TRANSPORT OF FAECAL BACTERIA FROM MANURE THROUGH THE VADOSE ZONE

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Abstract

Transport of faecal bacteria from manure through the vadose zone

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The movement of faecal coliforms, through the vadose zone following application of animal manure with contrasting dry matter contents, on two soils at contrasting initial soil water contents was researched.

Bacteria present in soil solution were collected using ceramic-porous-cup samplers. The development of a protocol for the calibration of these samplers is described.

An important conclusion of this study was that field application of animal manure can readily lead to ground water contamination with faecal bacteria. Macropore transport was more likely to occur in wet soils, but it was not necessarily restricted by the initial soil water content. The continuity of the soil's macropores was more important for the deep transport of faecal bacteria than the total porosity of the soil.

The potential for deep contamination with faecal bacteria was greater for the application of manure with higher water content.

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List of notations and acronyms:

BMV = Bacteria Migration Velocity

CFU = Colonies Forming Units

DL = Detection Limit

L/SL = Loam on Sand-Loam profile

L/ZL = Loam on Silt-Loam profile

LSM = Liquid Swine Manure

nd = not determined

NF = No bacterial Filtration by the soil

NTC = No bacterial Transport Confirmed

PV = Pore Volume

PWV = Pore Water Velocity

SBM = Solid Beef Manure

1. Introduction

Manure management has become a major concern in Ontario over the last decade because of issues of odour control, nutrient management and contamination of water resources.

Research on ground water contamination resulting from the use of manure has mostly been directed on contamination by excess nitrate, due to imbalances between the nitrogen inputs and outputs on cropped fields. However in the period from 1950 to 1992, although there was no evident change in the well water contamination with NO_3^-, the number of wells showing bacterial contamination increased from 15% to 25% (Goss et al., 1998).

Animal manure may constitute a point source for contamination (e.g. animal confinements, barns, exercise yards, and manure storage facilities). Goss et al., 1998, noted that the contamination of domestic wells was more likely to occur when they were situated close to a feedlot.

Manure may also constitute a diffuse source for water contamination after it has been applied to land surfaces. The most evident contamination is that of surface water resulting from the run off from fields to which manure was applied. A large number of manure spills associated with fish kills have been traced back to land spreading of liquid manure and the manure was found to enter the surface waters through the drain tile systems (Manure: farming & healthy fish habitat, 1997, pamphlet). Winter spreading on snow-covered or frozen fields has

been seen as increasing the potential for diffuse-source contamination by solid manure.

Although there is information on potential bacterial contamination of ground water being caused by septic systems and leaching beds (Klepper et al., 1987, Hagedorn, 1984), little information was found relating the presence of faecal bacteria in groundwater and the field application of manure (see Section 1.1.).

The main aim of the present thesis was to evaluate the potential for ground water contamination with faecal bacteria following land application of manure. One major problem in characterising the transport of bacteria to ground water following field application of manure results from difficulties in collecting the bacteria suspended in the soil solution. In the vadose zone generally the water is under negative pressure and energy is required to extract the solution. Soil samples can be collected from different locations and depths in the soil profile and the samples eluted for bacterial analysis (Natsch et al., 1996). However this method is destructive and the sampling cannot be repeated in the same location due to disturbance of the soil profile. Another way the movement of bacteria can be monitored through the vadose zone is by collection of samples from the tile drainage systems. This method has the advantage of being non-destructive and therefore the evolution in time of the bacterial movement can be evaluated. The fact that the drain tiles provide a direct path for water and bacteria movement can modify the pattern of transport through the vadose zone. Porous cups have been

used for many years to monitor the movement of different solutes through soil in field conditions (Wood, 1973). Krejsl et al. (1996) have tested a number of methods that can be potentially used for monitoring the bacterial transport in the vadose zone including the use of ceramic porous cups. However their tests with ceramic cups were performed only under saturated conditions, which rarely occur in the field. Furthermore, samples were collected over long periods of time using constant suction, which can lead to significant changes in the filtration characteristics of the cups (Hansen and Harris, 1974). This also required Krejsl et al. (1996) to average the bacterial transport rate over the whole period of the tests. No report of attempts to calibrate porous ceramic cups was found in literature. Therefore there was a real need to determine the most appropriate protocol for monitoring the bacterial movement within the vadose zone. In Chapter 2 of the present thesis, an evaluation of the potential use of the ceramic porous cups as sampling devices is presented. Although limitations for the use of such devices in detailed monitoring of bacterial transport were identified, the main finding was that they could be used to study to study the transport of bacteria through the vadose zone under well-defined boundary conditions.

The protocol developed in the first part of the study (as described in Chapter 2) was used in a field experiment that investigated bacterial movement after the field application of manure. Two manure types, liquid and solid, with very different dry matter contents were applied on two different soil profiles with contrasting initial soil-water content. Following the spreading of manure the equivalent of 50 mm of water was applied by drip irrigation. Soil solution was

sampled at various depth and time intervals using ceramic porous cup samplers (as described in Chapter 3).

The results of the field study (Chap. 3) indicated that the initial soil-water and the dry matter content of the applied manure contributed to the potential for bacterial contamination of ground water. However the transport of bacteria through macropore proved to be the most important parameter in the deep transport of bacteria from manure. A discussion of the results obtained in the field experiments considering the limitations of the sampling methods is presented in section 3.4., followed, in Chapter 4 and 5, by general discussion and the conclusions of the study.

1.1. Background

Manure is an inevitable by-product of livestock farming. The easiest way to dispose of manure is by spreading it on land. Manure is considered to be a useful amendment for improving the physical and chemical qualities of degraded soils and of soils with low organic matter content (Larney and Janzen, 1996, Martens and Frankenberger, 1992, Tester, 1990, Hornick, 1988). Numerous studies have focused on the nutrient content of animal manure, and its availability to crops.

In many regions the nitrogen content of manure has been used as the index for the quantity of manure that could be applied to a field. In part this developed as a means of stimulating the use of manure as a valuable economic

input to plant production (Wen et al., 1995, Bubb, 1987, Morison, 1981). On the other hand the nitrogen and phosphorus content of manure have also been used as factors to limit the amount of manure applied owing to the risk of contamination to surface waters and ground water by excess nutrients. For ground water contamination, nitrate is considered to be the most likely potential contaminant due to imbalances between the soil nitrogen input and output (Chang and Janzen, 1996, Goss and Goorahoo, 1995). Bacterial contamination hazard due to runoff into surface waters from fields after manure application is also considered as a potential problem (Pratt, 1979).

Investigation of the ground water contamination with bacteria, however, has focused primarily on point sources such as industrial sites, landfills, and septic systems (Malard et al., 1994, Steward and Reneau, 1982). Manure lagoons have been also considered as possible point source for pollution in the ground water (Westerman et al., 1995). When lagoons are emptied cracks may develop in the clay liner and newly added manure can seep out into the surrounding soil before the liner can reseal.

Increasingly, intensive agricultural activity in the recharge areas of urban well fields has been recognised as a potential threat to ground water quality because of diffuse or non-point source of contamination. Hence the impact of agricultural practices on surface and subsurface water quality has become a major concern in Ontario (Stone and Logan, 1988). There have been reports of greater contamination of ground water in areas where animal manure is applied

regularly (Ritter and Chimside, 1987). However the impact of agricultural land use practices on regional ground water is not well understood (Goss et al., 1998).

Goss et al., (1994), in a general assessment of the impact of animal manure on water quality in Ontario, reaffirmed the conclusions of previous studies that the pollutant of major concern for ground water quality is nitrate because of its mobility. However, when Goss et al. (1998) evaluated the rural ground water quality in Ontario, they found that bacterial contamination was the most wide spread with about 34% of the 120 wells studied having more than the permissible levels of coliform bacteria - faecal coliforms, or Escherichia coli, or total coliforms. Of these wells, 7% had unacceptable levels of both nitrate and coliform bacteria.

Bacteria may also enhance the transport of various chemical pollutants to the ground water acting as a vehicle for other organic and inorganic substances, which are attached to the bacteria surfaces (Choi and Corapcioglu, 1997, Kim and Corapcioglu, 1996, Saiers and Hornberger, 1996).

1.2. Factors influencing the potential for ground-water contamination with disease organisms from applied manure

The concentration of bacteria in manure applied to soil is a key parameter in determining the potential for contamination of water resources. The microbial population in manure undergoes considerable change during storage. The type

and density of microorganisms in manure may vary with animal species, age of animals, storage methods (liquid or solid), and storage period (Lachica, 1990, Nodar et al., 1992). Poultry excreta and cattle slurry, have been found to contain large numbers of microorganisms, but in pig slurry the numbers were greater by an order of magnitude. During the storage of liquid manure the population of viable organisms declines rapidly initially only to regain numbers later, up to five-fold the initial value after 14 weeks (Nodar et al., 1992). In solid manure there are gradients of temperature within the manure pile, which are the results of different rates and types of organic matter digestion (aerobic at the periphery, to more anaerobic toward the centre of the pile). Microorganisms have different rates of survival in these zones. The ones near the periphery have more chances to survive and form sources of contamination (Sutton, 1983).

Survival rate is another important factor influencing the potential for microorganisms to contaminate water sources. The survival rate depends on the species, and on the manure application method. When injected, microorganisms are less likely to be destroyed by the ultraviolet solar radiation. On the other hand, incorporation increases the possibility for microorganisms to be adsorbed by the soil particles (Patni et al., 1985). Biological activity in the superficial strata is higher in no-till than in conventional tillage, which results in better conditions for survival of the microorganisms (Levanon et al. 1994).

Competition between soil microorganism has been found to be a major factor in the reduction of the bacterial populations introduced in soils (Acea et al., 1988). Murry and Hinckley (1992), found that the number of Salmonella

enteritidis is more limited in the presence of earthworms (Eisenia foetida) – 8% reduction versus only 2% reduction without earthworms. They also noted that normal soil bacterial flora was reduced by 3% in the presence of earthworms. In contrast, in the earthworms' absence normal bacteria flora increased by 2%, compared to the initial levels. Other soil microorganisms such as protozoa, nematodes and Bdellovibrio - a soil bacterium - prey on soil bacteria and implicitly on the ones introduced with manure (Goss et al., 1996). The survival of faecal bacteria can extend over long periods after manure application; survival is possible 11-14 days after application of pig manure, and once bacteria reach the ground water the survival period can be extended to several months (Goss et al., 1996). Antibiotic resistant strains of Escherichia coli and Streptococcus faecalis were found to persist in high numbers over a period of at least 32 days in saturated soil conditions (Hagedorn et al., 1978). Recent research shows that Escherichia coli and Enteroccocus spp. from pig manure may survive in soil for even longer periods - 40 to 68 days after application (Cools et al., in press, Shresta et al., 1997).

Low temperature levels favour faecal bacteria survival, and bacteria are considered to be more likely to survive a longer period in soils with high water holding capacity (Gerba and Bitton, 1984). Low matric potential – high negative values - (i.e. dry conditions) seems to reduce the viability of bacterial cells in soil. However recent research suggests that the soil-water content has limited influence on the survival of enteric bacteria in soil (Cools et al., in press).

Survival rates may be also related to the level of available nutrients (Rattray et al., 1992). Survival of faecal coliforms is greatly extended in organic soil compared with that in mineral soils. This might have also to do with the higher water-holding capacity level of these soils (Gerba and Bitton, 1984). Laboratory tests performed by Cuthbert et al. (1950) showed that Escherichia coli and faecal streptococci have survived several weeks in limestone (pH 5.8-7.8) while dying in a few days in peat (pH 2.9-4.5). Under field conditions, it has been found that some regrowth may occur in the case of Escherichia coli and Streptococcus faecalis.

1.3. Contaminant pathways

1.3.1. General considerations

A substantial amount of research has been done on the transport of viruses and bacteria in porous materials. However the great majority of these studies have concentrated on the transport of microorganisms once they reached the ground water. Thus information on transport processes are very much limited to that for microorganisms within an aquifer, under conditions of saturation. There have been far fewer studies that have aided the understanding of bacterial transport mechanisms through the vadose zone above the ground water level.

The factors affecting the transport of bacteria through soils are those that dictate their concentration in soil and the flux of water available to move them.

These factors are very much the same factors that affect transport through soil of any other contaminant (Table 3.1.).

Table 1.1.

Characteristics that influence the transport of contaminants through soil (adapted after Wagenet and Rao, 1990):

I. Soil parameters	II. Climatological parameters
Dispersion coefficient	Evapotranspiration
Saturated water content	Temperature
Field-capacity water content	Snow melt
Wilting point water content	Hours of sunlight
Hydraulic properties	
Bulk density	III. Management parameters
Organic carbon content–	
pH	Crop-production systems variable
Cation Exchange Capacity	Soils variable
Heat flow parameters	

Under field conditions, soil-water content, soil structure and texture cause the transport and retention mechanisms to be significantly different for vertical and horizontal directions (Stotzky, 1985).

1.3.2. Water flow and microbial transport

The mechanisms that affect the movement of contaminants through soil are: a) **advection**; in which the contaminant is moved with the bulk of water.

Convection of the water (and therefore the advection of the suspended particles) is considered to be the main mechanism of transport in the unsaturated zone. In practice the flow patterns may be complicated by a number of factors: spatial variability of the physical properties of soils; coarse structure due to aggregates, cracks and channels; and secondary flows due to density gradient

in the liquid phase and to instability of the wetting fronts (Raats, 1984). Since the density of a bacterial cell is only slighter higher than that of water making it likely to move at the same speed as the water in which it is suspended.

b) **diffusion, dispersion;** the spreading of a solute due to the concentration gradients and mechanical mixing which occurs when water moving through the soil pores diverges around the soil particles and as a result the water front spreads out through the soil.

Transport of molecules and small particles (0.01 - 0.1 μm) may be described satisfactorily in terms of diffusion. Bacterial diffusion may be limited due to the pore size exclusion phenomenon. Particles of bacterial size are transported by a number of mechanisms, including dispersion, created by varying velocities in different pore size, which causes the front of water to spread out. and by fluid dynamic forces especially in a turbulent flow regime (Characklis, 1981). Hence some of the contaminant travels faster and some slower. In still water or in the viscous layer at the surface of soil particles Brownian movement could be of importance.

c) **adsorption-adhesion** – chemical and physical binding of the contaminant to the surface of the soil particles.

The result of adsorption-adhesion is retardation of the contaminant flow. In this case the microorganisms may be slowed down by reversible adsorption on the surface of soil minerals. Microorganisms are adsorbed at different degrees as function of the pH, organic matter content, and the characteristics of the microorganisms like wall structure and chemical composition.

Bacteria generally possess a net negative surface charge at most pH values found in nature. Soil particle surfaces have also a net negative charge. At first consideration this should indicate a repellent effect between the two. Because of the charge of these surfaces, a potential exists between them and the bulk aqueous phase. To counterbalance the surface charge, ions of opposite charge (gegen ions) are loosely attracted to the surface to form a diffuse double layer of ions. When two negatively charged bodies are in close association they may be repelled or attracted to each other. This effect depends on the thickness of the double layer, which, in turn, is dependent on the valence and concentration of the electrolyte (Marshall, 1985).

The DLVO[1] theory states that as rigid bodies of like charge approach each other, they are subject to attractive and repulsive forces that are additive, but vary independently with the distance of separation between the bodies. At relatively long distances the attractive forces are greater than the repulsive forces, resulting in attraction between the bodies. The forces of attraction at this distance (secondary minimum of potential energy) are weak and easily reversed by liquid shear. At shorter distances, the repulsive component prevails. At the potential energy maximum there is a strong repulsion between the two bodies. If the forces of repulsion can be overcome, at very short distances (1 nm), then there is a mutual attraction (primary minimum of potential energy). At this

[1] DLVO – Derjaguin-Landau-Verwey-Overbeek theory; the theory of stability for lyophobic colloids stating the balance between London (charge repulsion) and van der Waals forces (dispersive attraction) – also known as the theory of double ionic layer. Published by B. V. Derjaguin and L. Landau, 1941, in Acta Psysicochim., USSR vol. 14 and E. J. W. Verwey and T. G. Overbeek, 1948 in Theory of stability of lyophobic colloids, Elsevier, NY

distance the attraction between bodies is strong and not easily reversed - irreversible adhesion (Mills and Powelson, 1996, Marshall, 1985).

The overall charge and shape of the bodies are important and contribute significantly to the forces of attraction and repulsion. With increasing curvature (decreased radius), there is a decrease in the forces of attraction and repulsion. The forces of repulsion, however, decrease more rapidly than those of attraction do. Therefore, curved bodies come closer together at the secondary minimum and require less kinetic energy to get to the primary minimum (Christensen et al., 1985).

Bacteria should be attracted reversibly to the secondary attraction minimum at high electrolyte concentrations, but should be repulsed at lower electrolyte concentration.

Bacteria adhere to soil particles through chemical bonds, dipole interaction or hydrophobic bonding. Hydrophobic bacteria seem to adhere more firmly to solid surfaces than hydrophilic bacteria although other factors may modify this relationship. Clays are considered ideal adsorption sites for microorganisms. Thus, soils with higher clay content are more likely to adsorb a higher number of bacteria than sands (Stotzky, 1985).

The presence of certain metallic cations - Fe^{3+}, Cu^{2+}, Zn^{2+}, or NH_4^+, enhances the removal of bacteria from soil solution by reducing the repulsive forces between the two surfaces (soil particles and bacteria), thus allowing closer interaction between them, which permits adsorption to occur. This indicates that retention efficiency is higher in acid conditions. On the other hand soluble

organic compounds may compete with bacteria in soil for adsorption sites, although no significant competition has been found when wastewater effluent is passed through soil (Stotzky, 1985).

Rainfall affects bacteria adhesion by lowering ionic concentration and increasing infiltration rates. It appear that bacteria do not have enough energy to overcome the surface tension of the water associated with soil microaggregates (Stotzky, 1985). Water adsorbed on clay is less dense, more viscous, and freezes at a lower temperature than free water. This highly ordered water is unlikely to be available to microorganisms; it is more likely that microorganisms are associated with clay-associated water at some distance from clay minerals surface making it more likely for them to be removed from the adsorbed sites and released into the soil solution.

Therefore the attached bacteria are likely to be easily detached by changes in soil solution properties, like increased pH or decreased solute concentration.

If the contact between bacterial cells and soil particles is prolonged, bacteria may become attached by the means of polysaccharide slimes. Experiments, cited by Stotzky (1985), indicated that such adhesion might be enhanced by "starvation conditions". It is also known that in such conditions the bacteria tend to reduce their volume and therefore increase the surface's curvature. This means that in such situations bacteria will be more likely to adhere at the surface of soil particles reducing the likelihood to be transported to ground water.

While the adsorption of bacterial cells at the surface of soil particles due to surface electrical charges is reversible for the soil solution concentration range found in most soils, the attachment due to polysaccharide slime tends to be irreversible (Stotzky, 1985).

Field application of manure increases the amount of organic compounds that compete with bacteria for adsorption sites in soil. The alkaline pH of manure increases the chances for bacteria to be removed from the adsorption sites and released into the soil solution. Nutritional condition are also improved and therefore the chances for "starvation conditions" to occur are limited. All these factors restrict the chances for bacteria to be retained at the surface of soil particles.

Considering the mechanisms that affect bacterial transport in soil, the water flux (advection), bacterial cell characteristics, soil type and manure type (that control dispersion, diffusion, adsorbtion and adhesion) seem to be the major factors that influence the vadose zone transport of bacteria from manure.

1.3.3. Preferential flow

In general most measures of ground water contamination assume simple percolation from the land surface and ignore preferential flow paths in the vadose zone. These pathways result in a more direct and rapid movement of contaminants to ground water. The study of water and solute flow through soil has tended to concentrate on displacement flow.

The vast majority of researchers have considered that the water and solute flow follow Darcy's law considering the soil as a homogenous medium. Preferential flow or by-pass flow represents the flow that occurs not through the soil matrix but, as the name suggests, through channels which by-pass the matrix. Schumacher acknowledged this type of flow in 1864 (Beven and Germann, 1982). Lawes, in 1882, observed that "The drainage water may...be of two kinds: It may consist (1) of rainwater that passed with but little change in composition down the open channels of the soil, or (2) the water discharged from the pores of the saturated soil" (Beven and Germann, 1982). Hursh in 1944 affirmed that "...in upper soil horizons... the soil porosity is not a factor of individual soil particles size but rather of structure determined by soil aggregates which form a three dimensional lattice pattern"(Beven and Germann, 1982).

The existence of preferential flow has been studied with dye tracer observations and using chemical tracers experiments checking the speed at which they could be recovered at different soil depths. In an experiment with dye tracer on 14 soils from Switzerland, Flury et al. (1994) reached the conclusion that the occurrence of preferential flow is the rule rather than the exception.

Simpson and Cunningham (1982) in describing the mechanism for widely different water flow velocities through a Typic Hapludalf clayey, mixed, mesic soils reported the existence of "flow feature channels".

Experiments on a clayey soil (Sharkey clay soil) in Louisiana monitored the flux of atrazine and NO_3 - N applied at the soil surface. After a rain event the atrazine and nitrate were recovered in the drain tile in a short interval, in relation

to the interval predicted, using plug flow and the hydraulic conductivity. Thus such evidence provided an indication of preferential flow (Johnson et al., 1995).

Field studies of solute transport have shown that water flow may vary tremendously across a field. Further experiments made on undisturbed stony soil (gravel) monoliths (75 cm length and 30 cm diameter), have shown that the flow paths remained invariant, and may be an intrinsic property of the soil (Buchter et al., 1995).

In an experiment of Singh and Kanwar (1991) six undisturbed soil cores (61 cm length/ 15 cm diameter) were collected from three no-till and three conventional tillage field plots. The side walls were sealed with chemical inert materials (paraffin and plaster of Paris). The soil columns were saturated with $CaSO_4$ (0.005M); $CaCl_2$ (0.005M) was applied at the surface and the effluent was collected at the bottom. Later the samples were analysed for Cl^- (chloride) concentration. The Cl breakthrough curves (relative Cl concentration vs. relative pore volume) were analysed and the degree of preferential flow analysed. The results clearly suggested the occurrence of preferential flow through macropores in large undisturbed soil columns in both no-till and conventional-tillage.

No-till columns had larger values of immobile pore water fraction (56%) in comparison with conventional tillage (49%). The convective-dispersive equation (adjusting the hydrodynamic dispersion coefficient- D-, and the retardation factor -R) was used to develop breakthrough curves that compared well with observed breakthrough curves in all columns. Large values of D and a greater degree of deviation between observed and predicted breakthrough curves for no-till

columns in comparison with conventional tillage columns indicated a wider range of pore water velocities in no-till columns. Singh and Kanwar also concluded that because the laboratory studies, using soil columns, do not include the effect of large soil cracks, field studies are needed to monitor the effects of large and continuous cracks on water and solute transport processes.

The mechanisms that create preferential flow are relatively well understood, but cannot be absolutely predicted from the known characteristics of soil. The macropores through which macroflow occurs may be of different types:

- biopores - created by soil fauna and plant roots
- cracks and fissures (very often in dry soils with relatively high content of clay)
- natural soil pipes (erosion due to subsurface flow)

There is little accord between researchers as to what size of pore constitutes a macropore. Numbers cited by Beven and Germann (1982) reveal quite different views in understanding what macropores are. Thus the macropore are considered as the soil pores with a minimum equivalent diameter of 3000 μm (Beven and Germann, 1981), 60 μm (Bullock and Thomasson, 1979), or 30 μm (Marshall, 1959). Azooz and Arshad (1996), for the purpose of an experiment, considered as macropore everything with an equivalent diameter greater than 14 μm.

It is generally considered that for a microorganism to be transported through a pore by infiltrating water the size of that pore has to be at least 1.5 times greater than the microorganisms' major axis. That would suggest that the

minimum diameter for functioning macropores is dependent on the characteristics of a given microorganism. Thus if the capacity for transporting a microorganism is considered a major criteria for defining the term macropore then pores with a diameter as small as 3 to 5 μm can meet this definition even if only for short path lengths.

Experimental evidence also has shown that, under specific boundary conditions, preferential flow or bypassing of soil matrix may take place within capillary - sized pores. In a field investigation in a clayey soil pit-transects revealed that rapid saturated flow occurred through vertical zones of loose, porous, fine structured soil. The texture of the soil channels was clay, as was the matrix between channels (Simpson and Cunningham, 1982).

Boundary conditions for macropore flow can be very complex. A single solute pulse applied at the surface of a soil may split into many pulses of variable velocities. The faster pulse may carry a fraction of the applied chemical well below the biologically active zone in a very short time. It appears that a fast pulse starts when the intensity of infiltration exceeds a certain threshold. Preferential flow is generally considered to occur as the soil matrix becomes saturated with water. Hence, in the case of wetting soils, while the matric potential approaches zero, additional water is moved solely under the influence of gravitational force, which favour faster macropore flow.

In case of drying soils although the soil may be close to saturation a negative pressure may be exercised on the pore water limiting its flow. However soils that develop cracks in dry conditions, such as soils with high clay content,

can demonstrate preferential flow independently of the status of the matric potential. Depending on the initial content of water in soil, even rainfall events of 1 to 10 mm may be sufficient to initiate macropore flow. When the matrix is not saturated the water that flows through the macropores may infiltrate the macropores walls. Therefore greater initial soil-water content may allow deeper penetration along the macropores, infiltration along the macropore being reduced (Beven and Germann, 1982). However in an experiment carried by Flury et al. (1994) there was not a significant effect of the initial soil-water content on the flow pattern and the maximum penetration depth of the water. Nonetheless a greater preferential flow was observed in wet soils.

Preferential flow is also related to the soil structure and texture. It is usually considered that it is more likely to occur in structured soils than in coarse-textured, unstructured soils (Roth et al., 1991). Yet Kung, (1990), noted that preferential flow may also occur in sandy soils having high matrix permeability, but which also contain discrete fine textured lenses of porous material. When water reaches these lenses they act as a barrier for downward flow thus causing the water to focus, creating flow as through a funnel (fingering). Structural voids, such as cracks, can cause preferential flow at very high infiltration rates, different from the surrounding matrix. In fine-structured soils containing such structural spaces almost all of the convective transport may avoid the matrix (Beven and Germann, 1982).

This type of flow may be very significant for the deep transport of bacteria. In cases of instability of the water-front in coarse soils, the physical properties of

the preferential flow may be similar to the flow through matrix, only at different speed (Jury and Flühler, 1992). Great amounts of irrigation waters may enlarge the natural channels existing in soil (Simpson and Cunningham, 1982), although preferential flow, especially at saturation, is not conducive to macropore development except by eluviation and piping processes. Soil saturation inhibits the activity of animals and roots and will tend to lead to a breakdown of soil structure. Thus the role of macropores is limited to depths where saturation is a seasonal phenomenon (Beven and Germann, 1982).

On ploughed soils the water moves by Darcy's law through the layer of relatively high conductivity, and accumulates at the bottom of the tilled layer until the potential reaches about 0. Some of this water then apparently enters a few macropores initially through thin water films which become thicker as additional water moves into the macropores. This water then moves down the macropore due to gravitational potential. The initiation of water movement through macropores in the undisturbed soil under the ploughed layer is delayed because shearing, smearing and compacting due to tillage implements closes many of the macropores. Higher storage capacity of the ploughed layer can also delay the water reaching the macropores situated in the undisturbed layer below. If the quantities of additional water are lower than the storage capacity of the ploughed layer the deep macropore flow may be delayed indefinitely.

That is not the case in no-till soils (Thomas and Phillips, 1979). The development of the macropores to the soil surface can induce earlier macropore flow. In such conditions satisfying the conditions for macropore flow requires

lower amounts of water. Therefore the time to the start of macropore flow is reduced.

Due to highly heterogeneous nature of field soil the actual mechanisms involved in preferential flow may not be revealed exactly (even if theoretically they are known). To describe the transport process stochastic models have been created which ignore the actual mechanisms involved and treat the process as a black box (Li and Ghodrati, 1994). Therefore the problem of solute transport through soil cannot be characterised but it can be accounted for the volume of solutes by including the description of liquid transport through each zone and the transfer of solute mass between zones (Jury and Flühler, 1992).

A good quantitative prediction of flow through soil must consider the physical, chemical and biological components of soil. Field tests are very important for formulating equations that include preferential flow (Wagenet, 1990). Predictions of solute transport in the field using laboratory experiments and tests should be very carefully evaluated because there may be no account of the effect of adjacent sites. Laboratory investigations are mostly reduced to a two dimensional flow experiment. Therefore the most reliable estimates require a field study (Smith, 1995). Such studies account for differences in soil layering - variances in horizontal thickness of soil layers (Ward et al., 1995) and for the variability of the hydraulic properties on planes parallel and perpendicular to bedding (Yeh et al., 1985).

Saturated flow is more likely to favour the downward transport of bacteria because they will tend to confine their movement to macropore pathways. Under

unsaturated flow conditions the likelihood of macropores being filled with water is greatly reduced. The water is more likely to seep down in films along the macropore walls increasing the likelihood for bacteria to adhere to soil particles. Preferential flow is therefore important for bacterial transport as long as it refers to flow through channels with dimensions big enough to allow bacteria to pass.

1.3.4. The influence of soil characteristics on bacterial transport

The extent of microbial transport seems to be mostly related to soil structure. Soil physical properties, such as bulk density, influence bacterial displacement through its effect on soil porosity and pore size distribution. Increased soil bulk density decreases the volume of macropores thereby reducing the corresponding bacteria migration (Huysman and Verstraete, 1993).

Soil columns prepared from mixed, repacked soils were much more effective as bacterial filters (Smith et al., 1985). Suspended bacteria can move rapidly through the profiles of well-structured soils when moderate to high rates of water are added. This transport occurs in macropores with very small reductions in bacterial concentration (Natsch et al., 1996). Any field that receives water at a sufficient rate to fill these pores is likely to allow the rapid transport of suspended bacteria to the depth that these macropores are continuous. The degree of macropore flow influences the rate of movement of water and non-interacting solutes but determines the extent of bacterial transport. Non-interacting solutes and water flow through soil at a rate that is influenced by the

existence of macropores. In contrast bacteria can be transported through soil only through macropores (Abu-Ashour et al., 1994, Smith et al., 1985).

Under saturated conditions bacteria tend to move faster than the average water flow. This may be explained by pore size exclusion. Bacteria are only transported through the larger pores in the soil where the average pore water velocity is higher than the average of the entire soil column. Also anion exclusion may enhance bacterial transport. Negatively charged bacteria may be pushed away from the negatively charged soil particles keeping the bacteria in the middle of the pores where the flow velocity is highest. Therefore in the field, surface application of materials containing bacteria, especially when followed by series of rainfall events, can result in the rapid transport of microbes in large numbers through macropores. In such situations bacteria behave somewhat like a conservative ion, and consequently their transport is affected by cultural practices (Natsch et al., 1996)

Adsorption, adhesion, and straining processes will tend to slow down the movement of bacteria. Bacteria are believed to be largely removed through filtration processes while adsorption and adhesion are the major factors controlling retention. Filtration occurs when suspended particles including bacteria accumulate at the soil surface form a filtering mat, which restricts the movement of bacteria through soil.

When organic particles accumulate in macropore necks clogging restricts the downward movement of bacteria. Also the size of bacteria may influence the speed at which it is moved downward. Escherichia coli being a relatively large

bacterium may arrive faster at the water table than other smaller microorganisms (Gerba and Bitton, 1984). The fate of pathogenic bacteria and viruses in the subsurface is also determined by their survival and retention rates by soil particles. Both survival and retention are largely determined by three factors: climate, soil, and nature and the source of the microorganism.

1.4. Indicator organisms for faecal contamination

For a microorganism to be considered suitable for use as an indicator it should satisfy certain criteria: (a) the indicator should always be present in the source, (b) it should be present in numbers greater than the pathogen, (c) it should respond to the natural conditions similar to the pathogen, and (d) it should be easy to isolate, identify, and enumerate (Olivieri, 1982).

Animal faeces contain a large number of bacterial species of both gram negative and gram positive types, pathogens and non-pathogens. The largest represented groups are the faecal coliforms and streptococci. Faecal coliforms are always present in animal manure. Escherichia coli is considered the most common coliform in manure, and has both pathogenic and non-pathogenic strains. Escherichia coli is considered to survive in soil and water for periods similar to other contaminant bacteria as Pseudomonas aeruginosa and Salmonella spp. (Cools et al., in press, Burton. et al., 1988). Another organism widely used, as an indicator of faecal contamination, is Clostridium perifringens. Although it is more persistent in the environment as spores, it is found in

relatively smaller numbers in both pig and cow faeces (Geldreich, 1976). It is a very useful indicator for older contamination events (Olivieri, 1982). Other indicators, used in research mostly, are Pseudomonas aeruginosa and Bifidibacterium spp. The first one seems to be rather specific to humans than to animals. Bifidobacterium seems to have a greater sensitivity to environmental factors than Escherichia coli and consequently tends to die off faster. Therefore Bifidobacterium has been proposed as a useful indicator of fresh contamination (Carrillo et al., 1984).

Standard methods have been developed for the identification and enumeration of most of the possible indicators. Plate count tests for detecting and enumerating total coliforms and faecal coliforms are standard methods and these can be relatively quick tests. Supplementary confirmatory tests can be used only where deemed necessary.

Escherichia coli is considered to constitute the vast majority of organisms in the faecal coliforms group. Therefore the results of faecal coliform tests are very often considered to represent the incidence of E. coli (Charriere et al., 1994).

Nevertheless the presence of indicative faecal bacteria colonies in ground water does not necessarily reflect the field application of manure. Faecal bacteria in ground water may originate from point-sources as barns, animal exercise yards, manure storage facilities, septic systems, garbage dumps, soil fauna or wildlife activity. Therefore other organisms can be more useful indicators for determining the contamination source for a contaminated aquifer.

However the presence of indicative faecal bacteria in the vadose zone above the water table, for areas far away from any point source, after field manure application, is most likely to originate from the manure. Hence identification of Escherichia coli in the vadose zone may give an indication of the potential for ground water contamination with faecal coliforms from manure.

Considering this, Escherichia coli was considered a good indicator for the monitoring of bacterial transport through the vadose zone for the purpose of this experiment.

1.5. Collection, Detection and Enumeration of faecal coliforms

1.5.1. Bacteria collection

The Escherichia coli species includes numerous strains, pathogenic as well as non-pathogenic ones. The surface coats vary between strains so that the organisms behave in different ways at water-air or water-solid interfaces (Lachica, 1990). Hydrophobic strains are rejected from the aqueous phase and therefore attracted to any nonaqueous phase including the solid phase (Stenstrom, 1989, Marshall, 1985, Kjelleberg, 1985, McAneney et al., 1982, Marshall, 1980). The bacterium itself has little ability to overcome the repulsion barrier due to the double, soil-bacterium, negative charge at very small distances. Therefore if a bacterial cell is positioned close to a soil particle and in absence of strong shear forces it may attach irreversible through other

mechanisms like secretion of extracellular adhesive materials as polysaccharides.

Hydrophilic strains will be found in the bulk of the water. In saturated conditions, the water-air interface is very small or absent and, as noted above, the hydrophobic strains may tend to attach themselves to the solid-water interface. However attachment is reversible, with a time scale for detachment on the order of days or weeks. Slower attachment and detachment rates were observed for hydrophilic comparatively to the hydrophobic strains, suggesting that the former would move further before being removed by attachment to soil, but once attached, would be detached at a slower rate (McCalou et al., 1994). Consequently hydrophobic strains have been found to move slower than hydrophilic ones (Huysman and Verstraete, 1993).

Estimation of the presence and concentration of faecal coliforms in soil may be done by collection of soil samples followed by separation of the existing bacteria. In the case of the soil samples, bacteria are extracted from the sample by mechanical or manual shaking, trituration, sonication or mechanical blending (Klute et al., 1986). These methods may overestimate the potential for transport to depth of bacteria that were actually retained on the soil particle surfaces but were released and counted. Standardisation of the method used to extract the bacteria from the collected soil sample, however useful, cannot avoid errors due to the differences in the strength of the binding forces between bacteria and soil particles. These differences are function of the soil mineral, chemical and organic matter composition, and cell position (Richaume et al., 1993).

Potential contamination due to faecal coliform presence may be also estimated by analysing samples of soil solution. In this case only bacteria suspended in the soil solution bulk and the ones attached to the air-water interface are collected. As only the bacteria available to transportation are collected the method provides a more accurate estimate for potential for contamination. Still, bacteria that are reversibly attached to soil particles, and therefore able to move and potentially contaminate may not be accounted for. Repeat sampling can partly overcome this limitation.

Collection of samples from unsaturated soils may involve other difficulties mostly related to the potential small volumes of samples. By using porous cups filtration occurs (Krejsl et al., 1994) and therefore estimation of bacteria in soil solution requires calibration and indirect calculations.

1.5.2. Detection and Enumeration

There are two major standard techniques for the detection and enumeration of total and faecal coliforms in water samples. Faecal coliform membrane procedure also known as Membrane Filter technique -MF- and Most Probable Number technique -MPN- (Standard methods for the examination of water and wastewater, 1987). The MF technique is considered a faster alternative for the MPN technique. Multiple studies have been carried for testing the reliability of the MF technique for various situations. Seemingly, the results obtained by the two standard techniques are not significantly different from one another (Garcia et al., 1995), even if rosolic acid, which improves the detection

by colouring the Escherichia coli colonies, was missing from the MF mixture (Grabow et al., 1992). When very different levels of contamination were tested the MF technique was noted to have a higher accuracy at lower levels, < 100 Colonies Forming Units (CFU)/100 mL, while at the higher levels of over 500CFU /100 mL the MPN technique gave better results (Franzblau et al., 1982, Ingham and Moody, 1990). Other studies confirmed the fact that MPN technique may be inaccurate for very low levels of contamination so that the Multiple Polymerase Chain Reaction method -PCR- has been proposed as a better method (Jinneman et al., 1995). The PCR method seems to give statistically equivalent results to those obtained with plate counts (Bej et al., 1991).

It is reasonable to consider that the faecal bacteria population in soil contains a number of injured and physiologically-stressed cells. Their growth on a detection substrate may be diminished and therefore more difficult to count. A number of enrichment methods and substrate were developed for an improved recovery of these cells and therefore reducing the numbers of false negative results. However these enrichment procedures may tend to give a higher level of erroneous positive results (Johnson et al. 1995). Another choice for such cases would be to use a presence-absence test useful mostly for water contamination monitoring purposes (Rice et al., 1989).

For samples of low volume the MF plate count method is to be preferred over other detection methods which require higher volumes of water sample.

1.6. Hypotheses

Considering the characteristics of water and bacteria transport through the vadose zone, and the characteristics of animal manure, as previously presented, the following hypotheses were formulated regarding the vadose transport of bacteria from manure applied to field, and have been used to construct the field-based data collection that is described in chapter 3.

1. Escherichia coli is present in considerable numbers in the fresh manure applied to soil and thus is a suitable indicator organism to use to assess the relative susceptibility of groundwater to contamination due to transport of bacteria through the vadose zone.

2. Faecal coliforms collected from the vadose zone after field application of manure are an indication of transport of bacteria from manure through the vadose zone towards the ground water.

3. Due to the size of bacterial cells, soils with large porosity would be more likely to allow deep transport of bacteria.

4. Soils with low matric hydraulic conductivity are more likely to allow macropore water flow and consequently allowing deep transport of bacteria.

5. Enhanced surface filtration and pore clogging due to application of manure with higher content of dry matter are likely to slow down and limit the transport of the faecal coliforms from manure, comparatively to applications of liquid manure.

2. Development of protocol

The information found in the literature regarding the methods used for obtaining estimates of bacterial concentration in soil and soil solution, are scarce and divergent. Therefore in order to devise procedures suited to the testing of the hypotheses that were stated for this study it was necessary to conduct some laboratory studies of sampling equipment and to develop protocols for sample collection and analysis. In this chapter the work done to verify procedures is described.

2.1. Background

Estimation of bacterial movement in the vadose zone may be made by collection of soil samples over various depths (Natsch et al., 1996), collection of water drained from tile drainage systems or by collection of soil solution in the vadose zone using porous cups. While the soil sampling may be very detailed it can offer only a snapshot view of bacterial movement through the vadose zone; once the soil samples are collected the profile is disturbed and no further sampling can be done. By collecting water from drainage tiles the bacteria transport can be estimated over time by repeat sampling. However the results are averaged over the whole area drained by a certain drainage tile. Tile

drainage may also change the pattern of drainage through the unsaturated zone. Collection of soil solution directly from the vadose zone can be done by employing porous-cup suction samplers. This method has the advantage that sampling can be repeated, and the samples are representative for the local drainage flow around the samplers. This method can therefore give a more accurate description of the bacterial transport through the vadose zone over time and space.

Ceramic cups have been used for collection of soil solution from the vadose zone mostly for the purpose of evaluation of the movement of various solutes through soil (Wood, 1973, Hansen and Harris, 1974). Krejsl et al. (1994) tried to estimate the utility of ceramic cups for bacterial collection in a comparative study. Only 6% of the bacteria actually went through the cup walls. However no report was found in the literature considering the evaluation of the use of the ceramic cups for bacterial collection under field conditions, nor has any attempt at calibrating counts of colony forming units obtained from such samplers been found in the literature.

2.2. Theory

The efficiency of soil solution sampling using porous cups depends on the level of contact between porous cups and soil matrix and also on the soil-water content. As long as the negative pressure applied is lower than the cups' bubbling pressure the saturation state of the surrounding soil should not affect

the quality of the obtained samples, but only the quantity of solution collected. The porous cups contain kaolin, alumina, ball clay and other feldspar materials. Therefore they have a certain cation-exchange capacity. Yet over a longer contact period with the soil matrix the cup tends to reach cation exchange equilibrium and therefore this effect on sampling is considered minimal (Soil moisture equipment corporation, 1989).

The size of the Escherichia coli - the faecal bacteria considered as indicator of contamination due to manure– is between 0.5- 2 μm in diameter and 1-4 μm in length. A filtration effect could be expected (Krejsl et al, 1994). Bacteria of smaller dimensions should be able to travel through the cup walls at a higher rate than E. coli. Similarly E. coli strains with smaller dimensions are more likely to be collected. Therefore the size distribution of the bacteria would be the decisive factor in the relationship between the actual bacterial concentration in soil solution and apparent bacterial concentration collected by the porous cups samplers.

For the purpose of this experiment it was assumed that the bacteria size distribution did not change significantly over the range of dilutions attained in soil solution after the manure application, over the temporal and spatial dimensions involved in the experiment.

Constant suction over long periods of time may induce the pores in the cup walls to be excessively plugged (Hansen and Harris, 1974). Therefore limited suction periods are to be considered.

The surface of new ceramic cups can have a certain charge, that could interfere with movement of bacteria through the cup pores. After a period of cup-soil contact this charge can be reduced by the interaction with the ions existent in soil solution.

Sources of Error

A) Detection Limit (DL)

The DL represents the actual bacteria concentration level in the soil solution at which the apparent bacteria concentration levels in the sample reaches 0. Therefore the DL represents the minimum concentration of CFU in soil solution that may be detected with the ceramic-suction-cup method. The greater the sampling volumes the lower the chance for obtaining a plate count of 0 CFU in the sample.

The DL levels are estimated assuming a uniform distribution of bacteria in the soil solution and in the collected samples.

Sample size:

The minimum predictable value of CFU in soil solution is the value that gives a plate count of 1CFU per total sample volume collected with the porous cup samplers. Therefore the higher the sample volume the higher the accuracy of prediction at lower concentration levels.

B) Viable but non-culturable bacteria

The plate counting method determines accurately the active bacterial cells while the cells which entered the starvation-survival state may not produce

colonies (Chmielewski and Frank, 1995) and therefore the total count may underestimate the total viable cell count (Barer et al., 1993, Wilson and Lindow, 1992). Although these viable but non-culturable cells may maintain their virulence (Colwell, 1993), they can be detected only by direct detection methods (Huq and Colwell, 1996). However Bogosian et al. (1996), showed that decline in the Escherichia coli K-12 strain W3110 counts in sterile and non-sterile soil and water at different temperatures was not due to the cells entering the viable but non-culturable state, but is simply due to their death.

C) Interaction with other heterotrophic microorganisms

Presence of other heterotrophic microorganisms in the soil solution sample may obstruct the development of the faecal coliforms colonies by overgrowth, injuring the coliform cells and therefore reducing the number of coliform densities (LeChevalier and McFeters, 1983).

D) Bacteria in soil compared to bacteria in soil solution

Sampling of faecal bacteria using porous cups collects only bacteria existing in the soil solution at a given moment. Bacteria attached reversibly to soil particles may not be accounted for. Hence sampling cannot estimate the full potential for contamination that exists due to later bacteria detachment . This deficiency may be reduced by repeat sampling.

2.3. Methodology

Soil solution was sampled using ceramic porous cups (Soilmoisture Equipment Corp., Santa Barbara, CA) with an air entry value (bubbling pressure) of 1 bar (100 kPa). At this value the average pore size is calculated to be 2.9 μm. During collection of the solution, the porous cup assembly was connected to a vacuum pump generating a vacuum of 400-500 mm Hg (53 to 66 kPa). The vacuum was applied for limited periods and at similar values for all the samplers, using a manifold connection. For each repetition the first sample of soil solution collected was discarded, and only solution samples obtained subsequently, analysed.

For laboratory tests a bacterial-manure dilution was prepared as follows: 10 g of solid beef manure was added to 95 mL of 0.85% NaCl solution. Glass beads were added to facilitate the manure dispersion. This mixture was shaken mechanically for 20 min at 120-135 rpm. The solution obtained was diluted to an Escherichia coli CFU concentration of 10^3 to 10^4 mL^{-1}.

Samplers with new cups and with ones that had been previously used in field experiments on solute transport were used in the study. The samplers were subjected to three series of tests. The first experiment tested the possibility for bacterial diffusion to take place through the cups. A second series of tests was designed to estimate the possible effects of different contact periods between cups and bacterial solution (contact experiments). The final series was

performed to assess the effect of suction time that samplers were connected to the vacuum pumps (suction experiments).

Diffusion experiment: Nine solution samplers (6 new and 3 field-recovered ones) were left in a bacterial-manure solution up to 21 h with samples being taken at different intervals without applying supplementary suction apart from the suction due to use of vacutainers (pre-vacuumed collectors with a volume of 7 mL).

Contact experiments: For the contact experiments the solution samplers were inserted in a glass beaker containing a bacterial-manure solution and left to soak for periods of 1 min, 15 min or 30 min after which suction was applied for 5 min.

Suction experiments: For the suction experiment the solution samplers were inserted in a glass beaker containing a bacterial-manure dilution (see above). The suction started within the first minute of insertion and was applied for periods of 1 min or 15 min. Another set of samplers was under suction for 15 min with samplers inserted in solution only for the first 3 min, while for the rest of the time (12 min) the samplers were removed from solution (3 min+12 min). This last treatment (3 min +12 min) was designed to simulate the conditions when, in soil, under unsaturated conditions, the cup-soil solution contact is limited to lesser time periods than the actual suction period.

For each test samples were obtained with a volume of 2 to 7 mL. Escherichia coli presence was estimated by plate counting and the values were expressed as CFU/100 mL.

In parallel control samples of the manure dilution were taken without filtration with a pipette, for each experiment.

All experiments were conducted at room temperature (approx. 20° C).

A preliminary test was done to test the effect of new cups and field-recovered cups on bacteria sampling. Fifteen new cups and ten field-recovered cups were used. A contact period of less than 1 min was followed by a suction of 5 min. The results indicated that there was no significant difference between the two cup types (r^2=0.93) (fig. 2.1.). Therefore the results for new and used cups were not treated separately.

Fig 2.1.

Comparison of new and field-recovered cups

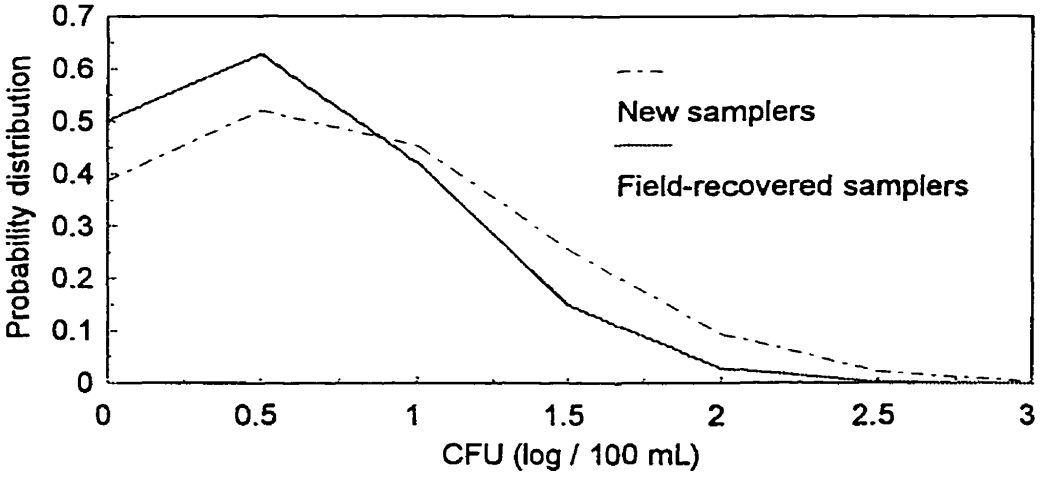


Table 2.2.

Number of cups used and number of samples obtained in the suction and contact tests

	Suction time			Contact time		
	15 min	3 min+12 min	1 min	30 min	15 min	1 min
Cups	12*	4*	20 (10*+10**)	4*	5*	3*
Sample	23*	17*	75 (65*+10**)	12*	14*	15*

Table 2.3.

Number of cups used and number of samples obtained in the diffusion test

Sampling intervals	1 hrs	2 hrs	4 hrs	6 hrs	8 hrs	21 hrs
Samples***	9(6*+3**)	9(6*+3**)	9(6*+3**)	9(6*+3**)	9(6*+3**)	9(6*+3**)

* New cups; ** Field recovered cups

*** One sample per cup for each sampling time

Faecal coliform numbers in the samples have been determined by the MF technique. One major reason for using this method was the small amount of sample obtained from the soil solution samplers. The MF technique may be performed with smaller volumes of sample (≤ 1 mL) while the MPN test requires higher volumes of sample to be split and analysed at a range of dilutions.

Soil solution samples were filtered under vacuum through a 0.45 μm pore-size membrane, and the filter was placed, in a Petri-dish, over a growth substrate of M-FC broth with rosolic acid solution, solidified through addition of granulated agar (Clesceri et al., 1989). This type of filter showed a very good capacity for retaining the Escherichia coli and total coliforms cells with no cell passing through (Shirey and Bissonnette, 1992). The Petri dishes were incubated for 24

hours ± 2 hours at 44.5° C. After incubation plate counts were performed. The formed blue colonies were considered faecal coliforms. When it was deemed necessary a confirmatory test was performed. The confirmatory test used was a presence absence test, which is a modification of the standard MPN test, known also as the Multiple-Tube Fermentation test (MTF). The presumed faecal coliform colonies from the MF plates were collected, with a thin wire loop, suspended in lauryl-tryptose broth and incubated for 24 to 48 hours until gas, from lactic fermentation, was collected into the tubes. The samples with growth, and the positive ones, were sub-sampled and mixed into an inositol brilliant green lactose bile broth. The samples that formed gas after 48 h were considered as confirmed positives containing faecal coliforms, specifically Escherichia coli.

2.4. Results

Diffusion of E coli through cups was virtually non-existent over a period of 21 h. The only way bacteria appeared to be transported in the ceramic cups was by advection with water under applied suction (53 to 66 kPa).

The plate-counts were $\log_{10}$ transformed and predictive values calculated using an inverse probability density function assuming a Poisson distribution. Thus the different treatments could be compared even if the number of replicates varied.

When the ceramic cup samplers were tested for their capacity for bacterial collection, samples between 1 and 7 mL were obtained. For clarity purposes the values were expressed as CFU 100 mL^{-1}. After the transformation of the results obtained with the lower sample amounts to CFU 100 mL^{-1} a 1 was added to all values. Thus the values of 0 CFU could be included in analysis as log 0.
Hence all the results are actual representation of the values obtained with low amount of samples.

Comparison between the actual and predicted cumulative probability distributions obtained by employing a Poisson distribution indicated that the predicted cumulative probabilities are a good representation of the actual cumulative probabilities and therefore it is appropriate to use them in further analysis (Tables 2.4 and 2.5).

Table 2.4.

Actual and predicted cumulative probability distributions of the numbers of CFU of faecal coliform bacteria in contact tests using porous cup samplers

Log(CFU/100mL)	1min contact + 5 min suction (15 samples)		15min contact + 5 min suction (14 samples)		30min contact + 5 min suction (12 samples)	
	Actual Cumulative Distribution	Predicted Cumulative Distribution	Actual Cumulative Distribution	Predicted Cumulative Distribution	Actual Cumulative Distribution	Predicted Cumulative Distribution
0	66.67%	60.05 %	21.43%	19.79 %	58.33%	29.23 %
1	73.33%	90.67 %	57.14%	51.85 %	66.67%	65.18 %
2.0	80.00%	98.48 %	78.57%	77.82 %	66.67%	87.29 %
3.0	100.00%	99.81 %	100.00%	91.84 %	100.00%	96.36 %
4.0	100.00%	99.98 %	100.00%	97.52 %	100.00%	99.14 %
Chi-test (critical value at P=0.05)	3.12(11.07)		0.2(11.07)		12.3(11.07)	
r^2	0.58		0.99		0.64	
Slope (std. Err. of slope)	0.84 (0.41)		0.97 (0.05)		1.16 (0.5)	
Std. Err. of r^2	12.84%		3.24%		20.04%	

Table 2.5.

Actual and predicted cumulative probability distributions of the numbers of CFU of faecal coliform bacteria in suction tests using porous cup samplers

Log(CFU/100mL)	1min suction (75 samples)		15 min suction but only 3 min in solution. (17 samples)		15 min suction (23 samples)	
	Actual Cumulative Distribution	Predicted Cumulative Distribution	Actual Cumulative Distribution	Predicted Cumulative Distribution	Actual Cumulative Distribution	Predicted Cumulative Distribution
0.0	74.67%	64.34%	58.82%	44.40%	47.83%	42.32%
1.0	94.67%	92.71%	76.47%	80.45%	91.30%	78.71%
2.0	96.00%	98.97%	100.00%	95.08%	100.00%	94.36%
3.0	100.00%	99.89%	100.00%	99.05%	100.00%	98.84%
4.0	100.00%	99.99%	100.00%	99.85%	100.00%	99.81%
Chi-test (critical value at P=0.05)	0.7(11.07)		1.9(11.07)		0.7(11.07)	
r^2	0.98		0.94		0.96	
Slope (std. err. of slope)	1.43 (0.11)		1.2 (0.18)		1.04 (0.1)	
Std. Err. of r^2	2.34%		6.74%		5.54%	

Regression equations were developed between actual (as prepared) bacterial concentrations and bacterial concentrations measured in the samples.

For the contact tests the results from the treatments with a longer contact period were closer to the actual values comparatively to the 1 min contact period. The standard error ranges were similar for the 15 min and 30 min treatments (Tables 2.6 and 2.7).

Table 2.6.

Regression equations describing the porous cups filtration effect for different contact intervals

Contact intervals	Regression equation		r^2 value
	Slope	y intercept	
1 min	0.436 (0.021)	-1.126 (0.661)	0.976
15 min	0.714 (0.018)	-1.281 (1.354)	0.993
30 min	0.636 (0.023)	-1.24 (1.156)	0.986

Note: Standard errors in parentheses

Each treatment in the contact experiment was distinctly significant compared to the other treatments, in terms of the regression slope.

Table 2.7.

Comparison of the regression coefficients (slope) for the contact experiment (DF=18)

Regression	t values	Significance
1 min vs. 15 min	3.101	**
1 min vs. 30 min	2.637	*
15 min vs. 30 min	2.358	*

*note: t-values for comparison of regression coefficients were calculated after Bailey (1959) using Mathcad™

The regression coefficient (slope) was closer to 1 for the treatments with longer suction periods (Tables 2.8 and 2.9).

Table 2.8.

Regression equations describing the porous cups filtration effect for different suction intervals

Suction intervals	Regression equation		r^2
	Slope	Intercept	
1'	0.387 (0.03)	-1.086 (0.65)	0.933
15'	0.528 (0.031)	-1.294 (0.966)	0.954
3'+12'	0.488 (0.031)	-1.193 (0.895)	0.96

Note: Standard errors in parenthesis.

Increase in the suction time resulted in differences in slope that were highly and very highly significant between treatments.

Table 2.9.

Comparison of the regression coefficients (slope) for suction experiment (DF = 18)

Regression	t values*	Significance
1' vs. 15'	4.608	***
1' vs. 312'	3.449	**
5' vs. 312'	3.135	**

*note: t-values for comparison of regression coefficients were calculated after Bailey (1959) using Mathcad™

Fig 2.2.a

The effect of different cup-solution contact intervals

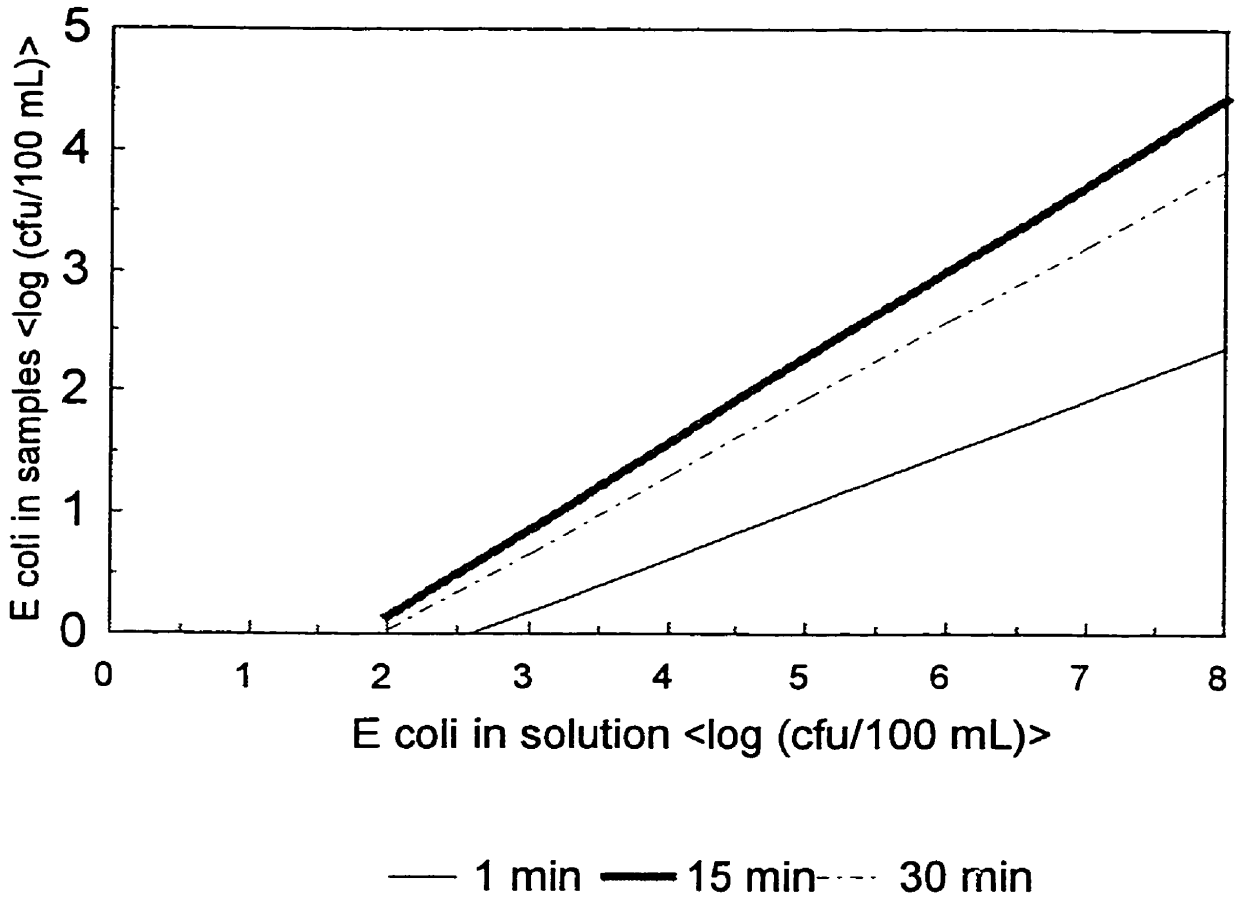
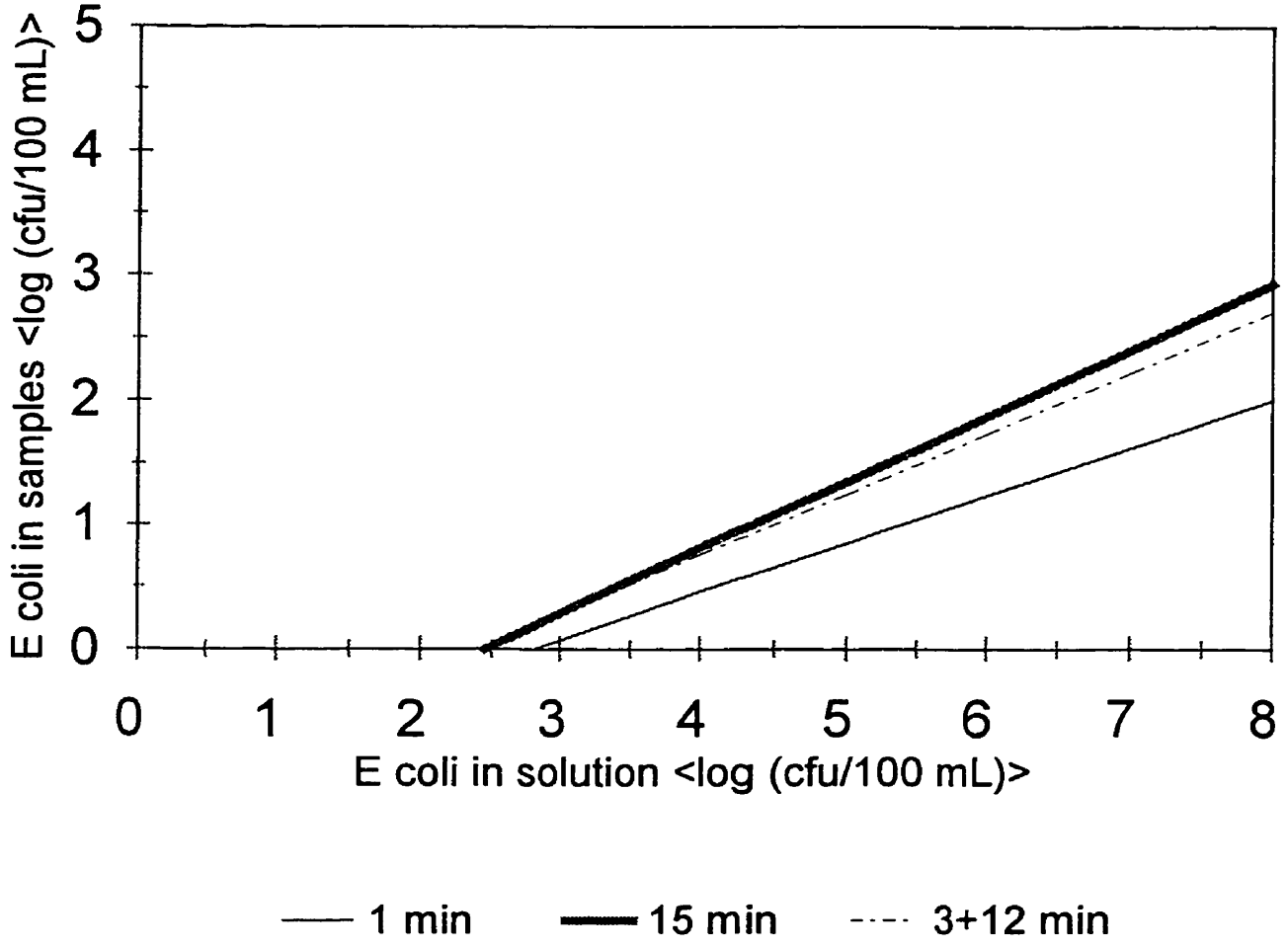


Fig. 2.2.b

The effect of different suction intervals with no previous contact



There was a significant difference between all treatments of the contact experiment. However the differences were less between the two treatments with the higher contact period (Table 2.10).

Table 2.10.

Contact experiment. Analysis of significance

Treatments	Anova: Two Way Without Replication		Paired t-Test, Two-Sample for Means, two-tailed		DF*
	P	Significance	t-values	Significance	
1vs15	0.0009	***	4.468	***	11
1vs30	0.0021	***	4.005	**	11
15vs30	0.0388	*	2.345	*	11

note: predicted values up to the pdf of 99% were considered.

Significance analysis, based on the t-test, revealed that there was no difference between the 3 min+12 min and the 15 min suction test results, while both were significantly distinct from the 1 min suction treatment (Table 2.11).

Table 2.11.

Suction experiment. Analysis of significance

	Anova: Two Way Without Replication		Paired t-Test, Two-Sample for Means, two tailed		DF*
	P	Significance	t-values	Significance	
1'vs15'	0.006	***	3.464	**	10
1'vs3'+12'	0.016	*	2.886	*	10
3'+12'vs15'	0.341	NS	1.000	NS	10

*note: predicted values up to a probability distribution function of 99% were considered.

The results were analysed by creating probability distribution functions for each treatment. By calculating the proportion of these functions that

corresponded to CFU counts lower than 0, the right hand column in table 2.12. was obtained. Conform to these calculations the lowest chances for obtaining a non-real 0 CFU count were for the contact treatments of 30 min and 15 min respectively. The chances for obtaining a count of 0 CFU were between one out of four for a sample with a volume of 100 mL and one out of two for a sample with a volume of 1mL - this being the average of calculated 0 CFU results, for the 15 min and 30 min contact tests- (Table 2.12).

The DL was calculated as being the bacterial concentration in solution at which a sample of a certain volume collected using a ceramic porous cup, as presented, might contain no CFU (Table 2.13.). In practice DL was calculated assuming a concentration of 1 CFU for different volumes of sample. For a sample of 1 mL the calculated detection limit was lowest for the 15 min and 30 min contact treatments. The detection limit was, as expected, predicted to decrease with increasing sample volume (Table 2.13).

Table 2.12.

Solution concentration levels at which the collected samples may indicate 0 CFU/100 mL due to filtration through the ceramic cups

Treatment	mean log CFU		Soil solution concentration at which the potential for CFU=0 is probable log (CFU/100mL)		Predicted % of samples indicating 0 CFU	
	sample size		sample size		sample size	
	1 mL	100 mL	1 mL	100 mL	1 mL	100 mL
Suction 1min	0.06	0.44	7.9	3.0	94.2	64.3
Contact 1 min	0.23	0.51	7.6	2.7	79.5	60
Suction 15 min	0.21	0.86	6.2	2.6	81.0	42.3
Suction 3+12 min	0.14	0.81	6.5	2.6	86.92	44.4
Contact 30 min	0.64	1.23	4.6	2.0	**52.8**	**29.2**
Contact 15 min	0.67	1.62	4.6	1.8	**51.1**	**19.8**

Table 2.13.

DL as function of sample size (values are expressed as log CFU/100 mL)

Sample size	Contact experiments			Suction experiments		
	1 min	15 min	30 min	1 min	15 min	3 min+12 min
1mL	7.6	4.6	4.6	7.9	6.2	6.5
3mL	6.3	4.0	3.9	6.7	5.3	5. 6
7mL	5.3	3.4	3.3	5.8	4.7	4.9
100mL	2.2	1.8	1.5	3.0	2.6	2.6

2.5. Discussion

Use of new versus field recovered solution samplers did not indicate any difference, suggesting that the neutralisation of the negative surface charge occurs very fast once the cups are in contact with manure solution (and presumably soil solution).

Tests showed that bacterial diffusion was insignificant even after 21 hours. Hence the only confirmed way Escherichia coli penetrated through cup walls was by advection with water.

Both predicted and actual cumulative distributions were highly skewed and therefore a chi-test was employed to compare the two. Chi-test results indicated that Poisson distribution gives an appropriate representation of the real data (tables 2.4. and 2.5.). There was a slight discrepancy in the case of 30 min contact treatment most probably due to the limited data available. However the

predictions were considered reasonable for the longer timings for both the contact and suction series of tests, which allowed further analysis using the obtained distributions.

Different contact periods between porous cups and bacterial dilution were considered (Fig. 2.1a). The contact periods before suction was applied, were of 1 min, 15 min, and 30 min respectively. Subsequent suction was applied for an interval of 5 min. In the contact experiments results clearly suggested that an initial contact period between cups and solution before suction was applied, greatly improved the chances for obtaining a representative bacterial solution sample. Initially the filtration effect declined markedly between the 1 min and 15 min treatments, but there was less change when the contact period was increased to 30 min (Table 2.10).

A comparison with results obtained in suction tests indicated that the contact period was more important than duration of the suction in influencing the plate counts. As there were no differences between new and used samplers the anion exclusion effect could be ignored as a factor.

Differences in the plate counts were more likely caused by superficial diffusion of bacterial cells into the cup-surface pores, so that they were readily available when suction was applied.

This suggests that although a minimum contact period is necessary its duration was such that it could be ignored in the context of field sampling.

The suction experiments (Fig. 2.2.b) revealed highly significant differences between the treatments with longer suction periods (15 min and 3 min+12 min)

and the 1 min treatment. Longer suction intervals showed an improved collection efficiency but there was no significant difference between the 15 min and 3 min+12 min treatments (Tables 2.8 and 2.11). Correlation coefficients between the predicted bacterial distributions in samples and control treatment were higher for the longer suction intervals (Table 2.9) indicating a better representation of the bacterial solution outside the cups for these treatments.

Sources of Error

Detection Limit (DL)

The actual intercept value on the x axis from the regression equation represents the DL for a 100 mL sample and therefore the lowest potential DL with this method.

Filtration effect:

The intercept values obtained (Table 2.6 and 2.8) indicated that the use of this particular type of cup did not allowed a precise estimation of the bacterial concentration in soil solution to be determined once it reached a lower level. This level was function of both suction time and contact period. The actual levels of bacteria concentration in soil solution below which plate counts were likely to be zero ranging from 10^3 to 10^2 CFU 100 mL^{-1} are presented in Table 2.12. However only a short period of contact between cups and solution reduced the levels of the possibly undetected concentrations to 10^2 or less if a potential sample of 100mL is considered.

The error level of the estimates was also lowered by linger contact periods.

By using the regression equation and giving different values to the plate counts (CFU/100mL) equivalent to a count of 1CFU per different volumes of sample, the effect of reduced sample volume could be estimated (table 2.13). As expected the DL increased with decreased sample volume.

2.6. Implications of laboratory tests' results on field sampling

The results of the protocol development were subsequently used for obtaining estimates of bacterial concentration in soil under field conditions.

1. As there was no significant difference between the new and field-recovered samplers the time the porous cup solution samplers have been in soil previously to the tests was considered as not having a significant influence on the sampling results.

2. As no diffusion effect was noted within 21 hours the effect of bacterial diffusion through the porous cup walls was ignored for the field tests.

3. The laboratory results indicated that the effects of contact period between cup and soil solution may be ignored for field tests on the assumption

that contact would have already occurred for a period much greater than 15 min previously to sampling.

4. While suction periods over one minute appeared necessary, a suction of approximately fifteen minutes was quite sufficient for a reasonable sample that was not greatly influenced by the filtration effect. However a suction of 15 minutes was not significantly different than the 3 min+12 min suction treatment. Based on this observation it was assumed that a suction of 5 minutes, as applied for the contact experiment, would be not significantly different too. In the field experiments suction periods between 15 and 30 min were used.

There was no significant difference between the 15 min and the 3 min+12 min suction treatments. Consequently the regression equation obtained for the 15 min contact treatment, followed by five minutes of suction was considered appropriate to be used as the predicting equation for the field soil-solution bacterial concentration.

5. The results indicated that the likelihood for contamination not to be identified was approximately one out of four. That means that the contamination may be detected within an error range of bacterial concentration in the soil solution of CFU log 1.15 to 1.35 (Table 2.6) in three cases out of four (Table 2.12).

Contamination resulting in less than 10^4 CFU 100 mL^{-1} (Table 2.13) is most likely not to be observed. This suggested that the use of ceramic cup

samplers might make it difficult to correctly estimate the bacterial concentration for situations when the contamination potential is low. Also the comparison of potential bacterial contamination between treatments is best done when the differences between the treatments that are compared are greater than the error due to sampling with ceramic cups.

3. Field experiment

3.1. Background

Although there is enough information about the point sources for bacterial contamination of groundwater, the information regarding the actual transport of bacteria through the vadose zone is limited. It is generally accepted that bacteria are most likely to be transported through the soil macropores. Transport of bacteria through the vadose zone has been investigated for the case of leaching from manure lagoons or septic systems (Westerman et al., 1995, Hagedorn, 1984, Steward and Reneau, 1982).

For assessing the potential contaminant effect of the field application of manure the study of vadose zone bacterial transport is essential. Natsch et al. (1996), showed that bacteria applied to the soil surface can be transported through the vadose zone to depth. Macropore flow was considered to facilitate the fast downward transport.

Even if some Escherichia coli strains are flagellated (Bergey et al., 1974) they do not move independently more than a few millimeters. Their movement is therefore limited mainly by the gravitational flow of water in which they are dispersed. Due to their relative large dimensions bacteria are most likely to be transported over significant distances through soil macropores. However several factors influence bacteria movement (Gannon et al., 1991):

- Flow characteristics

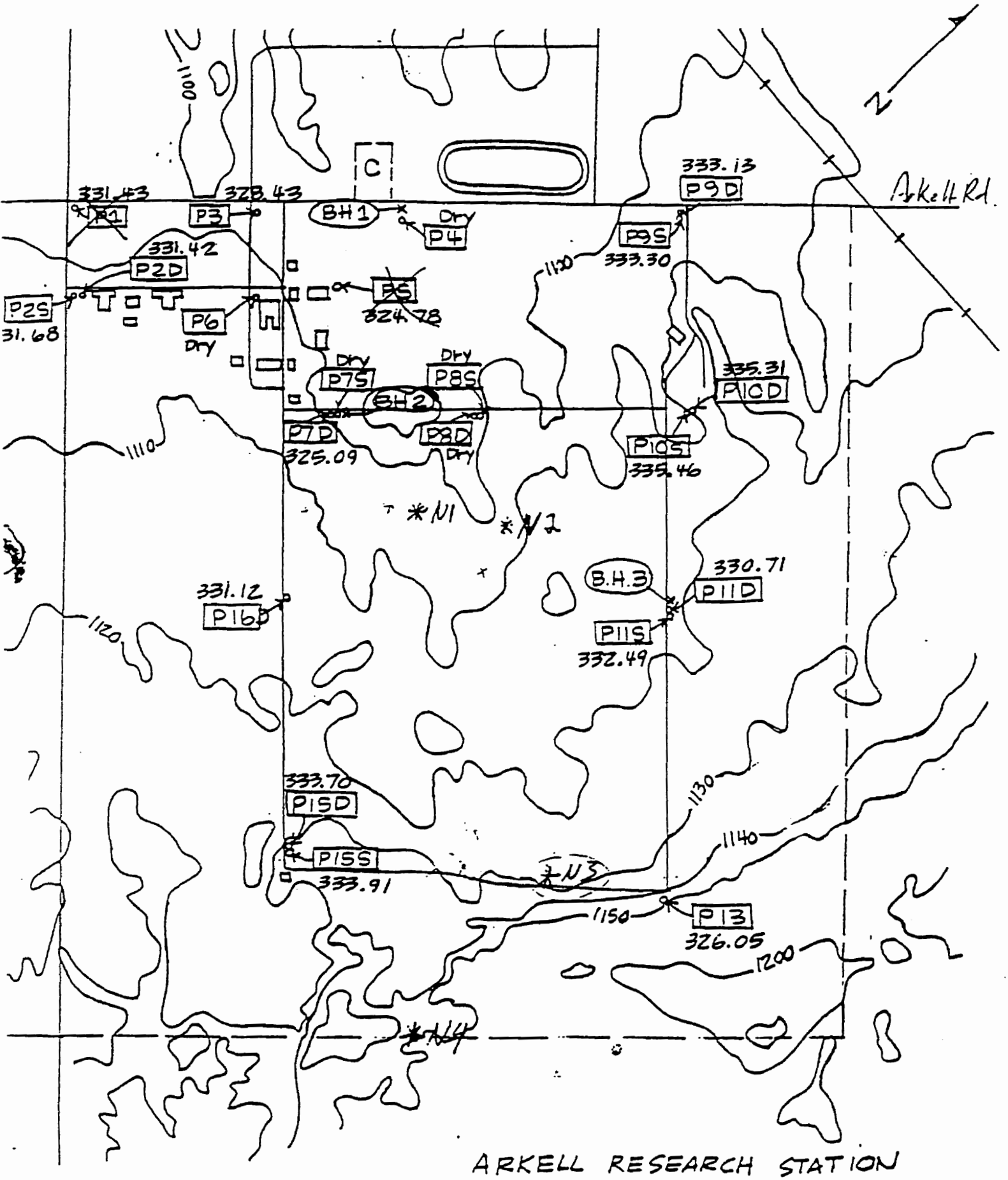
- Retention (adsorption, adhesion) on soil mineral and organic particles
- Filtration effects due to:
    - soil micropores,
    - clogging in macropores' necks,
    - filtration pads formed by solid components from applied manure (solid manure mostly)

The downward movement of the infiltrating water is very much a function of soil characteristics and initial soil-water content. It is generally accepted that in drier soils the flow is comparatively uniform with no flow through macropores. However macropore flow may occur even when relatively small volumes of water are added to soil (Beven and German, 1982). Once in the soil, bacteria transport at depth requires pores that have a great degree of continuity.

Retention of bacteria on soil particles surface is reversible. High ionic strength solutions facilitate attachment by reducing the thickness of the ionic double layer at particles surface. Hydrophobic bacteria attach to a much greater extent than hydrophilic bacteria. Attachment is increased on surface of clays or organic particles compared to other soil minerals.

To monitor the nitrate and bacterial contamination due to the management procedures, 24 wells were established in 1980 on the fields of the Arkell Experimental Station (43°32′ latitude and 80°11′ longitude) – University of Guelph (Fig. 3.1.). The soil is a loam or sandy-loam over glacial till. The farm has a long history of manure application. The wells were sampled periodically and analysed for

Fig. 3.1. Arkell. Location of test wells



bacteria between 1980 and 1982. The program restarted in 1995 when six new wells were installed.

The level and the temporal pattern of the bacterial contamination in the underlying groundwater were determined by testing the water samples from the wells for faecal coliforms. For the larger diameter wells sampling was done by lowering a bailer with a ball valve, into the wells. To avoid cross-contamination between wells, the bailer was washed with a chlorine solution and rinsed with water after each well. For six wells (the one noted with BH on Fig. 3.1) the sampling was done by using a high-density polyethylene tube which had a ball valve inserted at the lower end. This tube was inserted into the pipe to the bottom of the wells, and water was pumped by manual application of a piston movement to the tube. The tube was also washed with chlorine solution and rinsed with water.

Comparing results from the two sampling periods, 1980-1982 and 1995-1997, indicated no significant difference in the bacterial contamination frequency[1] of the wells (Fig. 3.2., Table 3.1.). Results indicated that bacteria contamination occurred at every depth (Fig. 3.3.a-c).

To establish whether the contamination was influenced by the sampling procedure a chlorinated solution was poured into six wells in February 1997. This was done to test if the bacteria present in the wells have been transported through the vadose zone or they were only a function of the well casing

[1] Contamination frequency was calculated as a ratio between the number of sampling events which produced contaminated samples and the total number of sampling events for a given location

contamination due to the well sampling technique. Two weeks after chlorinating no bacteria were found in the chlorinated wells. The faecal coliforms reappeared in these wells over a period of 8 weeks, which coincided with the period of major snowmelt. These results confirmed that the bacteria were moving to the wells, probably transported with the water drained through the vadose zone to the underlying aquifers (Table 3.2.).

These observations were consistent with the results of Goss et al. (1998), which showed that bacterial contamination of well water was more prevalent on farms where manure was applied than on other farms.

Table 3.1.

Number of contaminated wells in the 1980-1982 and the 1995-1997 periods

Contamination type	Well depth group (m)	Wells with at least one contaminated sample		Total wells compared*
		1995-1997	1980-1982	
Escherichia coli	0-10	6	7	8
	10-15	6	5	7
	Over 15	6	4	6
Total coliforms	0-10	8	8	8
	10-15	7	5	7
	Over 15	6	6	6

* Note: only wells that have been in place in both periods were considered

Table 3.2.
Bacterial concentrations level in the chlorinated wells

Sample time	Bacterial analysis	Well ID and depth (m)					
		P11S (9.00)	P11D (11.90)	BH2S (15.81)	BH2T (22.19)	BH3S (13.07)	BH3T (20.98)
Feb.19 '97	Total coliforms	<10	<10	20est	20est	o/g*	o/g
	E. coli	<10	<10	<10	<10	<10	<10
Feb.19 '97		Chlorination					
Mar. 03 '97	Total coliforms	0	0	4	0		
	E. coli	0	0	0	0		
Apr. 23 '97	Total coliforms	o/g	o/g	10	<10	<10	>800
	E. coli	<10	<10	<10	<10	<10	<10
July 16. '98	Total coliforms	<10	>80	80			>80
	E. coli	<10	<10	<10			>80

Note: o/g = overgrown

Fig. 3.2.

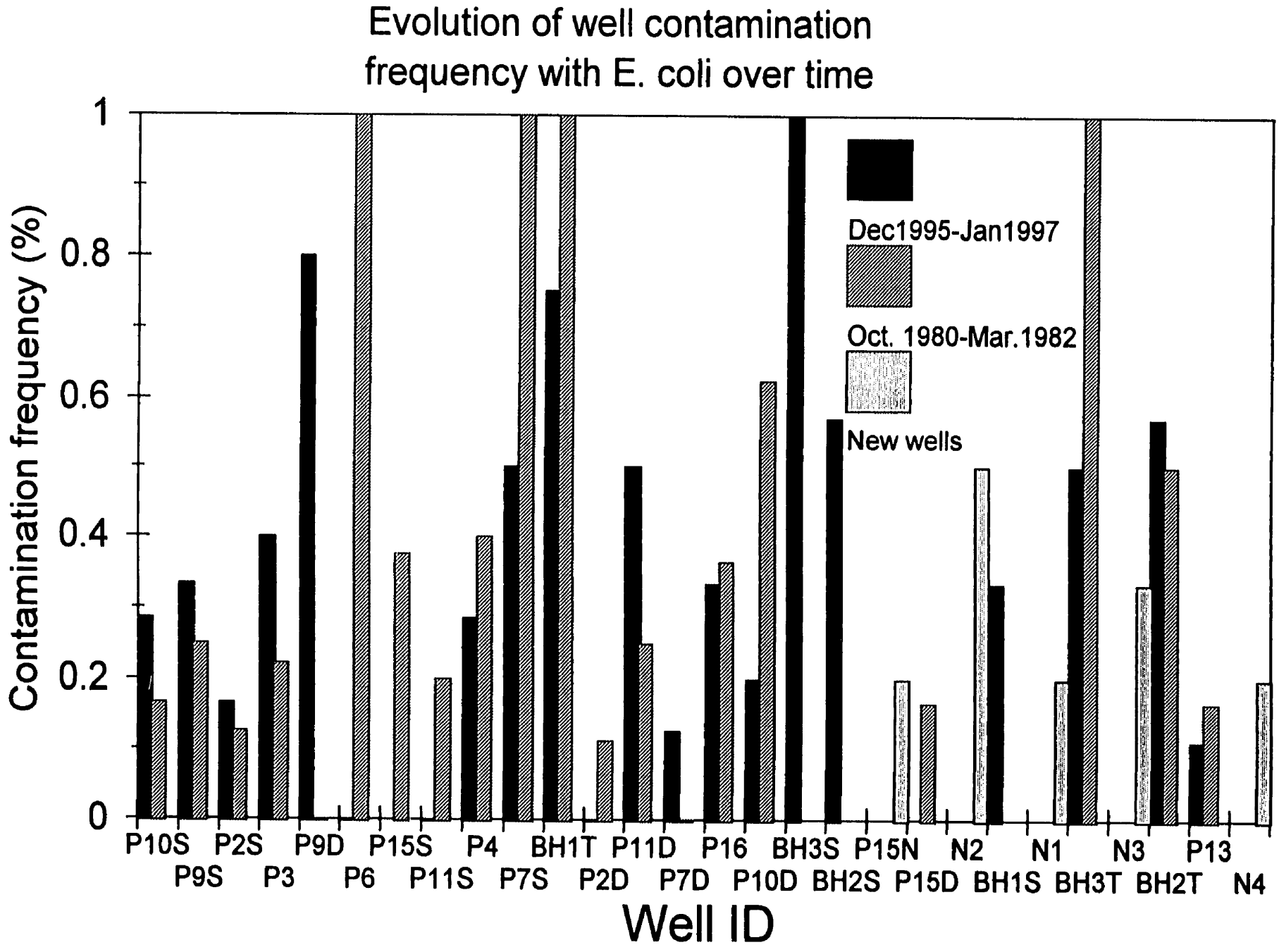

Evolution of well contamination frequency with E. coli over time
Contamination frequency (%)
1
0.8
0.6
0.4
0.2
0
Dec1995-Jan1997
Oct. 1980-Mar.1982
New wells
P10S
P9S
P2S
P3
P9D
P6
P15S
P11S
P4
P7S
BH1T
P2D
P11D
P7D
P16
P10D
BH3S
BH2S
P15N
P15D
N2
BH1S
N1
BH3T
N3
BH2T
P13
N4
Well ID

Fig. 3.3.a

Arkell: E. coli contamination frequency of wells <10m deep

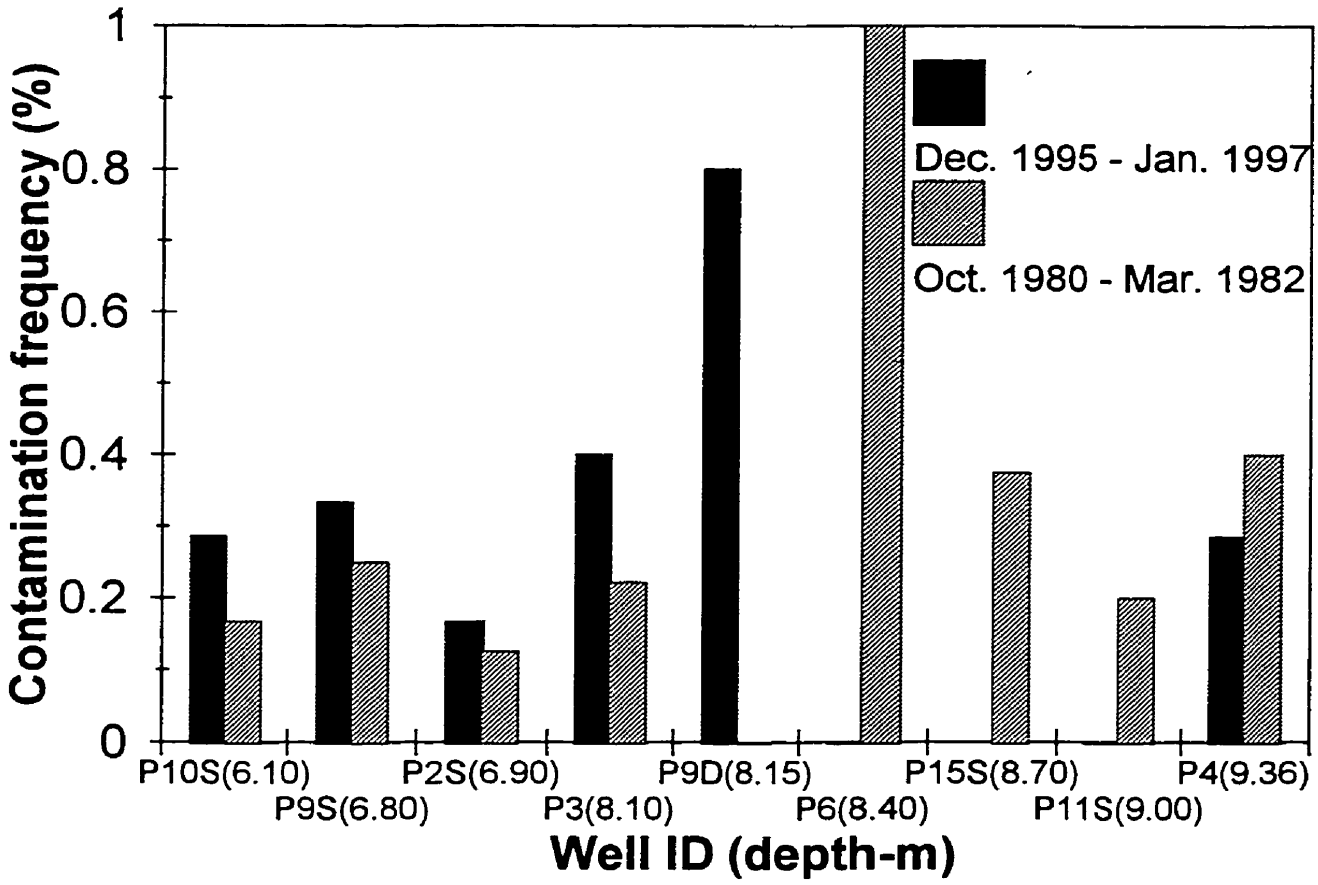


Fig. 3.3.b

Arkell: E. coli contamination frequency of wells 10 to 15m deep

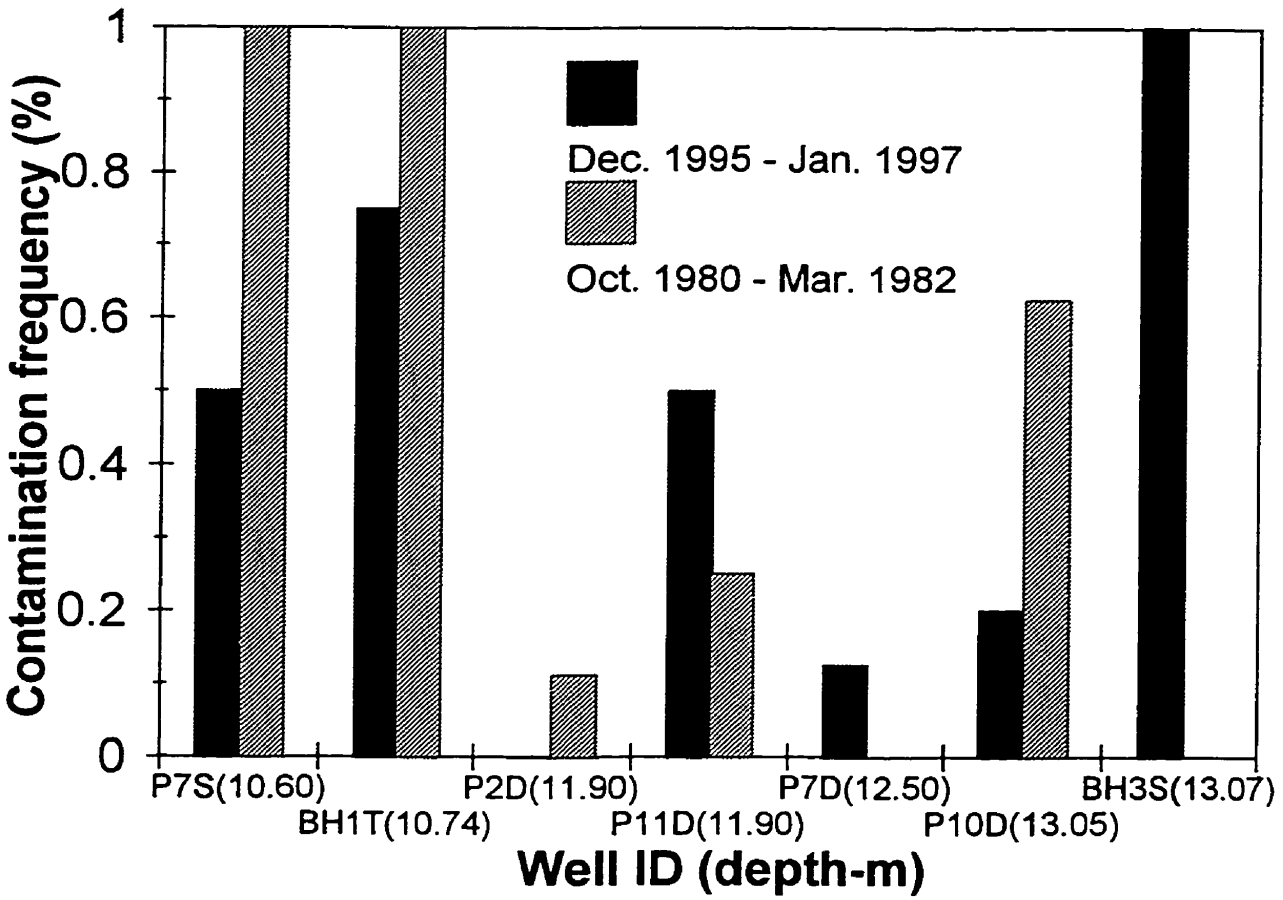
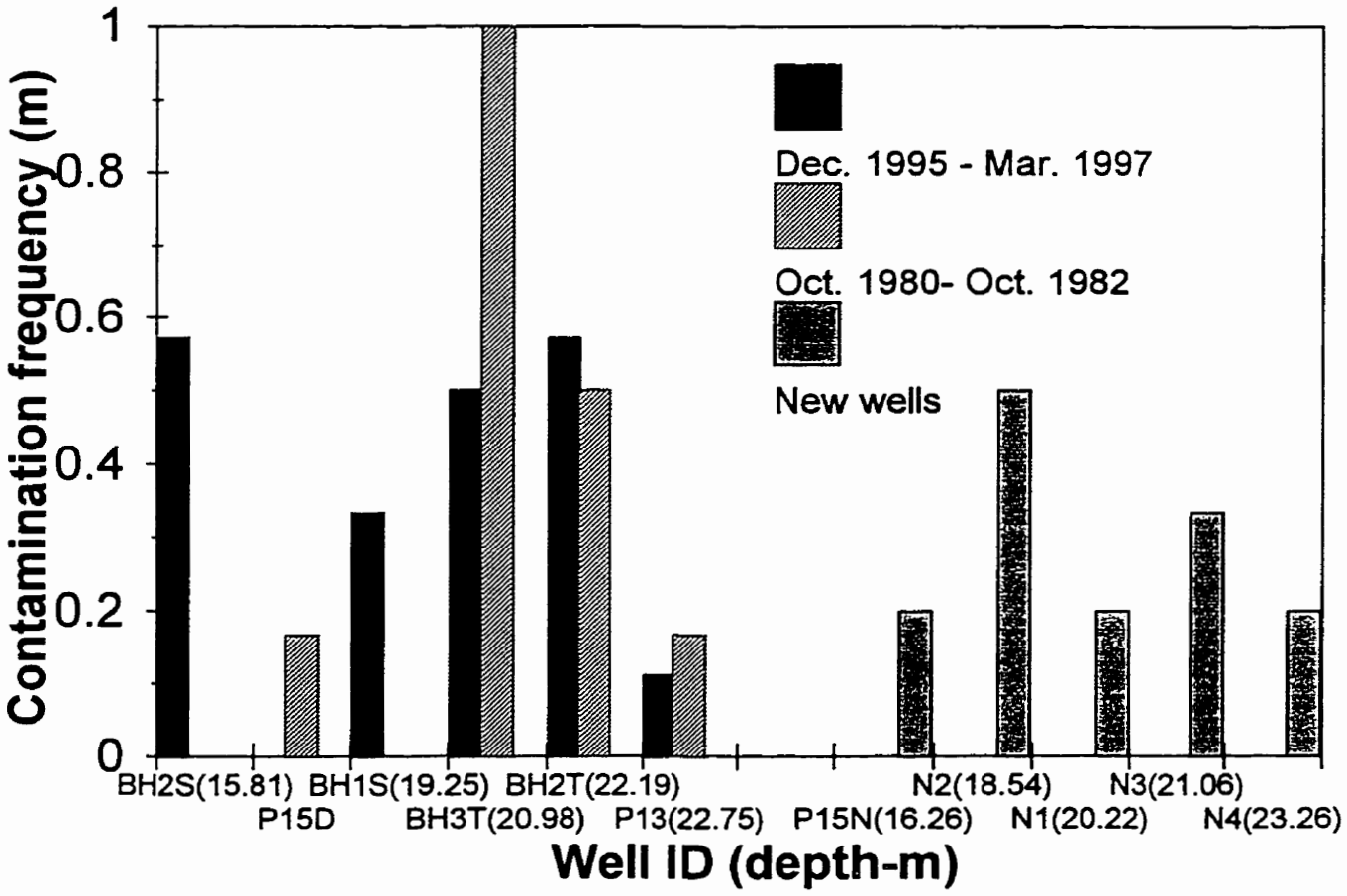


Fig. 3.3.c

Arkell: E. coli contamination frequency of wells >15m deep



Considering factors governing transport of bacteria originating from manure application soil solution ionic strength can be assumed to be relatively uniform over the whole area prior to manure application. The normal ionic strength of the soil solution at the pH of most soils is considered to have little influence on the bacterial adhesion to soil particles (Jewett et al., 1995, Kinoshita et al., 1993). Therefore differences in bacterial transport mediated by changes in the rates of bacterial attachment and detachment would most likely result only from the application of manure types with contrasting chemical characteristics.

It is unlikely for all the salts present in surface applied beef manure to be leached rapidly by precipitation or irrigation and enter the soil solution at high concentration. Initially water will be adsorbed partly by the manure and the remainder passes through, into soil, with minimal contact period with the manure. Subsequent water addition may saturate the manure and also the superficial soil surface. Organic colloids from manure can clog pores thereby creating a seal effect, which may induce a lower infiltration rate and consequently temporary water logging. This generates a longer contact period between manure and added water.

Application of manure with a large dry matter content would favour the development of filtration mats on the soil surface and also enhance the clogging processes of the soil pores creating supplementary barriers for bacteria in their descending pathway to the ground water. Application of manure with little content of dry matter on soils with high initial water content may favour the

downward transport of faecal coliforms leading to potential ground water contamination.

3.2. Methodology

When the observations of deep faecal bacterial transport from the test wells on the Arkell Experimental Farm were correlated with the general information regarding the potential bacterial transport through the vadose zone, the necessity of a field experiment to test the potential for ground water contamination with faecal coliforms from field applied manure, became evident.

3.2.1 Experimental setting

3.2.1.1. Site description

The experiment was conducted at two locations in southern Ontario. One location was at Arkell Research Station and the second was on a private farm near Petersburg, Ontario (approximately 43°27′ lat. and 80°23′ long.).

The Arkell site had a long history of application with both liquid and solid manure. The site at Arkell was on a Loam / Sandy-Loam (L/SL) profile over a glacial till rich in carbonates (Tables 3.3. to 3.5.). Sizeable stones were present over the whole profile depth, while below the depth of 55-60 cm they represented approx. 80% of soil volume. The surface in the immediate vicinity of the test area had zero slope. The ground water table was estimated to have been at 4 to 5 m

under surface during the tests. The ground water table level was estimated using measurements from two test wells located approximately 150-200 m from the experimental site.

Saturated hydraulic conductivity for the first 30 cm depth was estimated at 1.7 cm/h (after Saxton, 1986). Hydraulic conductivity showed an increase with an increase in depth reaching an estimated 3.8 cm/h over 60 cm depth due to an increase in the sand content of the soil. The soil was slightly compacted in the subsurface horizon (5 to 20 cm depth) having an average porosity of 45%. However biological activity, in the form of earthworms and root channels, was observed over the whole depth of the profile. The organic matter content of the 30 to 60 cm horizon was 0.035 g g^{-1} which is a very high level for this depth.

There was no recent history of manure application at the Petersburg site.

The Petersburg site was situated on a Loam/Silt-Loam (L/ZL) profile (Tables 3.6. to 3.8.) with a slight slope (circa 0.3%), and ground water table at about 1 m under the surface (less than 1 m after periods of rain). Although the sand content was greater in the first 0.5 m, compared to the Arkell site, the majority of sand particles were finer. The clay content was also greater. The subsurface strata were more strongly compacted (porosity 39%) indicating a lower incidence of macropores.

The saturated hydraulic conductivity for the first 20 cm depth was 1.8 cm/h (estimated after Saxton K., 1986), but decreases with depth up to the limit of 75 cm after which it increased sharply once the underlying sand is reached. Earthworms were found over the whole depth of the studied profile (1 m) but in a

fewer number than at the Arkell site. There was only 0.025 g g^{-1} organic matter in the first 20 cm with limited amounts in the lower horizons.

Both sites have a slight alkaline pH (Table 3.2. and 3.5.). Carbonates were present over the whole profile depth for both sites, but with a higher proportion at Arkell.

Both sites have been in cultivation for at least ten years previously. In the fall prior to the installation of this experiment both sites were ploughed. In the spring the soil surface was manually levelled using hand rakes.

The main differences between sites were in the clay content, and bulk density which were greater at the Petersburg site, while at the Arkell site total porosity and the soil organic matter content were larger.

Table 3.3.

Arkell – Soil bulk density and total porosity

Depth (cm)	Bulk density (g cm^{-3})	Assumed Particle Density (g cm^{-3})	Porosity (cm^3 cm^{-3})
0-5	1.31	2.65	0.51
5-10	1.45	2.65	0.45
10-20	1.45	2.65	0.45
20-30	1.36	2.65	0.49
30-45	1.34	2.65	0.49
45-60	1.34	2.65	0.49

Table 3.4.

Arkell – Soil texture, chemical and hydrological properties

Depth (cm)	Textural classification	Sand % by weight	Silt % by weight	Clay % by weight	pH ($CaCl_2$)	CaCO3 %	Org. mat. %	Saturated Hydraulic Conductivity ($cm\ h^{-1}$)
0-30	Loam	37.7	48.3	14.1	7.5	5.5	3.7	1.7
30-60	Loam	41.2	48.7	10.1	7.5	6.5	3.5	2.7
>60	Sandy-Loam	62.3	29.0	8.7	7.9	39.7	1.4	3.2

Table 3.5.

Arkell – Sand particle size analysis

Depth (cm)	VCS % by weight	CS % by weight	MS % by weight	FS % by weight	VFS % by weight
0-30	0.8	1.9	3.8	10.6	20.6
30-60	1.40	2.80	4.10	10.10	22.90
>60	7.00	16.40	9.90	13.80	15.30

Table 3.6.

Petersburg –bulk density and total porosity

Depth (cm)	Bulk density ($g\ cm^{-3}$)	Particle Density ($g\ cm^{-3}$)	Porosity ($cm^3\ cm^{-3}$)
0-5	1.43	2.65	0.46
5-15	1.54	2.65	0.42
15-30	1.66	2.65	0.38
30-45	1.61	2.65	0.39
45-70	1.55	2.65	0.42

Table 3.7.

Petersburg – soil texture, chemical and hydrological properties

Depth (cm)	Textural classification	Sand % by weight	Silt % by weight	Clay % by weight	pH (CaCl2)	CaCO3 %	Org. mat. %	Saturated Hydraulic Conductivity ($cm\ h^{-1}$)
0-22	Loam	51.9	35.4	12.7	7.3	1.3	2.5	1.9
22-40	Loam	41.7	40.8	17.4	7.5	7.2	0.7	1.1
40-60	Loam	30.4	49.2	20.4	7.6	22.0	0.5	0.9
60-75	Silt-Loam	22.4	55.5	22.1	7.6	29.0	0.4	0.9
>75	Loam Very Fine Sand	76.6	20.6	2.9	7.6	14.9	0.3	7.9

Table 3.8.

Petersburg - sand particle size analysis

Depth (cm)	VCS % by weight	CS % by weight	MS % by weight	FS % by weight	VFS % by weight
0-22	0.0	0.1	0.7	12.3	35.4
22-40	0.0	0.1	0.3	9.6	30.6
40-60	0.0	0.0	0.0	5.8	24.1
60-75	0.0	0.2	0.3	4.0	17.4
>75	0.2	0.0	0.4	16.2	59.7

3.2.1.2. Plot description

At each location an experimental plot was set up (Fig. 3.4.). Each plot has an area of 14 x 6 m. There were 4 subplots for each plot (A, B, C, and D). The size of each subplot was 3 x 1.5 m. The external border area acted as a buffer area surrounding the whole plot (1 m each direction), and separated the subplots from each other (2 m between AB and CD and 1 m between AC and BD). Each subplot was split into two to facilitate the work (Fig. 3.5.)

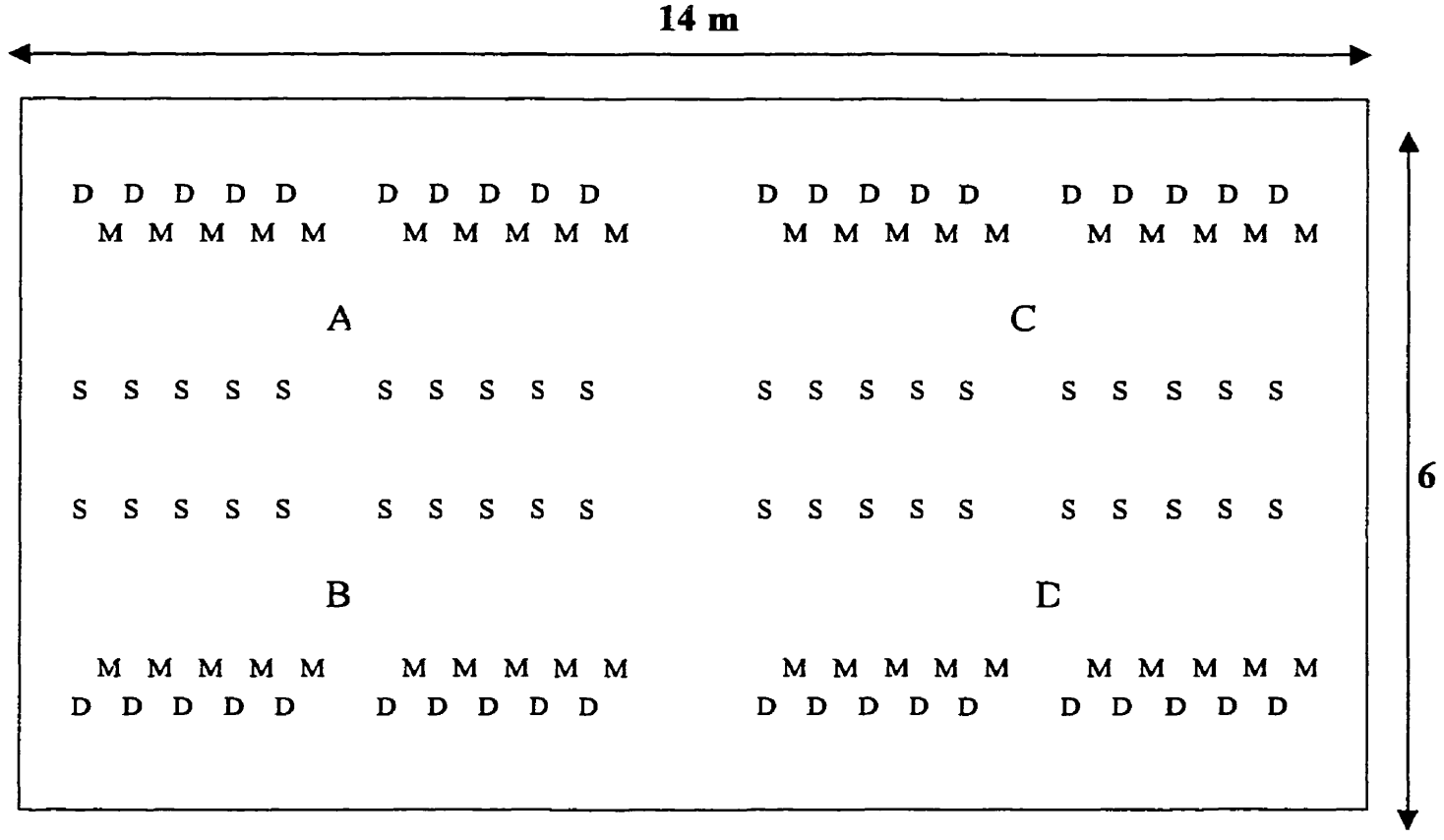
The solution samplers were inserted under the plot at an angle of 45 deg (fig 3.6.). There are three sampling depths - 30, 50, and 75 cm at Arkell, and 30,75, and 100 cm at Petersburg). At Arkell the depth of insertion was limited by the C-horizon which is extremely stony (>80%).

Each subplot had 10 solution samplers for each of the three depths.

Soil-water content was monitored using 2 pairs of TDR probes for each depth per subplot. At Arkell it was impossible to insert the probes to 75 cm depth and therefore readings were taken only for the depths of 30 and 50 cm.

Fig. 3.4.

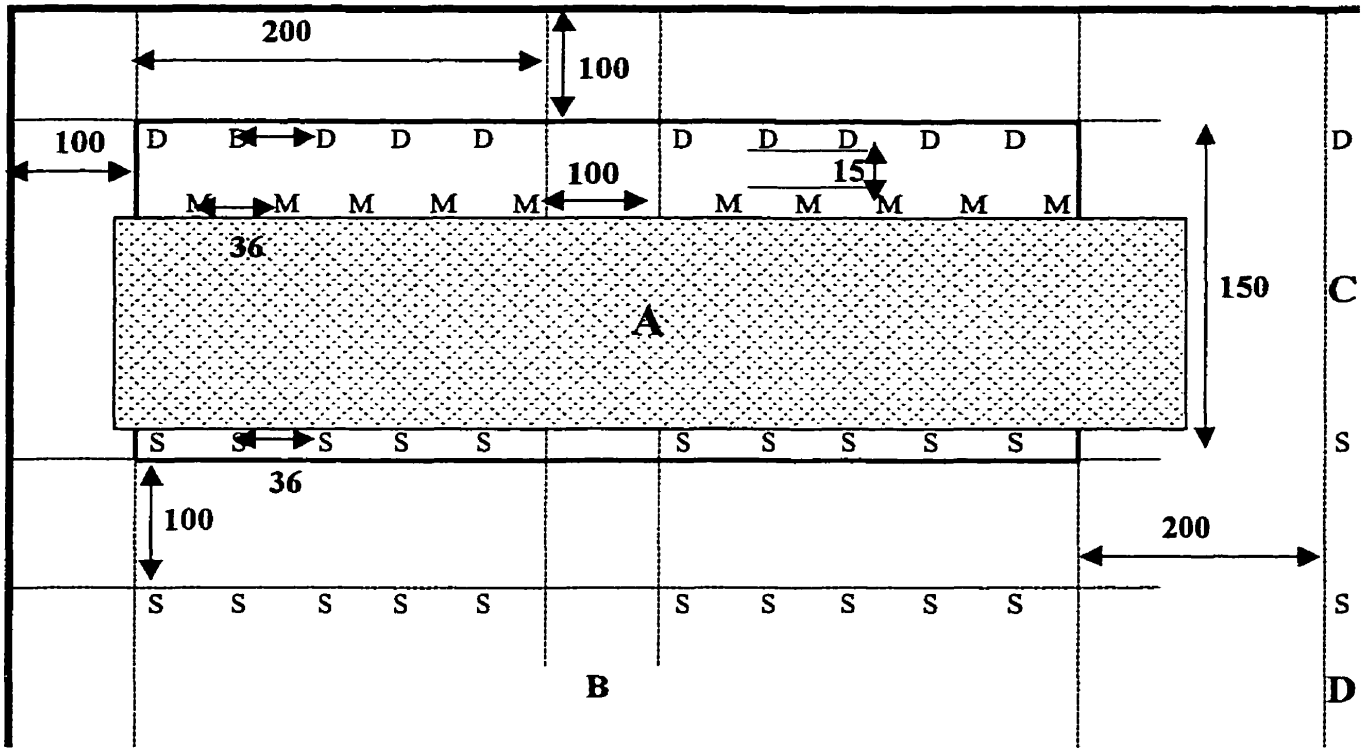
Experimental plot; dimensions and sampler locations



	Arkell	Petersburg
D = deep samplers	D = 75 cm	100 cm
M = medium deep samplers	M = 50 cm	75 cm
S = shallow samplers	S = 30 cm	30 cm

Fig. 3.5.

Subplot (not at scale)



Legend

D, M,S - insertion points of solution samplers

36 - Distance in cm

- Area where manure was applied

Fig. 3.5.a

Experimental setting at the Arkell site

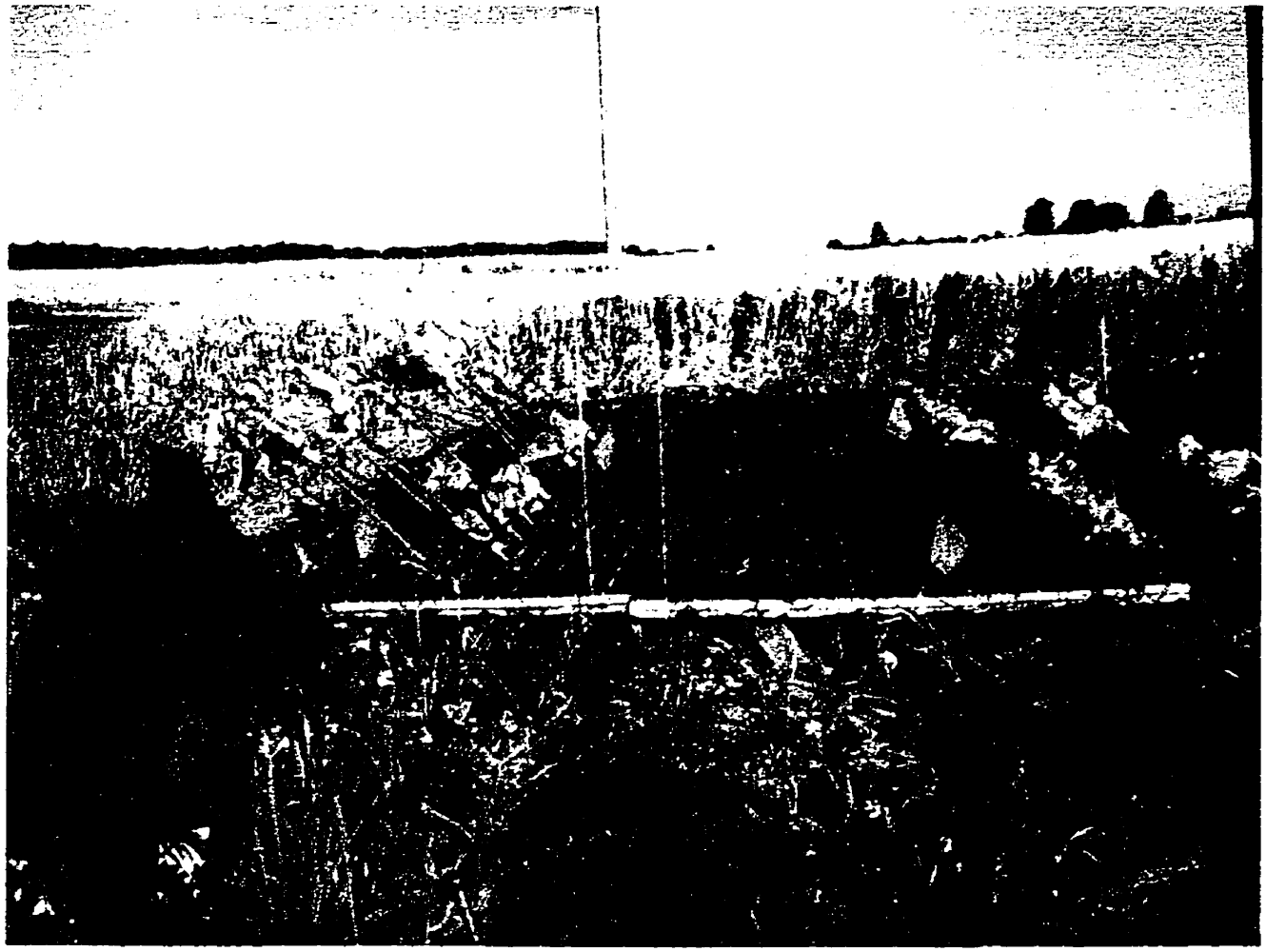
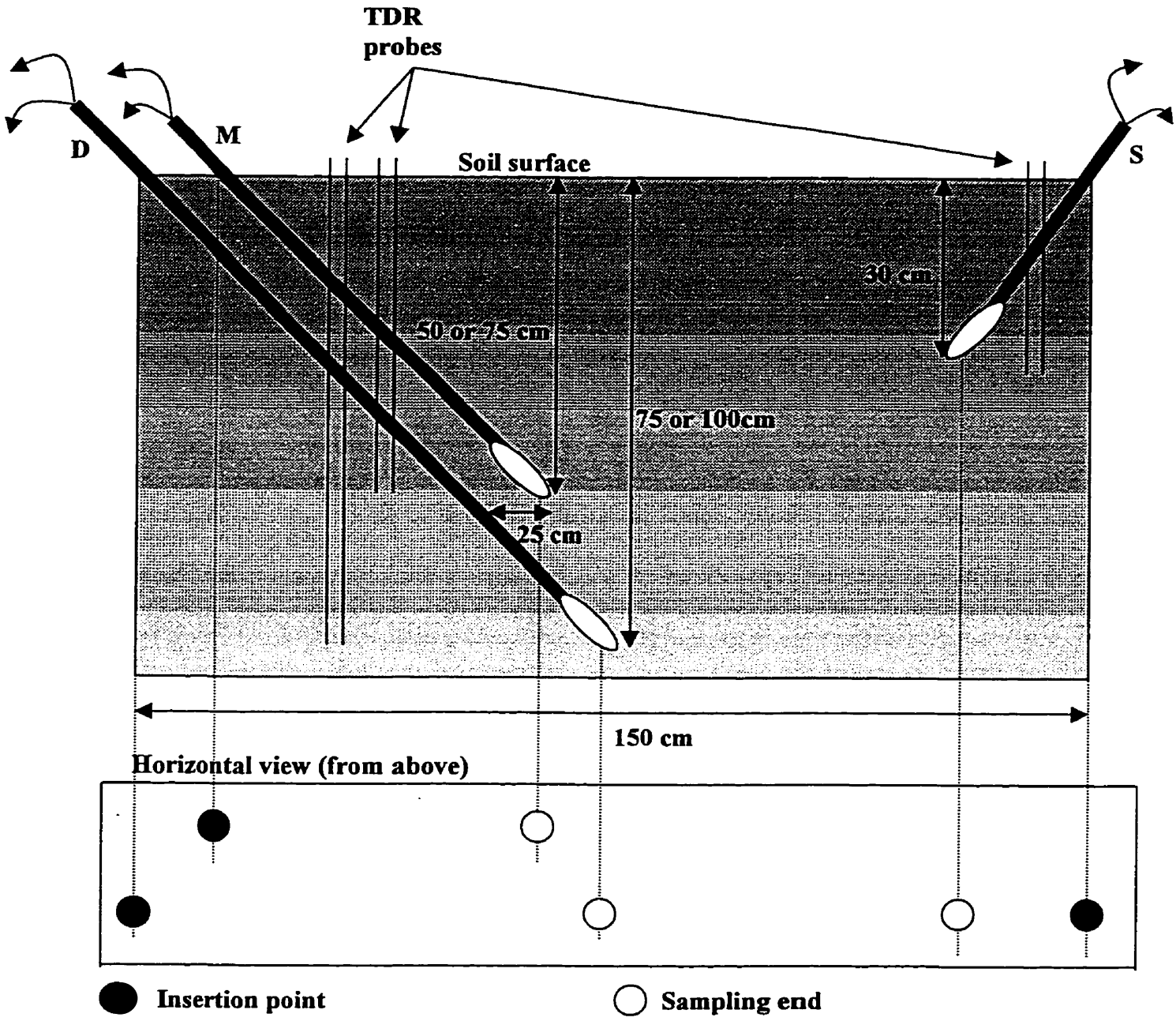


Fig. 3.6.

Solution samplers insertion in soil - Vertical section



3.2.1.3. Treatments

On each plot the following combinations (treatments) were used:

- liquid swine manure on dry soil (PD)
- liquid swine manure on wet soil (PW)
- solid beef manure on dry soil (BD)
- solid beef manure on wet soil (BW)

Note: Dry soil indicates the natural soil-water content at the time of the experiment. For the wet soil treatment the soil was irrigated with about 50-mm water over a period of approximately 2 hours and left to drain for 4-5 hours.

For each treatment a subplot was attributed.

Table 3.9.

Initial volumetric soil-water content (0-30cm), $m^3\ m^{-3}$

Treatment	Arkell			Petersburg		
	June '97	July '97	Oct. '97	June '97	July '97	May '98
LSM / dry soil	0.18	0.15	0.27	0.17	0.22	0.35
LSM / wet soil	0.25	0.35	0.35	0.24	0.32	0.36
SBM / dry soil	0.19	0.28	0.26	0.19	0.25	0.27
SBM / wet soil	0.24	0.34	0.37	0.24	0.34	0.42

Table 3.10.

Initial volumetric water-filled soil porosity (0-30cm)

Treatment	Arkell			Petersburg		
	June '97	July '97	Oct. '97	June '97	July '97	May '98
LSM / dry soil	0.34	0.31	0.57	0.43	0.54	0.85
LSM / wet soil	0.54	0.74	0.74	0.58	0.78	0.87
SBM / dry soil	0.38	0.60	0.55	0.46	0.62	0.68
SBM / wet soil	0.51	0.73	0.78	0.58	0.84	1.03*

note: value >1.0 due to variability in porosity estimate

Table 3.10.a

Average water-filled soil porosity in the first period following irrigation (0-30cm)

Treatment	Arkell			Petersburg		
	June '97	July '97	Oct. '97	June '97	July '97	May '98
LSM / dry soil	0.61 (6.5)	0.59 (12)	0.83 (6)	0.76 (10)	0.86 (12)	1.00* (6)
LSM / wet soil	0.65 (20)	0.91 (11.5)	0.94 (6)	0.89 (12)	0.97 (12)	1.00* (6)
SBM / dry soil	0.65 (6.5)	0.87 (12)	0.88 (6)	0.71 (10)	0.97 (12)	0.98 (6)
SBM / wet soil	0.65 (20)	0.54 (11.5)	0.75 (6)	0.86 (12)	0.97 (12)	1.00* (6)

Note: in parentheses - the time in hours over which the average was calculated
* values rounded to 1.00 with ponded water present

Table 3.11.

Average values of coliform bacteria in manure – (1997-1998)

Manure type	Bacterial group		Liquid swine manure	Solid beef manure
Dry matter		%	0.71(0.35)	24.69 (3.32)
Bacteria in fresh manure	Escherichia coli Total coliforms	Log CFU/100g Log CFU/100g	6.38 (0.75) 7.14 (0.67)	8.55 (0.28) 8.73 (0.28)
Relative counts (with bacteria in SBM as 100%)	Escherichia coli Total coliforms	% %	2.17 (2.84) 6.53 (8.24)	100.00 100.00
Percentage of Escherichia coli per Total coliforms		%	17.5 (3.54)	66.38 (1.09)
Bacteria per g of dry matter	Escherichia coli total coliforms	Log CFU/g dry matter Log CFU/g dry matter	6.58 (0.55) 7.16 (0.25)	7.34 (0.46) 7.34 (0.25)

Note: -Values obtained by plate counting method using diluted extracts of manure (dilution range - 10^{-4} to 10^{-6})
- standard deviation in parentheses

Two manure types were used, LSM (Liquid Swine Manure), from Arkell Research Station, and solid beef manure, from Elora Research Station (Table

3.11.). The bedding component for the SBM (Solid Beef Manure) was a mixture of sawdust and straws

Each manure type was applied on soil with contrasting water contents (dry and wet). Soil solution was collected using ceramic porous cups with an air entry value of 1 bar. The average pore size was between 1.4 and 2.9 μm.
In 1997 these treatments were repeated three times at Arkell (June, July, and Oct.), and only two times at Petersburg (June, July). At Petersburg the October repetition was cancelled due to weather conditions. In spring 1998 (May) a third repetition was completed at Petersburg. The soil was kept bare between the June and July repetitions while between July and October it was covered with oats in order to limit the high soil-water content expected due to fall rains by maximising evapotranspiration. The Petersburg site was kept covered with an impermeable tarpaulin over the winter season in order to limit additional water entry and therefore facilitate early entrance on the field for the spring repetition.

For the treatments that were not covered additional water input due to occasional rains was measured (Table 3.12.) and evapotranspiration was also calculated (Table 3.16.).

Table 3.12.

Additional water input from rain

Treatment	Arkell (L/SL)		Petersburg (L/ZL)	
	June '97	July '97	June '97	July '97
LSM / dry soil	27(116-212)*	16.2 (119-243.5)*	0	18 (82.5-109.5)*
LSM / wet soil	2(133-254)*	0	0	1(108.5-221.5)*
SBM / dry soil	27(116-212)*	16.2(119-243.5)*	0	18 (82.5-109.5)*
SBM / wet soil	2(133-254)*	0	0	1(108.5-221.5)*

Note: rain in mm (interval when rain(s) occurred-hours from manure application)

Manure was spread uniformly at a rate of 5 kg or L m^{-2}, equivalent to an application of 50 metric tones per ha. Following the manure application, 50mm water was added through drip irrigation at an approximate rate of 20 mm h^{-1} and left to infiltrate. The drip irrigation system was calibrated in field for each use by collecting the water from a known area over 5minute periods and then calculating the time necessary to attain the 50 mm irrigation level. For the LSM and SBM application on dry soil in June 1997 at Arkell only 44 mm of irrigation water was applied.

The total amount of water applied, considering the irrigation and the water added with the manure (Table 3.11.) was in the range of approximately 55 L m^{-2} for the liquid manure treatments and approximately 53.8 L m^{-2} for the solid beef manure.

Run-off was collected (it occurred only on wet treatments at Petersburg in June and July 1997), measured and analysed for bacteria.

Soil solution was sampled using ceramic porous cups, vacuum pumps, and vacutainers collectors with a volume of 7 mL. Vacuum was applied using a manifold connection ensuring that all the samplers from one treatment in the same initial soil-water content conditions were sampled at the same suction.

Sampling started at 12 hours (June '97 and July '97), and respectively at 6 hours (Oct. '97 and May '98) after irrigation stopped. The soil solution was sampled six times over a period of five days and once more ten days after application, and analysed for nitrate, ammonium, Escherichia coli and total coliforms. One sampling was initially performed before manure application.

The samples were transported in coolers with ice packs, and stored at 4° C, within 2 hours of collection. Bacterial analyses were performed as described in Chap. 1 within 24 hours of sampling. Within 20 days after collection the samples were analysed spectrophotometrically for nitrate and ammonium levels. Initial soil samples were taken for each plot from the space between subplots. Soil solution samples were also collected before application of manure. These samples – both soil and solution - were analysed for presence of faecal coliforms.

Soil sub-samples of 10 g each were taken from each field sample. The sub-samples were mixed with 95 mL of 0.55% NaCl solution. Glass beads were added to help the soil aggregates dispersion. The mixtures were then mechanically shaken for a period of 20 min at 125-135 rpm. Subsequently, the obtained mixture was sub-sampled in 10-mL volumes that were diluted by adding 90 mL of 0.55% NaCl solution. These steps were repeated to obtain a range of dilution from 10^{-2} to 10^{-5}. These solutions were then analysed for Escherichia coli using the plate count method, the same method as was used for the samples of soil solution.

Six manure samples were collected, two in each month of June, July and October 1997 for solid beef manure and five samples collected one in June and two in each of July and October, 1997, for liquid swine manure.

The ionic strength of manure-water mixture was estimated by means of electrolytic conductivity measurements and pH measurements.

For liquid swine manure electrolytic conductivity was measured on raw manure. In order to estimate the temporal release of ions by solid manure four solid beef manure-water mixture filtrates were analysed for each sample. In each case 25 mL of de-ionised water (equivalent to 1/4 of irrigation water used in experiment) were added over 20 g fresh solid beef manure. First mixture was shaken manually once, end over end, in order to simulate the water passing through manure at initial stages of rain. The other three mixtures were shaken mechanically for 20, 40, and 60 min at 130 rpm to simulate the effects of longer contact periods between manure and rain water once water ponding conditions appear. Subsequently the obtained slurries were filtered through filter paper (Whatmann 41) for 90 min in order to obtain the necessary solution for electrolytic conductivity measurements.

3.2.2. Calculations

3.2.2.1. Soil-water content

Soil-water content was measured using the Time Domain Reflectometry method as developed by Topp et al. (1980). This empirically developed method is based on the proportionality between the pulse travel time and apparent dielectric constant of soil, which is correlated to the volumetric soil-water content.

$$\theta_z = -5.3\times10^{-2} + 2.92\times10^{-2}\times\varepsilon - 5.5\times10^{-4}\times\varepsilon^2 + 4.3\times10^{-6}\times\varepsilon^3$$

eq. 1.

where: ε = apparent dielectric constant of soil-water mixture averaged over depth

Hence this equation gives the volumetric soil-water content over the insertion depth of the TDR probes.

3.2.2.2. Drainage

Drainage rates were calculated using a water balance approach using eq.:

$$q = \frac{\Delta W_t - E_t}{\Delta t}$$

(eq.2)

where: q = drainage rate - m^3 hr^{-1}

ΔW_t = difference between initial and final total soil-water content over the considered time period - m^3

E_t = evaporation cumulated over Δt - m^3

Δt = time - hr

For the initial intervals that followed the irrigation eq. 2 was modified to account for the added water, considering that TDR measurements were taken only before irrigation:

$$q = \frac{\Delta W_t - E_t + I}{\Delta t}$$

(eq.3)

where: I = irrigation water - m^3

Total soil water volume (W) was calculated as follows:

$$W = soil.volume \times \theta$$

(eq.4)

where: W = soil-water content - m^3

soilvolume - m^3

θ = measured volumetric water content - %

Evaporation was estimated using hourly temperature, dew point and air humidity with the method presented by Konstantinov, (1971).

3.2.2.3. Pore water velocity and bacteria migration velocity

By using the estimated drainage rate obtained with eq. 3, and the average soil volumetric water content over the considered period, an average pore water velocity was calculated (eq.5).

$$PWV_t = \frac{q_t}{(\theta_{t1} + \theta_{t2}) \times \frac{1}{2}} \times \frac{1}{A} \times 24 \times 100$$

(eq.5)

where: PWV_t = average pore water velocity over Δt - cm day^{-1}

q_t = drained water over the considered time period - m^3 hr^{-1}

$(\theta_{t1}+\theta_{t2})$ x 1/2 = average volumetric water content over the considered period - m^3 m^{-3}

A = area considered - m^2

24 & 100 = conversion coefficients (from hr to day and m to cm)

Drained Pore Volumes (PV) was calculated as:

$$\frac{Drained.volume}{Soil.pore.volume}$$

(eq.6)

where: Drained volume – m^3

Soil pore volume – m^3

Bacteria migration velocity was also roughly estimated:

$$BMV = \frac{depth.of.bacteria.recovery}{T_1 - T_0}$$

(eq.7)

where: BMV = bacteria migration velocity - cm day^{-1}

depth of recovery = depth of insertion for the ceramic cup - cm

T_1 = time of collection - day

T_0 = time of application - day

3.2.2.4. Filtration coefficient and estimation of potential contamination depth

The filtration efficiency of a soil can be defined as the removal of bacteria over a certain length (Mathess et al., 1988):

$$C = C_0 \times \exp(-\lambda_f \times x)$$

(eq.8)

where: C = observed concentration of bacteria (CFU/100mL)

C_0 = initial (applied) concentration of bacteria (CFU/100mL)

x = travel distance (m)

λ_f = filtration coefficient

Using the known initial and observed concentration at a certain depth the filtration coefficient was calculated:

$$\lambda_f = \ln(\frac{C_0}{C}) \times \frac{1}{x}$$

(eq.9)

Following, the maximum estimated depth of contamination was calculated as the depth at which the bacterial concentration (C) reaches a level of 1CFU/100mL:

$$x_{\max} = \ln(\frac{C_0}{1}) \times \frac{1}{\lambda_f}$$

(eq.10)

3.2.2.5. Contamination frequency

The frequency of contamination was calculated as the proportion of total active samplers showing contamination.

3.2.3. Analysis of significance for the factors influencing bacterial transport in the vadose zone

The significance of the factors was estimated using the predicted depth of contamination distributions; for the contamination frequency the raw probability data were used. The significance of the considered factors was estimated separately for the NF[2] occurrences. Factor significance was calculated using ANOVA. A two-tailed t-test was used to verify the significance levels. Only values obtained from equivalent treatments were compared. For example, initial soil-water content treatments with up to a 2% vol. difference were considered as being equal for the purpose of analysis of factor significance.

[2] NF notation stands for soil solution samples which were considered to represent soil solution with a bacterial concentration that showed No evidence of Filtration after passing through soil to the depth of collection

3.3. Results

Bacterial concentration of the two types of manure used for the experiment was correlated to the amount of dry matter of manure. Application of SBM gave a higher number of bacteria spread on the fields area compared to the LSM application (Table 3.12).

Preliminary soil and soil solution sampling showed no presence of Escherichia coli in soil. Hence it is assumed that the bacteria collected ulterior, after manure application, had the applied manure as source.

Sampling efficiency can be defined as representing the proportion of solution samples with a volume of ≥ 1 mL obtained for a number of total sampling attempts. Sampling efficiency was found to be less than 100% mostly because of difficulties in obtaining a sufficiently large volume of solution at low soil-water contents or because there was inadequate contact between soil and sampling cup, particularly in the horizons with high percentage of stones.

Table 3.13.

Sampling efficiency

Arkell (June '97, July '97 and Oct '97) –

total 30 samplers per treatment and depth(10*3)

	Depth (cm)	No. of working samplers	Total no. of samples	No. of working samplers	Efficiency of working samplers	Overall sampling efficiency
		Maximum 30	Maximum 240	%	%	%
PD	30	26	129	87	62	54
	50	25	153	83	77	64
	75	27	173	90	80	72
Means				87	73	63
PW	30	29	173	97	75	72
	50	26	135	87	65	56
	75	24	139	80	72	58
Means				88	71	62
BD	30	26	161	87	77	67
	50	30	193	100	80	80
	75	29	203	97	88	85
Means				94	82	77
BW	30	24	132	80	69	55
	50	30	175	100	73	73
	75	30	210	100	88	88
Means				93	76	72

Table 3.14.

Sampling efficiency

Petersburg(June '97, July '97 and May '98) –

total 30 samplers per treatment and depth (10*3)

	Depth (cm)	No. of working samplers Maximum 30	Total no. of samples Maximum 240	No. of working samplers %	Efficiency of working samplers %	Overall sampling efficiency %
PD	30	22.00	144	73	82	60
	75	24.00	139	80	72	58
	100	29.00	189	97	81	79
Means				83	79	66
PW	30	29.00	207	97	89	86
	75	30.00	208	100	87	87
	100	29.00	207	97	89	86
Means				98	88	86
BD	30	25.00	165	83	83	69
	75	28.00	137	93	61	57
	100	30.00	189	100	79	79
Means				92	74	68
BW	30	29.00	199	97	86	83
	75	28.00	186	93	83	78
	100	30.00	187	100	78	78
Means				97	82	79

Comparison between the actual and expected number of samples with a count of 0 CFU indicated that the actual 0 counts were significantly higher than would be expected due to the effect of filtration through the ceramic cups. This indicates that the differences between the expected and the obtained values are due to real zeroes, meaning samples from soil solution containing no bacteria.

Table 3.15.

Relationship between the measured and the predicted number of samples with CFU counts of 0 according to the Poisson distribution - Summary (detailed table for each repetition in Appendix 4)

Site	Manure type	Depth of collection (cm)	No. Total samples	Positive samples	Average probability for obtaining plate counts of 0 CFU due to filtration through the porous cups function of sample size (%)	Number of expected CFU counts=0	Number of confirmed CFU counts=0
Arkell (L/SL soil)	LSM	30	302	9	0.245 to 0.52	74 to 157	293
		50	288	7	0.245 to 0.52	71 to 149	281
		75	312	4	0.245 to 0.52	76 to 162	308
	SBM	30	293	7	0.245 to 0.52	72 to 152	286
		50	368	16	0.245 to 0.52	90 to 191	352
		75	413	7	0.245 to 0.52	101to 215	406
Petersburg (L/ZL soil)	LSM	30	351	2	0.245 to 0.52	86 to 183	349
		75	347	6	0.245 to 0.52	85 to 180	341
		100	396	3	0.245 to 0.52	97 to 206	393
	SBM	30	364	10	0.245 to 0.52	89 to 189	354
		75	323	20	0.245 to 0.52	79 to 168	303
		100	376	13	0.245 to 0.52	92 to 195	363

It was assumed that evaporation is occurring at significant values only as long as in the 0-30 cm horizon the water content is at or over the soil field capacity for water (estimated after Saxton, 1986). The total evaporation levels reflect the weather conditions along with the effect of the applied manure (Table 3.16.).

Table 3.16.

Estimated total evaporation over the period of the experiment (mm)

Treatment	Occasion					
	Arkell			Petersburg		
	June '97	July '97	Oct. '97	June '97	July '97	May '98
LSM – dry soil	12.42	63.75	0	39.49	35.06	0
LSM – wet soil	29.41	76.21	0	30.75	34.40	0
SBM – dry soil	99.54	90.74	0	43.82	35.06	0
SBM – wet soil	18.03	76.21	0	38.80	34.40	0

Drainage rate in the initial phases is a function of the initial soil-water content and manure type. For wet treatments the drainage rate peak appears earlier and at higher levels compared to dry treatments. Also under swine manure there are higher initial drainage rates than under solid beef manure (data in Appendix 3).

Drainage seems to have had two peaks, one early after irrigation, and later, after 50 to 100 hours, a smaller one (residual flow). This is consistent with both macropore and matrix flow.

Laboratory measurements indicated that the potential maximum ionic strength for the solution entering the soil after manure application occurred after application of raw liquid swine manure. The contact period between solid beef

manure and water had no influence on the ionic strength of the solution passing through manure and consequently entering the soil (Table 3.17.). The pH of the solution was similar for the two manure types.

Table 3.17.

Estimated ionic strength and pH of manure solution

Manure type	Shake time (min)	EC readings (μS/cm)		pH	
		Avg.	st. dev.	avg.	st. dev.
SBM	0	1880	330	8.63	0.14
	20	2490	140	8.67	0.14
	40	2440	240	8.67	0.09
	60	2450	180	8.63	0.14
LSM	Raw manure	9220	290	8.66	0.09

Although for the L/SL soil a greater number of macropores are present in the surface horizon, the contamination frequency indicated a greater number of macropores continuous to the depth of 75 cm being present in the L/ZL soil (Table 3.18.). This resulted in a higher contamination frequency in the surface horizon for the L/SL soil while the L/ZL soil was more prone to higher frequency of contamination in the deeper horizons (Table 3.18.).

Table 3.18.

Variation in contamination frequency with depth for liquid swine manure and solid beef manure on L/SL and L/ZL soils

Soil type	Manure type	Depth			
		0.30m	0.50m	0.75m	1.00m
L/SL	Liquid swine	0.15	0.16	0.08	
	Solid beef	0.24	0.20	0.08	
L/ZL	Liquid swine	0.12		0.20	0.16
	Solid beef	0.11		0.27	0.12

Note: frequency calculated as percentages out of total working samplers showing contamination

Contamination frequency was directly correlated with the initial water-filled porosity, especially for the deeper horizons. Contamination frequency at depth (75cm) was more strongly correlated with water-filled porosity on the L/ZL soil than on L/SL soil for both manure types (Table 3.19.).

Table 3.19.

Frequency of bacterial contamination as correlated to the initial water-filled soil porosity in the surface (0-30 cm) horizon (r^2)

Soil type	Manure type	Depth (cm)			
		30	50	75	100
L/SL	LSM	0.02	0.99	0.54	
	SBM	0.00	0.62	0.14	
L/ZL	LSM	0.06		0.64	0.08
	SBM	0.83		0.30	0.26

The average bacteria migration velocity was higher after application of LSM for both soils. On L/ZL soil bacteria migration velocity was higher than that in L/SL soil for both manure types. The variance of bacteria migration velocity was lower on L/SL. (Table 3.20. and Fig. 3.7.).

Table 3.20.

Average bacteria migration velocity (cm day^{-1})

Soil type	Depth (cm)	LSM			SBM		
		Mean	St. dev.	Variance	Mean	St. dev.	Variance
L/SL	30	31.61	28.11	790	22.04	28.50	812
	50	12.58	13.69	187	9.43	5.46	29
	75	54.63	49.04	2,404	11.99	4.70	22
L/ZL	30	32.06	20.02	400	63.25	30.64	938
	75	118.63	85.10	7,241	71.36	42.54	1,809
	100	141.52	85.70	7,345	111.73	70.44	4,961

For the similar time intervals (Δt), it was observed that the bacteria moved faster than the average pore water velocity for each level of initial soil-water content considered in the experiment (Table 3.21.).

Table 3.21.

Average bacteria migration velocity estimates, comparatively to average pore water velocity (relative values)

Soil type	Manure type	Depth (m)			
		0.30	0.50	0.75	1.00
L/SL	LSM	3.0 (1.2)	4.6 (0.9)	nd	
	SBM	3.7 (2.1)	4.2 (2.2)	nd	
L/ZL	LSM	3.0 (0.7)		9.4 (3.8)	34.8 (23.0)
	SBM	3.4 (1.3)		6.3 (3.2)	11.9 (7.1)

nd = not determined;

Fig. 3.7.

Predicted velocity distribution for bacteria migration– over depths and soil-water contents. Normal distribution; values calculated using the mean and standard deviation of the observed values.

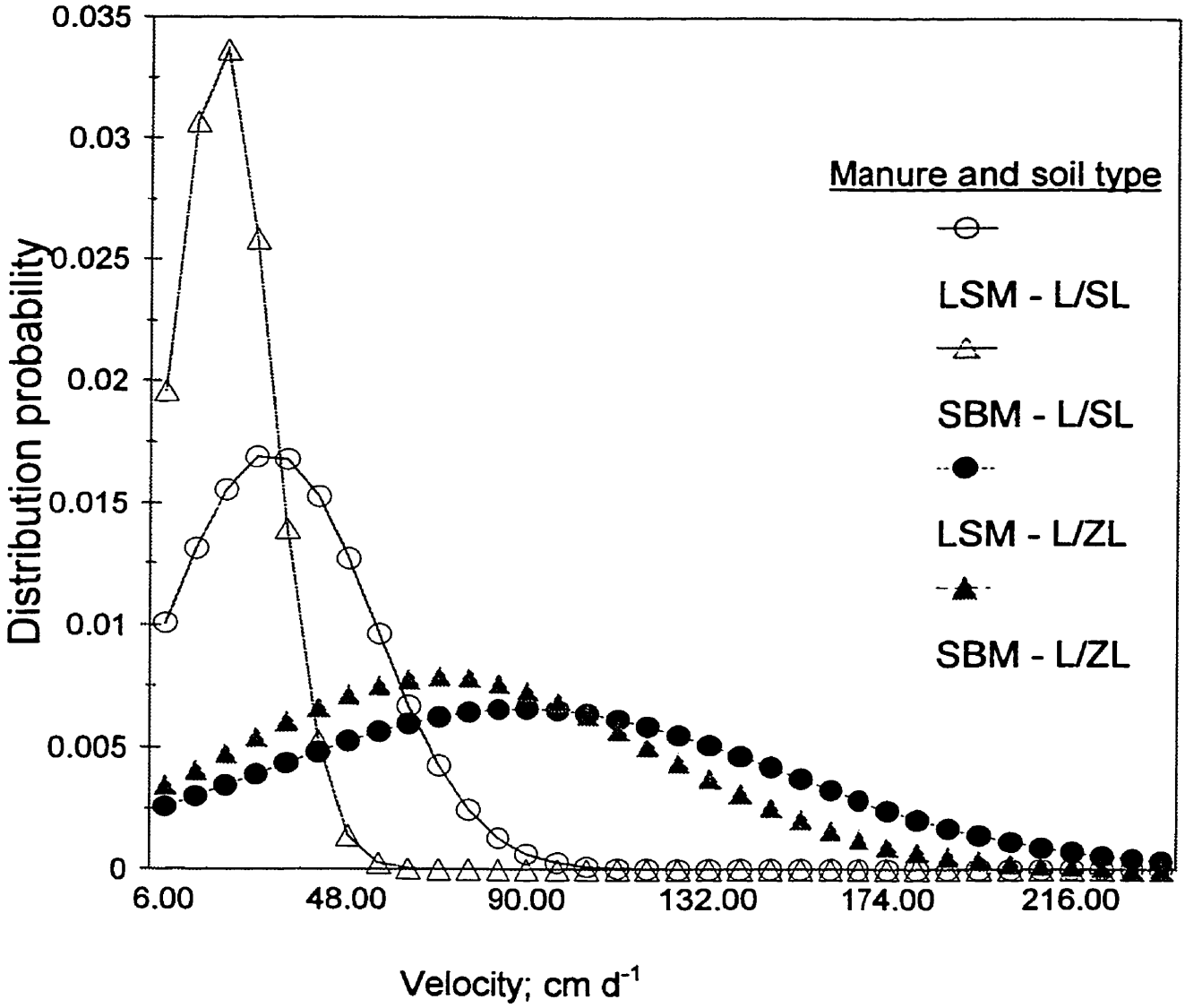


Fig. 3.8.

Predicted distributions of bacteria migration velocity as function of manure type and initial soil-water content

Fig. 3.8.a

Loam/ Sandy-Loam – liquid swine manure

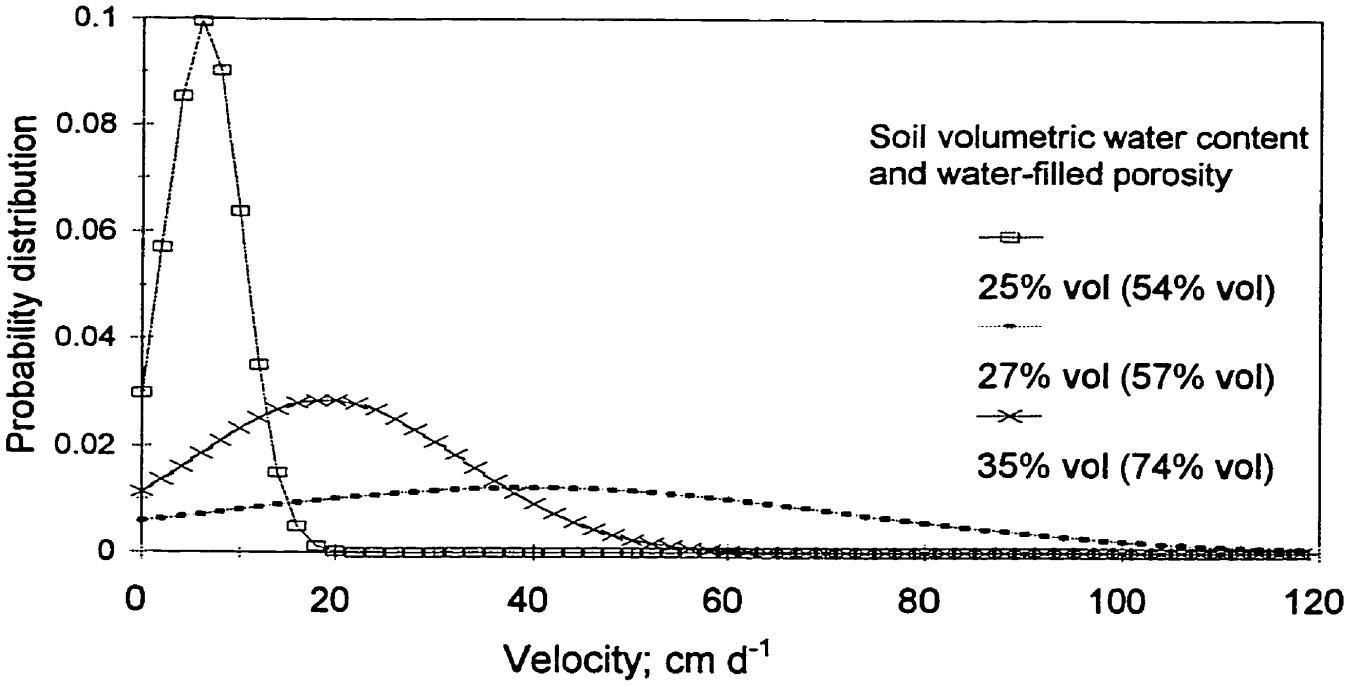


Fig. 3.8.b

Loam/ Sandy-Loam – solid beef manure

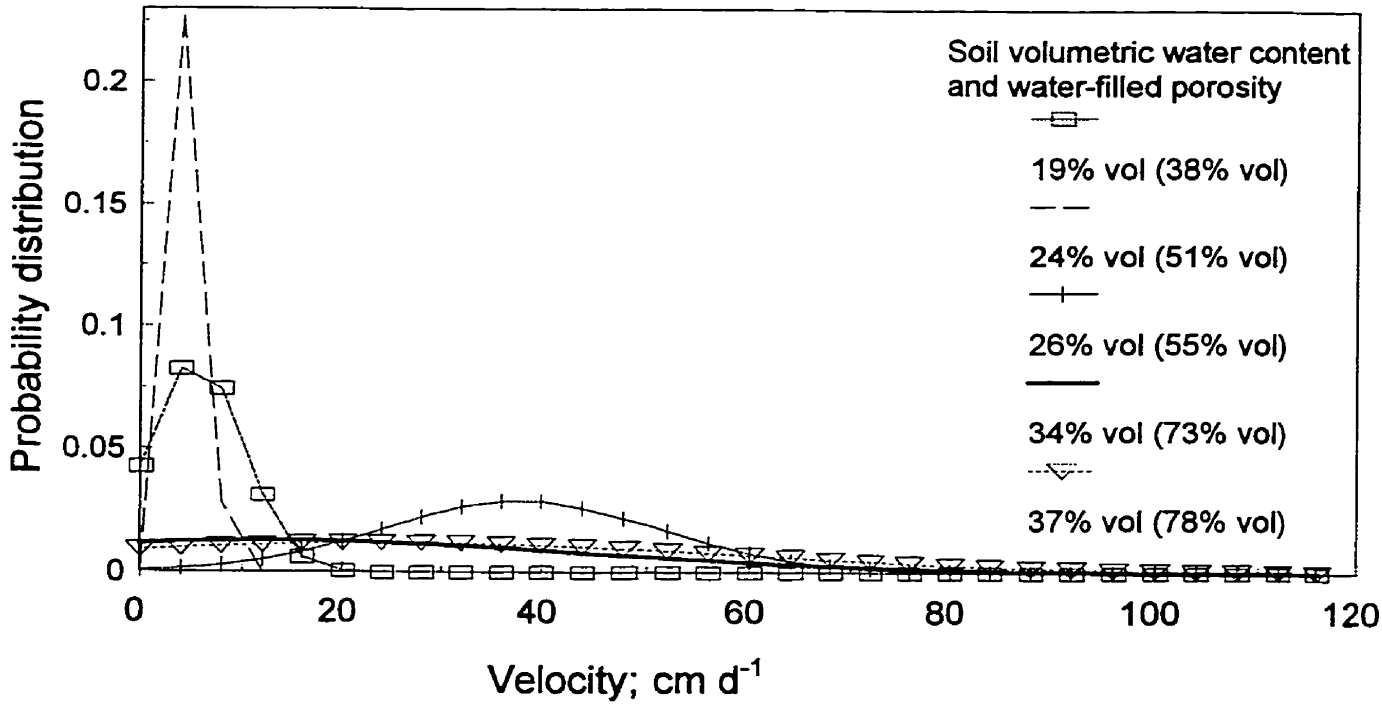


Fig. 3.8.c

Loam/ Silt-Loam – liquid swine manure

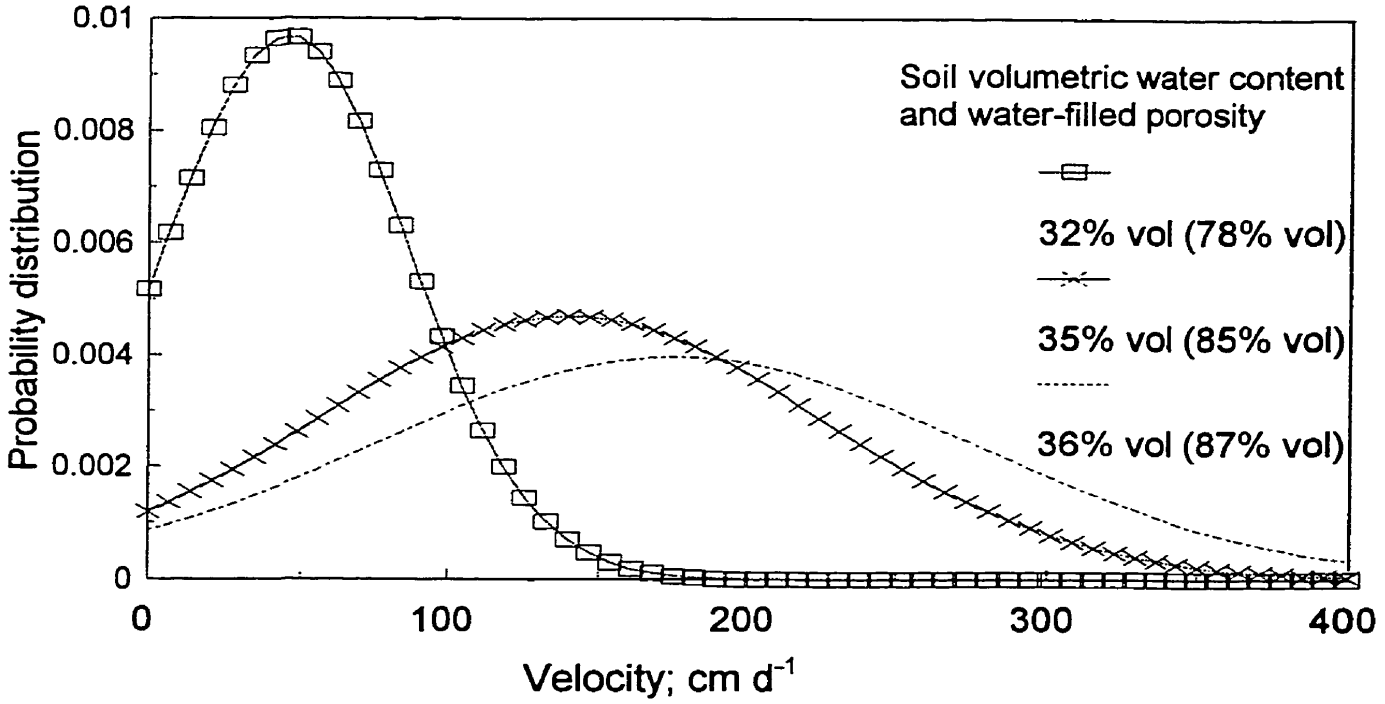
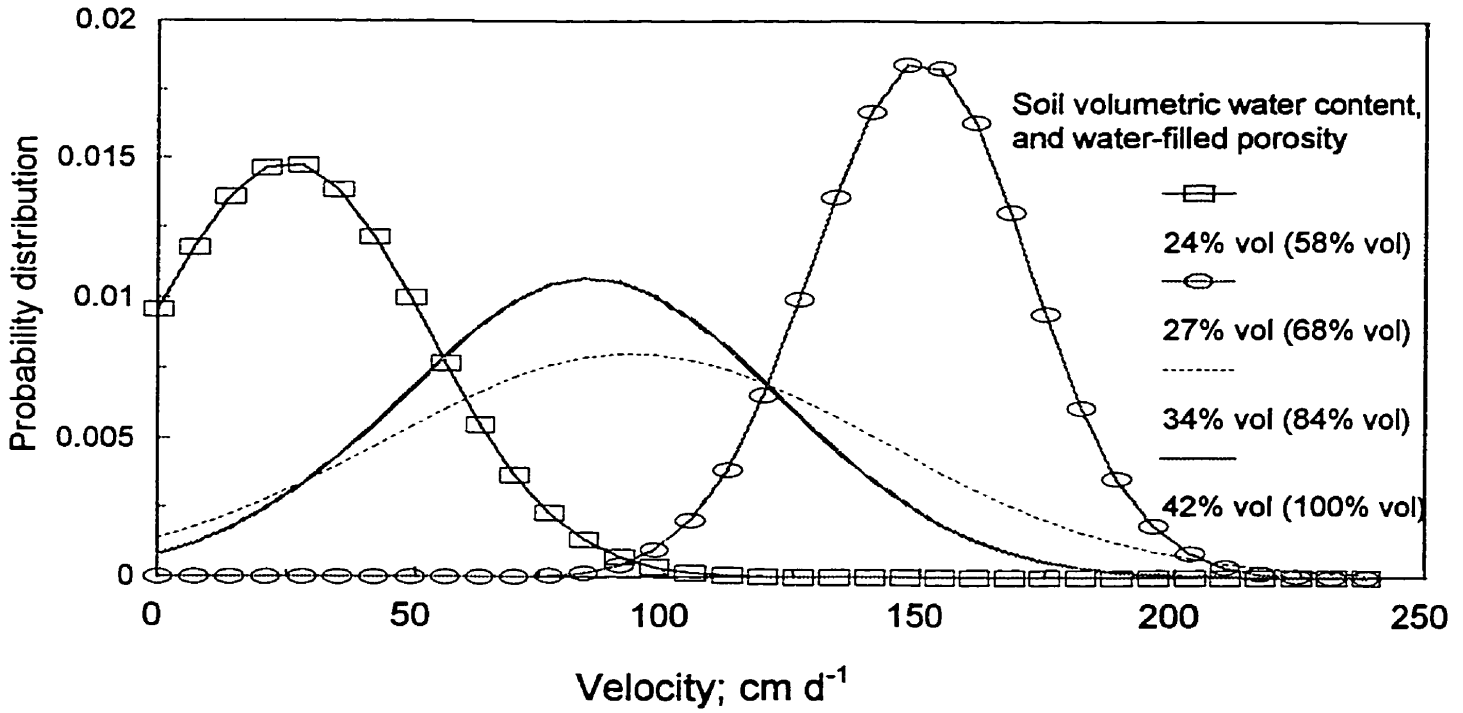


Fig. 3.8.d

Loam/ Silt-Loam – solid beef manure



Comparison between the average pore water velocity and the average bacteria migration velocity indicated that as average pore water velocity increased the average bacterial migration velocity also increased. This indicated that the two variables generally have a similar response for changes in the initial boundary conditions. It has to be mentioned that for some situations the average bacteria migration velocity was extremely high. This situation was most probably due to very fast preferential flow. These particular data points were not included in the general analysis (Table 3.22. and Fig. 3.9.a-d).

Table 3.22.

Comparison between the average pore water velocity and the average bacteria migration velocity (both measurements are expressed in cm day^{-1})

Soil type	Manure type	Soil initial water content (% vol.)	Average Bacteria Migration Velocity (BMV) (cm d^{-1})	Average Pore Water Velocity (PWV) (cm d^{-1})	proportion PWV/BMV
L/SL	LSM	0.18	66.73	1.12	**0.02***
		0.25	7.78	2.09	0.27
		0.27	39.72	8.71	0.22
		0.35	18.95	7.19	0.38
	SBM	0.19	7.69	2.69	0.35
		0.24	6.14	1.73	0.28
		0.26	37.71	4.08	**0.11**
		0.28	7.40	1.49	0.20
		0.34	13.77	2.48	0.18
		0.37	26.59	10.25	0.39
L/ZL	LSM	0.17	9.84	0.71	0.07
		0.22	45.00	3.33	0.07
		0.32	82.83	9.03	0.11
		0.35	140.25	6.64	0.05
		0.36	175.50	18.24	0.10
	SBM	0.24	25.15	7.59	0.30
		0.25	9.84	1.31	0.13
		0.27	150.00	6.87	**0.05**
		0.34	93.13	15.87	0.17
		0.42	84.86	14.70	0.17

Note: values considered as outliers are underlined

Fig. 3.9.
Comparison between the average pore water velocity (PWV) and the average bacteria migration velocity (BMV)

Fig. 3.9.a

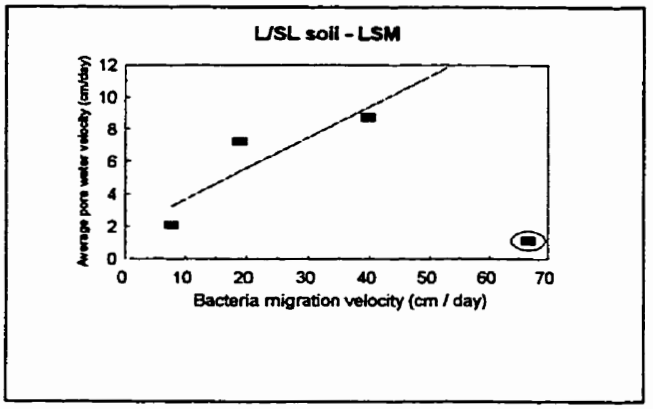


Fig. 3.9.b

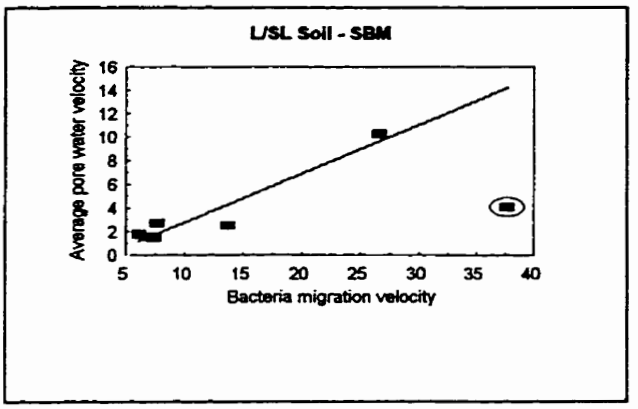


Fig. 3.9.c

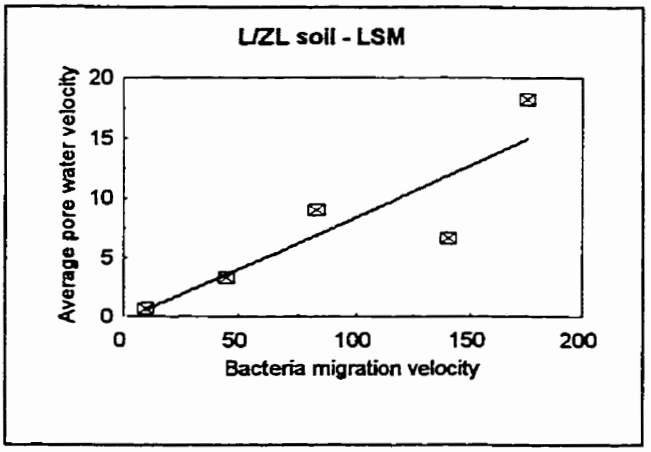
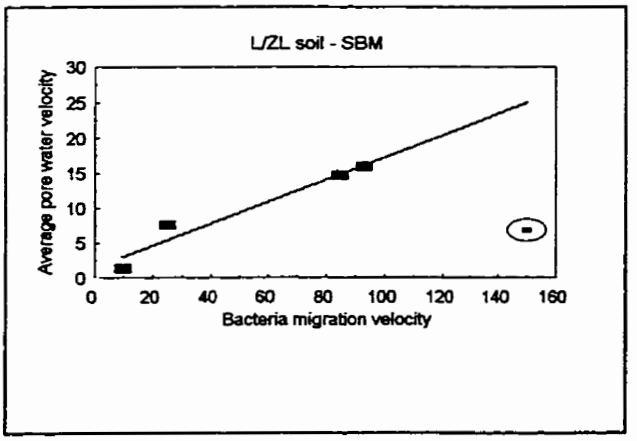


Fig. 3.9.d



Note: outliers encircled are not included in regression

Table 3.23.

Comparison between the average pore water velocity and the average bacteria migration velocity – outliers excluded (r^2)

	LSM	SBM
L/SL	0.79	0.91
L/ZL	0.76	0.94

Higher initial soil-water contents resulted in an increase in the range of pore sizes actively involved in bacterial transport, and generally an increase in pore water velocity (Table 3.24.).

Table 3.24.

Comparison between the pore water velocity and initial soil-water content (r^2)

	LSM	SBM
L/SL soil	0.54	0.39
L/ZL soil	0.66	0.66

On the L/SL soil, which had a higher total porosity, manure type was an important factor in the range of pore sizes that transported bacteria. On the other hand on the L/ZL soil the bacteria were transported through pores with a narrow size range. Soil type was important only in the case of LSM applications (Table 3.25).

Table 3.25.

Variance of bacteria migration velocity explained by the initial soil-water content (correlation coefficients and regression coefficients)

Soil type	Manure type	
	LSM	SBM
L/SL	0.39 (0.16)a	0.66 (0.55)b
L/ZL	0.58 (0.33)b	0.55 (0.31)b

Note: r^2 in parenthesis
Values followed by the same letter are not significantly different at $p<0.05$

Initial soil-water content in the first 30 cm had limited influence on the average bacterial migration velocity for both manure types on the L/SL soil. After application of liquid swine manure on L/ZL soil it was noted that the average bacterial migration velocity was directly proportional to the initial soil-water content in the surface (30 cm) horizon. However for the case of liquid swine manure application on L/SL soil there was only a slight correlation between the initial soil-water content in the 0 to 30 cm horizon and the average bacterial migration velocity. After application of SBM on the L/ZL soil, initial soil-water content was found to be directly correlated with the bacteria migration velocity up to a certain level – between 70% and 80% water-filled porosity. After that, further increase in the soil-water content slowed down the bacteria migration velocity (Fig. 3.8a-d).

Table 3.26.

Average bacterial migration velocity explained by the initial volumetric soil-water content (correlation coefficients and regression coefficients)

Soil type	Manure type	
	LSM	SBM
L/SL	0.37 (0.13)a	0.39 (0.15)a
L/ZL	0.86 (0.75)b	-0.02 (0.00)c

Note: r^2 in parenthesis

Table 3.27.

Average bacterial migration velocity explained by the initial soil-water-filled porosity (correlation coefficients and regression coefficients)

Soil type	Manure type	
	LSM	SBM
L/SL	-0.73 (0.53)a	0.35 (0.13)b
L/ZL	0.94 (0.89)c	0.38 (0.14)b

Note: r^2 in parenthesis
Values followed by the same letter are not significantly different at $p<0.05$

The effect of the soil-water-filled porosity on the average pore water velocity was very similar on both soil types and after both manure applications.

Table 3.28.

Average pore water velocity explained by the initial soil water-filled porosity (correlation coefficients and regression coefficients)

Soil type	Manure type	
	LSM	SBM
L/SL	0.73 (0.54)a	0.58 (0.34)b
L/ZL	0.81 (0.66)a	0.81 (0.65)a

Note: r^2 in parenthesis
Values followed by the same letter are not significantly different at $p<0.05$

Bacterial filtration coefficient as calculated with eq. 8, was higher under application of solid beef manure on both soils (Table 3.29.).

Table 3.29.

Estimated filtration coefficients (λ_f)

Soil type	Manure type	Filtration coefficient λ_f (m^{-1})	
		Average	St. dev.
L/SL	LSM	3.23	3.33
	SBM	15.09	7.44
L/ZL	LSM	5.98	6.40
	SBM	12.57	7.80

Predicted contamination

Potential contamination depth was calculated using the equation 10 (Section 3.3.2.4.). The obtained values represent the depth to which the bacterial concentration of the soil solution would reach the value of 1CFU/100 mL

soil solution, if the transport conditions are constant over the whole length of the transporting pore. This equation is based on assumptions of constant rates for bacterial filtration, adsorption and desorption on soil particles, and die-off.

Table 3.30.

Predicted general average contamination depth (m)– all depth and soil-water content levels (no-filtration samples were excluded)

Site	Manure type	
	LSM	SBM
L/SL	2.02(0.24)	1.65(0.31)
L/ZL	4.77(4.94)	2.04(0.21)

Note: standard deviation in parenthesis

Table 3.31.

Means of the predicted contamination depth maximums (m), (no-filtration samples were excluded)

Site	Manure type	
	LSM	SBM
L/SL	3.86(1.49)	3.21(1.14)
L/ZL	5.81(4.47)	3.16(1.23)

Note: - standard deviation in parenthesis
- maximum depths represent the highest potential depth value estimated for each repetition

Table 3.32.

Predicted depth of contamination

Site	TREATMENT	Depth of collection (cm)	Mean estimated contamination depth (m)	Maximum estimated contamination depth (m)	Time of confirmation for the maximum contamination -Δt- (hr)	No's of NF	Initial soil-water content for max. contamin (% vol.)	Total samplers with confirmed contamin.	Total active samplers
Arkell (L/SL)	PD	30	NTC	NTC	NTC	NTC	NTC	NTC	26
		50	n/a	NF	245.5	1	15	1	25
		75	NF	NF	14	1	15	1	27
	PW	30	1.42	NF	26.25	1	35	2	29
		50	2.37	NF	256	1	25	4	26
		75	n/a	5.35	135	0	25	1	24
	BD	30	n/a	0.77	238	0	19	1	26
		50	1.62	4.09	71.5	0	19	7	30
		75	n/a	1.70	238	0	19	1	29
	BW	30	1.49	3.92	110	0	24	4	24
		50	1.79	2.41	256	0	24	4	30
		75	2.74	3.59	98	0	34	2	30
Petersburg (L/ZL)	PD	30	NTC	NTC	-	NTC	-	NTC	22
		75	n/a	1.98	74	0	35	1	24
		100	8.68	14.55	244	0	20	2	29
	PW	30	1.29	NF	87	1	32	6	29
		75	3.34	NF	8	1	36	7	30
		100	3.77	NF	14	3	32	5	29
	BD	30	NTC	NTC	-	NTC	-	NTC	25
		75	n/a	1.61	14	0	27	1	28
		100	2.11	2.26	244	0	27	2	30
	BW	30	0.73	0.83	8	0	42	5	29
		75	2.27	3.04	15	0	42	12	28
		100	3.27	5.47	38.5	0	34	4	30

Note: NTC - no bacterial transport confirmed; NF - no-filtration by the soil occurred; n/a - no mean calculated (only one confirmed observation) and therefore not applicable; Mean values are calculated by excluding the NF observations; Δt = time from irrigation start to confirmed sampling

Fig. 3.10.

Predicted range of contamination depth with filtration

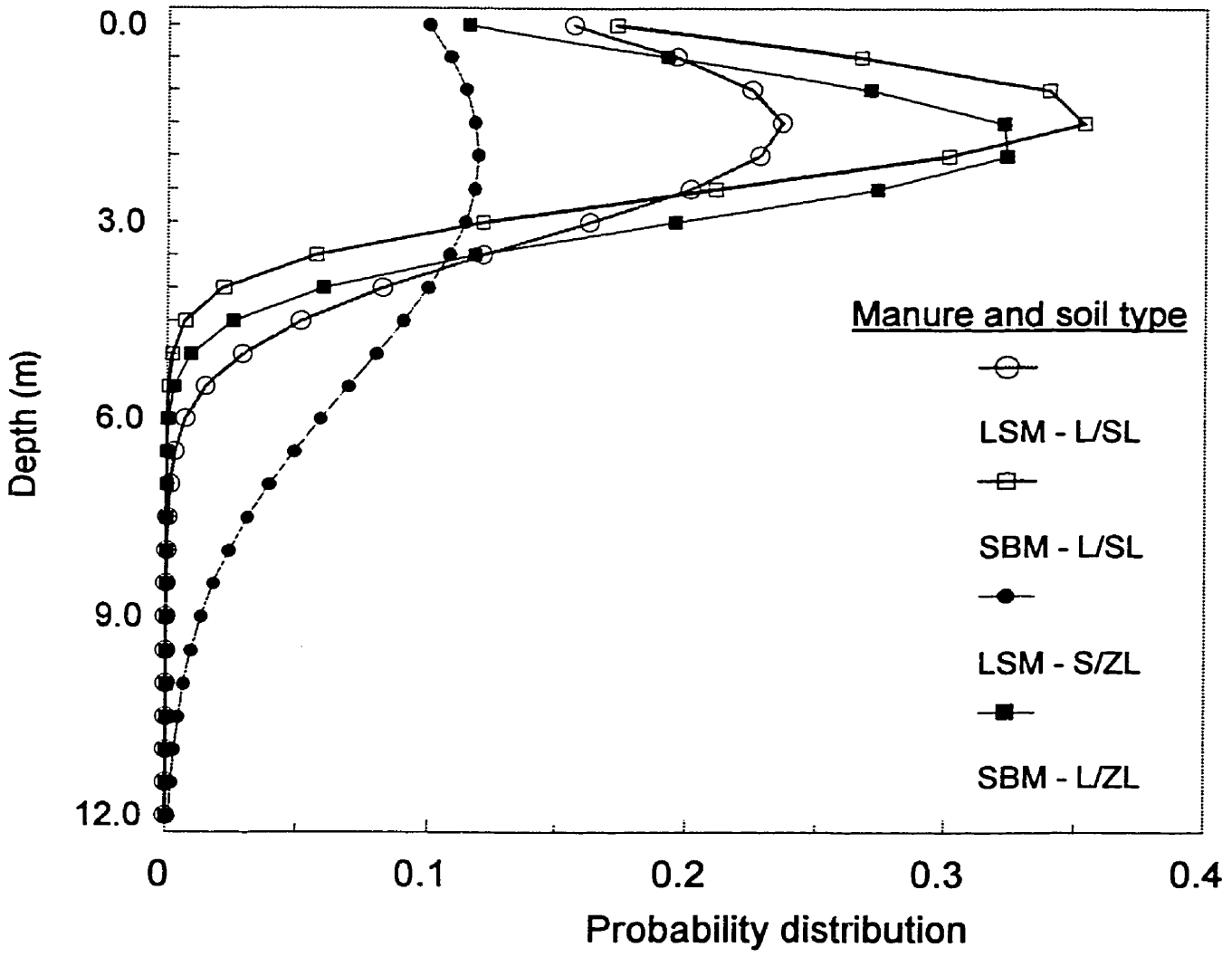


Fig. 3.11.

Estimated contamination depth with filtration as function of manure type and initial soil-water content (NF excluded)

Fig. 3.11.a

Loam/Sandy-Loam – liquid swine manure

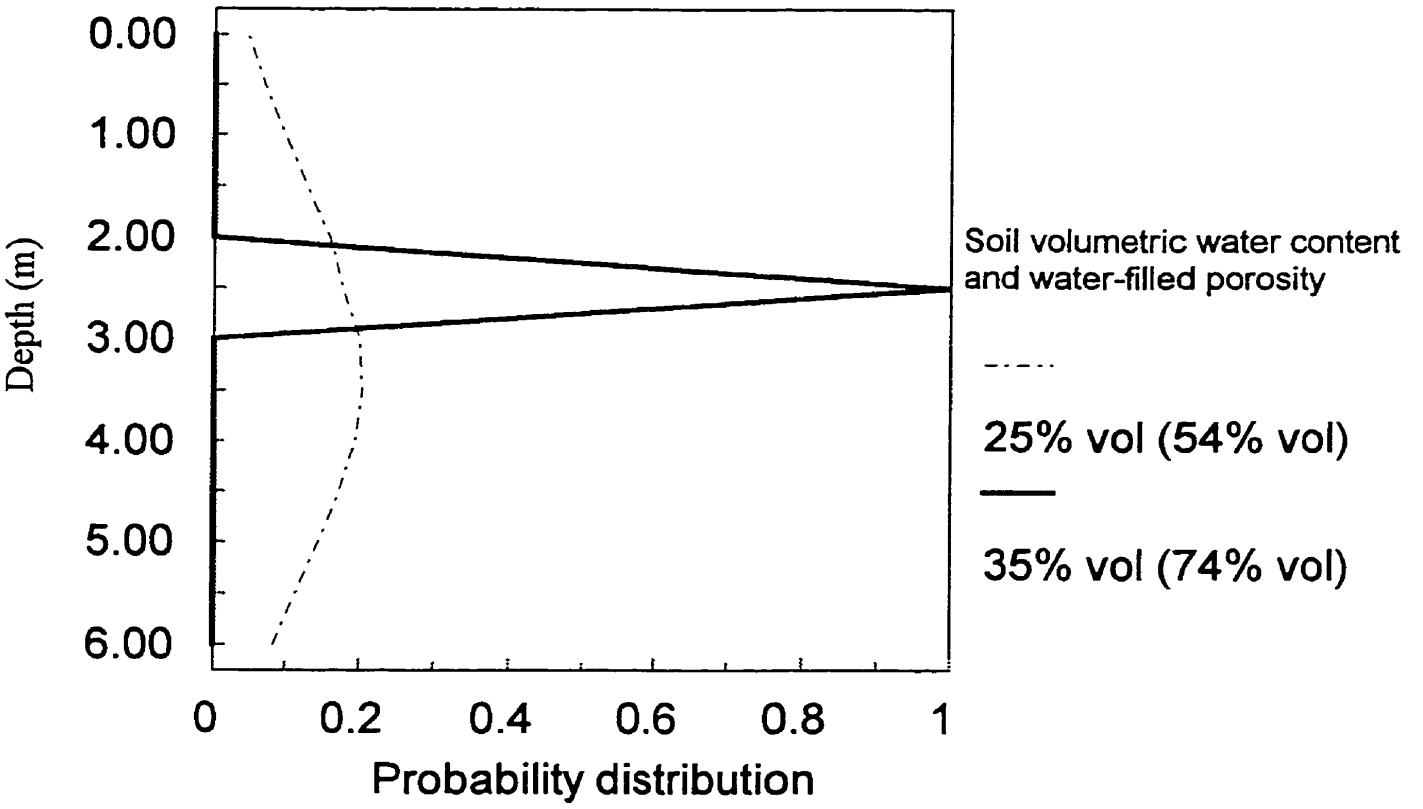


Fig. 3.11.b

Loam/Sandy-Loam – solid beef manure

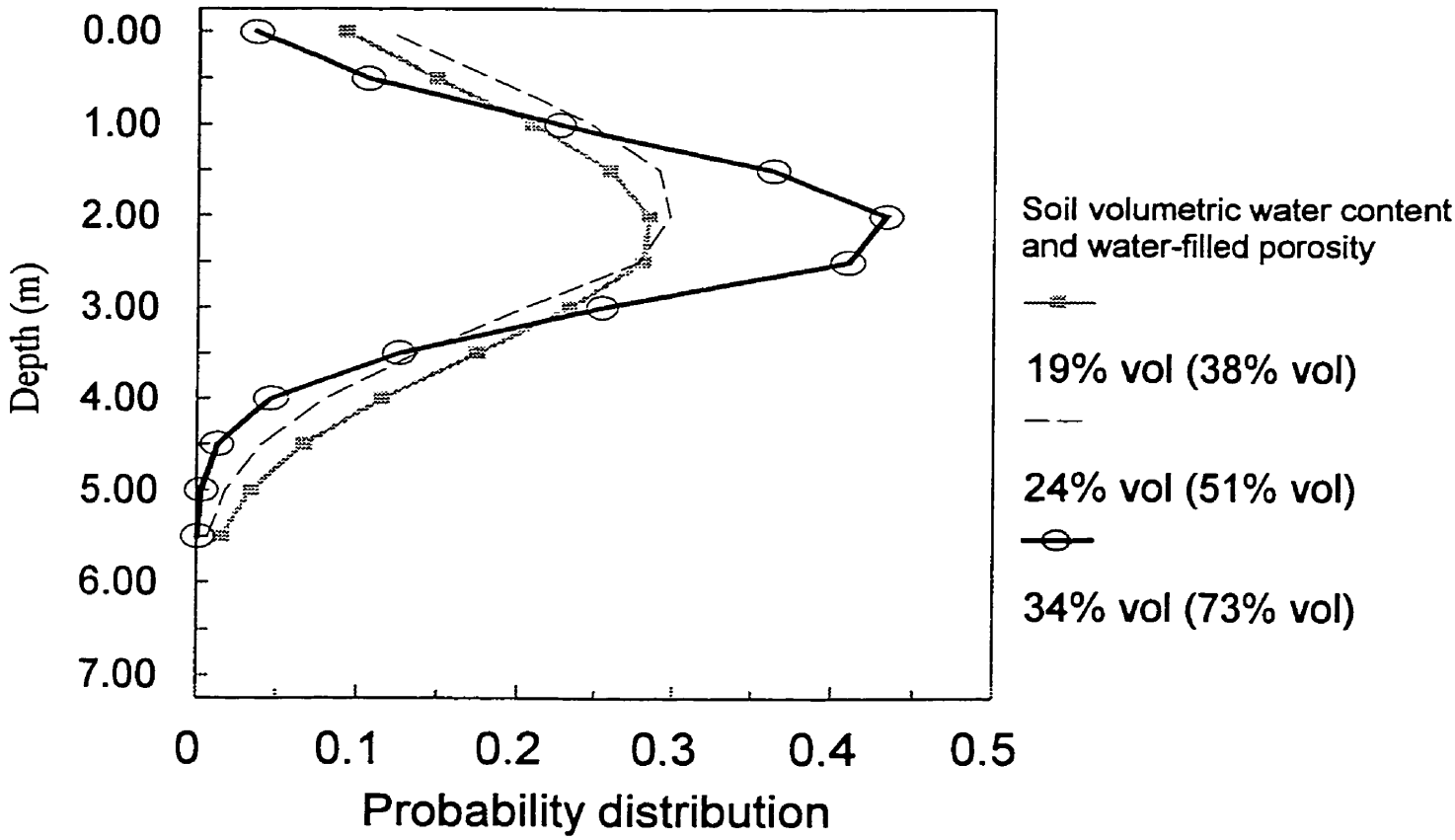


Fig. 3.11.c

Loam/Silt-Loam – liquid swine manure

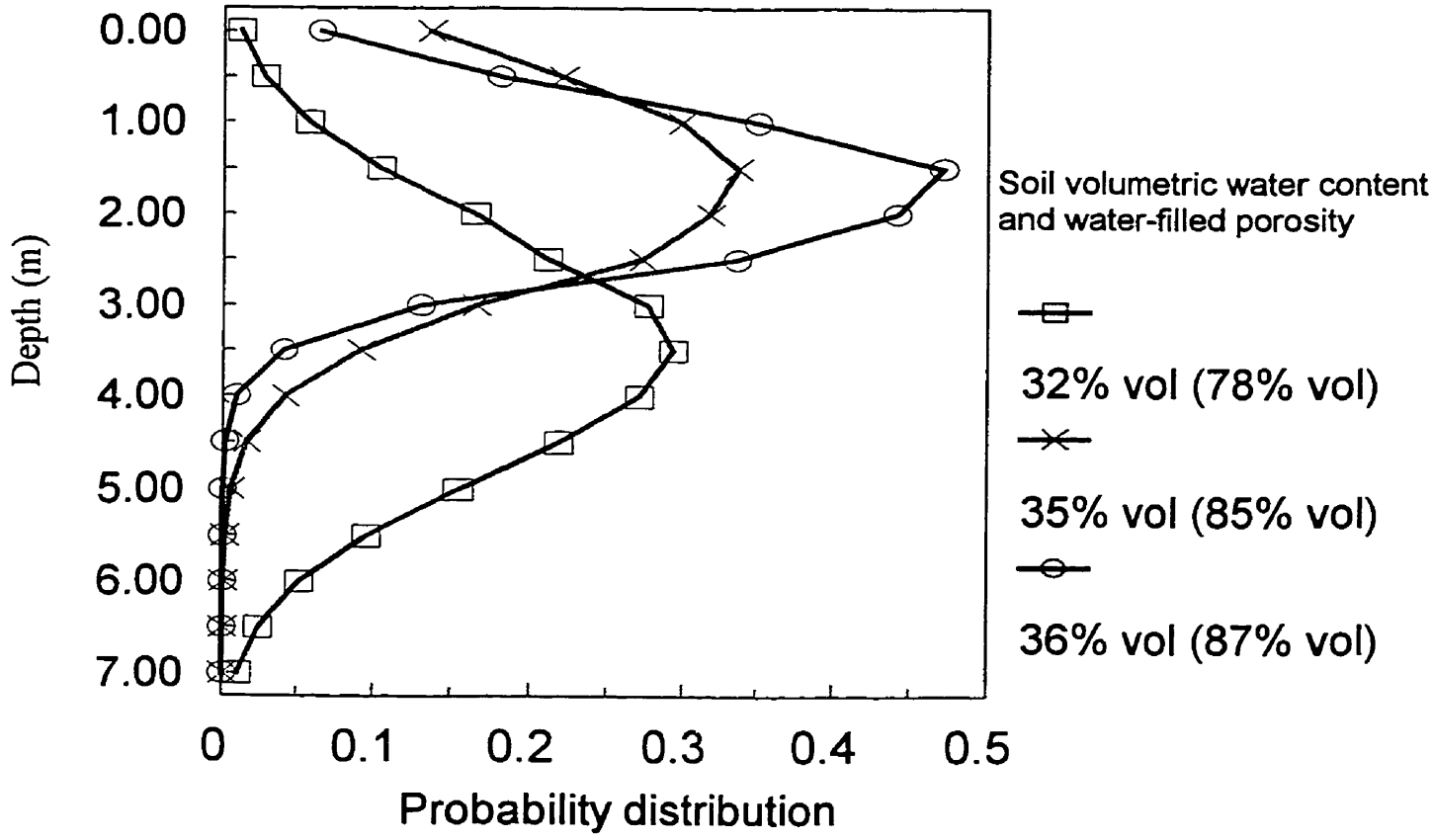
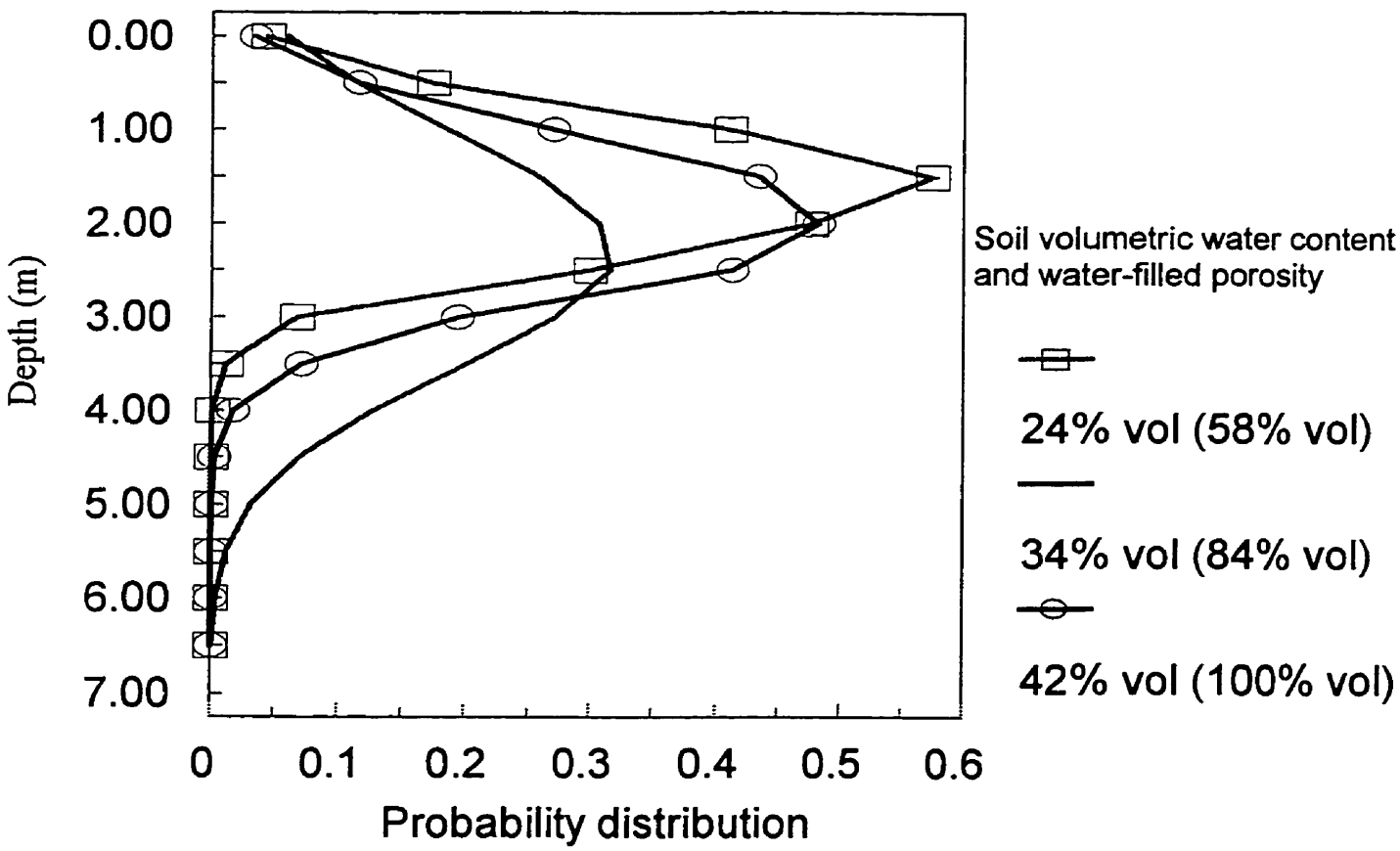


Fig. 3.11.d

Loam/Silt-Loam – solid beef manure



There was no correlation between the depth of collection and the concentration of faecal bacteria in the soil solution samples. Similarly there was no correlation between the depth at which bacteria were found and the time bacteria were collected.

Table 3.33.

Comparison between bacterial collection depth, time of collection and contamination level

Soil type	Manure type	Initial soil-water content (% vol.)	Collection depth and time of collection r^2	Collection depth and contamination level r^2
L / SL	LSM	0.27 0.25 0.35	0.00 0.00 0.58	0.00
	SBM	0.19 0.24 0.34 0.37	0.00 0.12 0.00 0.36	0.02 0.17 0.19
L / ZL	LSM	0.32 0.35 0.36	0.04 0.18 0.72	0.11 0.10 0.19
	SBM	0.24 0.34 0.42	0.00 0.03 0.49	0.30 0.26 0.01

Wetter soils were more susceptible to macropore flow resulting in higher contamination levels (Table 3.34.). For the case of LSM application on the L/SL profile the influence of the initial soil-water content was important only for the contamination in the surface horizon, while on the L/ZL profile it was also important for the deeper horizons too.

Table 3.34.

Bacterial contamination level explained by the initial volumetric soil-water content (correlation coefficients and regression coefficients)

Soil type	Manure type	Depth of confirmed contamination (m)			
		0.30	0.50	0.75	1.00
L/SL	LSM	0.98 (0.96)	0.23 (0.05)	-0.49 (0.24)	
	SBM	-0.76 (0.58)	-0.81 (0.65)	0.29 (0.09)	
L/ZL	LSM	0.61 (0.37)		0.81 (0.65)	0.56 (0.21)
	SBM	0.16 (0.03)		-0.18 (0.03)	0.38 (0.15)

Note: r^2 in parenthesis

Initial soil-water content was inversely correlated with the potential contamination depth when SBM was applied on L/SL profile. For SBM application on L/ZL profile the initial water content had no impact on the potential contamination depth.

While the significance of the initial soil-water content fades with depth the effect of the manure type gained in significance with depth for the L/SL profile.

Table 3.35.

Effect of initial soil-water content and manure type on the potential contamination depth for the L/SL profile

Depth (m)	Significance of initial soil-water content				Significance of manure type	
	LSM		SBM			
	P	Significance	P	Significance	P	Significance
0	0.0001	***	0.0001	***	0.57	NS
1	0.0044	**	0.0035	**	0.75	NS
2	0.0415	*	0.0476	*	0.22	NS
3	0.255	NS	0.178	NS	0.022	*

Initial soil-water had a significant impact on the potential depth of contamination for LSM applied on L/ZL profile. On the same soil under

application of SBM the effect of the initial soil-water content was not significant for most depths. Manure type influence declined with depth being of no statistical significance for depths over 3m.

Table 3.36.

Effect of initial soil-water content and manure type on the potential contamination depth for the L/ZL profile

Depth (m)	Significance of initial soil-water content				Significance of manure type	
	LSM		SBM			
	P	Significance	P	Significance	P	Significance
0	0.304	NS	0.426	NS	0.036	*
1	0.007	**	0.360	NS	0.007	**
2	0.0001	***	0.005	**	0.016	**
3	0.0016	**	0.022	*	0.074	NS
4	0.009	**	0.051	NS	0.158	NS

Table 3.37.

Effect of soil type effect on the potential contamination depth

Depth (m)	Treatments (manure type / initial soil-water content)					
	LSM - 35% vol.	Significance	SBM - 24% vol.	Significance	SBM - 34% vol.	Significance
Over 0	0.0013	**	0.269	NS	0.005	**
Over 1	0.006	**	0.560	NS	0.203	NS
Over 2	0.03	*	0.047	*	0.085	NS
Over 3	0.086	NS	0.117	NS	0.194	NS
Over 4	0.159	NS	0.170	NS	0.333	NS

The means and standard deviations for the predicted contamination depths obtained with of eq. 10 were used to develop a probability distribution of contamination over depth. For this purpose the inverse of the normal distribution

contamination over depth. For this purpose the inverse of the normal distribution technique was employed. A normal distribution was considered over other distributions since it accounts for the extreme values.

Table 3.38.

Predicted probability of contamination through continuous macropores with filtration

Soil type	Manure type	Initial soil-water content	Predicted depth of contamination (m)									
			0.5	1.0	1.5	2.0	3.0	4.0	5.0	6.0	7.0	8.0
L/SL	LSM	0.18	0.00%									
		0.25	81.5%	74.0%	65.1%	55.3%	35.3%	18.7%	8.1%	2.8%	0.8%	0.2%
		0.35	66.9%	50.3%	33.8%	19.9%	4.4%	0.5%				
	SBM	0.19	69.1%	53.9%	38%	23.9%	6.5%	1.0%				
		0.24	70.6%	56.6%	41.7%	27.9%	9.0%	1.8%	0.2%			
		0.34	95.4%	87.3%	72.5%	52.2%	15.1%	1.7%				
L/ZL	LSM	0.32	67.2%	57.6%	47.6%	37.7%	20.7%	9.3%	3.4%	1.0%	0.2%	
		0.35	82.2%	69.1%	53.0%	36.4%	11.6%	2.1%	0.2%			
		0.36	81.8%	69.1%	45.5%	26.7%	5.0%	0.4%				
	SBM	0.24	100.0	100.0	95.0%	9.80%						
		0.34	83.0%	69.0%	51.5%	33.7%	9.0%	1.2%				
		0.42	83.8%	73.9%	61.5%	47.8%	22.7%	7.4%	1.6%	0.2%		

The probabilities for estimated contamination depth were calculated for the treatments and water contents where at least three contaminated samples were obtained, which showed evidence of filtration. The samples that showed no-filtration (Table 3.40) were not considered for this estimation. Therefore the results summarised in Table 3.38 indicate the probability for contamination with faecal bacteria from the two manure types at different soil-water content in the presence of filtration, clogging and retardation processes. Also the samples that were obtained after supplementary water input through precipitation were

excluded (Table 3.43). Only the transport considered as being due to the initial irrigation was considered.

These probabilities indicate the chance of a continuous and potentially active pore allow bacteria transport to a certain depth. In other words this represents the predicted probability for the macropores to be continuous to a certain depth, while satisfying the hydraulic conditions that allow for water and bacteria transport.

Table 3.39.

Depth above which 95% of faecal bacteria were predicted to be located

Soil type	Manure type	Initial soil volumetric water content	Depth (cm)					NF
			All depths	30	50	75	100	
L/SL	LSM	0.18	nd	nd	nd	nd		Yes
		0.25	3.92	nd	nd	nd		Yes
		0.35	3.91	nd	3.99	nd		Yes
	SBM	0.19	3.21	nd	nd	nd		No
		0.24	3.35	nd	nd	nd		No
		0.34	3.53	nd	nd	4.34		No
L/ZL	LSM	0.18	nd	nd		nd	nd	Yes
		0.32	4.64	1.18		5.55	nd	Yes
		0.35	3.53	nd		nd	nd	No
		0.36	3.00	0.65		3.01	nd	Yes
	SBM	0.24	2.06	nd		3.22	nd	No
		0.25	1.50	nd		nd	nd	No
		0.27	1.06	nd		nd	nd	No
		0.34	3.33	0.66		2.71	5.79	No
		0.42	4.29	0.64		3.24	nd	No

Application of SBM resulted in bacterial transport that showed filtration effects for each positive collected sample. Only after applications of LSM was bacterial transport with no evidence of filtration by the soil observed.

Table 3.40.

Samples showing no evidence of bacterial filtration by the soil (NF samples)

Site	Treatment	Depth of collection (m)	Time of sampling -Δt- (hr)	Initial soil-water -% vol.-	Initial soil-water -% vol.-	Estimated potential contamination depth (m)
L/SL - June '97	LSM /wet soil	0.50	256*	0.25	0.54	∞
L/SL - July '97	LSM/dry soil	0.50	245.5**	0.18	0.34	∞
		0.75	14	0.18	0.34	∞
	LSM /wet soil	0.30	26.25	0.35	0.74	∞
L/ZL - June '97	LSM/dry soil	1.00	244	0.17	0.43	14.55***
L/ZL – July '97	LSM /wet soil	0.30	87	0.32	0.78	∞
		1.00	14	0.32	0.78	∞
		1.00	14	0.32	0.78	∞
		1.00	14	0.32	0.78	∞
L /ZL - May '98	LSM /wet soil	0.75	8	0.36	0.87	∞

* collected after additional 2 mm of rain;

** collected after additional 16.2 mm of rain

*** outlier and therefore considered closer to NF than to the predicted mean

3.3.1. Significance analysis for the NF (no-filtration) samples

Significance values for soil-initial water content, manure type, and soil type were calculated by using predicted probability distributions obtained by the mean of the Poisson distribution using the means of the observed probabilities. For each comparison only equivalent treatments were considered. The effect of the soil type was considered only in the case of the liquid swine manure, due to non-existence of the phenomenon in the case of the solid beef manure.

Occurrence of NF samples was not influenced by the initial soil-water content. The most important factor in NF occurrence was the manure type. The

NF phenomenon was not observed when SBM was applied. Soil type was important for the NF occurrence after LSM application.

Table 3.41.

Significance table for the occurrence of the NF samples

Site	Manure type	Factor	Range	P	Significance
L/SL	Liquid Swine	Initial soil-water content	(18%-25%-35%)	0.98	NS
	Manure type			0.00197	**
L/ZL	Liquid Swine	Initial soil-water content	(18%-32%-36%)	0.44	NS
	Manure type			0.00035	***
	Liquid Swine	Soil type		0.014	**

Contamination frequency on the L/SL profile was independent of the initial soil-water content or manure type. However, on the L/ZL profile the soil-water content had significantly influenced the frequency of contamination, without any significant effect due to the type of manure that was applied. The influence of the soil type was significant at the depth of 75cm.

Table 3.42.

Significance of the initial soil-water content, depth of bacteria confirmation, manure type, and soil type effects on the frequency of contamination.

Soil type	Manure type	Factor	P	Significance
L/SL	LSM	Initial soil-water content	0.32	NS
		Depth	0.73	NS
	SBM	Initial soil-water content	0.77	NS
		Depth	0.38	NS
	Manure type		0.47	NS
L/ZL	LSM	Initial soil-water content	0.0002	***
		Depth	0.227	NS
	SBM	Initial soil-water content	0.022	**
		Depth	0.11	NS
	Manure type		0.17	NS
Soil type[1)]			0.04	*

[1)] Significance of soil type was estimated for the depth of 75 cm

Transport of bacteria occurred after supplementary water addition due to natural rain events that occurred after the initial irrigation.

Table 3.43.

Bacteria transported with additional water from rain

Site	Treatment	Initial soil-water % vol.	Depth of collection (m)	Time of collection -Δt- (hr)	Rain amount (mm)	Estimated potential depth of contamination (m)
L/SL June '97	PW	0.25	0.50	256	2	NF
	BD	0.19	0.30	238	27	0.77
	BD	0.19	0.50	238	27	1.13
	BD	0.19	0.75	238	27	1.70
	BW	0.24	0.30	256	2	0.68
	BW	0.24	0.50	256	2	2.41
L/SL July '97	PD	0.18	0.50	245.5	16.2	NF
	BD	0.28	0.50	245.5	16.2	1.26
L/ZL July '97	PW	0.32	0.30	223.5	1	1.42

3.3.2. Comparison of contamination depth and bacteria migration velocity

A comparison was done using the average estimated potential depth versus the average bacteria migration velocity. Results of the analysis indicated that there was a positive correlation between the two after application of SBM on L/SL, and negative correlation after application of both manure types on L/ZL.

Table 3.44.

Predicted contamination depth explained by the predicted bacteria migration velocity

Soil type	Manure Type	Considered initial soil-water content levels - % vol. -	R	r^2
L/SL	LSM	nd	nd	nd
	SBM	19 & 24 & 28 & 34	0.66	0.44
L/ZL	LSM	22 & 32 & 35 & 36	-0.81	0.66
	SBM	24 & 25 & 34 & 27 & 42	-0.51	0.26

3.4. Discussion - field experiment

Both manure types used in this study, liquid swine manure and solid beef manure, had considerable concentrations of Escherichia coli over the whole period of the experiment (Table 3.11). Bacterial analysis of the two types of manure used for this experiment indicated that the amount of total coliforms per weight of manure dry matter was more or less equal. Escherichia coli was found to form a higher proportion of the total coliforms in the SBM compared to the LSM (Table 3.11). Therefore the actual total amount of Escherichia coli applied

on the field was of 2 to 3 orders of magnitude higher when SBM was applied compared to the LSM applications. This sort of direct comparison would indicate that if no other factors would be implicated in bacterial transport through the vadose zone, then the chances for contamination would be far greater for the field application of SBM.

The two manure types were tested for the potential ionic strength of the solution entering the soil. For the case of SBM the solution entering the soil was considered to be the irrigation water after it was in contact with the manure particles. In order to evaluate the ionic strength of the manure-water mixture, samples obtained after variable periods of contact between manure and water were tested.

Assuming that the molar conductivity remains the same (that is the ionic composition of the solution remains the same over different periods of manure-water contact), the changes in electrolytic conductivity are due only to changes in ionic concentration (Table 3.17.). The LSM was assumed to enter the soil undiluted and therefore its ionic strength was considered to represent the highest value for the ionic strength of the solution entering the soil under LSM treatments. The results indicated that the ionic strength was highest for the case of raw LSM. The ionic strength of the raw LSM was approximately 3 to 4 times higher than the SBM-water mixtures. The solutions obtained after mixing SBM with water showed little change for the four different manure-water contact periods. This suggested that SBM released its ions very rapidly after the initial contact with the irrigation water. This indicated that bacterial retention by soil

particles was probable to occur more significantly under the application of liquid swine manure on dry soils. The pH of the SBM-water mixture solutions and of the LSM was alkaline. The negative ions from solution compete with the bacteria for attachment sites on the surface of the soil particles, therefore favouring bacterial transport by reducing the rates of bacterial retention. They also may attach to the positive sites on the bacterial cell surface increasing its net negative charge. This might have lowered the bacterial potential attachment rates to the soil particles for both manure types.

Soil-water content at the time of manure application was expected to be an important factor in the velocity of downward migration of bacteria and was also expected to influence the number of bacteria moved to depth (Hegde and Kanwar, 1997). Comparison between the average pore water velocity and the bacteria migration velocity indicated that as the pore water velocity increased the bacteria migration velocity also increased (Tables 3.23 - 3.24 and Fig. 3.8.a-d), although the average pore water velocity was less than the average bacteria migration velocity (Table. 3.21). When the correlation between the pore water velocity and the bacteria migration velocity was analysed it was noted that, although there was a good correlation observed, for each treatment there were observed outliers for which the fraction between the bacterial migration velocity and the pore water velocity (BMV/PV) had very high values. This was an indication of the effect of preferential flow on the bacteria migration. Pore water velocity was also generally correlated with the initial soil-water content (Table 3.24). This suggested that as soils get wetter the hydraulic conductivity

increased, as expected, creating favourable conditions for the bigger pores to be active and therefore able to transport bacteria. Hence wet soils having a higher potential for macropore transport would have been expected to be more prone to deep bacterial transport than the dry ones. Results confirmed that in such cases bacteria moved downward at higher velocity (Table 3.27, 3.28, and fig 3.8.a -d). However after the soil-water content reached certain high levels of water-filled porosity the transport of bacteria was slowed down, as for liquid swine manure applied on the L/SL profile, or solid beef manure applied on L/ZL profile. In these cases bacteria movement was slower on very wet soils (Fig. 3.8.a-d). One explanation for this phenomenon would be that at high initial soil-water content the soil becomes saturated faster by the infiltrating water. Hence the infiltration rates are slowed down, and the transport of bacteria is likewise reduced. In such cases the water initially drained from the profile was the water already existent there before irrigation. Within the surface horizon, which has a higher saturated hydraulic conductivity, drainage is controlled mostly by the hydraulic conductivity of the deeper horizons, which tends to be lower than the surface horizon. Such a situation induces rapid ponding, creating conditions for the superficial soil aggregates to be dispersed. This leads to conditions favourable for sealing of the soil surface, impeding even more of the bacterial transport into the soil profile, and creating favourable conditions for run-off.

The bedding materials found in the SBM have a high water absorptive capacity, being able to retain twice to three times its own weight (Midwest Plan Service, 1975). Therefore, most probably due to its absorptive capacity, the

amount of water available for particle dispersion was reduced making the creation of a seal at the soil surface less likely when applying SBM.

Hence, for similar rates of irrigation or incoming rain the surfaces where SBM was applied were likely to satisfy the boundary conditions leading to ponding later compared to the surfaces where LSM was applied. This was noted by the reduced ponding that occurred where SBM was applied compared to the LSM applications for the same irrigation rates. Nevertheless application of SBM could have resulted in the formation of filtering mats on the soil surface if the applied water was sufficient to allow dispersion of manure material.

The greater clay content on the loam /silt-loam profile may have give more aggregate stability and therefore prevented disruption of the soil pores. Consequently, the contamination level, after application of liquid swine manure, was not significantly different between collection depth at the range of soil initial water content considered in experiment (Table 3.33). This suggested that, for increased initial soil-water content, the filtration capacity remained constant with depth.

As the initial soil-water content increased the velocity of bacterial migration became more variable (Table 3.25). Thus at higher soil-water contents a wider range of pores participated in bacterial transport. This correlation was stronger in the case of liquid swine manure, with higher average bacteria migration velocity after application of LSM for both soils. On L/ZL profile, bacteria migration velocity was higher than on L/SL profile for both manure types. The variance of the average bacteria migration velocity also indicated that bacteria moved

through a larger range of pore sizes after application of LSM on both soils as compared to applications of SBM. The lower variance of bacteria migration velocity on L/SL profile indicated a more uniform movement of the bacteria than was the case for the L/ZL profile where bacteria transport occurred with a large range of velocities (Table 3.25 and Fig. 3.7).

The correlation coefficient between the initial soil-water content and the variance of bacterial migration velocity showed no significant difference between the two soil types. However the effect of the manure type on the variance of the bacteria migration velocity was significant at $p<0.05$ for the L/ZL profile.

The greater stability of soil aggregates in the L/ZL profile may have also allowed bacteria movement to occur through a greater range of pore sizes than in the L/SL profile. The smaller total porosity in the L/ZL profile comparatively to the L/SL profile resulted in the matrix surrounding the macropores to become saturated more quickly creating favourable conditions for the infiltrating water to be funnelled through the bigger pores ending in higher bacteria migration velocity (Table 3.20).

Analysis of the effect of the initial soil-water content on the potential depth of contamination for the LSM application on the L/SL profile showed a very high correlation for the first horizon ($r^2=0.96$). Despite this relationship as the solution moves deeper the effect of the initial soil-water content was greatly reduced at 50cm depth and at a depth of 75cm the correlation became negative. This was not the case for the LSM applied on the L/ZL profile. There the initial soil-water

content was directly correlated to the potential depth of contamination for the whole depth of the soil profile (Table 3.34.).

One explanation could be that on the L/SL soil profile the aggregates are less stable at higher soil-water contents and therefore more likely to collapse, limiting the extent to which the macropores are continuous. As the bacterial suspension moved deeper the filtration was enhanced under wet conditions while passing thorough the suspected-collapsed sandy material. In such situations the bacteria filtration would be enhanced for wet soil conditions.

On the other hand for the application of SBM there was either a negative correlation with the initial soil-water content – on the L/SL profile – or no correlation at all – on the L/ZL profile. Therefore in the case of solid beef manure the filtration at higher initial soil-water content due to closed macropores was surmised to have been enhanced by supplementary pore clogging with the manure-originated colloidal particles. Hence the effect of the soil-water content on bacterial transport has been moderated by other factors like filtration and clogging due to the higher particulate content of the SBM[3].

The bacterial filtration coefficients were higher after application of SBM compared to the application of LSM on both soils.

Bacteria from solid beef manure moved through a more limited pore size range most probably due to limited length of the pores and likely also to clogging with manure material on the L/SL profile, and due to clogging with manure material but not pore lenght limitation on the L/ZL profile. It seems likely that

[3] note: When the SBM-water mixtures were prepared for the purpose of solution ionic strength measurements an inverse correlation between the length of shaking time and filtering speed was observed

clogging of pores was important in slowing down the bacterial migration velocity and enhancing the bacterial filtration on both soils (Tables 3.20, 3.27, 3.31, 3.32, 3.33, and 3.37).

Another phenomenon that has to be considered is the transport of bacteria with no-filtration or dispersion (Table 3.40). In this case the potential for contamination is very much increased by the lack of bacterial filtration. Application of liquid swine manure induced macropore flow of more or less pure manure, creating the potential for very deep contamination. Under solid beef manure no such effect was noticed. These observations are consistent with the hypothesis that higher dry matter content may favour reduction in the number of transported bacteria due to filtration. The soil initial water content was found not to have any significant effect on the occurrence of the NF bacterial transport (Table 3.42). However the correlation coefficient between confirmation time and soil initial water content in the case of NF samples was - 0.67. This indicated though that the higher the soil initial soil-water content the higher the likelihood for the matrix surrounding the macropores to be saturated allowing higher velocities for water flow and consequently for bacterial transport.

The soil type had a significant influence on the incidence of the NF occurrences, supposedly due to differences in the macropore continuity with depth over the two soil types. Another factor might have been the lower porosity and hydraulic conductivity of the L/ZL profile, characteristics that lowered the threshold for the amount of added water at which macropore flow starts.

Therefore the L/ZL profile type with a higher clay content was more prone to deep penetration than the L/SL profile.

The bacteria concentration levels were not correlated with the sampling depth indicating that the filtration rates are not uniform within the soil profile. Hence, because the equation used for the calculation of the filtration coefficient was based on the depth of sampling, the near surface sampling could not be used to predict correctly the potential maximum depth of contamination. Therefore in the case of macropore transport with minimal filtration, when the concentration levels were similar for different depths, the use of shallower collected samples may lead to an underestimation of the potential depth of contamination. In a non-structured soil the infiltrating water and the carried solutes are expected to move more or less uniformly. In such a scenario the transported contaminants are expected to first reach shallower horizons and later in time they are expected at deeper levels.

Results from the field experiment indicated that there is no correlation between depth and the time needed for bacteria to attain it. This suggested that the main flow split to many local flows allowing for very different transport speeds. Therefore the time needed for bacteria to reach a certain depth was not related to the travel distance. Similarly there was no correlation between the depth of contamination and contamination level indicating that filtration levels were also not a function of the travel distances. These results are similar with the findings of Natsch et al. (1996), who after field application of Pseudomonas, followed by 40mm irrigation, found similar concentration along the macropores

length between the depths of 30 and 150 cm. All this evidence pointed toward the importance of preferential flow for deep bacterial transport (Tables 3.34, 3.40).

Additional water from rain, enhanced the transport of bacteria for both manure types. In two occasions faecal bacteria survived in soil over the ten days of the experiment at very high concentrations, and the incoming additional water from rain transported it deeper. There might even have been growth of faecal coliform colonies in situ. Even smaller amounts of rain in range of 1 to 2 mm were enough to move the bacteria further downward. This confirmed that preferential flow may occur even if the amounts of incoming water is small (Beven and German, 1982), and if bacteria have been already moved below the soil surface, this localised flow may transport them stepwise to considerably greater depths.

Analysis of significance showed that, overall, the effect of the initial soil-water content in the first 30 cm on the contamination probability proved to be very highly significant for both manure types applied on the L/SL profile. For depths over 3 m the initial soil-water content becomes not significant. However frequency of deep contamination seemed to be significantly influenced by manure type, being higher for the liquid swine manure applications (Table 3.41.).

The lower porosity of the L/ZL profile makes the effect of the initial soil-water content in the first 30 cm very important for deep penetration of bacteria especially for manure with higher soil-water content as was the case for the liquid swine manure. For a soil volume of 0.75 m^3, equivalent to the volume of soil

contained in a microplot of 1 m^2 surface area and a depth of 75 cm, the total porosity for the L/SL profile was greater than for the L/ZL profile by 0.06 m^3. This is comparable to the amount of water applied with the irrigation after manure application.

Although the overall effect of manure type on contamination probability is highly significant, deep penetration did not seem to be significantly influenced by the manure type when filtration mechanisms were in place.

Soil type significance was tested only for three treatments where equivalent soil-water content levels could be matched. The results indicated highly significant differences due to soil type for the overall contamination probability for both manure types at high initial soil-water contents. However the contaminant potential for greater depth seems not to be significantly influenced by the soil type for both soil-water levels (24% - 34%) considered in the case of solid beef manure application (Table 3.37.).

The analysis of contamination frequency provided some information about the differences in pore size distribution between soil types, and the state of macropores following different treatments, hence their capacity to act as transport channels for bacteria. On the L/SL profile the frequency of contamination was not affected significantly by any of the factors considered by this experiment. This indicates that the percentage of pores active in bacterial transport does not change significantly with changes in depth, in initial soil-water content, or dry matter content of applied manure. In contrast, on the L/ZL profile the depth seems to gain more importance, although not reaching a significant

level. Also increases in initial soil-water content become a very highly significant factor in favouring a greater number of pores to be actively involved in bacterial transport. Differences between the two soil types were highly significant indicating that although the L/SL profile had a higher total porosity there were more chances for the macropores existent in the L/ZL profile to be continuous at depth.

4. General Discussion

Over an extended time period, after the bacteria have entered the soil, the size structure within the Escherichia coli population may change due to the stress factors in the new environment (Acea et al. 1988).

This may impede the accurate estimation of bacterial population at different stages after field application. However accounting for the fact that faecal coliforms may survive in soil for quite extended periods (Cools et al, 1998), it may be reasonable to assume that such effect is minimal over the first five days after application.

Collecting soil solution samples from an unsaturated vadose zone proved to be a difficult task. The low volumes of the samples increased the detection limit for bacterial concentration and also increased the error, which may occur especially at low levels of bacterial concentration. Therefore use of this sampling method for bacterial concentration monitoring over longer periods of time may not give accurate-enough results.

Nonetheless for estimating the potential for ground water contamination the higher values were of utmost importance. These values gave an indication about the maximum potential for contamination. Hence, in such a context, the errors within the lower concentration range lose their significance.

Use of this sampling method may result in high filtration rates. However comparison presented in Table 3.15 and appendix 4, indicated that the number of real zero counts was far greater than expected due to the sampling method. This indicated that estimation of the frequency of bacterial transport was possible.

Potential contamination was calculated considering the changes in the bacterial concentration with depth of transport. Therefore as long as the initial concentration of bacteria is considerably higher than the error associated with the estimating method, the evolution of the bacterial concentration with depth may be followed and estimated within the range of error due to the technique employed.

This assumed that the changes in bacterial concentration (C_0-C) with changes in the considered factor are higher than the error due to sampling technique. For the case of the field experiment the initial bacteria concentration in the applied manure was considerably higher than the error factor - log 6 for LSM and log 8 for SBM versus an estimated error of log 1.15 to 1.35.

Besides filtration and clogging, which refer to reduction of bacterial concentration over the transport length, the mechanisms involved in bacteria removal include the over-time die-off characteristics of the bacteria. Equation 7 (Mathess et al. 1988) and its variants, eq. 8 and 9, used here to estimate the

change in bacterial concentration, refers explicitly only to the spatial dimensions of the phenomenon, removal of bacteria over a certain length. The temporal dimensions is not explicitly considered by the equation. However, it is implicitly calculated through the fact that the actual measured concentration (C), which is part of the equation, is always a function of space and time. Therefore the die-off rate is indirectly accounted for in the calculation of the filtration coefficient. Hence this coefficient it is actually a reflection of both filtration over length and die-off, or, if there is the case, growth over time. If it is assumed that the die-off rate is not significant over the short period of the experiment then the filtration coefficient, as calculated, reflects only the change in bacterial concentration in soil solution over length.

Light reduction (UV radiation) of bacterial numbers (Whitelam and Codd, 1986) may have been an important factor in the case of solid beef manure where bacteria is exposed to solar radiation for a longer period as it does not infiltrate into soil untill rain or irrigation water is added. Liquid swine manure infiltrates comparatively faster in soil reducing the period for which the bacteria may be exposed to light. This factor was not directly studied by this experiment, but however if it had any significance in reducing the amount of bacteria available for transport into soil its effect was indirectly incorporated in the filtration coefficients.

Although the total porosity on L/SL profile was greater than on L/ZL profile the greater content of clay of the L/ZL profile supposedly created better conditions for the larger pores to be continuous at greater depths.

Bacteria were shown to move through macropores (Natsch et al., 1996), at higher velocity compared to the pore water velocity (Table 3.21.) and at less filtered or unfiltered concentrations (Table 3.29. and 3.40.). Knowing this it has been expected that there is a certain correlation between the average bacteria migration velocity and the average potential contamination depth. However the results of this comparison were not conclusive (Table 3.45). This may have to do with the errors due to the relatively lax schedule of sampling, cumulated with the errors due to the sampling procedure used.

5. Conclusions

The absence of E. coli in the soil solution samples collected prior to application of manure and their presence in later samples collected after manure application is a conclusive indication of transport of bacteria from manure through the vadose zone towards the ground water.

Bacteria migration velocities were higher than the average pore velocity; this indicates that bacteria were transported through the bigger pores where the water flux occurred at higher speeds. This confirms the assertion by Natsch et al. (1996), that bacteria can be transported downwards through the macropores after field application of manure followed by water addition, through irrigation or rain.

The field results show that macropore transport may be induced by small amounts of added liquids (as rain water and/or liquid manure), and that macropores become active even before the soil profile is saturated. This led to the transport of highly concentrated bacterial suspension even at soil-water contents lower than saturation. Such effects were very obvious under application of liquid swine manure.

Higher clay content favoured more pore continuity with depth and hence bacterial transport to greater depths..

As the surface soil stratum was saturated in the first period of liquid addition the water tended to penetrate through the existent macropores. While water was penetrating the macropores their walls were brought to saturation, favouring deeper penetration even if the bigger volume of matrix, which has a lower hydraulic conductivity, was not saturated. This local saturation allowed very high levels of local hydraulic conductivity, transporting the bacteria at speeds and to depths unattained by the average front of water. The wetter the soil the faster this localised saturation occurred and therefore the greater the potential depth of contamination. This was made obvious by the significant differences between the bacteria migration velocity and the average pore water velocity.

On the L/ZL profile with less porosity and lower hydraulic conductivity the soil matrix on the surface saturated faster. Therefore more water was available to penetrate the macropores and consequently the localised saturation of the pore walls could occur with a greater frequency. This resulted in deeper

penetration at higher velocities. The efficiency at which this transport mechanism occurred is most obvious for the treatments with liquid swine manure on drier soils. The frequency of confirmed contamination was very low there, and generally the filtration was very effective in restricting deep transport of bacteria. However even in these conditions some no-filtration transport occurred. This indicated that under liquid swine manure there is always a potential for deep transport, regardless of the initial soil-water content.

As the soil clay content and the bulk density increased and soil porosity decreased, the depth of bacterial transport increased considerably. Sandy horizons at depth allowed more bacterial dispersion and therefore higher filtration rates.

Higher soil-water content at the time of manure application led to bacterial movement through a greater number of pores. Pores within a larger size range participated in bacteria transport. However due to aggregate instability in the L/SL profile soil the pore continuity was reduced under increased soil-water content enhancing the filtration of bacteria. On the L/ZL profile higher soil-water contents increased the frequency and the level of contamination.

High total soil porosity was found not to be a good measure for the deep transport of bacteria. The uninterrupted length and the structural stability given by higher clay content was more important in facilitating macropore flow and therefore transport of bacteria through the vadose zone.

Although under solid beef manure more bacteria were applied to the field, owing to its high content in solids the filtration and probably clogging effects were

very pronounced. This reduced the range of the active pores and limited the potential for contamination to reach levels lower than the ones expected after liquid swine manure application especially since the no-filtration transport which occurred only under liquid swine manure and not with solid beef manure.

Hence liquid swine manure had the potential to produce deeper contamination than solid beef manure on both soil types and for every soil-water contents considered.

Die-off rates may have an important influence on the contamination over the long term. This study was focused only on the short-term contamination potential and therefore the die-off was assumed to have minimal impact over this short period.

Field application of manure proved to be a potential factor in ground water contamination. The closer the ground water table is to the surface the higher the contaminant potential.

Following the observations of these experiments manure should generally be applied on dry soils. Periods with high frequency of rains should be avoided if possible. However, specific recommendations are best to be done on an individual basis for each type of soil. Manure management practices that produce manure with higher content of dry matter are to be preferred.

The correlation between the bacteria migration velocity and bacteria contamination potential should be studied in more detail, using methods that are more sensible at relatively low variations in bacterial concentration. This could

lead to a more simple method for estimating the potential contamination of ground water with faecal coliforms under field conditions.

The characteristics of cell surface such as surface charge, cell size, and motility effect on vadose zone transport of faecal bacteria have to be assessed in order to improve the capacity for prediction of potential contamination with certain pathogenic bacterial strains.

Ground water contamination with bacteria from manure has to account for the vadose zone transport of faecal bacteria coupled with the survival rates of faecal bacteria in soil. Therefore more research has to be done to assess the die-off rate effects on potential bacterial contamination of ground water with time.

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Appendices

Appendix 1.

Arkell – Test wells depth

WELL ID	Surface Elevation (m)	Well Depth Below Surface (m)
P2S	336.08	6.90
P2D	336.11	11.9
P3	335.21	8.10
P4	333.72	9.36
P7D	335.94	12.15
P7S	335.94	9.94
P8S	335.67	9.80
P8D	335.67	7.60
P8N	335.67	12.03
P9S	337.10	6.80
P9D	337.12	8.15
P10S	339.48	6.10
P10D	339.51	13.05
P11S	340.87	9.00
P11D	340.91	11.9
P13	347.07	22.75
P15S	341.98	8.70
P15N	341.98	16.26
P16	340.17	12.16
BH1S	333.72	19.25
BH1T	333.72	10.74
BH2S	335.94	15.81
BH2T	335.94	22.19
BH3S	340.90	13.07
BH3T	340.90	20.98
N1		20.22
N2		18.54
N3		21.06
N4		23.26

Appendix 2.

Drainage from profile (L m^{-2})

Liquid swine and solid beef manure / dry soil treatment
Petersburg – June 1997

Manure type	Depth intervals (cm)	Sampling time and intervals (date and hour – hrs.)					
		June 25 hr 22^{00}	June 26 hr 12^{30}	June 27 hr 14^{00}	June 28 hr 14^{00}	June 29 hr 13^{00}	July 03 hr 14^{00}
		10.00	14.50	25.50	24.00	23.00	145.00
Liquid swine	0-30	20.32	8.72	24.31	(38.65)	2.42	(26.58)
	0-75	(34.23)	(1.45)	3.81	16.39	7.98	(30.22)
	0-100	(51.96)	(5.47)	(2.58)	15.52	(0.60)	(24.96)
Solid beef	0-30	13.35	(13.96)	13.28	(16.78)	(6.48)	(26.55)
	0-75	(15.76)	(22.17)	21.64	(4.88)	10.99	(23.27)
	0-100	(26.59)	9.57	(16.63)	(10.11)	17.66	(24.29)

Liquid swine and solid beef manure / wet soil treatment
Petersburg – June 1997

Manure type	Depth intervals (cm)	Sampling time and intervals (date and hour – hrs.)						
		June 27 hr 12^{30}	June 27 hr 21^{30}	June 28 hr 14^{00}	June 29 hr 13^{00}	June 30 hr 12^{30}	July 01 hr 10^{30}	July 05 hr 17^{00}
		12.00	9.00	17.00	25.00	21.50	22.00	126.50
Liquid swine	0-30	26.76	25.35	0.54	5.15	(6.44)	(5.47)	(36.81)
	0-75	9.03	(49.59)	10.98	10.19	9.27	0.55	(28.60)
	0-100	52.12	26.08	7.26	(0.45)	23.88	(16.29)	(28.87)
Solid beef	0-30	24.06	8.96	9.49	1.40	(1.72)	(6.76)	(17.63)
	0-75	52.02	23.83	19.02	9.49	(29.67)	21.96	(18.20)
	0-100	56.53	14.33	11.07	6.26	4.58	(14.18)	(20.32)
run-off		Liquid swine		≅ 10% (5.5 L m^{-2})				
		Solid beef		≅ 5% (2.5L m^{-2})				

Liquid swine and solid beef manure / dry soil treatment
Petersburg - July 1997

Manure type	Depth intervals (cm)	Sampling time and intervals						
		July 24 hr 8^{30}	July 24 hr 22^{30}	July 25 hr 9^{40}	July 26 hr 10^{00}	July 27 hr 6^{20}	July 28 hr 10^{00}	August 02 hr 10^{30}
		12.00	14.50	10.50	24.50	21.00	27.00	120.50
Liquid swine	0-30	27.84	(4.65)	3.25	(9.84)	4.56	(2.96)	7.60
	0-75	6.90	13.26	(18.16)	14.01	(0.42)	(3.14)	(5.19)
	0-100	6.18	13.91	0.50	(2.72)	0.17	(18.47)	11.31
Solid beef	0-30	13.68	9.30	1.78	(10.85)	(1.69)	(3.95)	16.82
	0-75	4.71	8.74	5.25	(2.70)	(3.36)	(13.13)	20.61
	0-100	(1.98)	6.57	12.23	4.68	(15.64)	(2.85)	21.02
Precipitation (mm)					1		18	

Liquid swine and solid beef manure / wet soil treatment
Petersburg - July 1997

Manure type	Depth intervals (cm)	Sampling time and intervals (date and hour – hrs.)						
		july 18 hr 12^{00}	july 18 hr 23^{00}	july 19 hr 12^{30}	july 20 hr 14^{00}	july 21 hr 13^{00}	July 22 hr 12^{30}	July 27 hr 5^{30}
		12.00	11.00	13.50	25.50	23.00	23.50	113.00
Liquid swine	0-30	45.03	(0.11)	3.01	0.95	2.03	(0.65)	(40.39)
	0-75	61.43	(2.38)	(13.91)	29.02	4.14	(11.32)	(26.17)
	0-100	65.64	0.92	(10.73)	31.97	3.87	(11.30)	(27.61)
Solid beef	0-30	57.23	(0.11)	5.71	6.21	(6.95)	(1.90)	(39.43)
	0-75	40.47	8.30	4.70	5.37	1.22	10.35	(20.33)
	0-100	40.24	14.17	7.40	6.47	1.77	10.32	(17.85)
Precipitation (mm)						1		1
run-off		Liquid swine	≅ 25% (13.5 L m^{-2})					
		Solid beef	≅ 10% (5 L m^{-2})					

Liquid swine and solid beef manure / dry soil treatment
Petersburg - May 1998

Manure type	Depth intervals (cm)	Sampling time and intervals (date and hour – hrs.)						
		May 20 hr 19^{45}	May 21 hr 03^{00}	May 21 hr 12^{00}	May 22 hr 14^{00}	May 23 hr 12^{30}	May 24 hr 15^{00}	May 30 hr 12^{00}
		6.00	7.00	9.00	26.00	23.00	26.00	141.00
Liquid swine	0-30	30.86	(1.18)	9.76	8.25	(3.61)	4.21	0.61
	0-75	15.95	7.64	4.39	12.61	4.76	4.76	(7.17)
	0-100	3.93	8.83	5.85	12.58	9.86	4.75	(14.21)
Solid beef	0-30	24.70	8.94	0.07	2.65	0.00	2.65	4.08
	0-75	26.62	0.51	(0.00)	12.95	9.87	0.00	(2.29)
	0-100	11.58	6.04	0.87	13.16	9.84	0.49	(4.95)

Liquid swine and solid beef manure / wet soil treatment
Petersburg - May 1998

Manure type	Depth intervals (cm)	Sampling time and intervals (date and hour – hrs.)						
		May 12 hr 20^{00}	may 13 hr 02^{00}	may 13 hr 13^{00}	may 14 hr 21^{00}	may 15 hr 13^{00}	may 16 hr 13^{00}	may 21 hr 11^{00}
		6.00	6.00	11.00	8.00	41.00	24.00	118.00
Liquid swine	0-30	36.37	6.34	3.81	1.33	16.35	(11.01)	2.72
	0-75	36.99	7.13	6.27	(2.06)	12.94	(1.13)	4.50
	0-100	35.56	10.93	7.76	(2.08)	16.49	(2.17)	4.53
Solid beef	0-30	32.10	13.85	5.86	4.86	7.60	(6.40)	10.30
	0-75	28.49	19.74	11.02	(1.12)	15.75	2.26	12.58
	0-100	24.24	26.96	12.01	0.97	15.71	0.68	16.26

Liquid swine and solid beef manure / dry soil treatment
Arkell - June 1997

Manure type	Depth intervals (cm)	Sampling time and intervals (date and hour – hrs.)						
		June 13 hr 20^{30}	June 14 hr 11^{00}	June 15 hr 10^{00}	June 16 hr 11^{30}	June 18 hr 10^{00}	June 22 hr 10^{00}	June 23 hr 10^{00}
		6.50	14.50	23.00	25.50	46.50	96.00	24.00
Liquid swine	0-30	2.49	(0.19)	0.06	0.58	(0.11)	(0.33)	0.21
	30-50	0.64	(0.58)	0.45	0.57	(0.16)	(0.38)	0.10
Solid beef	0-30	1.72	(0.77)	0.84	(0.40)	(0.20)	(0.49)	0.10
	30-50	(0.13)	(1.59)	0.02	1.25	0.70	(0.66)	0.20
Precipitation (mm)							27.00	
Note: only 44 mm irrigation applied								

Liquid swine and solid beef manure / wet soil treatment
Arkell - June 1997

Manure type	Depth intervals (cm)	Sampling time and intervals (date and hour – hrs.)						
		June 17 hr 17^{20}	June 18 hr 07^{10}	June 19 hr 7^{20}	June 20 hr 07^{10}	June 21 hr 08^{00}	June 22 hr 10^{00}	June 28 hr 11^{00}
		20.00	14.00	25.00	23.00	26.00	25.00	121.00
Liquid swine	0-30	3.54	(0.79)	(0.31)	(0.35)	0.21	0.16	(0.15)
	30-50	2.52	(1.22)	0.15	(0.62)	0.16	(0.08)	0.10
Solid beef	0-30	2.41	(0.52)	(0.41)	0.15	(0.18)	0.09	0.04
	30-50	1.74	(0.32)	(0.26)	0.07	(0.48)	(0.09)	0.31
Precipitation (mm)								2

Liquid swine and solid beef manure / dry soil treatment
Arkell – July 1997

Manure type	Depth intervals (cm)	Sampling time and intervals (date and hour – hrs.)						
		July 11 hr 09^{00}	July 11 hr 09^{00}	July 12 hr 09^{00}	July 13 hr 08^{30}	July 14 hr 08^{30}	July 15 hr 08^{00}	July 20 hr 08^{30}
		12.00	12.00	24.00	23.50	24.00	23.50	124.50
Liquid swine	0-30	1.94	0.31	0.10	0.15	0.15	(1.01)	(0.02)
	30-50	1.37	0.42	0.18	0.00	0.59	(0.35)	0.02
Solid beef	0-30	2.18	0.00	0.02	0.01	(0.04)	(0.36)	(0.04)
	30-50	1.36	0.33	(0.11)	0.03	0.02	(0.43)	(0.14)
Precipitation (mm)								16.2

Liquid swine and solid beef manure / wet soil treatment
Arkell – July 1997

Manure type	Depth intervals (cm)	Sampling time and intervals (date and hour – hrs.)						
		July 16 hr 07^{30}	July 16 hr 20^{15}	July 17 hr 07^{40}	July 18 hr 08^{00}	July 19 hr 08^{00}	July 20 hr 08^{30}	July 25 hr 07^{40}
		11.5	12.75	35	37.25	59	61.75	180
Liquid swine	0-30	4.63	1.07	(0.20)	0.01	(0.01)	0.14	(0.08)
	30-50	3.72	0.55	0.10	0.18	0.11	(0.01)	0.03
Solid beef	0-30	4.63	0.60	(0.20)	0.01	(0.11)	0.48	(0.28)
	30-50	4.13	0.72	(0.20)	0.31	(0.41)	0.13	

Liquid swine and solid beef manure / dry soil treatment
Arkell – October 1997

Manure type	Depth intervals (cm)	Sampling time and intervals (date and hour – hrs.)						
		Oct. 03 hr 23^{10}	Oct. 04 hr 05^{40}	Oct. 05 hr 18^{00}	Oct. 05 hr 18^{00}	Oct. 07 hr 20^{20}	Oct. 09 hr 16^{20}	Oct. 14 hr 09^{30}
		6.00	6.50	12.50	24.00	50.50	44.00	113.00
Liquid swine	0-30	5.37	0.27	0.00	0.24	(0.05)	(0.00)	0.17
	30-50	0.32	1.29	0.39	0.00	0.02	0.53	(0.09)
Solid beef	0-30	2.06	0.71	0.69	0.05	(0.04)	0.25	(0.18)
	30-50	4.72	1.22	(0.45)	0.11	(0.00)	0.52	(0.05)

Liquid swine and solid beef manure / wet soil treatment
Arkell – October 1997

Manure type	Depth intervals (cm)	Sampling time and intervals (date and hour – hrs.)						
		Oct. 10 hr 24^{00}	Oct. 11 hr 06^{00}	Oct. 11 hr 18^{00}	Oct. 12 hr 18^{00}	Oct. 14 hr 20^{30}	Oct. 16 hr 20^{00}	Oct 20 hr 20^{20}
		6.00	6.00	12.00	24.00	50.50	47.50	96.50
Liquid swine	0-30	8.37	(0.98)	0.60	0.32	0.08	0.00	0.01
	30-50	8.95	0.07	0.15	0.18	0.21	0.00	0.05
Solid beef	0-30	8.37	(0.95)	0.20	0.19	0.07	(0.11)	0.00
	30-50	6.89	0.52	0.76	0.19	0.23	(0.00)	0.01

Appendix 3. – **Summary of the analysis results for the samples with confirmed bacterial transport**

Site	Bacterial conc. of applied manure CFU/100ml	Treatment	Depth of sampling -Δz- m	Time of sampling -Δt- hours	Plate counts CFU/100ml	Estimated bacterial concentration in soil solution Log(CFU/100ml)	Estimated Bacteria migration velocity cm/day	Estimated pore water velocity over Δt cm/day	Bacterial migration velocity proportional to pore water velocity	Filtration coefficient m^-1	Estimated maximum potential depth of contamination m	Pore volumes to contamination $cm^3\ cm^{-3}$
Arkell-June		**PD**										
	1.9E+08	**BD**	0.30	238	200	5.03	3.03	1.11	2.74	24.86	0.77	0.44
	1.9E+08	**BD**	0.50	71.5	8000	7.26	16.78	3.55	4.73	4.66	4.09	0.15
	1.9E+08	**BD**	0.50	118	100	4.61	10.17	3.55	2.87	16.83	1.13	0.15
	1.9E+08	**BD**	0.50	214	400	5.45	5.61	3.55	1.58	12.99	1.47	0.23
	1.9E+08	**BD**	0.50	214	100	4.61	5.61	3.55	1.58	16.83	1.13	0.23
	1.9E+08	**BD**	0.50	238	100	4.61	5.04	3.55	1.42	16.83	1.13	0.23
	1.9E+08	**BD**	0.75	238	100	4.61	7.56	nd	nd	11.22	1.70	
	7.1E+05	**PW**	0.30	135	100	4.61	5.33	4.77	1.12	9.48	1.42	0.76
	7.1E+05	**PW**	0.50	256	65000	8.52	4.69	1.48	3.16	0.00	NF	0.25
	7.1E+05	**PW**	0.75	135	200	5.03	13.33	nd	nd	2.52	5.35	
	1.9E+08	**BW**	0.30	84	100	4.61	8.57	3.11	2.76	28.05	0.68	0.38
	1.9E+08	**BW**	0.30	110	15000	7.63	6.55	2.01	3.26	4.85	3.92	0.38
	1.9E+08	**BW**	0.30	135	100	4.61	5.33	1.64	3.25	28.05	0.68	0.40
	1.9E+08	**BW**	0.30	256	100	4.61	2.81	1.30	2.16	28.05	0.68	0.46
	1.9E+08	**BW**	0.50	135	1500	6.24	8.89	1.15	7.72	9.31	2.04	0.14
	1.9E+08	**BW**	0.50	256	2500	6.55	4.69	1.15	4.07	7.89	2.41	0.51
Arkell - July	7.1E+05	**PD**	0.50	245.5	800	5.87	4.89	1.12	4.35	0.00	NF	0.18
	7.1E+05	**PD**	0.75	14	1900	6.39	128.57	nd	nd	0.00	NF	
	2.2E+08	**BD**	0.50	121	100	4.61	9.92	1.49	6.66	17.17	1.12	0.19
	2.2E+08	**BD**	0.50	245.5	200	5.03	4.89	1.49	3.28	15.26	1.26	0.19
	7.1E+05	**PW**	0.30	26.25	10000	7.39	27.43	14.82	1.85	0.00	NF	0.47
	7.1E+05	**PW**	0.50	26.25	100	4.61	45.71	10.61	4.31	5.69	2.37	0.20

	7.1E+05	PW	0.50	122.75	100	4.61	9.78	1.67	5.86	5.69	2.37	0.24
	7.1E+05	PW	0.50	122.75	100	4.61	9.78	1.67	5.86	5.69	2.37	0.24
	2.2E+08	BW	0.50	98	300	5.27	12.24	2.74	4.47	14.13	1.36	0.26
	2.2E+08	BW	0.50	122	300	5.27	9.84	2.22	4.43	14.13	1.36	0.28
	2.2E+08	BW	0.75	98	2700	6.60	18.37	nd	nd	5.35	3.59	
	2.2E+08	BW	0.75	123	200	5.03	14.63	nd	nd	10.17	1.89	
Arkell - Oct.		PD	0.30	8	nd	nd	90.00	31.40	2.87	nd	nd	0.23
		PD	0.30	14	nd	nd	51.43	13.78	3.73	nd	nd	0.24
		PD	0.30	50	nd	nd	14.40	3.90	3.69	nd	nd	0.28
		PD	0.30	144.5	nd	nd	4.98	0.97	5.14	nd	nd	0.28
		PD	0.50	144.5	nd	nd	8.30	2.22	3.74	nd	nd	0.16
		PD	0.75	26	nd	nd	69.23	nd	nd	nd	nd	nd
		BD	0.30	14	nd	nd	51.43	5.45	9.44	nd	nd	0.08
		BD	0.50	50	nd	nd	24.00	2.71	8.86	nd	nd	0.14
		PW	0.30	26	nd	nd	27.69	9.71	2.85	nd	nd	0.40
		PW	0.50	244	nd	nd	4.92	1.00	4.90	nd	nd	0.31
		PW	0.75	244	nd	nd	7.38	0.00	nd	nd	nd	nd
		BW	0.30	8	nd	nd	90.00	41.88	2.15	nd	nd	0.32
		BW	0.30	26	nd	nd	27.69	7.23	3.83	nd	nd	0.34
		BW	0.30	244	nd	nd	2.95	0.72	4.10	nd	nd	0.39
		BW	0.50	244	nd	nd	4.92	1.41	3.50	nd	nd	0.27
		BW	0.75	244	nd	nd	7.38	0.00	nd	nd	nd	nd
Petersburg -June	7.1E+05	PD	1.00	244	400	5.45	9.84	0.71	13.94	0.93	14.55	0.04
	1.9E+08	BD	1.00	244	100	4.61	9.84	1.31	7.51	8.41	2.26	0.07
	7.1E+05	PW	0.75	40	100	4.61	45.00	3.33	13.51	3.79	3.55	0.10
	1.9E+08	BW	0.30	108.5	100	4.61	6.64	1.96	3.39	28.05	0.68	0.47
	1.9E+08	BW	0.75	23	100	4.61	78.26	20.03	3.91	11.22	1.70	0.10
	1.9E+08	BW	0.75	108.5	nd	nd	16.59	6.17	2.69	nd	nd	0.13
	1.9E+08	BW	0.75	108.5	600	5.69	16.59	6.17	2.69	7.91	2.41	0.13
	1.9E+08	BW	0.75	235	1500	6.24	7.66	3.60	2.12	6.21	3.07	0.13

Petersburg -July	7.1E+05	PW	0.30	87	1200	6.11	8.28	2.28	3.63	0.00	NF	0.42
	7.1E+05	PW	0.30	14	100	4.61	51.43	24.88	2.07	9.48	1.42	0.37
	7.1E+05	PW	0.30	14	200	5.03	51.43	24.88	2.07	6.30	2.14	0.37
	7.1E+05	PW	0.30	223.5	100	4.61	3.22	0.91	3.53	9.48	1.42	0.42
	7.1E+05	PW	0.75	14	100	4.61	128.57	28.13	4.57	3.79	3.55	0.20
	7.1E+05	PW	0.75	38.5	200	5.03	46.75	5.36	8.73	2.52	5.35	0.20
	7.1E+05	PW	0.75	25	nd	nd	72.00	10.64	6.76	nd	nd	0.20
	7.1E+05	PW	0.75	25	100	4.61	72.00	10.64	6.76	3.79	3.55	0.20
	7.1E+05	PW	0.75	110.5	100	4.61	16.29	2.97	5.48	3.79	3.55	0.31
	7.1E+05	PW	1.00	14	1200	6.11	171.43	2.90	59.14	0.00	NF	0.16
	7.1E+05	PW	1.00	14	1400	6.20	171.43	2.90	59.14	0.00	NF	0.16
	7.1E+05	PW	1.00	14	100	4.61	171.43	2.90	59.14	2.85	4.73	0.16
	7.1E+05	PW	1.00	14	2000	6.42	171.43	2.90	59.14	0.00	NF	0.16
	7.1E+05	PW	1.00	25	nd	nd	96.00	11.27	8.52	nd	nd	0.16
	7.1E+05	PW	1.00	223.5	nd	nd	10.74	1.91	5.63	nd	nd	0.25
	1.9E+08	BW	0.30	14	200	5.03	51.43	31.64	1.63	24.86	0.77	0.47
	1.9E+08	BW	0.30	14	100	4.61	51.43	31.64	1.63	28.05	0.68	0.47
	1.9E+08	BW	0.75	14	200	5.03	128.57	20.448	6.29	9.94	1.91	0.13
	1.9E+08	BW	0.75	25	300	5.27	72.00	11.128	6.47	9.19	2.07	0.16
	1.9E+08	BW	0.75	25	nd	nd	72.00	11.128	6.47	nd	nd	0.16
	1.9E+08	BW	0.75	25	200	5.03	72.00	11.128	6.47	9.94	1.91	0.16
	1.9E+08	BW	0.75	38.5	300	5.27	46.75	6.89	6.79	9.19	2.07	0.17
	1.9E+08	BW	0.75	38.5	1100	6.06	46.75	6.89	6.79	6.78	2.81	0.17
	1.9E+08	BW	1.00	14	nd	nd	171.43	17.32	9.90	nd	nd	0.10
	1.9E+08	BW	1.00	14	300	5.27	171.43	17.32	9.90	6.89	2.76	0.10
	1.9E+08	BW	1.00	14	200	5.03	171.43	17.32	9.90	7.46	2.55	0.10
	1.9E+08	BW	1.00	38.5	3500	6.76	62.34	7.56	8.24	3.48	5.47	0.15
Petersburg -May '98	2.7E+07	PD	0.75	74	100	4.61	24.32	2.87	8.48	8.67	1.98	0.15
	2.7E+07	PD	0.75	8	400	5.45	225.00	12.02	18.72	nd	nd	0.05
	2.7E+07	PD	1.00	14	133	4.78	171.43	5.04	34.03	6.11	2.80	0.03
	8.8E+08	BD	0.75	14	133	4.78	128.57	7.87	16.34	12.78	1.61	0.09
	8.8E+08	BD	1.00	14	67	4.37	171.43	5.88	29.15	10.54	1.95	0.04
	2.7E+07	PW	0.30	15	100	4.61	48.00	14.16	3.39	21.68	0.79	0.35
	2.7E+07	PW	0.30	24	50	4.19	30.00	8.39	3.58	24.91	0.69	0.38

	2.7E+07	PW	0.75	8	100	4.61	225.00	22.28	10.10	0.00	NF	0.12
	2.7E+07	PW	0.75	8	200	5.03	225.00	22.28	10.10	7.39	2.32	0.12
	2.7E+07	PW	0.75	8	50	4.19	225.00	22.28	10.10	9.96	1.72	0.12
	2.7E+07	PW	1.00	8	133	4.78	300.00	20.04	14.97	6.11	2.80	0.09
	8.8E+08	BW	0.30	8	nd	nd	90.00	19.81	4.54	nd	nd	0.36
	8.8E+08	BW	0.30	8	200	5.03	90.00	19.81	4.54	30.06	0.69	0.36
	8.8E+08	BW	0.30	8	634	5.72	90.00	19.81	4.54	24.71	0.83	0.36
	8.8E+08	BW	0.75	15	1334	6.17	120.00	17.10	7.02	8.51	2.42	0.16
	8.8E+08	BW	0.75	15	3400	6.74	120.00	17.10	7.02	6.77	3.04	0.16
	8.8E+08	BW	0.75	15	1400	6.20	120.00	17.10	7.02	8.42	2.45	0.16
	8.8E+08	BW	0.75	73	100	4.61	24.66	3.94	6.26	13.30	1.55	0.24
	8.8E+08	BW	1.00	99	200	5.03	24.24	2.89	8.39	9.02	2.28	0.20

nd – not determined
NF – samples showing no bacteria filtration by the soil

Appendix 4

Relationship between the measured and the predicted number of samples with CFU counts of 0 according to the Poisson distribution as function of sample size

Treatment		Depth -cm-	No. total samples	Positive samples	Probability for obtaining plate counts of 0 by the filtration through the porous cups %	Comfirmed probabilty for 0 CFU plate counts for field samples assuming Poisson distrib.	Field samples with a confirmed plate count of 0 CFU %	Expected 0's	Confirmed 0's
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
Arkell June '9 7	PD	30	27	0	0.245 to .52	1.00	1.00	7 to 14	27
		50	49	0	0.245 to .52	1.00	1.00	12 to 25	49
		75	45	0	0.245 to .52	1.00	1.00	11 to 23	45
	BD	30	25	1	0.245 to .52	0.96	0.96	6 to 13	24
		50	58	5	0.245 to .52	0.92	0.91	14 to 30	53
		75	61	1	0.245 to .52	0.98	0.98	15 to 32	60
	PW	30	47	1	0.245 to .52	0.98	0.98	12 to 24	46
		50	29	1	0.245 to .52	0.97	0.97	7 to 15	28
		75	54	1	0.245 to .52	0.98	0.98	13 to 28	53
	BW	30	22	1	0.245 to .52	0.96	0.95	5 to 11	21
		50	53	5	0.245 to .52	0.91	0.91	13 to 28	48
		75	66	2	0.245 to .52	0.97	0.97	16 to 34	64
Arkell July '9 7	PD	30	55	0	0.245 to .52	1.00	1.00	13 to 29	55
		50	57	1	0.245 to .52	0.98	0.98	14 to 30	56
		75	70	1	0.245 to .52	0.99	0.99	17 to 36	69
	BD	30	74	1	0.245 to .52	0.99	0.99	18 to 38	73
		50	71	2	0.245 to .52	0.97	0.97	17 to 37	69
		75	76	0	0.245 to .52	1.00	1.00	19 to 40	76
	PW	30	71	1	0.245 to .52	0.99	0.99	17 to 37	70
		50	63	3	0.245 to .52	0.95	0.95	15 to 33	60
		75	54	0	0.245 to .52	1.00	1.00	13 to 28	54

1	2	3	4	5	6	7	8	9	10
Arkell Oct. '9 7	BW	30	67	0	0.245 to .52	1.00	1.00	16 to 35	67
		50	66	2	0.245 to .52	0.97	0.97	16 to 34	64
		75	72	2	0.245 to .52	0.97	0.97	18 to 37	70
	PD	30	47	6	0.245 to .52	0.88	0.87	12 to 24	41
		50	47	1	0.245 to .52	0.98	0.98	12 to 24	46
		75	58	1	0.245 to .52	0.98	0.98	14 to 30	57
	BD	30	62	0	0.245 to .52	1.00	1.00	15 to 32	62
		50	64	2	0.245 to .52	0.97	0.97	16 to 33	62
		75	66	1	0.245 to .52	0.98	0.98	16 to 34	65
	PW	30	55	1	0.245 to .52	0.98	0.98	13 to 29	54
		50	43	1	0.245 to .52	0.98	0.98	11 to 22	42
		75	31	1	0.245 to .52	0.97	0.97	8 to 16	30
	BW	30	43	4	0.245 to .52	0.91	0.91	11 to 22	39
		50	56	0	0.245 to .52	1.00	1.00	14 to 29	56
		75	72	1	0.245 to .52	0.99	0.99	18 to 37	71
Petersburg June '9 7	PD	30	50	0	0.245 to .52	1.00	1.00	12 to 26	50
		75	52	0	0.245 to .52	1.00	1.00	13 to 27	52
		100	52	1	0.245 to .52	0.98	0.98	13 to 27	51
	BD	30	56	0	0.245 to .52	1.00	1.00	14 to 29	56
		75	35	0	0.245 to .52	1.00	1.00	9 to 18	35
		100	52	1	0.245 to .52	0.98	0.98	13 to 27	51
	PW	30	68	0	0.245 to .52	1.00	1.00	17 to 35	68
		75	69	1	0.245 to .52	0.99	0.99	17 to 36	68
		100	68	0	0.245 to .52	1.00	1.00	17 to 35	68
	BW	30	69	1	0.245 to .52	0.99	0.99	17 to 36	68
		75	56	4	0.245 to .52	0.93	0.93	14 to 29	52
		100	62	0	0.245 to .52	1.00	1.00	15 to 32	62
Petersburg July '9 7	PD	30	54	0	0.245 to .52	1.00	1.00	13 to 28	54
		75	34	0	0.245 to .52	1.00	1.00	8 to 18	34
		100	70	0	0.245 to .52	1.00	1.00	17 to 36	70
	BD	30	60	4	0.245 to .52	0.94	0.93	15 to 31	56
		75	51	5	0.245 to .52	0.91	0.90	12 to 27	46
		100	71	6	0.245 to .52	0.92	0.92	17 to 37	65

1	2	3	4	5	6	7	8	9	10
	PW	30	68	0	0.245 to .52	1.00	1.00	17 to 35	68
		75	70	0	0.245 to .52	1.00	1.00	17 to 36	70
		100	68	0	0.245 to .52	1.00	1.00	17 to 35	68
	BW	30	77	2	0.245 to .52	0.97	0.97	19 to 40	75
		75	62	6	0.245 to .52	0.91	0.90	15 to 32	56
		100	68	4	0.245 to .52	0.94	0.94	17 to 35	64
Petersburg May '98	PD	30	40	0	0.245 to .52	1.00	1.00	10 to 21	40
		75	53	2	0.245 to .52	0.96	0.96	13 to 28	51
		100	67	1	0.245 to .52	0.99	0.99	16 to 35	66
	BD	30	49	0	0.245 to .52	1.00	1.00	12 to 26	49
		75	51	1	0.245 to .52	0.98	0.98	12 to 27	50
		100	66	1	0.245 to .52	0.98	0.98	16 to 35	65
	PW	30	71	2	0.245 to .52	0.97	0.97	17 to 37	69
		75	69	3	0.245 to .52	0.96	0.96	17 to 36	66
		100	71	1	0.245 to .52	0.99	0.99	17 to 37	70
	BW	30	53	3	0.245 to .52	0.94	0.94	13 to 28	50
		75	68	4	0.245 to .52	0.94	0.94	17 to 36	64
		100	57	1	0.245 to .52	0.98	0.98	14 to 30	56

Appendix 5.

Estimated transported bacteria as fraction of the total applied bacteria (%)

Soil type	Treatment	Depth (cm)	June'97		July '97		Oct '98	
			Avg. (%)	Max. (%)	Avg. (%)	Max. (%)	Avg. (%)	Max. (%)
L/SL	LSM – dry soil	30	-*	-*	-*	-*	nd	nd
		50	-*	-*	-**	≅100	nd	nd
		75	-*	-*	-**	≅100	nd	nd
	LSM –wet soil	30	-**	5.77	≅100	-	nd	nd
		50	-**	≅100	5.77	5.77	nd	nd
		75	-**	15.18	-*	-*	nd	nd
	SBM – dry soil	30	-**	0.06	-*	-*	nd	nd
		50	1.10	98.36	0.03	0.05	nd	nd
		75	0.02	-*	-*	-*	nd	nd
	SBM – wet soil	30	5.16	23.06	-*	-*	nd	nd
		50	1.42	1.92	0.08	0.08	nd	nd
		75	-*	-*	0.75	1.81	nd	nd
L/ZL			June'97		July '97		May '98	
	LSM – dry soil	30	-*	-*	-*	-*	-*	-*
		75	-*	-*	-*	-*	4.60	11.04
		100	-**	39.93	-*	-*	-*	0.22
	LSM – wet soil	30	-*	-*	39.93	≅100	0.10	0.15
		75	-**	0.02	7.78	15.18	0.25	0.86
		100	-*	-*	≅100	≅100	-**	0.22
	SBM – dry soil	30	-*	-*	-*	-*	-*	-*
		75	-*	-*	-*	-*	-*	0.01
		100	-**	5.77	-*	-*	-*	0.00
	SBM – wet soil	30	-**	0.02	0.03	0.049	0.03	0.06
		75	0.35	0.94	0.16	0.52	0.86	3.59
		100	-*	-*	0.67	2.61	-**	0.30

Note: -* - no confirmation of bacterial transport
-** - only one confirmation; hence no average was calculated
nd - not determined