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Fecal Coliform Transport through Intact Soil Blocks Amended with Poultry Manure

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ABSTRACT

Poultry production in Kentucky increased almost 200% between 1991 and 1995. Their waste is typically land applied, and fecal pathogen runoff and infiltration may cause nonpoint source groundwater pollution. We looked at the preferential flow of fecal coliforms through undisturbed soil blocks since fecal bacteria typically infiltrate the soil profile to contaminate groundwater. Poultry manure was uniformly distributed on top of sod-covered or tilled (upper 12.5 cm) soil blocks and the blocks were irrigated. Drainage was collected in 100 uniformly spaced cells beneath each block and analyzed for fecal coliform content and drainage volume. The spatial distribution of drainage and fecal coliforms through the soil blocks was not uniform. Fecal coliforms appeared where most drainage flowed. Drainage water from each soil block consistently exceeded 200 000 fecal coliforms per 100 mL and was as great as 30 million fecal coliforms per 100 mL of leachate collected. Fecal coliforms leached as a pulse, but the breakthrough of fecal coliforms through tilled blocks was delayed with respect to the breakthrough of fecal coliforms through sod-covered blocks. Rainfall on a well-structured soil will cause the preferential movement of fecal bacteria, even with unsaturated flow conditions, and could contribute to fecal coliform concentrations in shallow groundwater that exceed standards for domestic discharge and primary contact water in Kentucky (200 fecal coliforms/100 mL).

BROILER PRODUCTION in Kentucky grew from 22 million birds in 1991 to 65 million birds in 1995 and is still expanding (Kentucky Agricultural Statistics, 1996). Since most poultry waste is land applied as the means of its disposal, one consequence of this increased production is that runoff and infiltration of bacterial pathogens from poultry waste-amended fields could degrade water quality. Groundwater contamination would be a significant problem for rural residents in areas where new production is sited (principally western Kentucky) since private water systems drawing from groundwater and springs are an important water source (Carey et al., 1993).

Escherichia coli (*E. coli*, that is, fecal coliforms) are indicators of enteric bacterial pathogens in fecal wastes. The greater the fecal coliform population, the greater the assumed potential that pathogens are present in water. Some *E. coli*, such as strain O157:H7, are serious pathogens in their own right. Bacterial pathogens must typically move through the soil profile to contaminate groundwater and springs although, in karst areas, runoff can contaminate groundwater directly. The potential for contamination depends on the depth of soil to the water table or bedrock. In central Kentucky, a karst region with shallow soils to bedrock, there is greater concern with land-applied animal wastes than elsewhere in the state, where deeper soils and water tables occur.

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In the absence of preferential water movement, fecal coliform motility is an unlikely transport mechanism through intact soils (Gammack et al., 1992). Preferential solute and water movement through intact soil occurs by two mechanisms (Poletika and Jury, 1994). Solution added to the soil surface may redistribute itself before leaching (based on small differences in elevation). Redistribution causes some regions of a soil column to receive more water than others, consequently, they have a higher average solution flow moving through the soil profile. Solutes and water may also move through macropores that exist in soils because of old root channels, insect and animal burrows, and natural structure (for example, interfaces between adjacent peds) (Gammack et al., 1992; Ritchie et al., 1972).

Macropores have tortuous paths that eventually coalesce nonuniformly with soil depth (Dexter, 1993; Wildenschild et al., 1994). Consequently, when solute moves through intact soil blocks, only a few regions discharge flow at any time (Quisenberry et al., 1994; Wildenschild et al., 1994). For example, Quisenberry et al. (1994) found that more than 50% of total drainage was collected in <20% of the area beneath a soil block.

When fecal coliforms move through intact soil columns, cell characteristics become inconsequential because of water flow within macropores. Smith et al. (1985) demonstrated that macropore flow through intact soil columns allowed *E. coli* to rapidly move through the soil profile, bypassing the soil matrix. McMurry and Coyne (1996) showed that water and bacteria traveled rapidly through intact soil blocks, and along the same paths, indicating preferential movement in well-structured soil.

Tillage disrupts structure and pores in the tilled layer (Quisenberry and Phillips, 1978). Smith et al. (1985) noted that structureless or repacked soils retarded *E. coli* movement compared to intact, well-structured soil. However, few studies have examined how tillage influences bacterial transport through intact soil blocks. Investigating these effects would improve our understanding of bacterial movement through soil, and its control by soil management. It would also facilitate evaluating soil types and soil structures that potentially favor rapid bacterial transport, thus, the potential for groundwater contamination by surface-applied animal manures in different soils. The objectives of this study were to examine preferential flow of fecal coliforms through intact soil blocks and its perturbation by soil disturbance.

MATERIALS AND METHODS

Field Site and Soil Block Extraction

We extracted Maury silt loam soil blocks (fine, mixed, mesic, Typic Paleudalfs) from the University of Kentucky Ag-

Abbreviations: CFU, colony forming units.

ricultural Experiment Station in Lexington during July 1995. The blocks were excavated from a site that was somewhat eroded, hence, the A horizon was shallower (15 cm) than is usual for a typical Maury soil (0–36 cm) and the Bt horizon was included in the soil blocks (USDA SCS, 1968). The A horizon (granular) and the Bt horizon (subangular blocky) both had soil structure consistent with a well-structured soil.

Our excavation methods were similar to those described by Bowman et al. (1994), Quisenberry et al. (1994), and Shipitalo et al. (1990). We carved three sod-covered blocks, 32.5 cm square by 32.5 cm deep, from undisturbed soil that had been in bluegrass sod (*Poa pratensis* L.) for more than 20 yr. We encased the soil blocks in plywood on four sides and poured liquid polyurethane foam into the gap between the soil block and wood casing. We let the polyurethane foam cure overnight, separated the soil blocks from the rest of the soil about 10 cm from the bottom of the casing, and transported them to a greenhouse for storage.

We similarly obtained three soil blocks of tilled Maury silt loam. We extracted these soil blocks from a field that had been chisel plowed to a depth of 12.5 cm and disked. Four-sided metal casings (32.5 cm by 32.5 cm by 17.0 cm height) were hammered into the soil to hold the tilled layer in place before carving the blocks. We excavated the blocks to a depth of 42.5 cm and removed them from the field as described above. All blocks were covered in plastic and periodically irrigated to ensure that the soil would not dry, crack, or pull away from the soil-foam interface.

Rainfall Application and Drainage Collection

For each individual soil block, we trimmed the bottom flush with the wood casing, placed it on a collection chamber, and caulked it with silicon to make an airtight and waterproof seal. The top of the chamber was a metal grid consisting of 144 cells in a 12 by 12 array that collected water leaching from the block. The collection cells were 3.05 cm square and tapered to a 3 mm diam. drain hole at the bottom. Glass wool, followed by a layer of sand, was placed in each of the cells. The metal ridges between each cell cut slightly into the bottom of the soil block and we assumed that this prevented lateral flow between cells at the bottom of the soil block. The outermost row of cells collected the outflow from the soil-foam interface. This ensured that no edge effects of flow were measured in the innermost 100 collection cells. Our measurements came from this 10 by 10 grid. Plastic trays held 100 plastic centrifuge tubes (50 mL volume) in place beneath the drain holes of the collection cells to collect soil block drainage and the drainage from the outermost row of cells. The procedures for installing a soil block on this collection chamber are described in greater detail by Quisenberry et al. (1994).

The rainfall applicator was also described by Quisenberry et al. (1994). The applicator was a square reservoir, 32 by 32 by 5 cm, constructed of acrylic plastic 0.32-cm thick. It was positioned 20 cm above the soil block. One hundred hypodermic needles, 0.25 mm in diameter (25 gauge), were connected to the bottom of the applicator at positions corresponding exactly with the centers of the innermost 100 collection cells of the collection chamber.

Experimental Procedures

The day before use, approximately one pore volume of 0.003 *M* $CaSO_4$ solution was applied to the block to thoroughly wet the soil matrix and remove extractable Cl^-. For sod-covered blocks, the height of the sod on the soil blocks was trimmed to 2 cm prior to irrigation. The block was drained overnight to approximate field capacity. Undercage poultry manure from layer production houses was obtained 4 d before each soil block study and stored at 4°C. The properties of the individual manure samples are listed in Table 1. Since the poultry manure was obtained at different times, the total fecal coliforms added to each soil block differed, although approximately the same weight of manure (106 g per block, wet) was applied. The poultry manure was evenly distributed on the surface of the soil block. The added manure was approximately equivalent to a 10 Mg ha^{-1} field application rate.

A peristaltic pump delivered solution with 0.001 *M* $CaCl_2$ to the rainfall applicator, which distributed it over the surface of the soil block at a rate of 1 cm h^{-1} (1056 mL h^{-1}). The $CaCl_2$ solution reduced clay dispersal and allowed us to monitor solute transport through the soil blocks via the Cl^- breakthrough curves (manuscript in preparation).

Soil block drainage was collected in 100 50-mL plastic centrifuge tubes beneath the drain holes of the collection chamber. Plastic trays held the 100 centrifuge tubes in place and also collected the drainage from the outermost row of cells. A vacuum of −2.0 kPa within the collection chamber was applied to the lower boundary of each soil block. The constant vacuum was maintained by regulating a water manometer attached to a vacuum source (Phillips et al., 1995). Based on the capillary equation (Danielson and Sutherland, 1986) this vacuum drained all pores >0.15 mm in diameter. The vacuum was constant for all of the sod-covered soil blocks and the first tilled block. Vacuum in the second and third tilled blocks could only be held at a maximum vacuum of −0.5 kPa, which would drain all pores >0.6 mm in diameter.

Each study lasted 36 h. Five trays of tubes collecting effluent from individual drain holes were alternated with four trays in which all drainage was composited. Two subsamples were taken from the composited sample to measure total fecal coliforms in drainage. Each tray stayed in the collection chamber 4 h. The trays with tubes were regularly checked for cells with high flow and these tubes were replaced if full. The tubes were weighed to determine drainage volume.

Fecal coliform distribution at the bottom of the soil blocks was determined by adding the total fecal coliforms for the five trays that contained centrifuge tubes. Each cell position was summed for fecal coliforms over the experiment period of 36 h, divided by the total fecal coliforms, and reported as the percent of total fecal coliforms for individual cells.

After each experiment, the soil blocks were drained overnight, removed from the collection chamber and incremental 5-cm sections were taken from the bottom of the soil block, at 32.5 cm depth, to within 7.5 cm of the block surface. An additional section was taken from the depth interval 0 to 7.5 cm.

Enumeration of Bacteria

Centrifuge tubes that contained more than 5 mL of leachate were stored at 4°C and fecal coliforms were enumerated within

Table 1. Characteristics of poultry manure applied to the soil blocks.

Treatment	Block no.	Percent total content, wet basis				Total fecal coliforms applied
		Water	N†	P†	K	
Sod-covered	1	58.0	3.1	1.1	0.7	4.6×10^9
	2	41.0	3.3	1.2	0.8	0.9×10^9
	3	37.5	3.9	0.8	0.9	0.2×10^9
Tilled	1	38.0	4.2	1.5	1.0	1.3×10^9
	2	34.0	4.4	1.6	0.8	1.5×10^9
	3	29.5	4.7	1.4	1.0	0.1×10^9

† Average % N and P are significantly greater in the tilled soil blocks than the sod-covered soil blocks ($\alpha = 0.05$).

48 h. Fecal coliforms were enumerated using a spiral plater that dispensed 48 μL of sample in an Archimedes spiral on a 100 mm diam. agar plate, leading to a 10^4-fold dilution of sample from the center to the edge of the plate. The colony forming units (CFU) that grew in defined areas were counted and divided by the known sample volume deposited in that area. This yielded CFU per mL. Samples were plated in duplicate on mFC agar (Difco, Detroit, MI) and incubated for 22 ± 2 h at 44.5°C. All blue and dark blue colonies were counted as fecal coliforms (Howell et al., 1995). Poultry manure samples were also enumerated with the spiral plater after 10-fold serial dilution in physiological saline (8.5 g NaCl in 1000 mL distilled water) (Table 1). We enumerated the fecal coliforms in soil in each 5 cm (or 7.5 cm) section by spiral plating diluted (10-fold) 10 g soil samples.

Statistical Analysis

Correlation coefficients and *t*-test analysis were performed using Microsoft Excel (Microsoft Corp., Redmond, WA).

RESULTS AND DISCUSSION

Drainage and Fecal Coliform Distribution

The fecal coliforms remaining in soil were uniformly distributed throughout the soil profile after 36 h of irrigation. Fecal coliform counts were typically 10 to 20 000 CFU g^{-1} soil in both sod-covered and tilled blocks. Considerably fewer fecal coliforms (<1000 CFU g^{-1} soil) were found in the third tilled soil block, which had the least number of fecal coliforms applied (Table 1). Considerably more fecal coliforms (>60 000 CFU g^{-1} soil in the upper 17.5 cm) were found in the first sod-covered soil block, to which the greatest number of fecal coliforms were applied.

The leachate collection pattern at the bottom of representative soil blocks is shown in Fig. 1 and 2. We expected the distribution of drainage in the collection cells beneath the soil blocks to be nonuniform if preferential flow occurred, and this was the case for all soil blocks. Quisenberry et al. (1994) and Phillips et al. (1995) both noted that at the bottom of similar-sized intact soil blocks, >50% of the flow was accounted for in <20% of the collection cells. Dexter (1993) and Wildenschild et al. (1994) postulated that nonuniform flow patterns were caused by macropores with tortuous paths coalescing nonuniformly with depth. We did not attempt to investigate the tortuosity of these flow paths, nor do we know if they are contiguous with the soil surface. Thomas and Phillips (1979), for example, suggest that the interface between A and B horizons in soils like the Maury, initiates water movement into macropores, which may simply be the interface between adjacent peds. Soil structure in the Maury changes from granular to subangular blocky as depth increases (USDA SCS, 1968).

Hagedorn et al. (1978) and Smith et al. (1985) suggest that macropore transport contributes to the rapid *E. coli* movement. As a general rule, the cells with the highest flow transported the highest percentage of fecal coliforms (Fig. 1 and 2). The correlation between percent of drainage and percent of fecal coliforms in drainage for individual collection tubes was significant ($P \leq 0.05$)

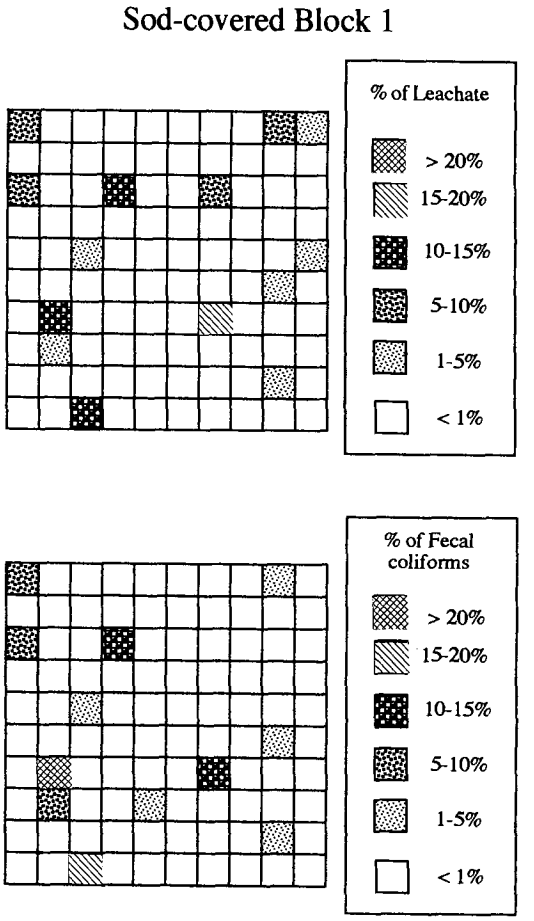

Fig. 1. Distribution of leachate and fecal coliforms at the bottom of an irrigated sod-covered soil block. Each square represents a surface area of 9.3 cm².

for both sod-covered (r^2 = 0.71) and tilled soil blocks (r^2 = 0.60).

Figure 3 illustrates this phenomenon for the 10 cells (in cumulative percent) in which most drainage and fecal coliforms were collected from the soil blocks. These locations were the outlets for between 63 and 99% of the total drainage and between 77 and 99% of the total fecal coliforms passing through these soil blocks. In every soil block for which data was collected, just three locations (2.6% of the soil block's surface area) were responsible for collecting >50% of the total fecal coliforms we enumerated in drainage.

Soil can be viewed as a filter and pathogens and chemicals as filterable agents that can be trapped in the soil matrix and impeded from contaminating groundwater. An obvious consequence of preferential flow is that it allows fecal pathogens to travel rapidly through soil and bypass much of the soil matrix that would trap them (Thomas and Phillips, 1979). An obvious consequence of the flow patterns we observed was the unpredictability of where that bypass might interface the groundwater table or bedrock. Preferentially flowing water can move below the root zone soon after water addition to the soil surface (Quisenberry and Phillips, 1978). Stoddard et al. (1993) demonstrated that open pan lysimeters 90 cm beneath undisturbed Maury soil collected fecal

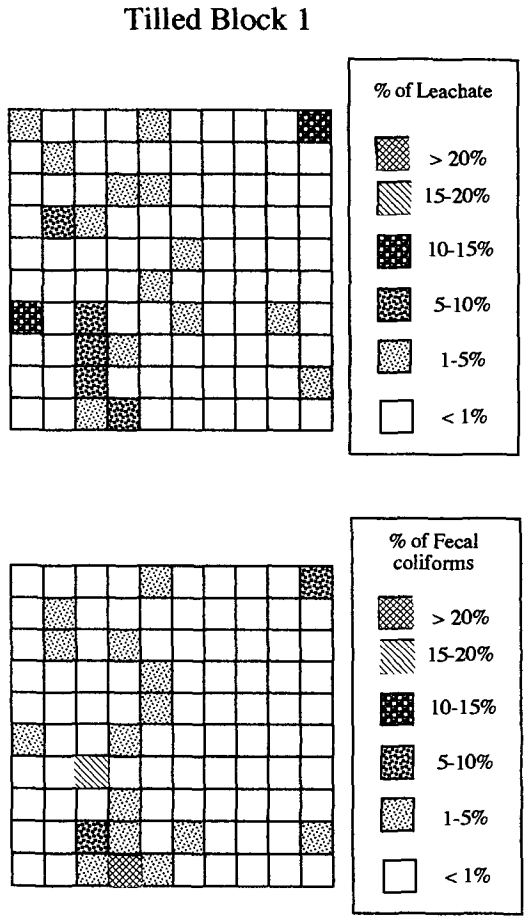

Fig. 2. Distribution of leachate and fecal coliforms at the bottom of an irrigated tilled soil block. Each square represents a surface area of 9.3 cm².

coliforms and fecal streptococci in the first rain following dairy manure application on tilled and untilled soils. Shallow groundwater may be similarly contaminated by fecal bacteria due to this preferential flow.

Fecal Coliform Breakthrough Curves

The fecal coliform breakthrough curves for two sod-covered soil blocks are shown in Fig. 4, while those for the tilled soil blocks are shown in Fig. 5. Fecal coliforms were too numerous to count in the third sod-covered block, and too few to be reflected by the graph of tilled soil blocks in Fig. 5.

In the initial leachate from sod-covered soil blocks (measured after approximately 4 cm of rain were applied) the fecal coliform concentration far exceeded Kentucky's regulatory water quality standards for domestic discharge and primary contact (bathing and swimming water, 200 fecal coliforms 100 mL^{-1}) (Kentucky Administrative Regulations, 1994). The concentrations were 28 million CFU/100 mL in block 1 and 18 million CFU/100 mL in block 2. The maximal fecal coliform concentrations were 33 million CFU/100 mL and 18 million CFU/100 mL in these blocks, respectively, after 8 cm of rain were applied. The fecal coliform concentrations declined thereafter. This suggests that a

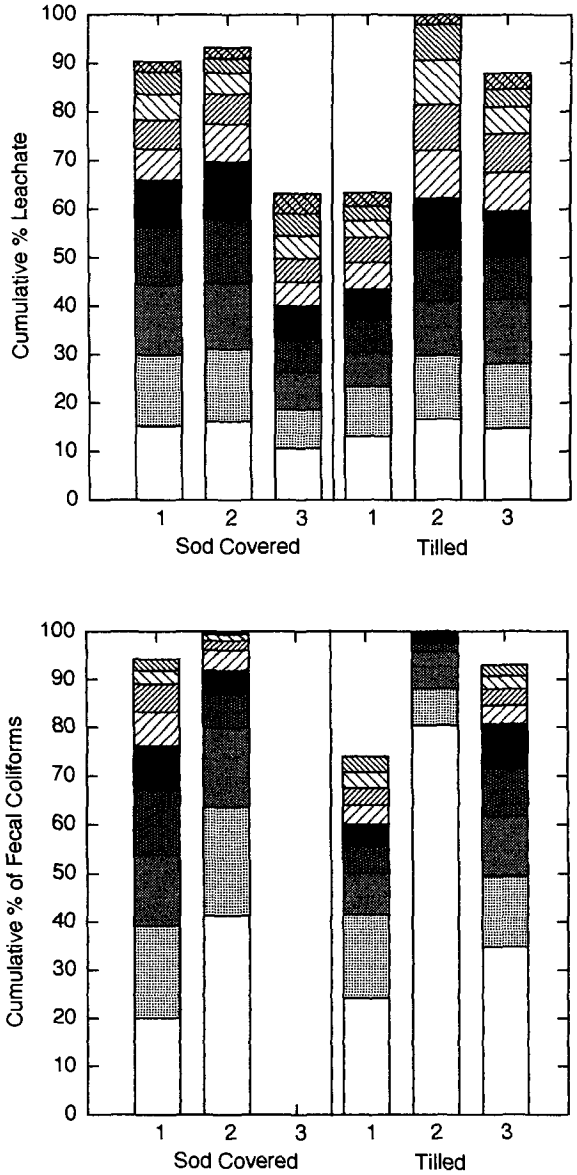

Fig. 3. Cumulative percent leachate and fecal coliforms, by cell, for the 10 most active cells of each soil block.

flush of fecal coliforms was eluted at the beginning of each irrigation.

The manure applied to the third sod-covered block was drier, and fewer total fecal coliforms were added to the soil block surface than the manure applied to the other sod-covered soil blocks (Table 1). Nevertheless, fecal coliforms in the drainage were too numerous to count. The total fecal coliforms eluted from each soil block exceeded the amount applied (data not shown), which suggests that the fecal coliform content of the initial manure was substantially underestimated, or that significant growth of bacteria occurred during the course of the experiments. The latter explanation is unlikely. Although fecal coliform populations will increase when manure is deposited into warm, moist environments (Howell et al., 1997) the speed with which most fecal coliforms were leached would probably have prohibited significant growth in soil purged with a nutrient-free

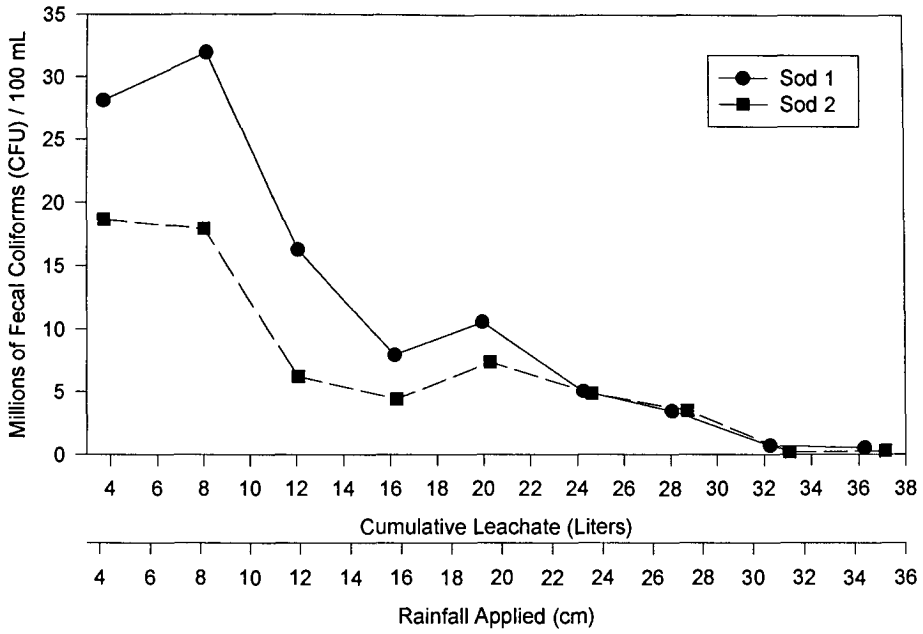

Fig. 4. Fecal coliform breakthrough curves for sod-covered soil blocks.

media. Although the mechanisms of bacterial sorption to solid surfaces have been thoroughly reviewed by Daniels (1980), extraction of fecal coliforms from complex substrates like manure, and the influence of moisture content on extractability (rather than simply survivability) are clearly pertinent areas for future research necessary to explore fecal bacteria transport.

The three tilled soil blocks showed a different pattern of fecal coliform elution than the sod-covered blocks (Fig. 5). Like them, the fecal coliform concentrations were well above water quality standards as soon as drainage was collected. However, fecal coliforms were retained in the soil profile for a period before the maxium concentrations eluted. The fecal coliform concentration in drainage from the first tilled block was maximal (20 million CFU/100 mL) after 22 cm of irrigation were applied, maximal for the second tilled soil block (12 million CFU/100 mL) after 14 cm of water were applied, and maximal for the third tilled soil block (1.0×10^5 CFU/100 mL—data not observable in Fig. 5) after approximately 10 cm of water were applied. The maximum concentrations of fecal bacteria corresponded to the initial number of fecal coliforms applied to the surface of the blocks (Table 1).

The irrigation required to elute the maximum fecal coliform concentration from the bottom of the soil blocks was significantly less in sod-covered soil blocks than tilled soil blocks ($P < 0.10$). Tilled soil blocks

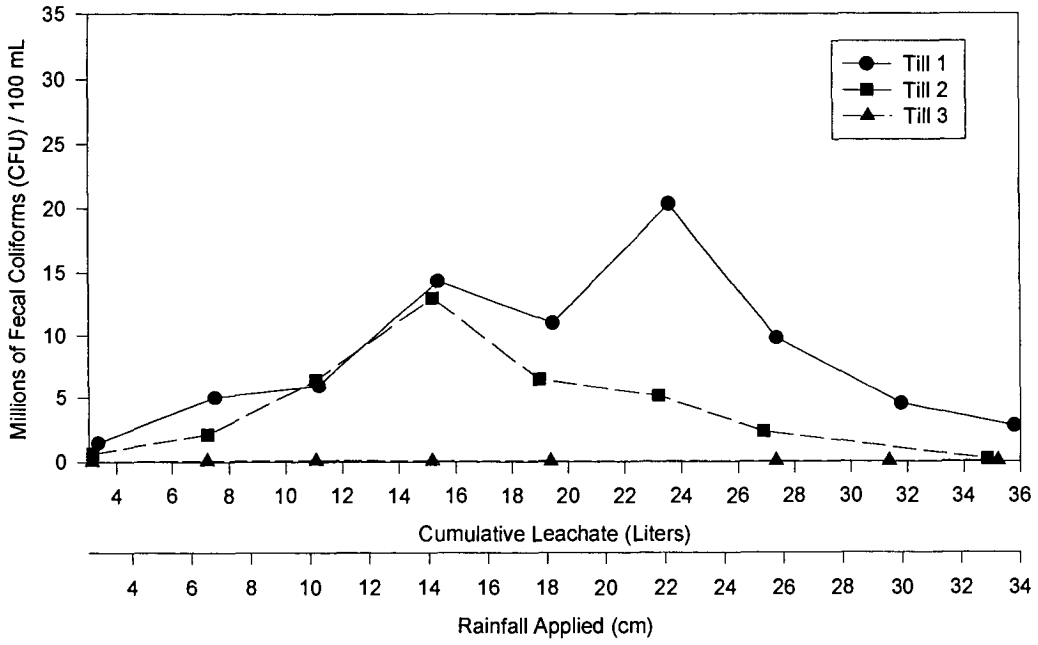

Fig. 5. Fecal coliform breakthrough curves for tilled soil blocks.

showed the greatest hindrance to the movement of fecal bacteria through the soil profile based on the breakthrough curves. The maximum fecal coliform concentrations took longer to elute in the tilled blocks probably because preferential flow paths were disrupted in the upper 12.5 cm of the soil. Wildenschild et al. (1994) suggested that a slow rise and fall of solute concentrations results from increasingly tortuous pore paths. This also appears to be the case for the tilled soil blocks in this experiment. Although this tillage practice did not hinder the flow of fecal coliforms sufficiently to meet water quality standards for primary contact water, the results are encouraging and suggest that more extensive tillage could further retard this movement.

The results are significant in several respects. The first rain after manure application produces the worst water quality (Edwards and Daniel, 1994). Rainfall or irrigation at an intensity of 1 cm h^{-1} would appear to be sufficient to drive manure-contaminated water to a depth of at least 32.5 cm in the Maury soil and presumably to as great a depth in similarly well-drained, well-structured soils. The potential to leach to greater depths with the same water input is present because of preferential flow, but we cannot extrapolate with confidence beyond the depths we sampled.

Over the period 1990 to 1995, at the Lexington site from which soil blocks were extracted, rain exceeded 4 cm on five occasions (1.2% of all measurable events, an event being considered as all consecutive days in which measurable precipitation occurred) (University of Kentucky Agricultural Weather Center). Rain exceeding 2 cm occurred 33 times (8.2% of all measurable events). Stoddard et al. (1993) showed that fecal bacteria applied to a Maury soil were driven to a depth of at least 90 cm by the first rain after application, when <2 cm of rain fell during a single event. Consequently, the potential for preferential flow to contaminate water supplies with fecal bacteria at this site would appear to be commonplace.

However, for tilled soil that has been chisel plowed and disked, as was the case in our soil block samples, the potential for displacing the peak concentration of fecal coliforms from poultry manure deposits is probably negligible. Maximum fecal coliform populations eluted after 10 cm of water were applied to the tilled soil block with the most rapid fecal coliform breakthrough. In the period 1990 to 1995, no events delivered this much rain.

CONCLUSIONS

Intact soil blocks were successfully used to evaluate the preferential water and bacteria flow through well-structured soil. The studies showed that preferential water movement occurred in each soil block, whether sod-covered or tilled. This caused a nonuniform distribution of drainage at the bottom of the blocks. Where preferential flow occurred, preferential fecal coliform movement also occurred. In a well-structured soil like the Maury, groundwater contamination by fecal coliform infiltration through soil may be significant during even modest rainfall (Howell et al., 1995).

Tillage retarded fecal coliform movement. The maximal fecal coliform transport through the tilled blocks was delayed compared to fecal coliform transport through undisturbed, sod-covered blocks. This delay suggests that tillage could possibly be used as a management practice to slow fecal coliform movement through the soil profile so that the magnitude, if not the incidence, of groundwater contamination represents a relatively infrequent event.

The method of applying fresh poultry manure to the surface of the soil blocks did not permit modeling the release of fecal coliforms. The experimental system, however, demonstrated the release of fecal coliforms as it might occur in field-like conditions, and it gave results that should be applicable to field studies predicting the potential infiltration of fecal coliforms after a poultry manure application.

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