MOVEMENT OF BACTERIA IN UNSATURATED SOIL COLUMNS WITH MACROPORES

J. Abu-Ashour, D. M. Joy, H. Lee, H. R. Whiteley, S. Zelin

ABSTRACT. *Rapid movement of bacteria through the soil has been observed after applications of manure to agricultural fields. Preferential flow through macropores has been suggested as the main reasons for these observations. Experiments with repacked soil columns were used to study the effect of artificially created macropores, soil type, soil water content, and simulated rain application on movement of a tracer bacterium, nalidixic acid-resistant Escherichia coli. Results form these experiments showed a significant increase in the number of biotracer cells passing through a soil column when macropores were present and the soil was wet. There was no passage of biotracer cells through a dry soil with macropores. No biotracer cells were eluted from columns without macropores even when the soils were wet. Simulated rainfall applied on the top of the soil columns caused bacteria to travel deeper into the soil. These results confirm the important role that macropores play in the movement of bacteria through heterogenous soils.*
Keywords. *Bacteria, Macropores, Transport.*

Environmental and public health problems associated with the spreading of sewage on land have been observed since the beginning of the twentieth century (Abu-Ashour et al., 1994). Land application of sewage is advantageous because it removes some of the pollutants from the sewage, constitutes a possible aquifer source, and increases crop yields by supplying essential nutrients and by improving soil properties (Lance et al., 1982; Tim et al., 1988). However, disadvantages of this practice may include degradation of surface and groundwater quality through chemical and microbial contamination, and accumulation of heavy metal in soils if application amounts are excessive or improperly timed.

Spreading agricultural liquid wastes on lands can cause environmental problems even when the application procedures are within the current guidelines. Problems have been demonstrated in Ontario by Dean and Foran (1990a,b, 1991), Fleming et al. (1990) and Palmateer et al. (1989) where applications of liquid manure to agricultural fields have resulted in rapid movement of a biotracer, nalidixic acid-resistant *Escherichia coli* (*E. coli* NAR), through the soil and subsurface drainage systems leading to contamination of surface waters. In another study, Reaume et al. (1993, 1994) used the same biotracer to mark liquid manure before application to tile-drained fields at four locations on southwestern Ontario. Their results showed the extent of biotracer movement to be dependent on pre-application conditions. They identified the soil water content and the presence of surface-connected macropores as the major factors determining bacterial movement from surface applied manure to the buried tile drains.

Bacteria and viruses have been shown to travel through porous media with the distance traveled being dependent on the type of porous medium. Gerba et al. (1975) reported studies in which coliform bacteria traveled from 0.6 m in fine sandy loam to 830 m in sand-gravel; bacteriophage T4 traveled up to 1600 m in a carbonate rock area. Stewart and Reneau (1981) detected migration of coliform bacteria from septic tank drainfields in both vertical and horizontal directions to monitoring wells of 152 and 305 cm depth located within 30 m of the drainfields. The extent of migration in both directions varied depending on the position of the monitoring well relative to the drainfield. They attributed these differences to variations in water flow.

Movement of microorganisms through soil can be rapid. Smith et al. (1985) compared the movement of a streptomycin-resistant *E. coli* K12 strain and a Cl^- tracer through soils of different texture. With the Huntington silt soil contained in 0.28 m undisturbed columns, about 90% of the *E. coli* applied initially moved through the column in 17 min, while about 70% of the applied Cl^- passed within the same time. The authors suggested that such rapid movement was due to the presence of continuous macropores. McCoy and Hagedorn (1979) found *E. coli* cells were transported in the subsurface at an apparent maximum speed of 17 cm/min. In another study in Humberside, U.K., bacteriophages were injected into an aquifer at boreholes 366 and 122 m from a pumping well (Skilton and Wheeler, 1988). The results showed that bacteriophages moved rapidly, reaching a maximum speed of 170 cm/min.

Many studies of bacterial movement through soil have been conducted in the field and the results generally show rapid movement and a high concentration of bacteria

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reaching receiving waters. The explanation normally provided is that the observed phenomena are due to preferential flow of microorganisms through macropores, cracks, fractures, worm holes and channels formed by plant roots and animals in the soil. In a review of literature White (1985) suggested that preferential pathways were the cause of the observed rapid movement of dissolved and suspended substances through soil. Preferential flow through macroscopes had been observed in both laboratory and field studies (Chandler et al., 1981; Thomas and Phillips, 1979; van Elsas et al., 1991).

While the work to date has suggested quite strongly the role that macropores play in the movement of water and substances through the soil, there is not enough information on the movement of bacteria under controlled conditions. In particular, there isn't information which specifically compares the relative contribution of macropores to the movement of bacteria and the effect of the other important parameters such as initial soil water content, soil type and rainfall. This study investigated bacterial movement through soil using repacked soil columns containing artificial macropores. Experiments were conducted to support the explanation of macropores as a dominant pathway for rapid movement of bacteria to buried drainage tiles in field experiments.

Materials and Methods

Soils

The focus of this research was to study under controlled laboratory conditions the movement of bacteria through a soil macropore such as those that occur under field conditions from worm holes, cracks, and fractures. Two soils, a silt loam and a loam, known to exhibit macropores under field conditions, were selected. Soil samples were collected from the top 0.3 m of fields near Guelph, Ontario. The silt loam was dark brown with an organic matter content of 4.7% while the loam was light brown with an organic matter content of 0.9%. Other soil characteristics are summarized in table 1.

Soils were collected at the start of the experiments in sufficient quantities to complete all the experiments. Prior to column preparation, soils were first passed through a mechanical separator to break up large clumps. After air-drying for three days, the soils were passed through a 2-mm sieve. Some of the larger clumps retained by the sieve were crushed and sieved again. Afterwards, the sieved soil was mixed thoroughly for 15 min using a Twin Shell Dry Blender and then divided into smaller batches, each with a sufficient mass (~1.5 kg) of soil to pack one column.

Table 1. Soil characteristics

Soil Type	Sand (%)	Silt (%)	Clay (%)	Organic Content (%)	Field Capacity (%)
Silt loam	29.8	51.5	18.7	4.7	28
Loam	28.3	48.5	23.2	0.9	21

Column Packing and Macropore Creation

Four identical plexiglass columns were constructed. Each column was 400 mm in length with an inside diameter of 89 mm. Columns were packed with soil to a height of 175 mm. Soil in the column was supported by a perforated plexiglass disc overlaid by a stainless steel mesh having an opening size of 38 μm. The base of the column had two ports; one for sampling the effluent and the other for allowing discharge.

Soil columns were packed to a dry bulk density of 1.2 g/cm^3 consistent with values measured in the field for these soils. The initial water content of the soil was measured by oven drying a sample at 103°C for 24 h. Initial water contents were prepared by first measuring the water content of the samples, adding the requisite water to bring the whole sample to the desired water content, mixing thoroughly and then placing the soil in the column. Water contents of the mixed soil were measured to confirm the desired water content was reached and these are reported in table 2 summarizing the experiments.

The soil was compacted in two layers each with a thickness of 87.5 mm after compaction. Two layers were selected after some initial experiments to determine the number of layers required to give a consistent packing and density throughout the soil column. Compaction was achieved by a rod attached to a plate just smaller than the inside diameter of the column (fig. 1). A 390 g weight sliding on the rod was dropped repeatedly (< 5 times) from a constant height to deliver constant energy to pack the soil. Correct compaction was determined by the mass of soil put into the column for each layer and the desired compaction volume associated with the bulk density of 1.2 g/cm^3. Total depth of soil in each column was 175 mm.

After testing several techniques for the creation of macropores, the following procedure was adopted. Metal rods, 2.4 mm in diameter were placed in the desired configuration in an empty column. The size was selected based on the typical size of macropores observed in the fields from which the soils were selected (Reaume et al.,

Table 2. Experimental conditions of soil columns

Column No.	Soil Type	Initial Soil Water Content (%)	No. of Macropores*	Rainfall Volume† (mL)	Total Volume (mL)
C-1	Silt loam	40	0	0	35
C-2	Silt loam	40	0	0	35
C-3	Silt loam	41	0	0	35
C-4	Silt loam	41	0	0	35
C-5	Silt loam	41	0	120	155
C-6	Silt loam	41	0	120	155
C-7	Silt loam	40	1	0	35
C-8	Silt loam	40	1	0	35
C-9	Silt loam	40	1	120	155
C-10	Silt loam	40	1	120	155
C-11	Silt loam	10	1‡	0	35
C-12	Silt loam	10	1*	120	155
C-13	Silt loam	10	0	0	35
C-14	Silt loam	10	0	120	155
C-15	Silt loam	9	1	0	35
C-16	Silt loam	9	1	120	155
C-17	Loam	35	0	0	35
C-18	Loam	5.8	0	0	35
C-19	Loam	35	0	120	155
C-20	Loam	5.8	0	120	155
C-21	Loam	32	1	0	35
C-22	Loam	32	1	0	35
C-23	Loam	36	1	0	35
C-24	Loam	11	1	0	35
C-25	Loam	36	1	120	155
C-26	Loam	11	1	120	155

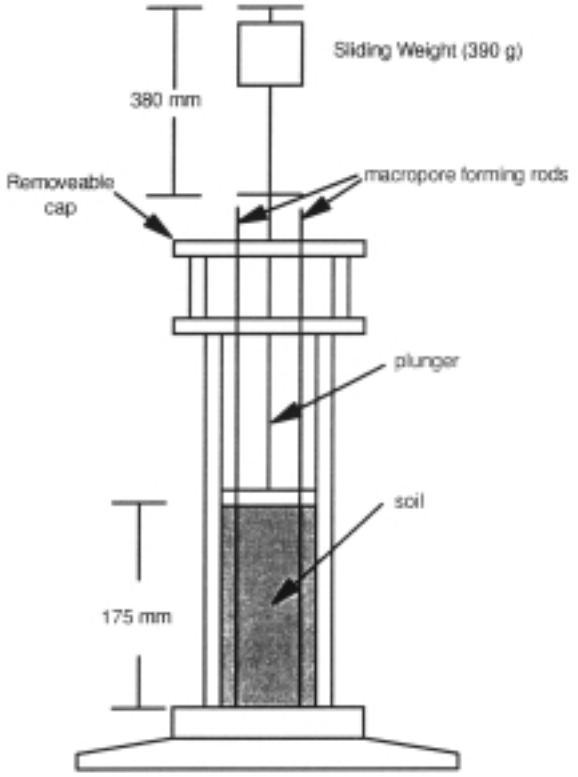

Figure 1–Column packing arrangement.

1994). The rods were firmly held in place at the bottom of the column by recesses in the permanent base plate. A removable guide plate maintained their position at the top during the addition and packing of soil around the rod, as shown in figure 1. The columns were filled with soil and packed in two layers. The rods were slowly pulled out from the soil leaving voids of the sizes typical in macropores. Initial experiments (Abu-Ashour, 1994) looked at the effect of the number of macropores present. These showed the results for 1 macropore to be representative of multiple macropores and thus only one macropore was used.

BIOTRACER

The microorganism used as a tracer in this study was a nalidixic acid-resistant *Escherichia coli* strain (*E. coli* NAR), provided by the Ontario Ministry of Environment and Energy. This biotracer has been successfully used in previous studies by Dean and Foran (1990), Fleming et al. (1990), Palmateer et al. (1989), and Reaume et al. (1994) to monitor bacterial contamination of receiving waters subsequent to farm application of liquid manure. In addition, laboratory studies conducted earlier (Joy et al., 1992) confirmed the suitability of using this tracer bacteria as an indicator of the soil transport characteristics of naturally occurring bacteria under various testing conditions.

E. coli NAR is a Gram-negative rod-shaped bacterium. It occurs singly or in pairs, and has a size of 1 μm × 1.25-2 μm as measured using a phase contrast microscope. The biotracer is classified as a facultatively anaerobic, chemoorganotrophic bacterium.

E. coli NAR was grown by adding a loopful of cells from an agar plate to a 125-mL Erlenmeyer flask containing 25 mL Tryptic Soy Broth (TSB; Difco Laboratories, Detroit, Mich.) and incubated at 20°C for 17 to 19 h with gyratory shaking at 200 rpm. Afterwards, the cells were harvested by centrifugation at 5,000 × g for 20 min. Cells were washed twice with 0.1 M phosphate buffer, pH 7.5, and resuspended in a phosphate buffer to a density of about 10^6 to 10^{10} Colony Forming Units (CFU)/mL.

EXPERIMENTAL PROCEDURE

A total of 26 soil columns, 14 with and 12 without macropores were prepared at different water contents as described earlier and summarized in table 2. A volume of 35 mL of inoculum described earlier was applied at the top of the soil column—equivalent to a depth of application of 6 mm. This inoculum was applied in a slug input distributed over the surface by a perforated plate 225 mm above the soil. Outflow was monitored for 24 h, although it was observed that water stopped dripping after the first hour in some columns and there was no effluent in others. All effluent was collected and measured volumetrically and a 1.5-mL sample was taken of the total effluent for analysis of the biotracer content.

Samples were serially diluted, spread on mTec-NA agar (Difco) plates (60 × 15 mm Petri Plates) supplemented with 100 μg/mL nalidixic acid, and incubated at 44°C for 24 h before being analyzed for the concentration of biotracer.

In 14 experiments, 24 h after inoculation, deionized water was applied at the top of the soil column at a rate of 60 mL/h for 2 h using a peristaltic pump, as shown in figure 2. The deionized water was pumped to a perforated plate, 225 mm above the soil surface to distribute the liquid evenly over the column cross-sectional area. This arrangement was designed to simulate a 10-mm rain storm with a duration of 2 h. Column outflow as monitored and collected if there was any outflow and a 1.5-mL sample taken of the total effluent. Concentrations of the biotracer in the sample collected were measured as described previously. After either the end of the simulated rainfall or the addition of the inoculum if no simulated rainfall was added, the columns were allowed to drain for a further 24 h before disassembly. In all cases all discharge from the column had ceased before disassembly of the column.

At the end of each experiment, the soil in the column was tested to determine both the viability of biotracer cells

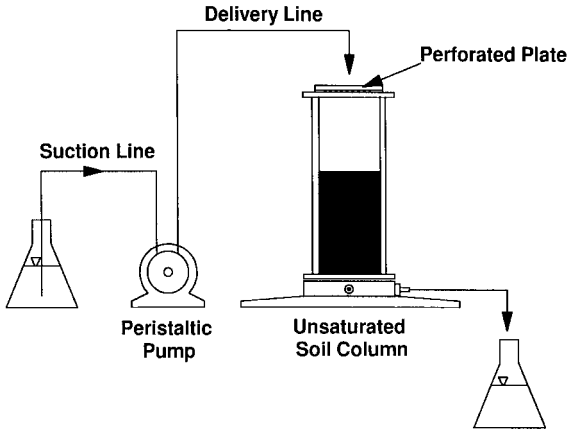

Figure 2–Rainfall simulation.

and the distribution of cells throughout the column. At this point the base of each column was removed and the soil was pushed out using a plunger. Five slices, each 10-mm thick, were taken, one from the bottom of the soil column, one from the top, and three at equidistant intervals between these two. Each slice was placed in a container and mixed well. From each slice, a 10-g soil sample was added to 95 mL of saline in a 250-mL Erlenmeyer flask. The flasks were shaken for 30 min at 200 rpm on a gyratory shaker before making serial dilutions in saline and plating on mTec-NA agar. The plates were incubated at 44°C for 24 h before enumeration.

Results and Discussion

A summary of the results of the 26 column experiments is given in table 3. The results are summarized in several ways. First, the effluent volumes and the number of cells eluted after the application of the inoculum and after the application of rain are given. To account for the varying input concentrations, these are given as a percent of the total number applied. Total number applied was determined by enumeration of an inoculum sample. Secondly, the number of cells enumerated in the columns after dissection—again as a percent of the amount added—are given. Finally, the distribution of cells throughout the soil column after the experiment is illustrated in figures 3-11. These results are first considered in light of the effect of presence or absence of macropores and secondly by initial water content. These are both discussed with and without rainfall.

Table 3. Summary of experimental results

Col. No.	Effluent Volume Before Rain (mL)	Effluent Volume After Rain (mL)	Cells Eluted Before Rain (%)	Cells Eluted After Rain (%)	Time of Soil Sampling (h)	Cells Recovered in Soil	
						Theoretical§ (%)	Measured (%)
C-1	13	N/A	0	N/A	24	100	100
C-2	13	N/A	0	N/A	24	100	84
C-3	35	N/A	0	N/A	24	100	140
C-4	35	N/A	0	N/A	24	100	22
C-5	23	110	0	0	48	100	9
C-6	21	130	0	0	48	100	110
C-7	35	N/A	67	N/A	24	2.7	26
C-8	35	N/A	48	N/A	24	4.7	21
C-9	32	100	70	1	48	1.1	18
C-10	32	100	83	1	48	1.3	18
C-11	0	N/A	0	N/A	24	100	90
C-12	0	0	0	0	48	100	15
C-13	0	N/A	0	N/A	24	100	59
C-14	0	0	0	0	48	100	17
C-15	0	N/A	0	N/A	24	100	120
C-16	0	0	0	0	48	100	45
C-17	20	N/A	0	N/A	24	100	23
C-18	0	N/A	0	N/A	24	100	150
C-19	35	100	0	0	48	100	8.4
C-20	0	0	0	0	48	100	290
C-21	18	N/A	74	N/A	24	26	13
C-22	18	N/A	73	N/A	24	27	32
C-23	16	N/A	34	N/A	24	66	15
C-24	0	N/A	0	N/A	24	100	64
C-25	35	110	65	1	48	34	3.3
C-26	0	0	0	0	48	100	120

* All macropores 2.4 mm diameter.
† Applied over a period of 2 h.
‡ Top 2 cm of soil were disturbed before application.
§ Theoretical (%) = 100% – cells eluted before rain (%) – cells eluted after rain (%).

Effects of Macropores

The effect of macropores on bacteria movement is considered by examining those columns with similar conditions with and without the macropore. For all columns without a macropore, regardless of initial water content, soil type or rainfall volume added, no biotracer was ever detected in the effluent. In general, columns with a macropore did have biotracer detected in the effluent.

Columns C-1 through C-4 are all without macropores and rain while Columns C-7 and C-8 have similar conditions but with a single macropore. All of these have the silt loam soil and a high initial water content of 40 to 41%. With or without a macropore water began dripping from the column within 2 min of application and ceased within 1 h of application of the inoculum. A volume equal to the inoculum amount was collected from the columns with a macropore while those without a macropore produced amounts as low as 1/3 of the amount added.

Not only did the presence of the macropore affect the amount of biotracer in the effluent it affected the distribution of the biotracer throughout the column. Figure 3 shows the average distribution of the cells as a % of those applied with and without a macropore. Without a macropore under these conditions the cells are retained very near the surface of the soil. Indeed nearly all the biotracer recovered in the soil was recovered in the top layer. In contrast to those columns without the macropore, the biotracer was found to be much more distributed in those columns (C-7 and C-8) with a macropore. Figure 3 shows that the biotracer has been able to penetrate the entire column length, although the majority retained is nearer the surface.

In some cases values of over 100% of the total number of cells estimated to have been added were recovered. This is clearly a problem in enumerating the number of cells added or the amount in the column and is, unfortunately, unavoidable. For cases were the amount is greater than 100% in a particular layer they are shown as 100%.

When rain was added to the columns at this high water content changes are seen to the situation with and without a macropore. Under these conditions effluent appeared within 1 min of applying the rain and ended within 1 h

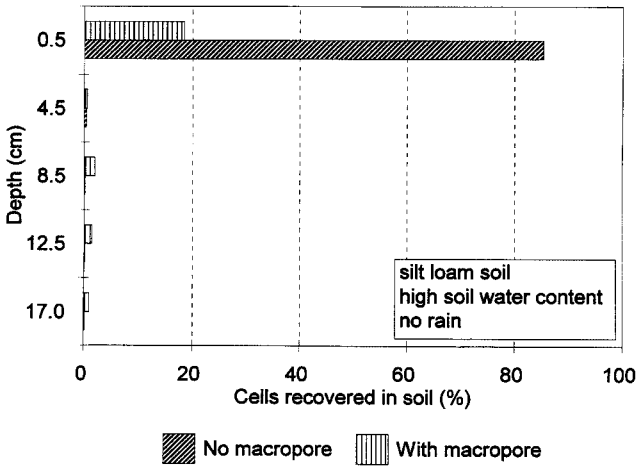

Figure 3–Distribution of cells in silt loam at high water content.

after the application of rain ended. Although significant amounts of the rain added appeared as effluent (110 and 130 mL) in these columns without a macropore, no biotracer was detected in the effluent. For the columns with a macropore the effluent after the inoculum alone again had significant amounts of cells eluted (70-83% of the total) prior to rain being added. Cells were also found in the effluent after the application of rain but these amounts were small (1%) due to the large numbers which had already passed through the system. The rain did, however, change the distribution of the bacteria within the column after the end of the experiment. Figure 4 shows the average distribution of cells at this water content with and without a macropore. With no macropore present the cells are distributed reasonably evenly over the top 3/5 of the soil column before dropping off. It is possible that with a longer period of rainfall or larger rainfall intensity that this distribution may have been extended further into the soil. The results in figure 5 also show that with a macropore the rain also helped to redistribute the cells throughout the column, although cells numbers are low throughout the column.

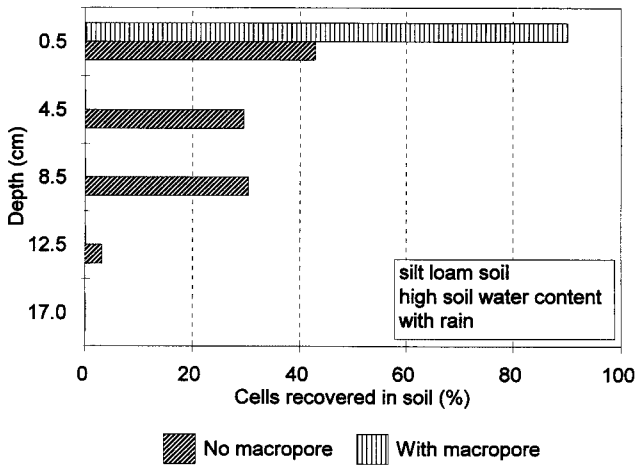

Figure 4–Distribution of cells in silt loam at high water content with rain.

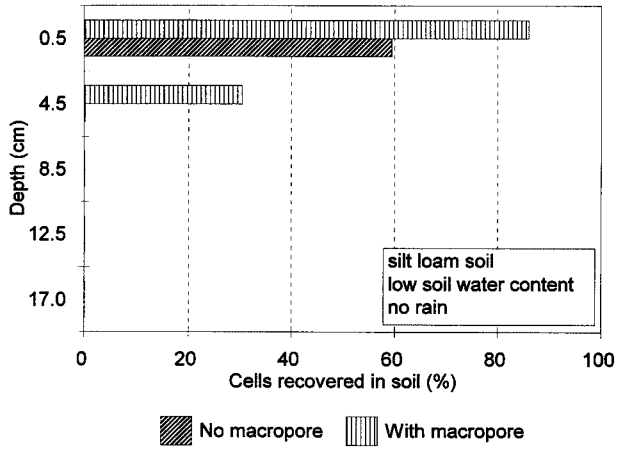

Figure 5–Distribution of cells in silt loam at low water content.

Effect of Initial Soil Water Content on Bacterial Movement

The soil water contents selected for the soils were those reported to occur at the locations where the soils were collected under wet and dry conditions (Reaume et al., 1994). The effect of initial water content can be seen by comparing the results from columns C-13 through C-16 at the low water content (9-10%) to the earlier columns at a high water content (40-41%). In this case only one experiment was conducted for each condition. For those soils at the low water content no effluent was ever noted under the conditions tested either with or without rain being added and with or without a macropore. This is due to the high suction pressures (approximately 10^5 mbar) at this water content. The possibility of the macropore having collapsed at this water content was investigated by doing some trials at higher rainfall rates and noting the rapidity that effluent appeared with a macropore compared to those without and also by during the dissection process noting the integrity of the macropore.

Without rain and without a macropore the distribution of bacteria in the column at both the low water content (column C-13, fig. 5) and at the higher water content (C-1 to C-4, fig. 3) are similar. In both situations nearly all the recovered cells were found in the top layer. The addition of a macropore to the dryer soil did not result in effluent either with or without rain. The distribution of the bacteria in the soil column for this situation is shown in figure 5. Without rain (C-15) all the cells recovered were in the top two layers. At the higher water content cells were found over the whole length of the column (fig. 3).

When rain was added to the low soil water content column without a macropore (column C-14, fig. 6) cells were detected down to the second layer of the soil column; whereas, in the higher water content soil columns bacteria were found over 4/5 of the soil column and were nearly uniform over the first 3/5 (fig. 4). The application of rain to the low water content soil with a macropore (Column C-16, fig. 6) helped to redistribute the cells so that they were recovered in four out of the five layers.

In summary, initial soil water appears to have a notable effect on bacterial movement through soils, especially in the presence of a macropore. The effect of a macropore in the soil on bacterial movement was not substantial when

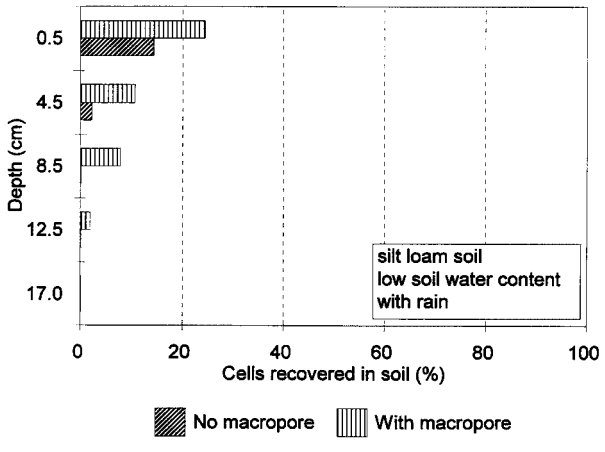

Figure 6–Distribution of cells in silt loam at low water content with rain added.

the soil was dry (9 and 10%), however the macropores were very effective at transporting bacteria when the soil was wet (40 and 41%).

Effect of Reworking Top Soil on Bacterial Movement

Columns C-11 and C-12 were packed with silt loam at a 10% soil water content and initially contained a single macropore of 2.4 mm diameter in each. The top 2 cm of soil in each column were disturbed in order to close the macropore inlet. This represents a possible method of reducing the macropores in the field prior to manure spreading. Inoculum solution was added on the top of each column. No outflow was observed from the bottom port. The dry soil absorbed the inoculum solution completely.

Post-experiment dissection of column C-11 (top soil disturbed) showed that all the cells recovered from the soil were in the top 3 cm (fig. 7). This result is similar to what has been found in column C-13 at a low soil water content without macropores and differs from the results of column C-15 (9% soil water content) which contained a fully open macropore. The cells reached the second layer from the top in column C-15 through the fully open macropore; whereas, with the top layer reworked they did not.

Rain applied on the top of column C-12 (top 3 cm of soil disturbed) was also totally absorbed by the soil. The biotracer distribution within the column (fig. 7) shows that infiltrating water carried a small percentage of the biotracer cells to the second layer of the soil column similar to the biotracer distribution in column C-14 (rain applied) which did not contain macropores. The biotracer distribution of cells in column C-12 differs from that in column C-16 (rain applied) where the biotracer cells reached lower layers through the fully open macropore.

It is clear that the reworked layer was an effective measure for reducing the extent of bacterial migration through the soil. The effect of macropores in a wet soil on bacterial movement was more noticeable than when the soil was dry. As a result, it is expected that if the soil water content was higher and macropores were present, blocking the inlets of these macropores by reworking the top soil would have been caused a more notable reduction in the extent of bacterial migration through the soil. This will need to be confirmed by additional work.

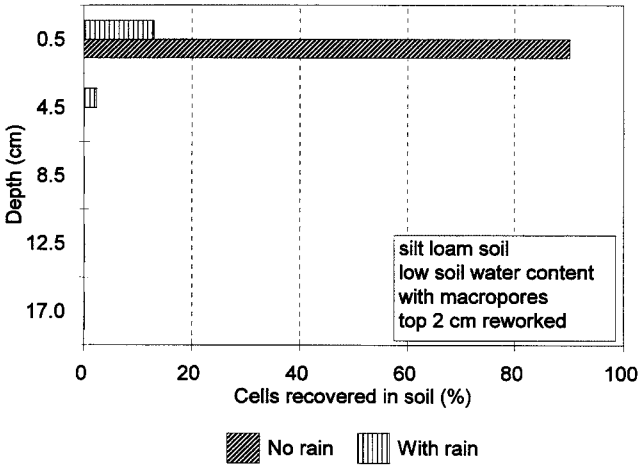

Figure 7–Distribution of cells in silt loam after reworking top layer.

Effect of Soil Type on Bacterial Movement Under Undersaturated Conditions

Columns C-17 through C-26 were packed with the loam soil under a range of macropore and soil water content conditions (table 2). The results shown in table 3 and in figures 8 through 11 were generally similar to the results of

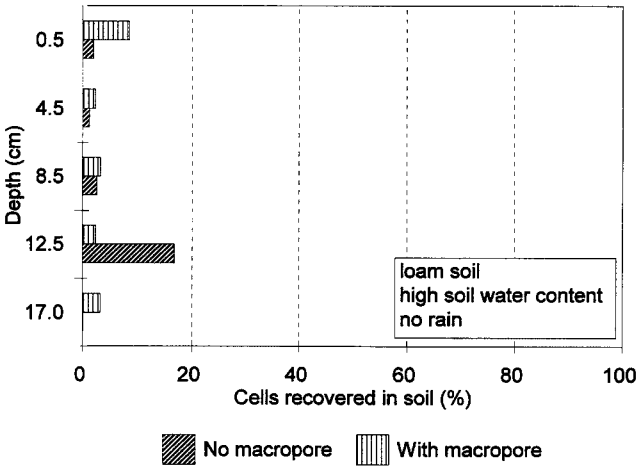

Figure 8–Distribution of cells in loam at high water content.

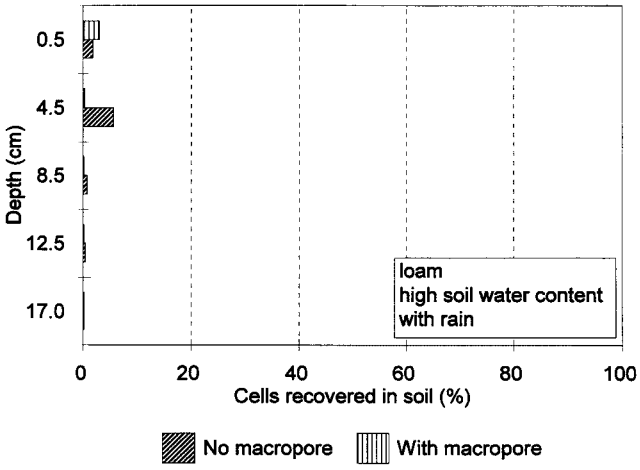

Figure 9–Distribution of cells in loam at high water content with rain added.

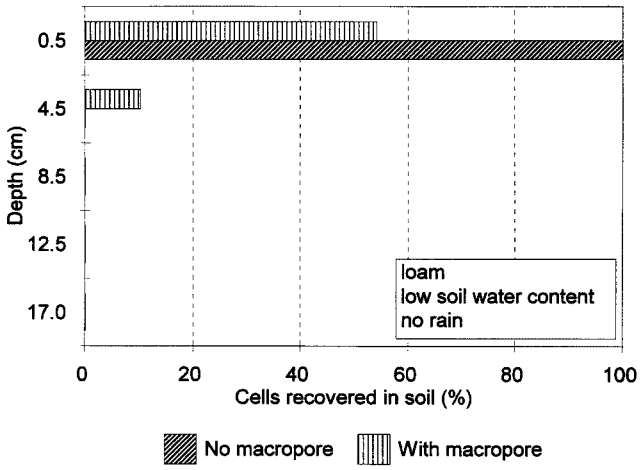

Figure 10–Distribution of cells in loam at low water content.

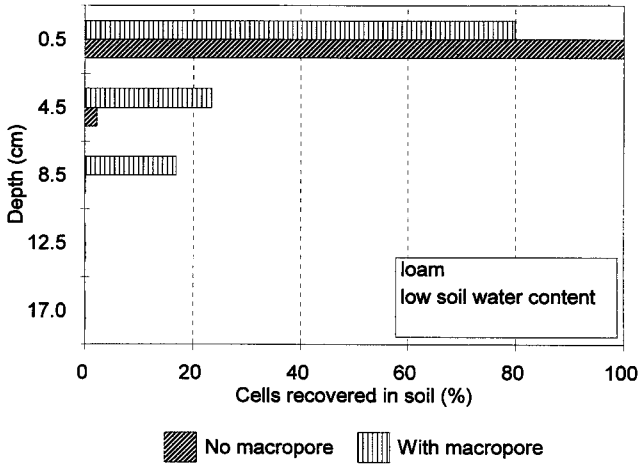

Figure 11–Distribution of cells in loam at low water content with rain added.

column experiments using silt loam under similar conditions. There was no notable effect of the soil type on bacterial movement through unsaturated soil. Under field conditions, the number and size of macropores have been observed to vary with the soil type as well as other factors (Reaume et al., 1994). Since the macropores created in the soil columns were controlled experimentally to a fixed number and size in both soil types, the effect of soil type was not clear and the results did not reflect its significance on the formation of macropores.

Finally, in all unsaturated soil column experiments, the percentages of biotracer recovery from the soils were higher when the soil was sampled 24 h after inoculation than found after 48 h. Decay of the biotracer cells appears to be the likely reason. Other experiments, not presented here (Abu-Ashour, 1994) showed that within these soils the bacteria survived with little or no die-off for 24 h but that after 24 h noticeable die-off occurs.

CONCLUSIONS

Soil water content and the presence of macropores have a pronounced effect on *E. coli* NAR movement through unsaturated soil columns. Macropores in wet soil significantly increase the ability of *E. coli* NAR to pass through for the conditions tested. Macropores allowed up to 83% of the cells to pass; whereas, without macropores no bacteria were able to travel through the soil. The extent of *E. coli* NAR movement through soil increases at higher soil water content. Macropores in dry soil increase the penetration of *E. coli* NAR into the soil in comparison to a dry soil without macropores. Reworking the top 2 cm layer of the soil blocks the upper portion of macropores and hence, reduces the extent of bacterial movement through soil. Infiltrating rainfall causes bacteria to penetrate deeper into the soil. If the amount and rate of rainfall is high enough after application, bacteria in the soil may reach the groundwater causing contamination, particularly if macropores are present. For the two soils tested, soil type does not appear to have an effect on bacterial movement through unsaturated soil columns prepared in the laboratory, although this may be a function of the way in which the macropores were created.

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