Bacteriological Quality of Surface and Subsurface Runoff from Manured Sandy Clay Loam Soil[1]

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ABSTRACT

The bacterial quality of surface waters determines their acceptability for both drinking and recreational uses. Since livestock activities have been implicated as sources of fecal contamination, information on the bacterial quality of runoff from manured cropland is required. Bacteriological parameters (total coliform [TC], fecal coliform [FC], and fecal streptococcus [FS]) were monitored in spring surface and subsurface discharge from continuously corn-cropped sandy clay loam that was amended with either liquid dairy manure, chemical N-P-K fertilizer at about recommended rates, or no fertilizer. Liquid manure was applied for 6 years at three rates, which averaged 105, 263, and 420 m^3 ha^{-1} year^{-1}, plowed under after harvest, in spring prior to seeding, or split between spring and fall. Liquid manure was also applied directly to snow or frozen ground.

With the exception of winter-applied treatments, neither rate nor time of manure application significantly affected organism contents in spring surface or subsurface discharge. Winter manure applications resulted in significantly higher FC and FS counts in surface runoff and FS counts in subsurface discharge when compared with other application times. Fecal coliform and FS counts did not increase with increased winter application rates. Fecal streptococcus populations of winter-applied manure changed little during the first 100 days after application, while both TC and FC counts declined in the manure to low levels 24–40 days after spreading.

Additional Index Words: **fecal coliform, stream pollution, liquid dairy manure.**

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Potential human and animal health risks from waterborne pathogens can exist in fecally contaminated water. Fecal contamination has been observed in runoff from feedlots (Miner et al., 1966), dairy farms (Janzen et al., 1974), grazed pastures (Kunkle, 1970; Doran and Linn, 1979), fallow and sod amended with poultry litter (Giddens and Barnet, 1980), grassland treated with dairy manure (McCaskey et al., 1971), and sewage-sludge treated land (Dunigan and Dick, 1980).

Development of management guidelines to minimize degradation of runoff quality from manured land requires that both times and rates of application be evaluated. In eastern Ontario, snowmelt usually occurs either in late March or early April. Since >50% of the total annual runoff from two of the major agricultural catchments, the Rideau and South Nation, occurs in March and April (Water Survey of Canada, 1977), particular attention to runoff water quality during this period is warranted.

The objectives of this study were to (i) investigate the bacteriological quality of snowmelt surface and subsurface discharge from corn (*Zea mays* L.) cropland receiving different rates of liquid dairy manure at different application times, and (ii) to measure survival rates of indicator organisms in winter-spread manure.

MATERIALS AND METHODS

Experimental Design

A gently sloping (0.8%) field of imperfectly drained Manotick sandy clay loam (Aquic Eutrochrept) was divided into 14 plots, each 75.6 by 11.6 m. Plots have been cropped in silage corn since 1973. Each plot was instrumented so that both surface and subsurface (plastic tile) waters could be measured and sampled. After an initial year when no manure was applied, liquid dairy manure treatments, at three rates and four different application periods, were randomized and assigned to each plot for a period of 6 years. During this 6-year period liquid manure (average solids 8.8%) application rates averaged 105, 264, and 420 m^3 ha^{-1} year^{-1}, and were either plowed under after corn harvest, plowed under prior to seeding, split between fall and the following spring, or applied to snow-covered or frozen ground during the winter. Selected chemical and microbiological properties of the manure, as listed in Table 1, were determined. Chemical fertilizer was

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Table 1—Nutrient (dry weight basis), solids, and bacterial contents in liquid dairy manure applied to sandy clay loam over 6-year experimental period.

	Nutrients					Bacteria				
	C	N	P	K	Solids	TC	FC	FS	Standard 20°C	Plate counts 35°C
	%					$\log_{10}$ (counts g^{-1})				
Mean	49.0	2.9	0.7	2.4	8.8	5.68	5.31	5.32	7.43	7.12
SD	3.6	0.3	0.1	0.8	1.4	0.79	0.90	1.16	0.45	0.41

Table 2—Microbial counts of indicator organisms in snowmelt surface runoff from sandy clay loam plots prior to commencement of manure applications.

Plot	TC	FC	FS
	count 100 ml^{-1}		
5	13 ab†	<2 a	<2 a
6	8 a	<2 a	<2 a
9	25 b	<2 a	<2 a
11	10 a	<2 a	<2 a
13	7 a	<2 a	<2 a

† Means within each column followed by the same letter are not significantly different at $p < 0.05$.

broadcast on one of the remaining two plots at annual rates of 134 kg ha^{-1} of N, 49 kg ha^{-1} of P, and 93 kg ha^{-1} of K, and disked-in prior to seeding. The final plot received neither fertilizer nor manure. A more detailed description of field layout, instrumentation, and treatments has been published elsewhere (Phillips et al., 1981).

Bacterial Count Procedures

SAMPLE COLLECTION

Manure and runoff samples were collected in autoclaved 200-ml Boston round bottles with cork stopper and paper cover. Samples were taken to the lab for immediate analysis. When evening sampling occurred samples were stored overnight at 1°C prior to analysis.

SAMPLE TREATMENT

Runoff—All samples were subjected to standard Membrane Filtration (MF) procedures for estimating total coliform (TC), fecal coliform (FC), and fecal streptococcus (FS) densities (APHA, 1971). Membrane filtrations of at least three appropriate volumes for each sample were done. Filters obtained for TC determinations were incubated on m-Endo LES[3] agar at 35°C for 20 ± 2 hours in a moisture-saturated environment. Negative results were expressed as <2 counts per 100 ml of sample to facilitate statistical analysis. M-FC Agar with rosolic acid was used for EC density determinations. Duplicate filters were incubated for 20 ± 2 hours in sealed plastic bags immersed in a recirculating water bath maintained at 44.5°C. Negative results were expressed as <2 counts per 100 ml. M-Enterococcus Agar was used for FS density determinations. Duplicate filters were incubated 48 hours at 35°C in a water-saturated environment.

Manure—Duplicate random samples of both liquid- (collected during application) and winter-applied manure were subjected to analytical procedures similar to those used for runoff samples. Ten-gram aliquots of well-mixed specimens were weighed and added to a standard, sterile Waring Blender jar with 90 ml of sterile standard phosphate buffer (APHA, 1971). Appropriate replicate decimal dilutions (in the 10^{-1}-10^{-7} range) of the blended manure samples were prepared in standard phosphate buffer; 1.0-ml aliquots of test dilutions were suspended in 40 ml of standard phosphate buffer suspension blanks for filtration through sterile membranes that were subjected to the same media and incubation regimes as were used for the runoff samples.

Similarly, 1.0-ml aliquots of the same decimal dilutions were plated on sterile Plate Count Agar. Standard Plate Counts per gram were determined from the average of two plates for the most appropriate dilution (30-300 colonies per plate) after incubation at 35°C for 48 hours or at 20°C for 72 hours. Solids contents of both liquid and winter-applied manures were determined by drying to constant weight at 105°C (about 24 hours).

STATISTICAL ANALYSIS

In order to reduce variances all bacteriological data were transformed to log values (base 10) prior to statistical analysis. Due to the size of the plots it was not possible to replicate treatments on one soil series. In order to obtain a preliminary estimate of between-plot variability no treatments were applied to the plots in the first year, and fairly extensive runoff sampling of selected plots was undertaken. After initiation of treatments the individual events throughout the 6-year experimental periods were used as measures of replication. Simple effects due to manure rate and time of application were tested by using the rate × time interation as the error term. Duncan's Multiple Range Test (with six degrees of freedom) was used to compare treatment means. The means listed in the tables have been back-transformed.

RESULTS AND DISCUSSION

Runoff Water Quality

Both FC and FS densities were below detectable levels in snowmelt surface runoff in the preapplication period (Table 2). This was not the case for TC; runoff from one plot contained significantly higher TC concentrations than the others. Nevertheless, TC densities were all well within the allowable level (100 100 ml^{-1}) for raw water used for human consumption (Health and Welfare Canada, 1969). Kunkle (1970) and Doran and Linn (1979) observed highly variable TC densities in surface runoff from ungrazed fields, and the authors suggested that FC would be a better indicator of the effects of grazing on runoff water quality.

Bacterial counts in snowmelt surface runoff (Table 3) were significantly ($p < 0.05$) affected by time of, but not rate of, liquid dairy manure applications during the 6-year experimental period. Indicator populations in surface spring-melt waters from the winter-applied treatments were significantly higher than those from all other manure treatments. The lack of manure-rate effects on indicator densities in surface runoff may be related to similar non-significant manure-rate effects on counts and die-off rates for FC and FS groups reported by Crane et al. (1980) in poultry-manure-amended clay and sand. Total coliform and FC, but not FS, densities in surface runoff collected during spring runoff were within maximum permissible raw water levels for drinking water supplies (Health and Welfare Canada, 1969).

Surface runoff from intense May-June storms occurred only twice during the experimental period. Samples from one of these events contained TC, FC,

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[3] All test media were Bacto Brand supplied by Difco Laboratories, Detroit, Mich.

Table 3—Microbial counts of indicator organisms in snowmelt surface runoff from sandy clay loam plots receiving: (i) liquid dairy manure at differing times of the year, (ii) mineral fertilizer at seeding, and (iii) no amendments.

	Time of manure application†				Controls	
Organism	Winter	Spring	Fall-Spring	Fall	Ferti-lized	Unferti-lized
	MF counts 100 ml^{-1}					
TC	337 a‡	208 ab	99 b	84 b	225	183
FC	7 a	<2 b	<2 b	<2 b	<2	<2
FS	2,442 a	113 b	104 b	72 b	62	67

† Averages of three rates of manure application.

‡ Means within each row followed by the same letter are not significantly different at $p < 0.05$.

and FS counts that were about 2,000, 5,000, and 10,000 times, respectively, those from the spring-melt period (Table 4). This was due, at least in part, to much higher sediment concentrations in non-snowmelt surface runoff and to the higher dilution factor during spring runoff. Kunkle (1970) also noted significant fluxes of TC and FC during storm events. Bacterial densities tended to be higher in June runoff from spring-manured treatments. However, runoff from the chemically fertilized plot had higher-than-expected bacterial counts.

Table 4—Indicator counts in June storm surface runoff from plots receiving (i) liquid dairy manure at differing times of the year, (ii) chemical fertilizer, and (iii) no amendments.

	Time of manure application†				Controls	
Indicator	Winter	Spring	Fall-Spring	Fall	Ferti-lized	Unferti-lized
	MF counts/100 ml^{-1}					
TC	91,000	214,000	148,000	126,000	220,000	64,000
FC	14,000	19,000	12,000	8,100	60,000	6,500
FS	53,000	72,000	59,000	55,000	100,000	46,000

† Means of three manure application rates.

As with surface runoff, time of, but not rate of, liquid dairy manure application significantly affected FS densities ($p < 0.05$) in spring subsurface tile drainage waters (Table 5). Differences in both TC and FC counts could not be related to treatments. Snowmelt subsurface waters from winter-applied plots contained much higher average FS populations than did either surface or subsurface discharge from all other treatments. These results indicate that substantial numbers of manure-FS bacteria not only survived winter weather conditions, but were also capable of movement through the soil profile to the drains (located at an average depth of 75 cm) during spring melt. However subsurface drainage waters from the non-winter treatments generally contained lower indicator densities than did surface runoff. Winter-plot tile discharge, collected about 10 days after the winter (1975) application, had FC and FS densities about 10 times those in subsurface drainage from non-winter treatments (data not presented). Total coliform contents in subsurface effluent from winter-manured plots were about double those in discharge from otherwise-treated plots. On the other hand, late-fall subsurface discharge (only one event) from fall-manured treatments contained marginally higher FS densities (about two times) and considerably higher FC counts (about six times) than did discharge from treatments consisting of non-fall manure applications (data not presented).

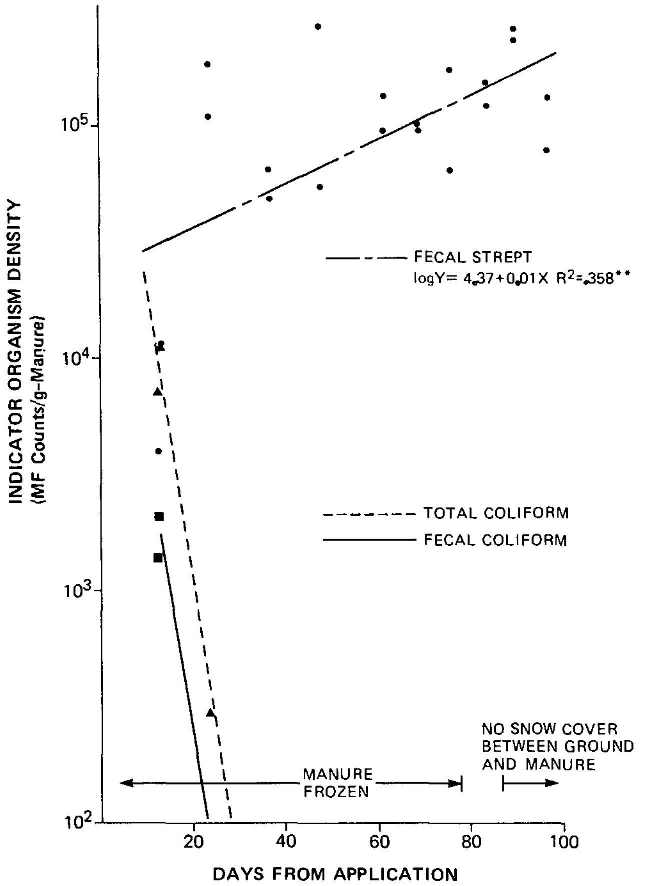

Fig. 1—Indicator organisms in dairy manure applied to snow-covered sandy clay loam.

Bacterial Densities in Winter-Applied Manure

Total and fecal coliform counts decreased to < 100 g^{-1} manure within 37 and 24 days of application, respectively (Fig. 1). Regression of FS ($\log_{10}$) against days from application yielded a significant ($p < 0.01$) positive relationship. However the positive relationship disappeared if the first data set (at 13 days) was dropped. Doran and Linn (1979) have reported poor recoveries of

Table 5—Microbial counts of indicator organisms in springtime subsurface drain discharge from sandy clay loam receiving (i) liquid dairy manure at differing times of the year, (ii) mineral fertilizer at seeding, and (iii) no amendments.

	Time of manure application†				Controls	
Indicator	Winter	Spring	Fall-Spring	Fall	Ferti-lized	Unferti-lized
	MF counts 100 ml^{-1}					
TC	46 a‡	42 a	37 a	35 a	17	36
FC	4 a	<2 a	3 a	3 a	<2	<2
FS	4,124 a	41 b	56 b	31 b	4	98

† Means of three application rates.

‡ Means within each row followed by the same letter are not significantly different at $p < 0.05$.

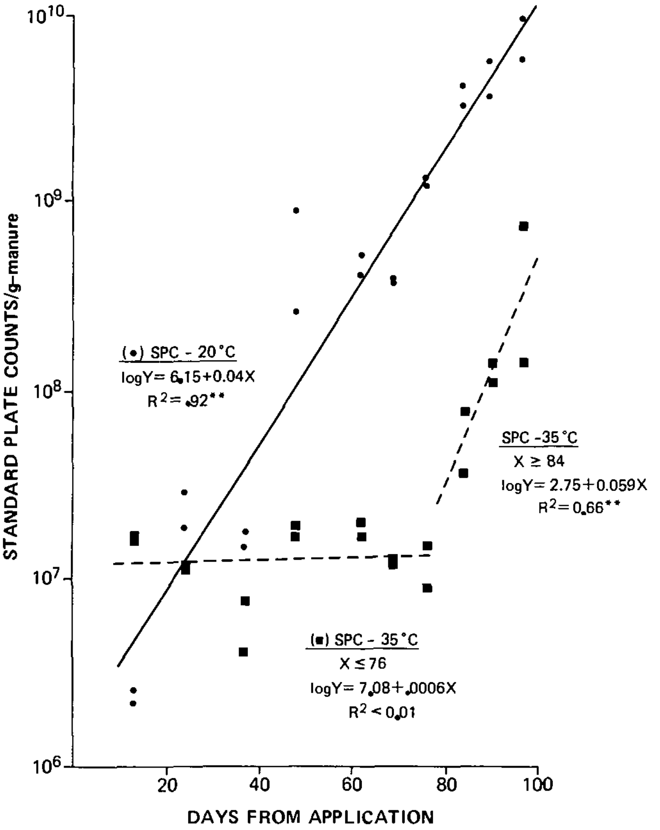

Fig. 2—Plate counts in dairy manure applied to snow-covered sandy clay loam.

FS bacteria from fresh cattle wastes when M-Enterococcus was used as the culture medium. Therefore even higher FS counts might have been obtained in this study if media with lower azide levels had been used. Nevertheless, the results of this study demonstrate the longevity of FS in frozen manure. These results contrast markedly with laboratory results of Kibbey et al. (1978), who observed that *S. faecalis* populations decreased by 98% when saturated soils were frozen for 8 weeks and then thawed. Several freeze-thaw cycles were found to be even more effective in reducing *S. faecalis* densities. It is doubtful that freeze-thaw cycles played a significant role in these results, as the frozen manure was snow-covered after several days and likely remained frozen until April.

Aerobic heterotrophs, as measured by standard plate counts incubated at 20°C for 72 hours, increased steadily between 13 and 97 days after application (Fig. 2). This was not so for plates incubated at 35°C for 48 hours. With the exception of the first sampling date, 35°C counts were lower than 20°C counts. No consistent change in 35°C counts was noted while the manure was frozen, however densities increased rapidly in the winter surface-applied manure once the manure thawed and came in contact with the soil surface.

CONCLUSION

The bacteriological quality of runoff from manured, corn-cropped, sandy clay loam indicated that both spring snowmelt surface and subsurface discharge from winter-applied treatments contained higher numbers of indicator organisms than did similar runoff from plots manured either the previous fall or spring. There was also some evidence that June storm surface runoff from spring-applied treatments contained higher bacterial densities than did plots that received manure the previous winter or fall. Survival of FS organisms in surface-applied frozen manure was noted, while both TC and FC densities declined to low levels within 40 days after application. Standard plate counts, incubated at 20°C for 72 hours, increased between the 13th and 97th day after application. Plate counts, incubated at 35°C for 48 hours, remained unchanged while the manure was frozen, then rose sharply once the manure thawed. Results of this study indicated that land should be manured and plowed in the fall, prior to freeze-up, in order to minimize microbial densities in runoff during the following spring.

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