

## The Influence of Vegetation in Riparian Filterstrips on Coliform Bacteria: I. Movement and Survival in Water

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### ABSTRACT

Swine (*Sus scrofa*) wastewater was applied to three separate 4 m wide × 30 m long riparian filterstrips consisting of 20 m grass and 10 m forest, 10 m grass and 20 m forest, and 10 m grass and 20 m maidencane (*Panicum hemitomon* Schult.) in Southern Georgia during each season. Total and fecal coliform numbers in the applied wastewater pulse did not decline as water moved downslope regardless of vegetation type or season. The pulse of applied wastewater did not move beyond 15 m in any treatment in autumn or summer (dry seasons) and only moved beyond 7.5 m in the 20 m grass–10 m forest treatment in the summer. Total and fecal coliform numbers in soil water and shallow ground water declined by approximately 10-fold every 7 d for the first 14 d regardless of vegetative treatment or season. Soil temperature and soil moisture correlated with total coliform bacteria in both 1.5 m wells ( $r^2 = 0.89$ ) and 2.0 m wells ( $r^2 = 0.89$ ), and with fecal coliform bacteria in 1.5 ( $r^2 = 0.82$ ) and 2.0 m ( $r^2 = 0.76$ ) wells. Animal production operations may need to locate in warm-dry climates so animal waste can be applied to lands to help ensure enteric bacteria input to surface and ground water will not occur.

AGRICULTURE is a major source of nonpoint-source pollution in lakes and streams in the United States (USEPA, 1994). The increase in the size and concentration of livestock units throughout the United States and the practice of manure disposal to agricultural lands has resulted in several instances of coliform bacteria in surface waters exceeding the limits set by the USEPA for recreational water quality standards (USEPA, 1998; Walker et al., 1990; Donnison and Cooper, 1989; Sorber and Moore, 1987). When animal waste applied to agricultural lands and subsequent surface runoff or leaching occurs due to overirrigation or rainfall, contamination of water resources by enteric bacteria may result (Entry et al., 2000). These same bodies of water are often used for sources of drinking water and for recreational activities; therefore, elevated concentrations of enteric bacteria pose a potential health hazard.

Total and fecal coliform bacteria are sensitive and commonly used indicators of bacterial pathogen contamination of natural waters. Their presence implies the potential presence of microorganisms that are pathogenic to humans. Runoff and ground water from agricultural land that has had animal waste applied shows that total and fecal coliform bacteria follow a pattern: (i)

higher numbers of coliform bacteria in water during spring flows, (ii) lower numbers of coliform bacteria in water during the summer dry period, (iii) higher numbers of coliform bacteria in water after applying wastewater by irrigation or after additional manure disposal, and (iv) a rapid decline of bacteria counts once manure application is halted (Fraser et al., 1998; Howell et al., 1996; Darling and Coltharp, 1973; Buckhouse and Gifford, 1976). Several investigators found that fecal coliform numbers declined rapidly when transported through dispersed soils, indicating that bacterial pollution occurs mainly by transport via water through soil macropores (Abu-Ashour et al., 1998; Howell et al., 1996; Huysman and Verstraete, 1993; Buckhouse and Gifford, 1976; Kunkle, 1970).

Riparian vegetation acts as a natural filter and removes nutrients and other contaminants through both ground- and surface water pathways (Hubbard et al., 1998; Snyder et al., 1998; Jordan et al., 1993; Lowrance et al., 1984). Peterjohn and Correll (1984) estimated an annual net  $\text{NO}_3^-$  removal of  $45 \text{ kg ha}^{-1}$  from subsurface waters moving out of fertilized crop land through 50 m of riparian forest. They also reported substantial decreases in particulate organic N and particulate P in surface waters. Riparian filterstrips provide an excellent sink for nutrients entering streams from surrounding agricultural lands. However, there has been little research on the effectiveness of forest riparian filterstrips to protect stream waters from coliform bacteria. The companion study (Entry et al., 2000) found that land application of swine waste to riparian vegetation increased total and fecal coliform concentrations in the 0 to 5, 5 to 15, and 15 to 30 cm soil layers from 10- to 1000-fold. Coyne et al. (1995), Walker et al. (1990), and Young et al. (1980) concluded that 10-m wide grass filterstrips can reduce the amount of fecal coliform bacteria in runoff by as much as 70%. The objective of this study was to determine the effectiveness of forest and grass vegetation growing in riparian filterstrips in filtering total and fecal coliform bacteria from surface and ground water in different climatic conditions.

### MATERIALS AND METHODS

#### Site Description

Plots were located at the Animal Science Research Farm at the Coastal Plain Experiment Station in Tifton, Georgia. The site included a grassed area that had formerly been the lowest end of a pasture for beef cattle (*Bos taurus*), an adjacent downslope riparian forest with slash pine (*Pinus elliotii* Engelm.), and accompanying underlying shrubby vegetation (Hubbard et al., 1998). The soil of the grassed area was Tifton loamy sand (fine-loamy, kaolinitic, thermic Plinthic Kandudults) while the riparian forest area was an Alapaha loamy sand (loamy, silicious, thermic Arenic Plinthic Paleaquults)

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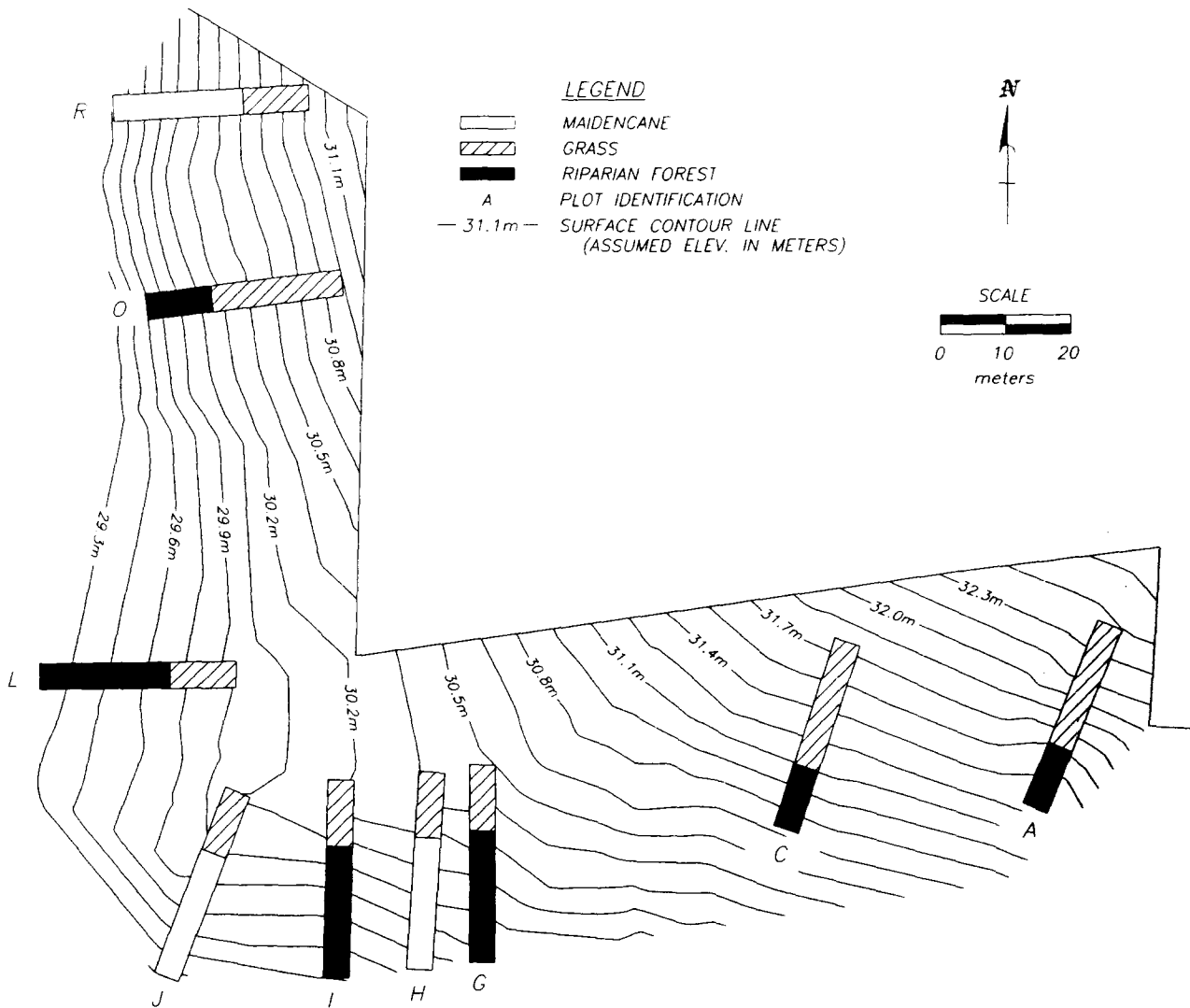


Fig. 1. Grass-riparian filterstrip treatments.

or an intergrade between it and Tifton loamy sand. These soils are underlain with plinthite and Miocene age materials of very low permeability. In the plinthic soils of the Tifton Upland, 99% of infiltrating water moves downslope as shallow lateral flow (Hubbard and Sheridan, 1983). The slope at the site ranged from 1.5 to 2.0%.

Three different vegetation types were used for the study (Fig. 1 and 2). Vegetative treatments were (i) 20 m grass buffer draining into 10 m existing forest riparian zone vegetation, (ii) 10 m grass buffer draining into 20 m existing forest riparian zone vegetation, and (iii) 10 m grass buffer draining into 20 m maidencane. Maidencane is a species recommended for constructed wetlands. The purpose of maidencane was to see if wetland plant species other than trees and understory vegetation would be effective in reducing survival of total and fecal coliform bacteria compared with grass and forest vegetation. Maidencane was planted as rhizomes during the summer of 1993. Three or four cuttings of the grassed zone were made each summer and the biomass was completely removed from the plots. Two cuttings (at approximately 30 cm height) were made of the maidencane during the summers. Maidencane is used as forage in Florida, so cutting and removal of the maidencane biomass was used to simulate potential cutting of this plant for hay by animal producers.

Coastal bermuda grass [*Cynodon dactylon* (L.) Pers; cv. Tifton 78] was planted in the grassed portion of the plots. During the fall of 1993, a heat-tolerant tall fescue (*Festuca arundinacea* Schreb.; cv. Georgia 5) was planted as a perennial winter cover in the grassed portion of the plots. During the winter of 1995–1998, crimson clover (*Trifolium incarnatum* L.) was seeded on the plots, since the fescue had not performed well in terms of cover during the previous winter (1994–1995). The forested part of the plots had slash pine that were 8 yr old and approximately 10 m tall by the end of the study in 1999. In this study grass was cut to a 10 cm height prior to each application of swine waste, while maidencane grew to a height of approximately 30 cm in spring and 2 m in summer.

### Plot Design

The overland flow-riparian buffer plots were each 4 m wide and 30 m long and were positioned on the landscape according to contour, so that flow of the wastewater downslope would be as uniform as possible (Fig. 2). The sides of each plot were bounded with plastic borders that extended 15 cm above ground and 15 cm below ground. An earthen berm at the top of each plot prevented surface runoff entering from upslope of the plot. At the top of each plot a gated pipe made of

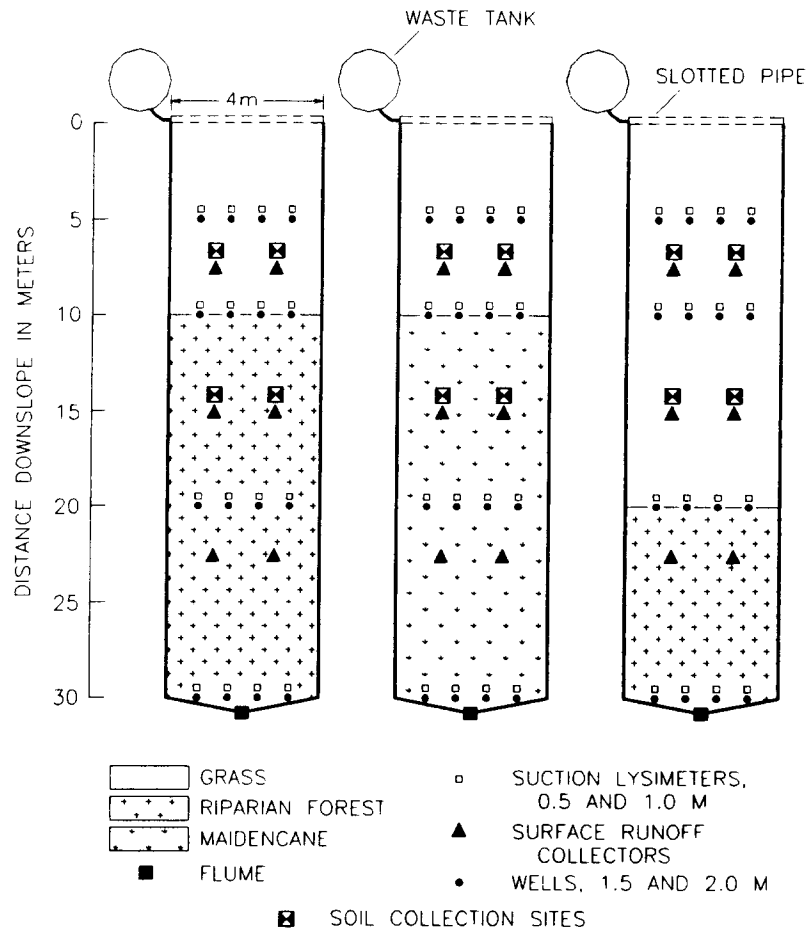


Fig. 2. Site diagram of swine waste grass-riparian filterstrip plots.

plastic was used to apply wastewater. Suction lysimeters were placed in pairs at depths of 0.5 and 1.0 m at four positions on a transect from the wastewater application pipe downslope to the bottom of each plot (Fig. 2). The suction lysimeters were constructed of 3.8 cm diameter (schedule 40) PVC pipe with porous cups on the end. Openings in the porous cups of the PVC pipe were 2.90  $\mu\text{m}$  diameter, large enough for bacteria to pass through. A vacuum system was applied to the lysimeters for approximately 12 h prior to collection of the samples. Wells were constructed of 5 cm diameter (schedule 40) PVC pipe and had 15 cm of slotted well screening at the bottom. A peristaltic pump was used to collect water samples from the suction lysimeters and ground water wells. The suction lysimeters and wells were completely evacuated during the sampling process to ensure that water for the next sampling would be fresh ground water.

The individual plots were positioned on the landscape to minimize cross-contamination of the shallow ground water and vegetative treatments (Fig. 2). Wastewater flowed from an individual tank above each plot into the gated pipe and then downslope. The pipe gates were spaced 46 cm apart and were adjusted on each plot so that overland flow would begin downslope movement as uniformly as possible (Fig. 2). Depending on soil moisture and vegetative conditions, wastewater flowed over one-half to two-thirds of each plot during application. Wastewater was not applied during rainstorms or if it appeared that rainfall was imminent. During the wet winter and spring months when the soil was nearly saturated, the wastewater was applied slowly, to minimize any potential for the waste to exit the plots via overflow of the plastic

borders. At other times the wastewater was applied as quickly as the tanks would drain completely, resulting in application times of approximately 10 min.

### Wastewater

The study was implemented using swine lagoon wastewater from the treatment-storage system at the University of Georgia Coastal Plain Experiment Station swine unit at Tifton, Georgia. The unit maintained an inventory of 350 to 550 head of swine during the course of the study. The lagoon system consisted of three lagoons, in series. The primary lagoon (12  $\times$  46 m) discharged into a secondary lagoon (15  $\times$  31 m), from which the liquid was pumped back into the barns (1800 L  $\text{min}^{-1}$ ) for flushing waste. The secondary lagoon discharged into a holding lagoon (18  $\times$  37 m) that was used as the source of the wastewater for the study. Features of the lagoon system have been further described (Hubbard et al. 1998; Newton and Haydon, 1985). Eight hours prior to application, liquid waste was pumped approximately 760 m from the holding lagoon into 2 m diameter  $\times$  1.5 m high plastic holding tanks located at the upper end of each plot (Fig. 2). Wastewater application consisted of 2570 L, from two tanks, per plot as one application each season, which is a typical amount of animal wastewater applied to a site to meet crop N demands (Hubbard et al. 1998).

Total coliform numbers in source wastewater ranged from  $4.92 \times 10^6$  to  $10.5 \times 10^5$  colonies 100 ml  $\text{water}^{-1}$ . Numbers of fecal coliform bacteria in source wastewater ranged from  $15.8 \times 10^5$  to  $7.0 \times 10^5$  colonies 100 ml  $\text{water}^{-1}$ . Analyses of

wastewater samples collected weekly during the study showed average N concentration of  $160 \text{ mg L}^{-1}$ , with most of the N in the  $\text{NH}_4^+\text{-N}$  form. Nitrate concentrations in the wastewater ranged from less than  $1 \text{ mg L}^{-1}$  to  $20 \text{ mg L}^{-1}$  with a mean of  $3 \text{ mg L}^{-1}$ .

### Experimental Design

The study was arranged in a completely random factorial design consisting of filterstrips with three vegetation types, climatic periods (season), distance from inflow source, and depth of soil water and ground water (Kirk, 1982). Each treatment was replicated three times. Wastewater was applied as a single pulse in winter, spring, summer, and autumn having four distinct climatic conditions (described below). Soil water and ground water was collected at four distances from the wastewater inflow source.

Total and fecal coliform bacteria were sampled in winter (wet-cool period; 14–28 January), spring (warm-wet; 16–30 March), summer (hot-dry; 6–21 July) and autumn (warm-dry; 11–25 November) 1998. In winter soil temperature ranged from  $13$  to  $14^\circ\text{C}$  and soil moisture ranged from  $1.8$  to  $2.8 \text{ g kg soil}^{-1}$ , in spring soil temperature ranged from  $17$  to  $22^\circ\text{C}$  and soil moisture ranged from  $1.5$  to  $3.3 \text{ g kg soil}^{-1}$ , in summer soil temperature ranged from  $30$  to  $45^\circ\text{C}$  and soil moisture ranged from  $0.1$  to  $0.7 \text{ g kg soil}^{-1}$ , and in autumn soil temperature ranged from  $13$  to  $18^\circ\text{C}$  and soil moisture ranged from  $0.3$  to  $1.1 \text{ g kg soil}^{-1}$ . Ground water temperature varied from  $13^\circ\text{C}$  in winter to  $22^\circ\text{C}$  in spring. Ground water temperature in the  $1.5$  and  $2.0 \text{ m}$  wells did not vary appreciably among filterstrip vegetation types in any season. There was no ground water in the  $1.5$  or  $2.0 \text{ m}$  wells during the summer sampling period.

### Sample Collection

Soil water was sampled from each lysimeter and shallow ground water was sampled from each well. Samples were analyzed for total and fecal coliform bacteria 2 to 4 d prior to each wastewater application. Ground water was also sampled in three areas of grass and forest vegetation at a nearby site that was neither grazed nor had animal manure applied to it as a control (Lowrance et al., 1998). Five separate samples were collected from each wastewater application tank (input) immediately prior to wastewater application.

Surface runoff samples were collected from the pulse of applied wastewater flowing over the soil surface at distances of  $7.5$ ,  $15.0$ ,  $22.5$ , and  $30.0 \text{ m}$  from the application pipe. Soil water (lysimeters) was sampled at  $0.5$  and  $1.0 \text{ m}$  depths and shallow ground water (wells) was sampled through wells at  $1.5$  and  $2.0 \text{ m}$  depths at  $5$ ,  $10$ ,  $20$ , and  $30 \text{ m}$  from the inflow source.

Survival of total and fecal coliform bacteria in each factorial combination of vegetation was determined in the pulse of wastewater as surface flow on the day of application (Day 0), soil water from suction lysimeters at 2 to 4 d prior to wastewater application, and at 1, 3, 7, 14, and 90 to 120 d after wastewater application. Shallow ground water was sampled 2 to 4 d prior to wastewater application and at 7, 14, and 90 to 120 d after wastewater application.

### Sample Analysis

Water in suction lysimeters and ground water wells was collected with a peristaltic pump into sterile  $500 \text{ mL}$  glass containers and transported at ambient temperature to the Agricultural Research Service Southeast Watershed Research Laboratory in Tifton, GA. Samples were stored at ambient temperatures and processed within 4 hr of collection. Total

and fecal coliform bacteria were analyzed with the membrane filter technique (Greenberg et al., 1992). Preliminary samples of the pulse of applied wastewater, soil water, and shallow ground water were taken to obtain the dilution that would grow approximately 20 to 200 total or fecal coliforms on each filter. A  $100 \text{ mL}$  of sample from each final dilution was vacuum-filtered through a sterile  $0.45 \text{ }\mu\text{m}$  filter and placed on m-Endo LES medium to determine total coliform bacteria or m-FC medium to determine fecal coliform bacteria. Total and fecal coliform bacteria were incubated at  $39.5^\circ\text{C} \pm 0.02$  and  $44.5^\circ\text{C} \pm 0.02$  respectively for 24 h. Three colony types of both total and fecal coliform bacteria from each sample date were identified by fatty acid analysis using the AEROBE library of the Microbial Identification System (Newark, DE).

### Statistical Analyses

All dependent variables were tested for normal distribution. Data were analyzed by means of analysis of variance (ANOVA) procedures for a completely random design with Statistical Analysis System (SAS Institute, 1996). Numbers of total and fecal coliform bacteria were log transformed to achieve normal distributions. Statistical comparisons were made of total and fecal coliform bacteria by vegetation type  $\times$  season  $\times$  time since application. Residuals were equally distributed with constant variances. Differences reported throughout are significant at  $p \leq 0.05$ , as determined by the Protected Least Squares Means test (Kirk, 1982; Snedecor and Cochran, 1980). Correlations were determined with soil temperature and moisture as independent ( $x$ ) variables and total or fecal coliform bacteria as dependent ( $y$ ) variables. Total and fecal coliform bacteria are reported in untransformed numbers.

## RESULTS

Total and fecal coliform numbers in the wastewater source immediately prior to application were usually 10-fold higher than in the pulse of applied wastewater at  $7.5$ ,  $15.0$ ,  $22.5$ , and  $30.0 \text{ m}$  from the inflow pipe on the day of wastewater application regardless of vegetation type or season. Total coliform numbers in source wastewater ranged from  $4.92 \times 10^6$  colonies  $100 \text{ mL water}^{-1}$  to  $11.6 \times 10^6$  colonies  $100 \text{ mL water}^{-1}$ . Numbers of fecal coliform bacteria in source wastewater ranged from  $2.9 \times 10^5$  colonies  $100 \text{ mL water}^{-1}$  to  $15.8 \times 10^5$  colonies  $100 \text{ mL water}^{-1}$ .

The pulse of applied wastewater moved as surface flow over-ground to a distance of  $30.0 \text{ m}$  in all treatments in the winter and in the  $20 \text{ m grass-10 m forest}$  treatment in spring. The pulse of applied wastewater as surface flow did not move beyond  $15 \text{ m}$  in any treatment in autumn or summer (dry seasons) and only beyond  $7.5 \text{ m}$  in the  $20 \text{ m grass-10 m forest}$  treatment in summer. Populations of total and fecal coliform bacteria in the pulse of applied wastewater did not decline as water moved downslope regardless of vegetation type or season of the year.

The general linear models procedure indicated that there was no significant difference in counts of total and fecal coliform bacterial numbers sampled in soil water (lysimeters) at  $0.5 \text{ m}$  and soil water (lysimeters) at  $1.0 \text{ m}$  depths in shallow ground water (wells) at  $1.5 \text{ m}$  and in shallow ground water (wells) at  $2.0 \text{ m}$  regardless of vegetative treatment, distance from the inflow source,



Table 2. Total and fecal coliform bacteria in 1.5-2.0 meter deep wells in soil growing three different types of riparian vegetation after application of swine waste water.<sup>†‡</sup>

Vegetation	Distance (m)	Total coliform bacteria											
		Winter			Spring			Autumn			98		
		Day 7	Day 14	Day 7	Day 14	Day 7	Day 14	Day 7	Day 14	Day 7	Day 14	Day 7	Day 14
colonies/100 ml water													
Control, grass§	0	47.5 × 10 <sup>b</sup>	14.9 × 10 <sup>c</sup>	47.5 × 10 <sup>b</sup>	28.9 × 10 <sup>b</sup>	10.1 × 10 <sup>b</sup>	36.0 × 10 <sup>d</sup>	15.0 × 10 <sup>c</sup>	71.2 × 10 <sup>d</sup>	2.3 × 10 <sup>e</sup>			
Control, forest§	0	14.5 × 10 <sup>b</sup>	68.8 × 10 <sup>c</sup>	14.5 × 10 <sup>b</sup>	17.4 × 10 <sup>b</sup>	58.5 × 10 <sup>c</sup>	71.6 × 10 <sup>d</sup>	43.3 × 10 <sup>b</sup>	43.4 × 10 <sup>d</sup>	1.1 × 10 <sup>e</sup>			
Source (Inflow)¶	0	11.6 × 10 <sup>b</sup>	24.8 × 10 <sup>d</sup>	4.92 × 10 <sup>a</sup>	16.2 × 10 <sup>b</sup>	16.7 × 10 <sup>c</sup>	10.4 × 10 <sup>a</sup>	48.8 × 10 <sup>c</sup>	97.0 × 10 <sup>d</sup>	2.5 × 10 <sup>e</sup>			
20 m grass, 10 m forest	5	51.5 × 10 <sup>a</sup>	36.5 × 10 <sup>b</sup>	11.2 × 10 <sup>d</sup>	14.7 × 10 <sup>bc</sup>	10.1 × 10 <sup>b</sup>	36.0 × 10 <sup>d</sup>	15.0 × 10 <sup>c</sup>	71.2 × 10 <sup>d</sup>	2.3 × 10 <sup>e</sup>			
	10	22.0 × 10 <sup>d</sup>	74.3 × 10 <sup>b</sup>	66.0 × 10 <sup>e</sup>	68.8 × 10 <sup>c</sup>	58.5 × 10 <sup>c</sup>	48.5 × 10 <sup>d</sup>	43.3 × 10 <sup>b</sup>	43.4 × 10 <sup>d</sup>	1.1 × 10 <sup>e</sup>			
	20	45.7 × 10 <sup>d</sup>	93.6 × 10 <sup>bc</sup>	53.0 × 10 <sup>e</sup>	24.8 × 10 <sup>d</sup>	16.7 × 10 <sup>c</sup>	76.3 × 10 <sup>d</sup>	48.8 × 10 <sup>c</sup>	97.0 × 10 <sup>d</sup>	2.5 × 10 <sup>e</sup>			
	30	14.3 × 10 <sup>c</sup>	45.0 × 10 <sup>c</sup>	13.9 × 10 <sup>d</sup>	36.5 × 10 <sup>c</sup>	11.3 × 10 <sup>c</sup>	10.3 × 10 <sup>d</sup>	34.4 × 10 <sup>c</sup>	46.5 × 10 <sup>d</sup>	5.7 × 10 <sup>e</sup>			
10 m grass, 20 m maidencane	5	33.1 × 10 <sup>d</sup>	33.1 × 10 <sup>c</sup>	46.3 × 10 <sup>d</sup>	40.4 × 10 <sup>c</sup>	17.1 × 10 <sup>b</sup>	46.5 × 10 <sup>d</sup>	50.3 × 10 <sup>c</sup>	92.5 × 10 <sup>d</sup>	5.9 × 10 <sup>e</sup>			
	10	17.3 × 10 <sup>d</sup>	76.5 × 10 <sup>cd</sup>	45.1 × 10 <sup>d</sup>	47.5 × 10 <sup>d</sup>	49.0 × 10 <sup>c</sup>	50.8 × 10 <sup>d</sup>	33.7 × 10 <sup>c</sup>	71.2 × 10 <sup>d</sup>	4.1 × 10 <sup>e</sup>			
	20	43.3 × 10 <sup>d</sup>	58.6 × 10 <sup>d</sup>	14.1 × 10 <sup>d</sup>	29.2 × 10 <sup>d</sup>	54.3 × 10 <sup>c</sup>	27.6 × 10 <sup>d</sup>	23.3 × 10 <sup>c</sup>	32.3 × 10 <sup>d</sup>	4.4 × 10 <sup>e</sup>			
	30	63.3 × 10 <sup>d</sup>	10.5 × 10 <sup>d</sup>	12.2 × 10 <sup>d</sup>	26.7 × 10 <sup>d</sup>	50.1 × 10 <sup>e</sup>	75.8 × 10 <sup>d</sup>	33.8 × 10 <sup>c</sup>	87.6 × 10 <sup>d</sup>	10.2 × 10 <sup>e</sup>			
10 m grass, 20 m forest	5	95.8 × 10 <sup>cd</sup>	34.6 × 10 <sup>b</sup>	17.7 × 10 <sup>d</sup>	67.2 × 10 <sup>bc</sup>	20.5 × 10 <sup>b</sup>	11.8 × 10 <sup>c</sup>	37.9 × 10 <sup>c</sup>	46.0 × 10 <sup>d</sup>	1.3 × 10 <sup>e</sup>			
	10	10.5 × 10 <sup>d</sup>	12.3 × 10 <sup>c</sup>	25.0 × 10 <sup>d</sup>	26.2 × 10 <sup>d</sup>	74.6 × 10 <sup>c</sup>	70.6 × 10 <sup>c</sup>	31.3 × 10 <sup>c</sup>	30.3 × 10 <sup>d</sup>	8.4 × 10 <sup>e</sup>			
	20	10.0 × 10 <sup>d</sup>	13.2 × 10 <sup>c</sup>	10.4 × 10 <sup>d</sup>	29.2 × 10 <sup>d</sup>	20.3 × 10 <sup>c</sup>	53.4 × 10 <sup>d</sup>	21.8 × 10 <sup>c</sup>	43.0 × 10 <sup>d</sup>	1.5 × 10 <sup>e</sup>			
	30	15.3 × 10 <sup>d</sup>	82.4 × 10 <sup>d</sup>	59.0 × 10 <sup>e</sup>	14.9 × 10 <sup>e</sup>	26.1 × 10 <sup>c</sup>	81.2 × 10 <sup>dc</sup>	27.1 × 10 <sup>c</sup>	18.1 × 10 <sup>d</sup>	2.3 × 10 <sup>e</sup>			
Fecal coliform bacteria													
colonies/100 ml water													
Control, grass§	0	58.3 × 10 <sup>c</sup>	15.2 × 10 <sup>c</sup>	58.3 × 10 <sup>bc</sup>	62.0 × 10 <sup>bc</sup>	33.7 × 10 <sup>c</sup>	18.8 × 10 <sup>c</sup>	17.8 × 10 <sup>c</sup>	25.3 × 10 <sup>c</sup>	15.4 × 10 <sup>c</sup>			
Control, forest§	0	83.0 × 10 <sup>cd</sup>	17.8 × 10 <sup>c</sup>	75.8 × 10 <sup>d</sup>	52.0 × 10 <sup>c</sup>	69.3 × 10 <sup>d</sup>	26.0 × 10 <sup>d</sup>	29.0 × 10 <sup>d</sup>	38.2 × 10 <sup>c</sup>	2.9 × 10 <sup>e</sup>			
Source (Inflow)¶	0	10.5 × 10 <sup>b</sup>	24.2 × 10 <sup>c</sup>	15.8 × 10 <sup>a</sup>	21.3 × 10 <sup>c</sup>	16.8 × 10 <sup>d</sup>	2.9 × 10 <sup>a</sup>	62.5 × 10 <sup>cd</sup>	39.2 × 10 <sup>c</sup>	3.4 × 10 <sup>c</sup>			
20 m grass, 10 m forest	5	2.3 × 10 <sup>d</sup>	39.1 × 10 <sup>c</sup>	18.7 × 10 <sup>d</sup>	15.2 × 10 <sup>c</sup>	33.7 × 10 <sup>c</sup>	17.8 × 10 <sup>c</sup>	17.8 × 10 <sup>c</sup>	25.3 × 10 <sup>c</sup>	15.4 × 10 <sup>c</sup>			
	10	29.3 × 10 <sup>d</sup>	67.7 × 10 <sup>c</sup>	56.5 × 10 <sup>d</sup>	17.8 × 10 <sup>c</sup>	69.3 × 10 <sup>d</sup>	29.0 × 10 <sup>d</sup>	43.3 × 10 <sup>c</sup>	38.2 × 10 <sup>c</sup>	2.9 × 10 <sup>e</sup>			
	20	0.0 × 10 <sup>d</sup>	33.8 × 10 <sup>c</sup>	0.0 × 10 <sup>e</sup>	24.2 × 10 <sup>c</sup>	16.8 × 10 <sup>d</sup>	62.5 × 10 <sup>cd</sup>	56.2 × 10 <sup>c</sup>	39.2 × 10 <sup>c</sup>	3.4 × 10 <sup>c</sup>			
	30	0.8 × 10 <sup>d</sup>	11.2 × 10 <sup>d</sup>	1.2 × 10 <sup>d</sup>	20.6 × 10 <sup>c</sup>	19.7 × 10 <sup>d</sup>	4.5 × 10 <sup>d</sup>	81.1 × 10 <sup>c</sup>	38.0 × 10 <sup>d</sup>	1.0 × 10 <sup>c</sup>			
10 m grass, 20 m maidencane	5	12.0 × 10 <sup>d</sup>	37.2 × 10 <sup>c</sup>	12.0 × 10 <sup>d</sup>	71.3 × 10 <sup>d</sup>	14.5 × 10 <sup>b</sup>	38.0 × 10 <sup>d</sup>	11.3 × 10 <sup>b</sup>	62.1 × 10 <sup>c</sup>	2.8 × 10 <sup>c</sup>			
	10	4.3 × 10 <sup>d</sup>	14.0 × 10 <sup>cd</sup>	20.7 × 10 <sup>d</sup>	18.9 × 10 <sup>cd</sup>	93.5 × 10 <sup>cd</sup>	0.0 × 10 <sup>d</sup>	88.8 × 10 <sup>c</sup>	85.0 × 10 <sup>c</sup>	4.2 × 10 <sup>c</sup>			
	20	9.7 × 10 <sup>d</sup>	37.0 × 10 <sup>d</sup>	0.0 × 10 <sup>e</sup>	12.8 × 10 <sup>d</sup>	23.2 × 10 <sup>d</sup>	23.0 × 10 <sup>d</sup>	24.3 × 10 <sup>c</sup>	13.0 × 10 <sup>d</sup>	1.3 × 10 <sup>c</sup>			
	30	2.2 × 10 <sup>d</sup>	32.8 × 10 <sup>c</sup>	0.3 × 10 <sup>e</sup>	37.0 × 10 <sup>c</sup>	27.0 × 10 <sup>d</sup>	0.8 × 10 <sup>d</sup>	27.0 × 10 <sup>c</sup>	83.0 × 10 <sup>d</sup>	0.0 × 10 <sup>d</sup>			
10 m grass, 20 m forest	5	44.5 × 10 <sup>d</sup>	12.8 × 10 <sup>bc</sup>	6.8 × 10 <sup>d</sup>	10.7 × 10 <sup>bc</sup>	56.5 × 10 <sup>d</sup>	3.6 × 10 <sup>d</sup>	12.3 × 10 <sup>c</sup>	30.7 × 10 <sup>c</sup>	5.5 × 10 <sup>c</sup>			
	10	18.9 × 10 <sup>d</sup>	18.8 × 10 <sup>d</sup>	35.5 × 10 <sup>d</sup>	28.6 × 10 <sup>bc</sup>	15.2 × 10 <sup>d</sup>	18.6 × 10 <sup>c</sup>	8.0 × 10 <sup>c</sup>	27.7 × 10 <sup>c</sup>	0.0 × 10 <sup>d</sup>			
	20	2.8 × 10 <sup>d</sup>	4.3 × 10 <sup>d</sup>	0.2 × 10 <sup>d</sup>	36.3 × 10 <sup>d</sup>	3.2 × 10 <sup>d</sup>	0.6 × 10 <sup>d</sup>	14.7 × 10 <sup>c</sup>	62.3 × 10 <sup>cd</sup>	2.0 × 10 <sup>c</sup>			
	30	5.3 × 10 <sup>d</sup>	1.8 × 10 <sup>d</sup>	0.7 × 10 <sup>d</sup>	12.3 × 10 <sup>d</sup>	1.2 × 10 <sup>d</sup>	0.5 × 10 <sup>d</sup>	27.1 × 10 <sup>c</sup>	2.8 × 10 <sup>d</sup>	2.2 × 10 <sup>c</sup>			

<sup>†</sup> In each season, in each column, values followed by the same letter are not significantly different as determined by the Least Square Means test ( $P \leq 0.05$ )  $n = 16$ . Tables can be read both vertically and horizontally beginning with the source value which was sampled immediately prior to waste application.

<sup>‡</sup> No water was in 1.5 or 2.0 m deep wells in the summer season.

<sup>§</sup> Controls were sampled at Gibbs Farm, where no animal waste has been applied.

<sup>¶</sup> Waste was applied at Day 0; on Day -1, water was sampled prior to application of swine waste and 63-130 days after the last application of swine waste on these plots.

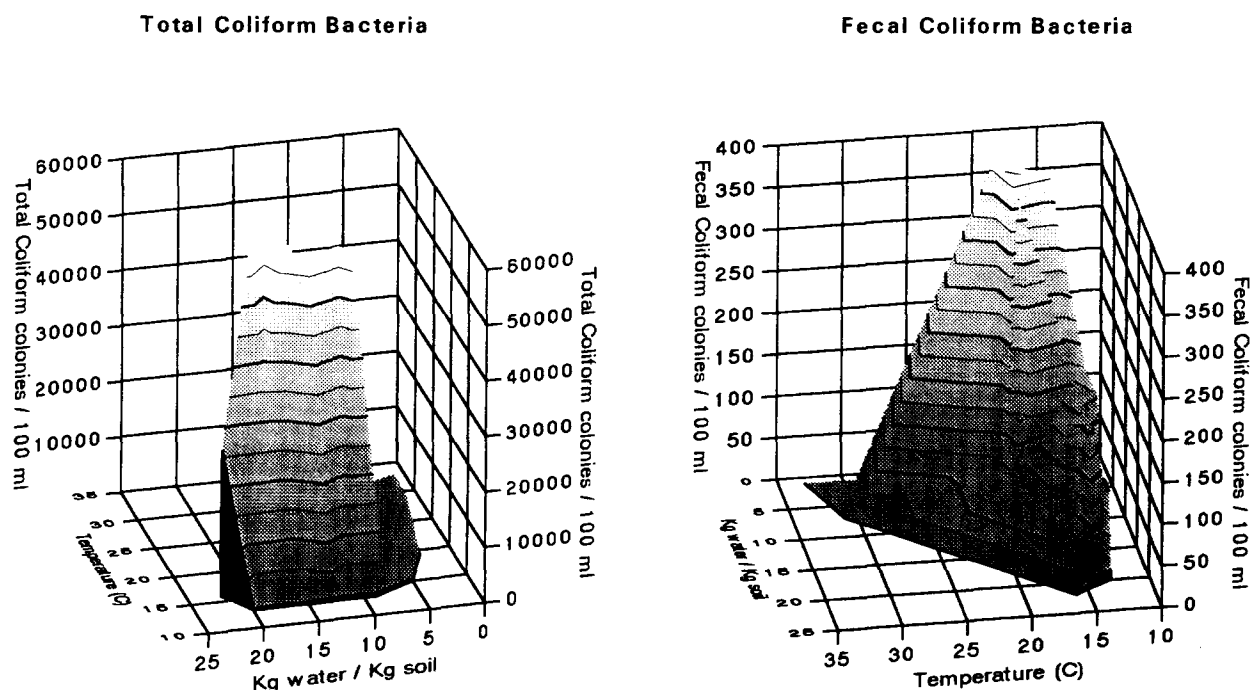


Fig. 3. Populations of total (left graph) and fecal (right graph) coliform bacteria in 1.5 m wells in riparian soils. Populations of total coliform bacteria in ground water in 1.5 m wells were explained by the following polynomial regression with ground water temperature (WT) and soil moisture taken at 6 to 30 cm deep (SM). Populations of total coliform bacteria =  $-13.1669 + 1.742 (WT) - 0.0497 (WT)^2 + 0.0000085 (WT)^3 - 0.568 (SM) + 0.0075 (SM)^2 + 0.000004 (SM)^3$ ,  $r^2 = 0.89$  ( $p < 0.0001$ ). Populations of fecal coliform bacteria =  $-6.668 + 1.2102 (WT) - 0.0428 (WT)^2 + 0.0000112 (WT)^3 - 0.07369 (SM) + 0.00740 (SM)^2 + 0.0000015 (SM)^3$ ,  $r^2 = 0.82$  ( $p < 0.0001$ ).

or season of year. Therefore, total and fecal coliform bacterial numbers will be discussed with regard to distance from inflow  $\times$  vegetative treatment  $\times$  season of year (Kirk, 1982; Snedecor and Cochran, 1980). Total and fecal coliform numbers in soil water (lysimeters) prior to sampling, which corresponded to 90 to 120 d since the wastewater application, were slightly higher in the 20 m grass–10 m forest filterstrip than the 10 m grass–20 m forest filterstrip in both winter and spring, regardless of distance downslope from the inflow pipe or lysimeter depth (Table 1). Total and fecal coliform numbers were usually higher in soil water sampled from the lysimeters at 5 and 10 m than soil water sampled at 30 m from the inflow point regardless of vegetative treatment, season of the year, or lysimeter depth. Total and fecal coliform numbers in soil water (lysimeters) declined by approximately 10-fold every 7 d for the first 14 d in winter and spring regardless of vegetative treatment.

Total and fecal coliform numbers in shallow ground water (wells) at 20 and 30 meters from the inflow pipe were slightly higher in the 20 m grass–10 m forest filterstrip than the 10 m grass–20 m maidencane filterstrip and 10 m grass–20 m forest filterstrip in winter, spring, and autumn regardless of well depth (Table 2). Numbers of total and fecal coliform bacteria were usually higher in ground water (wells) 5 and 10 m downslope of the inflow pipe than soil water sampled at 30 m from the inflow pipe. Numbers of total and fecal coliform bacteria in shallow ground water declined by approximately 10-fold every 7 d for the first 14 d regardless of vegetative treatment, season of the year, distance downslope from the inflow pipe, or well depth. Total and fecal coliform numbers in shallow ground water (1.5 and 2.0 m wells)

on the study plots immediately prior to wastewater application, which corresponded to 90 to 120 d since the wastewater application, were usually less than what was found in ground water in 1.5 and 2.0 m wells in grass and forest riparian vegetation that did not receive waste application or were not grazed (control treatments) regardless of vegetative treatment or season of the year.

Fourteen days after wastewater application, mortality of total coliform bacteria correlated with decreasing soil moisture and increasing ground water temperature in 1.5 m wells ( $r^2 = 0.89$ ) and in 2.0 m wells ( $r^2 = 0.89$ ) (Fig. 3 and 4). Fourteen days after wastewater application, mortality of fecal coliform bacteria also correlated with decreasing soil moisture and increasing ground water temperature in 1.5 m wells ( $r^2 = 0.82$ ) and 2.0 m wells ( $r^2 = 0.76$ ).

## DISCUSSION

This research revealed that: (i) total and fecal coliform numbers in the pulse of applied wastewater did not decline as water moved downslope regardless of vegetation type or season of the year; (ii) total and fecal coliform numbers in soil water and ground water at 20 and 30 m from the input source were slightly higher in 20 m grass–10 m forest filterstrips than the 10 m grass–20 m maidencane filterstrips and 10 m grass–20 m forest filterstrips in all seasons sampled; (iii) total and fecal coliform numbers in ground water declined by approximately 10-fold every 7 d for the first 14 d regardless of vegetative treatment or season of the year; (iv) total and fecal coliform numbers in soil water and in ground water positively correlated with soil water or ground water temperature and soil moisture; (v) total and fecal

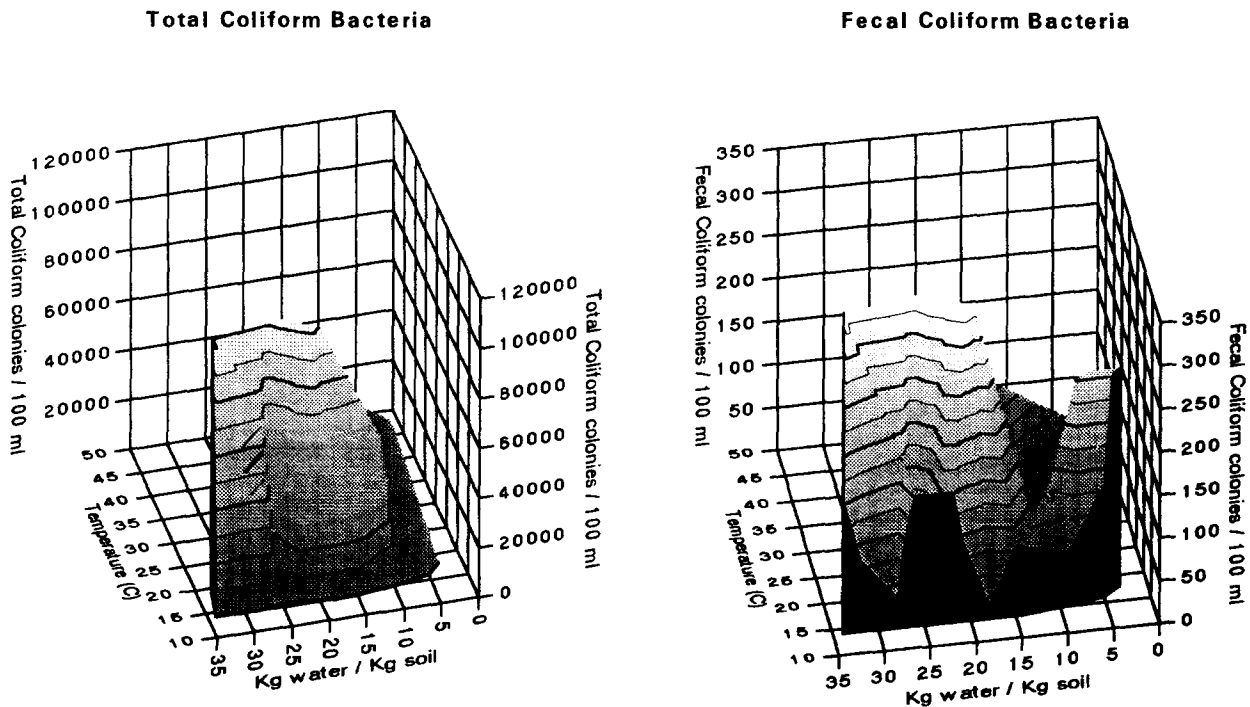


Fig. 4. Populations of total (left graph) and fecal (right graph) coliform bacteria in ground water in 2.0 m deep wells. Populations of total coliform bacteria in soil were explained by the following polynomial regression with ground water temperature (WT) and soil moisture (SM). Populations of total coliform bacteria =  $-13.1199 + 1.7034 (WT) - 0.048026 (WT)^2 - 0.0000081 (WT)^3 - 0.2091144 (SM) + 0.00914 (SM)^2 + 0.0000048 (SM)^3$ ,  $r^2 = 0.89$  ( $p < 0.0001$ ). Populations of fecal coliform bacteria =  $-0.12942 + 0.4867 (WT) - 0.02103 (WT)^2 + 0.0000058 (WT)^3 - 0.07672 (SM) + 0.0040 (SM)^2 + 0.0000099 (SM)^3$ ,  $r^2 = 0.76$  ( $p < 0.0001$ ).

coliform numbers in ground water immediately prior to application, which corresponded to 90 to 120 d since the preceding wastewater application, were usually less than in ground water in grass and forest riparian vegetation that did not receive wastewater application or was not grazed regardless of vegetative treatment or season of the year; and (vi) higher soil moisture and water temperature correlated in a curvilinear relationship with higher numbers of total coliform bacteria in soil water and shallow ground water.

Since filterstrips, regardless of vegetation type or season of year, did not reduce concentrations of total or fecal coliform bacteria in surface flow and higher soil moisture and water temperature correlated with higher numbers of both total and fecal coliform bacteria in soil water and shallow ground water, animal production operations should not apply wastewater to land when surface runoff or leaching (and thus, input of enteric bacteria) to a watercourse are likely to occur. Animal production operations may need a holding system (tanks and/or ponds) that can store both liquid and solid waste until they can apply waste to lands in a stable period of warm-dry weather. Animal production operations may need to locate in areas where there are long periods of warm-dry weather so they can apply waste to agricultural lands with reasonable assurance that the input of high concentrations enteric bacteria to surface and ground water will not occur. Since total and fecal coliform numbers in ground water declined by approximately 10-fold every 7 d for the first 14 d, and after 90 to 120 d fecal and total coliform bacteria were at or below background concentrations, the conclusion can be drawn that land application of wastewater is a viable

option of disposal that, if practiced with discretion, does not necessarily lead to contamination of surface or ground water. Because filterstrips, regardless of vegetation type, reduced total and fecal coliform populations by two to three log orders of magnitude compared with the source, animal confinement areas should have a 20 to 30 m vegetated filterstrip between the animals and watercourses. The filterstrips should be growing vegetative species best suited to the climate and soil type.

The grass and forest filterstrips that had no animal waste applied to them had total coliform bacterial populations ranging from 72 to 14 400 per 100 ml water and fecal coliform bacteria in soils ranged from 83 to 583 per 100 ml water. Forest and grass in riparian areas provide habitat for a large variety of warm blooded wildlife that will deposit enteric bacteria in soils and water. Several studies have found significant populations of enteric bacteria in soils and water in areas not influenced by human activity (Buckley et al., 1998; Niemi and Niemi, 1991; Gary et al., 1985; Doran and Linn, 1979). Therefore, we cannot expect soils or water in areas that are not influenced by anthropogenic activities to be completely free of enteric bacteria. Background concentrations of enteric bacteria in soils and water in areas that are not influenced by human or agricultural operations need to be established to determine if application of human or animal waste is polluting the environment.

Animal producers need to be aware of the potential for the spread of disease-causing microorganisms to themselves and others when handling and applying solid and or liquid waste. We isolated and identified 14 different species of bacteria that grew on total or fecal coli-



form media that can cause disease in humans. Most species of bacteria that were isolated and identified grow well in moist–warm habitats, but during this study many species were also isolated in the drier autumn and summer months. Diseases associated with enteric bacteria range from bacteria that cause mild to life threatening gastroenteritis, hepatitis, skin infections, wound infections, conjunctivitis, respiratory infections, and generalized infections (Moe, 1997). We also need to be aware of the potential for the spread of disease-causing microorganisms to agricultural lands and to the length of time these organisms can survive in soils even under adverse conditions for their survival. The opportunity for transfer of these organisms from soils after land application of animal waste to surface and ground water and ultimately humans will depend in part on their ability to survive in the soil environment.

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