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# Movement of Bacteria Through Macropores to Ground Water

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### RESEARCH REPORT NO. 139

## MOVEMENT OF BACTERIA THROUGH MACROPORES TO GROUND WATER

ΒY

M. Scott Smith Principal Investigator Grant W. Thomas Robert E. White Co-Investigators

### 1983



UNIVERSITY OF KENTUCKY WATER RESOURCES RESEARCH INSTITUTE LEXINGTON, KENTUCKY

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#### MOVEMENT OF BACTERIA THROUGH MACROPORES TO GROUND WATER

By

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### Grant W. Thomas and Robert E. White Co-Investigators

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#### Abstract

Effects of soil type, flow rate, antecedent soil moisture and other factors on transport of <u>E</u>. <u>coli</u> through soils was measured on disturbed and intact columns 20 cm in diameter by 25 to 30 cm in depth. Added <u>E</u>. <u>coli</u> were distinguished from indigenous microbes using an antibiotic resistance marker. Transport of Cl<sup>-</sup> and  ${}^{3}\text{H}_{2}$ 0 was also measured. Up to 96 percent of the bacteria irrigated onto the surface of intact columns were recovered in the effluent. Soil structure appeared to be related to the extent of transport. Columns prepared from mixed, repacked soil were much more effective bacterial filters than the intact soils. As rate of water input increased, the fraction of <u>E</u>. <u>coli</u> recovered in the effluent increased. We concluded that flow through soil macropores, which by-passes the adsorptive or retentive capacities of the soil matrix, is a common phenomenon. This conclusion was supported by the behavior of Cl<sup>-</sup> and  ${}^{3}\text{H}_{2}$ 0.

In waste disposal systems dependent on purification in the soil profile, this could significantly increase the probability of groundwater contamination by microbes. The effects of native soil structure (macropores) must be considered in the evaluation of these purification systems.

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#### CHAPTER I: INTRODUCTION

Objectives: initial project objectives were related to the direct observation of bacterial transport through soil surfaces:

- To determine the potential for groundwater contamination by bacteria moving through soil macropores using <u>Escherichia</u> coli labeled with an antibiotic resistance marker.
- 2. To observe the effect of rate of water addition, soil texture and structure, and concentration of bacteria on the ability of soils to filter organisms from contaminated water.

It became apparent as the project progressed that the experiments also revealed a great deal about the nature of water and solute movement through soils. Also, it was obvious that understanding of bacterial movement through soils was limited to the extent of our knowledge about water movement. Therefore, a third objective was added.

3. To observe and then explain the flow of water and solutes, specifically Cl<sup>-</sup>, through undisturbed soil columns as related to the transport of <u>E</u>. <u>coli</u>.

<u>Background and literature review</u>: Water flow through macropores in soil was noted as long ago as 1882 (Lawes et al., 1882) but often has been disregarded by sanitary engineers and soil scientists. Many studies of water movement and microbial and solute transport through soils have been conducted on sieved, uniformly packed soil columns. In these, of course, macropores and the native soil structure have been destroyed. Several observations of salt and water behavior under more realistic conditions suggest that much of the water applied to soils, and the dissolved or suspended matter in the water, by-passes the soil matrix, flowing rapidly down cracks and pores (Thomas and Phillips, 1979).

Whipkey (1967) and Aubertin (1971) working with forest soils and Thomas et al. (1973) on no-tilled soils provided convincing evidence that flow from the soil matrix itself was not very important in soil drainage immediately after a rain. Further work by McMahon and Thomas (1979), by Bouma (1981), Germann and Beven (1981), Tyler and Thomas (1977, 1981), Thomas and Phillips (1979), Wild and Babiker (1976), White et al. (1983), and many others has shown remarkable congruence.

By circumventing the adsorptive and filtering capacity of the soil matrix, macropore flow would reduce greatly the retention of pollutant bacteria and viruses in a soil profile, increasing the hazard of groundwater contamination. In evaluating the various soilbased waste disposal systems, this phenomenon has been appreciated infrequently. Studies by Hagedorn et al. (1981) and Rahe et al. (1978) demonstrate that rapid subsurface transport of enteric organisms may occur in many soils. Numerous press reports from regions of Kentucky depending heavily on septic disposal systems and underground water supplies suggest that drinking water contamination is common. A recently completed E.P.A. study, "National Statistical Assessment of Rural Water Conditions" (Francis, 1983), reported that 29 percent of rural households had sufficient bacterial contamination to pose a health hazard.

#### CHAPTER II: RESEARCH PROCEDURES

<u>Soils</u>: Soils used in the experiments were taken from three general areas in Kentucky. The Maury sil (Typic Paleudalfs) and the Lanton sicl (Cumulic Haplaquolls) were taken from the University of Kentucky research farm near Lexington. The Crider sil (Typic Paleudalfs) was collected at the Western Kentucky Experimental Station near Princeton. The Bruno sl (Typic Udifluvents) was taken from both a corn field on the Ohio River alluvial plain in Henderson Co. and from grass sod on alluvium of the Kentucky River. The Huntington sil (Fluventic Hapludolls) was also from Ohio River alluvium in Daviess Co., Kentucky. The Maury, Lanton and Crider sites were in old mixed sod. The Huntington was from an alfalfa field.

The soil columns were taken by driving a galvanized steel (duct pipe) cylinder 0.35 m in length by 0.2 m in diameter to a depth of about 0.28 m. A plywood and steel driving head supporting the top of the cylinder was repeatedly tapped with a sledge hammer. After driving the cylinders to the desired depth, a hole was dug next to the cylinder so that it and the soil it contained could be lifted out with little disturbance. Typically three to six cylinders were driven in a small area and removed, sealed in plastic bags and used within a month.

Soil moisture was not adjusted after taking the cylinders from the field. In most cases sampling was scheduled to obtain soils at or near "field capacity:" However, some drier samples were taken to determine the effect of antecedent soil water content on tracer behavior. Surface vegetation was clipped to a height of about 1 cm and the bottom of the soil was trimmed. Diameter and height of soil were measured.

Disturbed columns were prepared by removing the soil from the cylinder, mixing, and sieving through a 2 mm screen. The Bruno column was repacked to the original bulk density. The Crider and Maury

columns were packed to approximately 85% of their initial bulk density. Only the top 8 to 10 cm of the Crider column was disturbed, leaving the rest intact. The entire contents of the Maury and Bruno . cores were repacked. Soil water lost during these operations was replaced.

<u>Bacteria</u>: <u>Escherichia coli</u> K12 resistant to 100  $\mu$ g ml<sup>-1</sup> streptomycin were selected by plating dense suspensions of the parent culture on antibiotic containing nutrient agar (Difco). Resistance occurred in this population at a frequency of about 1 in 10<sup>7</sup> to 10<sup>8</sup>. Resistant isolates were purified by transferring three to four times on streptomycin nutrient agar. We selected one of the fastest growing isolates for use in all of these experiments. Resistance was sufficiently stable, as determined by the comparison of growth on media with and without antibiotics, after three transfers in antibiotic-free media.

<u>E. coli</u> suspensions were prepared by growing a culture in nutrient broth (Difco) for 36-48 hours at 26°C. This represents early stationary phase. Cells were harvested and washed by twice centrifuging and resuspending in 0.005 M CaCl<sub>2</sub>. Unless specified otherwise, there were approximately  $10^7$  cells·ml<sup>-1</sup> in the suspension applied to the soil columns. These suspensions were stirred and chilled in an ice bath as they were being applied to the soil, so the decrease in cell numbers was less than 5% during a 12 hour period. Clumping and sedimentation of cells was minimal in the CaCl<sub>2</sub> suspension, as determined by visual observation. The cells of this strain are rod shaped, approximately 1 µm in diameter by 2 µm long.

Experimental: During the laboratory experiments, the columns were supported on a large funnel. Water and tracers were applied at rates ranging from 5 to 40 mm per hour for 8 to 12 hours using a peristaltic roller pump (Auto-analyzer proportioning pump). These rates are representative of rainfall intensities in Kentucky. Irrigation suspensions or solutions flowed out of 20 hypodermic needles which were attached to a screen about 5 cm above the soil. This gave quite

uniform application to the soil surface. In most experiments, a total volume of effluent approximately equal to the pore volume of the core was collected. The effluent was caught in weighed beakers covered with parafilm to minimize evaporation. Chloride and <sup>3</sup>H were measured on duplicate subsamples of each effluent fraction. Chloride was determined using a Buchler-Cotlove Chloridometer and <sup>3</sup>H was determined on a Packard Tri-Carb liquid scintillation counter. Samples of the input solution were used to determine C<sub>0</sub> and all numbers for Cl and <sup>3</sup>H are reported as the ratio C/C<sub>0</sub>.

Soil columns were weighed prior to each experiment, after the last effluent had drained and after a week in a vented oven at 100°C. Antecedent volumetric water content, volumetric water content of the column during the experiment, void fraction and bulk density were calculated from these weights, the diameter and height of the soil.

Selected effluent fractions were subsampled for <u>E</u>. <u>coli</u> counts. Duplicate 10-fold dilution series of the effluent were prepared in sterile 0.85% NaCl. These were plated on nutrient agar containing 100  $\mu$ g·ml<sup>-1</sup> streptomycin and 50  $\mu$ g·ml<sup>-1</sup> cycloheximide, a fungal inhibitor. Antibiotics were filter-sterilized and added to the media after it was autoclaved. <u>E</u>. <u>coli</u> colonies were counted after 2 to 3 days. Coefficients of variation for replicate counts of the same effluent sample averaged 0.14. Mean <u>E</u>. <u>coli</u> effluent concentrations, C, for a column are derived from counts of at least five samples. The irrigation suspension itself was analyzed at the beginning and end of an experiment to determine the input concentration, C<sub>o</sub>.

Effluent from several control cores irrigated with <u>E. coli</u> - free solution was plated on antibiotic-containing agar. No more than 10 organisms per ml formed colonies and most of these were easily distinguished from E. coli.

#### CHAPTER 3: DATA AND RESULTS

To determine whether flow down the walls of the cylinder was a significant problem, a double funnel arrangement was set up on one soil column with water applied at 20 mm per hr (Fig. 1). The effluent from the inner portion of the column was kept separate from the outer effluent. Determination of  $C1^-$  and  ${}^{3}H$  showed that there was very little difference between the "inner" and "outer" effluents (Fig. 2a,b,c). Indeed, for these two tracers there was a slight trend for the outer effluent to be slightly lower in concentration than the inner effluent. The mean <u>E</u>. <u>coli</u>  $C/C_{0}$  of the effluent from the inner portion of the column was 0.61, the mean for the outer portion was 0.48. (These means are significantly different at the .05 level by ttest.) This indicates that, rather than introducing a gap for the transport of bacteria, the cylinder wall may block some of the conducting channels in the natural soil. In any case, there is no indication that there was leakage down the column walls. Therefore, it is assumed that the patterns for E. coli, Cl and  $\frac{3}{4}$  H in the column effluents represent a true picture of soil behavior and are not artifacts caused by wall leakage.

Cell density in the suspension applied to intact columns did not have a detectable influence on  $C/C_0$ . This was verified with the Crider, Lanton, and Maury soils with cell densities ranging from  $10^5$ to  $10^8$  cells per ml. In none of the three soils was there a consistent or significant effect of density (data not shown), suggesting that adsorption or filtration sites cannot be saturated by these relatively large doses of bacteria.

Elution patterns for <u>E</u>. <u>coli</u>, <u>Cl</u> and tritium: Figure 3 shows the concentration of <u>E</u>. <u>coli</u> and <u>Cl</u> in the effluent of intact Maury silt loam, irrigated at 20 mm  $\cdot$  h<sup>-1</sup>. The pattern for both <u>Cl</u> and <u>E</u>. <u>coli</u> is characteristic of most soils studied. Chloride C/C increased as



Figure 1: Illustration of the apparatus used to partition column effluent into inner and outer components to verify that

Figure 2a: C/Co of three different labels added to an intact Lanton column. Effluent was partitioned into inner (solid symbols) and outer (open symbols) components. V/Vo is cumulative effluent volume as a fraction of the soil pore volume. Tritium











Figure 3: Concentration of Cl  $(\bigcirc)$  and E. coli  $(\bigcirc)$  in the effluent as a fraction of their concentration in the input. The intact Maury silt loam core was irrigated at 2 cm·h<sup>-1</sup>.



irrigation proceeded and approached a C/C<sub>o</sub> of 1. Two aspects of the <u>E. coli</u> results should be emphasized. First, a very significant fraction of the bacteria passed through the column, 33% in this case. Second, <u>E. coli</u> C/C<sub>o</sub> was apparently constant as irrigation proceeded. No consistent increase or decrease in C/C<sub>o</sub> could be detected for most of the columns studied. In a few columns, the first 10 to 25 ml of effluent had higher cell density than all subsequent fractions which had approximately constant C/C<sub>o</sub> (Figure 6, for example). This was most common in columns which were initially dry. The different elution patterns for Cl<sup>-</sup> and <u>E. coli</u> demonstrate very different behavior in the soil profile.

The distribution of <u>E</u>. <u>coli</u> C/C<sub>o</sub> values in the initial effluent samples for all soil cores studied, and the relationship of this value to the Cl<sup>-</sup> breakthrough value (that is, the minimum value observed in the first three to four effluent fractions), is summarized in Fig. 4. (A plot with mean, rather than initial, <u>E</u>. <u>coli</u> C/C<sub>o</sub> would not be very different since initial and mean values differed in only a few initially dry columns.) For disturbed cores and the structureless Ohio River Bruno soil, <u>E</u>. <u>coli</u> C/C<sub>o</sub> was low regardless of the initial Cl<sup>-</sup> value. For all other intact soils, however, Cl<sup>-</sup> breakthrough was a reasonably good indicator of <u>E</u>. <u>coli</u> transport through the column. In the latter group of soils the correlation between <u>E</u>. <u>coli</u> and Cl<sup>-</sup> C/C<sub>o</sub> was significant (r = .93). The most important observation, however, is that a significant fraction of the cells applied passed through almost all of the soils studied.

We wondered how repeatable the breakthrough curves might be, given the randomness of macropores in soils. Figure 5 shows the Cl<sup>-</sup> breakthrough curves for three Maury columns with initial water contents ( $\theta = 0.33$ , 0.34 and 0.35) and water application rates (1.8 to 1.9 cm/hr) as uniform as possible. The C/C<sub>o</sub> at breakthrough varied from 0.02 to 0.08 but the remainders of the curves were practically identical. <u>E. coli</u> elution patterns also were remarkably uniform for soil columns taken from the same site at the same time. For example, four replicate columns of Maury soil irrigated at 20 mm·h<sup>-1</sup> gave a



<u>Figure 4:</u> Initial E. coli C/C vs. Cl<sup>-</sup> C/C for all cores assayed. Closed symbols are disturbed cores, numbers in open symbols are irrigation rates in cm·h<sup>-1</sup>, Maury ( $\bigcirc$ ), Crider ( $\bigcirc$ ), Lanton ( $\triangle$ ), Bruno ( $\bigcirc$ ), Huntington ( $\bigtriangledown$ ).

<u>Figure 5</u>: Chloride C/Co as a function of corrected effluent volume (V/Vo) for three replicate columns of Maury silt loam collected at the same time at the same site.



<u>Figure 6</u>: <u>E. coli</u> (**M**) and Cl<sup>-1</sup> (**@**) recovery in the effluent from an intact Crider silt loam core irrigated at 2 cm·h<sup>-1</sup>. E. coli application was discontinued at the point indicated.



mean <u>E. coli</u> C/C of 0.20 with a standard deviation of 0.05. These data suggest that the results for a given soil samples in a small area are surprisingly repeatable.

The largely irreversible nature of cell entrapment or adsorption was demonstrated by the experiment illustrated in Fig. 6. As before, <u>E. coli C/C</u> was constant initially. When the application of bacteria to the top of the column was stopped but Cl<sup>-</sup> solution application continued at the same rate, the <u>E. coli C/C</u> dropped quickly. The number of cells washed out was only a small fraction of the number not recovered during the initial application.

In general, it can be said that none of the soils used in the experiments showed anything approaching miscible displacement which is characteristic of ideal flow in uniformly packed columns. This nonideal flow in undisturbed columns is demonstrated best by the behavior of Cl<sup>-</sup> and tritium. Figures 7, 8 and 9 provide examples. Only the Bruno sl showed any "S-shaped" breakthrough (Figure 7) and this was weakly expressed and not located at one column pore volume (V/Vo = 1). Because the soils used in this study represent a large proportion of Kentucky, it seems safe to say that, in the undisturbed state, soils of Kentucky do not appear to conduct water in a way approaching the experiments of Bodman and Colman (1943), or of any other experiment done with packed columns. Obviously, macropore flow is very important in these soils.

<u>Soil Type</u>: Table 1 shows the soils used in the column studies, the county from which they were taken and the value of the  $C/C_0$  at break-through for both <sup>3</sup>H and Cl. It should be noted that the application rate for Bruno sl was one-half that of the other soils so the values are not comparable. For <sup>3</sup>H  $C/C_0$  at breakthrough, Maury sil had the lowest value (0.03), suggesting the least macropore flow (or most mixing with matrix water), followed by Lanton sil with 0.10, Crider sil with 0.20 and Huntington sil with 0.69. The behavior of the Huntington suggests that there are a few pipe-like macropores which carry all the water, whereas the other soils show considerably more



Figure 7: Elution of tritium from 2 replicate Bruno soil columns.





Figure 9: Elution of chloride (o) and tritium (•) from a Huntington column. Irrigation was stopped after V/Vo reached approximately 1.3.

		_		3							
Table	1.	C1	and	Ч	C/C	at	breakthrough	for	five	Kentucky	soils.
					0						

Soil	County	3 <sub>H</sub>	C1
Maury sil	Fayette	0.03	0.14
Lanton sicl	Fayette	0.10	0.38
Huntington sil	Daviess	0.69	0.73
Crider sil	Caldwell	0.20	0.38
Bruno sl	Henderson	0.08*	0.19*
•			

\* 1.0 cm per hr flow rate, others 2.0 cm per hr.

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mixing with water in the soil matrix.

The fourth column of Table 1, showing  $C/C_0$  for  $C1^-$  at breakthrough follows approximately the same order as for  $C/C_0^-$  (<sup>3</sup>H) but the  $C1^-$  values are higher. For the Huntington and Crider, which had the highest  $C/C_0^-$  values for <sup>3</sup>H, the percent difference is much less than for Maury (0.03 vs. 0.14) or Lanton (0.10 vs. 0.38).

Table 2 shows comparable results for E. coli elution from six different intact columns, representing five soil series and encompassing the range of soil textures found in this region. Except for the Bruno sandy loam from a site near the Ohio River, all permitted a large fraction of the bacteria to move through the column. No relationship between clay or organic matter contents and  $C/C_{c}$  is apparent. There was an apparent relationship between bacterial  $C/C_0$  and soil structure, qualitatively described. For example, the Kentucky River Bruno had obvious channeling due to roots and soil fauna. The Ohio River Bruno apparently is a more recent alluvial deposit in a field that had been cultivated a few months before sampling, and it was essentially structureless. The Huntington, with a C/C of 0.96, is an extremely well structured soil with large stable vertical cracks. The Maury also has stable structure but has a finer crumb-like structure at the surface than the Huntington.

Transport of <u>E</u>. <u>coli</u> through intact and disturbed soil cores is compared in Table 3. All soils became more effective bacterial filters when channels and pores were removed by mixing part or all of the soil in the column. Disturbed cores retained at least 93% of the cells applied. Intact cores retained 21 to 78% of the bacteria.

<u>Rate of water application</u>: The rate of suspension application had a large effect on <u>E</u>. <u>coli</u> transport through soil columns (Table 4). As application rate increased from 5 to 40 mm  $\cdot$  h<sup>-1</sup>, C/C<sub>0</sub> for the Maury soil increased more than six-fold. Similar trends were observed for three other soil series.

Table 5 shows the values of C/C (C1) for four soils at flow rates varying from 5 to 40 mm per hour. Note that in nearly every

Table 2.	Movement	of	<u>E</u> .	<u>coli</u>	through	intact	columns	of	various

Kentucky soils.\*

Soil Series	Texture	c/c
Maury	silt loam	0.22
Crider	silt loam	0.59
Lanton	silty clay loam	0.66
Huntington	silt loam	0.96
Bruno (Kentucky River)	sandy loam	0.79
Bruno (Ohio River)	sandy loam	0.003

\* Ohio River Bruno irrigated at 10 mm  $\cdot h^{-1}$ , all others at 20 mm  $\cdot h^{-1}$ .

	 c/c			
Soil ·	 Intact	Disturbed		
Crider	0.44	0.07		
Maury	0.22	0.002		
Bruno (Kentucky River)	0.79	0.05		

Table 3. Bacterial movement through intact vs. disturbed cores.\*

\* All at 20 mm  $\cdot$  h<sup>-1</sup> irrigation rate.

## Table 4. Influence of suspension application rate on transport of

		· · ·	Bacteri	al C/C	
Soil	Flow $(\mathbf{mm} \cdot \mathbf{h}^{-1})$ :	40	20	10	5
Maury		0.32	0.22	0.11	0.05
Huntington		n.d.*	0.96	n.d.	0,56
Crider		n.d.	0.59	n.d.	0.08
Bruno (Kentucky	River)	0.90	0.79	n.d.	n.d.

<u>E. coli</u> through intact soil columns.

\* n.d. - not determined.

	· · · · ·	Application R	ates (mm·h <sup>-1</sup> )	
Soil	40	20	10	5
н. - С.		c/c <sub>o</sub>	(C1)	
Maury	0.12	0.10	0.01	0.02
Crider	0.48	0.32	-	0.11
Lanton	0.68	0.38	0.28	-
Huntington	-	0.73	-	0.32

Table 5.  $C1^{-}C/C_{0}$  at breakthrough at different application rates for four Kentucky soils.

case, the value of  $C/C_0$  increases as application rate increases. However in the Maury soil the increase is moderate (0.02 at 5 to 0.12 at 40 mm/hr). The other three soils show a much greater increase in  $C/C_0$  as flow rate increases.

Flow down macropores should occur anytime the application rate exceeds the hydraulic conductivity of the soil matrix. These results have direct implication for the behavior of solutes located at the soil surface and their possible movement through the soil to groundwater. It can be expected that with intense bursts of rainfall solutes can be transported "past" the soil and directly into the groundwater. Of the soils used, the Huntington is most extreme in this regard, delivering 73% of the input solution concentration to the bottom of the column at breakthrough at a flow rate of 20 mm per hour.

Antecedent water content: Assuming that ideal piston flow occurred, it would be expected that antecedent water content of the soil would have no effect on the C/C<sub>o</sub> at breakthrough until the initial soil water content was very low. At that point, C/C<sub>o</sub> would approach a value of 1.0 (since there is no water to displace). However, with the soils used in this study, where less than complete displacement occurred, the situation is considerably more complicated. It appears that lowering the antecedent water content has an almost linear effect on C/C<sub>o</sub> for the Lantan and Crider but the Maury gives a rather erratic pattern. The ratio  $\theta_i/\theta_{sat}$  is plotted against C/C<sub>o</sub> (C1) at breakthrough (Figure 10). It is clear that below a  $\theta_i/\theta_{sat}$  value of 0.6, C/C<sub>o</sub> values increase very rapidly except for the Maury soil data points.

This too has implications for pollution. It is evident that in a dry soil the concentration of solute dissolved in the applied water will arrive at the bottom of the column at a considerably higher concentration than when the soil is near field capacity. If, however, the pollutant in question is in the soil rather than the solution, it will be somewhat protected from movement in clay soils.



#### CHAPTER 4: CONCLUSIONS

Our observations show that suspended bacteria can move rapidly through soil profiles and suggest that this transport occurs in macropores. This may occur in all structured soils. This would include virtually all Kentucky surface soils, except for some limited areas in which soils are very sandy and also would not apply to recently tilled soils, which are expected to behave more like our disturbed columns. The material underlying most Kentucky surface soils, particularly in the limestone regions, is not likely to act as a more effective filter than the surface soil. This material is cracked and porous and exhibits non-Darcian, macropore type of flow (for ex., Thrailkill et al., 1982).

Since replicate small columns give consistent results, macropore flow should not be considered an uncommon phenomenon to be observed in scattered spots containing a visibly large hole. Rather, an entire field or land area seems likely to exhibit consistent macropore flow.

The behavior of  $C1^-$  and  ${}^{3}H_{2}^{}0$  also suggests that much of the water movement in a soil profile is channeled through macropores. Macropore flow would minimize interaction between the soil matrix and solutes or suspended particles, such as <u>E. coli</u>. As the rate of rainfall or irrigation increases, water velocity within macropores would increase, as would the proportion of the total water channeled to macropores. This accounts for the effect of irrigation rate on <u>E. coli</u> C/C<sub>0</sub>.

We observed a correlation between  $Cl^- C/C_0$  at breakthrough and <u>E</u>. <u>coli</u>  $C/C_0$  in the initial effluent sample from the intact soils (Figure 4). Less expected were the very different effluent patterns for <u>E</u>. <u>coli</u> and  $Cl^-$  (Figure 3). Low initial  $Cl^-$  concentrations, any  $C/C_0$ values less than 1, are conventionally attributed to miscible displacement; that is dilution of the applied solution with soil water displaced by the piston-like flow of added water. If the initial effluent samples are diluted with regard to  $Cl^-$ , it might also be

predicted that they should be diluted with regard to <u>E</u>. <u>coli</u> or that <u>E</u>. <u>coli</u>  $C/C_0$  should be initially low and subsequently increase. This was not the case.

Chloride and <u>E</u>. <u>coli</u> effluent curves can be explained by either of two models. The first model is similar to a model previously proposed by one of us (Thomas et al., 1978). It assumes two components of water flow. One component moves through the soil matrix, essentially all <u>E</u>. <u>coli</u> are removed, Cl<sup>-</sup> passes in the water but this water also displaces soil water ahead of it. A second component moves through macropores; Cl<sup>-</sup> and <u>E</u>. <u>coli</u> move in this water with little interaction with the soil. Mostly, these large pores are drained initially so there is relatively little water displaced ahead of this component. The initial Cl<sup>-</sup> and <u>E</u>. <u>coli</u> values are generally similar because these values are related to the fraction of water released from macropores. Since effluent released from the matrix never contains a significant number of cells but contains a progressively increasing concentration of Cl<sup>-</sup>, <u>E</u>. <u>coli</u> C/C<sub>0</sub> remains constant but Cl<sup>-</sup> C/C<sub>0</sub> increases, approaching a value of one.

The second possible model assumes that nearly all of the water transmitted through a soil moves in macropores. In this model miscible displacement is of minimal significance. Initial dilution of  $Cl^-$  in the effluent is attributed to diffusive exchange between irrigation water and  $Cl^-$  free soil water. A process similar to dialysis can be envisioned. Removal of <u>E. coli</u> is by sedimentation and entrapment along macropore walls. This is controlled only by pore geometry and by water velocity. This model is diagrammed in Figure 11.

Very different conclusions about the effectiveness of soils as a bacterial filter can be drawn, depending on whether the soils observed are intact or disturbed. Transport of cells through most sieved or mixed soils will be insignificant relative to transport through most structured soils. In repacked soil columns, it is likely that the clay and organic matter content of soils are critical determinants of bacterial transport, because of the capacity of these materials to



water flow through soils. Net downward water transport occurs only in macropores. Exchange between matrix and macropore water accounts for initial

Figure 11: Diagrammatic representation of diffusion-based model of

adsorb cells. For intact columns, we conclude that soil structure and velocity of water flow are the important factors. Because of the extreme differences between intact and disturbed cores, we question the relevance to many field situations of bacterial and virus transport studies in sieved, packed soil columns.

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