Using Bacteria To Monitor the Influences of Cattle Wastes on Water Quality

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USING BACTERIA TO MONITOR THE INFLUENCES OF CATTLE WASTES ON WATER QUALITY[1/]

By S. H. Kunkle[2/]

ABSTRACT

Four bacterial indices of water pollution--total coliforms, fecal coliforms, fecal streptococci, and enterococci--were sampled in the surface runoff from a 0.1 ha sprinkle-irrigated field plot in Vermont. The field study was made on mowed-grass pasture during spring to late summer. The runoff was sampled during three irrigations before and ten irrigations after treating the field plot with a single application of recently excreted dairy cattle manure.

Results showed that the fecal coliform group, especially if used together with the total coliform group, was a good index for discriminating between contamination from new as compared with old manure.

KEYWORDS: Monitoring, overland flow, non-point source pollution, water quality index, FC/FS ratio, coliforms, bacterial indicators, animal wastes, surface runoff.

INTRODUCTION

Surface waters in rural areas must be reasonably free of contamination by pathogenic organisms to protect water supplies and to safeguard public health in recreation areas. One source of biological contamination of water is field-applied or stored animal wastes that may wash into streams during storms. From a health viewpoint, the flushing of recently excreted animal wastes into streams is of greatest concern because older wastes lose many of their pathogens through natural die-off processes. Although several bacterial groups are good indices of fecal contamination as determined in laboratory studies, these groups are not always so good for detecting contamination under

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field conditions. For example, the "total coliforms" include many nonenteric organisms that are mainly soil organisms. Thus, surface runoff from almost any farm field contains a high total coliform count, even from experimental fields devoid of animal wastes, as shown in studies by this author (Kunkle 1970) and others. Consequently, an index--bacterial or other--is needed that clearly establishes the presence of recently excreted animal wastes in water. Many laboratory studies have shown that the fecal coliform bacterial group can be used as such an index for human or animal contamination and can serve as a good indicator for the potential presence of pathogens spread by human or animal wastes. Most of these were laboratory based or municipal pollution studies.

The general objective of this study was to determine which of four common bacterial groups is the best index to monitor contamination by recently excreted animal wastes in surface runoff. There has been little field investigation of the fate of bacterial indicators in farm surface runoff despite the routine use of these same indicators for monitoring the water quality of streams and water supplies.

The specific objectives were (1) to evaluate the relative concentrations of four common bacterial indicators in runoff from a field which was free of animal wastes as compared with those found in runoff from the same field after cattle manure was applied, and (2) to observe the relative die-off (reduction in numbers) of these four bacterial groups in surface runoff for several weeks after manure application. The runoff was generated approximately once a week by sprinkle irrigation and, occasionally, by natural storms.

The study was part of a series of stream and plot studies on runoff and water quality made on the Sleepers River Research Watershed in Danville, Vt. This plot was used in earlier related studies (Kunkle 1970, 1971).

MATERIALS AND METHODS

A surface runoff plot of about 30 x 35 m ($\sim$0.1 hectare) was constructed in a hilly area consisting of a deep and permeable soil, the moderately well-drained Cabot silt loam. The plot had a 14 percent slope and faced southwest. Vegetative cover was a mixture of timothy, bluegrass, red clover, and red top and was maintained at a 10-20 cm height by hand cutting. The plot elevation was 300 m above mean sea level. During the period of this study (spring and summer 1970) daily maximum air temperatures were typically 25-30 ^{o}C (only rarely above 33 ^{o}C), and night air temperatures dropped to the 10-20 ^{o}C range.

"Rainbird" type sprinklers were set on supports in a grid fashion 1.2 m above ground, with the sprinkler heads about 6 m apart. The plot was sprinkle irrigated 13 times during June-September at a rate of about 25 mm/h. Each irrigation lasted for 1-1/2 to 3 hours. Figure 1 gives the total irrigation and runoff amounts for each date. All natural storms that produced runoff (June 27 and 30) were sampled. The amount of runoff on June 30 was very small (<100 liters) and is suspected to be unrepresentative. The analysis of the June 27 runoff (also minor) fits between those observed for the June 25 and July 1 irrigation induced runoff events.

Plastic runoff gutters, inserted just under the top 5-10 cm of soil on the downhill edge, served to catch the surface runoff. Runoff was measured with H-type flumes. Rain or irrigation depths were determined at 32 collectors scattered over the plot. For each irrigation event, up to 20 sample bottles of runoff were collected, plus several control samples of the irrigation water.

For each sample bottle at least duplicate analyses at various dilutions were made for total coliform bacteria (M-Endo broth), enterococci (M-Enterococcus agar), fecal streptococci (KF-Streptococcus agar), and fecal coliforms (M-FC broth) using membrane filter procedures. For each of the four bacterial groups there were at least 364 individual readings; 13 irrigations x ∿14 sample bottles (observations)/irrigation x 2 or more analyses (plates)/bottle. Four to six control samples of the irrigation water for each irrigation were analyzed in the same way. All samples were iced immediately upon collection and then filtered within a few hours. Sediment concentrations in the runoff water were very minute, so no prefiltration was needed.

The fecal coliforms were incubated in a water bath at $44.5 \pm 0.5\ ^{o}C$. Other bacteria groups were incubated at $35 \pm 0.5\ ^{o}C$, according to "Standard Methods" (American Public Health Association 1971). Colorimetric analyses of phosphate were made (Bausch and Lomb Spectronic-20)using a stannous chloride method.

Manure Application

The runoff plot was ideal for observing background levels before treatment because it had not been grazed, fertilized, or manured for about 10 years before the study. The bacterial contamination in runoff before manuring was monitored in 1968, 1969, and 1970 to establish background levels for the four bacterial groups. Three irrigations were made before applying manure. On June 22, 800 kg of manure was spread over the plot 1-2 hours before the fourth irrigation. There were nine more irrigations over a 3-month period (fig. 1).

About 1 m^3 (0.8 ton) of wet dairy manure was applied to the 0.1 ha plot; i.e., an 8 ton/ha application rate. The manure was 1-5 days old and contained 10 percent sawdust and 56 percent water. It was spread evenly over the plot but never closer than 4 m from the gutters on the downhill edge.

The manure was analyzed (one sample, five aliquots) for fecal coliform/fecal streptococci (FC/FS) and fecal coliform/total coliform (FC/TC) ratios. It was hauled, applied to the plot, sampled, and analyzed on the same day. Each sample was iced immediately and then prepared for analysis within a few hours. Based on a serial dilution, FC/FS averaged about 0.5. In three parallel dilutions per aliquot, FC/FS ratios ranged from 0.05 to 1.37, averaging 0.53. The same five aliquots and dilutions also showed the total coliform bacteria to be comprised of about 71 percent fecal coliforms with a FC/TC ratio ranging from 0.46 to 1.00, averaging 0.71.

Since the manure had about 300,000 fecal coliforms/g wet manure, according to analysis, the 1 m^3 application contained $240,000 \times 10^6$ fecal coliforms. According to Geldreich (1966), this would be about 44 cow days of fecal coliform bacteria applied. (Cow days are defined as the estimated *per capita* contribution of indicator microorganisms from a single cow, which averages $5,400 \times 10^6$ fecal coliforms/day). Assuming a cow produces 23 kg/day of manure, the 800 kg of manure that had been spread on the plot would equal about 35 cow days. In summary, according to the manure volume or bacterial numbers analyzed in the manure, between 35 and 44 cow days of manure were applied to the plot.

RESULTS AND DISCUSSION

The results of the study are presented in figures 1 and 2. Figure 1 shows concentrations for the bacterial groups by irrigation event during the study period. Each point represents an average concentration of approximately 14 observations (bottles) for that event. The individual observation was the best quality plate of at least two isolated plates. Figure 2 shows individual observations for fecal coliform and phosphate (PO_4-P) for some of the events. Phosphate analyses for the samples provided an index for comparing the bacterial concentrations to the nutrient levels in the runoff water before and after the manuring. The time of manuring in both figures is designated by the arrow. The phosphate levels were almost zero before manuring, rose sharply to about 2.5 mg/l in the first irrigation event after manuring, then fell to about 0.5 mg/l or below for all following events.

The irrigation water remained very clear throughout the study, with typically zero or near-zero fecal coliforms/sample. Concentrations for the other bacterial groups were so low as to be insignificant by any comparison to the runoff water. For example, on the first irrigation event after manuring (June 22), the concentrations in irrigation water averaged total coliform-40, fecal coliform-15, fecal streptococci-35, and enterococci-20/100 ml, compared to values in the 10^4 and 10^5 range in the runoff water as shown in figure 1.

Pretreatment Concentrations

The pretreatment (background) bacterial concentrations in runoff before June 22 were very similar for three of the four bacterial groups--total coliforms, fecal streptococci, and enterococci--ranging mainly from 10,000 to 100,000/100 ml. On the other hand, fecal coliform concentrations were mainly at the less-than-100/100 ml level (fig. 1). The pretreatment bacterial concentrations (June 11, 16, and 18) resembled those found in 200 runoff samples taken from the same plot during the previous summer.

Since soil and vegetation are the source of large numbers of nonfecal coliforms (Geldreich 1966, Kunkle 1970 and 1971, van Donsel et al. 1967), the large numbers of total coliforms observed in the runoff are not surprising. Analysis of a few surface soil samples on the study plot showed nonfecal coliform concentrations up to 30,000/g of soil.

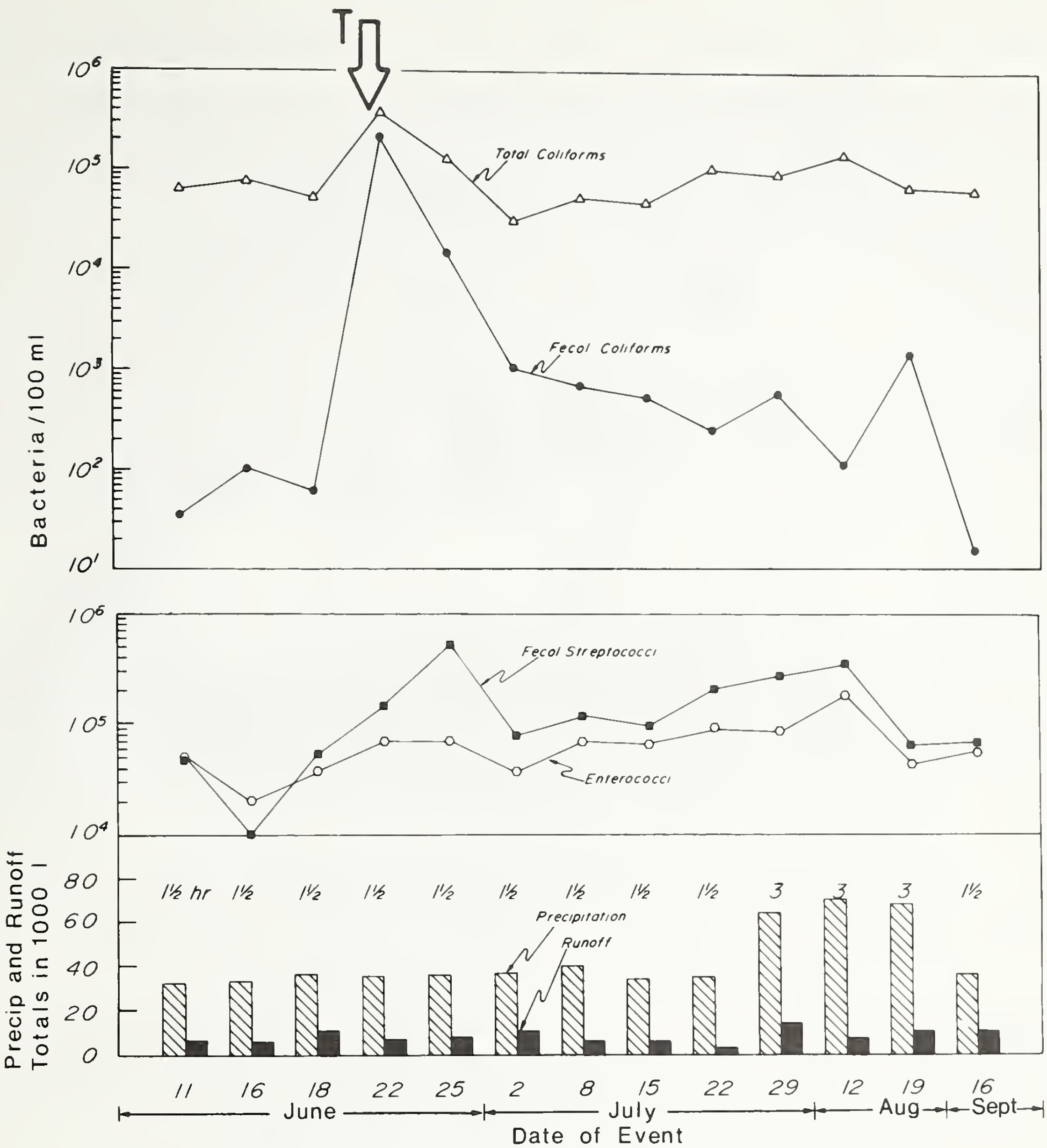

Figure 1.—Average concentrations for the four bacterial groups for the 13 irrigation events. Each point is the average of about 14 samples. The large arrow and "T" show the time of manure application.

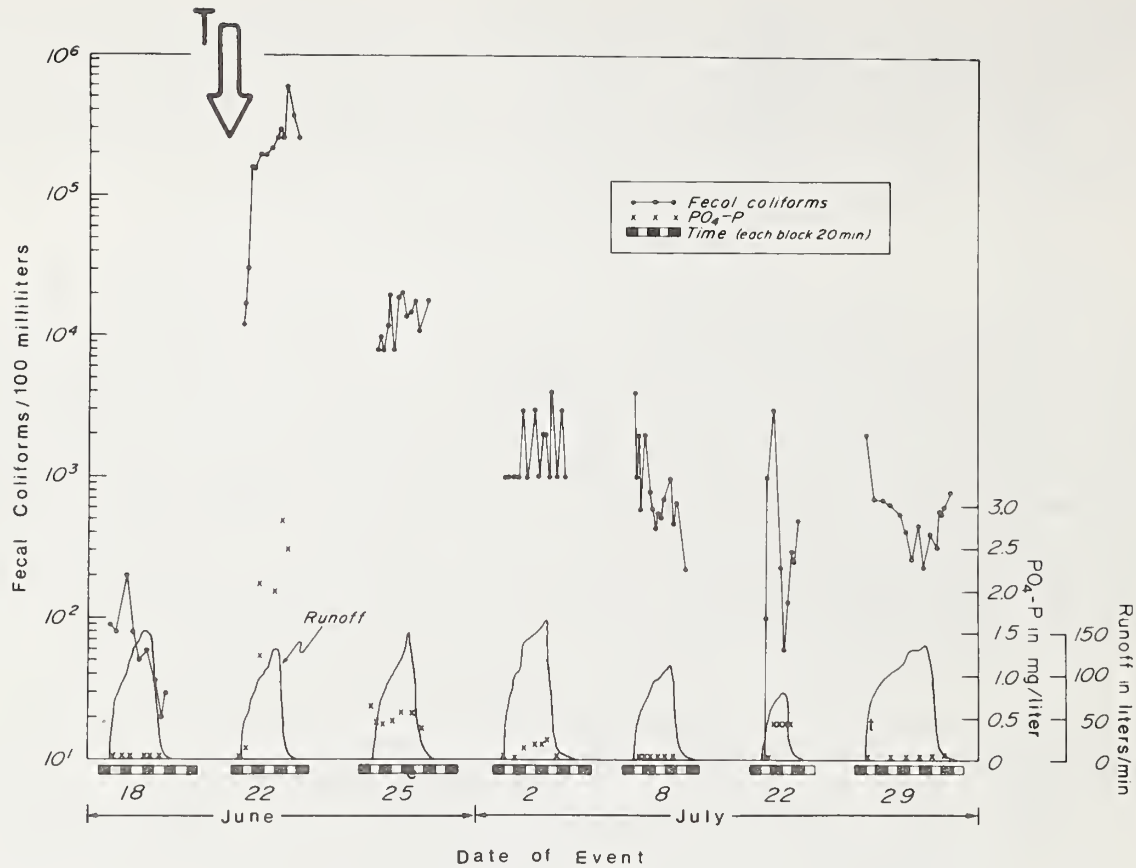

Figure 2.--Concentrations of fecal coliform bacteria and phosphate (PO_4-P) for selected events showing individual observations.

Background concentrations of fecal coliforms were quite low but always present in surface runoff from the plot. Small mammals, birds, and other wildlife transmit some of these bacteria to soil. Their subsequent survival depends on climate and other factors (Geldreich 1966, van Donsel et al. 1967).

Immediate Effects of Manuring

Concentrations of all four bacterial groups increased after the manure treatment on June 22, but only the fecal coliform group increased by several orders of magnitude (fig. 1). The fecal coliform counts approached those of the total coliforms, indicating that many of the coliforms after manure application were of fecal origin, as indicated by analysis of the manure. Concentrations of all four groups similarly increased immediately after treatment, but the relative increase in fecal coliforms was the greatest between pretreatment and posttreatment. The trends in the fecal coliform concentrations resembled those observed for phosphate levels in the runoff water (fig. 2).

Long Term Effects - Die-Off

Which bacterial group or combination of groups appeared to be the best indicator of recent animal excrement? This question was answered by analyzing the relative decline in bacterial concentrations in the runoff during the weeks after manuring. As the manure aged, the irrigation was repeated and, presumably, organisms died off during exposure to the elements and predators. Among the four bacterial groups, the fecal coliform counts declined much more rapidly than did those of the other three groups. After about 70 days, the fecal coliform concentrations returned to pretreatment levels. The total coliform concentrations also decreased and returned to pretreatment levels although the recession was less rapid. On the other hand, fecal streptococci and enterococci remained at essentially the same concentration for many weeks following the manure application. Therefore, these latter two groups couldn't be used to distinguish the recent animal excrement in the plot's runoff from that found after several weeks of ageing.

Other Comparisons

Geldreich (1966) compared fecal coliforms to fecal streptococci using an FC/FS ratio and found, in controlled laboratory studies, that generally a ratio under 1 indicates animal fecal influences, whereas a ratio of 4.0 or more indicates contamination by human waste. In this study, mean ratios were calculated for the individual runoff samples after manuring. Ratios in the applied waste averaged 0.53. As shown in table 1, the individual ratios were nearly all under 1.0, which substantiated Geldreich's findings.

Table 1.--Averages for the fecal coliform/fecal streptococcus (FC/FS) ratios for samples taken during 13 irrigation events

Date	Mean FC/FS 1/	Date	Mean FC/FS
	Pretreatment		
June 11	0.015 (.002-.051)	July 8	0.005 (.002-.010)
June 16	0.132 (.000-.300)	July 15	0.007 (.003-.014)
June 18	0.002 (.001-.004)	July 22	0.006 (.000-.021)
		July 29	0.003 (.000-.006)
	Posttreatment	Aug. 12	0.001 (.000-.002)
		Aug. 19	0.017 (.002-.036)
June 22	0.748 (.071-1.579)	Sept. 16	0.001 (.000-.004)
June 25	0.040 (.015-.106)		
July 2	0.029 (.011-.051)		

1/ Minimum and maximum values of ∿14 samples in parenthesis.

SUMMARY

The main study objective was to evaluate some common bacterial indices for discriminating between recently excreted vs. old animal wastes under field conditions. In addition, I wanted to further substantiate and define previously published studies which showed that fecal coliform concentrations in streams could be used to distinguish grazed from ungrazed catchments (Kunkle 1971).

The results showed that fecal coliform populations in runoff waters increased substantially more than did three other bacterial groups after newly excreted cattle manure was added to the plot. Fecal coliforms were an insignificant fraction of total coliforms in runoff before manuring, but their concentrations approached those of the total coliforms immediately after manuring. Concentrations then decreased quickly during the weeks after the manure had been applied. In about 70 days, their numbers returned to pretreatment levels. Conversely, the other bacterial groups did not show a similar decrease in concentrations. The fecal streptococci and enterococci remained at about the same concentrations in runoff for many weeks after manuring.

In conclusion, the fecal coliform group, especially when used in conjunction with the total coliforms, was a very useful index for discriminating between runoff contamination from new as compared with old or no manure from a field. The other bacterial groups tested did not discriminate as well.

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