AN ABSTRACT OF THE THESIS OF

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in	Microbiology	presented onJune 29, 1979				
Title:						
	Hillslope Soils	q				
Abstra	ct approved:F	Redacted for Privacy				

Three isolates of Escherichia coli were labeled by their resistance to sodium azide and, separately, to novobiocin, nalidix acid, and tetracycline. The strains exhibited a high degree of persistence in the soil environment and were recoverable on strain specific media at levels within the 95% confidence interval of the numbers recovered on nonselective media. The E. coli strains were subsequently used to evaluate the events which would occur when a septic tank drainfield became submerged in a perched water table and effluent-borne bacteria escaped into the groundwater. Field experiments were conducted in a Dixonville soil and in a soil representing a transition between the Steiner and Hazelair soil series, by introducing the tracer strains into horizontal lines installed into the A., B, and C horizons of the soil profiles. Bacterial transport was evaluated by collecting groundwater samples from rows of piezometers (sampling six separate depth zones/row) located downslope from the injectionlines and enumerating the tracer organisms present in the water samples. In addition, surface water samples were collected from the transition site at the furthest upslope location of observed overland flow.

The Dixonville series site was located on a uniform 14% slope and bacterial penetration proceeded at a relatively constant velocity within a single layer throughout the site. Also, the maximum bacterial density in the groundwater, observed at each sampling distance downslope, was used to produce a mathematical relationship which described the overall decrease in numbers of organisms with increased distance through the soil. In the transition series, however, bacterial translocation patterns varied as the downslope flow was directed upward in the soil profile by hydraulic gradients and a restrictive clay layer. Also, a transition from predominantly matrix to flow to predominantly "pipe" flow occurred and a large volume of the water passing downslope was intercepted and conducted by these chan-In both experimental sites, subsurface bacterial transport progressed nels. at high apparent velocities and large numbers of organisms were carried substantial distances downslope. These results demonstrate the extent of incomplete septic-effluent treatment as these wastewaters migrate through saturated hillslope soils, and provides a basis for assessing the potential health hazards which were created.

Tracing Septic System Effluent Movement Through Saturated Hillslope Soils

by

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A THESIS submitted to Oregon State University

in partial fulfillment of the requirements for the degree of Master of Science .

Completed 29 June 1979 Commencement June 1980

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TABLE OF CONTENTS

			<u>P</u>	age
1.	INTRO	DUCTION	•	1
2.	LITER	ATURE REVIEW	•	3
	 2.1. 2.2. 2.3. 2.4. 	Bacterial Purification of Septic System Effluents Bacterial Transport Through Soils Bacterial Migration from Septic-Tank Absorption Fields Literature Cited	•	
3.		ITATIVELY TRACING BACTERIAL TRANSPORT IN ATED SOIL SYSTEMS	•	21
	3.1. 3.2. 3.3. 3.4. 3.5	Introduction Materials and Methods Results Discussion Literature Cited	• • •	24 29 33
4.	STRAI	PORT OF RESISTANCE-LABELED <u>ESCHERICHIA COLI</u> NS THROUGH A TRANSITION BETWEEN TWO SOILS IN OGRAPHIC SEQUENCE	•	49
	4.1. 4.2. 4.3. 4.4. 4.5.	Introduction and Methods Materials and Methods Results Discussion Literature Cited	•	51 54 57
5.	APPEN	DICES	•	70
	5.1. 5.2.	Appendix I		

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TRACING SEPTIC SYSTEM EFFLUENT MOVEMENTS THROUGH SATURATED HILLSLOPE SOILS

1. INTRODUCTION

Domestic wastewaters accommodate a miscellaney of human pathogenic microorganisms representing the bacteria, virus, protozoa, and helminth biological groups (Burge and Marsh, 1978). In addition, these infectious agents are widely distributed in all manner of waste effluent and are commonly present in large populations. Therefore, untreated domestic wastes embody a substantial health hazard, and proper wastewater purification and disposal is a primary concern (Hoadley and Goyal, 1976). Septic-tank soil-absorption treatment systems serve as the principal disposer of waste effluent to the soil environment (Geraghty and Miller, 1978). The Office of Water Supply (1977), in a U. S. Environmental Protection Agency report to Congress estimates that three billion cubic meters of domestic wastewaters annually enters the soil subsurface. Soil disposal of septic effluent serves to replenish groundwater tapped by individual and public water supply wells, and soil percolation is required for bacterial purification of drainfield effluent. However, many shallow groundwater supplies are displaying increased pollution levels from contaminated recharge waters, and many investigators (Brooks and Cech, 1979; Hackett, 1965; Maynard, 1969; Robeck, 1969; Sandhu et al. 1979; Wall and Webber, 1970) attribute this decline to indiscriminate use of septic tank systems in soils unsuited for adequate domestic waste purification. In addition, septic-tank soil-absorption systems are not confined to rural areas of low population density. Across the U.S. there are four counties (Nassa and Suffolk, NY; Dade, FL; and Los Angeles, CA) each with more than 100,000 housing units served by septic tanks and there

are an additional 23 counties with more than 50,000 household installations (Geraghty and Miller, 1978).

The development of new homesites beyond municipal boundaries and sewerage facilities is -- by regional land use regulations -- restricted to the marginal agricultural lands on the low hills surrounding the Willamette Valley floor. Yet, the soils in these upland positions are on slopes of varying degrees and often develop seasonally perched water tables over the less permeable subsoil and bedrock formations. If these elevated water tables rise above the septic system absorption trenches located on a hillside, potentially pathogenic microorganisms may pass directly out of the soil treatment zone and into the rapidly moving groundwater. Therefore, a high percentage of these upland soils do not meet minimum standards for the subsurface disposal of sewage from standard septic-tank drainfield systems as set by the Oregon Department of Environmental Quality. However, these standards are developed empirically and little is known about the fate of fecal populations introduced into soils during periods of heavy rain and elevated water tables. This thesis is a report on the investigations tracing effluent-borne bacterial populations through selected hillslope soils to assess the health hazards involved with septic system installations in Willamette Valley terrace soils.

2. LITERATURE REVIEW

2.1 Bacterial Purification of Septic System Effluents

Septic-tank soil-absorption treatment systems serve as the conventional means for the disposal of domestic wastes in areas lacking centralized sewerage facilities. Households located in these outlying areas also generally do not receive municipally purified water and, therefore, rely upon individual water supply systems collecting untreated groundwater as a potable water source. In most cases, these individual wastewater treatment and water supply systems are located on the building site, adjacent to each other, and theoretically function as a small hydrologic loop. Therefore, in order to maintain drinking water quality and protect the health of household members, the on-site wastewater treatment process must mineralize the organic matter and remove the toxic and infectious agents found in domestic wastewaters and restore the effluent to its previous potable-water quality.

On-site sewage treatment systems are composed of two individual units, the septic tank which functions under anaerobic conditions, and the percolation field which in unsaturated soil operates aerobically. The septic tank is designed to slow the movement of raw sewage and promote the removal of solids either by settling or liquefaction, and the organic load and fecal populations are reduced only to a limited extent. As an example of the minimum purification afforded by passage through the septic tank, Ziebell <u>et al</u>. (1974) enumerated selected fecal populations in septic tank effluents from five systems and observed mean population densities of: $3.4 \times 10^6/100$ ml for total coliforms, $4.2 \times 10^5/100$ ml for fecal coliforms, $3.8 \times 10^3/100$ ml for fecal streptococci, and $1.0 \times 10^4/100$ ml for <u>Pseudomonas aeruginosa</u>. Also, salmonellae species were detected in 59 percent of 17 different septic tank pump-out sludges and BOD₅ reductions of only 30-50 percent were noted subsequent to passage of wastewaters through the tank. Therefore, distribution of the septic tank effluents into unsaturated soil is necessary to complete the treatment process, and if the entire system is to function properly, the soil delivers the bulk of the wastewater treatment and must act as a physical filter, chemical reactor, and biological transformer (Goldstein et al. 1972).

The single most comprehensive study on the soil absorption of septic system effluent was conducted by the Small Scale Waste Management Project Group at the University of Wisconsin. In the report of investigations on the purification efficiency of 19 subsurface soil disposal systems, Bouma et al. (1972) concluded that septic systems which exhibited proper hydraulic functioning also served to purify the septic effluent. Bacterial filtration was determined by dissecting a septic drainfield system and enumerating the indicator organisms present in the soil at various distances below the drainfield trench. The large populations of total coliforms, fecal coliforms, and enterococci present in the septic tank effluent were reduced to levels associated with control soil samples within two feet below the percolation trench. In addition, the most abrupt population decreases occurred in the so-called biological mat or clogged zone located at the interface of the drainfield trench and the soil. The importance of this clogged zone in the proper functioning of septic tank drainfield systems was emphasized in their report: Since this layer of only a few centimeters deep was highly efficient in trapping and holding bacterial species present in the wastewater it served as the primary barrier to subsurface escape of fecal organ-However, if the mat developed too heavy and restricted the hydraulic isms.

functioning of the system, then the effluent could not enter the soil and became ponded in the trench and subsequently spilled out onto the soil surface. Shallow wells were installed strategically in and near the absorption fields, and groundwater samples were assayed for indicator organism density and total bacterial counts. Although total bacteria counts were higher in samples taken from wells adjacent to the absorption field and downfield in the direction of groundwater flow, in most cases no indicator organisms were detected from these samples. Thus these results indicated that groundwater collected adjacent to these treatment systems rendered minimal disease hazard.

The transition from high to low numbers of coliform and streptoccal indicator bacteria with depth below the distribution trench was also observed by Ziebell <u>et al</u>. (1974). Upon dissection of a "typical" absorption field located in sandy soil, indicator bacteria enumerations indicated that the high levels of organisms present in the septic tank effluent were increased approximately five-fold in the soil of the clogged zone at the base of the trench. However, soil samples collected 30 cm lateral to, and 8 cm below the trench produced indicator values 100-fold less than the septic tank effluent, and samples collected 30 cm lateral to, and 38 cm below the trench yielded values 3000-fold less than the tank effluent enumerations. Therefore, in a well functioning absorption field the indicator and potential pathogenic bacteria were almost completely removed after a relatively short travel through unsaturated soil.

Viraraghavan and Warnock (1976) constructed an experimental tile line, into which household septic tank effluent was diverted, to further investigate the efficacy of the soil-absorption system. Pourous cup sampling probes were placed in a row approximately 0.76 m adjacent to and parallel with

the test tile line, and groundwater sampling wells were installed at increasing distance down gradient. The results of weekly lysimeter samplings over a nine-month interval indicated, as observed earlier, that unsaturated soil removed a high percentage of the indicator organisms present in the sewage effluent. Also, groundwater samplings indicated that the organisms which reached the row of wells exhibited a decreasing trend with increased distance from the septic tile, although absolute values were not presented in this report. During spring snowmelt periods the water table was observed to approach near the soil surface, and the wastewater treatment efficiency was reduced.

More recently, Brown et al. (1979) reported the results of a two-year study on the migration of effluent populations into undisturbed soils of 7.6%, 41%, and 80% sand. The soils were enclosed in lysimeters of 153 by 204 by 180 cm deep, and septic effluent was metered equally into a 15 cm diameter drain tile enclosed in a 30 cm square layer of gravel located 30 cm below the soil surface of each lysimeter. Weekly soil leachate samples were collected through porous cups located 120 cm below the septic tile line and the fecal coliform density was determined in the leachate samples. Of the 545 composite leachate samples analyzed, only 26 were positive for fecal coliforms with the majority of the positive results occurring near the beginning of effluent application to the tile lines. In addition, the lysimeters were dissected after varying periods of time and soil samples were collected from an array of points located adjacent to and below the trench. An inventory of the fecal coliforms present in the soil samples demonstrated that indicator populations decreased greatly with increased distance through the soil profile, and reductions of 10- to 100-fold per 5 cm distance were not uncommon. In some cases, however, fecal coliforms exhibited higher

counts in isolated locations distant from the tile line, and thus appeared to move through the soil or survive in root channels and soil cracks.

An interesting observation was reported in a note by Dazzo and Rothwell (1974) where porcelain porous cup soil water samplers were examined to determine their validity in obtaining samples for fecal coliform analysis. When fresh cow manure slurry was drawn through porous cups, the fecal coliform numbers were reduced 100- to 10,000,000-fold as compared with the external slurry; and 65 percent of the cups yielded coliform free samples. Hence, the authors concluded that porous cup samplers did not yield valid water samples for fecal coliform analysis. However, the two previous papers (Viraraghavan and Warnock, 1976; Brown <u>et al</u>. 1979) included results which relied upon porous cup samplers of similar construction, pore size, and conductivity and therefore the validity of this portion of their results must be questioned.

In the studies by Bouma, Ziebell, Brown and their coworkers, absorption field dissection results produced a similar distribution of the fecal indicator populations in the unsaturated soil beneath the drainfield trench. This distribution delineates a soil transition zone from high populations in the clogged zone which were equal to or exceeding septic-tank effluents, to population levels of deeper depths that were associated with unpolluted soil. In addition, the transition zone indicated the soil depth required for retention of the wastewater microorganisms and, hence, the depth of soil necessary for purification of the effluent. Based on this line of reasoning, therefore, approximately one to three feet of soil below the base of the drainfield trench was adequate for complete bacterial purification of septic effluents provided the soil was both permeable to effluent flow and adequately restrictive to form a clogged zone. Therefore, published guidelines for the installation of conventional septic systems (U.S. Public Health Service, 1967) which recommend from four to five feet of "suitable" soil as an adequate zone for the protection of groundwater, falls well in line with experimental data.

2.2 Bacterial Transport <u>Through</u> Soils

The soil depth required for bacterial purification of septic effluents is derived in the preceeding section from a somewhat limited body of information. Indeed, research investigations on the purification efficiency of a much wider variety of soils subjected to many different waste application techniques results in a less close agreement on the degree of bacterial retention by soil. The land application of human wastes includes topics from the disposal of raw excreta in pit latrines to application of oxidation pond effluents into rapid infiltration basins. In addition, investigations of these various disposal methods serve as the large body of knowledge necessary to further define the conditions in which wastewaters are purified by land application, and as such are the subject of several excellent reviews (McGauhey and Krone, 1967, Romero, 1970; Gerba <u>et al</u>., 1975; Hutchinson, 1974). Table 1 is adapted from these reviews and outlines the content of a variety of studies on land application of domestic wastes.

Although a wide diversity of disposal methods were studied in the papers reviewed in Table 1, and large variations were observed in the results reported in these papers, some pertinent conclusions are ascertained from this information. 1) Coliforms and other microorganisms move only a few feet with the percolating waters in unsaturated soil layers and generally a few hundred feet under saturated flow conditions. 2) Under all soil water conditions, the degree of bacterial retention by the soil is inversely proportional to the size of the component particles in the unstructured

Table 1. A summation of the results of selected studies on the transport of bacteria through the solumn in relation to land application of domestic wastewaters.

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Nature of Pollution	Organ i sms	<u>Medium</u>	Maximum Distance Traveled	Time of Travel	Reference
Sewage trenches intersecting groundwater	<u>Bacillus coli</u>	Fine sand	19.8 m (65 ft)	27 wks	Stiles and Crohurst (1923)
Sewage trenches intersecting groundwater	Coliforms		70.7 m (232 ft)		Warrick and Muegge (1930)
Sewage in pit latrine inter- secting groundwater	<u>Bacillus coli</u>	Fine and coarse sand	24.4 m (80 ft)		Caldwell (1937)
Sewage in bored latrine inter- secting groundwater	Bacillus coli	Sand and sandy clay	10.7 m (35 ft)	8 wks	Caldwell and Parr (1937)
Sewage in pit latrine inter- secting groundwater	Bacillus coli	Fine and medium sand	3.1 m (10 ft)		Caldwell (1938)
Primary and treated sewage in infiltration basins	Coliforms	Fine sandy loam	0.6-4 m (2-13 ft)		Butler <u>et al</u> . (1954)
Diluted primary sewage injected subsurface	Coliforns	Aquifer	30 m (98 ft)	33 hrs	McGauhey and Krone (1954)
Canal water in infiltration basins	<u>Escherichia</u> coli	Sand dunes	3.1 m (10 ft)		Baars (1957)
Subsurface injection	Enterococci		15 m (44 ft)		Fournelle (1957)
Secondary sewage in infiltration basins	Col i fornis	Sandy gravels	0.9 m (3 ft)		McMichael and McKee (1956)
Tertiary treated wastewater in percolation beds	Fecal coliforms and fecal streptococci	Coarse gravels	457.2 m (1500 ft)	15 da	Merrell (1967)
Primary sewage injected subsurface	Coliforms	Sand and pea gravel aquifer	30.5 m (100 ft)	35 hr	Krone <u>et al</u> . (1968)
Secondary sewage injected sub- surface	Fecal coliforms	Fine to coarse sand aquifer	30.5 m (100 ft)		Wesner and Baier (1970)
Tertiary treated wastewater in percolation beds	Coliforns	Sand and gravel	830 m (2723 ft)		Ana'ev and Demin (1971)
Inoculated water and diluted sewage injected subsurface	Bacillus stearothermophilis	Crystalline bedrock	28.7 m (94 ft)	24-30 hrs	Allen and Morrison (1973)
Tertiary treated wastewater in infiltration basins	Coliforms	Fine to medium sand	6.1 m (20 ft)		Young (1973)
Secondary sewage in infiltration basins	Fecal coliforms	Fine loamy sand to gravel	9.1 m (30 ft)		Bower <u>et al</u> . (1974)
Primary sewage in infiltration basins	Fecal streptococci	Silty sand and gravel	183 m (600 ft)		Schaub and Sorber (1977)

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matrix. 3) The physical straining or filtration of organisms at the soil surface appears as the main limitation to travel through soils, and sedimentation of bacterial clusters occurs throughout the zone of saturated flow. 4) Adsorption is a factor in the retention of bacteria by soil and contributes increasing activity in unstructured soils which contained clay. 5) Death of the microorganism plays an important role during the longer retention periods only. Thus, in these studies, both physical removal and biological antagonism characterize the change in bacteriological quality of wastewaters percolating through the soil.

2.3 Bacterial Migration from Septic-Tank Absorption-Fields

Precipitation falling to the earth was shown by Geldreich et al. (1968) to contain less than one coliform per 100 ml sample, possibly derived from dust, pollen grains, or insects. Yet, once these waters reached the earth, contamination occurred from numerous sources in the urban and rural environments of man. Septic systems submerged in stormflow saturated soils represented a potentially large source of fecal contamination, however, the magnitude of these additions were only assessed to a limited extent. Reneau et al. (1975) investigated the interaction of 1) soils of various suitability for septic tank effluent disposal and 2) seasonal precipitation creating perched water tables on the bacterial contamination of a watershed from septic sources. The small watershed covered an area of 80 ha and contained within its boundaries approximately 30 single-family dwellings and an elementary school. The watershed soils were classified according to their suitability for septic system effluent disposal as suitable, marginal, and unsuitable; and of the total setpic systems placed in this area, 17 percent were in suitable soil, 41 percent were in marginal soil, and 42 percent were in unsuitable soil. Nine surface and seven subsurface sampling sites

were established throughout the watershed and samples were collected periodically during the first half of the years 1972 and 1973 when streamflow was continuous and seasonal water tables were present. The bacteriological contamination of the watershed from failing septic systems was evaluated by determining the total fecal coliform density in the water samples. Bacterial enumeration of a surface water sample which was subjected to minimal contamination yielded fecal coliform MPN ranging from 930 - 3 /100 ml sample, whereas a surface sample in direct contact with septic effluent contained from 24,000,000 to 5,300 fecal coliform MPN/100 ml. Also, the surface sample not in contact with any effluent source had a mean fecal coliform MPN of 230/100 ml while the watershed outflow produced a mean fecal coliform MPN of 5,600/100 ml sample. Subsurface water samples generally displayed a decrease in total and fecal coliform MPN with increased distance from individual septic-tank soil-absorption installations. Contamination of the watershed outflow was particularly noticeable during or following a rainstorm when septic tank effluent was flushed by movement of runoff water into surface flow. Thus, this watershed containing septic tank systems installed in unsuitable soil produced marginal bacteriological quality runoff waters.

In an effort to further resolve the processes involved with the failure of septic systems by the subsurface escape of fecal organisms, Reneau and Petry (1975) expanded on a prior observation that coliform and fecal coliform numbers decreased with increasing subsurface distance from a septic drainfield. Three septic tank drainfields located on a Varian sandy loam, a Goldsboro loamy sand, and a Beltsville sandy loam were each instrumented with a series of piezometers at selected distances from the drainfield in the direction of groundwater flow. The soils were located on the western edge of the coastal plain in Virginia and were considered marginally suitable

for the sanitary disposal of domestic wastes. In addition, these soils were underlain by water restrictive layers and developed high water tables during periods of seasonally high rainfall. Total and fecal coliforms present in the groundwater were enumerated and the results indicated that although there was a large reduction in bacterial numbers with horizontal distance from the drainfield, considerable populations were present at all sites even at the further distances sampled. Thus, at 28 m from the drainfield in the Beltsville soil, up to 430 fecal coliform MPN/100 ml were recovered; and in the Goldsboro soil 2,900 fecal coliform MPN/100 ml were recovered at 13.5 m distance; with 110,000 fecal coliform MPN/100 ml recovered at 6.1 m from the drainfield in the Varian soil series. In all cases, the maximum coliform density was observed in the saturated zone directly above the water restricting layers present in these soils and abrupt decreases were observed below these layers. Since few total and fecal coliform were observed at the deeper depths in these field sites, and then only on a limited number of sampling dates, the authors presumed that the groundwater supply was not becoming contaminated by vertical movement of fecal bacteria. Yet, very large surface areas were required to remove the fecal indicator organisms from effluent as it penetrated horizontally through the soil, and all possible routes of groundwater recharge were not investigated.

Artificial drainage has improved the hydraulic functioning of septic systems located in soils underlain by slowly permeable soil horizons and subjected to seasonally high water tables. Yet health guidelines which specify the minimum distance between the disposal area and intercepting tile lines or drainage ditches were established intuitively with little data support. To remedy this situation, Reneau (1978) investigated the influence of artificial drainage on the penetration of total and fecal coliforms into wet tile drained soils, and drew conclusions to assess the health

hazard involved. Sampling wells were installed (at three household systems) to depths of approximately 1.5 m in the area between the disposal field and the drainage tile. The wells were arranged in two rows positioned perpendicular to the septic system drainfield and were composed of four wells per row. The four wells in each row were, additionally, placed at increasing distance from the disposal trench with the final well located on the opposite side of the drainage tile to collect control groundwater samples. The drainage tile was located 11.9 m from the disposal trench in site 1, 11.6 m at site 2, and 19.2 m at site 3. Water samples collected during high water table periods displayed a rapid decrease in total and fecal coliforms with increased distance from the disposal area. However, waters in the drainage tiles contained considerably greater total and fecal coliform populations than the control well samples, indicating that horizontal penetration of bacteria in saturated soil could continue for large distances. In addition, an adjacent stream receiving the tile flow contained moderately high concentration of total and fecal coliforms which reflected the influx of septic effluent bacterial populations. Based on the data from this study, the author constructed several hypothetical situations to demonstrate the discharge of fecal organisms into receiving streams from various drainage systems, and emphasized the necessity for maintaining an adequate distance between the disposal area and intercepting tile lines.

Viraraghavan (1979) positioned a row of 2 m deep groundwater sampling wells as distances of 0, 3, 6, 9.2, 12,2, and 15.2 m downgradient from the end of an experimental septic tile line. Viraraghavan and Warnock (1976) utilized this same experimental set-up in their previously reported paper and noted efficient removal of fecal populations from septic effluents during periods of low water tables. However, the experimentation discussed in this later paper was conducted during periods of elevated watertables when only 15 cm of unsaturated soil occurred below the drainfield trench. The results of enumeration of total coliforms, and fecal coliforms, and fecal streptococci from groundwater samples indicated that these populations experienced a decline with distance from the septic tile line. Yet, the microorganism level at a horizontal distance of 15.2 m from the tile line were between $10^2 - 10^4/100$ ml sample.

The papers of Reneau and Viraraghavan established that fecal populations present in septic effluent have the potential to penetrate saturated soil, and the fecal coliform density in groundwater sampled at increasing distances from functioning systems exhibited an initial rapid decrease. However, the flow processes transporting microorganisms through the soil were not investigated in these static system studies.

An early attempt to study the dynamics of the subsurface migration of fecal organisms from failing septic systems was conducted by Hagedorn <u>et al</u>. (1978). In these studies, antibiotic resistant strains of <u>Escherichia</u> <u>coli</u> and <u>Streptococcus faecalis</u> were injected as a tracer slug into gravel pits positioned in a Veneta soil series of 2 percent slope. Sampling wells were located radially around the pit at 0.5 and 1 m distances, and additional wells were located at up to 15 m from the pit in the direction of groundwater flow. The characteristics of tracer penetration through the saturated soil profile was determined by enumerating the resistant organisms present in the groundwater samples at successive intervals after injection. The results demonstrated that tracer organisms were intercepted and carried from the gravel pit by saturated flow since successive peaks of bacterial numbers at increasing distance from the pit appeared only after periods of precipitation creating elevated water tables. Although the bacterial density

peaks were lessened at increasing sampling distances from the pit, $10^3 - 10^4$ tracer cells per milliliter were detected at even the furthest sampling distance. Also, a second rainfall event produced a secondary set of peaks in the enumeration of tracer with time, however, the bacterial numbers were somewhat less. In addition, the organisms survived in the soil water at relative high concentrations for up to 32 days after introduction into the soil.

To conduct a more refined study of the subsurface escape of fecal microorganisms from septic system absorption fields, Rahe et al. (1978) established simulated drainfield installations on natural hillslope soils, and monitored downslope bacterial penetration through saturated soil. The experimental sites were constructed on soils of the Hazelair and Dixonville series with the Hazelair soil characterized as moderately deep and poorly drained with a heavy clay layer starting at 80 cm below the surface, and the Dixonville soil described as moderately deep, well drained, and underlain by fractured saprolite and bedrock. Antibiotic-resistant Escherichia coli were introduced into saturated soil via a horizontal injection line representing a drainfield tile, and groundwater was collected at successive periods of time from an array of sampling piezometers located at various depths and distances downslope. In both sites, the flow of tracer bacteria through the soil was characterized by a rapid penetration of the saturated soil with high numbers of organisms contributing to the peak flow. The bulk of bacterial transport through the soil occurred in specific depth-zones with the primary transmitting zone located directly above the water restricting layer in the Dixonville soil series and near the surface layers in the Hazelair soil. The results indicated that pathogenic species potentially present in septic effluents are disseminated through the soil in high numbers, over large

distances, and after relatively short periods of time; and thus face minimal reductions from death while maintaining a high degree of virulence.

The studies of Hagedorn and Rahe have examined some of the initial processes involved with the transport of microorganisms through saturated soils. Their results demonstrated that water flow in saturated soils was responsible for the translocation of microorgamisms. Additionally, transport was shown to occur at rapid rates, in high numbers, and through specific zones in the saturated soil. The reports which follow further investigated these phenonema in additional soil series to more thoroughly describe bacterial transport through hillslope soils as a dynamic physical process.

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3. QUANTITATIVELY TRACING BACTERIAL TRANSPORT

IN SATURATED SOIL SYSTEMS

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3.1 INTRODUCTION

One-third of all waterborne disease outbreaks reported in the United States from 1971-1976 were traced to the consumption of water from untreated groundwater sources (Craun et al., 1976; Craun, 1978). In addition, a 1970 nationwide projection for marginally treated public and private water supplies estimated that approximately 60 million consumers relied upon the absence of microbial pathogens in groundwater (Allen and Geldreich, 1975). Septic-tank/soil-adsorption disposal systems ranked highest in total volume of wastewater discharged directly into the groundwater and were also the most frequently reported source of groundwater contamination (Geraghty and Miller, 1978). However, properly installed and correctly functioning septic systems do not contribute to pathogen contamination of groundwater supplies because the soil, in this case, serves as an efficient filtering and adsorptive media. Consequently, fecal organisms are retained in the treatment zone directly adjacent to the drainfield trench where aerobic conditions contribute to rapid destruction of effluent-borne enteric pathogens (Bouma et al., 1972; Viraraghaven and Warnock, 1976; Zibell et al., 1974).

The purification of drainfield effluent during soil percolation is the optimal condition necessary for maintenance of groundwater quality, yet improper site selection and/or poor installation can result in a malfunctioning of this soil percolation system. The manifestation of soil adsorption dysfunction includes: surfacing of untreated effluent, incomplete treatment due to anaerobic conditions, or the subsurface escape of fecal organisms from the treatment zone; all of which create potential health hazards (Reneau and Pettry, 1975; United States Environmental Protection Agency, 1973). In this study antibiotic/azide resistant strains of <u>Escherichia coli</u> were used to assess the hazard potential associated with the subsurface escape of pathogens from the treatment zone. In these circumstances, seasonally high water tables inundate the soil adjacent to the drainfield trench and rapid water movement transports the organisms with little filtering or adsorptive effects (Rahe et al., 1978).

The use of resistance-labeled organisms for natural ecosystem analysis is well documented (Danso et al., 1973; Habte and Alexander, 1975; Hagedorn et al., 1978; Rahe et al., 1978) although, in these studies, few safeguards were taken to insure that the observed results were not the consequence of spontaneous reversion of the organisms used or reduced recovery on selective media. Therefore, the <u>E</u>. <u>coli</u> strains used in our research were suspended in diffusion chambers in situ to determine survival, apparent reversion and selective recovery rates over a hypothetical maximum experimental period.

3.2 MATERIALS AND METHODS

The bacteria used in this study were isolated from the Corvallis, Oregon Municipal Wastewater Treatment Plant. Purified cultures were identified as <u>Escherichia coli</u> by growth and gas production in EC broth (Difco) after 24 h at 44.5 C, characteristic colonies on Difco Eosin Methylene Blue (EMB) agar, and IMViC patterns of [+ + - -]. Spontaneously-resistant mutants were selected with the gradient plate procedure (Miller, 1972) and the three strains used in this study were all resistant to 100 µg/ml sodium azide (Azi) and, individually, to 100 µg/ml novobiocin (Nov), nalidixic acid (Nal), and tetracycline (Tet). These test strains were therefore designated: Nov^r Azi^r <u>E</u>. coli, Nal^r Azi^r <u>E</u>. coli, and Tet^r Azi^r <u>E</u>. coli.

Survival Recovery Evaluations

The survival characteristics of the <u>E</u>. <u>coli</u> strains were determined using membrane filter chambers (McFeters and Stuart, 1972). Cells were grown in the nutrient constituents of M-FC broth (Difco) for 16-18 h at 37 C, washed twice by centrifugation (4000 x g for 10 min) in a filtersterilized soil extract, and diluted to the desired population density. The chambers, bounded by Millipore membrane sheets (HAWP 304 F0, 0.45 μ m pore size, Millipore Corp., Bedford, MA) were filled with the bacterial suspensions, submerged into a beaker of freshly collected groundwater and, after a one hour equilibration period, the first sample was removed. The chambers were then transported to a field site and immersed (in a previously constructed trench) into the water table so that subsurface water movement provided a constant exchange of the soil solution bathing the cells. Samples were taken every twelve hours for the 84 h duration of the experiment and one-half ml samples were removed from each chamber and immediately diluted in cold phosphate buffer (American Public Health Association, 1975) with 0.1% peptone. The temperatures recorded during the sampling periods ranged from 9 to 13.5 C.

Enumeration was initiated within 15 min of sampling and a comparison of the effects of using selective vs nonselective media was determined by parallel platings from the same dilutions. A modification of the single drop plating technique (Miles and Misra, 1938) was used to determine total surviving cells. Five 0.025 ml drops were dispensed (Oxford Micro-Doser Pipette) on pre-dried plates of Tryptic Soy Agar (Difco) with 0.3% yeast extract (TSYA), and the strain specific (SS) M-FC agar containing rosolic acid, 100 µg/ml Azi, and 100 µg/ml of the appropriate antibiotic (SS-MFC). All plates were incubated for 12-14 h at 37 C and colonies were counted with the aid of 7x to 45x magnification. The reduction in bacterial numbers on the SS-MFC media as compared to TSYA was taken to be a measure of the apparent reversion of an organism to a non-resistant state and/or the inability of a cell to tolerate selective media. This assessment was termed the selective recovery rate.

A second membrane filter chamber experiment was conducted in the laboratory with cell suspensions prepared as described. The three <u>E</u>. <u>coli</u> strains were combined and diluted 1000x in both a sterile and nonsterile soil solution and each suspension was placed in a single filter chamber. The chambers were submerged in a beaker of recently collected groundwater and the contents of the beakers were mixed continuously while the temperature was maintained at 15-18 C. Samples from both chambers were collected daily for four days with the bathing solution changed after each sampling. Enumeration of the individual strains was

accomplished by the single drop technique on SS-MFC media. Also, an identical sample volume was passed through a membrane filter (Millipore HAWG 025 00) and placed on SS-MFC media (1.0% agar). By comparing bacterial counts derived from these two techniques the recovery rate by membrane filtration was obtained.

Site Description and Water Table Characteristics

The experimental site was located on a hillslope soil (upper backslope position) in Benton Co., Oregon, with a 14% north east facing relief. The surface horizon was a dark brown silty clay loam which overlaid a dark reddish brown silty clay to a depth of 34 cm. The subsoil was a dark brown heavy clay from approximately 35 to 60 cm in depth. This was underlain by a substratum of reddish and yellow-brown saprolite which graded into CaCO₃ cemented sandstone at 100 cm. The thickness of the saprolite varied from a few cm to 70 cm over the experimental site and the depth to lithic sandstone varied from 70 to 150 cm from the surface. The soil series was described as a moderately welldrained transition between the Philomath and Dixonville series.

The elevated water table varied in depth with distance downslope from 55 cm at the furthest upslope sampling line to 85 cm at the furthest downslope sampling position. The height of the perched water table in the soil profile was well within the range of the water table monitored at this location during the winter of 1975-76 by Hammermeister (D. Hammermeister. 1978. Water and Anion Movement in Selected Soils of Western Oregon. Ph.D. Thesis, Oregon State University, Corvallis).

Bacterial Translocation Studies

Transport of Escherichia coli through the soil was measured by introducing the resistance-labeled strains into the soil profile through injection lines located in depths which generally corresponded to the A, B, and C horizons of the soil. The organisms were subsequently recovered from piezometers located at various distances and depths downslope from the injection lines. Details on the installation of the experimental site were described previously by Rahe et al. (1978). Briefly, three 9.15 m injection lines were installed (0.5 m apart) at 12, 30 and 60 cm depths in the experimental site with the 12 cm deep line occupying the furthest downslope position while the 60 cm deep line was installed upslope from the other two. Thirty-six piezometers were installed on the experimental site to depths of 12, 30, 60, 110, 150 and 200 cm in lines at distances of 2.5, 5.0, 10.0, 15.0 and 20.0 m downslope from the A horizon injection line. The 12 and 30 cm deep piezometers sampled a 10 and 15 cm zone in the soil while the remaining piezometers each sampled a 20 cm zone. The installation sequence of the six piezometers at each sampling line was randomized. One additional sampling line which served as a control was installed 2.5 m upslope from the C horizon injection line.

Inoculum for the field experiments was prepared identically for each strain. A 1200 ml volume of the nutrient constituents of M-FC broth was inoculated (10% v/v) and incubated for 14-16 h at 37 C. The optical density (Bausch & Lomb Spectronic 20,520 nm) of a 10 fold dilution of each culture was measured for determination of the initial inoculum levels and the individual cultures were divided among six 500 ml aspirator bottles for transport to the field and injection into the soil. Inoculation of the field site was accomplished by attaching an aspirator bottle to each of the injection line access ports. The Nov^r Azi^r <u>E</u>. <u>coli</u> was injected into the A horizon, the Nal^r Azi^r <u>E</u>. <u>coli</u> was injected into the B horizon, and the Tet^r Azi^r <u>E</u>. <u>coli</u> was injected into the C horizon of the experimental site.

Samples were vacuum extracted through the downslope piezometers at 20, 40, 60, 90, 120, 150, 190, 230, 290, and 350 min post injection and control samples collected just prior to injection (designated time zero). Samples were recovered in 250 ml bottles, transported to the laboratory and stored no longer than 24 h at 4 C before analysis. All samples from the field experiment (350 min duration) were collected before examination of the samples was initiated. Enumeration of the tracer bacteria present in each water sample was accomplished by the membrane filtration method described above.

Bacterial Survival and Recovery

The resistance-labeled <u>E</u>. <u>coli</u> strains possessed a high level of persistence in the physiochemical environment of the soil solution as determined by both field and laboratory survival patterns (Figure 1). Also apparent were the effects of interactions with the associated microbiota when a nonsterile soil solution was used as the suspending medium. The <u>E</u>. <u>coli</u> populations in this case survived in parallel with those in the sterile soil solution for up to 48 h, after which a rapid decrease in the nonsterile populations reduced the number of cells to below detection limits by the 96 h sampling.

During the field survival studies the assumption was made that the arithmetic mean of the results obtained from TSYA counts represented the actual number of viable bacteria present in the sample (Table I). Therefore, at every sampling the mean of the results of parallel plating on SS-MFC agar were compared with the TSYA determined population density. The mean selective recovery rate of SS-MFC relative to the TSYA counts of all samples taken over the 84 h duration of the experiment were: 102.3% for the Nov^r Azi^r \underline{E} . coli, 93.3% for the Nal^r Azi^r \underline{E} . coli, and 104.2% for the Tet^r Azi^r <u>E</u>. <u>coli</u> (Table I). The counts obtained on SS-MFC for all three tracer strains in all samples were well within the 95% confidence limits of the TSYA counts. The recovery rate using membrane filter methods was determined by comparing the arithmetric mean of the counts from SS-MFC single drop platings with the mean of the membrane filter counts. For all samples over the entire 96 h survival experiment, the membrane filtration technique recovered 97.5% of the Nov^r Azi^r E. coli, 98.2% of the Nal^r Azi^r <u>E. coli</u>, and 96.7% of the Tet^r Azi^r <u>E</u>.

<u>coli</u>; also well within the 95% confidence limits of the numbers derived from direct plating.

Bacterial Translocation Studies

Computer-generated three-dimensional plots which displayed the translocation of resistance-labeled <u>E</u>. <u>coli</u> in the groundwater of the experimental site were constructed using, as the axes, times in minutes after injection, bacterial counts/ml, and the depths of the six sampling zones (Figure 2a-e). The individual plots depict the results from the five sampling lines located at increasing distances downslope. The translocation patterns of the individual strains injected into the A, B, and C horizons were of such similarity that only the data from the B horizon inoculations is shown.

Prior to inoculation of the site, no background antibiotic-resistant <u>E</u>. <u>coli</u> were detected from any of the piezometers. After injection into the profile at a depth of 30 cm (B horizon) the tracer strain was first detected in the 40 min sample from the 300 and 550 cm downslope sampling lines (Figure 2a, b). This initial appearance occurred at both distances in the 90-110 cm zone. Bacterial transport in the 90-110 cm zone continued downslope through the 1050 and 1550 cm sampling lines to the 2050 cm downslope position where the labeled strain was found in the 130-150 cm zone (Figure 2c, d, e). The bulk of bacterial translocation for this soil series occurred almost exclusively within these specific zones in the soil and in all cases the bacterial counts showed an increase over time to a maximum value (which differed for each distance downslope). The instances where transport was detected in zones either above or below the specific zone in which maximum movement rate and

bacterial numbers occurred demonstrated a vertical widening of the zone of influence in the soil profile. However, the reduced rate of transport in these zones along with the greatly reduced bacterial counts obtained suggested that this effect was only secondary and influenced a comparatively small portion of the total number of \underline{E} . coli cells.

The sampling times which corresponded to the first appearance of tracer bacteria in each sampling line as compared to the apparent downslope distance traveled by the bacteria are plotted in Figure 3. The linear relationship between the time of first appearance and distance traveled established the apparent maximum velocity of bacterial transport through the site at 17.0 cm/min, and this velocity remained constant over the entire site. In comparison, the relationship between the sampling times which corresponded to the appearance of the maximum bacterial counts vs the downslope distance (Figure 3) increased disproportionately with distance. This observation was characteristic of the hydrodynamic dispersion phenomena observed in liquid-porous systems which (with constant velocity) lowered the rate of increasing counts with additional distance. The time of first appearance and time of maximum peak development from the furthest upslope sampling line did not fit well into this relationship. Also, inspection of the data plots (Figure 2a) indicated that the movement patterns at this distance (300 cm) were split between the 90-110 and 130-150 cm sampling zones. This evidence can be used to suggest that the passage of the maximum rate of bacterial flow occurred either vertically between the detection zones (i.e. in the 110-130 cm zone), or was directed away from the individual sampling piezometers by the horizontal spatial variability of the soil

system. In either case, the maximum flow was intercepted further downslope as suggested by the close agreement of the recovery times among subsequent sampling lines.

The maximum bacterial concentrations in the groundwater decreased with distance downslope from the injection line (Figure 4). The individual points were determined from the mean value (N) of the maximum bacterial counts observed over the range of sampling times beginning with the initial appearance of the maximum value, and continuing through to the final sampling. Subsequently, all mean maximum values were divided by the injection density of the individual tracer strains (N_0) to normalize for injection density differences and obtain the percent reduction. Therefore, the variation in the three relationships observed in Figure 4 must have resulted from differences in the horizonal locations of injection of the three individual strains relative to the zone of transport. The vertical distance in the soil profile between the depth of injection (12, 30, and 60 cm) and the zone of maximum transport was thought to largely affect the numbers of organisms that entered into that zone. However, there was no apparent correlation between the maximum numbers observed downslope and the depth of injection. This indirectly suggested that structural variations with depth in the soil profile could also influence the ability of the organisms to leave the injection depth and enter the zone of maximum transport.

The reduction of maximum bacterial counts as a function of distance downslope for the tracer strains injected into the A, B, and C horizons was represented by logarithmic equations (Table II). These equations were generated from the mean maximum values (N), and the relationships fitted to the transformed values by linear regression analysis.

3.4 DISCUSSION

Any tracer material suitable for application in the soil system should be: 1) physically similar to the material being simulated, 2) stable throughout the period of testing, 3) exclusively selectable from a heterogenous sample, and 4) detectable in low concentrations. The three <u>E</u>. <u>coli</u> strains tested in these studies can be compared favorably against the above criteria as demonstrated through their survival patterns and ability to respond to selective recovery.

Knowledge of survival and recovery characteristics is essential for the application of resistance-labeled organisms to ecosystem analysis. Previous research using membrane filter chambers to determine E. coli survival (Faust, et al., 1975; McFeters et al., 1974; McFeters and Stuart, 1972) and recovery on selective media (Bissonnette et al., 1975) have indicated rapid death rates and greatly reduced recovery after equivalent periods and in physiochemical environments similar to those in our study. However, when care was taken to reduce the stress placed upon the E. coli strains in preparation for survival studies (unpublished data) and in recovery from natural environments (Klein and Wu, 1974), significant increases in percent survival and recovery over time were realized. The \underline{E} . <u>coli</u> tracer experiments in our study were not designed to simulate the survival of fecal organisms naturally introduced into the soil environment, but were conducted only to determine if survival and recovery of specific bacterial strains could be maximized for a particular purpose.

This study utilized resistance-labeled strains of <u>E</u>. <u>coli</u> to present a phenomenological view of the characteristics of bacterial transport through a saturated soil system. In a similar fashion, effluent-borne bacterial populations from septic systems are also thought to move through the soil in zones of maximum hydraulic conductivity and at rates limited by: 1) the ability of the soil to conduct water, and 2) the hydraulic gradient or slope of the system. Granted, these characteristics will vary from soil to soil and from site to site, however, unifying principles do exist. Reneau (1978) and Viraraghaven (1978) determined the total and fecal coliform densities in the groundwater downslope from three operating septic systems inundated by high water tables. Although the data was based on the numbers of bacteria/100 ml and resulted from naturally occurring populations, the results described similar reductions in bacterial numbers with distance downslope from the drainfield tile line as in our study. Therefore, even though initially large reductions in bacterial numbers occur as the populations enter the soil system (Figure 4), once the organisms reach a highly conductive zone, relatively long distances are necessary for the further reduction of bacterial densities. Effluent-borne pathogens in these circumstances have the opportunity for rapid transport either horizontally to surface receiving waters, or vertically to aquifers through saturated recharge pathways. Our evidence indicated that serious contamination of individual water supply systems could occur when a high density of housing units with individual on-site waste disposal systems is located on soils which are generally unsuitable due to the occurrence of seasonally-perched water tables.

In sparsely populated areas, the subsurface escape of fecal organisms from the treatment zone of a septic system drainfield is highly localized and rapidly diluted in the groundwater system. However, groundwater that meets current coliform standards and is considered safe against

outbreaks of bacterial disease may not be protected against low levels of virulent pathogens (National Academy of Sciences, 1977). Therefore, proper septic system location and design, restricting the development of elevated water tables and the saturated flow conditions which make fecal organism transport possible, is necessary for the adequate protection of this potable water source.

Many states have adopted standards establishing the minimum soil depth to bedrock or highest level reached by a water table in which a septic system can be installed (Baker, 1978). Such standards are based on little or no information concerning the maximum depth of unsaturated soil necessary for proper treatment under all environmental conditions as might exist at any particular location. Results reported here indicated that the maximum bacterial densities recovered downslope in one soil series varied little from injection into the A horizon (12 cm depth) vs injection into the C horizon (60 cm depth) with a water table approximately 50 cm from the surface, and emphasized the need for a large zone of unsaturated soil around waste treatment systems to prevent the release and rapid transport of large populations of fecal microorganisms.

ACKNOWLEDGMENTS

Contribution from the Oregon Agricultural Experiment Station, Technical Paper No. . Research supported by the Office of Water Research and Technology, U.S. Dept. of the Interior, by the provisions of the Water Resources Research Act of 1964, as amended.

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Experimental conditions	<u>E. coli</u> strain	Initial density	Final density	Duration (h)	Relative recovery (%)	
					SS-MFC ^a direct plating	SS-MFC ^b membrane filtration
Field site ^C	Nov ^r Azi ^r	5.8 x 10 ⁸	4.2×10^8	84	102.3	
	Nal ^r Azi ^r	2.4×10^{8}	1.8×10^8		93.3	
	Tet ^r Azi ^r	6.5×10^8	3.2×10^8		104.2	
Laboratory ^d	Nov ^r Azi ^r	8.5 x 10 ⁵	1.4×10^5	96		97.5
	Nal ^r Azi ^r	6.9×10^5	1.2×10^{5}			98.2
	Tet ^r Azi ^r	4.3×10^5	3.4×10^5			96.7

Table I. The maximized survival and recovery rates of the three resistance-labeled \underline{E} . <u>coli</u> strains under field and laboratory conditions.

^aBacterial counts relative to TSYA counts.

^bBacterial counts relative to SS-MFC direct plating counts.

^CIndividual suspension of <u>E</u>. <u>coli</u> strains in membrane filter chambers.

 $d_{\text{Mixed suspension of all } \underline{E}. \underline{\text{coli}}$ strains in a membrane filter chamber.

Table II. Equations describing the reduction in the maximum bacterial densities as observed with injections into the A, B, and C horizons (12, 30 and 60 cm in depth) and subsequent transportation downslope.

Linear Transformation	r ²
A horizon injection: log (cells/ml) = 8.57 - 1.91 log (distance ^b)	0.97**
B horizon injection: log (cells/ml) = 8.79 - 1.66 log (distance)	0.88*
C horizon injection: log (cells/ml) = 9.00 - 1.81 log (distance)	0.93**

^aDetermined as the arithmetic mean of the maximum bacterial densities observed from the time of first appearance of the maximum numbers of bacteria and continuing to the final sample.

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^DDistance in cm.

**Significant at P = 0.005.

*Significant at P = 0.01.

- Figure 1. The survival of resistance-labeled <u>E</u>. <u>coli</u> strains. The cells were washed and suspended in a soil extract and were contained within membrane filter chambers placed in a saturated soil at a field location (using a high bacterial density) or in a laboratory setting (with a low bacterial density). Symbols: (△) Nov^r Azi^r <u>E</u>. <u>coli</u>, (□) Nal^r Azi^r <u>E</u>. <u>coli</u>, (0) Tet^r Azi^r <u>E</u>. <u>coli</u>. The open characters indicate that the suspending solution was sterile while the closed characters indicate that the suspending solution was nonsterile.
- Figure 2. The B horizon injection plots showing the saturated zones of translocation of <u>E</u>. <u>coli</u> through the soil profile at increasing distances downslope. The open planes represent the magnitude of the populations recovered (y-axis) at each sampling time (x-axis) from the soil depths sampled (z-axis). Symbols: (0-0 or 0---0) indicate zones where a water sample could be recovered, $(-\cdot-\cdot)$ marks the location of the water table at each distance downslope.
- Figure 3. The first appearance where the tracer strains could be detected (open characters) and first appearance of the maximum bacterial numbers (closed characters) of the resistance-labeled <u>E</u>. <u>coli</u> strains (for all depths) at increasing distance downslope. The symbols are located in terms of specific sampling times at the various sampling distances downslope relative to the horizontal injection of: (Δ) Nov^r Azi^r <u>E</u>. <u>coli</u> in the A horizon, (\Box) Nal^r Azi^r <u>E</u>. <u>coli</u> in the B horizon, (0) Tet^r Azi^r E. coli in the C horizon.

Figure 4. The percent reduction with distance downslope of the tracer <u>E</u>. <u>coli</u> strains injected into the soil profile of the experimental site. Symbols: (△) A horizon injection, (□) B horizon injection, (0) C horizon injection.

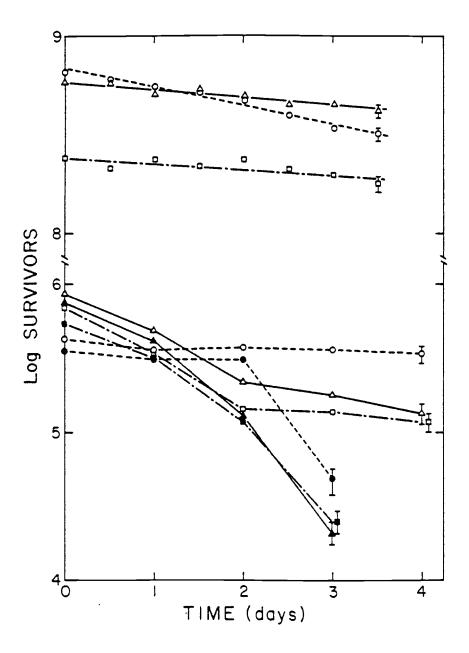
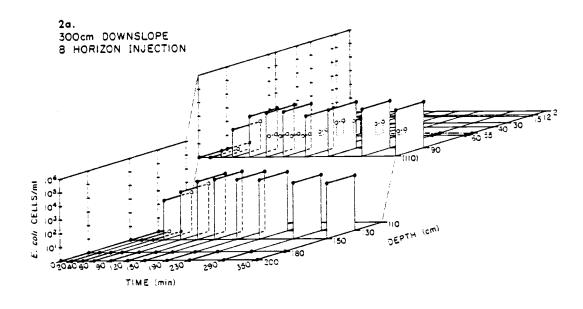


Figure 1



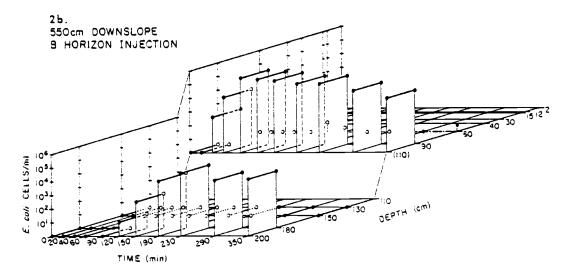


Figure 2 a,b

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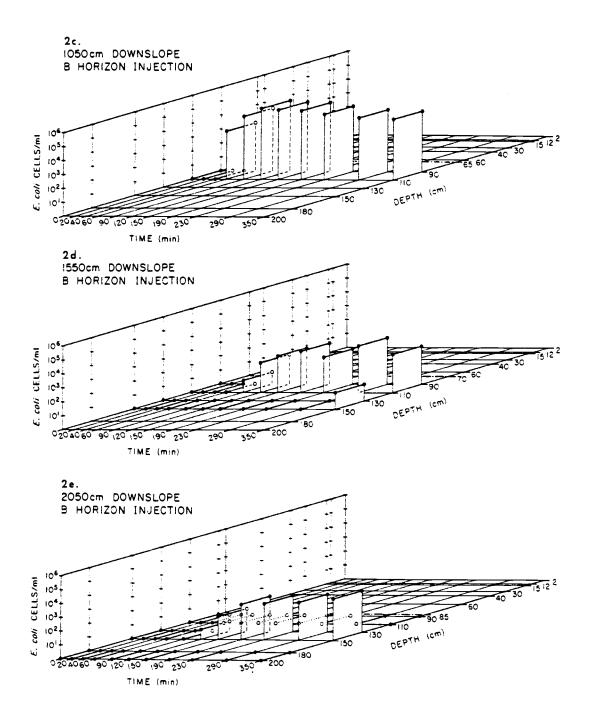
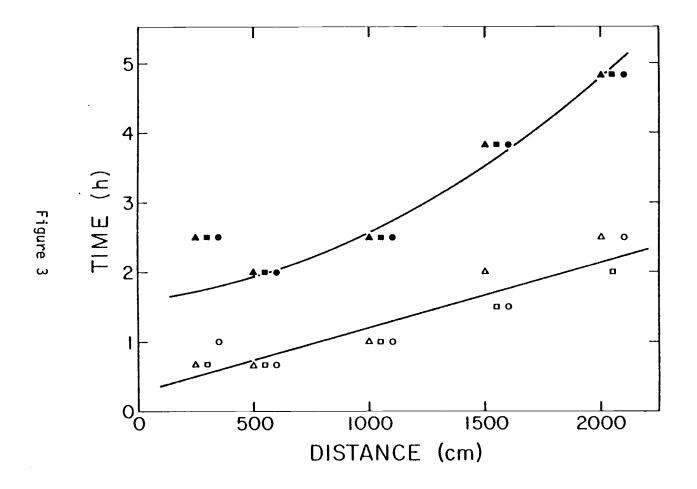


Figure 2 c-e



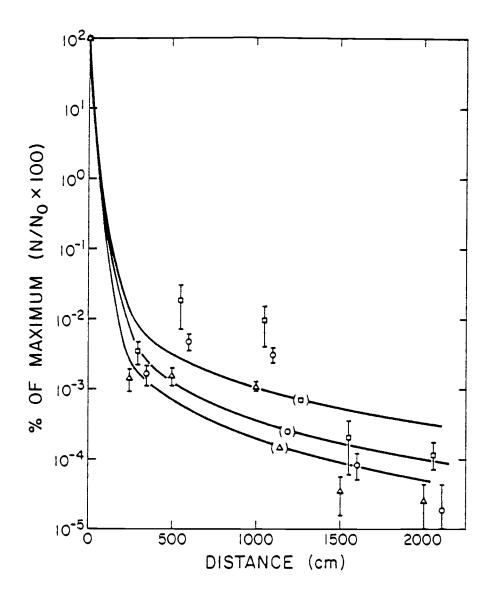


Figure 4

4. TRANSPORT OF RESISTANCE-LABELED <u>ESCHERICHIA</u> <u>COLI</u> STRAINS THROUGH A TRANSITION BETWEEN TWO SOILS IN A TOPOGRAPHIC SEQUENCE

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4.1 INTRODUCTION

The hydrologic behavior of the soil area positioned on a hillslope regulates, in part, the upward extent of saturation occurring at a given slope location during rainstorm/snowmelt events (Weyman, 1973). In many areas of western Oregon, water restrictive layers of clay or bedrock corroborate the seasonally high rainfall to produce widespread areas with elevated water tables (Simonson and Boersma, 1972). These seasonally perched water tables which reduce the volume of unsaturated soil also foster the improper purification of waste waters applied to the soil through septic tank-drainfield systems (Bouma, 1975). In addition, the hydrology of an individual drainfield installation will determine the manner in which improperly purified effluent migrates from the disposal area and poses a health threat. Storm runoff processes are, however, complex and encompass a variety of flow pathways which may exist singly or in combination varying with space and time (Freeze, 1974). Yet, the flow pathways and soil physical processes governing storm runoff systems on a hillslope, as observed through tracer studies, have been examined only to a limited extent (Pilgrim, 1978).

Studies tracing the transport patterns of fecal microorganisms introduced into saturated soils from either functioning (Reneau, 1978; Reneau and Pettry, 1975; Viraraghaven, 1978) or a simulated septic system drainfields (Rahe <u>et al</u>. 1978; McCoy and Hagedorn, 1979) have been limited to uniform slopes of uniform soil series and fail to describe effluent migration on the hillslope scale. Hydrologic models which describe hillslope outflows classically combine the flow components and generalize the internal structure of the system and as such can be misleading if used as the basis for the study of transport mechanism (Woolhiser, 1975).

in this study we investigated the hydrologic processes and physical parameters of a transition sequence located in a concave slope position between two diverse soil series. The storm runoff mechanisms which transported fecal microorganisms introduced into the soil at this location coalesced the results of our previous studies and more completely described septic system effluent migration on a hillslope scale. Also, our findings lend support to subsurface storm flow as being a mechanism in initiating saturated overland flow in a variable source area.

4.2 MATERIALS AND METHODS

Bacterial transport through the soil was measured by introducing resistance-labeled <u>Escherichia coli</u> strains into the soil through injection lines located at depths which generally corresponded to the A, B, and C horizons of the soil profile. The organisms were subsequently recovered in water samples from piezometers located at various distances and depths downslope from the injection lines, and from samples of surface water collected on both sides of the experimental site.

Site Description:

The experimental site was located in Benton County, Oregon at the Oregon State University Poultry Research Facility, and lies in a westfacing upper footslope position with slopes ranging from 13-24% (Fig. 2). A contour plan of the experimental site (constructed with 0.6 m contour intervals) is presented in Figure 1. Details on the installation of the experimental site were described previously (Rahe et al., 1978). Briefly, three 9.15 m horizon injection lines were installed (0.5 m apart) at 12, 35, and 70 cm depths in the experimental site with the

12 cm deep line occupying the furthest downslope position while the 70 cm deep line was installed upslope from the other two. Thirty piezometers were installed on the experimental site to depths of 12, 35 or 40, 70 or 75, 110, 150, and 200 cm (Fig. 1) with the 12 cm deep piezometers sampling a 10 cm zone of soil and the remaining piezometers sampling a 20 cm zone. The piezometers were arranged in rows at distances of 2.5, 5.0, 10.0, and 15.0 m downslope from the 12 cm deep horizon injection line corresponding to sampling rows 1, 2, 3, and 4 (Fig. 1) respectively. The installation sequence of the six piezometers in each sampling row was randomized. One additional sampling row (sampling row 0, Fig. 1) which served as a control was installed approximately 2.5 m upslope from the 70 cm deep horizon injection line. Rodent tunnels of an approximate diameter \geq 3.0 cm were partially mapped (in Fig. 1) by techniques outlined by Atkinson (1978).

Soil Descriptions

The experimental site is positioned in the transition zone between a Hazelair (very fine, mixed, mesic Aquultic Haplaxeroll) and Steiwer (fine, loamy, mixed, mesic Ultric Haploxeroll) soil series. Soil descriptions were made from pits located adjacent to the hillslope site (Table 1). The Hazelair soil series, located at the lower end of the experimental site, was characterized by the grayish-brown and dark yellowishbrown massive clay which extended from between 40 and 70 cm from the surface downward to at least a 200 cm depth. The Steiwer soil series was located upslope on the experimental site and was a dark-brown to yellowish-brown silt loam underlain at approximately 100 cm by finely

Therefore, laminated siltstone mixed with areas of more weathered silty clay saporlite.

The position of the major soil textural zones was determined by soil corings taken subsequent to the completion of all experimentation at this hillslope location (Fig. 2), and variation in the vertical position of the boundaries between the individual soil textural zones range with horizontal location along the contours as much as 20 cm from that indicated. The height of the perched water table, as determined from water levels in the shallow piezometers and several adjacent observation wells, also exhibited vertical variation but to a lesser degree than the soil textural boundaries.

Bacterial Translocation Studies

The <u>Escherichia coli</u> strains used in this study were collectively resistant to 100 mg/l sodium azide (Azi) and individually resistant to 100 mg/l novobiocin (Nov), nalidixic acid (Nal), and tetracycline (Tet). The tracer strains were therefore designated: Nov^r Azi^r <u>E</u>. <u>coli</u>, Nal^r Azi^r <u>E</u>. <u>coli</u>, and Tet^r Azi^r <u>E</u>. <u>coli</u>. By incorporating these selective agents into a recovery medium an individual tracer strain could be selected from a heterogenous sample containing both the natural bacterial populations present in the soil and the two associated introduced strains.

Inoculum for the field experiment was prepared identically for each strain. A 1200 ml volume of the nutrient constituents of M-FC broth was inoculated (10% v/v) and incubated for 14-16 h at 37 C. The initial bacterial population present in the inoculum was determined optically and the individual cultures were divided among six 500 ml aspirator bottles for transport to the field and injection into the soil. Horizon

inoculation was accomplished by attaching an aspirator bottle to each of the injection line access ports and allowing the contents to drain into the injection line. The Nov^r Azi^r <u>E</u>. <u>coli</u> was injected into the A horizon, the Nal^r Azi^r <u>E</u>. <u>coli</u> was injected into the B horizon, and the Tet^r Azi^r <u>E</u>. <u>coli</u> was injected into the C horizon at the experimental site.

Water samples were vacuum extracted from the downslope piezometers at 20, 40, 60, 90, 120, 150, 195, and 240 minutes post injection. Control samples (designated time zero) were collected just prior to site injection. Samples were recovered in 250 ml bottles, transported to the laboratory and stored no longer than 24 h at 4 C before analysis. All samples from the field experiments (240 minute duration) were collected before examination of the samples was initiated. Enumeration of the tracer bacteria present in each water sample was accomplished by membrane filtration on to M-FC broth base plus 1.0% agar, rosolic acid, 100 mg/l sodium azide, and 100 mg (of the appropriate antibiotic)/l.

Surface samples were collected during the experiment from the furthest upslope region of observed surface flow as indicated by the points 'N' and 'S' in Fig. 1. The surface flow on the south side of the experimental site (S) originated from a rodent tunnel while the surface flow on the north side (N) originated from a general seepage in the immediate area of the indicated collection point. No surface water was observed in the immediate vicinity of the sampling piezometers.

4.3 RESULTS

Computer generated three-dimensional plot diagrams which display the translocation of resistance-labeled E. coli through the experimental site were constructed using, as the axes, time in min after injection, bacterial counts/ml, and the depths of the six sampling zones. The individual 3-D plots depict the results from sampling row 1 and 2 at 250 and 500 cm downslope from the A horizon injection (Fig. 3a, b), and 300 and 550 cm downslope from the B horizon injection (Fig. 4a, b). The translocation patterns of the labeled <u>E</u>. <u>coli</u> injected into the C horizon were of such similarity to those of the B horizon injection that only data from the B horizon inoculation is shown. Tracer <u>E</u>. <u>coli</u> strains from the individual horizon injections were not detected in water samples from sampling row 3 or 4 over the course of the 240 min experiment. The concentration of <u>E</u>. <u>coli</u> in the water samples collected from surface flow which percolated up from the rodent tunnel on the south side of the experimental site are shown in Figs. 3c and 4c. No labeled strains were detected in surface samples collected on the north side of the experimental site.

Injection of <u>E</u>. <u>coli</u> into the A horizon (12 cm depth) of the experimental site resulted in a detectable number of organisms in the 2-12 and 55-75 cm depth zones 250 cm downslope from the horizon injections line after 60 and 120 min. respectively (Fig. 3a). The upper surface of the perched water table reduced the actual zone of soil sampled from the shallow piezometers to approximately 1.0 cm and resulted in the single points displayed in Fig. 3a. The initial arrival of the bacterial transmitting flow at this distance also occurred in the upper level of the saturated zone with the observed magnitude of bacterial counts/ml, approximately 10 times greater than in the 55-75 cm deep zone. Bacterial transport observed at the 500 cm downslope sampling position was

restricted exclusively to the 26-35 cm zone, once again at the upper level of the zone of saturation.

A large volume of water was observed flowing from the rodent tunnel which surfaced on the south side of the experimental site. Enumeration of the labeled <u>E</u>. <u>coli</u> strain recovered at this location from the A horizon injection is presented in Fig. 3c. In relation to the flow patterns from the piezometer samples (Fig. 3a, b) and considering the distance of this sampling point from the injection line; the arrival time and numbers of organisms observed demonstrate the relatively large influence of subsurface channel flow within this transition series. Indeed, as suggested by the bacterial density of these samples, the upslope penetration of the subsurface channel network may exceed, albeit in a more branched and minor form, that which has been mapped (Fig. 1)

Bacterial translocation following injection into the B horizon (35 cm depth) of the experimental site differed considerably from A horizon injection at sampling row 1; 300 cm downslope (Fig. 4a). There was an overall wider zone of bacterial spread through the soil profile and maximum translocation occurred in the 90-110 cm deep zone where the tracer initially appeared after 40 minutes. Also, whereas the 20-40 cm zone represented a secondary but notable flow pathway from the B horizon injection, no bacteria were detected in this zone from the A horizon injection. Nevertheless, as bacterial penetration from the B horizon injection approached sampling row 2; 550 cm downslope the zone of bacterial migration narrowed considerably (Fig. 4b) and in fact, displayed an identical pattern as that for the A horizon injection. Therefore, the primary streamlines generated by bacterial transport from injection into the B horizon, as determined from piezometer samplings, follow

through the 90-110 cm zone at sampling row 1 followed by a surfacing to the upper level of the zone of saturation at sampling row 2. In addition, the flow pattern observed from B horizon injection at the channel outflow (Fig. 4c) displays nonuniformity with the piezometer samplings in a similar fashion as discussed above for the A horizon injection results.

4.4 DISCUSSION

Hillslope landforms possessing concave slopes generally present toposequences of more uniform soil series towards the backslope or summit of a slope and more highly differentiated series with thicker B horizons at the footslope position. This sequence of profile development has been explained hydrologically by Zaslavsky and Rogowski (1969) in their model of unsaturated flow in anisotropic hillslope soils; and therefore suggest the more widespread nature of the herein reported flow pathways. Also, inherent in their model was the convergence of unsaturated streamlines below a concave slope which, with increased precipitation, forms a saturated pocket and subsequent surface seepage even in the absence of impermeable layers and widespread saturated conditions. Moreover, the relatively impermeable clay layer present in the Hazelair soil series serves to accentuate this initiation of surface flow by directing the water moving through the Steiwer profile in toto into a very shallow zone of soil positioned above the clay layer in the Hazelair series. Therefore, the bacterial migration observed at this field site interfaces well with these flow systems and apparently, both hydraulic gradients and a restrictive clay layer contribute to upward movement with subsequent surfacing and saturated overland flow.

Translatory channel flow or through-pipe flow in saturated conditions apparently transports microorganisms in a manner which by-passes interaction with the bulk of the soil matrix. This same phenomena has been observed by Reneau (1978) where a relic agricultural tile intercepted the disposal area of a septic system drainfield and quantitatively reduced further purification of the transmitted effluent. Also, subsurface pipe flow was inferred by Rahe et al. (1978) as the causative agent when bacterial penetration in saturated soils displayed localized variability and rapid flow rates. Conversely, relatively uniform flow through a well delineated section of the solumn was observed in a previous study (McCoy and Hagedorn, 1979). In fact, these two somewaht conflicting views were noted on the same hillslope. The results reported herein, however, supply the connection between these previously conflicting studies: Flow at a depth in the soil below the biologically active zone passes through the soil matrix and intercepts few root channels or rodent tunnels. Then, as observed through the transition series in this study, when hydraulic gradients and/or restrictive layers direct the flow toward the surface, the interception of the flow by a pipe or root channel becomes a more likely consequence. Hence, the level of the phreatic surface along with any vertical hydraulic gradients regulates the depth of bacterial transmission in the soil, and the degree of spatial variability observed.

Lastly, the variable source area concept of Hewlett and Hibbert (1967), maintains that during a storm period an expanding surface channel network reaches out from the stream to tap subsurface flow systems which have exceeded their capacity to transmit water beneath the soil surface. This converts the primary flow pathway in an area from a relatively slow subsurface route to rapid saturated overland flow. However, the reservoir

of excess water producing overland flow is still a source of controversy. Similar studies by Dunne and Black (1970) and Whipkey (1965) support the conflicting mechanisms, rainfall in excess of the soil's water holding capacity and subsurface recharge from further upslope, respectively as the primary mode of initiation of overland flow. Also, Freeze (1972), through a mathematical model, determined that upslope recharge from concave slopes would be insufficient to supply excess water at a rate necessary to produce overland flow. However, pipe flow was not considered in construction of the model. Our results which demonstrate the large influence of pipe flows in draining a concave slope, therefore, support the presumption of Jones (1971) and the measurements by Whipkey (1965) and indicate that an overland flow network is interconnected with a substantial subsurface pipe network which penetrates further upslope and contributes large volumes of rapidly flowing water.

ACKNOWLEDGMENTS

Contribution from the Oregon Agricultural Experiment Station, Technical Paper No. . Research supported by the Office of Water Research and Technology, U.S. Dept. of the Interior, by the provisions of the Water Resources Research Act of 1964, as amended.

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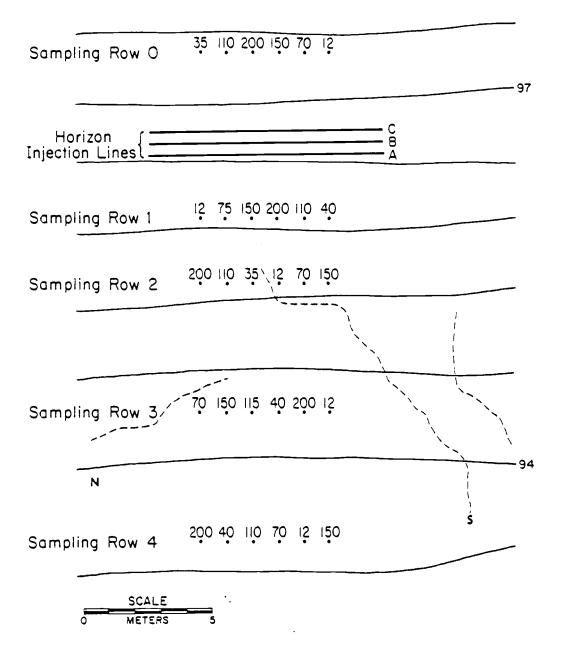
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Table 1. Profile descriptions of the adjacent soil series which formed the experimental transition site.

Horizon	Depth	Profile Description			
	<u>cm</u>				
		Steiwer series/upslope position			
A1	0-12	Dark brown (10YR 4/3) silt loam; moderate, medium granular structure; friable, slightly sticky, and slightly plastic; many fine and very fine roots; many fine interstitial pores; medium acid; abrupt smooth boundary.			
A3	12-46	Dark brown (10YR 4/3) silt loam; moderate, medium subangular blocky structure; friable, slightly sticky and slightly plastic common fine and few medium roots; many fine tubular pores; medium acid; clear, smooth boundary.			
82	46-70	Yellowish brown (10 YR 5/5) silt loam; moderate, medium sub- angular blocky structure; friable, slightly sticky and slightl plastic; few medium and very few coarse roots; common fine tubular pores; slightly acid; clear, wavy boundary.			
83	70-100	Dark brown (7.5YR 4/4) silt loam; moderate, medium subangular blocky structure; friable, slightly sticky and slightly plastic; few fine and medium and very few coarse roots; common fine tubular pores; medium acid; clear, irregular boundary.			
С	100-	Very pale brown (10YR 7/4) and yellowish red (7.5YR 4/6), laminated, fractured, folded, and weatnered siltstone with micaceous and clay minerals mixed with areas of more weathered silty clay saprolite; can be dug with shovel when wet; very few medium and coarse roots.			
		Hazelair series/downslope position			
Al	0-12	Dark brown (10YR 3/3) silt loam; few faint dark yellowish brown (10YR 4/4) mottles; moderate, medium granular structure; slightly hard, slightly sticky and slightly plastic; many fine and very fine roots; many fine and very fine interstitial pores; medium acid; abrupt smooth boundary.			
A3	12-47	Dark brown (10YR 3/3) silty clay loam; many distinct dark yellowish brown (10YR 4/4) mottles; moderate, medium sub- angular blocky structure; hard, slightly sticky and slightly plastic; many fine roots; many fine and very fine tubular pores; slightly acid; gradual, smooth boundary.			
IIB2	47-80	Grayish brown (10YR 5/2) clay; many distinct brownish yellow (10YR 6/6) mottles; moderate, medium prismatic to moderate, medium subangular blocky structure; very hard, very sticky and very plastic; very few fine roots; common fine and very fine tubular pores; medium acid; clear, smooth boundary.			
I IB3	80-110	Dark yellowish brown (10YR 4/4) clay, massive structure; very firm, very sticky and very plastic; very few fine roots; common very fine tubular pores; medium acid; gradual, wavy boundary.			
IIC	110-	Dark brown (10YR 4/3) clay, massive structure; very firm, very sticky and very plastic; very few fine roots; few very fine tubular pores; slightly acid.			

- Figure 1. The contour plan (in meters above sea level) of the experimental site showing the A, B and C horizon injection lines, and rows of sampling piezometers installed to the depths indicated (in cm). A partial map of the rodent tunnels present at this location are indicated (dashed lines), along with the north and south sampling positions (N, S).
- Figure 2. A cross sectional view of the experimental site showing the major soil textural zones in the transition between the Steiwer (located upslope) and Hazelair (located downslope) soil series. The soil surface is indicated by the dashed line, and the numerals designate the location of the five rows of sampling piezometers.
- Figure 3. The A horizon injection plots showing the saturated zones of translocation of <u>E</u>. <u>coli</u> through the soil profile at increasing distances downslope. The open planes represent the magnitude of the populations recovered (y-axis) at each sampling time (x-axis) from the soil depths sampled (z-axis). Symbols: (0-0, or 0---0) indicate zones where a water sample could be recovered, ('-'-') marks the location of the water table at each distance downslope. a) Samples collected at sampling row 1. b) Samples collected at sampling row 2. c) Samples collected at sampling point 'S'.
- Figure 4. The B horizon injection plots showing the saturated zones of translocation of <u>E</u>. <u>coli</u> through the soil profile at increasing distances downslope. The open planes represent the magnitude of the populations recovered (y-axis) at each sampling time

(x-axis) from the soil depths sampled (z-axis). Symbols:
(0----0, or 00) indicate zones where a water sample could be recovered, ('-'-'-) marks the location of the water table at each distance downslope. a) Samples collected at sampling row
1. b) Samples collected at sampling row 2. c) Samples collected at sampling point 'S'.





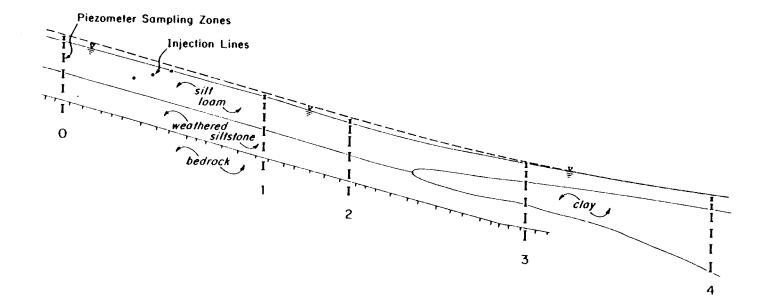


Figure 2

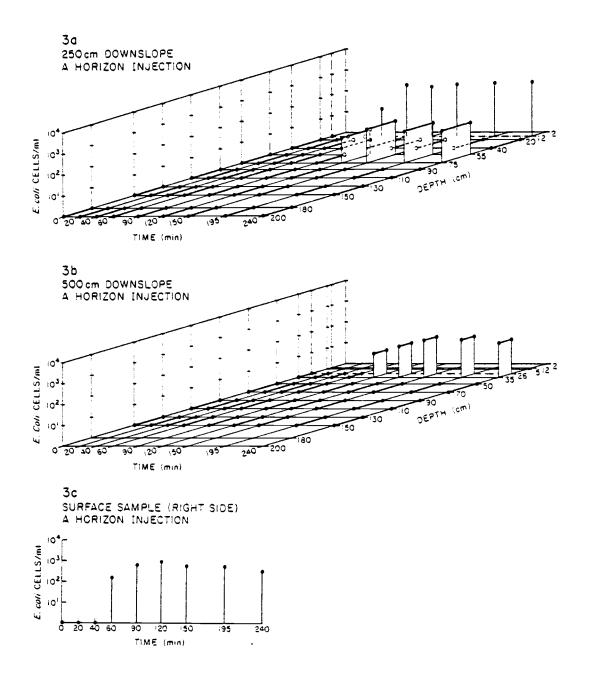


Figure 3 a-c

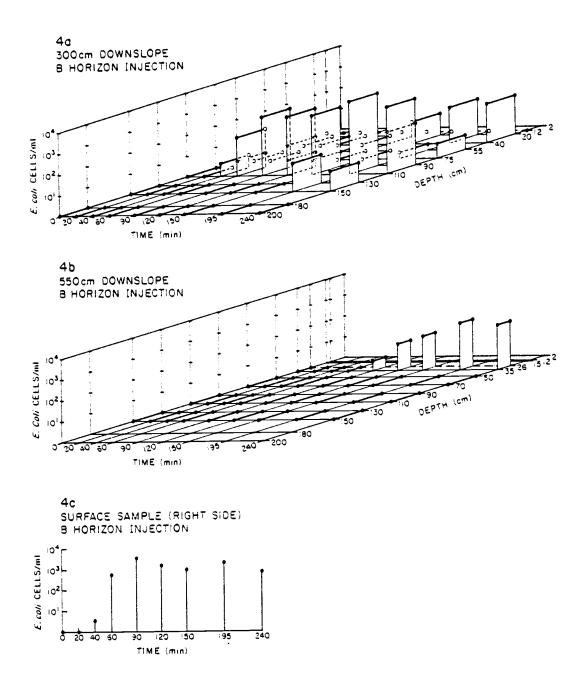


Figure 4 a-c

APPENDICES

APPENDIX I

AN ILLUSTRATIVE SUMMARY: PHENOMENA ASSOCIATED WITH THE TRANSPORT OF FECAL BACTERIA THROUGH SATURATED SOIL SYSTEMS AS A THREAT TO PUBLIC HEALTH.

ABSTRACT

Bacteriological purification of septic system effluents occur as these liquid wastes percolate through the soil. However, elevated water tables which inundate the treatment zone adjacent to a drainfield trench reduces the soil's purification efficiency. To simulate the failure of this treatment system, three resistance-labeled tracer Escherichia coli strains were injected into the A, B and C horizons of a saturated hillslope soils (Fig. 1.). Subsurface water movement subsequently transported the tracer populations as determined by consecutive groundwater samplings at various distances downslope and depth-zones in the soil, and enumerating the tracer organisms present in the water samples. Bacterial transport at several locations on a hillside occurred at relatively high velocities and within sepcific depth-zones in the soil. Also, the reduction in the tracer populations observed at increasing distance from the point of injection obeyed uniform logarithmic relationships. These results demonstrated the extent of incomplete treatment of septic system effluents which can occur in a saturated soil system.

Figure 1

Aerial view of experimental site no. 4 showing the plot layout with horizontal injection lines installed to depths which correspond to the A, B, and C horizons of the soil profile; and rows of sampling piezometers installed to the depths indicated (in centimeters). Contour intervals are indicated in meters above sea level (solid line) and a partial map of the rodent tunnels present at this location are included (dashed line).

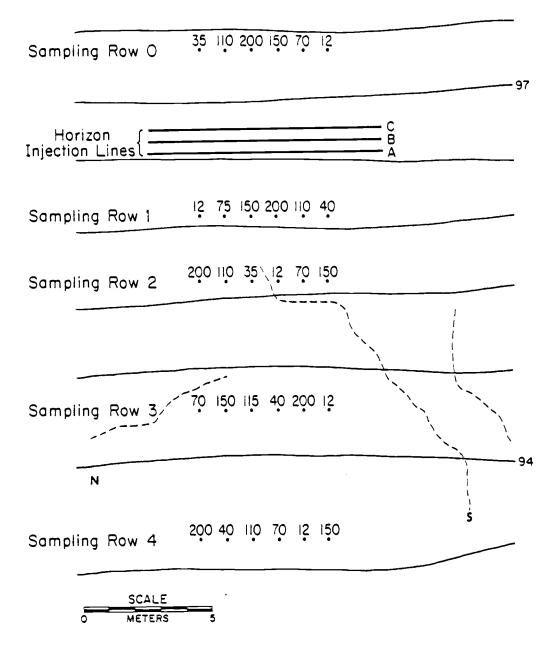
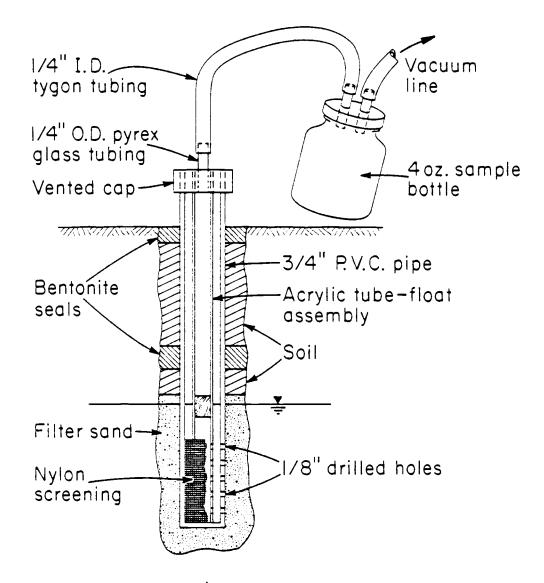


Figure 2

Piezometer design for groundwater sample collection.

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THEORY

The transport of tracer E. coli populations laterally through the soil display distinctive trends when plotted as cfu/ml vs time after injection (Fig. 3.). In each case there is a characteristic increase in tracer cfu/ml until a maximum value is obtained although the rate and magnitude of this increase differ for the various distances downslope. These flow patterns are therefore described by classical methods for the dispersion of a solute during flow through porous media. Dispersion of a solute in the direction of flow is described by equation (1), which can be solved for a singlestep input of tracer yielding (2) where K₁ is the longitudinal dispersion coefficient, \bar{v} the apparent mean velocity, t the time in minutes, x the downslope distance in centimeters and C the concentration of tracer. The experimental design also allows the further simplification of equation (2)to give equation (3) from which (4) follows for the determination of bacterial cfu/ml (N) with time at a given distance downslope. Equation (4) is then fit to the experimental data (Fig. 3.) by determining K_{i} and \bar{v} from plots of the tracer cfu/ml vs time via equations (5), (6) and (7) where t.16, t.5 and t.84 are the times at which the cfu/ml were 16%, 50% and 84% of the maximum observed at each distance respectively. In addition, the individual K, and ∇ values used to fit equation (4) to experimental data appear in Table 1. This treatment was subsequently used to plot the appearance of tracer E. coli populations at given distances downslope (Figs. 4-7.).

Longitudinal Dispersion Equation (Fried, 1975)

$$\frac{\partial C}{\partial t} = K_{L} \frac{\partial^{2} C}{\partial x^{2}} - \bar{v} \frac{\partial C}{\partial x}$$
(1)

$$C(x, 0) = 0$$

 $C(0, t) = C_0$
 $C(\infty, t) = 0$

$$\frac{C(x,t)}{C_0} = \frac{1}{2} \left[\operatorname{erfc}\left(\frac{x-\overline{v}t}{2\sqrt{K_L t}}\right) + \exp\left(\frac{\overline{v}x}{K_L}\right) \operatorname{erfc}\left(\frac{x+\overline{v}t}{2\sqrt{K_L t}}\right) \right] \quad (2)$$

$$\frac{C(x,t)}{C_0} = \frac{1}{2} \operatorname{erfc}\left(\frac{x-\overline{v}t}{2\sqrt{K_L t}}\right)$$
(3)

$$N = N_{MAX} \cdot \frac{1}{2} \operatorname{erfc}\left(\frac{x - \overline{v}t}{2\sqrt{K_{L}t}}\right)$$
(4)

$$K_{L} = \frac{1}{2} \sigma^2 x \bar{v}$$
 (5)

$$\sigma = \frac{\frac{1.84^{-1.16}}{21.5}}$$
(6)

$$\bar{\mathbf{v}} = \frac{\mathbf{x}}{\frac{1}{5}} \tag{7}$$

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Exptl. _site_	Distance downslope (cm)	к _L	⊽	Mean site ⊽ ± std. dev.
4	300	30.0	3.3	3.45 ± 0.21
	550	55.0	3.6	
5	250	22.0	3.0	4.35 ± 1.80
	500	17.0	2.6	
	1500	25.0	5.8	
	2000	30.0	6.0	
7	600	152.0	10.5	9.83 ± 1.80
	1100	175.0	12.0	
	1600	439.0	8.0	
	2100	727.0	8.8	
8	300	11.0	10.4	8.23 ± 3.20
	550	14.5	4.0	
	1050	22.0	7.5	
	1550	63.0	11.0	

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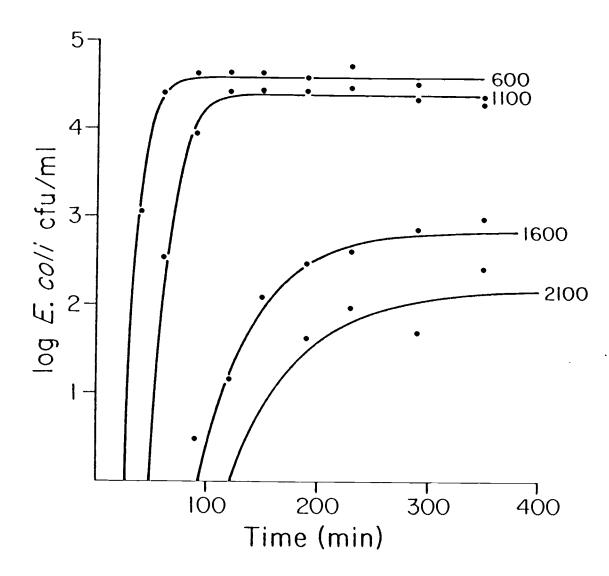
Table 1. Parameters derived from experimental data and used via equation (4) to construct the appearance of tracer with time curves (figures 4-7).

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Figure 3.

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The tracer <u>E. coli</u> density in water samples recovered from the zone of maximum transport as a function of time demonstrating the fit of equation (4) to experimental data by adjusting K_L and ∇ .



RESULTS

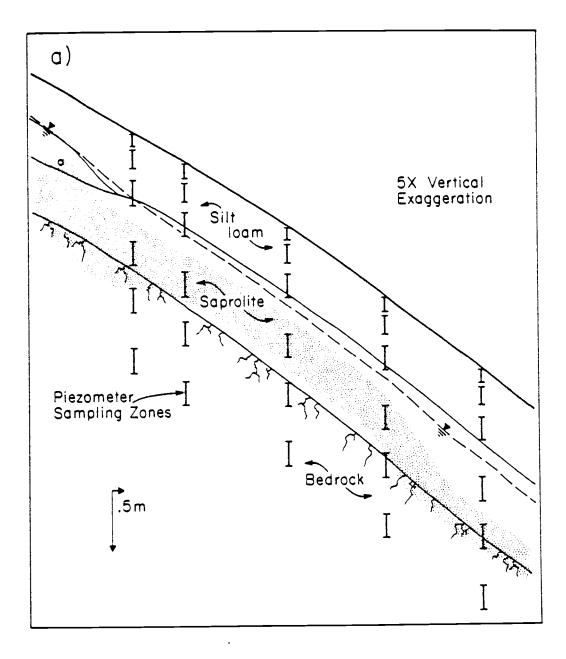
The relationships for the appearance of tracer <u>E. coli</u> with time (solid line, Fig. 4-7) were individually truncated at the point which corresponds to the downslope distance (bottom axis) from which the samples were recovered. Also, the values of the maximum cfu/ml recovered at each distance fell within the brackets indicated on each of these curves.

Figure 4.

The pathways and characteristics of bacterial transport through site number 7, a Dixonville soil series located in an upper backslope position.

a) Cross section of the experimental site displaying the zone of bacterial penetration from the C horizon injection.

b) The appearance of tracer <u>E. coli</u> with time at increasing distance downslope (solid line) from equation (4) using experimental K_L and site mean \bar{v} values. The decrease in the mean maximum bacterial density as a function of distance downslope both before (dashed line) and after (dotted line) correction for dispersion.



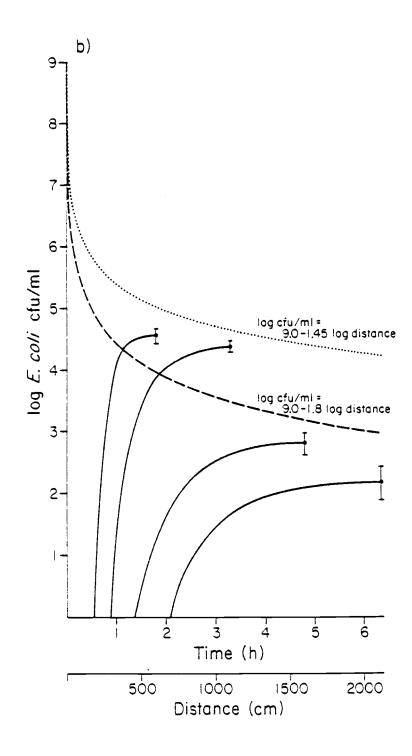
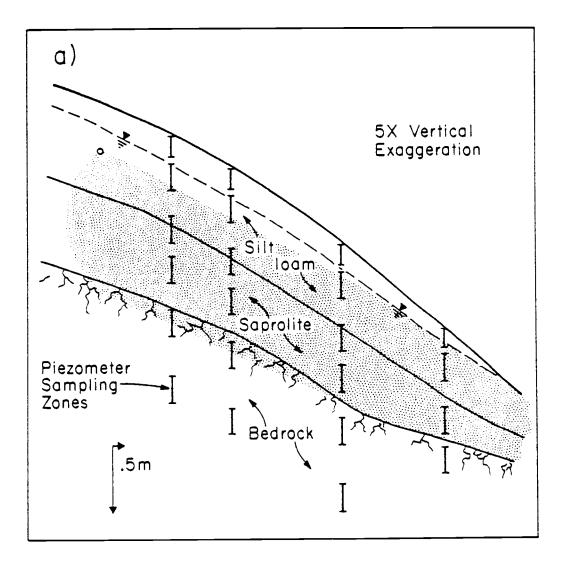


Figure 5.

The pathways and characteristics of bacterial transport through site number 8, a Dixonville soil series located in a lower backslope position (Rahe <u>et al.</u>, 1978).

a) Cross section of the experimental site displaying the zone of bacterial penetration from the B horizon injection.

b) The appearance of tracer <u>E. coli</u> with time at increasing distance downslope (solid line) from equation (4) using experimental K_L and site mean \bar{v} values. The decrease in the mean maximum bacterial density as a function of distance downslope both before (dashed line) and after (dotted line) correction for dispersion.



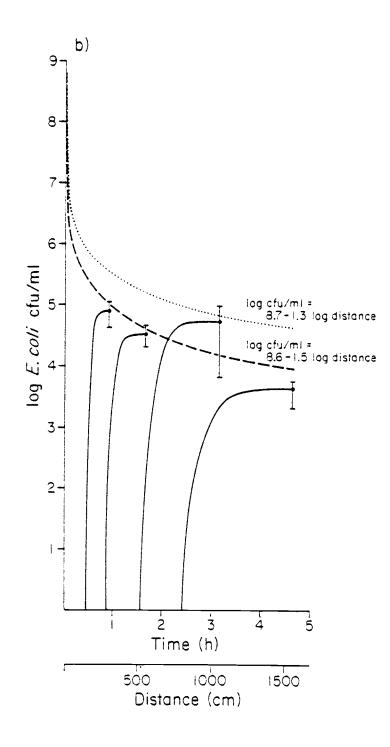
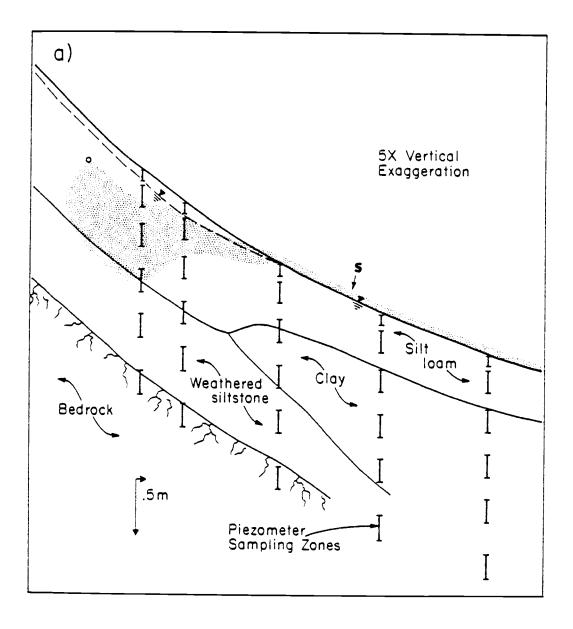


Figure 6.

The pathways and characteristics of bacterial transport through site number 4, a Steiwer/Hazelair transition soil series located in an upper footslope position.

a) Cross section of the experimental site displaying the zone of bacterial penetration from the B horizon injection.

b) The appearance of tracer <u>E. coli</u> with time at increasing distance downslope (solid line) from equation (4) using experimental K_{L} and site mean \bar{v} values. The decrease in the mean maximum bacterial density as a function of distance downslope both before (dashed line) and after (dotted line) correction for dispersion. The curve labeled 'S' (Fig. 6b) was produced from the tracer enumeration of water samples collected at the surface from the outflopw of a rodent tunnel. The position of this sampling point is indicated in figure 6a and also in figure 1 where a partial map of the extent of rodent tunneling is displayed (dashed line).



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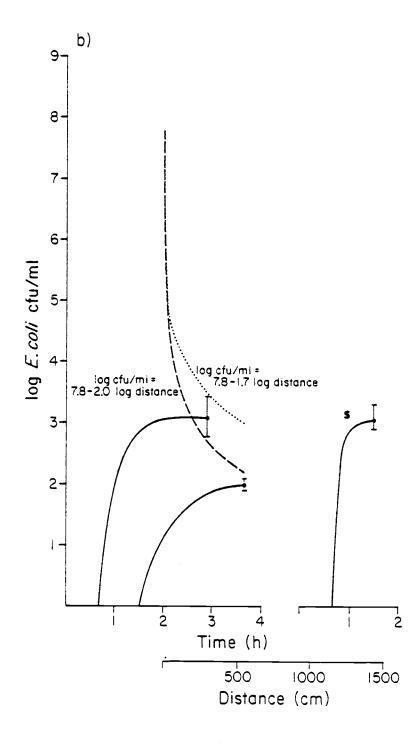


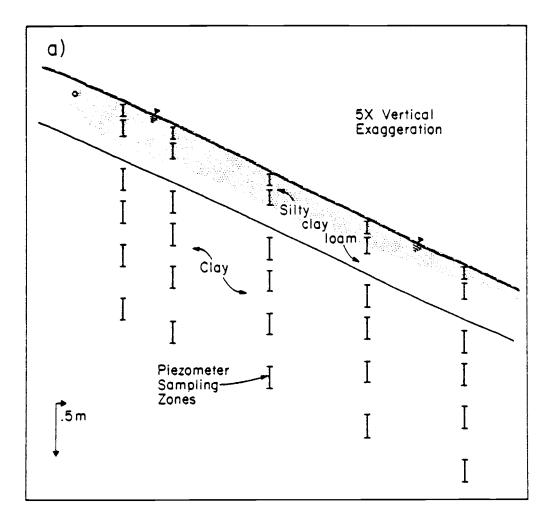
Figure 7.

The pathways and characteristcs of bacterial transport through site number 5, a Hazelair soil series located in a lower footslope position.

a) Cross section of the experimental site displaying the zone of bacterial penetration from the A horizon injection.

b) The apeparance of tracer <u>E. coli</u> with time at increasing distance downslope (solid line) from equation (4) using experimental K_L and site mean \bar{v} values. The decrease in the mean maximum bacterial density as a function of distance downslope both before (dashed line) and after (dotted line) correction for dispersion.

c) The appearance of tracer <u>E. coli</u> with time from surface samples taken at increasing distance downslope (cm). Values indicated are means of right and left side samples.



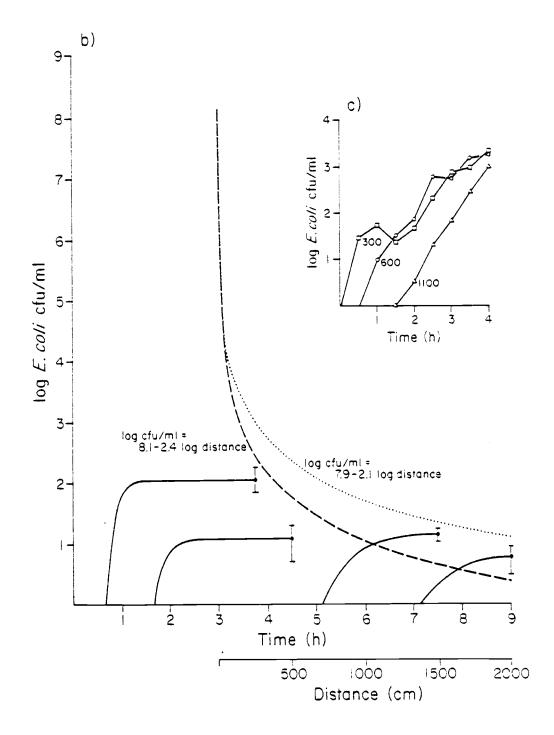
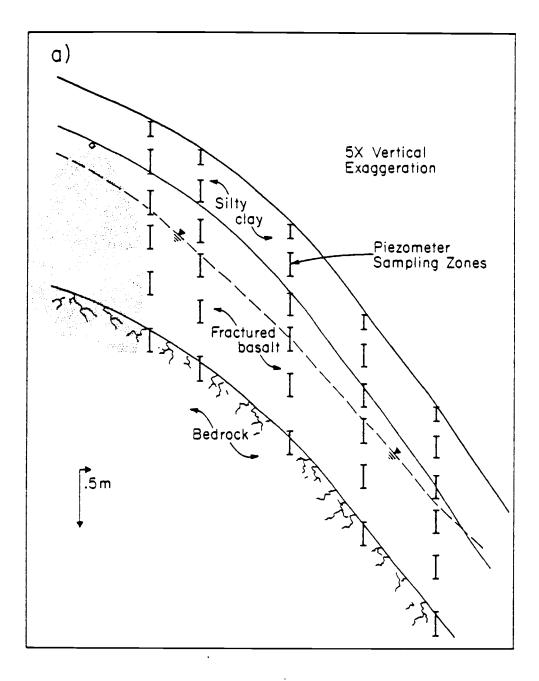


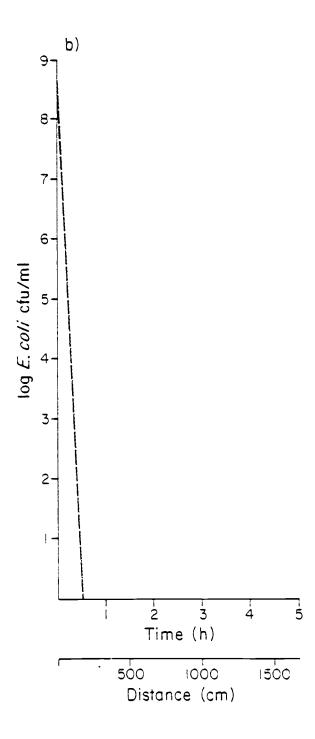
Figure 8.

The pathways and characteristics of bacterial transport through site number 6, a Philomath soil series located in a summit position.

a) Cross section of the experimental site displaying the zone of bacterial penetration from the B horizon injection.

b) The appearance of tracer <u>E. coli</u> with time at increasing distance downslope (solid line) from equation (4) using experimental K_L and site mean \bar{v} values. The decrease in the mean maximum bacterial density as a function of distance downslope both before (dashed line) and after (dotted line) correction for dispersion.





CONCLUSIONS

Water flow in saturated hillslope soils occur predominately through the zones of maximum hydraulic conductivity. These highly conductive soil layers within a soil profile are, under saturated conditions, highly inefficient for the renovation of contaminated groundwater. Some of the phenomena associated with the hillslope hydrologic processes which serve to support this claim include: 1) The leveling of the decrease in maximum tracer cfu/ml vs distance curves presented in figures 4b-7b (dashed line), and the more pronounced leveling when a correction for longitudinal dispersion is made (dotted line). 2) The tremendous transmitting power of large continuous pipes or tunnels in the soil (Fig. 6). 3) The surfacing of large numbers of tracer bacteria originally injected below the surface (Fig. 7). Therefore, the ability of septic system effluent-borne bacterial populations to travel large distances and in relatively high numbers demonstrates a threat to the purity of surface and groundwater used as a potable water source.

LITERATURE CITED

Fried, J.J. 1975. Groundwater Pollution: Theory, Methodology, Modelling, and Practical Rules. Elsevier Pub. Amsterdam.

Rahe, T.M., C. Hagedorn, E.L. McCoy, and G.F. Kling. 1978. Movement of antibiotic-resistant <u>Escherichia coli</u> through western Oregon hillslope soils under conditions of saturated flow. J. Environ. Qual. <u>7</u>:487-494.

Table 1. The soil water relationships expressed as the depth to the saturated zone measured in individual piezometers prior to site inoculation.

SITE 7: DIXONVILLE SERIES, 9 March, 1978.

<u>Piezometer No.</u>	Depth (cm)	<u>Depth to Water (cm)</u>
1 2 3 4 5 6	30 110 200 150 60 12	^a 38 87 65 35
11 12 13 14 15 16	12 60 150 200 110 30	58 77 173 57
21 22 23 24 25 26	200 110 30 12 60 150	98 48 58 128
31 32 33 34 35 36	60 150 110 30 200 12	
41 42 43 44 45 46	200 30 110 60 12 150	175 80 81
51 52 53 54 55 56	12 150 30 60 200 110	102 175 93

^ano water present in piezometer

Table 2. Data used to generate 3-D plots from the experimentation conducted at site 7, 9 March, 1978.

^aplot number, sampling time in minutes after injection.

^b-1 indicates that a water sample was not recovered.

^Csampling depth, tracer density in cfu/ml; note, depth values are given for the upper and lower limits of the depth-zone sampled.

 d_{\star} indicates the level fo the perched water table.

^einitial tracer density: A horizon - 3.5 x 10⁸ B horizon - 5.8 x 10⁸ C horizon - 7.9 x 10⁸

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Table 3. The soil water relationships expressed as the depth to the saturated zone measured in individual piezometers prior to site inoculation.

SITE 4: STEIWER/HAZELAIR TRANSITION SERIES, 7 JANUARY, 1978.

Piezometer No.	Depth (cm)	<u>Depth to Water (cm)</u>
1	35	^a
2	110	54
3	200	80
4	150	78
5	70	55
6	15	
11	15	12
12	75	22
13	150	77
14	200	9
15	110	22
16	40	22
21 22 23 24 25 26	200 110 35 15 70 150	25 16 18 16
31	70	19
32	150	124
33	110	102
34	40	25
35	200	
36	15	10
41 42 43 44 45 46	200 40 110 70 15 150	23 76 20 5 29

^ano water present in piezometer.

Table 4. Data used to generate 3-D plots from the experimentation conducted at site 4, 7 January, 1978.

^aplot number, sampling time in minutes after injection.

^b-1 indicates that a water sample was not recovered.

^csampling depth, tracer density in cfu/ml; note, depth values are given for the upper and lower limits of the depth-zone sampled.

^d*indicates the level of the perched water table.

^einitial tracer density: A horizon - 1.0×10^7 B horizon - 5.5×10^7 C horizon - 5.9×10^7

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250 CM DOWNSLOPF, A HOPIZON INJECTION<sup>e</sup>

1.0.20,40.60.70.120.150.195.24na

2.-1.-1.-1.-1.-1.-1.-1.-1.-1.

12.-1.-1.-1.-1.-1.-1.-1.-1.

12.0.0.0.20.248.240.300.360.490*d

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300 CM DOWNSLOPE, B HORTZON INJECTICN 1.0.2C, 40.6C.90.120.150.195.240 2.-1.-1.-1.-1.-1.-1.-1.-1.-1 12.-1.-1.-1.-1.-1.-1.-1. 20.0.0.0.0.0.0.0.0.0 20.0.0.0.0.0.0.0.0.0 40.9.0.0.0.0.0.0.0.1.4 75.0.0.0.0.0.0.0.0.1.4 90.0.0.3.66.610.590.640.2920.1510 11C.0.0.3.66.610.590.640.2920.1510 130.C.0.0.0.0.0.0.22.9 150.C.0.0.0.0.0.0.22.9 150.C.0.0.0.0.0.0.0.22.9 150.C.0.0.0.0.0.0.0.0.0 200.0.0.0.0.0.0.0.0.0 550 CM DOWNSLOPE. B HORIZON INJECTICN 2.0.20.40.60.90.120.150.105.240 2.1.1.1.1.1.1.1.1.1.1.1.1.1 12.-1.1.1.1.1.1.1.1.1.1.1.1 25.0.0.0.0.0.0.0.0.0.0 550 CM DOWNSLOPE. B HORIZON INJECTICN 2.0.20.0.0.0.0.0.0.0.0 550 CM DOWNSLOPE. B HORIZON INJECTICN 2.0.20.0.0.0.0.0.0.0.0 550 CM DOWNSLOPE. B HORIZON INJECTICN 2.0.20.0.0.0.0.0.0.0 550 CM DOWNSLOPE. B HORIZON INJECTICN 2.0.20.0.0.0.0.0.0 550 CM DOWNSLOPE. B HORIZON INJECTICN 2.0.20.0.0.0.0.0.0 550 CM DOWNSLOPE. B HORIZON INJECTICN 2.0.20.0.0.0.0.0 550 CM DOWNSLOPE. B HORIZON INJECTICN 2.0.20.0.0.0.0 550 CM DOWNSLOPE. B HORIZON INJECTICN 2.0.20.0.0.0.0 550 CM DOWNSLOPE. B HORIZON INJECTICN 2.0.20.0.0.0 550 CM DOWNSLOPE. B HORIZON INJECTICN 2.0.20.0.0 550 CM DOWNSLOPE. B HORIZON INJECTICN 2.0.20.0 550 CM DOWNSLOPE. B HORIZON INJECTICN 550 CM DOWNSLOPE. 150,0,0,0,0,0,0,0,0,0,0,0 180,-1,-1,-1,-1,-1,-1,-1,-1,-1 200,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1 350 C* POWNSLOPF, C HORIZON INJECTION 1, C, 20, 40, 60, 90, 120, 150, 195, 240 2, -1, -1, -1, -1, -1, -1, -1, -1 12, -1, -1, -1, -1, -1, -1, -1, -1 12, 0, 0, 0, 0, 0, 0, 0, 0 20, 0, C, 0, 6, 28, 58, 85, 101, 86 40, 0, C, 0, 6, 28, 58, 85, 101, 86 40, 0, C, 0, 0, 0, 0, 0, 0 75, 0, 0, 0, 0, 0, 0, 0, 0 90, C, 5, 95, 270, 230, 66, 58, 89, 63 110, 0, 5, 95, 270, 230, 66, 59, 89, 63 130, C, 0, 0, 0, 0, 0, 0, 0 150, 0, 0, 0, 0, 0, 0, 0, 0 180, 0, 0, 0, 0, 0, 0, 0, 0 150,0,0,0,0,0,0,0,0,0,0,0,0 180,0,0,0,0,0,0,0,0,0,0,0 290,0,0,0,0,0,0,0,0,0,0,0,0,0

Table 5. The soil water relationships expressed as the depth to the saturated zone measured in individual piezometers prior to site inoculation.

Piezometer No.	Depth (cm)	Depth to Water (cm)
1 2 3 4 5 6	30 110 200 150 80 12	0 a 40 0
11 12 13 14 15 16	12 80 150 200 110 30	0 32 100 0
21 22 23 24 25 26	200 110 30 12 80 150	 2 0 50
31 32 33 34 35 36	80 150 110 30 200 12	 5 3
41 42 43 44 45 46	200 30 110 80 12 150	0 90 56 0
51 52 53 54 55 56	12 150 30 80 200 110	5 5 70

SITE 5: HAZELAIR SERIES, 20 FEBRUARY, 1979.

^ano water present in piezometer.

Table 6. Data used to generate 3-D plots from the experimentation conducted at site 5, 20 February, 1979.

^aplat number, sampling time in minutes after injection.
^b-1 indicates that a water sample was not recovered.
^c sampling depth, tracer density in cfu/ml; note, depth values are given for the upper and lower limits of the depth-zone sampled.
^d* indicates the level of the perched water table.
^e initial tracer density: A horizon - 2.2 x 10⁸

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250 CM DOWNSLOPE, A HORIZON INJECTION e
1,0,20,50,80,120,180,240,300,360,420,480,540 a
2,0,0,1,41,94,104,116,84,71,52,44,35*
12,0,0,1,41,94,104,116,84,71,52,44,35
15,0,0,1,2,3,3,1,1,1,0,0,0
30.0.0.1.2.3.3.1.1.1.0.0.0
60,0,0,0,0,0,0,0,0,2,3,0,1
80,0,0,0,0,0,0,0,0,2,3,0,1
90,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1
180,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1
200,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1
500 CM DOWNSLOPE, A HORIZON INJECTION
2,0,20,50,80,120,180,240,300,360,420,480,540
2,0,0,0,1,0,3,9,20,7,5,7,0*
12,0,0,0,1,0,3,9,20,7,5,7,0
15.0.0.0.0.0.0.0.0.1.0.0.0
30,0,0,0,0,0,0,0,0,1,0,0,0
60,0,0,0,0,0,0,0,0,0,0,2,0
80,0,0,0,0,0,0,0,0,0,0,2,0
110,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1
150,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1
180,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1
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1000 CM DOWNSLOPE, A HORIZON INJECTION

3,0,20,50,80,120,180,240,300,360,420,480,540

2,0,0,0,0,0,0,0,5,3,2,3,1*

12,0,0,0,0,0,0,0,0,0,0,0,0

30,0,0,0,0,0,0,0,0,0,0,0,0

60,0,0,0,0,0,0,0,0,0,0,0,0

80,0,0,0,0,0,0,0,0,0,0,0,0

90,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1

110,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1

130,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1

150,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1

180,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1

200,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1
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1500 CM DOWNSLOPE, A HORIZON INJECTION

4,0,20,50,80,120,180,240,300,360,420,480,540

2,0,0,0,0,0,0,1,12,14,13,11,16

12,0,0,0,0,0,0,0,0,4,3,0,1

30,0,0,0,0,0,0,0,0,4,3,0,1

60,0,0,0,0,0,0,0,0,0,0,0,0

80,0,0,0,0,0,0,0,0,0,0,0

90,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1

110,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1

150,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1

180,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1

200,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1
```

```
2000 CM DOWNSLOPE, A HORIZON INJECTION

5,0,20,50,80,120,180,240,300,360,420,480,540

2,0,0,0,0,0,0,0,1,9,4,3,7 *

12,0,0,0,0,0,0,0,0,0,0,0,0

30,0,0,0,0,0,0,0,0,0,0,0,0

60,0,0,0,0,0,0,0,0,0,0,0,0

80,0,0,0,0,0,0,0,0,0,0,0,0

90,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1

110,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1

130,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1

150,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1

180,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1

200,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1,-1
```

Table 7. The soil water relationships expressed as the depth to the saturated zone measured in individual piezometers prior to site inoculation.

SITE 6: PHILOMATH SERIES, 16 FEBRUARY, 1979.

Piezometer No.	<u>Depth (cm)</u>	<u>Depth</u> to Water (cm)
1 2 3 4 5 6	45 110 200 150 80 12	^a 78 98 84 70
11 12 13 14 15 16	12 80 150 200 110 45	67 81 94 75
21 22 23 24 25 26	200 110 45 12 80 150	98 77 71 82
31 32 33 34 35 36	80 150 110 45 200 12	77 64 80 86
41 42 43 44 45 46	200 45 110 80 12 150	134 78 68
51 52 53 54 55 56	12 150 45 80 200 110	 76 84 82

^ano water present in piezometer.