

Survival and Movement of Fecal Indicator Bacteria in Soil under Conditions of Saturated Flow¹

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ABSTRACT

Antibiotic-resistant fecal bacteria were used to monitor the degree of movement and subsequent ground water contamination by septic-tank effluent discharged into a drainfield under saturated conditions. Two pits of different depths were constructed to simulate drainfield beds, and ground water samples were removed during 32-day sampling intervals from sampling wells installed at set distances from each inoculation pit. The bacteria added to the deep pit were released into a B2t horizon which contained a higher clay content than the A horizon in which the shallower pit was installed. Streptomycin-resistant strains of *Escherichia coli* and *Streptococcus faecalis* amended to each pit site moved in a directional manner, required more time to reach sampling wells when inoculated into the deeper of the two pits, and moved relatively long distances when considering that the area where the sites were located had only a 2% slope. Bacterial numbers peaked in the sampling wells in association with major rainfall patterns and the populations required longer periods to peak in the wells furthest from the inoculation pits. The results indicated that antibiotic-resistant bacteria eliminated the problem of differentiating between the amended bacteria and those nonresistant strains already in the soil, and the potential is excellent for including this type of microbiological procedure for assessing the suitability of a soil site for septic-tank and waste water drainfield installations.

Additional Index Words: environmental pollution, ground water contamination, septic-tank discharge.

Limited information is available on the potential for ground water contamination from recharged sewage plant or septic tank effluents. Site ratings for septic tank and drainfield installations traditionally have been determined through evaluating such factors as soil texture and structure, degree of slope, depth of soil to an impermeable layer, and depth to a water table and filtration rates (Weibel et al., 1954; Bouma et al., 1972). Research on the environmental effects of septic tanks was reviewed by Patterson et al. (1971). They found that, except for a few studies on the average composition of septic tank effluent (which included fecal bacteria), pollution of ground water by effluent has been monitored largely through the use of fluorescein dyes or halogen salts.

Fecal bacteria have received little emphasis as effluent tracers because of the difficulties in differentiating between those which represent recent contamination from septic drainage and those from sources such as livestock or rodents. The problem is further enhanced by the limited survival time of the fecal bacteria in soil. Kaufman and Orlob (1956) evaluated ground water tracers and stated that the ideal tracer should correctly depict

the movement of water through a porous medium without modifying the transmission characteristics of the system. By these criteria, the fecal bacteria would hardly qualify as ground water tracers. However, there is good evidence to indicate that the fecal bacteria do survive long enough in soil and water (Van Donsel et al., 1967) to allow their use as a tracer to assess the potential for ground water contamination. Our objectives were to examine the potential use of antibiotic-resistant fecal bacteria to monitor subsurface flow under saturated conditions and to assess health hazard potentials resulting from ground water contamination by septic tank effluents.

MATERIALS AND METHODS

SITE DESCRIPTIONS

The soil at the specific location used in this study was a somewhat poorly drained variant of the Veneta series (Land County, Oreg.) with a 2% east-facing slope near the center of a broad, nearly level interfluvium on terraces above the main floor of the Willamette Valley. The profile contains a slowly permeable layer at about 70 cm (Table 1). Two experimental sites were located in one field that had an elevated water table with occasional surface flooding during the winter rainy season.

At Site I a pit 30 cm deep and 75 cm wide was filled with 2.0 to 5.0 cm drainfield gravel, while Site II was 75 cm wide and 60 cm deep with the lower 30 cm filled with drainfield gravel and the upper 30 cm refilled with the removed topsoil. A 15-cm soil mound was placed over the top of each pit. As the pits were filled, one end of a 1.0-cm diam polyethylene tube was placed half-way down into the center of each gravel layer, while the other end was located above the soil mounds on top of the pits. This end was capped and the tube was used to place the fecal bacteria into the gravel beds. Site II was installed approximately 12 m northwest of Site I. The two inoculation pits were installed to simulate drainfield trenches at two different depths (30 cm at Site I and 60 cm at Site II) and to assess the movement, with respect to both distance and time, of fecal bacteria out of these simulated drainfields and through the soil under saturated flow conditions.

Sampling wells were installed in eight compass directions in concentric rings at 0, 50, and 100 cm from the edge of each pit. Initial experiments demonstrated that the direction of subsurface flow was to the east-northeast. Additional sampling wells were then installed at 300, 500, 1,500, and 3,000 cm from the edge of each pit in the east-northeast directions. Each sampling well was 50 cm deep with a diameter of 10.5 cm. A section of plastic PVC pipe (10-cm diam) was placed in the upper 20 cm of each well. The top of the PVC pipe extended 5 cm above the well and the pipe was sealed in each pit with bentonite packing clay to prevent surface flow contamination of the wells. Each well was covered by a 20 by 20 cm plastic pot. A glass tube (1.0-cm diam) 70 cm long was inserted through the pot into each well until the end of the glass tube was about 5.0 cm from the bottom of the well. A sampling bottle and hand-operated vacuum pump connected in series were attached to the 30-cm plastic tube extending from each well. Water samples of 50 ml from each well were collected by applying negative pressure with the vacuum pump. Precipitation (rain gauges) and the ground water depth was recorded on each sampling date. All precipitation recorded was from rainfall and temperatures were cool (2 to 15°C) with only occasional frosts.

INDICATOR BACTERIA

Streptococcus faecalis and *Escherichia coli* were isolated from raw sewage (Corvallis, Oreg., Wastewater Treatment Plant) and were identified according to *Standard Methods* (1971). *S. faecalis* belonged

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Table 1—Soil profile description at the experimental site used in this investigation

Poorly drained variant of Veneta Soil Series; classified as a fine, mixed, mesic, Ultic Haploxeralf			Characteristics						
Horizon	Depth, cm	Profile description	pH	% Organic matter	CEC, meq/100 gms	% Sand	% Silt	% Clay	Saturated conductivity, cm/hour
A	0-36	Very dark grayish brown (10 YR 3/2) silt loam; few fine faint mottles; moderate fine granular structure; friable, slightly sticky, slightly plastic; many fine and very fine roots; few medium, many fine and very fine tubular pores; clear smooth boundary.	6.0	5.2	35.1	20.4	52.4	27.2	0.92
B2t	36-50	Dark grayish brown (10 YR 4/2) silty clay loam; common fine faint mottles; weak fine subangular blocky structure parting to weak fine granular structure; friable, slightly sticky, slightly plastic; many fine and very fine roots; few medium many fine and very fine pores; clear smooth boundary.	5.9	4.8	26.3	7.9	55.5	36.6	0.33
B22t	50-71	Yellowish brown (10 YR 5/6) moist clay loam; common distinct mottles; weak medium subangular blocky structure; friable, slightly sticky, plastic; several fine and very fine roots; many fine and very fine pores; clear smooth boundary.	5.5	1.3	18.5	16.3	44.2	39.5	0.16
IIB3	71-95	Variegated colors of light brownish gray (10 YR 6/2) and yellowish brown (10 YR 5/4) moist clay loam; many large prominent mottles with yellow exterior (2.5 YR 8/8) grading into red masses (2.5 YR 5/8); strong coarse subangular blocky structure; firm, sticky and plastic; few medium, fine and very fine roots; few medium, fine and very fine pores; abrupt smooth boundary.	5.4	0.6	11.2	12.1	43.1	44.8	0.04

to the enterococcus group according to the scheme by Geldrich and Kenner (1969). Antibiotic resistant mutants were selected from cultures of *E. coli* on Difco Eosin Methylene Blue agar (EMB) and *S. faecalis* on Difco m-Enterococcus Agar (ENT). Each medium was amended with 40 µg/ml streptomycin. The most rapidly growing sub-cultures of these sewage isolates in the presence of the antibiotic was used as the assay organism. The streptomycin-resistant cultural isolates were transferred several times until their level of resistance was determined on several general purpose media containing streptomycin.

Inoculum of each organism was grown in Difco Brain Heart Infusion broth (BHI) at 37°C on a rotary shaker at 150 rpm for 24 hours. Concentration (no./ml) of the inoculum was determined by dilution plate procedures and ranged from 3 to 5 × 18⁸ for each inoculation period. Site I was inoculated with *E. coli* on 8 Dec. 1975 and with *S. faecalis* on 19 Jan. 1976, while Site II was inoculated with *S. faecalis* on 8 Dec. 1975 and with *E. coli* on 19 Jan. 1976. Four liters of cultural inoculum were amended to each site and the two dates were chosen because they preceded the onset of major storm events. The *E. coli* inoculation of Site II on 19 Jan. 1976 was done with a hand-pump, as the gravel portion of the pit was beneath the water table on that date.

SAMPLE ANALYSIS

Following inoculation, samples were removed from each well on 14 occasions during a 32-day sampling period. Sample bottles were placed in coolers on ice, transported to the laboratory, and stored at

4°C until used. The fresh water samples of 10.0, 1.0, and 0.1 ml were used to inoculate three presumptive enrichment tubes containing streptomycin (40 µg/ml). Azide dextrose broth (Difco) was used for *S. faecalis* and tryptone mannitol ricinoleate broth (TMRB) was used for *E. coli* (Pugsley et al., 1973). Before the sites were inoculated, sample wells were assayed for the presence of streptomycin-resistant bacteria.

Inoculated presumptive enrichment tubes were incubated at 37°C for 48 hours. Positive water samples were serially-diluted in 0.5% peptone broth, vigorously shaken, and aliquots of the dilutions were spread over the surface of pre-poured and dried agar plates. EMB agar was used for *E. coli* while ENT agar was used for *S. faecalis*. Streptomycin at 40 µg/ml was added to both media. All plates were incubated at 37°C for 48 hours before counting colonies.

RESULTS

DIRECTION OF BACTERIAL MOVEMENT UNDER CONDITIONS OF SATURATED FLOW

The *E. coli* numbers in the E and NE directions were larger, moved greater distances, and persisted longer than in the other directions (Table 2). On days 1 and 2, *E. coli* was found in all eight wells 50 cm from the edge of the inoculation pit. No indicator organisms were ever recovered from the 100-cm wells in the SE, S, SW, or W directions. Also, *E. coli* was not detected at the 50-cm

Table 2—Plate counts of *Escherichia coli* in sampling wells 50 and 100 cm from the edge of the injection pit at Site I

Direction†	Days following inoculation									
	1		2		4		6		8	
	50 cm	100 cm	50 cm	100 cm	50 cm	100 cm	50 cm	100 cm	50 cm	100 cm
N	4.4 × 10 ⁴	2.6 × 10 ³	8.3 × 10 ³	7.4 × 10 ²	1.4 × 10 ³	2.5 × 10 ²	7.5 × 10 ²	—‡	—	—
NE	5.0 × 10 ⁶	8.2 × 10 ⁶	2.7 × 10 ⁶	1.0 × 10 ³	8.2 × 10 ⁴	4.5 × 10 ⁴	6.5 × 10 ⁴	3.6 × 10 ³	9.4 × 10 ³	1.2 × 10 ³
E	7.5 × 10 ⁴	3.2 × 10 ³	6.6 × 10 ⁴	1.1 × 10 ³	5.0 × 10 ⁵	7.0 × 10 ⁴	7.8 × 10 ⁵	6.1 × 10 ⁶	4.7 × 10 ⁶	3.9 × 10 ⁴
SE	2.9 × 10 ³	—	1.2 × 10 ³	—	7.4 × 10 ²	—	2.6 × 10 ²	—	—	—
S	4.6 × 10 ³	—	3.0 × 10 ²	—	—	—	—	—	—	—
SW	3.6 × 10 ³	—	2.3 × 10 ²	—	—	—	—	—	—	—
W	5.2 × 10 ³	—	6.4 × 10 ²	—	1.4 × 10 ²	—	—	—	—	—
NW	2.3 × 10 ⁴	1.2 × 10 ²	5.2 × 10 ³	—	3.6 × 10 ³	—	1.4 × 10 ³	—	3.9 × 10 ²	—

† All data are from the first five sampling days on the 8 Dec. 1975 loading.

‡ (-) signifies that no indicator bacteria were detected.

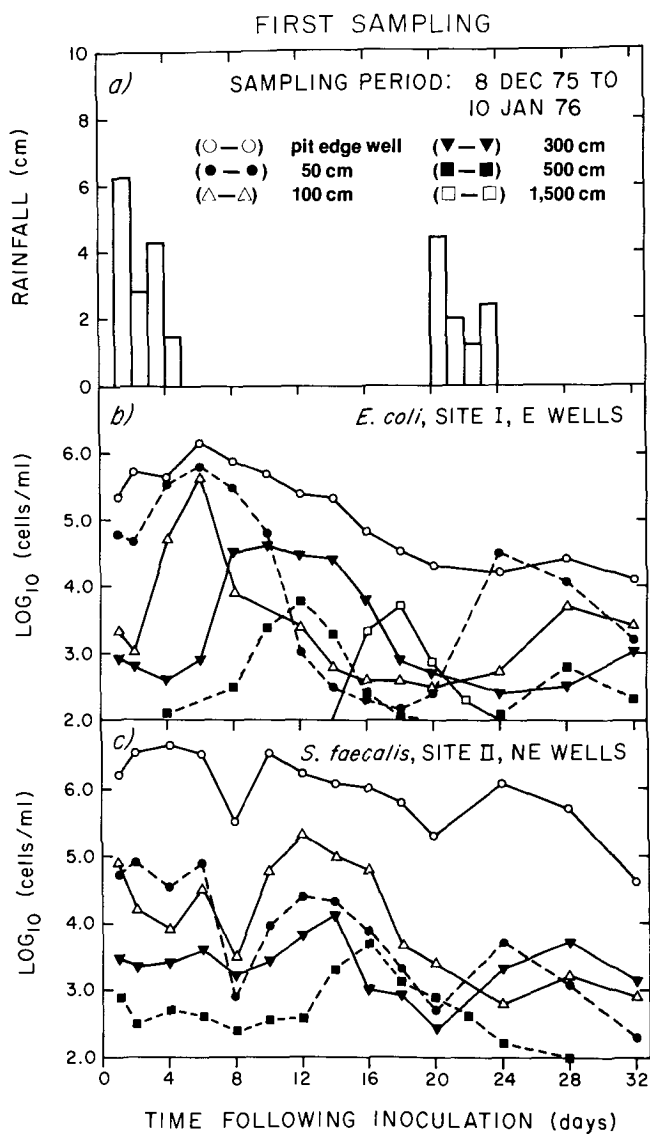


Fig. 1—Major rainfall intervals during the first sampling period (a); *E. coli* population data at Site I, E wells (b); and *S. faecalis* populations at Site II, NE wells (c).

distance wells in these same four directions on day 8. There were some moderate numbers of *E. coli* detected in the N and NW directions, although by day 8 the N direction wells were negative and there was only a residual population remaining at the 50-cm well in the NW direction. Results clearly indicate that the direction of subsurface flow at Site I was towards the E and NE. Similar results (data not shown) were obtained for Site II except that the major direction of movement was only in the NE direction.

INFLUENCE OF RAINFALL AND GROUND WATER FLOW ON BACTERIAL MOVEMENT

On comparing the rainfall data (Fig. 1a) to the bacterial populations during the first sampling period (Fig. 1b,c), it can be seen that there were two major precipitation periods from days 1 to 5 and 20 to 24 and that these periods closely correspond to the peaks recorded for the

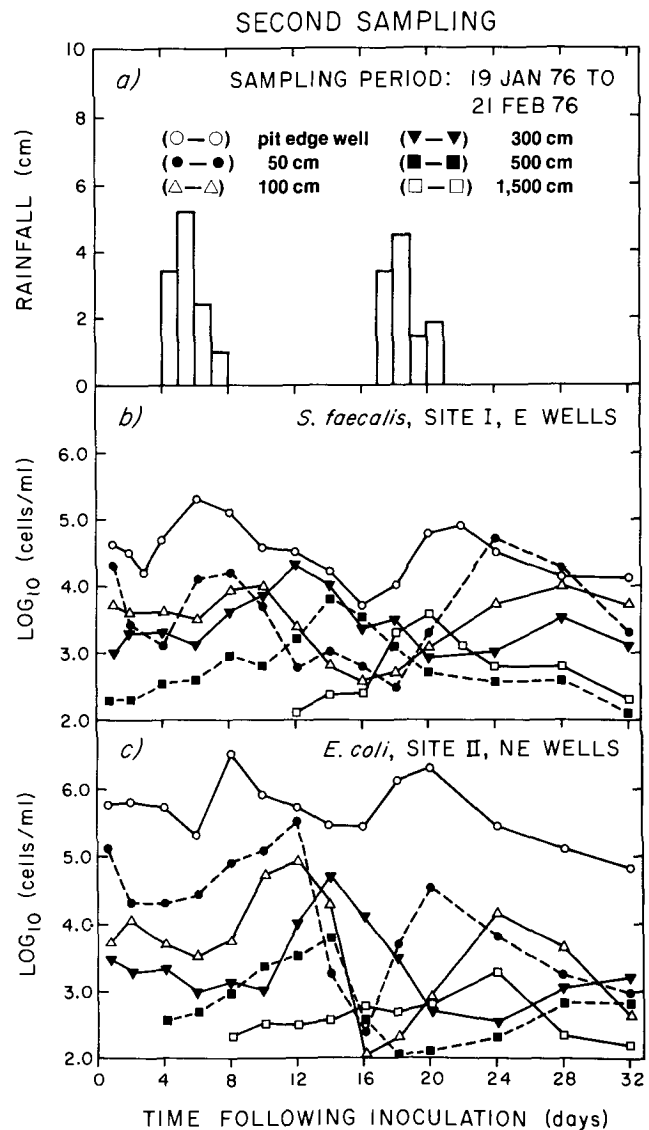


Fig. 2—Major rainfall intervals during the second sampling period (a); *S. faecalis* population data at Site I, E wells (b); and *E. coli* populations at Site II, NE wells (c).

bacteria in the various sampling wells. The peaks at Site I occurred within 2 or 3 days after heavy rain during both precipitation periods and, while this was true for Site II during the second rain period, there was a lag of around 8 days after the first heavy rain before the enterococcus numbers increased. At inoculation time, the water table was at 45 cm and after 4 days was within 15 cm of the soil surface. The water table level fluctuated but remained within this range of 15 to 45 cm throughout the first sampling period.

Similar results were obtained for the second sampling period (Fig. 2). The initial heavy rain period (day 4-8) was followed by the first peaks as the bacterial populations were carried through the soil under the saturated flow conditions. The second peaks in bacterial numbers accompanied the second major rain interval (days 17 to 19) although second peaks were not detected in the 500- and 1,500-cm wells during the sampling period. The water table was at 27 cm and rose to within 5 cm of the

Table 3—Days at which the first peaks in the bacterial indicator populations appeared in the various wells for both sites during the two sampling periods†

Sampling well	Sampling period 1			Sampling period 2		
	Site I		Site II	Site I		Site II
	N	NE	NE	E	NE	NE
pit edge	6‡	1	10	6	4	8
50 cm	6	1	12	8	4	12
100	6	1	12	10	6	12
300	10	4	14	12	14	18
500	12	6	16	14	16	20
1,500	18	12	—‡	20	24	24

† Site I was inoculated at a depth of 15 cm while Site II was inoculated at 45 cm.

‡ Refers to the day in which a given peak was recorded.

§ (-) indicates no fecal bacteria were detected in this well.

soil surface 8 days after the first major rainfall period. The water table level fluctuated throughout the remainder of the second sampling period but was never greater than 40 cm.

INFLUENCE OF SOIL PROFILES ON BACTERIAL MOVEMENT

During both sampling intervals (Table 3), the indicator bacterium which was inoculated into Site I moved through the soil faster than the bacterium inoculated into Site II. This was indicated by the detention times for the bacterial peaks in the various wells. As the bacteria inoculated into Site I were released into the A horizon (a silt loam) and those added to Site II were released into the B2t horizon (a silty clay loam), the results indicate that the rate of subsurface flow, and hence the movement of the amended bacteria, was faster in the A than in the B2t horizon. The results also indicate that, during the first sampling period, the bacteria moved through the soil in the NE direction more rapidly than in the E direction although this trend was only true for three of the six wells (pit edge, 50, and 100 cm) for the second sampling period. It is difficult to compare these trends between the first and second sampling periods because rainfall amounts, which affect water table fluctuations and, subsequently, the rate of subsurface water flow, were different. Also, the rates of bacterial movement and die-off could not be assessed from the data in Fig. 1 and 2 and Table 3.

DISCUSSION

Three concepts of major importance can be derived from data on the presence of the indicator bacteria in the test wells (Fig. 1 and 2). First, the bacteria moved long distances in a relatively short period of time in a soil with a surface gradient of only 2%. Second, the populations of indicator bacteria in the various wells reached maxima during intervals closely associated with the rise of the water table following major rainfall periods. Third, both *E. coli* and *S. faecalis* survived in appreciable numbers in the saturated soil throughout a 32-day sampling schedule.

Two prominent developments were associated with the movement of the indicator bacteria through the soil

in the 24 hours following inoculation. At both sites the inoculated bacteria always reached the 300-cm well and in two cases (Fig. 1c and 2b) they traveled as far as the 500-cm well. Also, the strong directional subsurface movement to the E at Site I and to the NE at Site II suggests that subsurface flow under saturated conditions is more rapid and more directed than would have been expected from a location with a 2% surface gradient and located several hundred feet from a discernable drainageway. This rapidity of movement is difficult to explain since macropore measurements and the hydraulic gradient of the subsurface flow were not determined. Examinations of soil cores revealed no unusually large channels or pores within the soil and, hence, the possibility of subsurface channels being responsible for the movement of the bacteria seemed remote.

It appears that the bacteria diffused out in all directions (up to 50 cm) when first added to the inoculation pits as the water table rose and the inoculum became submerged (Table 2). At the first sampling, the microbial numbers were several orders of magnitude higher in the direction of subsurface flow. These data also indicate that rainfall washed large numbers of bacteria from the inoculation pits and that they moved as a front (pulse) through the soil in the direction of water flow (Fig. 2) and took a longer period of time to reach the more distant sampling wells. In addition, the more distant the sampling well from the inoculation pit, the lower were the peak numbers of bacteria found. This trend was consistent for all the major rainfall periods, for both bacterial populations, and at both sampling sites. Likewise, the numbers of indicator bacteria at the second peak were not as great as the first peak. This can probably be attributed to a combination of dilution in the ground water, by removal through soil filtration, death, and by the fact that the bulk of the cells had been washed past the sampling wells following the first peak.

The bacterial population peak reaching the 1,500-cm wells appeared to be associated with the first rainfall period, while the other, closer, sampling wells were already reaching their second peak. Sampling intervals would probably have to have been extended past 32 days in order to detect a second peak in the 1,500-cm wells. No indicator bacteria were found in the 3,000-cm wells during either of the sampling periods as it may have taken longer than 32 days for the bacteria to arrive at the 3,000-cm well.

Our results indicate that both indicator bacteria survived in appreciable numbers throughout 32 days and, with the wet and cool soil conditions, it is highly probable that their survival would extend considerably beyond 32 days.

The incorporation of antibiotic-resistant strains of fecal bacteria is a useful approach to use in a system containing any type of fecal contamination. Only in this manner could the amended strains be differentiated from other fecal bacteria and flow/movement rates be determined. Even in uncontaminated soils, there are a variety of other bacteria of nonfecal origin (for example, *Klebsiella*, *Enterobacter*) and several common fungi which can grow on some of the routinely used selective media. These interferences can be eliminated by the incorporation of the appropriate antibiotic in the

media to allow only the survival of the antibiotic-resistant indicator bacteria.

Research has shown antibiotic-resistant bacterial strains move through the soil under saturated soil conditions and provide an excellent microbiological method for assessing the suitability of soils for the installation of septic tank-drainfield systems. We are currently conducting research on a variety of different soils using several antibiotic-resistant fecal bacteria. The methods developed should provide management criteria for evaluating environmental contamination of ground water, streams, and domestic wells in the event of drainfield failures.

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