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ARTICLE SIZE AND TEMPERATURE AFFECT FECAL BACTERIA SURVIVAL IN SEDIMENT

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

INTRODUCTION

When cattle have direct access to streams, fecal bacteria concentrations in stream sediments increase. If these bacteria persist, and if the sediments are resuspended, fecal bacteria may also appear in surrounding water for extended periods. Why do fecal bacteria persist, since dry conditions, high acidity or alkalinity, sunlight, competition from native microbes, and extreme temperatures all diminish their populations in soil? The effects of these environmental factors are much reduced in sediment. Water protects fecal bacteria from desiccation and ultraviolet light. High temperatures can promote their regrowth in wet environments. Fecal bacteria also survive on fine-sized sediments in streams because the sediments have a high surface area. These factors may help explain our observations that streams flowing through pastures typically exceed Kentucky standards for primary contact water (200 fecal coliforms/100 ml) long after cattle depart.

The fecal coliform/fecal streptococci ratio (FC/FS), is a tool in water quality assessment that diagnoses the source of fecal contamination, whether from people (FC/FS > 4) or animals (FC/FS < 0.1). The ratio is extremely variable and sensitive to the persistence of the indicator

bacteria used in it. For example, we observed in central Kentucky streams that as the temperature increased during spring, the FC/FS ratio also increased. Fecal coliform growth shortly after manure deposition might explain some of the variability we have observed in our water monitoring studies. In this study we tried to account for the seasonal variability of FC/FS ratios in agricultural watersheds, and determine whether sediment particle size and water temperature interacted to influence fecal bacteria persistence and the FC/FS ratio.

MATERIALS AND METHODS

Our experiment had a factorial design of 3 incubation temperatures (39, 77, and 95°F) and 4 substrates: physiological saline (0.85% NaCl in water), and sediments of 3 textural classes (sand, loam, and clay) (Table 1).

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

Physiological saline or sediment was put in plastic bottles and a slurry containing fresh cattle (*Bos taurus*) manure in sterile physiological saline was added. The only source of fecal bacteria and organic matter was the manure slurry. We mixed the bottles on a shaker and let them settle for 2 hours. We then enumerated the fecal coliforms and fecal streptococci to determine their starting concentrations based on the number of colony forming units (CFU) that grew on selective agar media. Each treatment was incubated 40 days in saturated conditions and was replicated three times.

At 0, 3, 8, 15, 20, 30, and 40 days, we removed sediment samples from each bottle (a supernatant sample in the physiological saline treatment) with minimal disturbance to the remaining sediment, and measured the fecal coliform and fecal streptococci concentration in terms of CFU/g (CFU/ml for the physiological saline). Bacteria increased from day 0 to day 3, but decreased thereafter, so we analyzed the data from day 3 to day 40 for differences in bacterial death rates. We calculated half lives (the number of days required to reduce culturable cells to 50% of their maximum concentration) assuming that the fecal bacteria death rate was exponential once it began.

RESULTS AND DISCUSSION

There was no interaction between sediment and temperature on fecal coliform or fecal streptococci survival. Consequently, some of our results were averaged across either all sediment particle sizes or all temperatures.

Fecal coliforms survived longer in water with sediment than in sediment-free water (an additional reason to reduce soil erosion from manured cropland). Fecal coliform survival increased as particle size decreased from sand-sized to clay-sized (Figure 1). At 77°F, for example, fecal coliforms survived significantly longer in clay-sized sediment (37 day half-life) than in sandy sediment or physiological saline (7 to 14 day half life) (Table 2). By comparison, fecal coliforms in dairy manure applied to the

soil surface in spring or fall have a half life ranging between 6 and 8 days. Fecal streptococci also survived significantly longer in clay-sized sediment than in physiological saline. Other particle sizes had little effect. Fecal streptococci survival was significantly greater than fecal coliform survival in physiological saline, sand, and loam but the survival rates of both fecal bacteria were similar in clay (Table 2). As a rule, when the temperature increased from 35°F to 95°F, fecal coliform and fecal streptococci survival decreased (Figure 2). An exception to this rule was the survival of fecal coliforms in clay-sized sediment. Fecal coliforms had higher mortality rates than fecal streptococci at 39°F and 95°F but lower mortality rates at 77°F (Table 2).

Once fecal coliforms contaminated sediment, it took at least 30 days for the fecal coliforms to fall below 200 CFU/g if the sediment was sand-sized and > 40 days to do so if the sediments were silt- or clay-sized. Runoff from eroded land will typically have < 0.1g sediment/100 ml, so 200 CFU/g, if it was resuspended, would fall below Kentucky's primary water contact standard of 200 CFU/100 ml. If water temperatures were 95°F, fecal coliforms fell below 200 CFU/g in about 20 days. At lower temperatures, it took > 40 days to fall to the same level. Persistence in these terms entirely depends on the initial fecal bacteria loading rate. Since death rates are independent of the starting population (no matter how many bacteria you start with, they die off at the same rate), the more bacteria you have, the longer their apparent survival, even for the same magnitude of reduction.

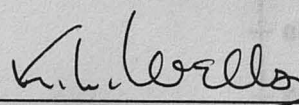
On day 0, all FC/FS ratios indicated a domestic animal source of feces. However, after 3 days at 77°F and 95 °F, fecal coliform concentrations increased more than 10-fold and greatly exceeded fecal streptococci (which also grew, but not nearly as much). At 39 °F, the FC/FS ratio remained between 0.1 and 4, except when the sediment was clay-sized. At 77 °F and 95 °F, the FC/FS ratio usually exceeded 4. This

was caused by the elevated fecal coliform concentrations after 3 days of incubation. So, the variability of FC/FS ratios in sediment was mainly a function of sediment particle size and temperatures allowing fecal bacteria regrowth, particularly fecal coliforms, and not necessarily a reflection of the original source of these bacteria (animal or human).

CONCLUSIONS

Our study showed that fecal bacteria persisted in sediment. Persistence increased as the sediment sizes become finer and as the temperature decreased. Persistence of these bacteria means that streams can appear contaminated with fecal material if sediments are resuspended. Thus, fecal contamination can be measured long after obvious sources of fecal contamination are gone.

Temperature, the presence of sediment, and sediment particle size influence the FC/FS ratio. During warmer weather, fecal coliform regrowth increases FC/FS ratios to levels indicating human contamination, even where none exists. This complicates the interpretation of the FC/FS ratio and explains why it is a poor independent measure of water quality in agricultural settings. Consequently, the FC/FS ratio is not currently recommended to identify the sources of fecal contamination.



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Table 2. The influence of substrate type and temperature on the half-lives of fecal coliforms and fecal streptococci in sediment.

Substrate	Temperature (F)	Half life (days)	
		Fecal coliforms	Fecal streptococci
Water	39	15.6	35.9
	77	13.9	6.4
	95	4.3	4.4
Sand	39	13.1	41.5
	77	7.3	5.4
	95	4.2	4.6
Loam	39	18.1	40.6
	77	15.7	11.5
	95	4.3	6.2
Clay	39	24.7	39.1
	77	36.9	31.9
	95	6.4	9.3

Figure 1. Relative survival of fecal coliforms in substrates of different particle size.

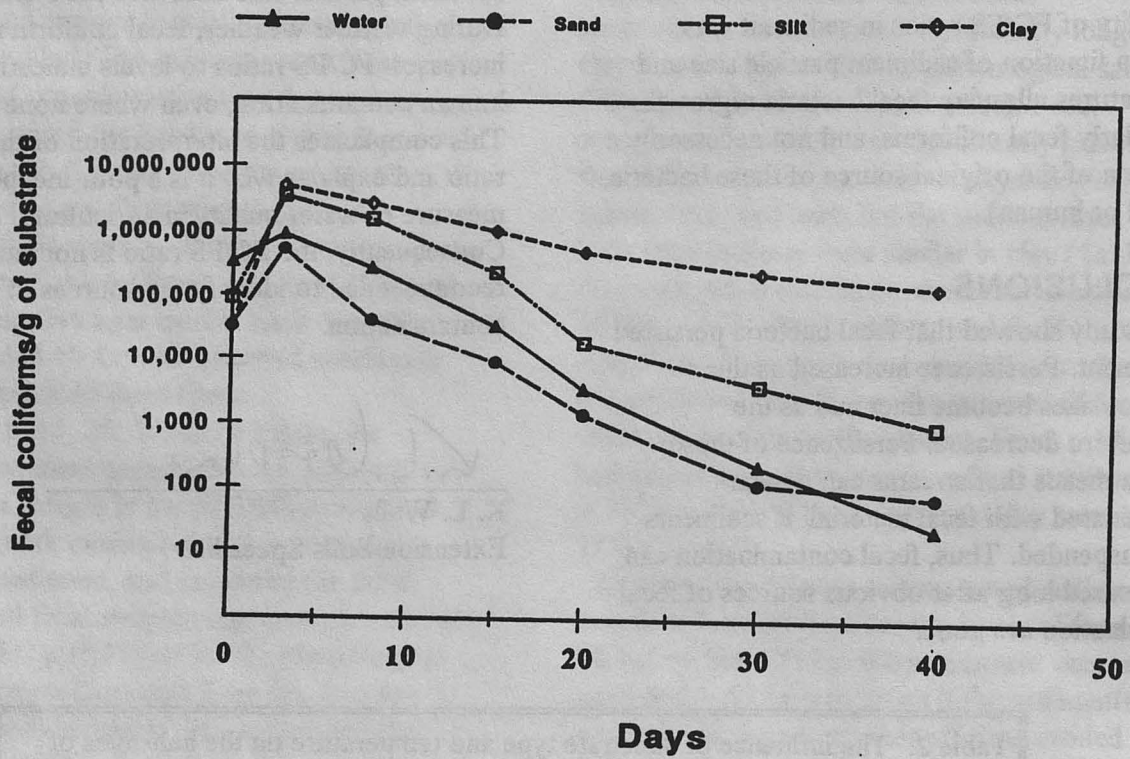
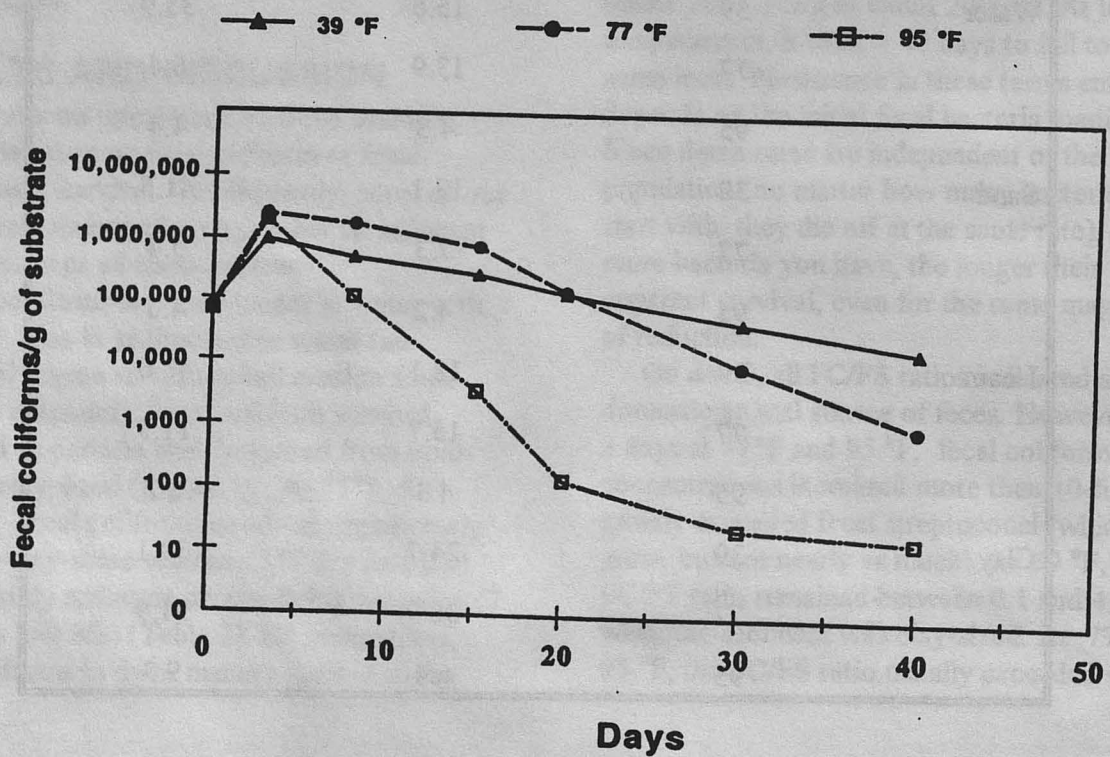


Figure 2. Relative survival of fecal coliforms in sediment at different temperatures.



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