.0-97.0 FTU (Appendix B, Tables 34-36).

E.3. Well Water Quality for Winter

Observations for winter included the months January, February, and March 2000 (Table 9). For January 0 of 7 well water samples were positive. However 1 of 7 well samples were positive in February (XC-39) with a low value of <10 FCs/100ml. Zero of 7 well samples were positive for the month of March (Appendix A, Tables 25-27).

Chemical Analysis was not problematic for the winter. There was a pH range of 7.0-7.75, PO_4–P had a range of 0.02-0.07 mg/L, NO_3-N had a range of 0.03-7.54 mg/L, NH_4-N had a range of 0.05-0.30 mg/L, BOD_5 had a range of 0.2-2.8 mg/L, and turbidity had a range of 0.0-521 FTU (Appendix B, Tables 37-39).

E. 4. Well Water Quality for Spring

Spring observations included the months April 2000, May 2000, May 1999, and June 1999 (Table 9). For May 1999, 0 of 8 well samples were positive. For June 1999, 1 of 8 well samples were positive (XC-31). This sample was low with <10 FCs and no FS. In April 2000, 1 of 10 well samples was positive (XC-39). This sample had a moderate value of 300 FCs per 100ml. For May 2000, none of the 10 well samples were positive (Appendix A, Tables 17, 18, 28 and 29).

Chemical analysis for spring was not problematic. There was a pH range of 7.38-8.20. Phosphate had a range of 0.01-0.13 mg/L, NO_3 -N had a range of .15-9.39 mg/L. Ammonium had a range of 0.04-0.34 mg/L, BOD_5 had a range of 0.19-1.4 mg/L, and turbidity had a range of 0.0-16.11 FTU (Appendix B, Tables 30 and 40).

E.5. Summary

Generally speaking contamination in well water was not problematic as only 12 (11%) of 109 samples were positive (Tables 9 and 10). There were periodic events of contamination in wells and were primarily associated with the seasonal drought during the fall 1999, where 8 of 12 positive well samples (66%) were found (Table 10).

Table 10. Comparison of positive well water samples by well and by season. Millwood Clarke County, VA.

Sample	Summer	Fall	Winter	Spring
XC-09	0	0	0	0
XC-28	0	1	0	0
XC-29	0	0	0	0
XC-30	0	1	0	0
XC-31	0	1	0	1
XC-33	0	1	0	0
XC-34	0	0	0	0
XC-35	0	1	0	0
XC-36	0	0	0	0
XC-37	1	1	0	0
XC-38	0	2	0	0
XC-39	0	0	1	1
Total	1	8	1	2

F. Well Water Quality Comparisons for the Year

F.1. Seasonal Comparisons

On a seasonal basis well water samples were most problematic during fall 1999 following the summer 1999 drought (Tables 9 and 10).

F.2. Overall Comparisons

Overall there was not a significant difference between the seasons with the exception of fall (Table 10).

F.3. Summary

The homes that participated in this study were strictly on a volunteer basis. Of the homes used in this study neither the chemical constituents analyzed in this study nor fecal coliforms pose a significant threat to well water quality (Tables 9 and 10, Appendix A and Appendix B). Fecal coliforms in wells were problematic during the drought, and appear to be susceptible to surface runoff.

G. Antibiotic Resistance Analysis (ARA)

ARA was used to determine the source of fecal contamination in Spout Run. The sources fall into three categories: human, livestock, and wildlife.

G. 1. The ARA Database

For a database to be able to correctly classify bacteria in a polluted stream, accuracy of classification is important, but other issues factor in as well. The database must also contain enough isolates to be representative of the organism being classified. It is not actually a question of a specific number of isolates need to provide better source identification, high average rate of correct classification (ARCCs), but rather a question of representativeness of the database. The

best way to determine if a database is representative is to regularly add samples of known source isolates to an existing database. If the ARCC and/or the individual correct classification do not change significantly as new samples are added, then the library should be representative (Hagedorn et.al., 1999).

The ARCC for human versus animal for the database used in this research is 96.29%. The ARCC for human versus livestock versus wildlife is 92.02% (Table 11). Other Literature is almost entirely reporting ARCCs in the range of 70% to 80% (Wiggins et al., 1999). The Spout Run watershed is small in size and the area was intensely sampled for fecal isolates from known sources to get the highest possible ARCC.

Table 11. The antibiotic resistance analysis (ARA) database of 1,174 fecal streptococcal isolates from unknown sources in the Spout Run watershed, Clarke County, VA.

	Human		Animal	
Category	No. of isolates	% identified	No. of isolates	% identified
Human	194	**95.57**	129	2.99
Animal	9	4.43	942	**97.01**
Total	203	100	971	100

	Human		Livestock		Wildlife	
Category	No. of Isolates	% identified	No. of Isolates	% identified	No. of Isolates	% identified
Human	192	**94.58**	14	1.91	12	5.07
Livestock	9	4.43	688	**93.73**	17	7.17
Wildlife	2	0.99	32	4.36	208	**87.76**
Total	203	100	734	100	237	100

G.1. ARA for Summer

Samples for the month of July were analyzed using ARA. Samples FC-60, FC-61, and FC-62 are found in upper Millwood (Fig.1). Of the 48 isolates from FC-60, 14 or 29% were identified as human, 11 or 23% were identified as livestock, and 23 or 48% were identified as wildlife. Of the 48 isolates from FC-61, 1 or 2% were identified as human, 22 or 46% were identified as livestock and 25 or 52% were identified as wildlife. Of the 48 isolates form FC-62, 7 or 15% were identified as human, 20 or 42% were identified as livestock and 21 or 43% were identified as wildlife (Table 12).

Samples FC-55, FC-56, and FC-59 were found in middle Millwood. Of the 48 isolates from FC-55, 0 were identified as human, 30 or 63% were identified as livestock and 18 or 37% were identified as wildlife. Of the 48 isolates from FC-56, 0 were identified as human, 7 or 35% were identified as livestock and 31 or 65% were identified as wildlife. Of the 48 isolates from sample FC-59, 0 were identified s human, 14 or 29% were identified as livestock and 34 or 71% were identified as wildlife (Table 12).

Samples FC-53, FC-54, and FC-58 are found in lower Millwood. Of the 48 isolates from sample FC-53, 1 or 2% was identified as human, 35 or 73% was identified as livestock and 12 or 25% was identified as wildlife. Of the 48 isolates from FC-54, 3 or 6% was identified as human, 13 or 27% was identified as livestock, and 32 or 67% was identified as wildlife (Table 12).

Isolates from livestock and wildlife were relatively even for July 1999. Livestock isolates were in highest concentration at FC-59. There was also a significant human signature found in upper Millwood (Table 12).

Table 12. Antibiotic resistance analysis of fecal streptococci isolates from Spout Run (July 1999).

Site No.	No. of	Human		Wildlife		Livestock	
Upper Millwood	Isolates	No. of Isolates	%	No. of Isolates	%	No. of Isolates	%
FC-60	48	14	29	11	23	23	48
FC-61	48	1	2	22	46	25	52
FC-62	48	7	14	20	42	21	44
Middle Millwood							
FC-55	48	0	0	30	62	18	38
FC-56	48	0	0	17	35	31	65
FC-59	48	0	0	14	29	34	71
Lower Millwood							
FC-53	48	1	2	35	73	12	25
FC-54	48	3	6	13	27	32	67
FC-58	48	2	4	13	27	33	69
Total	432	28	7	175	41	229	53

G.2. ARA for Fall

Samples for the months October and December were analyzed using ARA. A few sites from October were selected for ARA because of very high FC counts (Table 3 and Appendix A), while December was chosen as a seasonal late fall representation for ARA. For the month of October samples FC-61, FC-56, FC-59, FC-57, and FC-58 were analyzed using ARA. FC-61 is found in upper Millwood and of the 48 isolates from FC-61, 10 or 21% were identified as human, 34 or 71% was identified as livestock, and 4 or 8% was identified as wildlife (Table 13).

FC-56 and FC-59 are found in middle Millwood. Of the 48 isolates from FC-56, 10 or 21% were identified as human, 26 or 54% were identified as livestock, and 12 or 25% were identified as wildlife. Of the 47 isolates from FC-59, 11 or 23% were identified as human, 21 or 45% were identified as livestock, and 15 or 32% were identified as wildlife.

FC-57 and FC-58 are found in lower Millwood (Table 13). Of the 42 isolates fromm FC-57, 15 or 36% were identified as human, 21 or 50% were identified as livestock, and 6 or 14% were identified as wildlife. Of the 48 isolates from FC-58, 9 or 19% were identified as human, 14 or 29% were identified as livestock and 25, or 52% were identified as wildlife.

Livestock isolates predominate throughout Millwood except at FC-58 in October, 1999. The percentage of human isolates increased as compared to the summer season. Isolates of human origin were found at all locations (Tables 12 and 13).

Table 13. Antibiotic resistance analysis of fecal streptococci isolates from Spout Run (October 1999), Clarke County, VA.

Sample No. Upper Millwood	No. of isolates	Human		Livestock		Wildlife	
		No. of Isolates	%	No. of Isolates	%	No. of Isolates	%
FC-61	48	10	21	34	71	4	8
Middle Millwood							
FC-56	48	10	21	26	54	12	25
FC-59	47	11	23	21	45	15	31
Lower Millwood							
FC-57	42	15	36	21	50	6	14
FC-58	48	9	19	14	29	25	52
Total	233	55	24	116	50	62	27

For the month of December, samples FC-60, FC-61, FC-62, FC-55, FC-56, FC-59, FC-57 and FC-58 were analyzed using ARA (Table 14). FC-60, FC-61, and FC-62 are found in upper Millwood. Of the 12 isolates from FC-60, 0 were identified as human, 5 or 42% were identified as livestock and 7 or 58% were identified as wildlife. Of the 12 isolates form FC-61, 0 were identified as human, 4 or 33% were identified as livestock and 8 or 67% were identified as wildlife. Of the 12 isolates from FC-62, 1or 8% was identified as human, 7 or 58% were identified as livestock, and 4 or 33% were identified as wildlife.

FC-55, FC-56, and FC-59 are located in middle Millwood (Table 14). Of the 12 isolates from FC-55, 0 were identified as human, 11 or 92% were identified as livestock and 1 or 8% was identified as wildlife. Of the 11 isolates from FC-56, 0 were identified as human, 10 or 91% were identified as livestock and 1 or 9% were identified as wildlife. Of the 12 isolates from FC-59, 0 were identified s human, 11 or 92% were identified as livestock and 1 or 8% were identified as wildlife.

FC-57 and FC-58 are located in lower Millwood (Table 14). Of the 12 isolates from, FC-57, 5 or 42% were identified as human, 5 or 42% were identified as livestock and 2 or 16% were identified as wildlife. Of the 12 isolates from FC-58, 0 were identified as human, 10 or 84% were identified as livestock and 2 or 16% were identified as wildlife.

There were so few isolates available for December 1999, resultant of an increased dilution of the stream due to increased precipitation and snowmelt. Livestock isolastes were heavy in the middle Millwood and likely to be due to surface runoff. FC-57 has a high percentage of isolates of human origin and this is only because of the small number of isolates used in ARA (Table 14).

Table 14. Antibiotic resistance analysis of fecal streptococci isolates from Spout Run (December 1999), Clarke County, VA.

Sample No.	No. of Isolates	Human		Livestock		Wildlife	
		No. of Isolates	%	No. of Isolates	%	No. of Isolates	%
Upper Millwood							
FC-60	12	0	0	5	42	7	58
FC-61	12	0	0	4	33	8	67
FC-62	12	1	8	7	59	4	33
Middle Millwood							
FC-55	12	0	0	11	92	1	8
FC-56	11	0	0	10	92	1	8
FC-59	12	0	0	11	92	1	8
Lower Millwood							
FC-57	12	5	42	5	42	2	17
FC-58	12	0	0	10	83	2	17
Total	95	6	6	63	66	26	27

G.3. ARA for Winter

Samples from the month of March were analyzed using ARA (Table 15). FC-60, FC-61, and FC-62 are found in upper Millwood. Of the 48 isolates from FC-60, 3 or 6% were identified as human, 34 or 71% were identified as livestock and 11 or 23% were identified as wildlife. Of the 48 isolates from FC-61, 2 or 4% were identified as human, 38 or 79% were identified as livestock and 8 or 17% were identified as wildlife. Of the 48 isolates form FC-62, 1 or 2% were identified as human, 31 or 65% were identified as livestock, and 16 or 33% were identified as wildlife.

FC-56 and FC-59 are found in middle Millwood. Of the 48 isolates from FC-56 0 were identified as human, 45 or 94% were identified as livestock and 3 or 6% were identified as wildlife. Of the 46 isolates from FC-59 2 or 4% were identified as human, 37 or 81% were identified as livestock and 7 or 15% were identified as wildlife (Table 15).

FC-53, FC-54, and FC-58 are found in lower Millwood. Of the 48 isolates from FC-53, 1 or 2% were identified as human, 42 or 88% were identified as livestock, 5 or 10% were identified as wildlife. Of the 48 isolates from FC-54, 4 or 8% were identified as human, 40 or 84% were identified as livestock, and 4 or 8% were identified as wildlife. Of the 25 isolates from FC-58, 0 were identified as human, 15 or 60% were identified as livestock, and 10 or 40% were identified as wildlife (Table 15).

Isolates of livestock origin were predominate in March 2000 (Table 15). The findings for March 2000 were similar to those in July and December 1999 (Tables 12 and 14). Only a few human isolates were found in middle Millwood.

Table 15. Antibiotic resistance analysis of fecal streptococci isolates from Spout Run (March 2000), Clarke County, VA.

Sample No.	No. of isolates	human		livestock		wildlife	
Upper Millwood		No.of Isolates	%	No. of Isolates	%	No. of Isolates	%
FC-60	48	3	6	34	71	11	23
FC-61	48	2	4	38	79	8	17
FC-62	48	1	2	31	65	16	33
Middle Millwood							
FC-56	48	0	0	45	94	3	6
FC-59	46	2	4	37	81	7	15
Lower Millwood							
FC-53	48	1	2	42	88	5	10
FC-54	48	4	8	40	84	4	8
FC-58	25	0	0	15	60	10	40
Total	359	13	4	282	79	64	18

G.4. ARA for Spring

Samples from May 1999 were analyzed using ARA (Table 16). Sites FC-61 and FC62 are found in upper Millwood. Of the 48 isolates from FC-61, 7 or 15% were identified as human, 18 or 37% were identified as livestock, and 23 or 48% were identified as wildlife. Of the 48 isolates from FC-62, 7 or 15% were identified as human, 20 or 42% were identified as livestock, and 21 or 43% were identified as wildlife.

Sites FC-55, FC-56, and FC-59 are found in middle Millwood. Of the 47 isolates from FC-55, 0 were identified as human, 25 or 53% were identified as livestock, and 22 or 47% were identified as wildlife. Of the 47 isolates from FC-56, 1 or 2% were identified as human, 21 or 45% were identified as livestock, and 25 or 53% were identified as wildlife. Of the 48 isolates form FC-59, 4 or 8% were identified as human, 21 or 44% were identified as livestock and 23 or 48% were identified as wildlife (Table 16).

Sites FC-53, FC-54, and FC-58 are found in lower Millwood. Of the 48 isolates from FC-53, 2 or 4% were identified as human, 19 or 40% were identified as livestock and 27 or 56% were identified as wildlife. Of the 48 isolates from FC-54, 1 or 2% were identified as human, 35 or 73% were identified as livestock, and 12 or 25% were identified as wildlife. Of the 25 isolates from FC-58, 0 were identified as human, 24 or 50% were identified as livestock and 24 or 50% were identified as wildlife (Table 16).

Livestock and wildlife isolates were relatively even for May 1999 (Table 16). There was a human signature evident as was in July and December 1999 (Tables 12 and 15).

Table 16. Antibiotic resistance analysis of fecal streptococci isolates from Spout Run (May 1999), Clarke County, VA.

Sample No.	No. of Isolates	Human		Livestock		Wildlife	
		No. of Isolates	%	No. of Isolates	%	No. of Isolates	%
Upper Millwood							
FC-61	48	7	14	18	38	23	48
FC-62	48	7	14	20	42	21	44
Middle Millwood							
FC-55	47	0	0	25	53	22	47
FC-56	47	1	2	21	45	25	53
FC-59	48	4	8	21	44	23	48
Lower Millwood							
FC-53	48	2	4	19	40	27	56
FC-54	48	1	2	35	73	12	25
FC-58	48	0	0	24	50	24	50
Total	382	22	6	183	48	177	46

G.5. Summary

Antibiotic resistance patterns (ARA) of isolates of fecal streptococci, analyzed with DA were a suitable method to differentiate and identify sources of fecal pollution in Spout Run. Discriminate analysis was important in determining sources of contamination in stream water where isolates came from multiple sources. The high rate of correct classification for the known-source isolates from Spout Run was an important result, as it meant that the database could successfully be used to identify unknown isolates.

Fecal coliform and fecal streptococcus populations in the stream samples reflected the activities of cattle and wildlife that had unrestricted access to Spout Run. Cattle were in the streams regularly during warm weather and this resulted in the high ARA results from livestock obtained during the spring, summer and fall seasons.

Page Brook and Roseville Run are the two tributaries that form Spout Run. There was no evidence of human signature in Page Brook (Hagedorn et al., 1999) nor was there evidence of a human signature in Roseville Run. Therefore, the problem with fecal contamination of human origin, originates within Millwood. There are 32 homes in upper Millwood, 21 homes in middle Millwood, and 13 homes in lower Millwood. All of these homes have individual septic systems. The human signature found in Spout Run is probably attributed to surface runoff following very dry periods.

V. CONCLUSIONS

There is a human signature in Spout Run, but it is small compared to the proportion of isolates from livestock and wildlife. The sporadic instances where well water samples were positive appeared primarily during very dry periods. Restricting livestock access to streams can dramatically lower fecal coliform counts during the unusually hot and dry periods. Reducing FC counts to below recreational water standards for Virginia (1000 per 100ml for any one sample) may be achievable, however to maintain streams below standards may prove to be difficult, as Spout Run is in an area where there are large populations of Canada geese, deer, and other wildlife, and will be hard to restrict these animals.

The results presented here affirms the works of Hagedorn and Wiggins by showing that antibiotic resistance patterns of fecal streptococci are a suitable means to determine sources of fecal pollution in water. The knowledge of the sources of pollution is an important factor in determining the degree of risk for humans exposed to contaminated water. It is desirable to be able to determine the sources of fecal contamination, both to assess the risk to the people who are exposed to the waters and to assist in the development of BMPs to reduce fecal loading. Some recommended BMPs include stream fencing, establishing riparian buffers, installing in-pasture watering stations, and improved waste treatment system for Millwood.

We have the potential to provide agencies responsible for water quality and public health with a resource, based on new methodology to determine sources of fecal contamination. Until sources of pollution can be reliably identified, risk to communities can not be accurately assessed, and water quality improvement will remain a hit -or-miss affair that is not effective or cost efficient.

VI. LITERATURE CITED

American Public Health Association, Inc. 1995. Standard methods for the examination of water and wastewater. 19th ed. New York, New York. Pp 1:18-25, 2:8-9, 9:24-72.

Bancroft, K., E. Nelson, and G. Childers. 1988. Comparison of the Presence-Absence and Membrane Filter Techniques for Coliform Detection in small, Nonchlorinated Water Distribution Systems. Appli. Environ. Microbiology 55:507-510.

Becker, J.M., G.A., Caldwell, and E.A. Zachgo. 1996. Biotechnology: A laboratory Course. 2nd edition. Academic Press, New York.

Bernhard, A. E., and K.G. Field. 2000. Identification of Nonpoint Sources of Fecal Pollution in Coastal Waters by using Host-Specific 16S Ribosomal DNA Genetic Markers form Fecal Anaerobes. Appli. Environ. Microbiology 63:2607-2612.

Brady, N.C. and R.R. Weil. 1999. The Nature and Properties for Soils 12th edition. Prentice-Hall, Inc. Upper Saddle River, New Jersey.

Clausen, E. M., B. L. Green, and Warren Litsky. 1977. Fecal Streptococci: Bacterial Indicators of Pollution. Health Hazards associated with Water ASTM STP635, A.W. Hoadely and B.J. Dutka, ed. American Society for Testing and Materials. p 247-264.

Conservation Technology Information Center. 2000. Know your Watershed: TMDL Facts. Purdue University. http://ww.ctic.purdue.edu/kyw/tmdl/tmdlfact.html .

Craun, G.F. 1981. Outbreaks of waterborne diseases in the United States: 1971-1978. J. Amer. Water Works Assoc. 73:360-369.

Craun, G.F. 1985. A summary of waterborne illness transmitted through contaminated groundwater. J. Environ. Health 48:122-127.

DiPaola, T.S. 1998. Biological and Chemical Renovation of Wastewater with a Soil Infiltrator Low-Pressure Distribution System. Virginia Tech University Press, Virginia.

Geldreich, E.E. 1996. Sanitary Significance of Fecal Coliforms in the Environment. Publication No. WP-20-3. Federal Water Pollution Control Administration, Cincinnati, Ohio. 122p.

Geldreich, E.E. 1996. Microbial Quality of Water Supply in Distribution Systems. Lewis Publishers. CRC Press Inc. Boca Raton, FL.

Geraghty, J.J. and D.W. Miller 1978. Status of groundwater contamination in the U.S. J.Am. Water Works Assoc. 70:162-167.

Gleeson, Cara and N. Gray. 1997. The Coliform Index and Waterborne Disease: Problems of microbial drinking water assessment. E&FNSPON, London.

Graves, L. and B. Swaminathan. 1993. Universal Bacterial DNA Isolation procedure, pp. 617-621. *In* Diagnostic Molecular Microbiology Principles and Applications, D.H. Persing, T.F. Smith , F.C. Tenover, and T.J. White, eds. ASM Press, Washington, DC.

Hagedorn, C., S. L. Robinson, J.R. Filtz, S. M. Grubbs, T. A. Angier, R. B. Reneau. 1999. Determining Sources of Fecal Pollution in a Rural Virginia Watershed with Antibiotic Resistance Patterns in Fecal Streptococci. Appli. Environ. Microbiology 65:5522-5531.

Hagedorn, C. 2000. Bacterial Source Tracking. [online] http://www.bsi.vt.edu/biol_4684/BST/BSTover.html [26 April 2000, last date accessed]

Keeling, W.G. Bacterial Source Tracking. 2000. Virginia Department of Conservation and recreation; Division of soil and Water Conservation, Virginia.

Kott, Yehuda. 1977. Current concepts of indicator Bacteria. *In* Bacterial Indicators/Health hazards Associated with Water, ASTM STP^#%, A.W. Hoadley and B.J. Dutka, Eds., American Society for Testing and Materials, pp. 3-13.

McFeters, G.A. and D.G. Stuart. 1972. Survival of Coliform bacteria in Natural Waters: Field and Laboratory Studies with Membrane-Filter Chambers. Appl. Microbiology., 24:805-811.

Miesfeld, R.L. 1999. Applied Molecular Genetics. John Wiley and Sons, Inc., New York.

Miner, R.J. 1990. Will a Water Trough Reduce the Amount of Time Hay-Fed Livestock Spend in the Streams. Office of International Research and Development, Oregon State University. Oregon.

Moberg, R. and K. Rice. 1999. Measuring Streamflow in Virginia.[online] http://va.water.usgs.gov/online_pubs/OFR95-713.html [27 June 2000, last date accessed]

Parveen, S., R.L. Murphree, L. Edmiston, C.W. Kaspar, K.M. Portier, and M.L. Tamplin. 1997. Association of Multiple-Antibiotic-Resistance Profiles with Point and Nonpoint sources of *Escherchia coli* in Apalachicola Bay. Appli, Environ. Microbiology 63: 2607-2612

Prescott, L., J. harley, and D. Klein. 1996. Microbiology. 3rd ed. WCB Publishers. Chicago, IL. Pp. 811-877.

Ptak, D.J., and W. Ginsburg. 1977. Bacterial Indicators of Drinking Water Quality. *In* Bacterial Indicators of Pollution /Health Hazards Associated with Water, ASTM STP635, A.W. Hoadley and B.J. Dutka, Eds., American Society for Testing and Materials. Pp. 247-264.

Reneau, R.B., Jr. and D.E. Pettry. 1975. Movement of Coliform bacteria from septic tank effluent through selected coastal plain soils of Virginia. J. Environ. Qual, 4:41-44.

Sall, J., and A. Lehman. 1996. Start Statistics. SAS Institute Inc. New York, New York. Pp. 117-239.

USDA Natural Resources Conservation Service. 1997. Water Quality and Agriculture: Status, Conditions, and Trends. USDA Natural Resources Conservation Service, Washington, DC.

US Environmental Protection Agency. 1999. [online] Total Maximum Daily Load (TMDL) Program http://www.epa.gov/owowwtr1/tmdl/tmdlfs.html [21 April 2000, last date accessed].

US Environmental Protection Agency. 1995. Section 319 National Monitoring Program: An Overview. [online] http://www.epa.gov/owowwtr1/NPS/ [9 June 2000, last date accessed]. http://www.epa.gov/owowwtr1/NPS/Section319/enc3.htm

US Environmental Protection Agency. 1997. Recreational Water Quality. [online] http://www.epa.nsw.gov.au/soe/97/ch3/18_1.htm .[30 June 2000, last date accessed].

US Environmental Protection Agency. 1 September 1999. National Watershed Database 305b. [online] http://www.epa.gov/surf3/states/VA [31 October 1999, last date accessed].

Wiggins, B. A, R. W. Andrews, R. A. Conway, C.L. Corr, E. J., Dobratz, D. P. Dougherty, J. R. Epprd, S. R. Knupp, M. C. Limjoco, J. M. Mettenburg, J. M. Rinehardt, J. Sonsino, R.L. Torrijos, and M.E. Zimmerman. 1999. Identification of sources of Fecal Pollution using Discrimnant Analysis: Supporting Evidence from Large Data Sets. Appl. Environ. Microbiology 65: 3483-3486.

Youston, A. 1999. Microbial Physiology. Virginia Tech University Printing Services. Virginia.

APPENDIX A

TABLES 17-29, FECAL COLIFORM AND FECAL STREPTOCOCCUS COUNTS BY MONTH AND SAMPLE

Appendix A Table 17. Fecal coliform and fecal streptococcus counts for May, 1999, Spout Run, Clarke County, VA[a]

SAMPLE ID. NO.	COLILERT TEST	FECAL COLIFORMS (FCs) 10ML	FECAL COLIFORMS (FCs) 1.0ML	FCs 100ML	FECAL STREPS 100ML
XC-28	NEGATIVE			NEG.	NEG.
XC-29	NEGATIVE			NEG.	NEG.
XC-30	NEGATIVE			NEG.	NEG.
XC-31	NEGATIVE			NEG.	NEG.
XC-32	NEGATIVE			NEG.	NEG.
XC-33	NEGATIVE			NEG.	NEG.
XC-34	NEGATIVE			NEG.	NEG.
XC-35	NEGATIVE			NEG.	NEG.
FC-35	**POSITIVE**	**114**	**18**	**1,140**	**130**
FC-53	**POSITIVE**	**5**	**0**	**<10**	**114**
FC-54	**POSITIVE**	**0**	**1**	**<10**	**84**
FC-55M	**POSITIVE**	**10**	**0**	**100**	**176**
FC-56	**POSITIVE**	**8**	**0**	**<10**	**300**
FC-57	NEGATIVE			NEG.	NEG.
FC-58	**POSITIVE**	**63**	**5**	**630**	**112**
FC-59	**POSITIVE**	**6**	**0**	**<10**	**150**
FC-60	**POSITIVE**	**63**	**7**	**630**	**144**
FC-61	**POSITIVE**	**10**	**7**	**100**	**188**
FC-62MR	**POSITIVE**	**53**	**8**	**530**	**220**
FC-63	**POSITIVE**	**8**	**0**	**<10**	**134**

[a]XC= well samples FC= stream samples

Appendix A Table 18. Fecal coliform and fecal streptococcus counts for June, 1999, Spout Run, Clarke County, VA[a]

SAMPLE ID. NO.	COLILERT TEST	FECAL COLIFORMS (FCs) 10ML	1.0ML	FCs 100ML	FECAL STREPS 100ML
XC-28	NEGATIVE			NEG.	NEG.
XC-29	NEGATIVE			NEG.	NEG.
XC-30	NEGATIVE			NEG.	NEG.
XC-31	**POSITIVE**	**0**	**0**	**<10**	**0**
XC-33	NEGATIVE			NEG.	NEG.
XC-35	NEGATIVE			NEG.	NEG.
XC-36	NEGATIVE			NEG.	NEG.
XC-37	NEGATIVE			NEG.	NEG.
FC-35	**POSITIVE**	**26**	**0**	**260**	**334**
FC-53	**POSITIVE**	**40**	**1**	**400**	**242**
FC-54	**POSITIVE**	**26**	**0**	**260**	**294**
FC-55MR	**POSITIVE**	**128**	**0**	**1,280**	**502**
FC-56	**POSITIVE**	**185**	**22**	**1,850**	**8,000**
FC-57	NEGATIVE			NEG.	NEG.
FC-58	**POSITIVE**	**96**	**3**	**960**	**7,600**
FC-59MR	**POSITIVE**	**116**	**7**	**1,160**	**5,500**
FC-60	**POSITIVE**	**7**	**7**	**<10**	**398**
FC-61	**POSITIVE**	**83**	**10**	**830**	**340**
FC-62MR	**POSITIVE**	**102**	**5**	**1,020**	**356**

[a]XC= well samples FC= stream samples

Appendix A Table 19. Fecal coliform and fecal streptococcus counts for July, 1999, Spout Run, Clarke County, VA[a]

SAMPLE ID. NO.	TEST	COLILERT	FECAL COLIFORMS (FCs) 10ML	1.0ML	FCs 100ML	FECAL STREPS 100ML
XC-28		NEGATIVE			NEG.	NEG.
XC-29		NEGATIVE			NEG.	NEG.
XC-30		NEGATIVE			NEG.	NEG.
XC-31		**NEGATIVE**			**NEG.**	**NEG.**
XC-33		NEGATIVE			NEG.	NEG.
XC-37		**POSITIVE**	**1**	**0**	**<10**	**10**
FC-35		**POSITIVE**	**TNTC**	**31**	**3,100**	**1,852**
FC-53		**POSITIVE**	**TNTC**	**33**	**3,300**	**284**
FC-54		**POSITIVE**	**37**	**4**	**370**	**224**
FC-55MR		**POSITIVE**	**143**	**12**	**1,430**	**576**
FC-56		**POSITIVE**	**78**	**6**	**780**	**374**
FC-57		**POSITIVE**	**1**	**0**	**<10**	**<10**
FC-58		**POSITIVE**	**58**	**6**	**580**	**342**
FC-59MR		**POSITIVE**	**20**	**9**	**200**	**280**
FC-60		**POSITIVE**	**72**	**3**	**720**	**390**
FC-61		POSITIVE	85	14	850	332
FC-62MR		**POSITIVE**	**93**	**10**	**930**	**304**

[a]XC= well samples FC= stream samples

Appendix A Table 20. Fecal coliform and fecal streptococcus counts for August, 1999, Spout Run, Clarke County, VA[a]

SAMPLE ID. NO.	COLILERT TEST	FECAL COLIFORMS (FCs) 10ML	1.0ML	FCs 100ML	FECAL STREPS 100ML
XC-28	NEGATIVE			NEG.	NEG.
XC-29	NEGATIVE			NEG.	NEG.
XC-30	NEGATIVE			NEG.	NEG.
XC-31	NEGATIVE			NEG.	NEG.
XC-33	NEGATIVE			NEG.	NEG.
XC-35	NEGATIVE			NEG.	NEG.
XC-36	NEGATIVE			NEG.	NEG.
XC-37	NEGATIVE			NEG.	NEG.
XC-38	NEGATIVE			NEG.	NEG.
FC-35	**POSITIVE**	**77**	**1**	**770**	**430**
FC-53	**POSITIVE**	**80**	**11**	**800**	**990**
FC-54	**POSITIVE**	**20**	**2**	**200**	**490**
FC-55MR	**POSITIVE**	**40**	**4**	**400**	**430**
FC-56	**POSITIVE**	**60**	**0**	**600**	**1,050**
FC-57	NEGATIVE			NEG.	NEG.
FC-58	**POSITIVE**	**14**	**1**	**140**	**680**
FC-59MR	**POSITIVE**	**38**	**1**	**380**	**460**
FC-60	**POSITIVE**	**30**	**0**	**300**	**520**
FC-61	**POSITIVE**	**40**	**5**	**400**	**410**
FC-62MR	**POSITIVE**	**62**	**2**	**620**	**430**

[a]XC= well samples FC= stream samples

Appendix A Table 21. Fecal coliform and fecal streptococcus counts for September, 1999, Spout Run, Clarke County, VA[a]

SAMPLE ID. NO.	COLILERT TEST	FECAL COLIFORMS (FCs) 10ML	FECAL COLIFORMS (FCs) 1.0ML	FCs 100ML	FECAL STREPS 100ML
XC-28	NEGATIVE			NEG.	NEG.
XC-29	NEGATIVE			NEG.	NEG.
XC-30	NEGATIVE			NEG.	NEG.
XC-31	NEGATIVE			NEG.	NEG.
XC-33	NEGATIVE			NEG.	NEG.
XC-35	NEGATIVE			NEG.	NEG.
XC-36	NEGATIVE			NEG.	NEG.
XC-37	NEGATIVE			NEG.	NEG.
XC-38	NEGATIVE			NEG.	NEG.
FC-35	**POSITIVE**	**20**	**0**	**200**	**1,204**
FC-53	**POSITIVE**	**0**	**0**	**<10**	**554**
FC-54	**POSITIVE**	**54**	**0**	**540**	**430**
FC-55MR	**POSITIVE**	**0**	**0**	**<10**	**632**
FC-56	**POSITIVE**	**53**	**8**	**530**	**652**
FC-57	NEGATIVE			NEG.	NEG.
FC-58	**POSITIVE**	**62**	**0**	**620**	**492**
FC-59MR	**POSITIVE**	**0**	**0**	**<10**	**432**
FC-60	**POSITIVE**	**78**	**3**	**780**	**654**
FC-61	**POSITIVE**	**16**	**0**	**160**	**554**
FC-62MR	**POSITIVE**	**9**	**0**	**90**	**534**

[a]XC= well samples FC= stream samples

Appendix A Table 22. Fecal coliform and fecal streptococcus counts for October, 1999, Spout Run, Clarke County, VA[a]

SAMPLE ID. NO.	COLILERT TEST	FECAL COLIFORMS (FCs) 10ML	FECAL COLIFORMS (FCs) 1.0ML	FCs 100ML	FECAL STREPS 100ML
XC-28	**POSITIVE**	**14**	**2**	**140**	**979**
XC-29	NEGATIVE			NEG.	NEG.
XC-30	NEGATIVE			NEG.	NEG.
XC-31	**POSITIVE**	**0**	**0**	**<10**	**0**
XC-33	**POSITIVE**	**0**	**0**	**<10**	**0**
XC-35	**POSITIVE**	**0**	**0**	**<10**	**0**
XC-36	NEGATIVE			NEG.	NEG.
XC-37	**POSITIVE**	**0**	**0**	**<10**	**0**
XC-38	**POSITIVE**	**0**	**0**	**<10**	**0**
FC-35	**POSITIVE**	**119**	**12**	**1,190**	**580**
FC-53	**POSITIVE**	**119**	**10**	**1,190**	**872**
FC-54	**POSITIVE**	**195**	**9**	**1,950**	**864**
FC-55MR	**POSITIVE**	**144**	**7**	**1,440**	**1,228**
FC-56	**POSITIVE**	**307**	**74**	**3,070**	**852**
FC-57	**POSITIVE**	**202**	**11**	**2,020**	**50**
FC-58	**POSITIVE**	**263**	**64**	**2,630**	**872**
FC-59MR	**POSITIVE**	**208**	**62**	**2,080**	**742**
FC-60	**POSITIVE**	**257**	**77**	**2,570**	**748**
FC-61	**POSITIVE**	**261**	**54**	**2,610**	**852**
FC-62MR	**POSITIVE**	**225**	**70**	**2,250**	**712**

[a]XC= well samples FC= stream samples

Appendix A Table 23. Fecal coliform and fecal streptococcus counts for November, 1999, Spout Run, Clarke County, VA[a]

SAMPLE ID. NO.	COLILERT TEST	FECAL COLIFORMS (FCs) 10ML	FECAL COLIFORMS (FCs) 1.0ML	FCs 100ML	FECAL STREPS 100ML
XC-28	NEGATIVE			NEG.	NEG.
XC-29	NEGATIVE			NEG.	NEG.
XC-30	**POSITIVE**	**0**	**0**	**<10**	**0**
XC-31	NEGATIVE			NEG.	NEG.
XC-35	NEGATIVE			NEG.	NEG.
XC-36	NEGATIVE			NEG.	NEG.
XC-37	NEGATIVE			NEG.	NEG.
XC-38	**POSITIVE**	**0**	**0**	**<10**	**0**
FC-35	**POSITIVE**	**241**	**25**	**2,410**	**688**
FC-53	**POSITIVE**	**260**	**23**	**2,600**	**404**
FC-54	**POSITIVE**	**227**	**21**	**2,270**	**512**
FC-55MR	**POSITIVE**	**231**	**28**	**2,310**	**612**
FC-56	**POSITIVE**	**292**	**33**	**2,920**	**315**
FC-57	**POSITIVE**	**3**	**1**	**<10**	**0**
FC-58	**POSITIVE**	**345**	**35**	**3,450**	**624**
FC-59MR	**POSITIVE**	**240**	**30**	**2,400**	**568**
FC-60	**POSITIVE**	**230**	**25**	**2,300**	**576**
FC-61	**POSITIVE**	**208**	**21**	**2,080**	**632**
FC-62MR	**POSITIVE**	**224**	**31**	**2,240**	**592**

[a]XC= well samples FC= stream samples

Appendix A Table 24. Fecal coliform and fecal streptococcus counts for December, 1999, Spout Run, Clarke County, VA[a]

SAMPLE ID. NO.	COLILERT TEST	FECAL COLIFORMS (FCs) 10ML	FECAL COLIFORMS (FCs) 1.0ML	FCs 100ML	FECAL STREPS 100ML
XC-28	NEGATIVE			NEG.	NEG.
XC-29	NEGATIVE			NEG.	NEG.
XC-30	NEGATIVE			NEG.	NEG.
XC-31	NEGATIVE			NEG.	NEG.
XC-35	NEGATIVE			NEG.	NEG.
XC-36	NEGATIVE			NEG.	NEG.
XC-37	NEGATIVE			NEG.	NEG.
XC-38	NEGATIVE			NEG.	NEG.
FC-35	**POSITIVE**	**1**	**0**	**<10**	**<10**
FC-53	**POSITIVE**	**1**	**0**	**<10**	**16**
FC-54	**POSITIVE**	**0**	**0**	**<10**	**<10**
FC-55MR	**POSITIVE**	**2**	**0**	**20**	**30**
FC-56	**POSITIVE**	**14**	**0**	**140**	**38**
FC-57	NEGATIVE			NEG.	NEG.
FC-58	**POSITIVE**	**3**	**1**	**30**	**22**
FC-59MR	**POSITIVE**	**9**	**0**	**90**	**40**
FC-60	**POSITIVE**	**5**	**0**	**50**	**20**
FC-61	**POSITIVE**	**15**	**0**	**150**	**76**
FC-62MR	**POSITIVE**	**16**	**1**	**160**	**48**

[a]XC= well samples FC= stream samples

Appendix A Table 25. Fecal coliform and fecal streptococcus counts for January, 2000, Spout Run, Clarke County, VA[a]

SAMPLE ID. NO.	COLILERT TEST	FECAL COLIFORMS (FCs) 10ML	FECAL COLIFORMS (FCs) 1.0ML	FECAL FCs 100ML	STREPS 100ML
XC-28	NEGATIVE			NEG.	NEG.
XC-29	NEGATIVE			NEG.	NEG.
XC-30	NEGATIVE			NEG.	NEG.
XC-31	NEGATIVE			NEG.	NEG.
XC-35	NEGATIVE			NEG.	NEG.
XC-36	NEGATIVE			NEG.	NEG.
XC-37	NEGATIVE			NEG.	NEG.
FC-35	**POSITIVE**	**18**	**4**	**180**	**64**
FC-53	**POSITIVE**	**5**	**0**	**50**	**20**
FC-54	**POSITIVE**	**4**	**0**	**40**	**20**
FC-55MR	**POSITIVE**	**13**	**0**	**130**	**50**
FC-56	**POSITIVE**	**18**	**0**	**180**	**34**
FC-57	NEGATIVE			NEG.	NEG.
FC-58	**POSITIVE**	**1**	**1**	**<10**	**28**
FC-59MR	**POSITIVE**	**12**	**0**	**120**	**60**
FC-60	**POSITIVE**	**7**	**1**	**70**	**62**
FC-61	**POSITIVE**	**12**	**1**	**120**	**70**
FC-62MR	**POSITIVE**	**10**	**4**	**100**	**50**

[a]XC= well samples FC= stream samples

Appendix A Table 26. Fecal coliform and fecal streptococcus counts for February, 2000, Spout Run, Clarke County, VA[a]

SAMPLE ID. NO.	COLILERT TEST	FECAL COLIFORMS (FCs) 10ML	FECAL COLIFORMS (FCs) 1.0ML	FCs	FECAL STREPS 100ML	FECAL STREPS 100ML
XC-28	NEGATIVE				NEG.	NEG.
XC-29	NEGATIVE				NEG.	NEG.
XC-30	NEGATIVE				NEG.	NEG.
XC-31	NEGATIVE				NEG.	NEG.
XC-36	NEGATIVE				NEG.	NEG.
XC-37	NEGATIVE				NEG.	NEG.
XC-39	**POSITIVE**	**1**	**1**		**<10**	**<10**
FC-35	**POSITIVE**	**12**	**2**		**120**	**36**
FC-53	NEGATIVE				NEG.	NEG.
FC-54	**POSITIVE**	**3**	**0**		**30**	**20**
FC-55MR	**POSITIVE**	**3**	**0**		**30**	**38**
FC-56	**POSITIVE**	**8**	**2**		**80**	**38**
FC-57	NEGATIVE				NEG.	NEG.
FC-58	**POSITIVE**	**4**	**0**		**40**	**12**
FC-59MR	**POSITIVE**	**6**	**1**		**60**	**14**
FC-60	**POSITIVE**	**8**	**0**		**80**	**50**
FC-61	**POSITIVE**	**5**	**0**		**50**	**46**
FC-62MR	**POSITIVE**	**12**	**0**		**120**	**70**

[a]XC= well samples FC= stream samples

Appendix A Table 27. Fecal coliform and fecal streptococcus counts for March, 2000, Spout Run, Clarke County, VA[a]

SAMPLE ID. NO.	COLILERT TEST	FECAL COLIFORMS (FCs) 10ML	FECAL COLIFORMS (FCs) 1.0ML	FCs	FECAL STREPS 100ML
	100ML				
XC-28	NEGATIVE			NEG.	NEG.
XC-29	NEGATIVE			NEG.	NEG.
XC-30	NEGATIVE			NEG.	NEG.
XC-31	NEGATIVE			NEG.	NEG.
XC-35	NEGATIVE			NEG.	NEG.
XC-36	NEGATIVE			NEG.	NEG.
XC-37	NEGATIVE			NEG.	NEG.
FC-35	**POSITIVE**	**60**	**3**	**600**	**90**
FC-53	**POSITIVE**	**8**	**1**	**80**	**66**
FC-54	**POSITIVE**	**21**	**12**	**210**	**70**
FC-55MR	**POSITIVE**	**13**	**2**	**130**	**41**
FC-56	**POSITIVE**	**19**	**1**	**190**	**42**
FC-57	NEGATIVE			NEG.	NEG.
FC-58	**POSITIVE**	**12**	**0**	**120**	**40**
FC-59MR	**POSITIVE**	**20**	**2**	**200**	**80**
FC-60	**POSITIVE**	**28**	**0**	**280**	**92**
FC-61	**POSITIVE**	**23**	**5**	**230**	**98**
FC-62MR	**POSITIVE**	**16**	**0**	**160**	**74**

[a]XC= well samples FC= stream samples

Appendix A Table 28. Fecal coliform and fecal streptococcus counts for April, 2000, Spout Run, Clarke County, VA[a]

SAMPLE ID. NO.	COLILERT TEST	FECAL COLIFORMS (FCs) 10ML	FECAL COLIFORMS (FCs) 1.0ML	FCs 100ML	FECAL STREPS 100ML
XC-09	NEGATIVE			NEG.	NEG.
XC-28	NEGATIVE			NEG.	NEG.
XC-29	NEGATIVE			NEG.	NEG.
XC-30	NEGATIVE			NEG.	NEG.
XC-31	NEGATIVE			NEG.	NEG.
XC-33	NEGATIVE			NEG.	NEG.
XC-35	NEGATIVE			NEG.	NEG.
XC-36	NEGATIVE			NEG.	NEG.
XC-37	NEGATIVE			NEG.	NEG.
XC-39	**POSITIVE**	**30**	**10**	**300**	**38**
FC-35	**POSITIVE**	**74**	**7**	**740**	**398**
FC-53	**POSITIVE**	**1**	**1**	**<10**	**24**
FC-54	**POSITIVE**	**8**	**0**	**80**	**92**
FC-55MR	**POSITIVE**	**3**	**0**	**30**	**42**
FC-56	**POSITIVE**	**7**	**5**	**70**	**50**
FC-57	NEGATIVE			NEG.	NEG.
FC-58	**POSITIVE**	**10**	**2**	**100**	**<10**
FC-59MR	**POSITIVE**	**18**	**0**	**180**	**56**
FC-60	**POSITIVE**	**14**	**0**	**140**	**504**
FC-61	**POSITIVE**	**29**	**0**	**290**	**110**
FC-62MR	**POSITIVE**	**4**	**0**	**40**	**68**

[a]XC= well samples FC= stream samples

Appendix A Table 29. Fecal coliform and fecal streptococcus counts for May, 2000, Spout Run, Clarke County, VA[a]

SAMPLE ID. NO.	COLILERT TEST	FECAL COLIFORMS (FCs) 10ML	FECAL COLIFORMS (FCs) 1.0ML	FCs 100ML	FECAL STREPS 100ML
XC-28	NEGATIVE			NEG.	NEG.
XC-29	NEGATIVE			NEG.	NEG.
XC-30	NEGATIVE			NEG.	NEG.
XC-31	NEGATIVE			NEG.	NEG.
XC-35	NEGATIVE			NEG.	NEG.
XC-36	NEGATIVE			NEG.	NEG.
XC-37	NEGATIVE			NEG.	NEG.
FC-35	**POSITIVE**	**63**	**4**	**630**	**298**
FC-55MR	**POSITIVE**	**154**	**22**	**1,540**	**896**
FC-56	**POSITIVE**	**125**	**12**	**1,250**	**604**
FC-57	NEGATIVE			NEG.	NEG.
FC-58	**POSITIVE**	**103**	**18**	**1,030**	**644**
FC-59MR	**POSITIVE**	**133**	**0**	**1,320**	**592**
FC-60	**POSITIVE**	**150**	**9**	**1,500**	**440**
FC-61	**POSITIVE**	**117**	**16**	**1,170**	**720**
FC-62MR	**POSITIVE**	**157**	**9**	**1,570**	**600**

[a]XC= well samples FC= stream samples

APPENDIX B

TABLES 30-40, CHEMICAL ANALYSES BY MONTH AND SAMPLE

Appendix B Table 30. Chemical analyses for June, 1999, Spout Run, Clarke County, VA.[a]

Sample ID	pH	PO_4	NO_3	NH_4	BOD_5
		mg/l	mg/l	mg/l	mg/l
XC - 31	7.81	0.13	0.15	0.19	2.0
FC - 53	8.07	0.19	2.51	0.22	0.8
FC - 54	8.02	0.16	2.61	0.24	2.3
FC - 55	8.07	0.25	2.34	0.27	0.8
FC - 56	8.04	0.19	2.29	0.25	0.5
FC - 58	8.06	0.10	2.21	0.19	0.8
FC - 59	8.05	0.21	2.43	0.32	0.8
FC - 60	8.20	0.19	2.37	0.25	0.8
FC - 61	8.04	0.16	2.40	0.32	0.8
FC - 62	8.09	0.18	2.38	0.25	2.0

[a]XC= well samples FC= stream samples

Appendix B Table 31. Chemical analyses for July, 1999, Spout Run, Clarke County, VA.[a]

Sample ID	pH	PO_4	NO_3	NH_4	BOD_5	Turbidity
		mg/l	mg/l	mg/l	mg/l	FTU
XC - 28	7.61	0.18	0.91	0.27	0.3	0.00
XC-29	7.67	0.18	2.38	0.28	0.2	0.00
XC-30	7.49	0.14	5.75	0.24	0.5	0.00
XC-31	7.38	0.19	0.27	0.33	0.8	148
XC-33	7.37	0.14	0.36	0.30	0.2	0.00
XC-37	7.27	0.16	5.17	0.29	0.5	1.92
FC-53	8.00	0.21	2.63	0.35	2.8	5.05
FC-54	7.97	0.21	2.80	0.36	0.5	1.57
FC-55	8.01	0.19	2.62	0.36	0.8	46.8
FC-56	8.02	0.22	2.54	0.33	1.4	10.3
FC-57	7.62	0.20	3.43	0.34	0.0	0.00
FC-58	7.86	0.17	2.21	0.34	1.1	16.6
FC-59	7.91	0.17	2.65	0.38	0.5	26.3
FC-60	7.95	0.21	2.64	0.51	4.1	10.1
FC-61	7.94	0.21	2.60	0.33	0.9	5.18
FC-62	7.92	0.17	2.59	0.31	0.6	8.88

[a]XC= well samples FC= stream samples

Appendix B Table 32. Chemical analyses for August, 1999, Spout Run, Clarke County, VA.[a]

Sample ID	pH	PO_4	NO_3	NH_4	BOD_5	Turbidity
		mg/l	mg/l	mg/l	mg/l	FTU
XC-28	7.48	0.20	0.89	0.20	0.0	1.67
XC-29	7.67	0.18	2.90	0.21	1.2	0.00
XC-30	7.53	0.13	5.72	0.15	0.0	0.00
XC-31	8.17	0.17	0.17	0.20	1.1	179
XC-33	7.49	0.18	0.38	0.22	0.9	0.00
XC-35	7.84	0.21	2.50	0.19	0.8	0.00
XC-36	7.28	0.17	3.55	0.16	0.8	0.00
XC-37	7.50	0.18	4.72	0.14	2.0	4.33
XC-38	7.53	0.17	1.76	0.13	1.1	0.00
FC-53	8.13	0.19	2.78	0.21	1.2	7.44
FC-54	8.04	0.18	2.90	0.20	0.5	0.76
FC-55	8.05	0.15	2.71	0.17	1.1	27.3
FC-56	8.12	0.17	2.59	0.17	1.2	7.92
FC-57	7.78	0.17	2.42	0.14	0.6	0.00
FC-58	8.05	0.15	2.39	0.19	1.1	28.3
FC-59	8.05	0.19	2.74	0.20	1.2	39.2
FC-60	8.10	0.22	2.79	0.26	1.2	21.9
FC-61	8.12	0.22	2.80	0.26	1.1	21.2
FC-62	8.11	0.17	2.74	0.20	1.1	25.5

[a]XC= well samples FC= stream samples

Appendix B Table 33. Chemical analyses for September, 1999, Spout Run, Clarke County, VA.[a]

Sample ID	pH	PO_4	NO_3	NH_4	BOD_5	Turbidity
		mg/l	mg/l	mg/l	mg/l	FTU
XC-28	7.25	O.18	1.12	0.26	2.7	16.5
XC-29	7.51	0.21	2.15	0.23	3.2	0.30
XC-30	7.36	0.17	6.09	0.22	2.6	1.92
XC-31	7.82	0.16	0.17	0.22	2.6	3.55
XC-33	7.22	0.13	0.53	0.18	2.0	0.00
XC-35	7.49	0.17	3.77	0.17	2.6	1.41
XC-38	7.14	0.13	2.00	0.20	2.3	1.18
FC-53	7.91	0.18	2.30	0.24	3.3	49.6
FC-54	7.88	0.20	2.49	0.28	3.6	26.0
FC-55	7.97	0.18	2.35	0.23	3.3	82.0
FC-56	8.03	0.15	2.16	0.20	7.7	0.00
FC-57	7.64	0.15	2.51	0.26	3.2	0.00
FC-58	7.92	0.17	2.08	0.22	4.1	51.0
FC-59	7.88	0.17	2.37	0.24	4.2	49.8
FC-60	7.97	0.15	2.33	0.21	3.6	37.0
FC-61	7.89	0.15	2.38	0.20	3.8	28.9
FC-62	7.85	0.18	2.38	0.22	3.2	45.4

[a]XC= well samples FC= stream samples

Appendix B Table 34. Chemical analyses for October, 1999, Spout Run, Clarke County, VA.[a]

Sample ID	pH	PO_4	NO_3	NH_4	BOD_5	Turbidity
		mg/l	mg/l	mg/l	mg/l	FTU
XC - 28	7.47	0.10	0.86	0.22	0.8	0.46
XC - 29	7.71	0.08	4.26	0.19	0.3	0.25
XC - 30	7.52	0.07	5.83	0.21	0.2	0.20
XC - 31	7.62	0.08	0.13	0.24	0.8	24.63
XC - 33	7.35	0.07	0.50	0.22	0.2	0.00
XC - 35	7.46	0.10	2.37	0.25	0.6	0.00
XC - 36	7.29	0.07	2.63	0.39	0.6	1.73
XC - 37	7.33	0.09	4.37	0.23	0.3	3.94
XC - 38	7.35	0.09	1.75	0.28	0.2	1.78
FC - 53	8.17	0.09	1.94	0.23	1.2	19.68
FC - 54	8.12	0.12	2.00	0.24	0.8	11.45
FC - 55	8.15	0.12	1.78	0.23	0.8	12.84
FC - 56	8.10	0.10	1.74	0.21	0.9	16.74
FC - 57	7.50	0.10	3.36	0.23	0.0	0.31
FC - 58	8.18	0.11	1.69	0.23	1.2	21.80
FC - 59	8.02	0.10	1.79	0.28	1.1	11.41
FC - 60	8.08	0.11	1.80	0.23	1.1	8.21
FC - 61	8.01	0.09	1.79	0.24	1.4	4.38
FC - 62	7.96	0.08	1.80	0.25	1.2	4.81

[a]XC= well samples FC= stream samples

Appendix B Table 35. Chemical analyses for November, 1999, Spout Run, Clarke County, VA.[a]

Sample ID	pH	PO_4	NO_3	NH_4	BOD_5	Turbidity
		mg/l	mg/l	mg/l	mg/l	FTU
XC 28	7.32	0.01	0.47	0.11	2.3	7.82
XC 29	7.55	0.13	4.12	0.13	1.7	2.89
XC 30	7.46	0.09	6.03	0.12	2.1	1.70
XC 31	7.44	0.11	0.08	0.13	1.4	97
XC 35	7.61	0.11	1.93	0.10	1.7	0.05
XC 36	7.13	0.08	2.93	0.12	1.5	53
XC 37	7.35	0.05	4.44	0.11	2.1	6.88
XC 38	7.31	0.08	1.62	0.12	2.0	0.29
FC 53	8.02	0.07	2.08	0.15	2.0	4.70
FC 54	8.02	0.06	2.11	0.12	2.0	3.48
FC 55	8.06	0.07	1.72	0.12	2.3	5.83
FC 56	8.11	0.06	1.76	0.24	9.6	22.41
FC 57	7.76	0.09	3.05	0.12	2.0	0.57
FC 58	8.09	0.06	1.69	0.09	2.3	3.71
FC 59	8.11	0.15	1.81	0.19	2.4	2.51
FC 60	8.12	0.05	1.81	0.10	2.4	4.25
FC 61	8.00	0.14	1.85	0.12	2.1	7.17
FC 62	7.97	0.15	1.88	0.12	2.1	9.47

[a]XC= well samples FC= stream samples

Appendix B Table 36. Chemical analyses for December, 1999, Spout Run, Clarke County, VA.[a]

Sample ID	pH	PO_4	NO_3	NH_4	BOD_5	Turbidity
		mg/l	mg/l	mg/l	mg/l	FTU
XC - 28	7.37	0.09	0.84	0.20	0	0.42
XC - 29	7.57	0.09	4.40	0.22	0.6	0.52
XC - 30	7.37	0.08	7.48	0.19	0.2	0.26
XC - 31	7.48	0.07	0.14	0.27	0	31.42
XC - 35	7.65	0.09	2.37	0.20	0	0.28
XC - 36	7.25	0.08	3.10	0.26	0	17.26
XC - 37	7.23	0.07	5.17	0.13	0	5.39
XC - 38	7.24	0.07	1.99	0.18	0	0.30
FC - 53	8.07	0.09	2.61	0.18	0	1.21
FC - 54	7.95	0.11	2.77	0.19	1.2	0.86
FC - 55	8.06	0.10	2.36	0.16	0.8	2.55
FC - 56	8.06	0.10	2.43	0.16	1.4	1.66
FC - 57	7.60	0.10	3.41	0.22	0	0.19
FC - 58	8.05	0.10	2.28	0.18	0	2.22
FC - 59	8.06	0.10	2.36	0.15	1.1	3.48
FC - 60	8.06	0.10	2.47	0.17	0.5	2.51
FC - 61	7.88	0.09	2.53	0.18	0.9	3.41
FC - 62	7.99	0.12	2.65	0.24	1.5	4.10

[a]XC= well samples FC= stream samples

Appendix B Table 37. Chemical analyses for January, 2000, Spout Run, Clarke County, VA.[a]

Sample ID	pH	PO_4	NO_3	NH_4	BOD_5	Turbidity
		mg/l	mg/l	mg/l	mg/l	FTU
XC 28	7.74	0.03	1.08	0.18	0.2	3.56
XC 29	7.75	0.03	3.57	0.05	0.6	0.26
XC 30	7.65	0.03	7.54	0.07	0.2	0.14
XC 31	7.52	0.02	0.27	0.18	3.6	521
XC 35	7.44	0.07	2.49	0.22	0.5	0.78
XC 36	7.21	0.03	2.88	0.10	0.5	71
XC 37	7.38	0.03	5.42	0.08	0.6	25
FC 10	7.84	0.03	1.93	0.12	0.8	2.88
FC 12	8.03	0.03	2.14	0.04	1.4	4.05
FC 15	7.57	0.03	3.01	0.11	0.8	0.92
FC 16	7.99	0.03	1.99	0.11	0.2	2.33
FC 35	7.99	0.03	2.43	0.13	1.2	9.79
FC 53	8.05	0.03	2.62	0.02	0.6	1.95
FC 54	8.07	0.03	2.68	0.02	0.5	2.28
FC 55	8.05	0.04	2.40	0.12	0	2.01
FC 56	8.07	0.04	2.49	0.05	0.6	2.21
FC 57	7.71	0.03	3.44	0.05	0.8	1.71
FC 58	8.07	0.04	2.37	0.06	0.5	1.97
FC 59	8.12	0.04	2.38	0.01	1.4	4.20
FC 60	8.11	0.04	2.49	0.19	1.1	2.83
FC 61	8.08	0.04	2.42	0.05	0.8	3.30
FC 62	8.05	0.04	2.48	0.07	1.2	1.90

[a]XC= well samples FC= stream samples

Appendix B Table 38. Chemical analyses for February, 2000, Spout Run, Clarke County, VA.[a]

Sample ID	pH	PO_4	NO_3	NH_4	BOD_5	Turbidity
		mg/l	mg/l	mg/l	mg/l	FTU
XC - 28	7.40	0.03	0.91	0.20	1.2	3.43
XC - 29	7.68	0.02	4.45	0.13	1.0	0.00
XC - 30	7.55	0.02	6.77	0.21	0.9	0.00
XC - 31	7.45	0.02	0.20	0.09	1.0	18.39
XC - 36	7.39	0.00	3.07	0.06	21.0	48.27
XC - 37	7.16	0.00	5.54	0.06	0.8	7.60
XC - 39	7.32	0.01	11.36	0.08	1.4	43.33
FC - 35	7.82	0.02	2.41	0.15	1.0	5.20
FC - 53	8.14	0.03	2.85	0.19	1.5	2.46
FC - 55	8.40	0.03	2.51	0.10	2.0	2.51
FC - 56	8.16	0.02	2.62	0.12	1.5	4.50
FC - 57	8.19	0.02	3.64	0.13	1.0	0.21
FC - 58	8.10	0.02	2.61	0.12	1.5	1.92
FC - 59	8.02	0.03	2.64	0.16	1.0	2.32
FC - 60	8.10	0.05	2.68	0.13	1.5	3.48
FC - 61	8.17	0.05	2.77	0.22	3.4	6.25
FC- 62	7.94	0.04	2.71	0.13	1.8	7.08

[a]XC= well samples FC= stream samples

Appendix B Table 39. Chemical analyses for March, 2000, Spout Run, Clarke County, VA.[a]

Sample ID	pH	PO_4	NO_3	NH_4	BOD_5	Turbidity
		mg/l	mg/l	mg/l	mg/l	FTU
XC - 28	7.09	0.04	0.03	0.14	2.0	0.00
XC - 29	7.50	0.04	4.51	0.10	1.8	0.00
XC - 30	7.43	0.03	7.24	0.11	1.8	0.00
XC - 31	7.36	0.04	0.17	0.11	1.8	0.52
XC - 35	7.00	0.04	2.25	0.09	1.5	0.00
XC - 36	7.00	0.02	2.88	0.09	1.2	21.50
XC - 37	7.30	0.04	5.49	0.30	2.8	14.38
FC - 43	7.90	0.04	2.04	0.10	1.5	7.06
FC - 44	8.05	0.15	2.97	0.34	3.3	7.16
FC - 53	8.03	0.03	2.32	0.08	1.4	3.50
FC - 54	7.97	0.04	1.86	0.09	1.8	3.93
FC - 55	7.98	0.04	2.38	0.14	1.5	4.21
FC - 56	7.99	0.04	2.08	0.05	2.1	14.19
FC - 57	7.66	0.03	3.43	0.11	1.5	0.00
FC - 58	7.88	0.04	2.02	0.09	1.6	4.31
FC - 59	7.94	0.05	2.23	0.16	2.0	5.96
FC - 60	7.94	0.04	2.16	0.09	2.1	9.31
FC - 61	7.94	0.06	2.22	0.20	2.7	16.04
FC- 62	7.88	0.04	2.13	0.08	1.8	0.58

[a]XC= well samples FC= stream samples

Appendix B Table 40. Chemical analyses for April, 2000, Spout Run, Clarke County, VA.[a]

Sample ID	pH	PO_4	NO_3	NH_4	BOD_5	Turbidity
		mg/l	mg/l	mg/l	mg/l	FTU
XC - 09	7.64	0.02	2.62	0.22	0.2	0.00
XC - 28	7.54	0.02	0.75	0.07	0.3	0.68
XC - 29	7.76	0.02	3.94	0.34	0.3	1.97
XC - 30	7.70	0.02	5.97	0.25	0.3	1.28
XC - 31	7.68	0.01	0.15	0.10	0.2	4.50
XC - 33	7.56	0.02	0.34	0.12	0.2	1.49
XC - 35	7.57	0.03	2.14	0.04	0.3	0.00
XC - 36	7.40	0.02	0.31	0.21	0.4	0.73
XC - 37	7.39	0.02	5.03	0.11	1.4	3.02
XC - 39	7.38	0.02	9.39	0.24	0.2	16.11
FC - 53	8.07	0.02	2.30	0.13	0.4	6.59
FC - 54	7.98	0.02	2.10	0.11	1.2	7.43
FC - 55	8.12	0.02	1.67	0.11	3.0	6.23
FC - 56	8.13	0.02	1.81	0.15	1.0	11.21
FC - 57	7.68	0.03	3.17	0.11	1.4	0.00
FC - 58	8.10	0.02	1.82	0.10	0.8	3.05
FC - 59	8.10	0.02	1.70	0.16	2.4	2.90
FC - 60	8.14	0.01	1.78	0.13	0.6	3.81
FC - 61	8.11	0.01	1.72	0.14	0.3	3.21
FC- 62	8.05	0.02	1.78	0.15	0.8	3.12

[a]XC= well samples FC= stream samples

VITA

The author was born on July 12, 1976 to Alexander and Claretta Graves and raised in Yanceyville, NC. She graduated from Bartlett Yancey Senior High School in 1994 and began her undergraduate studies at Winston-Salem State University in August 1994. In 1998 the author received a Bachelor of Science in Biology with a minor in Chemistry from Winston-Salem State University.

In the fall of 1998 the author began her Master of Science in Crop and Soil Environmental Science at Virginia Tech under the guidance of Dr. Charles Hagedorn. She also worked with Dr. Hagedorn on determining sources of fecal pollution in water for a rural Virginia community and this project was sponsored by the Virginia Department of Conservation and Recreation. Her research project focused on the use of a new methodology, bacterial source tracking, to determine the source of fecal contamination in water.

After finishing all required coursework , the author was accepted to begin her studies in the Doctorate program, in the Department of Crop and Soil Environmental Sciences at Virginia Tech.