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MICROBIAL IMPACTS OF ANIMAL WASTES AND OTHER FECAL WASTES ON WATER RESOURCES

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ABSTRACT

The microbial impacts of animal wastes and other fecal wastes on surface water quality were studied. Surface water samples were collected quarterly for three years in Gaston and Wake counties, NC, in watersheds having different levels of agricultural, residential, and urban development. In the Long Creek watershed (Gaston County) factors influencing enteric microbe levels in surface water samples collected at a dairy farm, a municipal biosolids land application site and an urbanized region were investigated. In the Lake Wheeler watershed the potential impact of animal agriculture systems on stream water microbial quality was investigated.

Geometric mean microbial concentrations varied with land usage, sampling site, season, total suspended solids (TSS) and rainfall events. Geometric mean concentrations of fecal coliforms, *E. coli*, and somatic coliphages were generally higher at sites impacted by animal and human wastes compared to rural residential and background stations. Fecal coliform levels declined at the Gaston County dairy stations after implementing best management practices. Runoff from a cattle pasture and feedlot at Lake Wheeler Road Farm contained high concentrations of microbial indicators. The levels of microbial indicators and TSS increased significantly during rainfall events at all sites. Microbial indicators were positively correlated with TSS, but correlation strengths differed for base flow and storm samples. Of the microbial indicators, concentrations of enterococci and somatic coliphages were most influenced by season and were highest in summer.

When fecal coliform results were compared to NC's standard for stream water quality, only 2 of 6 rural residence/background sites complied. The percentage of samples complying varied from 0% (livestock-impacted site) to 60% (rural/background site).

Animal feeding operations (AFOs), biosolids land application areas and urban development impacted the microbial quality of nearby surface waters. Microbial impacts on stream water quality were not generally significant for base flows but they were for storm flows.

KEY WORDS
(Water Quality, Microbiology, Microbial Indicators, Fecal Contamination, Animal Wastes, Wastewater, Land Use, Storm Events)

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SUMMARY AND CONCLUSIONS

Animal feeding operations, municipal biosolids land application and urban land uses, including municipal waste water effluent discharges, resulted in increased levels of microbial contamination of fecal origin in adjacent or nearby stream waters. Increased fecal contamination of stream waters was associated with all three types of activities, despite the fact that waste management systems for AFOs and biosolids application sites are considered to be of the "non-discharge" type. In general, urban-impacted and livestock-impacted stations had higher bacterial densities than rural/background stations. However, these differences were only statistically significant between livestock-impacted stations and rural/background stations.

The densities of viral indicators of fecal contamination were influenced differently by the various inputs and sources than were the bacterial indicators of fecal contamination. The somatic coliphages were highest at the livestock-impacted sites, achieving statistically significant ($p<0.05$) differences when compared to rural/background stations. In contrast, the F+ coliphage concentrations were lowest at the rural/background stations and highest at the urban-impacted sites. These differences in F+ coliphage levels were statistically significant between the urban-impacted sites and both the livestock-impacted and rural/background sites. In this study, F+ RNA coliphages were more commonly isolated at urban-impacted sites than both rural/background sites and livestock-impacted sites. The proportion of Type I F+ RNA coliphages isolated was highest for the background/rural sites (76%), followed by urban sites (60%). The proportion of Type II F+ RNA coliphages was lowest at the rural/background stations (18%) and highest at the urban stations (32%). Type IV F+ RNA coliphages were detected primarily at the agricultural sites.

Specific Impacts on Microbial Indicator Concentrations in Water

Land Usage- Only 2 (MS19 and S0) of the 6 rural/background monitoring stations studied had geometric mean fecal coliform levels within the North Carolina standard for stream water quality (geometric mean of 200 fecal coliforms/100mL). The fecal coliform standard was met by 7 of 22 (32%) samples at the rural/background sites upgradient from the dairy (MS12 and MS13), 3 of 10 (30%) samples at the background site for the urban watershed (MS22) and 4 of 24 (17%) of the samples at the rural/background sites for the Lake Wheeler Road Farm cattle and swine operation areas (S1 and S7).

Significant ($p<0.05$) increases in the geometric mean concentrations of 5 of the 6 measured fecal indicators were observed between upgradient surface water sampling stations and the Long Creek Watershed dairy farm stations. Implementation of Best Management Practices (BMPs) on this dairy significantly ($p=0.0006$) decreased the fecal coliform densities at the dairy sampling site downgradient from the heavy animal use area (MS17). At the western cattle pasture of Lake Wheeler Road Farm, the geometric mean concentrations of microbial indicators at the station immediately downgradient of the pasture were higher than the concentrations at the background station immediately upgradient of the cattle pasture areas, although none of these increases were statistically significant at the 5% level. However, the geometric mean concentrations of fecal

coliforms at livestock-impacted sites MS17, S4 and S11 greatly exceeded (10,300, 600 and 1200 CFUs/100mL, respectively) the North Carolina standard for stream water quality. None of the samples collected from MS17, one quarter of the S4 samples, and one sixth of the samples collected from S11 met this criterion. In the northern cattle and swine operations area at Lake Wheeler Road Farm, there was a general trend toward increasing microbial indicator concentrations between the background surface water station and downstream stations, with the exception of *C. perfringens*; however, these increases were statistically significant only for somatic and F+ coliphages.

Runoff from the western cattle pasture contained fecal coliforms at concentrations greater than 10^5 CFU/100 mL during one of the precipitation events studied. This is a level at and below which vegetative buffer techniques can be effective for the removal of enteric bacteria (Crane et al., 1983), although it is not certain that adequate control of fecal bacteria also results in adequate control of fecal viruses and parasites. Other management techniques, such as keeping grass short on pastures and land application sites and adjusting the frequency of land application, can increase the die-off and retention of enteric microbes in fecal wastes applied to land. This increased die-off is expected to occur due to increasing exposure of the microbes to environmental degradation by mechanisms such as sunlight irradiation and desiccation (Dazzo et al., 1973; Crane et al., 1983). The extent to which cattle waste runoff may have contributed to the increased concentrations of fecal bacteria observed in stream water samples from the Lake Wheeler Farm Road stations potentially impacted by the dairy operation is not known and was not specifically investigated in this study.

At the municipal biosolids recovery station in the Long Creek watershed, increases in the mean microbial indicator densities were observed downgradient from the land application sites. The difference in the fecal coliform, *E. coli,* and somatic coliphage geometric mean $\log_{10}$ concentrations between the upgradient and downgradient sites increased following land application of biosolids, although not significantly ($p>0.05$). The geometric mean concentration of fecal coliforms at the two downgradient sites (660 CFUs/100mL) exceeded the North Carolina stream quality standard. Only 3 of the 24 (13%) samples collected downgradient from the biosolids land application sites had fecal coliform densities within the North Carolina stream water quality standard.

Comparing the four urban watershed sampling stations, the station downgradient from the wastewater treatment plant (WWTP) outfalls of the cities of Dallas and Gastonia (MS23) exhibited the highest geometric mean concentrations of fecal coliforms, *E. coli*, *Cl. perfringens*, somatic coliphages, and male-specific (F+) coliphages. However, there were not statistically significant changes in these microbial concentrations when the city of Gastonia WWTP outfall was moved to a new location during the study period (in 1998). The geometric mean fecal coliform concentration at this site was 600 CFUs/100mL, exceeding the NC stream water quality standard. Only 2 of the 11 (18%) samples taken during the study period met this criterion. The station immediately downstream of the City of Gastonia urban area (MS22) had the highest geometric mean concentration of enterococci.

Of all the stations monitored in the Long Creek Watershed, the two sampling stations near the dairy farm had the highest geometric mean concentrations of fecal coliforms, *E. coli*, enterococci, *Cl. perfringens*, and somatic coliphages.

Weather- Sampling during storm events revealed statistically significant increases in microbial indicator densities and total suspended solids (TSS). Overall, the microbial densities were significantly correlated ($p<0.05$) with TSS measurements. However, the correlations between TSS and geometric mean microbial concentrations were less strong for some indicators when only storm event results were analyzed. This could be due to the lower numbers of samples analyzed, which reduces the power of the statistical analyses. However, it could also be due to the existence of other possible mechanisms or routes of microbial transport to streamwater stations besides stormwater runoff.

Seasonal effects were observed for the levels of several microbial indicators in both the watersheds studied. These differences were the greatest between winter and summer for *C. perfringens*, fecal coliforms, and enterococci. Microbial indicators that were highest in the winter relative to other seasons included F+ coliphages and *C. perfringens*. In contrast, fecal coliforms, enterococci, and somatic coliphages were highest in the summer relative to other seasons.

The mechanisms by which microbial fecal contamination of stream waters occurs from the studied sources and land uses were not specifically investigated. Therefore, further investigation would be required to elucidate the pathways through which fecal contamination from these sources reaches surface waters, especially for the "non-discharge" sources.

RECOMMENDATIONS

The results of this study suggest that greater efforts are needed to prevent fecal contamination of stream waters from confined animal feeding operations, municipal biosolids application sites and urban activities, including municipal wastewater effluent discharges. This is because all three types of activities were associated with increased fecal contamination of stream waters, despite the fact that waste management systems for AFOs and biosolids application sites are considered "non-discharge" systems. Therefore, more intensive and broader investigations of this type are strongly recommended in order to gain a better understanding of the microbial impacts of animal and human fecal contamination on North Carolina water resources.

The use of a range of microbial indicators of fecal contamination as used in this study is also recommended for future studies of this nature. This is because different microbial indicators showed somewhat different patterns of occurrence with respect to fecal waste sources and land use, seasons and precipitation events. It is also recommended that future studies of this nature include measurements of enteric microbial pathogens, including viruses, bacteria and parasites, in addition to the microbial indicators of fecal contamination that were measured. This is because the risks to human health that may occur as a result of exposure to or ingestion of fecally contaminated stream water are directly related to the concentrations and loads of pathogenic microorganisms present. However, the levels and amounts of pathogens can not be quantitatively predicted from the levels of microbial indicators.

The routes and mechanisms by which microbial contamination by fecal wastes from AFOs and municipal biosolids land application systems reach adjacent or nearby stream waters requires further investigation and is recommended. This is because the transport routes and mechanisms of such contamination were not specifically investigated in this study. Furthermore, only the concentrations of microbial contamination were measured in this study. While the concentrations of fecal microbes in water is essential information for understanding human exposure risks to waterborne fecal contamination and is the basis for regulatory criteria and standards, further information about microbial contamination also would be useful, such as microbial loads and streamflows. Due to limited financial and human resources, data for microbial concentrations in stream waters were not linked to stream flow data to calculate or estimate total microbial loads to stream reaches, sub-basins or watersheds. In addition, the data for microbial concentrations in stream waters were not used in hydrological or other models to assess or otherwise analyze the impacts of fecal contamination on streamwater quality within the watersheds or their sub-basins. Such analyses would undoubtedly provide further insights into the microbial impacts of the fecal contamination that was investigated on water quality in a broader environmental context, and they are recommended for future studies.

INTRODUCTION

Background

Fecal contamination of water is the most common cause of human waterborne illness (Barwick et al., 2000; Kramer et al., 1996), and continues to be a problem in the United States in spite of regulations designed to protect surface water quality for primary contact recreation and for drinking water supplies. Several widely publicized outbreaks of waterborne disease resulting in significant morbidity and deaths in immune-compromised individuals have raised public awareness of the risks of microbially contaminated drinking and recreational waters. The potential sources of this contamination include human waste inputs, feral animals, and animal feeding operations (AFOs). Efforts to determine the sources of contamination have been largely unsuccessful because detection methods have been limited for distinguishing between these fecal wastes. In addition, specific risk factors for waterway impairment by fecal waste sources are poorly characterized. Nonetheless, the USDA/USEPA estimates that AFOs impact at least 10% of the nation's impaired waterways.

Animal waste occupies a unique niche in the landscape of waste management approaches and strategies. Historically viewed by the farming community as a resource used to reduce the use of chemical fertilizers, the predominant management practice that has evolved consists of disposing concentrated animal feeding operations (CAFO) -generated animal wastes through land application. This is a "non-discharge" method that does not allow the direct discharge of the wastes to surface water, as is the tradition for the disposal of most treated municipal wastewater. On farms where animal stocking rates on pastures are sufficiently low, the fecal waste is not collected but, instead, is left on the fields to be microbially degraded through natural processes. There is a limited body of evidence suggesting that these current, government-approved waste management systems of cattle and swine facilities may not consistently or adequately protect surface waters from microbial non point source (NPS) pollution under all animal production and management scenarios.

Untreated animal wastes contain high concentrations of enteric microorganisms and can contain microbial human pathogens (Hill & Sobsey, 1998; Hrubant et al., 1972; Crane et al., 1980; Overcash et al., 1983; Cole et al., 1999). Furthermore, enteric microbes have been shown to survive for weeks on pasture land soil and vegetation, depending on factors such as ambient temperature, humidity, exposure to sunlight, soil pH, soil moisture content, and land application frequency (Crane and Moore, 1986; Brown et al., 1980; Larkin et al., 1976; Dazzo et al., 1973). Microbes can potentially survive and be transported to groundwater through infiltration or to surface water through runoff from agricultural lands (Moore et al., 1981; McMurry et al., 1998). However, published studies have reached conflicting or inconsistent conclusions regarding the microbial impacts of animal production activities on stream water quality. Some studies observe correlations between cattle grazing and enteric microbial indicator concentrations in stream water and others show little difference in water quality between control

watersheds and those containing animal production or manure-spreading activities (Baxter-Potter et al., 1988; Crane et al., 1983).

Other studies have observed trends or patterns in microbial water contamination and have attempted to link these to various watershed impacts. A review of human cryptosporidiosis implicated agricultural pollution using seasonal and temporal trends in human infection that frequently coincided with increased rainfall and certain agricultural practices such as lambing, calving, and the application of wastes to cropland (Meinhardt, 1996). Other studies of water contamination with human pathogens have also noted increased fecal microbe concentrations in surface water during times of increased rainfall (Patz, 1998; Ashford, 1998; Mager, 1996). A small panel study (Mager, 1996) evaluating the presence of human pathogens in various U.S. watersheds found seasonal variation in the occurrence of waterborne pathogens. In this study, *Salmonella* spp. isolation ranged from 10-20% in 109 tested waters. Positive samples found in March and April came from agricultural and pristine watersheds, while all positive samples found in July were from an urban watershed. Biweekly sampling found the urban watershed to be more frequently contaminated with *Salmonella* than the other two types of watersheds, and the pristine watersheds were more frequently positive for the parasites *Cryptosporidium* and *Giardia*. *E. coli* O157:H7 was not found at any time in the watersheds. Weather event sampling found microbial contamination to be increased early during a storm event, then decreased, then increased again towards the end of the increased flow in all watersheds.

In general, agricultural runoff contains microbial concentrations exceeding the recommended standards for primary contact recreational water use (Crane et al., 1983), and for this reason improved waste management practices are being investigated and implemented to control point source and non-point source transport of animal waste constituents from agricultural lands. These best management practices (BMPs) include the use of buffer areas and vegetative strips to remove animal waste constituents in runoff from land application sites, improved land application methods, storage and/or treatment of feedlot runoff, and improved waste treatment prior to land application.

Study Overview

This report describes the results of quarterly and storm event monitoring of enteric microbial indicator concentrations in two watersheds. Sampling sites included streams flowing through animal production areas, urban-impacted rivers, human biosolids land application areas, and rural (background) streams in the Gaston County, NC, Long Creek watershed and at the North Carolina State University (NCSU) Lake Wheeler Road Farm located in Wake County, NC. Descriptions of the sampling station locations for the Long Creek and Lake Wheeler studies is provided in Tables 1 and 2, respectively. Maps showing the sampling locations for the Long Creek and Lake Wheeler studies are provided in Figures 1 and 2, respectively. The dairy farm located in the Long Creek watershed has implemented the following waste management actions in the past four years: new animal waste lagoon (Fall 1994); alternative animal watering system (Winter 1995); livestock stream exclusion, streambank stabilization and riparian planting (February 1996). Anaerobically digested, Class B biosolids were land applied at the

resource recovery farm during the study period. Beginning in October 1996, approximately 1 million gallons of the biosolids were land-applied per month between the months of March and December.

Samples of runoff from a cattle pasture and feedlot at Lake Wheeler Road Farm were analyzed for enteric indicator microbes. Samples were also collected from the swine and bovine lagoon treatment systems at Lake Wheeler Road Farm. In addition, storm samples were collected by automatic sampling units at the Long Creek watershed dairy farm, upgradient from this farm, and at an urban, industrialized site during the last 6 months of the study.

Samples were analyzed for six microbial indicators of fecal contamination: fecal coliforms, *Escherichia coli*, enterococci, *Clostridium perfringens*, somatic coliphages, and male-specific (F+) coliphages. Microbial indicators of fecal contamination are used to evaluate microbiological water quality and treatment effectiveness because they are typically present at greater concentrations than enteric pathogens when fecal contamination is present, are absent from water when fecal contamination is absent,

TABLE 1. Gaston County, Long Creek Watershed Sample Location Descriptions

Sample Number	Sample Location Description
DAIRY STATIONS	
MS12	"Background"; rural residential (low-density; on septic), agricultural area (some cattle)
MS13	Rural residential (higher-density than MS12), agricultural area
MS16	Upstream of heavy use area of dairy, on Kaiser Branch
MS17	Downstream of heavy use area of dairy, on Kaiser Branch
BIOSOLID RESOURCE RECOVERY SITE STATIONS	
MS19	Rural residential and forest; "Background" for biosolids site; housing development on septic located upgradient
MS20	Rural forested area; downgradient of biosolids site (best catchment)
MS21	Rural residential (large development on septic) and forest, some agriculture; downgradient of biosolids site (not as good a catchment as MS20)
URBAN WATERSHED STATIONS	
MS22	Urban, industrialized watershed outlet on Kaglor Branch, no NPDES points upstream
MS23	Park and recreation area; Long Creek outlet; Gastonia WWTP discharge point was upstream, but moved (beginning of 1998) to between MS24 and MS25; City of Dallas wastewater discharge located further upstream
MS24	"Background" for this project land use; area of former WWTP, rural setting; Upstream of confluence of Long Creek and South Fork Catawba River
MS25	Downstream of textile factory in rural residential area; downstream of confluence of Long Creek and South Fork Catawba River; WWTP discharge currently upstream

TABLE 2. Lake Wheeler Sample Location Descriptions

Sample Number	Sample Location Description
WEST CATTLE OPERATIONS	
S0	"Background;" some medium-density residential on septic
S1	"Background;" some nearby agricultural activities
S3	Within animal operations area; cattle pasture, currently no direct access
S4	Within animal operations area; cattle pasture, direct animal access excluded in March 1998
NORTH CATTLE AND SWINE OPERATIONS	
S7	"Background;" headwaters of stream north of animal operations area
S9	North of animal operations area; low-density calf and cattle pasture, no direct access
S10	East of animal operations area; low-density calf and cattle pasture, no direct access; upgradient of swine waste land application area
S11	East of animal operations area and S10; cattle pasture, no direct access; downgradient of swine waste land application area
LAGOON AND RUNOFF SAMPLES	
SL1	Swine Lagoon #1 grab sample; Aquaculture waste discharged to this pond
SL2	Swine Lagoon #2 grab sample
BL1	Dairy Lagoon #1 grab sample
BL2	Dairy Lagoon #2 Grab Sample
R1	Runoff from dairy cattle pasture
R2	Runoff from dairy cattle feedlot

and respond similarly to pathogens during treatment in the environment. However, some indicators are thought to be more representative of potential pathogen responses than others under different environmental conditions. Fecal coliforms, *E. coli* and enterococci are traditional bacterial indicators of enteric bacterial pathogens. *E. coli* are more specific indicators of fecal contamination than fecal coliforms because the fecal coliform test yields positive results for some non-fecal bacteria, such as *Klebsiella* species arising from woody vegetation. Enterococci are possibly more representative of the survival of more environmentally resistant bacterial pathogens. *Clostridium perfringens* is a spore-forming anaerobic bacterium that has been suggested as a possible indicator for the removal and persistence of environmentally stable protozoan parasite cysts and oocysts, such *as Giardia lamblia* and *Cryptosporidium parvum*. Somatic and male-specific (F+) coliphages are bacteriophages infecting *Escherichia coli* and are considered potential indicators for enteric viruses in water and wastewater. The two groups of indicator coliphages analyzed in this study are differentiated by how they infect their host bacteria. The bacteriophages can infect host bacteria either by attaching to the bacterial cell wall (somatic coliphages) or by attaching to appendages ("male-specific," F+ pili) protruding from the bacterial cell wall. F+ RNA coliphages are considered promising enteric virus indicators (Sobsey, 1990) because they superficially resemble the small (25-30 nm diameter), non-enveloped animal enteric viruses containing single-stranded RNA genomes (enteroviruses, caliciviruses, astroviruses and hepatitis A and E viruses). Furthermore, F+ RNA coliphages may distinguish between animal and human fecal contamination based on which groups of them are detected (Furuse, 1987; Havelaar et al., 1990; Hsu et al., 1995). There are four groups or types of F+ RNA coliphages (I, II, III, IV) of which types II and III predominate in human wastes, type IV predominates in animal wastes, and type I is found in both human and animal wastes.

MATERIALS AND METHODS

SAMPLE COLLECTION, HANDLING AND STORAGE

Stream samples from the Long Creek watershed (Figure 1) and Lake Wheeler Road Farm area (Figure 2) were collected quarterly between June 1996 and September 1999. These baseflow stream samples were collected manually and were immediately chilled in coolers. Between December 1998 and June 1999, stormwater samples were collected at the Long Creek Watershed dairy using Isco, Inc. automatic samplers. The samplers were programmed to collect water at four points during streamflow response to storm events: 2 samples during the rising portion, 1 near the first peak, and one on the falling limb of the hydrograph. Stormwater samples collected at the background sites (MS12 & MS22) were collected when the water level rose to the stage of the sampler intake. Animal waste runoff samples were collected manually during three storm events from channels draining a cattle pasture and a dairy feedlot at Lake Wheeler Road Farm. Lagoon wastewater samples were collected manually on a quarterly basis from each lagoon comprising the two-stage waste storage systems at the Lake Wheeler Road Farm swine unit and dairy unit. Stream, runoff and wastewater samples from Lake Wheeler Road Farm were hand-delivered on the same day of collection. Samples from Gaston County were shipped overnight and received in the laboratory the morning after collection.

MICROBIAL INDICATOR ASSAYS

Samples were collected aseptically in sterile polypropylene wide-mouth bottles, shipped and stored under chilled conditions, and analyzed for bacterial and viral indicators within 24 and 48 hours of collection, respectively. Bacterial indicators were enumerated by membrane filtration methods. Fecal coliforms were enumerated by incubating membranes on plates of mFC agar media for 2 hours at 37°C and then 44.5°C for another 20 ± 2 hours. *E. coli* were enumerated by transferring membranes with countable colonies from mFC plates to plates containing nutrient agar and 4-methylumbelliferyl-β-D-glucuronide (MUG), incubating the plates for 3-4 hours at 37°C, and observing colonies under long-wavelength UV light for blue fluorescence. Enterococci were enumerated after incubating plates of modified mE agar for 48 hours at 41°C. *C. perfringens* were enumerated by incubating plates of mCP agar in an anaerobic jar for 18-24 hours at 41°C, followed by exposure to ammonium hydroxide fumes.

Coliphages were concentrated from water samples by supplementing the water with 0.05 M $MgCl_2$ and filtering the water through 47- or 90-mm diameter, 0.45 :m pore-size cellulose ester membrane filters using a vacuum filter assembly for coliphage adsorption to the filter medium (Sobsey et al., 1990; Sobsey et al., 1998). Coliphages adsorbed to the filters were then eluted off of the filters using small (10- to 20-ml) volumes of 3% beef extract-0.3% Tween 80, pH 7-8. Following filter adsorption and elution, viral indicators in the elution medium were enumerated by the plaque technique using a double-agar layer, pour plate assay (Adams, 1959). Coliphages in wastewater samples were enumerated by the single agar layer (SAL) method using sample volumes of 8-10 ml per plate (Grabow and Coubrough, 1986; Sobsey et al., 1998). Somatic and F+ coliphage analyses were performed using the host bacteria *E. coli* CN-13 and *Salmonella typhimurium* WG49, respectively. Some bacteriophage isolates on the F+ RNA

coliphage host, *S. typhimurium* WG49, were recovered from plaques by aspirating the material with a micropipettor and adding it to a small volume of PBS. These bacteriophage isolates were tested for sensitivity to RNase and those that were RNase-sensitive and therefore F+ RNA coliphages were serotyped using previously described methods in order to determine if they were Type I, II, III or IV (Hsu et al., 1995). Briefly, 5-10 :l volumes of F+ RNA coliphage suspension were spotted into 5 separate plates with pre-poured lawns of host bacteria in agar media containing either: no coliphage antiserum, anti-Group I, anti-Group II, anti-Group III or anti-Group IV sera. F+ RNA coliphages are typed when they produce zones of lysis on the plate with no antiserum and on 3 of the 4 plates with Group antisera, but not on 1 plate with one of the 4 antisera. These results identify them as belonging to the Group of the antiserum plate where no zone of lysis was produced.

STATISTICAL ANALYSES

Following the confirmation of a log-normal distribution of all microbial indicator concentrations at the 95% confidence level (Kolmogorov-Smirnov test), log-transformed data was statistically analyzed utilizing parametric methods (Student's T-test) to evaluate significant differences in microbial indicator concentrations between sampling locations and other grouping variables. For differences within sites, a correlated Student's T-test was used. A chi-square test was used to evaluate differences in proportions. Correlations between geometric mean microbial concentrations and other covariate data were evaluated utilizing a Pearson Product-Moment Correlation statistic using log-transformed concentration data. The significance of all tests was evaluated at the 95% confidence level using a two-tailed criterion.

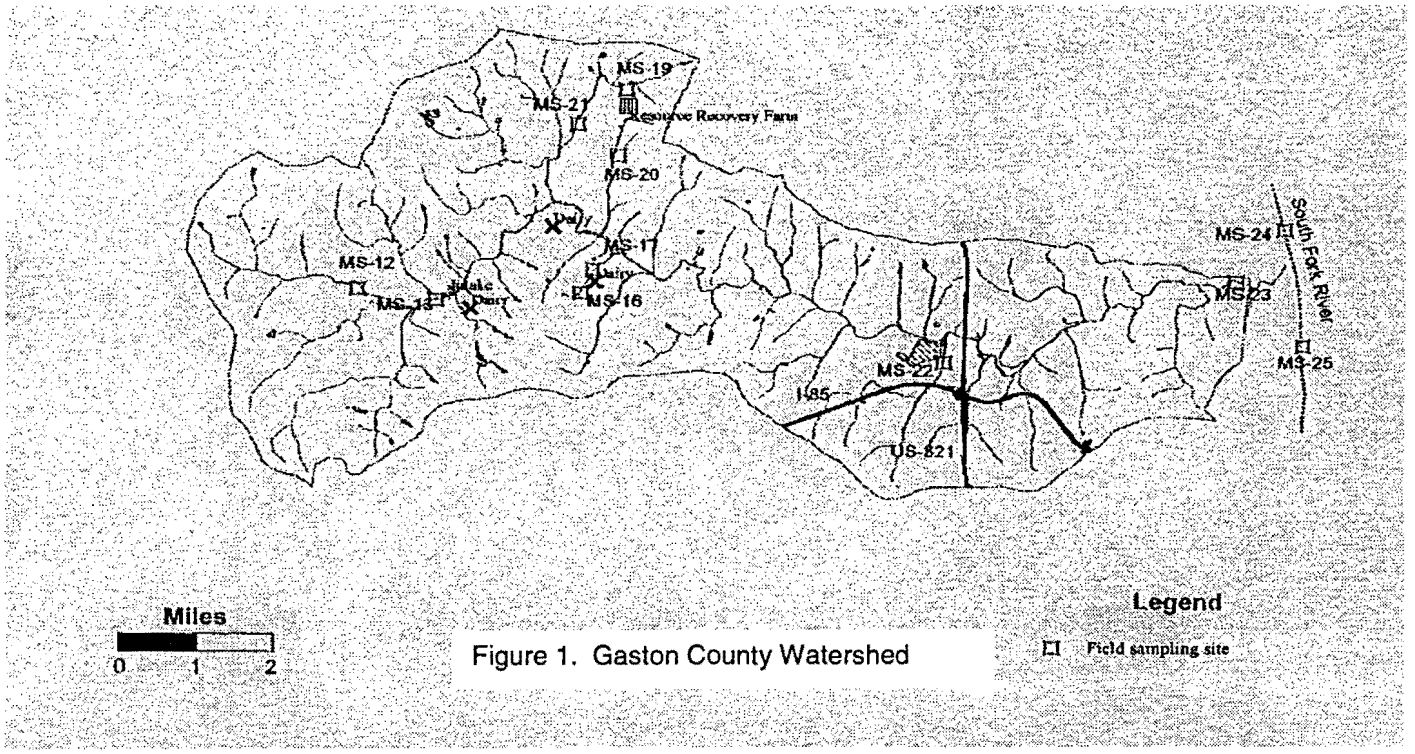


Figure 1. Gaston County Watershed

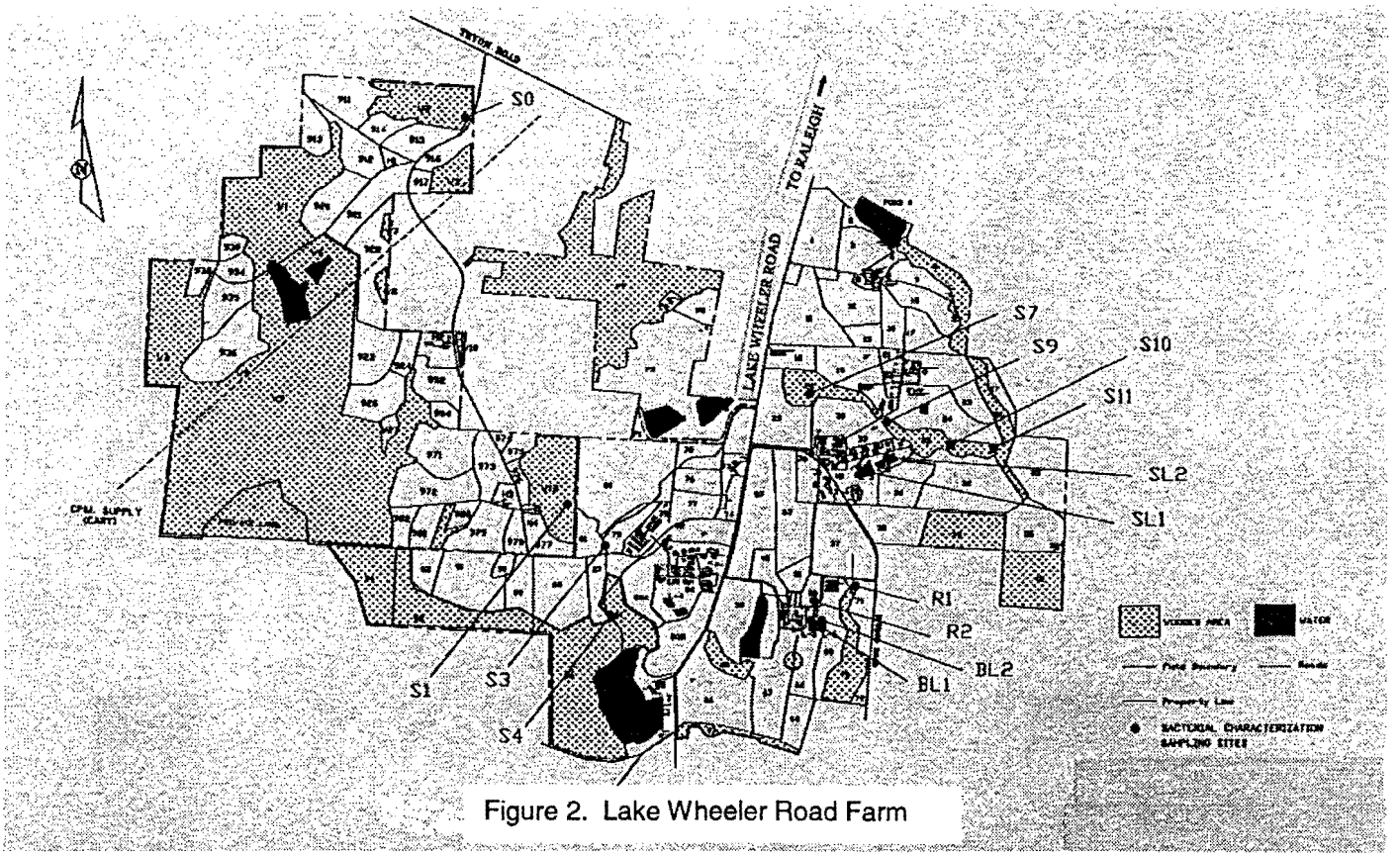


Figure 2. Lake Wheeler Road Farm

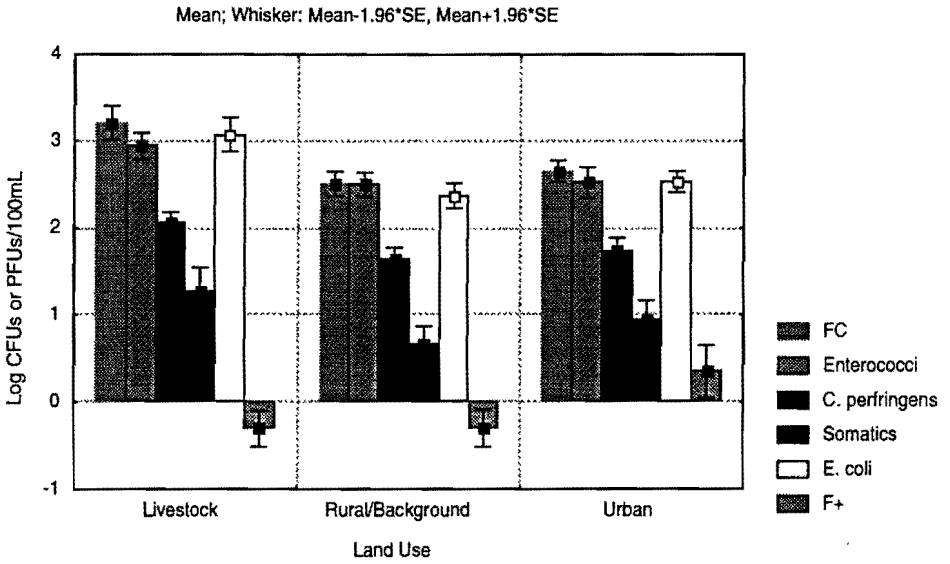
RESULTS AND DISCUSSION

MICROBIAL QUALITY OF STREAM WATERS RELATIVE TO LAND USES

Data from the two watersheds were pooled to assess the relative differences in the geometric mean microbial densities between livestock-impacted sites, urban-impacted sites, and rural/background sites (Figure 3). The data are presented as box-and-whisker plots, with bars (boxes) indicating geometric mean values and whiskers indicating 95% confidence intervals. For the three types of fecal waste sources and land uses, microbial indicator concentrations were highest for the bacterial indicators of fecal coliforms, *E. coli* and enterococci Concentrations of *Cl. perfringens*, the spore-forming anaerobe, were lower than those of the other fecal indicator bacteria. Concentrations of somatic coliphages were lower than those of the indicator bacteria, and those of F+ RNA coliphages were the lowest of all indicators tested.

The mean $\log_{10}$ concentrations of all microbial indicators were higher at the urban-impacted sites compared to the rural/background sites during base flow conditions, but most of these differences were not significant. However, the densities of F+ coliphages were significantly higher ($p<0.05$) at the urban-impacted sites compared to both the rural/background and livestock-impacted sites. There were no statistically significant differences in the F+ coliphage densities between livestock-impacted sites and rural/background sites. Livestock-impacted sites had significantly higher mean $\log_{10}$ concentrations of fecal coliforms, *E. coli*, enterococci, and *Cl. perfringens* than either the urban-impacted or rural/background sites during base flow conditions. The somatic coliphage mean $\log_{10}$ concentrations were higher at livestock-impacted sites compared to both the urban-impacted and rural/background sites, but this difference was only statistically significant for the comparison with the rural/background sites.

Figure 3. Land Use Impacts on Microbial Densities



Geometric Mean Microbial Densities (CFUs or PFUs/100mL) and 95% CIs			
	Rural/Background (n=77)	Livestock-impacted (n=82)	Urban-impacted (n=52)
Fecal Coliforms	321 (234, 447)	1634 (1047, 2570)	447 (339, 589)
E. coli	235 (170, 324)	1188 (759, 1862)	338 (257, 447)
Enterococci	316 (229, 427)	868 (603, 1230)	330 (219, 490)
C. perfringens	43 (31, 59)	113 (85, 151)	53 (37, 76)
Somatic coliphages	5 (3, 7)	19 (10, 35)	9 (6, 14)
F+ coliphages	0.5 (0.3, 0.8)	0.5 (0.3, 0.8)	2 (1.1, 4.5)

F+ RNA coliphages have been suggested as possible fecal indicators viruses capable of distinguishing between human and animal sources of fecal contamination on the basis of which of four serogroups (I, II, III, IV) are found. The occurrence of the different types of F+ RNA coliphage types found at the different study sites and in the different types of wastes are summarized in Table 3.

Table 3. Serotypes of F+ coliphages					
Sample Site	Type I, # (%)	Type II, # (%)	Type III, # (%)	Type IV, # (%)	Total RNA phages isolated
Rural/Background	101 (78)	20 (16)	5 (4)	3 (2)	129
AFO-impacted	64 (52)	30 (24)	19 (15)	10 (8)	123
Urban-impacted	90 (60)	47 (32)	12 (8)	0	149
Swine Lagoons	17 (68)	4 (16)	4 (16)	0	25
Bovine Lagoons	18 (75)	1 (4)	1 (4)	4 (17)	24

The occurrence of different F+ RNA coliphage groups varied somewhat with fecal waste sources and land use practices at sites. Group IV F+ RNA coliphages were detected mostly at sites impacted by animal waste sources and in bovine lagoon waste, but not in urban-impacted samples or in swine lagoon waste. This is consistent with previous evidence that Group IV F+ RNA coliphages are present in various animal fecal wastes but not human fecal wastes. Both the livestock-impacted sites (29%) and the urban sites (32%) had significantly higher proportions of Group II F+ RNA coliphage isolates than did the background/rural stations (18%). Although previous studies have shown that Group II F+ RNA coliphages are present primarily in human fecal waste, they have also been found at low concentrations in swine and cattle waste. Group III F+ RNA coliphages were found in samples from all sites, but were found mostly in AFO-impacted sites and in swine lagoon wastes. In previous studies, Group III F+ RNA coliphages were found mostly in human wastes, but not exclusively so. Type I F+ RNA coliphages were most frequently isolated from all stations (Figure 4), which is consistent with the presence of this F+ RNA coliphage group in both human and animal fecal waste sources. Background/rural sites had a significantly ($p<0.05$) higher proportion of Type I F+ RNA coliphage isolates (76%) than both the livestock-impacted (46%) and the urban stations (60%). In addition, urban sites had a significantly greater proportion of Type I F+ RNA coliphages isolated than the livestock-impacted sites. The reasons for the differences in the levels of occurrence of Group I F+ RNA coliphages at sites with different fecal waste impacts and land uses in unclear. Recently, laboratory studies have shown that some strains of Group I F+ RNA coliphages survive for long periods of time in water, and much longer than Group IV F+ RNA coliphages (Meschke and Sobsey, unpublished results). This raises the possibility that higher proportions of Group I F+ RNA coliphages occur in waters which have been exposed to environmental conditions long enough for the other F+ RNA coliphage Groups to decline in numbers as a result of die-off. Of the F+ RNA coliphage grouping results of this study, the most definitive finding was the presence of Group IV isolates at AFO-impacted sites and the absence of Group IV isolates at urban sites. This provides evidence of the lack of association of Group IV F+ RNA coliphages with human wastes and the association of these phages with at least some animal waste sources, especially cattle.

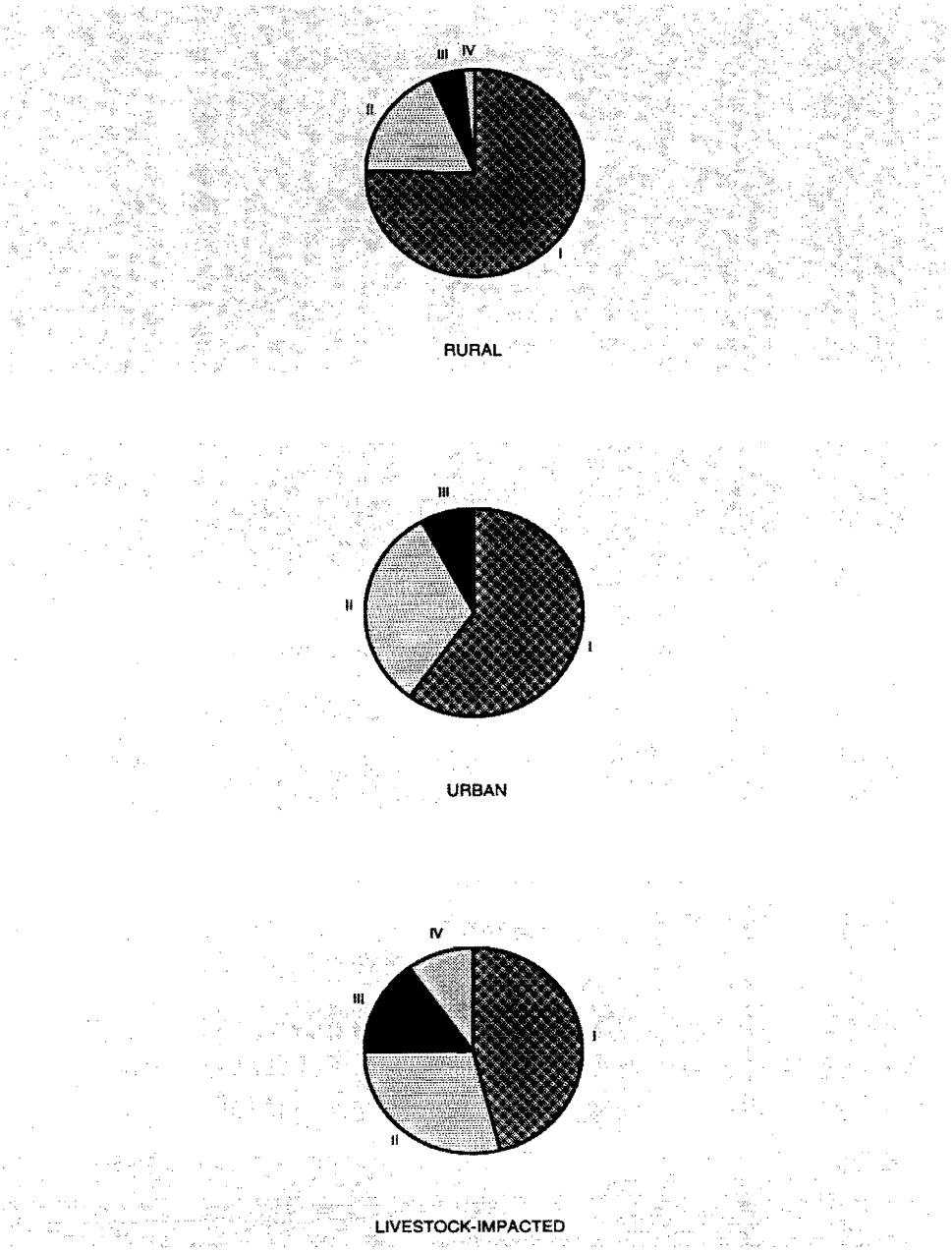


Figure 4. Proportions of F+ RNA Coliphage Serotypes

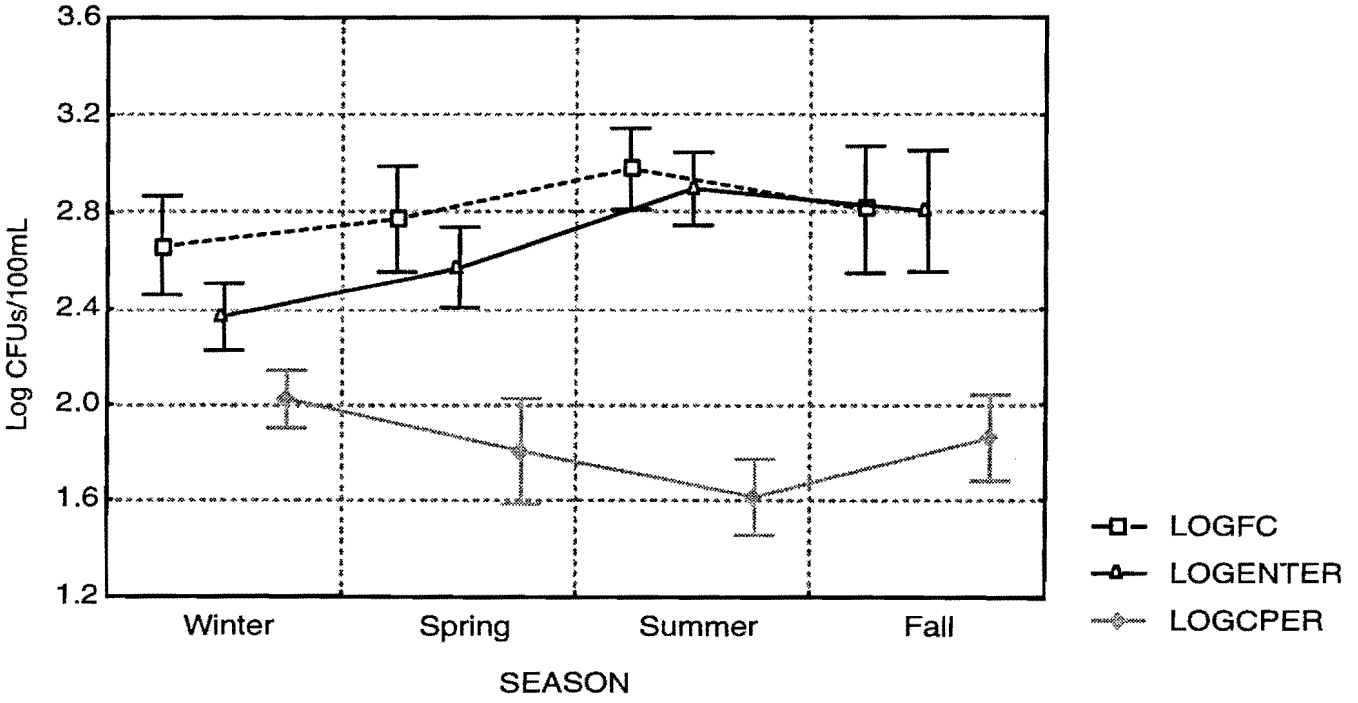
SEASONAL EFFECTS ON THE MICROBIAL QUALITY OF STREAM WATERS

To evaluate the effect of season on microbial indicator densities, the seasonal trends in the $\log_{10}$ mean concentrations for the different indicator organisms from each watershed were compared.

At both watersheds, the mean $\log_{10}$ concentrations of *Cl. perfringens* were higher in the winter (December-February) than in the summer (June-August), but the mean $\log_{10}$

concentrations of enterococci and fecal coliforms were higher in the summer compared to winter. The sampling data for these indicators were pooled and grouped according to the season of collection to evaluate the effect of season on the geometric mean microbial concentrations. In the pooled analyses, the fecal coliforms and enterococci were significantly higher ($p \leq 0.019$) in the summer compared to winter (Figure 5). In contrast, the observed densities of *Cl. perfringens* were significantly higher ($p < 0.05$) in the winter than in the summer and fall (Figure 5). Without considering seasonal variability in source loading to the watersheds, it is noteworthy to measure higher enteric microbe concentrations in stream water during summer months than during winter months. This is because enteric microbe die-off rates in environmental samples tend to increase with increasing temperature. The reasons for this finding are uncertain, but could possibly be due to the proliferation of non-fecal enterococci and fecal coliforms in environmental media during the warmer months. It is well know that some fecal coliform, such as Klebsiella species are associated with non-fecal sources, such as woody vegetation. Therefore, it is possible that higher fecal coliforms during warmer months could be caused by increased levels of these non-fecal bacteria in watershed vegetation and soil. For the sampling sites impacted by the swine unit, increased enteric microbial densities observed in the summer months may reflect increased land application of wastes. Waste from the swine unit is land applied more frequently during warmer months than during cold months.

Figure 5. Seasonal Effects on Microbial Indicators



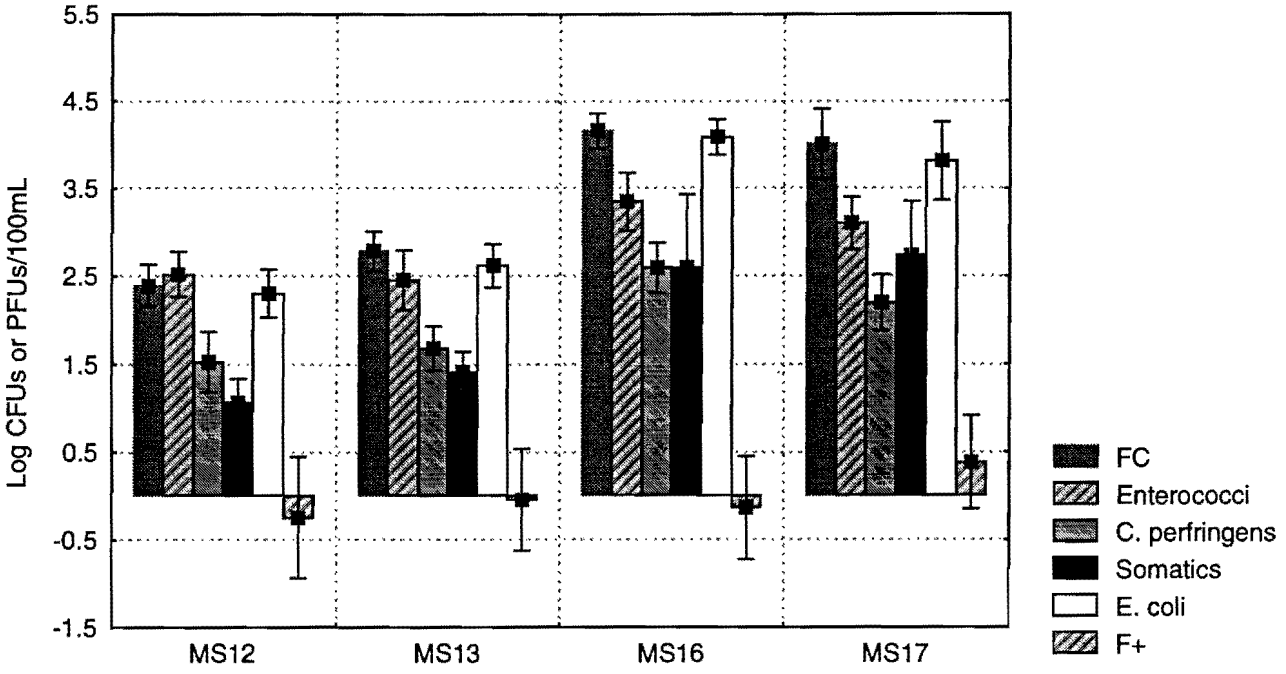
Geometric Mean Microbial Densities (CFUs /100mL) and 95% CIs				
	Winter (n=57)	Spring (n=38)	Summer (n=65)	Fall (n=51)
Fecal Coliforms	460 (288, 741)	594 (363, 977)	953 (645, 1380)	650 (355, 1175)
Enterococci	233 (170, 324)	375 (257, 549)	790 (562, 1122)	640 (363, 1122)
C. perfringens	105 (79, 138)	63 (38, 105)	41 (29, 59)	73 (48, 110)

LONG CREEK WATERSHED STUDY

Microbial Quality of Stream Water from Base Flow Sampling

Dairy Collection Sites. The results for microbial indicator concentrations at Long Creek watershed-sampling stations in the vicinity of the dairy are summarized in Figure 6 and its accompanying data table. These data show that there is a general pattern of increased concentrations of microbial indicators going from stations MS12 and MS 13, the two background stations, to MS 16 and MS17, the two livestock stations. The concentrations of most microbial indicators are more than an order of magnitude (10-fold) higher at the two dairy stations than at the two background stations. There were no statistically significant differences in the mean $\log_{10}$ microbial concentrations between the two-upgradient rural residential collection sites (MS12 and MS13). Likewise, there were no statistically significant differences in the mean $\log_{10}$ microbial concentrations between the two dairy-impacted sites (MS16 and MS17) (Figure 6). However, the geometric mean concentrations of 5 of the 6 microbial indicators studied were significantly higher at the dairy cattle area sampling stations (MS 16 and MS17) compared to the upgradient rural residential areas (MS12 and MS13). Only the F+ coliphages, which were found at very low concentrations in general, were not significantly greater at MS16 and MS17 compared to the two upgradient sites (MS12 and MS13) (Figure 6).

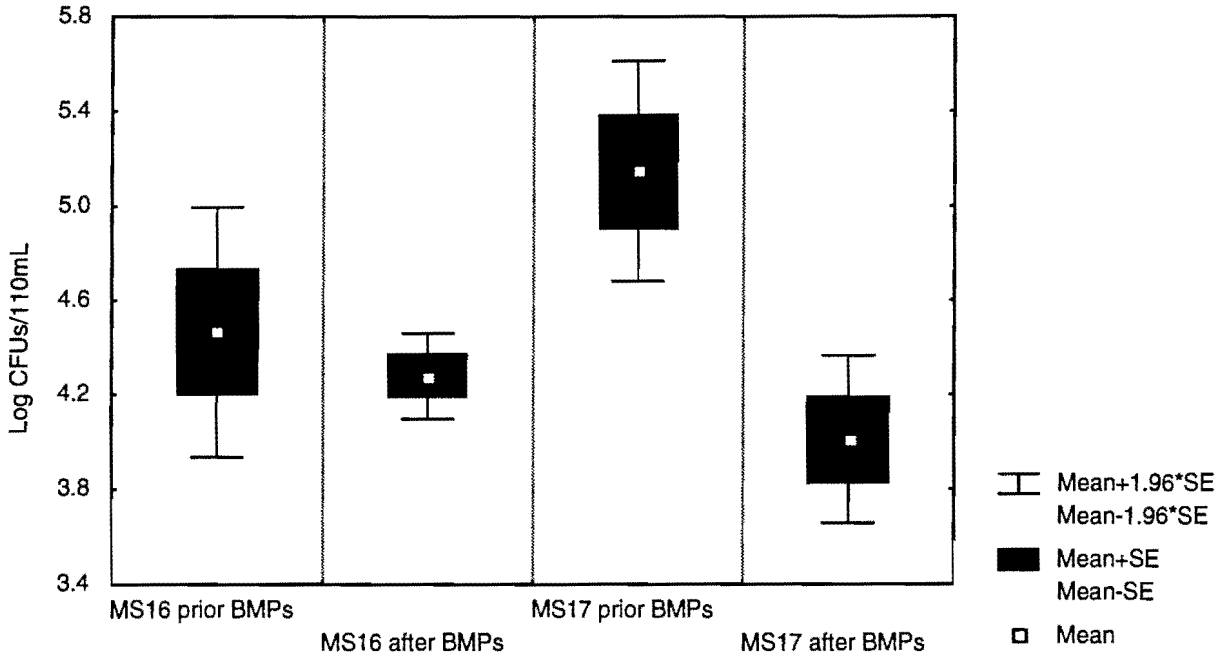
Figure 6. Gaston County Background and Livestock Sites
Mean; Whisker: Mean-1.96*SE, Mean+1.96*SE



Geometric Mean Microbial Densities (CFUs or PFUs/100mL) and 95% CIs				
	MS12	MS13	MS16	MS17
Fecal Coliforms	246 (n=11) (145, 427)	608 (n=11) (363, 1023)	14,332 (n=11) (9120, 22909)	10,265 (n=11) (4073, 25704)
E. coli	200 (n=11) (107, 372)	413 (n=11) (234, 741)	12,150 (n=10) (7586, 19498)	6,461 (n=10) (2291, 18197)
Enterococci	332 (n=11) (182, 603)	283 (n=11) (129, 617)	2,221 (n=11) (1023, 4786)	1,265 (n=11) (631, 2512)
C. perfringens	34 (n=11) (16, 74)	48 (n=11) (27, 85)	391 (n=11) (204, 759)	159 (n=11) (76, 331)
Somatic coliphages	12 (n=11) (6, 21)	25 (n=11) (14, 45)	390 (n=7) (56, 2692)	550 (n=9) (135, 2239)
F+ coliphages	0.6 (n=11) (0.1, 2.8)	0.9 (n=11) (0.2, 3.5)	0.7 (n=9) (0.2, 2.8)	2.4 (n=11) (0.7, 8.3)

Since June of 1993, the Gaston County Health Department has measured fecal coliform concentrations at sites MS16 and MS17. These data were pooled with the fecal coliform data of this present study to evaluate the effects of Best Management Practices (BMPs) implemented at the dairy. Prior to pooling of the data, the fecal coliform analytical results for samples evaluated by both the county health department and our laboratory within 24 hours of each other were compared for significant differences. No significant difference was found between the fecal coliform results of the two labs. Following completion of BMP implementation, there was no significant change in the geometric mean concentration of fecal coliforms at the site upgradient from the heavy use area (MS16) as compared to the fecal coliform levels prior to BMP implementation. However, comparing fecal coliforms levels before and after BMP implementation, there was a statistically significant decrease in the geometric mean fecal coliform concentration at the downgradient site MS17 ($p=0.0007$). The significant changes in fecal coliform concentrations before and after the implementation of BMPs on the dairy suggest beneficial effects of BMPs by successfully reducing the previously high levels of microbial fecal contamination at the downgradient site (Figure 7).

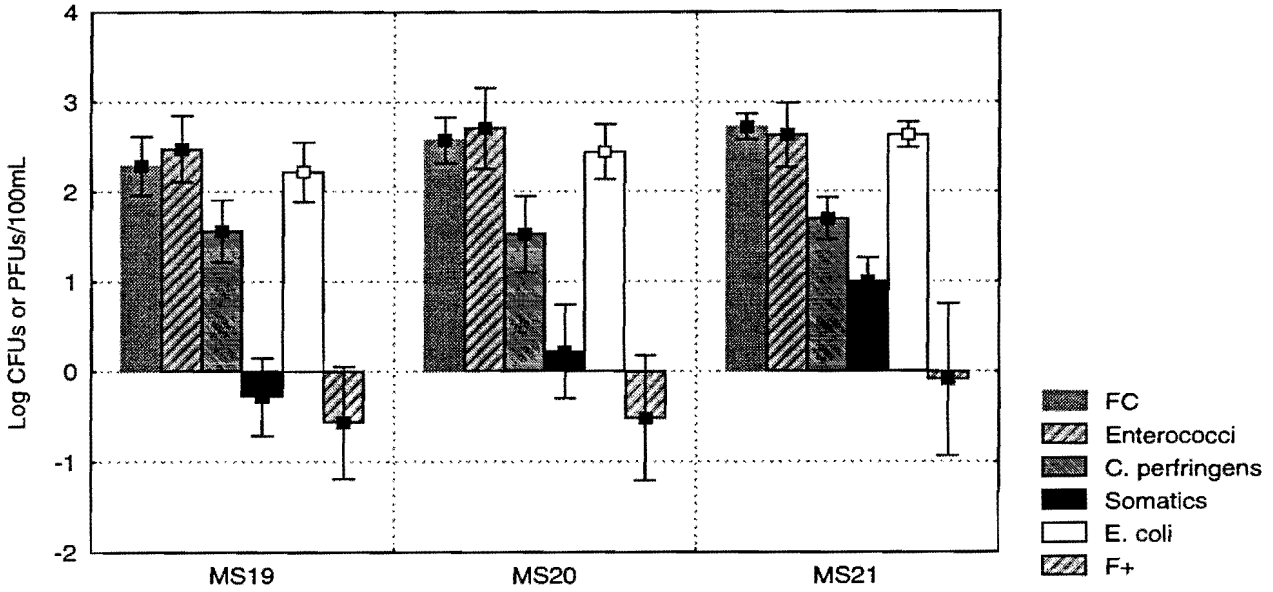
Figure 7. Effect of BMP Implementation on Fecal Coliforms at Dairy Sites



Biosolids Recovery and Land Application Sites. The effects of land application of class B municipal biosolids on stream water quality were investigated, and the microbial data for sampling stations in the vicinity of this land use are summarized in Figure 8 and its accompanying Table. No significant differences were noted in the microbial indicator geometric mean $\log_{10}$ concentrations between the background site (MS19) and downgradient site MS20. However, the $\log_{10}$ mean concentrations of fecal coliforms (p=0.029), *E. coli* (0.04), and the somatic coliphages (p=0.000092) were significantly higher at the downgradient site MS21 compared to upgradient site MS19. These results suggest that there are modest but demonstrable effects of land application of Class B municipal biosolids on the microbial quality of nearby stream water. However, the magnitude of the effect, based on geometric mean $\log_{10}$ microbial indicator densities, is relatively small, ranging from about 2.5- to 3-fold for some indicator bacteria (fecal coliforms and *E. coli*) to 10-fold for an indicator virus (somatic coliphages. Furthermore, there was no demonstrable effect for some of microbial indicators, such as enterococci, *Cl. perfringens* and F+ coliphages. The geometric mean $\log_{10}$ concentration of somatic coliphages was significantly elevated at downstream station MS21 compared to downstream station MS20 (p=0.016), but no other indicators were significantly different between these two sampling sites, both of which are located downgradient from the biosolids application lands (Figure 8). The reasons for the significant differences in somatic coliphage concentrations between these two downstream stations are not known, but some other microbial indicators (fecal coliforms, E. coli and F+ coliphages) also were higher, although not significantly so, at downstream site MS21 compared downstream site MS20. It is possible that microbial water quality at site MS21 is a more complete measure of the total impact of upstream land application of municipal biosolids because it

is somewhat further downstream and allows for better integration and mixing of microbial inputs from the biosolids land application site.

Figure 8. Gaston County Biosolid Recovery Station Sites
Mean; Whisker: Mean-1.96*SE, Mean+1.96*SE

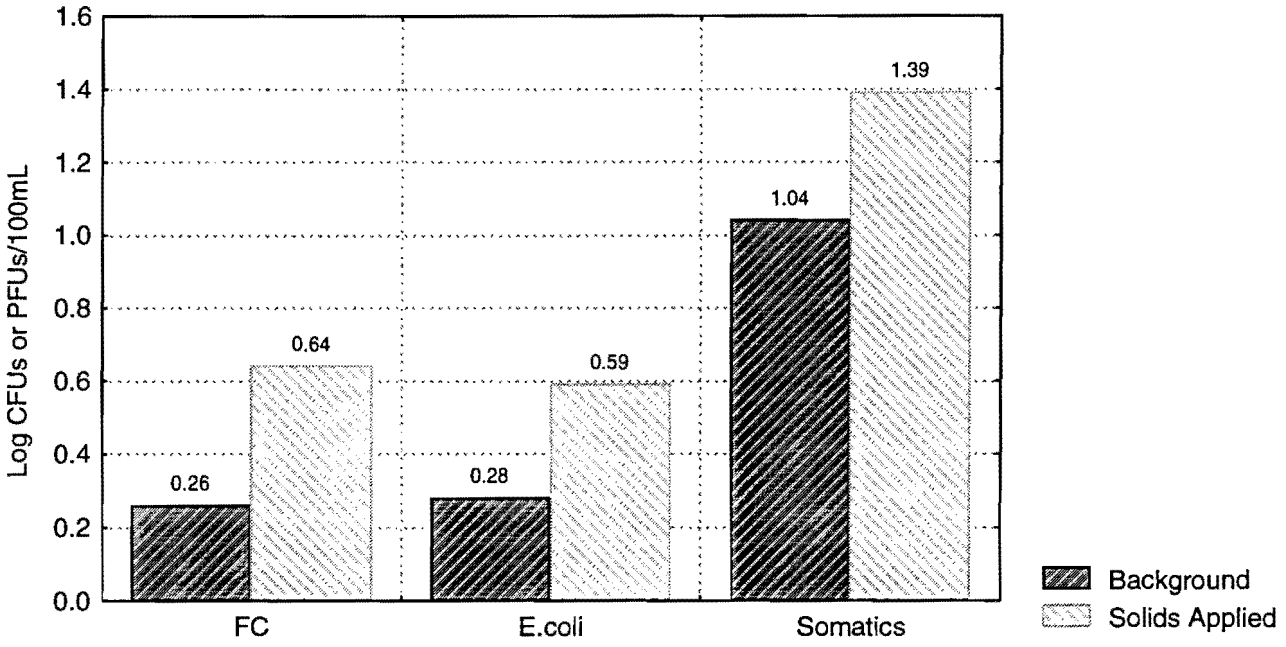


Geometric Mean Microbial Densities (CFUs or PFUs/100mL) and 95% Cis			
	MS19	MS20	MS21
Fecal Coliforms	194 (n=10) (93, 417)	380 (n=10) (209, 676)	525 (n=10) (380, 741)
E. coli	166 (n=10) (78, 363)	282 (n=10) (138, 575)	427 (n=10) (309, 589)
Enterococci	302 (n=10) (129, 708)	513 (n=10) (182, 1445)	427 (n=10) (186, 977)
C. perfringens	36 (n=10) (17, 81)	48 (n=10) (27, 85)	34 (n=10) (13, 89)
Somatic coliphages	0.5 (n=10) (0.2, 1.4)	1.7 (n=10) (0.5, 5.5)	10 (n=10) (5.5, 18.6)
F+ coliphages	0.3 (n=5) (0.1, 1.1)	0.3 (n=4) (0.1, 1.0)	0.8 (n=6) (0.1, 5.6)

To evaluate the overall effect of biosolids application on microbial indicator concentrations, the data from the 2 downgradient sites were pooled and averaged and the difference in geometric mean indicator concentrations between this average downstream value and the upstream background site value were calculated for each indicator organism. The differences in downstream and upstream microbial indicator concentrations were calculated when biosolids were applied within a week of sample collection, and they were compared to the upstream and downstream differences calculated when biosolids had not been applied during this time period. Although there were greater differences in fecal coliform, *E. coli,* and somatic coliphage geometric mean concentrations between the upgradient and downgradient sites following biosolids

application as compared to non-application periods (Figure 9), there were not enough replicate data to demonstrate statistically significant effects for the individual indicators.

Figure 9. Difference in Microbial Concentrations Between Upstream and Downstream Sites Following Land Application of Municipal Biosolids



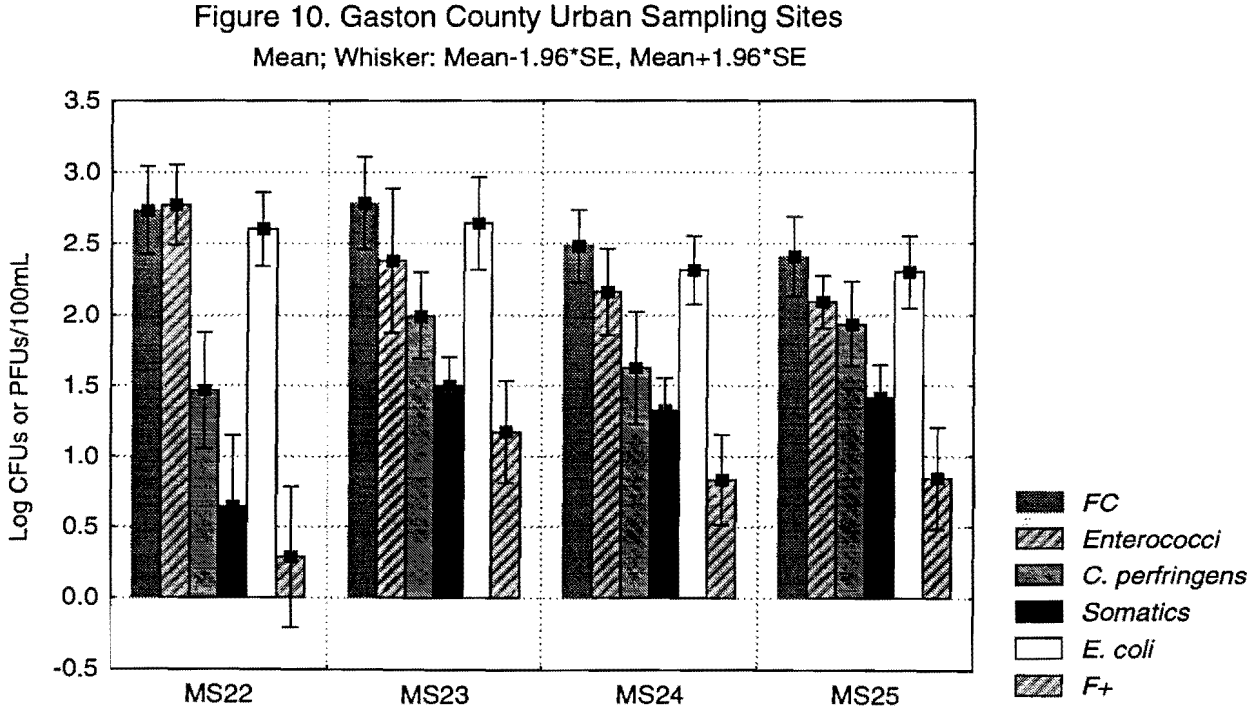
Urban Watershed Area. The microbial quality of streamwaters was determined for a series of four stations flowing through urban and suburban communities of the Long Creek watershed. The $\log_{10}$ mean concentrations of fecal coliforms, *E. coli* and enterococci in the urban watershed stream samples were lower at MS24 and MS25 than at MS22 and MS23. Both MS22 and MS23 are in urban areas. MS22 is in an industrialized watershed outlet on Kaglor branch, although there are no NPDES permitted discharges upstream of this station. MS23 is in a park and recreation area, near the outlet of Long Creek, and the wastewater effluent discharge of the City of Dallas WWTP is upstream from this station. The City of Gastonia WWTP also was upstream from this station, but the outfall was moved at the beginning of 1998 to between MS24 and MS25. Both MS24 and 25 are in rural areas. MS24 is effectively the background station for the urban samples, as it is located on the South Fork River, upstream of its confluence with Long Creek (Figure 1). MS25 is downstream of the confluence of Long Creek and the South Fork of the Catawba River and there is currently a WWTP discharge from the City of Gastonia upstream of this station.

Of the bacterial indicators, a statistically significant difference ($p=0.000986$) in geometric mean $\log_{10}$ concentrations was found for enterococci between sites MS25, the rural downstream station in the South Fork of the Catawba River and MS22, the station associated with the urbanized area of the City of Gastonia (Figure 10). The lowest $\log_{10}$ mean concentrations of *C. perfringens*, somatic coliphages and F+ coliphages were

observed at MS22, the urban, industrialized station on Kaglor branch. The geometric mean $\log_{10}$ concentrations at MS22 were significantly different than those for all other urban region stations. The geometric mean $\log_{10}$ concentrations of F+ coliphages between sites MS22 and MS23 also were significantly different. In the urban area, the highest geometric mean $\log_{10}$ concentrations of all microbial indicators, except enterococci, were observed at MS23, the station downstream of the City of Dallas wastewater treatment plant (WWTP) outfall and the City of Gastonia WWTP outfall (until February 1998, when the Gastonia WWTP outfall was moved to the South Fork River between MS24 and MS25). The highest $\log_{10}$ mean concentration of enterococci was observed at MS22 and it was statistically significant from the other urban stations, except MS23. Station MS22 is on a branch of the Long Creek watershed (Kaglor Branch) that drains a highly urbanized area.

The microbial indicator geometric mean $\log_{10}$ concentrations were similar at MS24 (rural, "background" station) and MS25 (rural residential station). Levels of microbial indicators at MS23, an urban station on Long Creek just before it joins with the South Fork River, were greater than the levels of indicators at both stations MS24 and MS25. Although none of the differences in indicator concentrations were statistically significant, the trend suggests that the levels of microbial indicators at the outlet of the Long Creek watershed are higher than the levels of indicators in the South Fork River. However, microbial loading from Long Creek may not be contributing greatly to the enteric microbe levels in the South Fork Catawba River (Figure 10).

Figure 10. Gaston County Urban Sampling Sites



Geometric Mean Microbial Densities (CFUs or PFUs/100mL) and 95% CIs				
	MS22	MS23	MS24	MS25
Fecal Coliforms	537 (n=11) (269, 1096)	603 (n=11) (288, 1288)	302 (n=10) (170, 550)	257 (n=10) (135, 490)
E. coli	398 (n=12) (219, 724)	437 (n=11) (209, 912)	204 (n=10) (118, 355)	200 (n=10) (112, 355)
Enterococci	589 (n=11) (309, 1122)	240 (n=11) (76, 776)	145 (n=10) (72, 288)	123 (n=10) (81, 186)
C. perfringens	30 (n=11) (11, 76)	98 (n=11) (49, 200)	42 (n=10) (17, 105)	87 (n=10) (44, 170)
Somatic coliphages	4 (n=11) (1, 14)	32 (n=10) (20, 50)	21 (n=10) (12, 36)	26 (n=10) (16, 45)
F+ coliphages	2 (n=11) (0.6, 6)	15 (n=11) (6, 34)	7 (n=10) (3, 14)	7 (n=9) (3, 16)

The impact of the Gastonia WWTP outfall on waterborne microbial concentrations was evaluated by comparing the measured geometric mean concentrations at downgradient sampling stations (MS23 before February 1998 and MS25 after that) before and after the plant was moved. There were no statistically significant changes in the observed microbial densities at either impacted site (MS23 or MS25). Furthermore, there were no statistically significant differences in microbial densities between upgradient and downgradient stations (MS24 and MS25) as a result of the Gastonia WWTP outfall location, although the data had poor statistical power to detect a difference.

Storm Water Sampling

Beginning December 1998 water samples were collected from dairy farm stations MS16 and MS17, the background site MS12, and the urban site MS22 during rainfall events. The geometric mean $\log_{10}$ concentrations of all microbial indicators were significantly elevated during rainfall events at all sampling sites except MS12, where there were not enough data to detect statistically significant differences in F+ coliphages and enterococci (Figures 11a. and 11b.).

Figure 11a. Effects of Precipitation Events on Log_{10} Geometric Mean Concentrations of Microbial Indicators at a Rural Background (MS12) and Urban Stream Water Stations

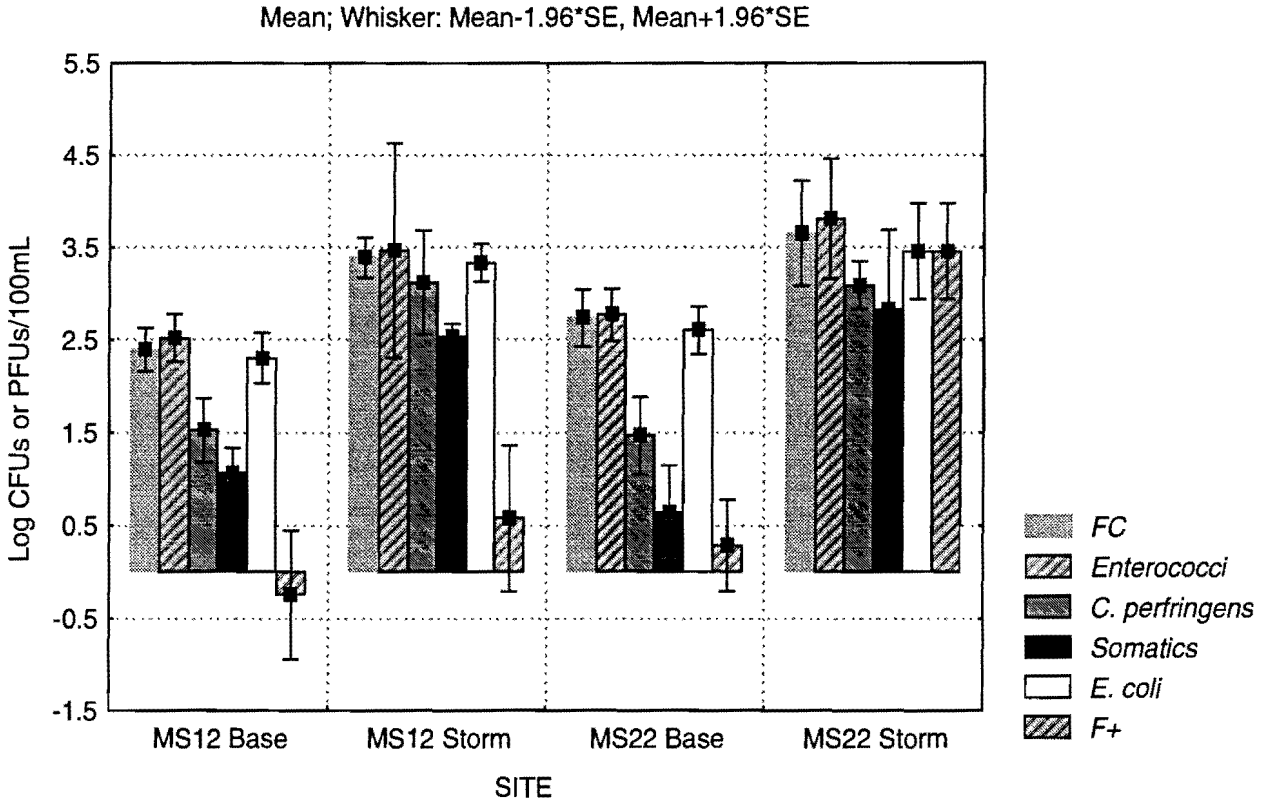
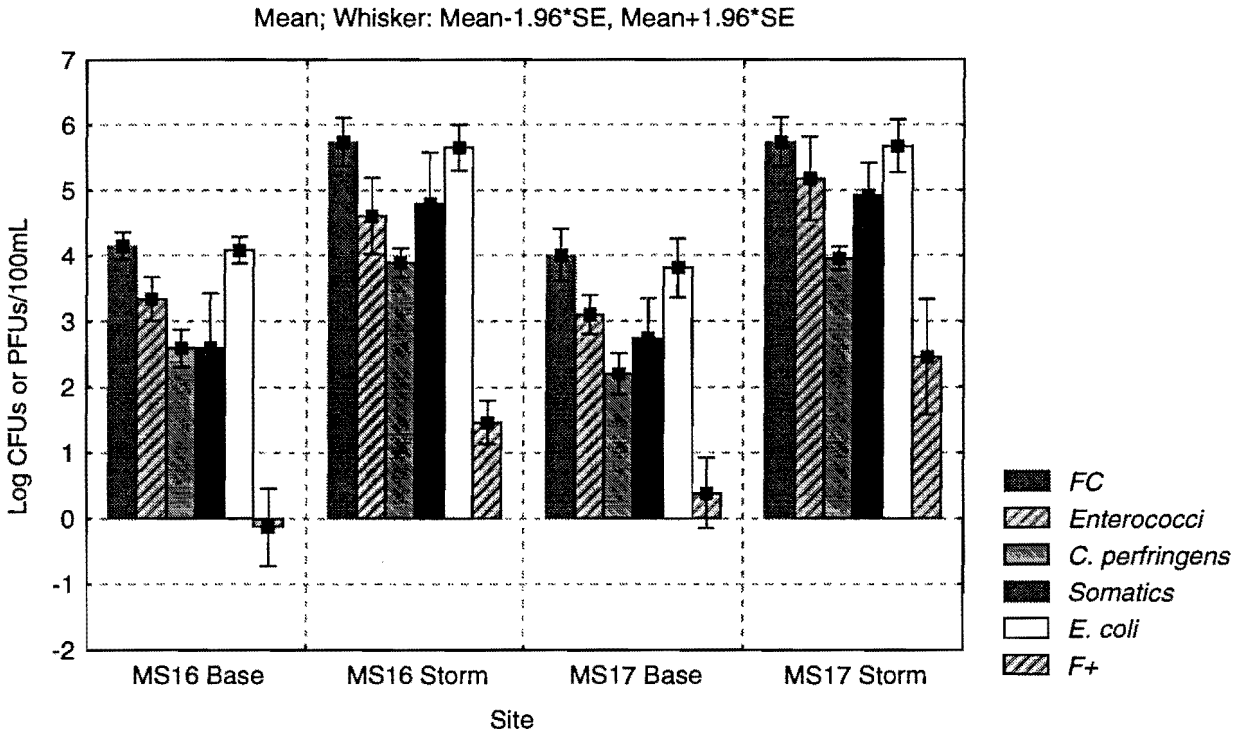


Figure 11b. Effects of Precipitation Events on Log_{10} Geometric Mean Concentrations of Microbial Indicators at Stream Water Stations in a Dairy Cattle Area



During storm events, the measured total suspended solids (TSS) concentrations were also significantly higher than during base flow. Pearson Product Moment correlations between log_{10} TSS concentrations and the microbial log_{10} concentrations were calculated to evaluate the possibility that microbial elevations were associated with a greater input of sediment during storm events. All microbial indicator concentrations exhibited statistically significant positive correlations with TSS concentrations. However, when the data was stratified, the significant correlations between concentrations of TSS and all microbial indicators except *C. perfringens* and somatic coliphages disappeared during storm events. These results suggest that mechanisms of microbial transport independent of sediment movement or possibly other factors may be responsible for some of the observed increases in microbial concentrations during storm events (Table 4).

Table 4. Correlations Between Log TSS and Log Microbial Concentrations

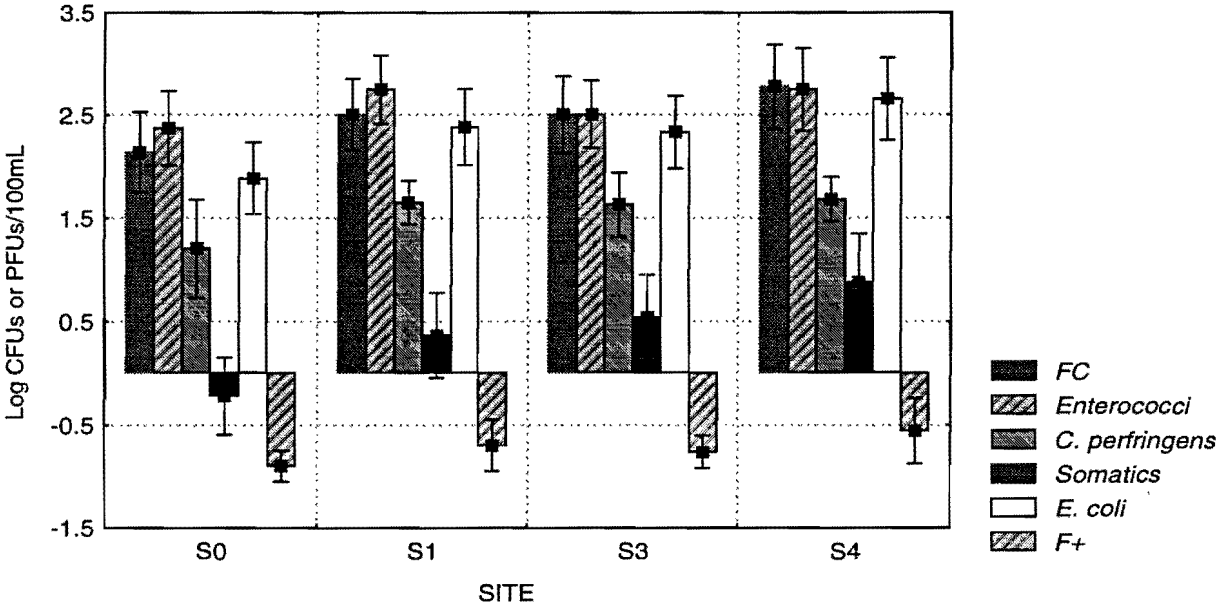
	All samples	**Storm Events**
Fecal Coliforms	0.67 ($p<0.05$)	0.55
E. coli	0.65 ($p<0.05$)	0.51
Enterococci	0.64 ($p<0.05$)	0.15
C. perfringens	0.75 ($p<0.05$)	0.57 ($p<0.05$)
Somatic coliphages	0.69 ($p<0.05$)	0.67 ($p<0.05$)
F+ coliphages	0.74 ($p<0.05$)	0.09

Baseflow Sampling

West Cattle (Beef) Operations Area. The concentrations of microbial indicators of fecal contamination were determined at a total of four sampling stations in the western cattle operations area of Lake Wheeler Farm: two stations (S0 and S1) were "background" stations and two stations (S3 and S4) were within the animal operations area (Figure 2 and Table 2). An increase in $\log_{10}$ geometric mean microbial indicator concentrations was apparent between background station S0 and downstream station S4 for all the indicators studied (Figure 12). Increases in $\log_{10}$ geometric mean microbial concentrations ranged from 3-fold to 13-fold, depending on the microbial indicator. The increases between S0 and S4 were significant for fecal coliforms ($p = 0.04$), *E. coli* ($p = 0.01$) and somatic coliphages ($p = 0.001$). However, the increases in microbe concentrations between S0 and S4 were not significant for enterococci ($p = 0.13$), *C. perfringens* ($p = 0.14$, by Mann-Whitney), and F+ coliphages ($p = 0.07$). The geometric mean concentrations of fecal coliforms in stream water at sampling stations S0 and S4 were 140 and 590 CFU/100 mL, respectively. North Carolina has a fecal coliform surface water quality standard of 200 CFU/100 mL. The geometric mean concentrations measured at stations S1, S3, and S4 exceeded this surface water quality standard. A similar trend of increasing enteric microbial concentrations also was generally apparent between station S1, the station immediately upgradient of the cattle pasture area (but near some other agricultural activities), and station S4, the station immediately downgradient of the pasture area. However, none of the increases in microbial indicator concentrations between these stations were significant.

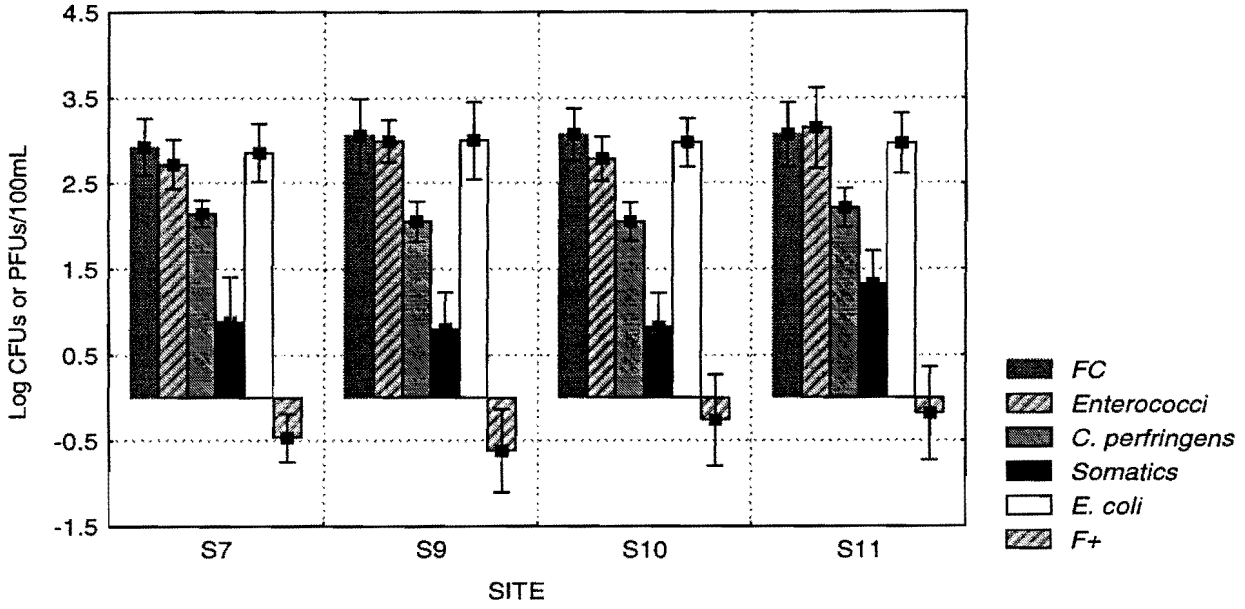
Northern Cattle and Swine Operations Area. The four sampling stations in this area were S7, a background station, S9, a low-density calf and cattle pasture with no direct animal access to the stream, S10, another low-density calf and cattle pasture with no direct animal access to the stream and upgradient of the land application area for swine waste, and S11, a cattle pasture with no direct animal access to the stream and down gradient of the land application area for swine waste. In the northern cattle and swine operations area, there was a general trend toward increasing microbial indicator concentrations in stream water between background station S7 and station S11, the station downstream of the land application area for swine waste (Figure 13). The extent of increase of microbial concentrations from S7 to S11 varied from only slight (<2-fold) to 2.6-fold, depending on the microbial indicator. The increases in microbial indicator concentrations were significant only for somatic coliphages ($p = 0.02$), and not for fecal coliforms ($p = 0.32$), *E. coli* ($p = 0.36$), enterococci ($p = 0.11$), *C. perfringens* ($p = 0.34$), or F+ coliphages ($p = 0.11$). It should be noted that the microbial levels in S7, the baseline station for this sampling area of the farm, were appreciably higher (by factors of 2.2 to 13-fold) than those at background station S0. Therefore, it is appears that increased levels of fecal contamination already existed before the stream flowed through this area of the farm.

Figure 12. Lake Wheeler Road Western Cattle Operations Area
Mean; Whisker: Mean-1.96*SE, Mean+1.96*SE



Geometric Mean Microbial Densities (CFUs or PFUs/100mL) and 95% Cis				
	S0	S1	S3	S4
Fecal Coliforms	138 (n=12) (58, 339)	324 (n=11) (148, 708)	316 (n=12) (135, 741)	589 (n=12) (234, 1514)
E. coli	78 (n=12) (35, 170)	240 (n=11) (102, 562)	214 (n=12) (95, 479)	447 (n=12) (178, 1122)
Enterococci	234 (n=12) (102, 537)	562 (n=11) (263, 1202)	324 (n=12) (151, 676)	562 (n=12) (219, 1413)
C. perfringens	16 (n=12) (5, 48)	45 (n=11) (28, 72)	43 (n=12) (20, 87)	48 (n=12) (29, 79)
Somatic coliphages	0.6 (n=11) (0.3, 1.4)	2 (n=10) (0.9, 6)	4 (n=11) (1, 9)	8 (n=12) (3, 22)
F+ coliphages	0.1 (n=11) (0.1, 0.2)	0.2 (n=10) (0.1, 0.4)	0.2 (n=11) (0.1, 0.3)	0.3 (n=11) (0.1, 0.6)

Figure 13. Lake Wheeler Road Northern Cattle and Swine Operations Area
Mean; Whisker: Mean-1.96*SE, Mean+1.96*SE



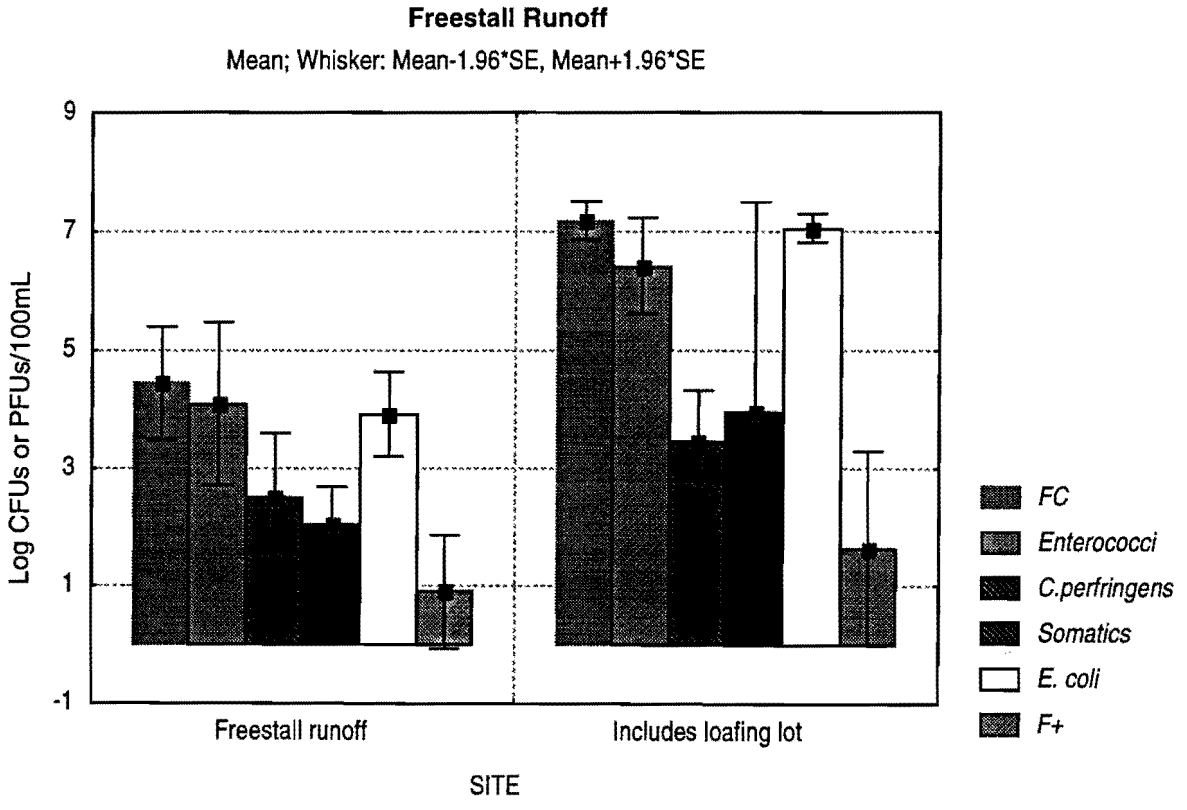
Geometric Mean Microbial Densities (CFUs or PFUs/100mL) and 95% Cis				
	S7	S9	S10	S11
Fecal Coliforms	832 (n=12) (389, 1820)	1122 (n=12) (417, 3090)	1175 (n=12) (589, 2344)	1175 (n=12) (490, 2754)
E. coli	708 (n=12) (331, 1549)	1000 (n=12) (347, 2818)	933 (n=12) (490, 1778)	912 (n=12) (407, 2089)
Enterococci	525 (n=12) (269, 1023)	977 (n=11) (550, 1738)	603 (n=12) (339, 1097)	1380 (n=12) (468, 4169)
C. perfringens	141 (n=12) (98, 200)	115 (n=12) (66, 195)	112 (n=11) (68, 191)	162 (n=12) (98, 275)
Somatic coliphages	8 (n=11) (2, 25)	6 (n=11) (2, 17)	7 (n=11) (3, 17)	21 (n=11) (9, 51)
F+ coliphages	0.3 (n=10) (0.2, 0.7)	0.2 (n=10) (0.1, 0.7)	0.5 (n=10) (0.2, 1.9)	0.7 (n=10) (0.2, 2.3)

Cattle Waste Runoff

Cattle waste runoff samples associated with precipitation events were collected on three occasions from a cattle pasture and dairy feedlot at Lake Wheeler Road Farm. Efforts are made to contain and manage this waste runoff by various techniques, including collection systems, riparian buffers, etc., so that the waste does not impact nearby surface waters. In general, $\log_{10}$ geometric mean microbial indicator levels were much higher in runoff from the feedlot than from the pasture. The mean concentrations of fecal coliforms (>7 $\log_{10}$/100 ml), *E. coli* (>7 $\log_{10}$/100ml), and enterococci (>6 log10/100 ml) were over two orders of magnitude greater in feedlot runoff and approached the levels found in flushed wastes. Previous studies of fecal contamination from various sources suggest that

increased amounts of impervious surface are associated with increased levels of fecal contamination of surface waters. Because the cattle feedlot area has a large impervious surface, this may have contributed to the higher concentrations of microbial indicators in the runoff from this source compared to the runoff from the cattle pasture. However, runoff from the cattle pasture also contained much higher geometric mean concentrations of all six microbial indicators than were observed in stream water at the eight sampling stations at Lake Wheeler Road Farm. It was observed that concentrations of microbial indicators in stream waters were higher during storm events than during dry weather (baseflow) conditions. Therefore, it is possible that waste runoff from the cattle operations impacted the stream water stations near the dairy cattle area of Lake Wheeler Road Farm. Because concentrations of microbial indicators in all stream water stations were always higher during precipitation events than during dry weather, regardless of land use or waste source, the role of cattle waste runoff in increased microbial concentrations in stream waters can not be specifically determined without more detailed investigations.

Figure 14. Geometric Mean $\log_{10}$ Concentrations of Microbial Indicators in Cattle Waste Runoff in a Dairy Cattle Area



Cattle and Swine Waste Lagoon Samples

The results of swine and dairy cattle wastewater lagoon sampling and analysis show that the raw wastes contains high concentrations of fecal indicator microbes (>10,000,000 or >8 $\log_{10}$ organisms per 100 ml), as could be expected (Figure 15). These microbial levels are comparable to or in excess of those observed for municipal or domestic raw sewage. Treatment of the waste in a primary anaerobic lagoon reduces the fecal indicator microbial concentrations of the raw waste by about 90-99% or 1-2 $\log_{10}$. However, wastewater from single stage anaerobic lagoons or the first lagoon cells in the swine and dairy lagoon treatment systems of this farm still contain high levels of enteric indicator bacteria, with levels in excess of 100,000 or 5 $\log_{10}$ per 100 ml. The levels of most fecal indicator microbes are further reduced (by about 90-99% or 1-2 $\log_{10}$) in the second stage anaerobic lagoon. The exception is the reduction of *Cl. perfringens*, which was reduced by <90%. Microbial indicator levels remaining in secondary lagoon liquids are in excess of 10,000 per 100 ml for most of the bacterial indicators as well as somatic coliphages (Figure 15 and Table 5). The microbial concentrations in the primary and secondary lagoons of these swine and dairy units are representative of the levels present in the wastewaters applied to land adjacent to these facilities. In North Carolina stored anaerobic lagoon liquid is land applied by spray irrigation or other methods with no further pre-treatment.

Figure 15. Geometric Mean Concentrations of Microbial Indicators in Raw, Primary Lagoon and Secondary Lagoon Swine Waste

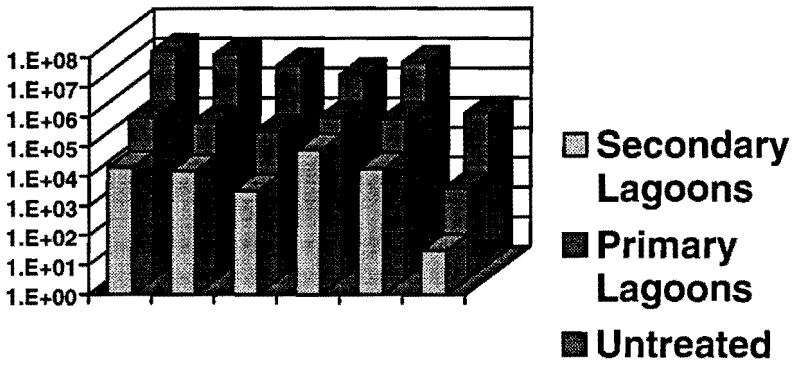


Table 5. Geometric Mean Concentrations of Microbial Indicators in Raw, Primary Lagoon and Secondary Lagoon Swine Waste

Waste Type	Geometric Mean Microbial Concentrations per 100 ml					
	Fecal Coliform	*E. coli*	Entero-cocci	*Cl. perfringens*	Somatic Coliphages	F+ Coli-phages
Untreated	13,000,00	10,000,000	4,300,000	2,100,00	5,500,000	110,000
Primary Lagoon	270,000	190,000	90,000	270,000	250,000	1100
Secondary Lagoon	20,000	15,000	3,300	78,000	18,000	31

It is noteworthy that relatively high concentrations of microbial indicators of fecal contamination remain the in treated swine and dairy cattle waste after treatment in a single anaerobic lagoon or by two anaerobic lagoons in series. Geometric mean microbial concentrations of fecal coliforms, *E. coli*, enterococci and *Cl. perfringens* are still in excess of 100,000 per 100 ml and 10,000 per 100 ml in primary and secondary lagoon liquids, respectively. Therefore, any further reductions of microbial indicators in the treated wastes must occur after the wastes are land- applied. The extent to which these indicator microbes and enteric pathogens die off or persist in the wastes after they are land- applied was not specifically investigated in this study. It is generally assumed that the enteric microbes remaining in the land-applied wastes are retained and/or inactivated on the land and they do not enter surface or ground waters. Some previous studies on the fate of fecal coliform bacteria in land-applied manures support this assumption (Wilson, 1997; references). However, other studies have shown that bacteria and other enteric microbes in land applied manures can contaminate ground and surface waters and can cause waterborne outbreaks of enteric illness in humans, under some circumstances. Further studies of the fate of enteric microbes in land applied manures under BMPs are recommended in order to determine if current practices are adequate to adequately prevent or minimize microbial contamination of ground water and surface water. Microbial contamination of surface water in the vicinity of animal agriculture systems using BMPs was observed in this present study. However, the sources of this contamination and the mechanisms and pathways by which is occurs were not specifically investigated. Such studies are recommended to better understand and quantify the extent to which animal agriculture systems impact the microbial quality of surface and ground waters.

REFERENCES

LITERATURE CITED

Adams, M.H. 1959. Bacteriophages. Wiley Interscience, New York.

Ashford DA, Bragg SL, Weyant RS, Sanders EJ, Bodnar UR, Smythe L, Shieh WJ, Zaki SR, Perkins BA, Spiegel RA, 1998. Leptospirosis and climate: recent experience at the National Center for Infectious Diseases (NCID). In *Program and abstracts of the International Conference on Emerging Infectious Diseases*. Poster 21.22 Atlanta GA. March 8-11. P. 135.

Barwick, R.S., D.A. Levy, G.F. Craun, M.J. Beach and R. L. Calderon (2000) Surveillance for Waterborne-Disease Outbreaks —United States, 1997–1998. Morbidity and Mortality Weekly Reports - Surveillance Summary, Vol. 49 / No. SS-4 MMWR 1. 44 pages.

Baxter-Potter, W.R. and M.W. Gilliland. 1988. Bacterial pollution in runoff from agricultural lands. J. Environ. Qual. 17:27-34.

Brown, K.W., S.G. Jones and K.C. Donnelly. 1980. The influence of simulated rainfall on residual bacteria and virus on grass treated with sewage sludge. J. Environ. Qual. 9:261-265.

Cole DJ, Hill VR, Humenik FJ, Sobsey MD. 1999. Health, safety and environmental concerns of farm animal waste, In *Occupational Medicine State of the Arts Review Series on Health and Safety Concerns of Animal Handlers*, R. Langley (ed.). Hanley & Belfus: Philadelphia, PA.

Crane, S.R., J.A. Moore, M.E. Grismer and J.R. Miner. 1983. Bacterial pollution from agricultural sources: a review. Trans. ASAE, Journal Paper No. 6446, 858-866.

Crane, S.R., P.W. Westerman and M.R. Overcash. 1980. Die-off of fecal indicator organisms following land application of poultry manure. J. Environ. Qual. 9:531-537.

Crane, S.R. and J.A. Moore. 1986. Modeling enteric bacterial die-off: a review. Water, Air, and Soil Pol. 27:411-439.

Dazzo, F., P. Smith and D. Hubbell. 1973. The influence of manure slurry irrigation on the survival of fecal organisms in Scranton fine sand. J. Environ. Qual. 2:470-473.

Furuse, K. 1987. Distribution of coliphages in the environment: general considerations. *In* Phage Ecology, S.M. Goyal, C.P. Gerba and G. Bitton (eds.), Wiley-Interscience, New York. 87-124.

Gerba, C.P. and J.B. Rose. 1990. Viruses in source and drinking water. *In* Drinking water microbiology, G.A. McFeters (ed.), Springer-Verlag, New York, 380-396.

Grabow, W.O.K. and P. Coubrough (1986) Practical Direct Plaque Assay for Coliphages in 100 Milliliter Samples of Drinking Water. Appl. Environ. Microbiol., 52 (3): 430-433.

Havelaar, A.H., W.M. Pot-Hogeboom, K. Furuse, R. Pot M.P. and Hormann. 1990. J. Appl. Bacteriol., 68:30-37.

Hill, VR and MD Sobsey. 1998. Microbial indicator reductions in alternative treatment systems for swine wastewater. *Water, Science and Technology* 38:119-122.

Hrubant, G.R., R.V. Daugherty and R.A. Rhodes. 1972. Entero-bacteria in feedlot water runoff. Appl. Microbiol. 24:378-383.

Hsu, F.C., Y.S.C. Shieh, J. van Duin, M.J. Beekwilder, and M.D. Sobsey. 1995. Appl. Environ. Microbiol., 61:3960-3966.

Kramer M.H., Herwaldt B.L., Craun G.F., Calderon R.L., and Juranek D.D. 1996. Surveillance for waterborne-disease outbreaks—United States, 1993-1994. *MMWR* 45(SS-1):1-33.

Larkin, E.P., J.T. Tierney and R. Sullivan. 1976. Persistence of virus on sewage-irrigated vegetables. J. Environ. Eng. Div., Proc. ASCE 102:29-35.

Mager AL, Standridge J, Kluender SM, and Peterson LL. 1996. Source and Occurrence of Pathogens in Watersheds. In Proceedings of the Water Quality Technology Conference, American Water Works Association, Boston, MA.

Meinhardt PL, Casemore DP, Miller KB. 1996. Epidemiologic aspects of human cryptosporidiosis and the role of waterborne transmission. *Epidem Rev* 18(2):118-136.

Moore, B.E., B.P. Sagik and C.A. Sorber. 1981. Viral transport to ground water at a wastewater land application site. J. WPCF 53:1492-1502.

McMurry, S.W., M.S. Coyne and E. Perfect. 1998. Fecal coliform transport through intact soil blocks amended with poultry manure. J. Environ. Qual. 27:86-92.

Overcash, M.R., F.J. Humenik and J.R. Miner. 1983. Livestock waste management, Volume I. CRC Press, Boca Raton, FL.

Patz J.A., Rose J.B., Curriero F.C., Lele S.R., Daeschner S., and Craun G.F. 1998. Association between rainfall and water-borne disease outbreaks in the US: 1972-1994. In *Program and abstracts of the International Conference on Emerging Infectious Diseases.* Poster 8.3 Atlanta GA. March 8-11, P. 96

Sobsey, M.D., Schwab K., and Handzel T. 1990. J. Amer. Water Works Assoc., 82:52-59.