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Effect of Sediment Particle Size and Temperature on Fecal Bacteria Mortality Rates and the Fecal Coliform/Fecal Streptococci Ratio

J. M. Howell, M. S. Coyne,* and P. L. Cornelius

ABSTRACT

Extended survival of fecal bacteria in sediment can obscure the source and extent of fecal contamination in agricultural settings. The variability in fecal coliform/fecal streptococci (FC/FS) ratios with time and discrepancies between observable fecal sources and measured FC/FS ratios in shallow surface water from agricultural watersheds may be explained by examining FC and FS mortality rates in response to ambient temperature and sediment particle size. We measured FC and FS mortality rates at three different temperatures and in three feces-amended sediments with different particle size in a laboratory study. In controlled conditions, using physiological saline to reduce cell death by osmotic shock, FC mortality rates exceeded FS mortality rates. These rates declined as sediment particle-size shrank and as temperature decreased. There was no interaction between these two factors in determining fecal bacteria persistence. The apparent half-lives of FCs exceeded those of FS, even though mortality rates were higher, because of FC regrowth shortly after deposition. The FC/FS ratio is influenced by temperature, the presence of sediment, and sediment particle size. In warm conditions, FC regrowth increases FC/FS ratios to levels indicative of human contamination even where none clearly exists. These factors interfere with the interpretation of the FC/FS ratio and contribute to the difficulty of its use in agricultural settings.

FECAL BACTERIA concentrations in underlying sediment increase when cattle (*Bos taurus*) have direct access to streams. These bacteria survive for prolonged periods in stream sediment. Monitoring programs in agricultural watersheds that use indicator bacteria, such as FCs and FS, to assess water quality, may be biased as a result. Since bottom disturbance resuspends fecal bacteria in sediment (Gary and Adams, 1985; Sherer et al., 1988), they may also appear in overlying water for extended periods (Biskie et al., 1988; Jawson et al., 1982; Stephenson and Rychert, 1982). Consequently,

streams flowing through pastures typically exceed EPA limits for primary contact water (200 FCs/100 mL) long after cattle are removed (Howell et al., 1995; Jawson et al., 1982; Stephenson and Street, 1978).

Small sediment particle size increases *Escherichia coli* (FC) survival (Tate, 1978). Extended survival of *E. coli* and *Salmonella* in sediments with high clay contents has been attributed to organic matter and nutrients held by the clay (Burton et al., 1987) and to physical protection in macropores. Moisture content, pH, sunlight, competition from indigenous soil microflora, and ambient temperature are other environmental factors that affect fecal bacteria survival in soil (Gerba et al., 1975). Higher temperature usually increases bacterial mortality but it can also promote FC regrowth in aquatic environments (Doran and Linn, 1979; Stephenson and Street, 1978).

Howell et al. (1995) noted that the FC/FS ratio increased in streams of central Kentucky as temperature increased during spring. Ideally, the FC/FS ratio could be used to assess whether nonpoint source pollution comes from fecal contamination by humans (FC/FS > 4), domestic animals (FC/FS between 0.1 and 0.6), or wild animals (FC/FS < 0.1) (Geldreich, 1976). However, the ratio has had questionable applicability in agricultural settings (Doran and Linn, 1979; Howell et al., 1995; Jawson et al., 1982) and is no longer recommended to distinguish between human and animal sources of contamination (APHA, 1992). This is due, in part, to the variability of FC/FS ratios.

We were interested in accounting for the variability in FC/FS ratios that we have observed on a seasonal basis in agricultural watersheds (Howell et al., 1995). While the individual effect of ambient temperature and particle size on fecal bacteria survival have been studied, the interactive effect of these parameters has not. Furthermore, FC growth shortly after manure deposition may explain some of the variability we have observed in

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water-monitoring studies (Howell et al., 1995). Our objectives were to observe whether interactive effects between sediment particle size and temperature influenced fecal bacteria growth and mortality in sediment. We also wanted to demonstrate that these effects, individual and interactive, confound use of the FC/FS ratio as an indicator of fecal contamination sources in agricultural settings.

MATERIALS AND METHODS

Treatment design for the experiment was a factorial arrangement of four substrates (physiological saline, sand, loam, and clay) and three incubation temperatures (4, 25, and 35°C). Particle-size distribution in each sediment type was determined by sieving and the hydrometer method (Table 1).

Two hundred and fifty grams of substrate was placed in 500-mL Nalgene bottles and a slurry containing 10 ± 0.05 g of fresh cattle manure and 200 mL of sterile physiological saline (8.5 g NaCl/L water) was added to each bottle. Sediment were nonsterile but contained no fecal bacteria prior to adding the manure slurry. The mixtures were shaken on a mechanical flatbed shaker for 30 min to ensure thorough mixing and allowed to settle for 2 h. Fecal coliforms and FS were subsequently enumerated to determine initial concentrations. Each particle size and water treatment was incubated at 4, 25, and 35°C for 40 d. All sediment treatments were saturated and maintained at a constant volume by adding sterile physiological saline. The bottle caps were loosened during the incubations to permit air exchange while minimizing evaporation. The experiment was replicated three times with each replicate done in a different 40-d period.

Data Collection

At 0, 3, 8, 15, 20, 30, and 40 d, 10-g subsamples from each bottle were collected with a spatula with minimal disturbance to the sediment. Ten milliliter subsamples of the physiological saline treatment were also collected. Fecal coliforms and FS in each subsample were enumerated by spiral plating after 10-fold dilution in sterile physiological saline. Fecal coliforms were incubated on mFC agar (Difco Corp., Detroit, MI) for 22 ± 2 h at 44.5°C. Fecal streptococci were incubated on KFS agar (Difco) for 44 ± 4 h at 35°C. All dark blue colonies growing on mFC agar were counted and assumed to be presumptive FCs. All red and light red colonies growing on KFS agar were counted and assumed to be presumptive FS.

The dry weight of sediment was determined 2 h after initial mixing and on Day 15 of incubation. The initial dry weight of each sediment was used to calculate the fecal bacteria per gram of dry sediment for the sample at Day 0. Dry weights determined 15 d later were used to calculate fecal bacteria populations per gram dry sediment for samples taken from Day 3 to 40. Since the samples stayed at a constant volume, the percent dry weight of each substrate remained approximately constant after the initial sample. Fecal bacteria in the physiological saline treatment are reported as organisms per milliliter of water.

Statistical Analysis

Since bacterial counts increased from Day 0 to 3, but decreased thereafter, the data from Day 3 to 40 were analyzed for differences in bacterial mortality rates. For this analysis, bacterial counts were transformed to the natural logarithm (ln) of count + 1. For each experimental unit, the linear regression of ln (count + 1) on number of days incubation was computed

Table 1. Particle-size distribution of coarse, medium, and fine sediment.

Sediment type	Clay	Silt	Sand	Textural class
	%			
Coarse	0	0	100	Sand
Medium	10	49	41	Loam
Fine	100	0	0	Clay

as a measure of mortality rate for FCs and FS. Regressions of the difference between FCs and FS with respect to ln (count + 1) were also computed. Treatment differences with respect to such regressions were evaluated by analysis of variance of the regression coefficients obtained from the individual experimental units.

Bacterial half-lives, that is, the number of days, X₅₀, required to reduce culturable cells by 50% from their maximum concentration (assumed to have occurred on Day 3), were estimated for each treatment using the model

$$\ln(\text{count} + 1) = \beta_0 + \beta_1 X^{\beta_2} + e \quad [1]$$

where β₀, β₁, and β₂ are model parameters to be estimated, X is the number of days after deposition, and e is a random error. Note that Eq. [1] postulates an exponential decay curve of the form y = ae^{-γz} where a = e^{β₀}, γ = -β₁, and z = X^{β₂}. The solution for X₅₀ is

$$X_{50} = [\beta_1^{-1} \ln 0.5 + 3\beta_2]^{1/\beta_2} \quad [2]$$

Least squares fitting of the nonlinear model, Eq. [1], was accomplished using SAS procedure NLIN (SAS Institute, Inc., 1990). The exponential decay curve would be equivalent to Eq. [1] with β₂ = 1. To show that Eq. [1] was a better fit, all that was needed was to test H₀/β₂ = 1. Rejection of H₀ implied that Eq. [1] was more appropriate than the exponential decay curve.

RESULTS AND DISCUSSION

Effects of Sediment Type and Temperature on Mortality

There was no evident interaction between sediment and temperature on fecal coliform (P = 0.659) and fecal streptococci (P = 0.243) mortality rates. Consequently, for some comparisons, the results were averaged across either all sediment types or all temperature regimes.

Table 2. Fecal coliform and fecal streptococci mortality rates as influenced by substrate type and temperature.

Treatment	Fecal coliform mortality rate† (FC)	Fecal streptococci mortality rate (FS)	H ₀ /FC - FS = 0‡ P > T
Substrate			
Water	0.128a	0.087a	0.0004
Sand	0.104a	0.078ab	0.0153
Loam	0.103a	0.068ab	0.0020
Clay	0.043b	0.046b	0.7576
Temperature			
4°C	0.047a	0.018a	0.0024
25°C	0.097b	0.079b	0.0453
35°C	0.139c	0.113c	0.0056

† Mortality rates are in units of ln cells d⁻¹. Mortality rates in the same column with the same letters are not significantly different. The effect of substrate on mortality rate is averaged across the three incubation temperatures and the effect of temperature on mortality rate is averaged across each substrate type.

‡ The null hypothesis, H₀, is that fecal coliform (FC) mortality rates are equal to fecal streptococci (FS) mortality rates.

Table 3. The influence of substrate type and temperature on the mortality rates and half-lives of fecal coliforms and fecal streptococci.

Substrate type	Temperature, °C	Fecal coliforms		Fecal streptococci	
		Mortality rate†	Half-life, d	Mortality rate	Half-life, d
Water	4	0.077abc	15.6	0.029a	35.9
	25	0.135cde	13.9	0.109a	6.4
	35	0.172e	4.3	0.124bc	4.4
Sand	4	0.043ab	13.1	0.013a	41.5
	25	0.124cde	7.3	0.119bc	5.4
	35	0.146de	4.2	0.103bc	4.6
Loam	4	0.043ab	18.1	0.010a	40.6
	25	0.108bcde	15.7	0.064ab	11.5
	35	0.156e	4.3	0.130c	6.2
Clay	4	0.025a	24.7	0.019a	39.1
	25	0.022a	36.9	0.025a	31.9
	35	0.083abcd	6.4	0.095bc	9.3

† Mortality rates are in units of \ln cells d^{-1} . Mortality rates in the same column followed by the same letters are not significantly different.

Overall, sediment type significantly affected fecal coliform mortality ($P = 0.001$) but not FS mortality ($P = 0.131$). Fecal coliform mortality rates were significantly less in clay-sized sediment than in coarser sediment or physiological saline (Table 2). Fecal streptococci mortality rates were also significantly less in clay-sized sediment than in physiological saline but not significantly less than in other sediment types (Table 2). Sherer et al. (1992) also observed that FCs survived longer in fine sediments than in coarse sediments and found no differences in FS survival between fine and coarse sediments. Grimes (1980) suggested that higher FC populations in clayey sediments are the result of surface area and particle-charge differences.

Fecal coliforms survive longer in sediment-laden than sediment-free water (Sherer et al., 1992). In our study, FC and FS mortality rates were higher in physiological saline than in other treatments, but this difference was not significant when compared to sediment types other than clay (Table 2). Bacterial survival in physiological saline may have been affected by particulates in the manure. In effect, the particulates provided a range of particle sizes on which the fecal bacteria could attach and grow.

Temperature significantly affected both FC and FS mortality ($P = 0.001$). As temperature increased, mortality rates significantly increased (Table 2). Other studies have shown that temperature affects fecal bacteria survival in water, soil, and sediment (Faust, 1982; Gerba et al., 1975; Von Donsel et al. 1967; Zibilske and Weaver, 1978). *Salmonella typhimurium* mortality in soil, for example, increases with increasing temperature (Zibilske and Weaver, 1978).

We calculated bacterial half-lives using nonlinear models because mortality was not predicted well by simple exponential decay [$\ln(N/N_0) = -kt$] (Table 3). As temperature increased or as sediments became coarser, fecal bacteria mortality became increasingly nonlinear (Fig. 1). Davies et al. (1995) also reported that simple exponential decay did not accurately reflect the mortality of fecal bacteria in freshwater and marine sediments. They attributed this to complications because of predation and growth. These are also potential explanations for our

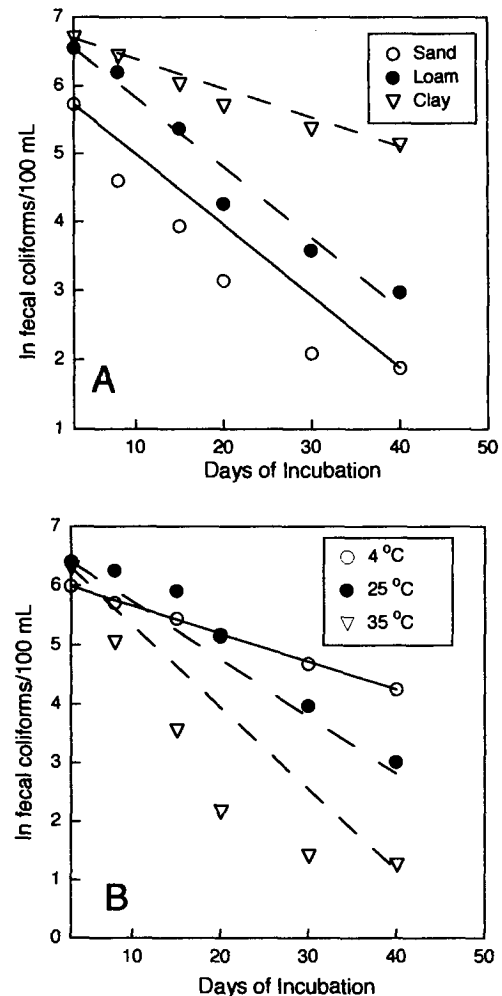


Fig. 1. Nonlinear mortality of fecal coliforms in sediment of various particle size (A) or at different temperature (B). Symbols represent the mean concentration of FCs averaged across temperature (A) or particle size (B), respectively. The plotted regression lines are the predicted exponential decay using a simple first-order model with rate constants derived from Table 2.

system since predators, such as protozoa, were not excluded from the manure used in the study and conditions were favorable for bacterial growth at the higher incubation temperatures.

Our data reflect culturable rather than total viable cells. This underestimates the true half-life of the fecal bacteria since the initial population is underestimated. We assume that it does not affect the mortality rates unless viable but nonculturable bacteria differ in this respect. Davies et al. (1995) reported a study in which the proportion of culturable to viable but nonculturable *E. coli* remained constant. So, the culturable cells in our study are probably a reasonable indicator of overall FC mortality rates. In the absence of information to the contrary, we assume the same is also true for FS, though this clearly may not be valid.

Fecal Coliform Mortality vs. Fecal Streptococci Mortality

Fecal streptococci mortality rates were significantly less than FC mortality rates in physiological saline, sand, and loam but the mortality rates of both fecal bacteria

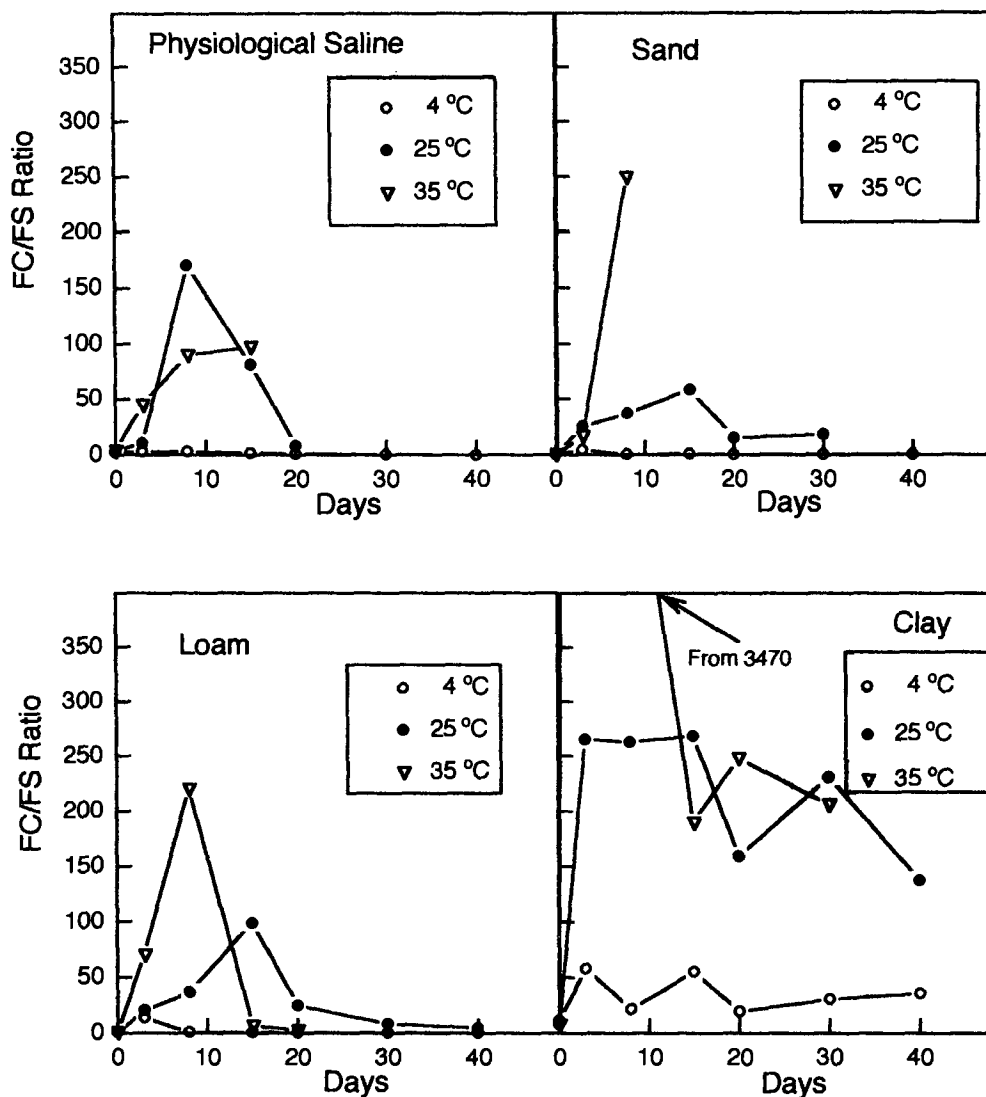


Fig. 2. Effect of sediment type and incubation conditions on the fecal coliform/fecal streptococci (FC/FS) ratio of cattle manure-amended sediment.

were similar in clay (Table 2). This is in contrast with observations by Sherer et al. (1992) that FS survive longer than FCs in fine sediment and FCs survive longer in coarse sediment. Fecal coliform survival was also significantly less than FS survival at each incubation temperature in our study (Table 2).

Fecal streptococci mortality rates were less than FC mortality rates for all treatment combinations except for clay-sized particles at 25 and 35°C (Table 3). Although FS mortality rates were lower for most treatment combinations, FC half-lives exceeded FS half-lives at 25°C and were approximately equal at 35°C in all substrates but clay (Table 3).

Fecal coliform concentrations increased until Day 3 of incubation (data not shown). After 3 d incubation at 25 and 35°C, FCs greatly exceeded FS. This caused greater FC half-lives even though their mortality rates were higher than that of FS. Because death is a rate process, and is independent of the actual number of organisms, the higher the initial number of organisms, the longer the apparent survival (Von Donsel et al., 1967).

Fecal coliform regrowth in soil, sediment, and runoff

water has frequently been reported (Crane et al., 1980; Doran and Linn, 1979; Von Donsel, 1967). Doran and Linn (1979) noted that FS concentration in runoff water containing fresh cattle feces changed little over a 40-h incubation at 4 and 21°C. However, while *S. bovis* initially made up 81% of the FS population, after a 40-h incubation at 4 and 21°C, *S. bovis* made up 19 and 1% of the population, respectively. They suggested that while the *S. bovis* population declined during incubation, non-starch hydrolyzing FS increased to maintain a constant FS population.

Fecal Coliform/Fecal Streptococci Ratio

On Day 0, all FC/FS ratios were indicative of the domestic animal source of feces except in the clay substrate where the ratio exceeded 7. The high ratio in the clay substrate may be caused by different adsorption characteristics of FCs and FS (Biskie et al., 1988). Bacterial cell surface hydrophobicity influences adhesion of bacteria to solid surfaces and streptococci have been found with greater hydrophobicity than *E. coli* (Huysman and Verstraete, 1993). The greater hydrophobicity of

FS may increase their adherence to clay. Consequently, on Day 0 and thereafter, fewer FS than FCs would be extracted from clay during enumeration and this could contribute to an elevated FC/FS ratio.

At 4°C, the FC/FS ratio remained between 0.1 and 4, except when the sediment was clay sized (Fig. 2). The low temperature may have impeded FC regrowth and slowed *S. bovis* mortality, thereby maintaining a ratio indicative of contamination by cattle. At 25 and 35°C, the FC/FS ratio almost always exceeded 4 even though FC mortality rates exceeded FS mortality rates. This was caused by the elevated FC concentrations after 3 d of incubation. Doran and Linn (1979) found the FC/FS ratio of runoff water containing fresh bovine feces increased from approximately 1 to 315 within 40 h at 21°C. This was attributed to a 330-fold increase of FCs while FS counts remained constant.

CONCLUSIONS

Our study indicates, as have other reports, that fecal bacteria have prolonged survival in sediment. Under controlled conditions, in which physiological saline was used to reduce cell death by osmotic shock, FC mortality rates were higher than FS mortality rates. These rates decreased as sediment particle size became finer and as temperature decreased, but there was no evidence for interaction between these two factors in determining fecal bacteria persistence. The apparent half-lives of fecal coliforms exceeded those of FS, even though mortality rates were higher, because of FC regrowth shortly after deposition.

The FC/FS ratio is influenced by temperature, the presence of sediment, and sediment particle size. During warmer weather, FC regrowth increases FC/FS ratios to levels indicative of human contamination even where none clearly exists. These factors interfere with the interpretation of the FC/FS ratio and contribute to the difficulty of its use in agricultural settings.

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