The Transport of *Escherichia coli* Through Soil

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Abstract of the Thesis

Sorption is an important process in the retention of pathogens by clay barriers. Batch sorption experiments were conducted to investigate the sorption of non-pathogenic *E. coli* to illite, kaolinite, and montmorillonite clays, a natural red clay, a silt and a sand. The Freundlich isotherm model provides the best fit for the sorption data. The sorptive capacity (SC) of each soil for *E. coli* was calculated at an equilibrium *E. coli* concentration of 1×10^8 CFU/mL. The SC values correlate strongly with the measured cation exchange capacity (having an R^2 of 0.92), as well as the weighted average particle size (having an R^2 of 0.83), of the soils.

Columns of compacted and normally consolidated high plastic clay and compacted non-plastic silt soils were exposed to cycles of freezing and thawing to simulate conditions of physical weathering, in turn creating a fracture network in the specimens. To determine bacterial transport properties, these columns, as well as intact sand, were permeated with a nonpathogenic *E. coli* strain suspended in a minimal salt medium in a constant head permeability mode. As a result of freezing and thawing, the permeability of the soil columns was found to increase by one order of magnitude for the silt and two to three orders of magnitude for the normally consolidated and compacted clay columns. Full (100%) breakthrough of the *E. coli* was observed after three pore volumes of flow in the sand, 35 pore volumes of flow in the silt, and was not observed in both compacted and normally consolidated clay columns, even after as many as 55 pore volumes of flow. However, as many as 5×10^5 CFU/mL of *E. coli* were found to permeate through clay columns, indicating a possible public health risk from bacterial transport through freeze-fractured soil liners. Both sorption and filtration

through soil were found to be important factors in determining bacterial transport through fine grained soils.

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Chapter 1: Literature Review

1.0.0 Bacterial Transport Through Soil

Understanding bacterial transport through soil is important in order to protect groundwater sources. Groundwater is an important source of drinking water, especially for rural populations. Recent surveys of spring and well water in Canada and the USA show that up to half of drinking water wells had evidence of fecal bacteria contamination, resulting in 750,000 to 5.9 million cases of illness and 1400 to 9400 deaths per year (Macler and Merkle, 2000).

Protective soil barriers, typically composed of at least 20% fines (fine silt and clay particles), are constructed to be practically impermeable, having a permeability of less than 10^{-7} cm/s (Qian et al, 2002). However, values of permeability depend on many factors, including moisture content and the degree of fracturing in the soil. Theories and models for liquid flow through soils "have been validated, if at all, and only in highly artificial sets of laboratory produced conditions" (Hillel, 1982). Hydrologic heterogeneity is observed in real soils, where flow paths are created through fractures induced by either drying or freezing (Brady, 1984; Eigenbrod, 1996). Under saturated conditions, a swelled sample of montmorillonite clay will have a very low permeability. However, at unsaturated conditions, it will have a very high permeability due to shrinkage (Brady, 1984). Hence, some sections of a soil field may swell more than others due to differences in water and mineral content, and quantifying permeability in the field is difficult.

The transport of bacteria through soil liners is not only proportional to the permeability of the liner, but depends on many factors (Foppen and Schijven, 2006). A general model of bacterial transport was generated by Foppen and Schijven in 2006 based on a literature review of *E. coli* transport studies. Their model estimates transport by calculating the number of bacteria retained in a soil sample per unit time, and is calculated according to:

$$\delta S/\delta t = \pi a_p^2(k_a + k_{str})C - k_r S_2 - k_{is} S \qquad \textbf{(Equation 1.1)}$$

Where

$\delta S/\delta t$ = Rate expression for the fractional surface coverage of attached bacteria

a_p = The diameter of the bacteria (mm)

C = Concentration of suspended bacteria in the aqueous phase (CFU/mL)

S = Dimensionless fractional surface coverage, defined as the total cross-sectional area of the deposited bacteria per interstitial surface area of the porous medium solid matrix (no units)

S_2 = Fraction of S available for kinetic sorption (%)

k_a = Attachment rate coefficient

k_{str} = Straining rate coefficient

k_r = Detachment rate coefficient

k_{is} = Inactivation and die-off rate coefficient

(Foppen and Schijven, 2006)

Equation 1.1 describes the inhibition of bacterial transport in terms of cell size, concentration, and inactivation and die-off rate (a_p, C, k_{is}) sorption rate (S, S_2, k_a, k_f),

and filtration, or straining rate (k_{str}). The coefficients used in this equation are only mathematical models, and have yet to be verified in field experiments, but they provide a starting point for modeling bacterial transport (Foppen and Schijven, 2006).

Calculations for the sorption and filtration rate coefficients described in Equation 1.1 are based on data gathered only from coarse-grained soils, and do not take into account the strong negative charges on clay materials or preferential flow through fracture networks in clay materials (Foppen and Schijven, 2006). Hence, although Equation 1.1 may prove to be an accurate predictor of bacterial transport through sandy aquifers and other coarse-grained soil materials, additional modeling may be necessary to predict bacterial transport through fine-grained clay materials.

1.1.0 Bacterial Interactions with Soil

Bacteria are often enumerated in terms of colony forming units (CFU). This unit is measured by spreading bacterial samples onto nutrient agar plates and then incubating them. As a result, any live bacteria which are well suited to the growing conditions will continually divide and form a colony, which is visible to the naked eye. It is impossible to determine if only 1 bacterial cell was responsible for the formation of a colony, because several may have been clumped together before incubation. Hence, one colony forming unit does not necessarily represent one bacterial cell in the sample, but is the closest estimate which is easily measured (Tortora et al, 2004).

Culturable microorganism concentrations in surface soils can reach 10^8 CFU/g dry soil, and have been estimated to represent over 10,000 species of bacteria (Turco and Sadowsky, 1995). However, despite these large numbers, bacteria typically make

up only approximately 0.001% of the total volume in most soils (Maier et al, 2000). The distribution of microorganisms in soils is heterogeneous due to variability in the distribution of nutrients (primarily in organic content), water, and suitable pore sizes for bacterial reproduction (Maier et al, 2000).

Bacterial surfaces and clay particles are both negatively charged, so the binding of bacteria to clay particles requires a positively charged intermediate, or "bridge". This bridge can be composed of cations, organic matter such as humus, or extracellular metabolites released by the bacteria themselves (Maier et al, 2000). It has been estimated that approximately 80 to 90% of cells in porous media are sorbed to solid surfaces.

Groundwater and rainwater availability increase bacterial density and activity in both surface and subsurface soil environments. However, compared to subsurface soil, surface soil contains more weathered minerals (which serve as inorganic nutrients) and organic matter (which serve as carbon and nitrogen sources), both of which allow for higher cell density and more uniform distribution of microorganisms (Maier et al, 2000).

Movement of bacteria through soil may be partially attributed to bacterial motility by flagella, but most of bacterial transport through soil is by passive movement along water flow paths (Unc and Goss, 2003). These authors found that travel through macropores in the soil structure is responsible for almost all of the bacterial transport through their samples. The same study also concluded that because bacterial transport requires water flow, bacterial transport is lower in soils with low water content than in wet soils.

Sand, silt and clay particles are the substratum of extremely active bacterial processes in surface soils. These processes result in the formation of microbial gums, polysaccharides and other extracellular bacterial metabolites. These metabolites, along with the bacteria themselves, act as an adhesive which binds together sand, silt and clay particles into aggregates which can protect bacteria from desiccation and provide them with a means to retain water and organic nutrients (Maier et al, 2000).

Sorption of bacteria onto particle surfaces enhances bacterial survival for several reasons. First, attachment onto surfaces, particularly in intraaggregate pores, allows bacteria to avoid predation by protozoa and utilize water and nutrients sorbed onto soil particle surfaces (Maier et al, 2000). Secondly, sorption allows bacteria to congregate into colonies (Gilbert et al, 1993). Colony formation allows for the containment and recycling of limiting nutrients among attached cells. Colony formation also increases the proximity of soil bacteria, allowing for much more frequent genetic exchange. It has also been shown that colonies can alter the microsite environment's pH in order to optimize growth conditions (Gilbert et al, 1993). Finally, cells in colonies can produce biofilms consisting of extracellular polymeric substances (EPS), which offer protection to the bacteria from environmental stresses such as temperature changes, pH changes, and the presence of toxins in the soil. Certain types of fine clay particles have also been found incorporated into complex biofilm structures known as "clay hutches". These hutches are thought to be formed due to the clay particle's ability to sorb and attract carbon sources in carbon-starved environments (Lunsdorf et al, 2000).

Free-living (unsorbed) bacterial cells are less commonly found in soil than sorbed cells, but are important for cell dispersion in the environment (Maier et al, 2000).

Dispersion is essential for bacterial survival because colonies may eventually use up all of the nutrients at a given microsite and die. There is evidence that bacterial cells on the outer surface of colonies undergo changes in their surface properties that allow newly formed daughter cells to be released from the colony after cell division (Gilbert et al, 1993). As these daughter cells grow, their surfaces undergo various biochemical changes that make attachment at a new site more favorable (Gilbert et al, 1993).

1.2.0 Factors affecting bacterial sorption to soil particles

Sorption of bacteria to particle surfaces is important in determining their transport in the soil environment, as sorption reduces bacteria's rate of transport (see section 1.0.0). The following discussion introduces a basic sorption modeling system, and then the factors which complicate complete and accurate modeling of bacterial sorption onto soil particle surfaces are analyzed.

1.2.1 DLVO Theory to Model Sorption

In 1989, Loosdrecht *et al* attempted to model bacterial adhesion to soil via a physicochemical approach and utilized the DLVO theory to model bacterial sorption to soil. This modeling approach, named after Derjaguin, Landau, Verwey and Overbeek, describes the force between surfaces in the presence of a liquid medium. The model combines the effects of Van der Waals forces and repulsion due to ionic forces.

Loosdrecht et al generated the model by analyzing the adhesion of *Pseudomonas* strains to negatively charged polystyrene. The DLVO model indicated that bacterial adhesion is reversible, and thus is relatively weak, until cells come into

contact in such a way as to pass the energy barrier (Loosdrecht, 1989). This can be accomplished by molecular interactions such as hydrogen, ionic and covalent bonding, as well as interactions involving extracellular structures such as EPS (Extracellular polymeric substances, which are polymers that typically consist of acidic heteropolysaccharides) (Parent et al, 2004). Since this study, it has been found that the DLVO model of bacterial adhesion is not sufficient to explain adhesion events in natural systems, due to the complexities of biological surfaces and natural soils (Parent et al, 2004).

According to a study by Scholl et al (1992), the dependence of bacterial sorption upon environmental conditions suggests that bacterial immobilization "could change substantially over relatively short distances." This indicates that simple modeling of bacterial sorption within a soil system may be impossible due to heterogeneity of soil particles and their environment. The following sections will investigate factors that complicate the possibility of developing an all-encompassing sorption model.

1.2.2 Particle size

The particle size distribution of soils plays an important role in the sorption of bacteria. Bengtsson and Ekere (2001) found that particle size correlated strongly with groundwater bacteria sorption rates in sandy aquifer materials. Likewise, Marshall (1971) showed that sorption of *Bacillus cereus* and *Serratia marcescens* was significantly higher in soils that contained 10% clay or more, probably due to increased initial attachment on the charged clay particles. Increased sorption of bacteria to clay soils may also be due to the smaller average pore size found in clay. McGechan and

Lewis (2002) found that smaller soil pores provide more opportunities for sorption to occur between bacterial cells and soil particles, partially due to initial contact from physical filtration. Increased nutrient availability on smaller soil particles due to a higher surface area to volume ratio may also explain the increased sorption seen in McGechan's study. Heukelekian and Heller (1940) also found that increasing the surface area per unit mass of a synthetic soil (consisting of glass beads) allowed for better nutrient aggregation and better survival of *E. coli.*

Particle size also affects the pore distribution in a soil. Coarse soils such as sand have larger pores than fine grained soils such as clay (Hillel, 1982). Therefore, bacteria are much more likely to become physically filtered in clay materials than in sand materials. Physical filtration will result in the bacteria having a longer exposure time to soil particles, and a subsequent increase in sorption rates (Foppen and Schijven, 2006).

1.2.3 Mineral Type, pH, and Cation Exchange Capacity

The dependence of pH on bacterial sorption to soil was investigated by Yee et al (2000). Part of this study investigated the sorption of *Bacillus subtilis* bacterial membranes to corundum, an aluminum based mineral which is abundant in natural soils. This soil was chosen because it is well characterized and known to be rich in aluminum sites, which are known to be strongly involved in sorption (Yee et al, 2000). The study found that bacterial membrane adsorption onto corundum surfaces was highly pH dependent, with much higher sorption occurring at lower pH values. Varying other parameters such as ionic strength, and bacterial concentration strongly affected the sorption of bacterial membranes onto corundum, but did not have any effect on their

sorption to quartz, which sorbed below the detection limit of the experiment in all conditions (Yee et al, 2000).

A study by Hassen et al (2003) also found a pH dependent effect on bacterial sorption. Their study investigated the adsorption of *Pseudomonas aeruginsa* and *Bacillus cereus* to smectitic clay, and found that lower pH increases retention efficiency in their system (Hassen et al, 2003).

Roberts (2004) continued investigation into the effects of pH on sorption of bacteria onto different soil minerals. Her study found that the colonization of soil minerals by bacteria was highest when the minerals were positively charged at an experimental pH of 8.6 (Roberts, 2004). Iron oxide and aluminum oxide (positively charged) soils exhibited strong colonization across a wide pH range while quartz and plagioclase (negatively charged) only exhibited strong colonization at low pH ranges (4 and lower) (Roberts, 2004). Foppen and Schijven (2006) also found that a soil mineral's charge strongly affects its ability to sorb bacteria in a study investigating the sorption of *Escherichia coli* to activated charcoal, calcite and goethite.

These results all suggest that mineral type plays an important role in bacterial sorption, based on the mineral's surface charge, which is dependent on the pH of the liquid in the soil.

Sorption of bacteria is largely accomplished by binding to cations bound to the negatively charged clay particles (Marshall, 1971). The binding is due to the attraction of negatively charged cell surfaces to the positively charged cations. Because this bridging requires cations to bind two negatively charged surfaces, multivalent cations are much more efficient at sorbing bacteria than monovalent cations (Marshall, 1971). Walker et al

(1989) found that multivalent cation bridging aided in *E. coli* cell wall sorption by increasing the incidence of planar surface binding orientations. Without these multivalent cations present (i.e. when replaced by sodium), binding was almost exclusively to the positively charged edges of the clay particles, and far fewer *E. coli* were bound (Walker et al, 1989). A study by Hall et al (2005) also found that the presence of the multivalent cations such as aluminum and iron in a natural aquifer promoted attachment of the bacteria *Paenibacillus polymyxa.*

Marshall (1971) points out that there are some positively charged components of cellular membranes that may also be responsible for some sorption, but that since the overall charge on the bacterial cell is negative, cation bridging is the most important mechanism of sorption.

1.2.4 Soil Organic Content and Dissolved Oxygen Content

Organic content has a strong effect on the ability of *E. coli* to sorb to soils (Guber, 2005). Data from experimental studies by Guber (2005) indicate that increasing manure content in soil resulted in decreased bacterial attachment, while a study by Parent et al (2004) indicated that bacterial attachment increased in the presence of humic acid, a component of manure. These contradictory findings may be the result of using different soil substrates, different strains of bacteria and different conditions such as pH.

Dissolved oxygen content (DOC) has also been shown to play a role in bacterial attachment. Irreversible binding of *Comamonas sp.* to iron oxyhydroxide-coated columns was shown to occur only in groundwater in which the DOC was at or below 1ppm (Hall et al, 2005). The effect of DOC on bacterial attachment in this study was

thought to be due to oxygen's electrostatic interactions in the columns, which inhibit bacterial binding to iron, manganese, and aluminum oxyhydroxides. Oxygen's interactions with these molecules reduce the number of sites to which bacteria can bind and become adsorbed.

1.2.5 Cell properties

Many components of cellular membranes are non-polar, and tend to self-associate in the presence of aqueous solution. These are known as hydrophobic components because they tend to repel water. The hydrophobicity and net electrostatic charges on the cellular membranes of bacteria play an important role in the sorptive process (Gannon et al, 1991). For this reason, different bacteria will have different capacities for sorption onto soil particle surfaces. Hydrophobicity and net electrostatic charge are altered by changes in the surrounding environment and by the development of biofilms, which can increase sorption rates and enhance the strength of adhesion (Yee et al, 2000, Loosdrecht et al, 1989).

Cell surface charge is often measured by a bacteria's zeta potential, derived from electrophoretic mobility tests (Foppen and Schijven, 2006). As described in section 1.2.1.4, the difference between the cell surface charge and the soil surface charge is an important factor in determining sorptive rates. However, cell surface charge has been found to vary considerably between bacteria even of the same strain, and is affected by environmental factors such as the ionic strength of the suspension medium (the liquid entering the soil, in which the bacteria are transporting) and the pH of the soil solution (Foppen and Schijven, 2006). Thus, it is very difficult to accurately quantify a bacteria's

zeta potential during transport simply by measuring the zeta potential before introducing the bacteria into the soil, as the soil solution can often drastically change it.

Variations in water content and nutrient availability in the microenvironment of pore spaces and aggregates further complicates attempts at quantifying surface charge because such variation can lead to biofilm development, which in turn alters the hydrophobicity and charge on cellular surfaces. Biofilms are also often enhanced or inhibited by the presence of other bacteria. This introduces further heterogeneity in the state of cells inside of a soil (Subramanian et al, 1999).

A study by Jordan et al (2004) investigated the influence of system complexity on *Pseudomonas putida* transport through saturated soils. This study demonstrated that natural bacterial populations (comprising only 7% of the total bacteria in the soil after the addition of the *P. putida*) created very high variability in the transport parameters and survival of *P. putida*. In soils that were sterilized previous to the transport, variability was very low (Jordan et al, 2004). The author states that the variability in transport rates introduced by the biological component of the soils would be even greater in field-scale systems, and that more focus should be given to the uncertainty associated with microbial processes in natural systems.

1.3.0 Factors Affecting Bacterial Filtration in Soil

The transport of *E. coli* through soil liners is primarily retarded by sorption and filtration. Here, filtration will be defined as the physical blockage of bacterial transport by pores smaller than the bacteria. In some studies, filtration is defined to include "physicochemical" filtration, which is essentially sorption (Tufenkji et al, 2004). Often, it

is difficult to differentiate filtration and sorption, because filtered cells will be stopped, which allows time for more sorption to occur, increasing the overall rate of sorption in the system. Also, sorbed cells on the soil structure can act as filters by blocking open soil pores, so increased rates of sorption can also increase filtration.

Different types of soil have different average pore sizes, and the pore sizes within a soil material may vary considerably depending on the heterogeneity, moisture content and dry density of the soil (Hillel, 1982). A study by Gannon et al (1991) indicated significant filtration of bacterial cells through a soil consisting of 22.5% clay. In the study of 20 different bacterial strains, those that were smaller than 1um in diameter were able to move through the soil in significantly greater numbers than cells larger than 1um in diameter (Gannon et al, 1991). The difference in transport rates in this experiment was suspected to be due to filtration, because the chief difference between the strains was the cellular size (Gannon et al, 1991). However, the authors state that sorption seems to be primarily responsible for most of the retention of cells inside of soil barriers.

Attempts to mathematically model bacterial filtration have been based on pore geometry (Matthess and Pekdeger, 1985), colloid geometry (Herzig et al, 1970), and contact efficiency (Neumann, 1983). In 2006, Foppen and Schijven combined these models into one all encompassing mathematical model of filtration:

$$k_{str} = \frac{\eta_S v\varepsilon \alpha_{str}}{4} = 0.68(a_p/a_c)^{3/2} v\varepsilon \alpha_{str}$$ **(Equation 1.6)**

Where

k_{str} = straining rate coefficient (mm^4/s) (probability of being strained in a pore)

η_S = straining contact efficiency (probability of entering a pore in which straining can occur)

v = pore water flow velocity (mm/s)

ε = effective porosity (mm^3)

a_p= diameter of bacteria (mm)

a_c = median of the grain size distribution (mm)

α_{str} = dimensionless straining correction factor to account for fluid velocity variations at pore level, grain geometry variations and filter bed porosity. (unitless)

This model was based on an extensive literature review, and allows for an approximate quantification of the number of bacterial cells that will be strained in a soil system, but has yet to be verified with experimental data (Foppen and Schijven, 2006, see Section 1.0.0). The data used to generate this model were based on sandy aquifer systems, in which sorption is significantly lower than systems with clay due to a lack of charge on the soil particles. In systems with higher amounts of sorption, quantifying straining mathematically would also require quantifying sorption. As discussed, quantifying sorption is a very difficult task due to the many factors which affect it.

1.4.0 Conclusions on Bacterial Transport

Bacterial transport through soil is clearly a very complicated process involving the contributions of many factors. Simple quantification of the effect of any given factor is very difficult because controlled systems are very difficult to generate in soil and microbiology experiments. For example, results may be complicated by the use of different soils, soil preparation methods, liquid mediums, background bacteria, tracer bacteria, temperatures, experimental scales, and sampling methods. All of these factors make it difficult to compare results between experiments.

Bacterial transport through soil is especially important for sites containing heavily contaminated waste, such as landfills, sewage lagoons and manure piles. The protective soil barriers laid under these waste sites are designed to prevent bacterial transport, but given the wide variability in transport parameters seen in experimental data, the safety of these liners should be scrutinized. Groundwater used as drinking sources often flows under areas of contaminated waste, and can become contaminated if protective soil barriers fail.

The effects of freezing and thawing on the ability of protective soil barriers to prevent bacterial transport has not been studied in any of the literature, even though freezing and thawing have been shown to induce fracturing and fracture-flow is the primary mode of transport for bacteria through soil. Our study will investigate the effects of freezing and thawing of fine-grained soils on bacterial transport, and will further analyze some factors affecting bacterial sorption to clay materials.

1.5.0 Objectives of this study

Our long term goal is to identify the factors affecting the transport of *E. coli* through freeze-fractured soils. The amount of bacterial sorption occurring in the test soils will be measured in order to quantify the theoretical amount of *E. coli* sorbed during transport studies.

The objectives of this study are:

1. To quantify the sorption characteristics of a range of soils, from sand to clay
2. To assess the transport characteristics of *E. coli* and bromide tracer through freeze-fractured clay barrier materials (normally consolidated and compacted freeze-fractured clay)
3. To compare the transport characteristics of *E. coli* and bromide tracer through clay barrier materials with sand and silt soils in order to observe the effect of particle size on bacterial retention in freeze-fractured systems

Chapter 2: Sorption of *Escherichia coli* to Soil Surfaces

2.0.0 Abstract

Sorption is an important process in the retention of pathogens by clay barriers. Batch sorption experiments were conducted to investigate the sorption of non-pathogenic *E. coli* to illite, kaolinite, and montmorillonite clays, a natural red clay, a silt and a sand. The Freundlich isotherm model provides the best fit for the sorption data. The sorptive capacity (SC) of each soil for *E. coli* was calculated at an equilibrium *E. coli* concentration of 1 x 10^8 CFU/mL. The SC values correlate strongly with the measured cation exchange capacity (having an R^2 of 0.92), as well as the weighted average particle size (having an R^2 of 0.83), of the soils.

2.1.0 Introduction

Contamination of groundwater drinking sources by pathogenic microorganisms such as *Escherichia coli* released from farm operations, landfills or sewage lagoons is a serious health threat. Recent surveys of spring and well water in several countries, including Canada and the USA, reveal widespread occurrence of pathogenic bacteria and viruses in groundwater, with the incidence commonly exceeding 20% of the wells tested (Taylor et al. 2004). Soil liners, typically composed of at least 20% fines (fine silt and clay), are designed to contain liquid wastes and leachates generated from solid wastes (Das, 2006). The ability of clay barriers to retain and sorb pathogenic

microorganisms such as *E. coli* is an important factor in designing clay barrier systems to prevent groundwater contamination.

Two major bacteria retention processes are filtration (straining) and sorption. In filtration, bacteria are retained in dead-end pores or by pore throats that are too small for them to pass through. Sorption involves the attachment or adhesion of bacteria to soil particle surfaces. This study focuses on the bacterial sorption capacity of various soils.

A study of bacterial filtration by Gannon et al. (1991) determined that the bacterial size relative to the pore size of the soil material was the greatest indicator of filtration rates, but also found that sorption is primarily responsible for most of the retention of bacterial cells inside of soil materials. Walker et al. (1989) note that in nature, it is rare that soil is found that is not enshrouded with microorganisms and their debris. Sorption of bacteria is primarily accomplished by the binding of the negatively charged bacterial surface to cations bound on the negatively charged soil particles (Marshall 1971). A comprehensive review of *E. coli* transport through aquifers by Foppen et al. (2006) concluded that sorption is one of the most important factors that determine the bacterial transport rates of the soils. Observations also suggest that multivalent cation bridging was of high importance in bacterial sorption to clay (Walker et al, 1989). However, quantification of the effect of CEC was not performed in these studies.

The particle size distribution of soils plays an important role in the sorption of bacteria. Bengtsson and Ekere (2001) found that particle size correlated strongly with groundwater bacteria sorption rates in sandy aquifer materials. Likewise, Marshall

(1971) showed that sorption of *Bacillus cereus* and *Serratia marcescens* was significantly higher in soils that contained 10% clay or more, probably due to increased initial attachment on the charged clay particles. Increased sorption of bacteria to clay soils may also be due to the smaller average pore size found in clay. McGechan and Lewis (2002) found that smaller soil pores provide more opportunities for sorption to occur between bacterial cells and soil particles, partially due to initial contact from physical filtration. Increased nutrient availability on smaller soil particles due to a higher surface area to volume ratio may also explain the increased sorption seen in McGechan's study. Heukelekian and Heller (1940) also found that increasing the surface area per unit mass of a synthetic soil (consisting of glass beads) allowed for better nutrient aggregation and better survival of *E. coli*.

Recently, we have examined the transport of an *E. coli* strain through sand, silt and clay soil columns. The study showed that the clay columns had the highest attenuation of the *E. coli*, followed by the silt and sand columns (Kjartanson et al. 2005, Scott and Nguyen 2006, Lukacs et al, 2007). In order to better understand and quantify the fate and transport of *E. coli* through soils, it is crucial to examine the sorption capacity of various soil types in relation to their CEC and grain size characteristics. The objectives of this study are to determine: (1) the sorptive capacity of an *E. coli* strain, S17gfp, to illite, kaolinite, montmorillonite, a natural red clay, a silt and a sand, and (2) the correlations of the sorptive capacity of these soils with their CEC and average grain sizes. This data may be incorporated into the bacterial transport model developed by Foppen and Schijven (2006), in order to help to predict sorption rates based on soil properties instead of deriving them experimentally.

2.2.0 Materials and methods

2.2.1 Preparation of Bacteria

The bacterium used in the experiments was a genetically modified, non-pathogenic *Escherichia coli* S-17-1λ strain containing a pJB29 green fluorescent protein plasmid, herein referred to as *E. coli* strain S17gfp. The plasmid includes a green fluorescence protein gene (*gfp*), an ampicillin resistance gene (β-lactamase) and a kanamycin resistance gene (*nptII*). These modifications allow for simple and effective assay to differentiate the target *E. coli* S17gfp from other background bacteria.

Batches of *E. coli* S17gfp were prepared by inoculating sterile Tryptic Soy Broth (TSB) containing 50 µg/mL of kanamycin monosulphate and 100 µg/mL of ampicillin sodium salt with a loopful of the *E. coli* cells, and then incubating the culture at 37°C for 20 hours with shaking at 120 rpm. The volume of TSB inoculated varied considerably (depending on the amount of cells needed) but was within 200 to 500mL per trial. After growing the bacteria, the cells were harvested by centrifugation at 8000 rpm for 10 minutes at 4°C. After removing the supernatant, the cell pellet was suspended in a sterile minimal salt medium (MSM). The MSM solution contains 1.25 mM KH_2PO_4, 0.4 mM $MgSO_4 \cdot 7H2O$, 0.02 mM $FeSO_4 \cdot 7H_2O$, 1.4 mM NH_4Cl, and 3.73 mM K_2HPO_4. The final salt concentration in this solution is approximately 999.2 ppm, and its pH was 6.9. Washing with MSM was repeated twice and the cells were suspended in MSM at an optical density (OD_{600nm}) of 1.0 units measured by a Novaspec II Visible Light Spectrophotometer (Biochrom, 22 Cambridge Science Park, Cambridge, United Kingdom). This OD of the *E. coli* cells corresponded to a cell concentration of approximately 1.25×10^9 colony forming units (CFU)/mL.

2.2.2 Sorption Testing

Soil samples used for the sorption testing ranged from sand to highly active clay. The sand was medium grained, industrial grade silica sand with particles ranging from 0.85 to 0.18 mm in size, as described in Meyer and Wilson (2005). The silt soil, acquired from a landfill in Thunder Bay, was a non-plastic silt and contained about 22% fine sand and 78% silt (Kjartanson et al, 2005). The red clay was also local to Thunder Bay and of preglacial-lake origin (Eigenbrod, 1996). The other clay soils tested were processed illite, kaolinite and bentonite clay soils (provided by Atomic Energy of Canada Limited), containing predominantly illite, kaolinite and montmorillonite clay minerals, respectively. Hereafter, the bentonite soil will be termed "montmorillonite" in this paper. For the purposes of this study the soils were characterized by their CEC values and average grain size, as described later in the paper.

A representative mass of 0.25 g of air-dried soil was placed into a sterile polypropylene tube with a sealable lid. To this soil, 0.5 mL of cell suspension at various cell densities was added and each tube was vortexed for 10 seconds to ensure sufficient and consistent mixing. The tubes were then sealed and allowed to reach sorption equilibrium for 1 hour, which according to a literature review (Marshall, 1971), should be more than twice as long as necessary for complete sorption to occur. The sorption experiments were carried out at room temperature (21°C).

Five mL of sterile sucrose solution (60% wt/vol) was added to each tube by injecting it into the bottom of the soil-cell solution mixture. A 5mL sterile syringe was used to inject the sucrose solution. The needle and syringe were sterilized between each injection by aspirating and ejecting several full volumes of 95% ethanol and drying by repeatedly pumping air through the syringe. Once injected with the sucrose solution,

the tubes were spun in a horizontal centrifuge at 3200 x g for 30 minutes at 4°C. Under centrifugation, the sucrose forms a density gradient which allows for the separation of the free (unsorbed) cells from the soil and the sorbed cells. Without sucrose, the free cells would spin down to the bottom of the tube along with the soil and sorbed cells.

In some clay samples, distinct layers consisting of particles of varying densities were visible after spinning in the centrifuge. The layers included a bottom layer consisting of soil aggregates and bacteria, a cloudy layer consisting of fine clay particles bound to bacterial cells, and a clear layer, which appeared to contain bacterial cells that were unbound to clay particles (Fig 2.1). The clear layer was removed from the tube by aspiration with a sterile 5mL syringe and needle, and was transferred to a sterile 10mL glass tube. Care was taken to avoid removing any of the cloudy layer with the clear layer, so a very thin clear layer was left on top. In silt and sand samples, only a clear layer was present and was separated from the soil layer by means of a sterile 5mL syringe.

2.2.3 Bacterial Enumeration

The cell concentration of the layer containing *E. coli* was measured by drop plating. First, the separated layers were serially diluted with sterile MSM in order to reduce cell concentration. LB Miller agar plates (EMD Biosciences, 10394 Pacific Center Ct, San Diego, CA, USA) containing 50 µg/mL of kanamycin monosulphate and 100 µg/mL of ampicillin sodium salt were prepared, and 5µl of each cell dilution were dropped on the plates 6 times. The kanamycin monosulphate and ampicillin sodium salt were used to prevent growth of background soil bacteria other than the target *E. coli.*

After incubation at 37°C for 20 hours, the number of colonies in each drop was recorded in order to determine the sample cell density. The number of cells sorbed to the soil was calculated by subtracting the number of unsorbed cells remaining in the sucrose gradient from the number of cells inoculated in the soil at the beginning of the experiment.

2.2.4 CEC and Particle Size Analyses

Soil samples were dried at 35°C for 24 hours and crushed in a similar manner as in the sorption experiments in order to obtain a dry and uniform sample. Samples were then placed into 50 mL Erlenmeyer flasks, and 25 mL of 1M ammonium acetate solution at pH 7 was added. The flasks were then shaken at 65 rpm for 15 minutes in order to ensure complete mixing. The samples were then filtered with Fisher Q5 filter paper into 15mL centrifuge tubes. Samples were then analyzed using a Varian Vista Pro radial inductively coupled argon plasma spectrometer (Varian Inc, 3120 Hansen Way, Palo Alto, CA, USA) in order to determine the amount of extractable calcium, magnesium, potassium and sodium ions in each sample. The weight of each cation in each soil was converted to moles and then to milliequivalents. Milliequivalents for each cation were added together in order to determine the CEC of each soil.

In order to determine the particle size distribution of the sand, dried soil samples were passed through a stack of sieves ranging from 7.5 cm to 75 μm, shaken, and the weight of each particle size retained on each sieve was recorded, according to the methods described in Bardet (1997). For finer soil samples, hydrometer tests were used to determine the particle size distribution. For a hydrometer test, distilled water and a

dispersion agent consisting of 4% sodium hexametaphosphate buffered with 0.8 M Na_2CO_3 are added to the soil, and the soil suspension is poured into a 1 L graduated cylinder and mixed well. A hydrometer is placed into the cylinder at selected time intervals, and the distance that the bulb sinks is measured (Bardet, 1997). The particle size distribution is calculated according to Stoke's equation:

$$D = \sqrt{\frac{30\eta H_R}{981(G_s - 1)\rho_w t}} \qquad \textbf{(Equation 2.1)}$$

Where,

D = particle diameter (cm)

η = viscosity of water (g/cm * s)

H_R = depth of hydrometer fall, corrected for displacement caused by the bulb (cm)

G_S = specific gravity of solids

ρ_w = unit mass of water (g/cm^3)

t = time (s)

2.3.0 Results

The particle size distributions shown in Figure 2.2 were generated using methods described in Section 2.2.4.

The goodness of fit of two sorption models, the Freundlich and Langmuir models, was evaluated by calculating the coefficient of correlation (R^2) associated with the line of best fit for the sorption data for each soil. The sample size for each soil's sorption isotherm is at least 25 points, meaning that an R^2 value of more than 0.38 is necessary in order to be 95% confident that there is a correlation in the data (McBean and Rovers,

1998). Sorption data were found to have higher R^2 values with a Freundlich sorption isotherm model (average R^2 of 0.92) than with a Langmuir sorption isotherm model (average R^2 of 0.85). This suggests that sorption of bacterial cells occurred in multiple layers on soil particles, as opposed to occurring as only a monolayer (Watts 1998). Because multiple layers of *E. coli* can bind to each soil particle, there is no theoretical maximum sorptive capacity for *E. coli* for soils under this model.

The equation that describes this study's Freundlich system is:

$$S = K_f C_e^n \qquad \textbf{(Equation 2.2)}$$

Where

S = number of *E. coli* sorbed per gram of dry soil (CFU/g soil)

K_f = empirical constant ((CFU/g)/(CFU/mL)^n)

C_e = concentration of *E. coli* remaining in suspension at equilibrium (CFU/mL)

n = empirical constant (unitless)

By plotting the amount of cells sorbed (S) against the equilibrium concentration (C_e) on log scales and determining the line of best fit with a power function regression analysis, the empirical constants K_f and n for each soil were determined (Fig 2.3, 2.4, 2.5, and Table 2.1). A "sorptive capacity" of each soil was calculated to compare the *E. coli* sorption characteristics of the different soils and to correlate the sorption capacity of each soil with CEC and with particle size distribution characteristics. This sorptive capacity (SC) was calculated for each soil at a C_e value of $1*10^8$ CFU/mL using the K_f and n parameter values determined for each soil. A C_e value of $1*10^8$ CFU/mL was

chosen because this represents the upper limit of the data for sand and silt (Fig 2.3). The SC values for each soil are given in Table 2.1.

The CEC values are given in Table 2.1. The sand and silt soils have low CEC values, as expected. The values for kaolinite, illite and montmorillonite are within the ranges reported by Mitchell and Soga (2005) for these types of clay minerals. The natural red clay contains a mixture of clay minerals, and has a cation exchange capacity between illite and montmorillonite (Table 2.1).

Table 2.1. Soil properties and Freundlich model parameters of soils used in sorption experiments.

Soil Type	Soil Properties			Freundlich Model Parameters		
	Air-Dry Moisture Content (%)	CEC (mEq / 100g soil)	Weighted Average Grain Size (mm)	K_f (CFU/g)/ (CFU/mL)^n	n (unitless)	Sorptive capacity (SC) (CFU/g)
Sand	0.364	0.08	0.600	8.52E+01	0.832	3.82E+08
Silt	0.240	0.54	0.0520	7.57E+03	0.617	6.54E+08
Kaolinite	0.656	3.07	0.0346	9.66E+05	0.446	3.57E+09
Illite	0.316	20.13	0.0196	3.34E+04	0.630	3.68E+09
Red Clay	1.68	37.85	0.00863	4.09E+07	0.290	8.55E+09
Montmo-rillonite	6.92	85.43	0.00412	2.20E+03	0.816	7.47E+09

2.4.0 Analysis

The SC values were plotted against the CEC of each soil in order to examine potential correlation between these variables (Fig 2.6). The R^2 between these variables was 0.924, indicating a strong relationship. The line of best fit on this plot was

calculated in order to determine the relationship between sorptive capacity (SC) and CEC. The data were fit best with a power function regression having the formula:

$$SC = 1.23*10^{9} * (CEC)^{0.458} \quad \textbf{(Equation 2.3)}$$

Similarly, a weighted average of the grain sizes within each soil was calculated and plotted against the soil's sorptive capacity (Fig 2.7). This graph shows that the SC is inversely correlated with the average grain sizes for the soils. There was a slightly weaker correlation between these two variables than between SC and CEC. The data were best fit with a power function regression, with an R^2 of 0.834, and the equation:

$$SC = 2.25*10^{8} * (\text{Average Grain Size})^{-0.68} \quad \textbf{(Equation 2.4)}$$

2.5.0 Discussion

This study presents a novel method for assessing the ability of a soil liner material to sorb bacterial cells based on the CEC of the soil material. Previously, there have been bacterial sorption studies attempting to quantify the effect of the ionic strength and pH of the suspension medium (Yee et al, 2000, You et al, 2003), the shape, size, and composition of the cell surface (Marshall, 1971), as well as the particle size of the soil (Bengtsson and Ekere, 2001), which was also examined in this study.

The generally better fit of the Freundlich model to the sorption data indicates that these soils do not display a maximum sorptive capacity for *E. coli*, at least in the range

of *E. coli* concentrations examined. This also implies that multiple layers of *E. coli* likely build up around soil particles.

Figure 2.6 depicts a strong correlation between the CEC and the sorptive capacity (SC) for *E. coli*. CEC values ranged from 0.08 mEq/100g for sand to 85.43 mEq/100g for montmorillonite clay soil. The correlation of SC with the CEC was stronger (having an R^2 of 0.92) than the correlation of SC with the weighted average grain size (which had an R^2 of 0.83).

As discussed in section 2.1, cation bridging between the soil particles and the cell surface of the *E. coli* is thought to be responsible for most of the sorptive processes on the soil particle surface. Roberts (2004) suggests that negatively charged soil particles may attract positively charged ions such as aluminium and iron, onto which bacteria preferably colonize. This may suggest an explanation for the strong relationship between CEC and bacterial sorption. However, quantification of sorption in relation to the CEC of a soil has not been studied. Equation 2.3 may be useful in building on the mathematical bacterial transport model outlined in Foppen and Schijven (2006).

A weighted average of the grain sizes within the soils was calculated and correlated with the SC, and was found to have an R^2 of 0.83 (Fig 2.7). Likewise, Bengtsson and Ekere (2001) calculated an R^2 of approximately 0.85 in a study of the correlation between grain size and groundwater bacteria sorption coefficients. The inverse correlation between the variables shows that as the weighted average grain size gets smaller, the sorptive capacity of the soil increases. This is likely related to the increase in specific surface area with decreasing particle sizes, as described in Bardet

(1997) and Bengtsson and Ekere (2001). Correlation with specific surface area of the soils should be examined in future studies.

The mathematical bacterial transport model outlined in Foppen and Schijven (2006) is also lacking in data for the sorption of *E. coli* to clay materials, and equation 2.4 may be useful in further refining their model of sorption based on particle size. Predicting sorption rates from soil characteristics would be a valuable method of reducing the number of experiments needed to predict *E. coli* transport properties based on their model.

The trend of montmorillonite sorption data is somewhat anomalous and unexpected. The amount sorbed in the lower *E. coli* concentration range is quite low when compared with the red clay (Fig 2.5). Further work should be carried out to investigate this.

Because alkaline substances can reduce a soil's cation exchange capacity, the ability of a clay material to retain bacterial cells may be altered by the presence of alkaline substances in the waste being stored. Dairy manure pH is typically alkaline (ranging from 7.0 to 8.5) which can reduce retention efficiency on farming waste sites by altering the CEC of soil liners. The effect of adding alkaline substances to soil material on the maximum sorptive capacity of the soil should also be investigated by further testing.

Results from these sorption experiments, column permeation experiments and future experiments will be used in a program of numerical modelling in order to quantify factors affecting *E. coli* transport through soil barriers. The numerical model will aid in

developing procedures to assess the containment ability of soil liners on landfills, farms, and other waste storage sites.

2.6.0 Figures

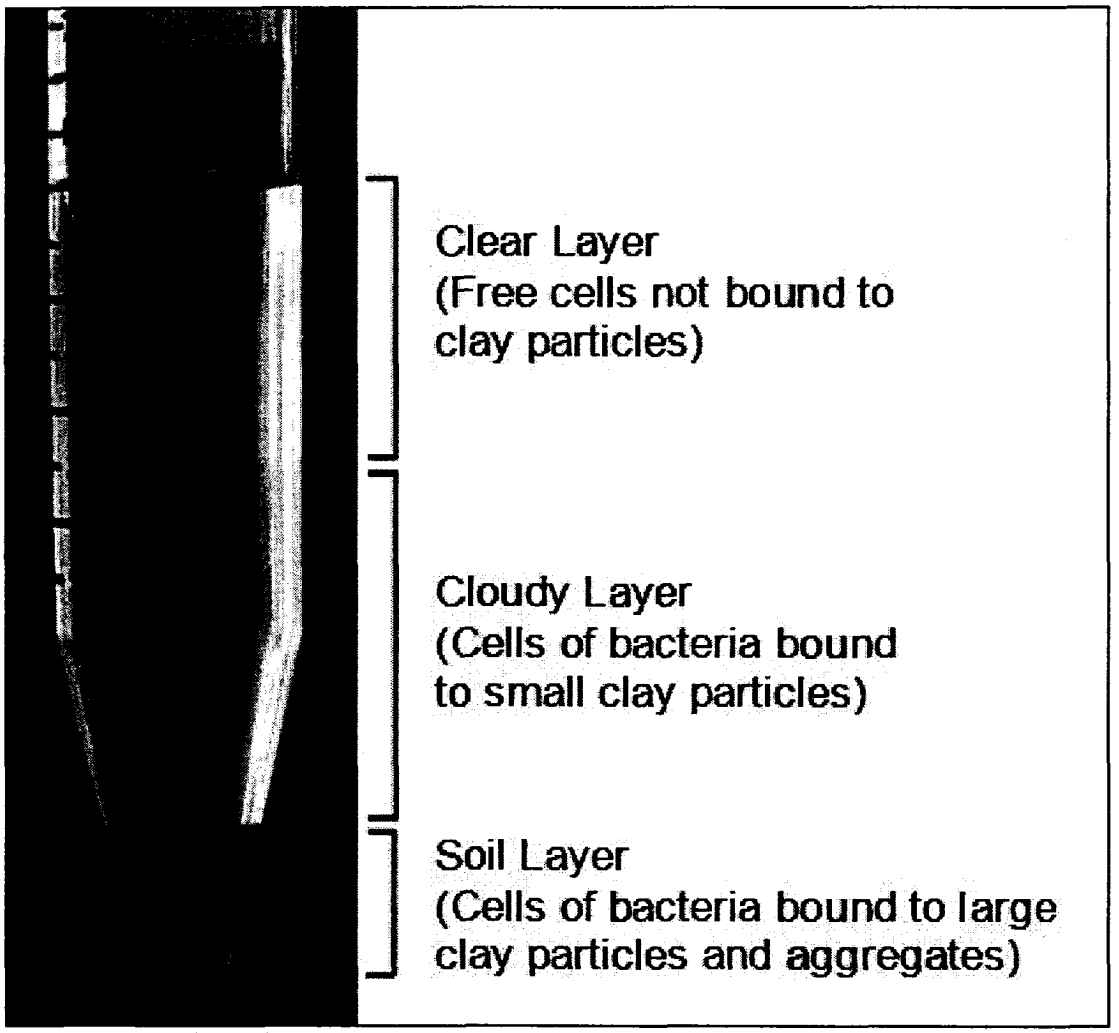

Figure 2.1. Diagram of layers in sorption experiments

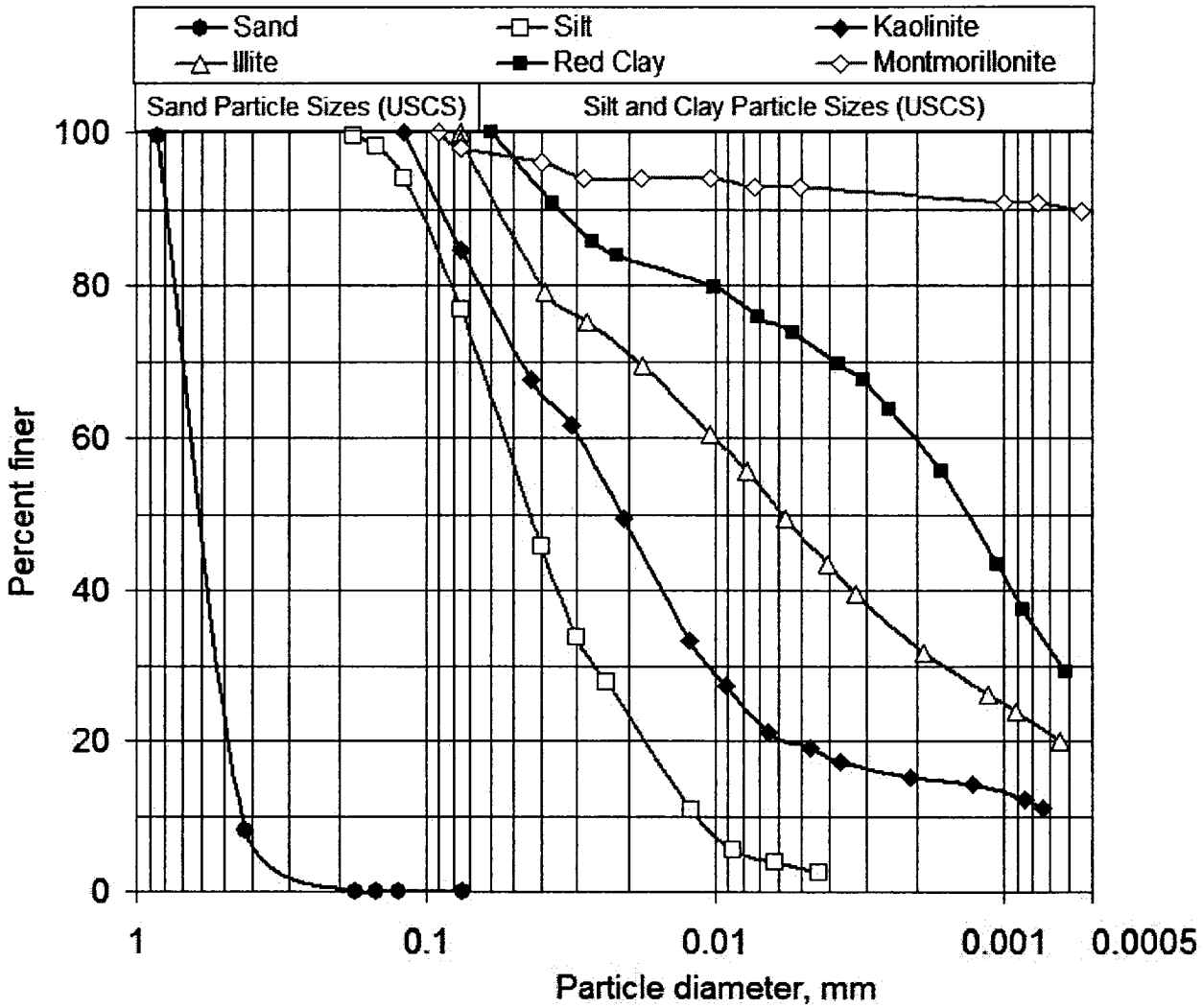

Figure 2.2. Grain size distribution of soils tested.

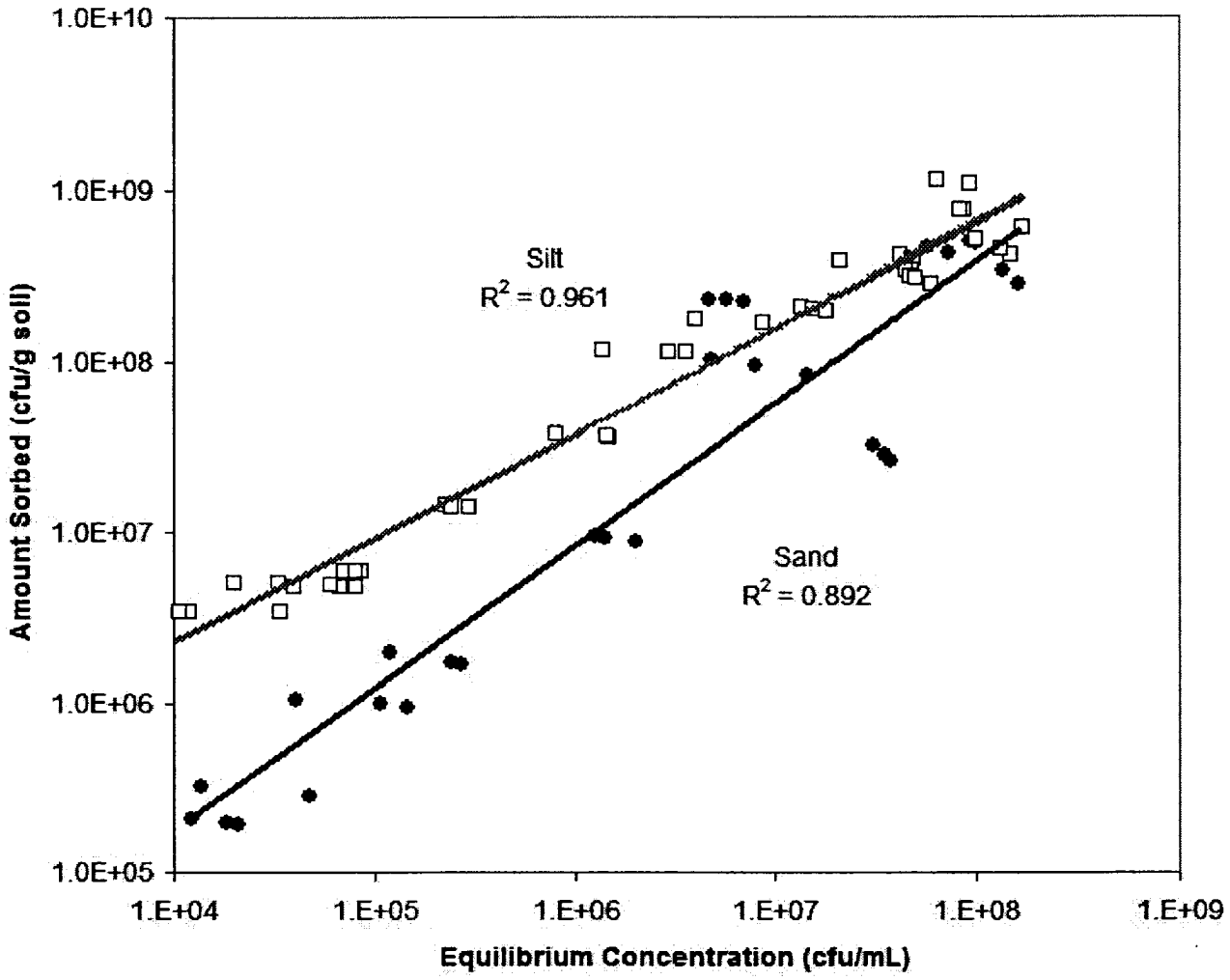

Figure 2.3. Freundlich isotherm plot for sand (solid circles) and silt (hollow squares).

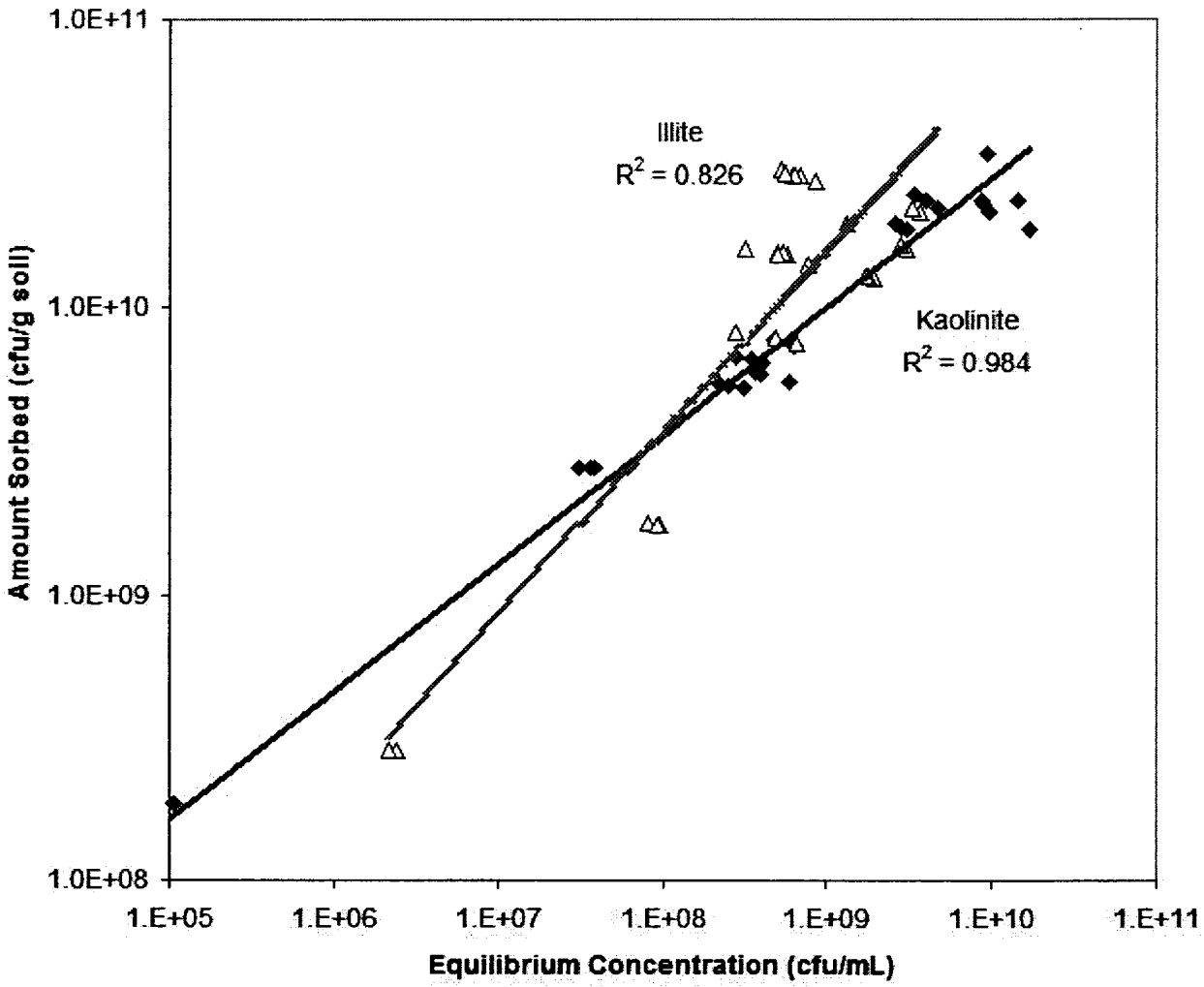

Figure 2.4. Freundlich isotherm plot for kaolinite (solid diamonds) and illite (hollow triangles).

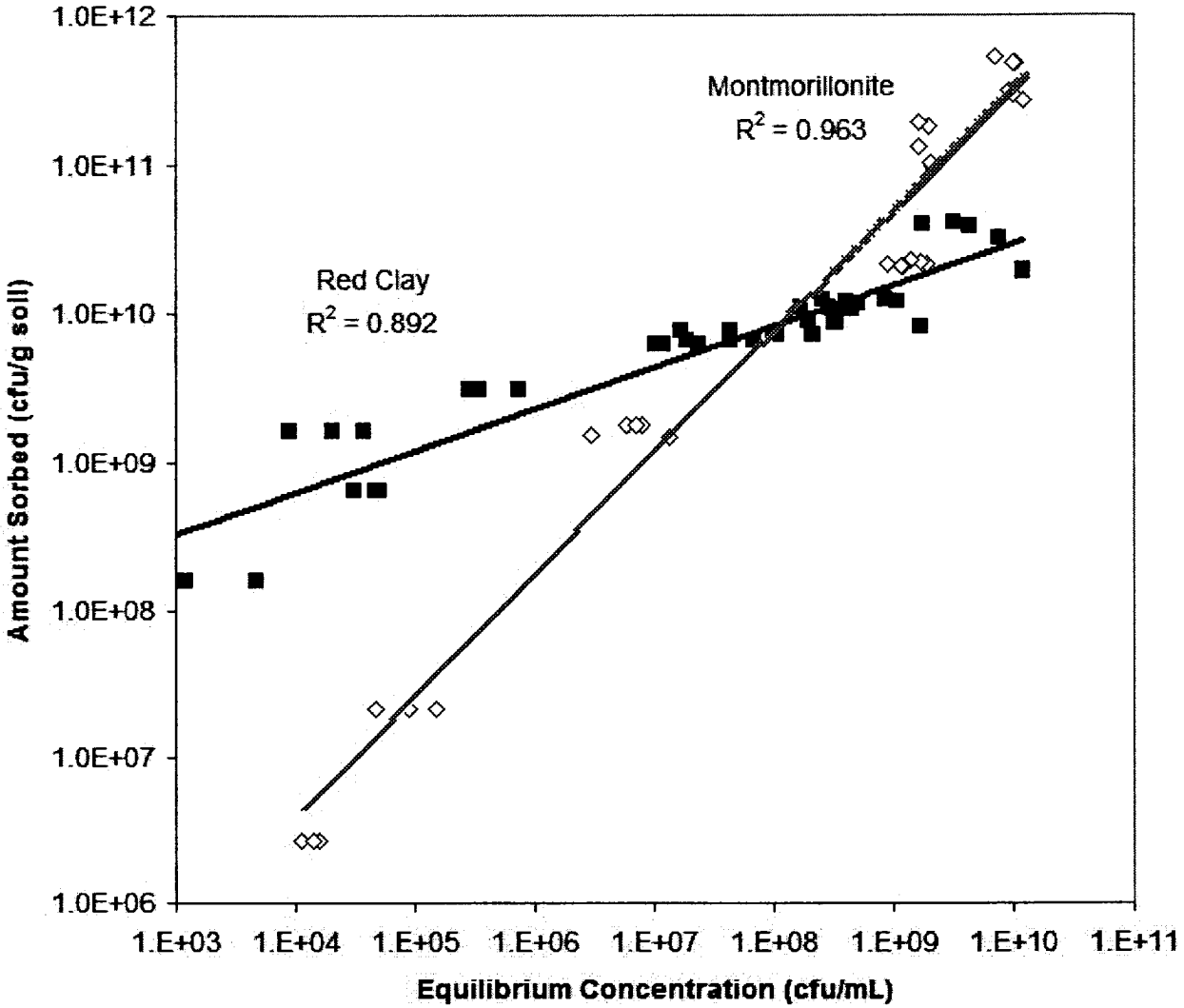

Figure 2.5. Freundlich isotherm plot for red clay (solid squares) and montmorillonite (hollow diamonds).

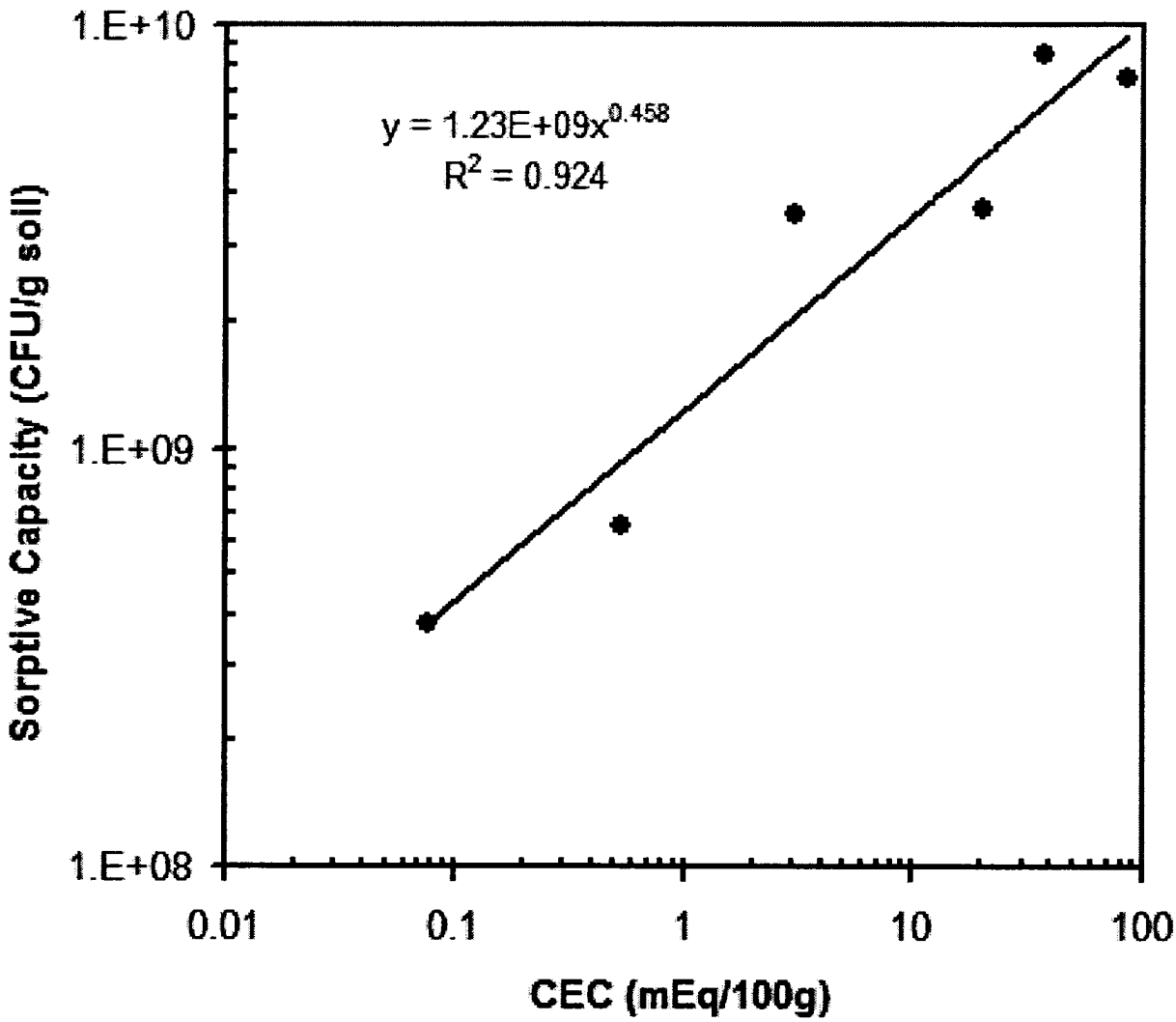

Figure 2.6. Sorptive capacity (SC) versus CEC for soils tested.

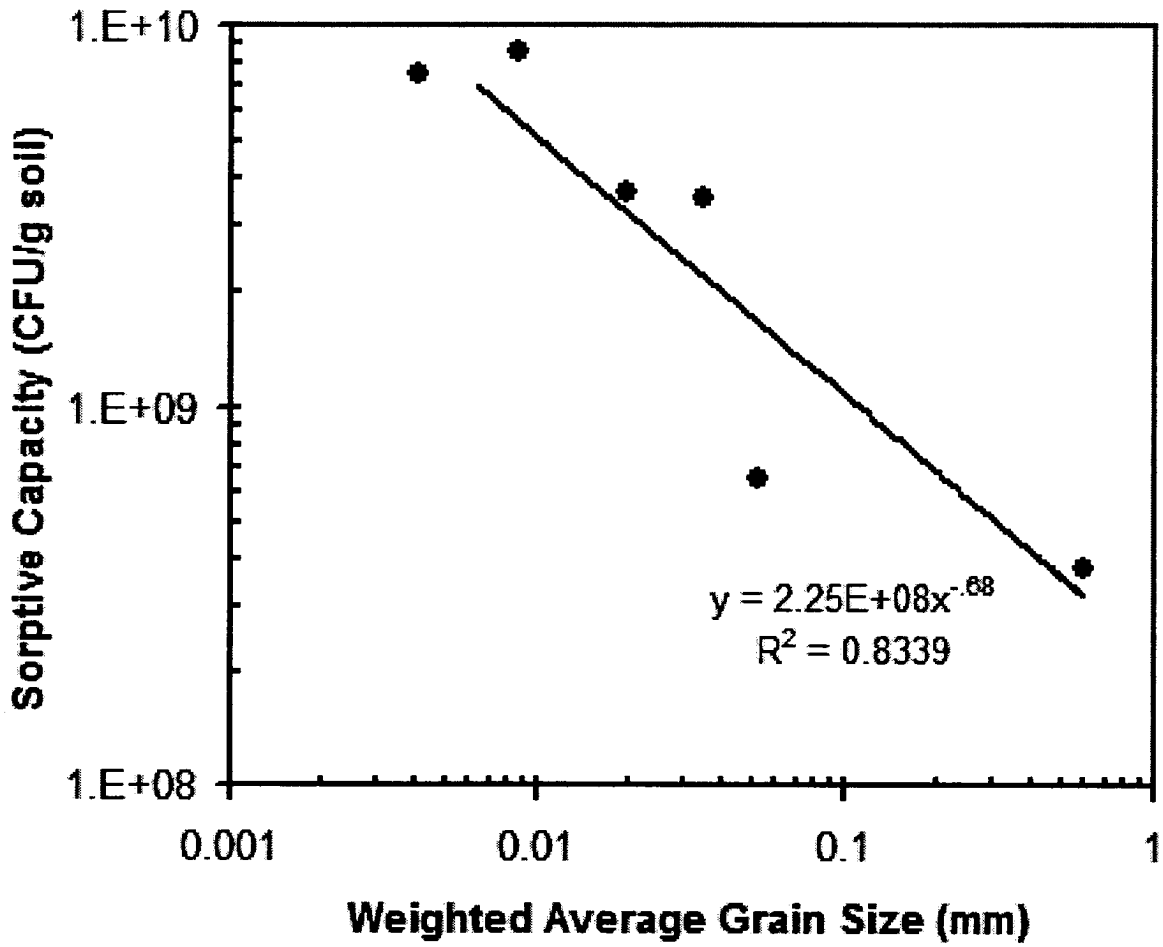

Figure 2.7. Sorptive capacity (SC) versus average grain size for soils tested.

Chapter 3: Transport of *Escherichia coli* Through Clay, Silt and Sand Columns

3.0.0 Abstract

Columns of compacted and normally consolidated high plastic clay and compacted non-plastic silt soils were exposed to cycles of freezing and thawing to simulate conditions of physical weathering, in turn creating a fracture network in the specimens. To determine bacterial transport properties, these columns, as well as intact sand, were permeated with nonpathogenic *E. coli* suspended in a minimal salt medium in a constant head permeability mode. As a result of freezing and thawing, the permeability of the soil columns, as compared with literature values, was found to increase by one order of magnitude for the silt and two to three orders of magnitude for the normally consolidated and compacted clay columns. Full (100%) breakthrough of the *E. coli* was observed after three pore volumes of flow in the sand, 35 pore volumes of flow in the silt, and was not observed in both compacted and normally consolidated clay columns, even after as many as 55 pore volumes of flow. However, as many as 5 x 10^5 CFU/mL of *E. coli* were found to permeate through clay columns, indicating a possible public health risk from bacterial transport through freeze-fractured soil liners. Both sorption and filtration through soil were found to be important factors in determining bacterial transport through fine grained soils.

3.1.0 Introduction

Groundwater is an important source of drinking water, especially for rural populations. Recent surveys of spring and well water in Canada and the USA, show that up to half of drinking water wells had evidence of fecal bacteria contamination, resulting in 750,000 to 5.9 million cases of illness and 1400 to 9400 deaths per year in these countries (Macler and Merkle, 2000).

Potential sources of pathogenic microorganisms include animal feedlot operations, manure spreading, leaking sanitary sewers, septic tanks, sewage and manure lagoons and landfills. Lagoons and landfills are typically lined with compacted clay liners. Beyond the engineered barrier system, natural clay deposits are also relied on for attenuation of pathogens. Physical weathering processes, such as freeze-thaw and desiccation, cause the formation of fractures and fissures in clay liners and natural clays (Eigenbrod, 1996), creating potential preferential migration pathways for pathogens. A field study by Taylor et al (2004) indicated that there is high groundwater velocity variability in natural soils, resulting in increased microbial transport, but this study did not separate the effects of freeze fracturing, desiccation fracturing, or soil heterogeneity.

Most drinking water standards and guidelines have a limit of zero colony forming units (CFU) of fecal coliforms per 100 ml of water. A recent survey of rural groundwater quality in Canada showed that drinking water wells located in clayey soils had a higher frequency of bacterial contamination than wells in granular soils (Conboy and Goss 2000). These clay soils may have been exposed to natural freeze-thaw cycles, resulting in bacteria transport through fracture flow paths, resulting in the contamination.

However, the effect of freeze fracturing on the transport of bacteria in these soils has not been studied.

This chapter presents the results of a study that examines and compares the fate and transport of a non-pathogenic *Escherichia coli* through several soils: an active, high plastic clay, a non-plastic sandy silt, and a silica sand. The effects of freeze-thaw cycles on the permeability of the clay and silt soils are evaluated, and the resulting effect on *E. coli* transport is evaluated. The clay soil columns were prepared from either from a slurry (representing a normally consolidated, natural clay barrier) or from compaction (representing a compacted, engineered barrier). Sand columns were tested in order to compare *E. coli* and bromide transport characteristics to clay and silt soils and to provide additional data for development and calibration of microbe transport models.

3.2.0 Materials and Methods

3.2.1 Soil Materials

The clay soil used is local to Thunder Bay, Ontario and is of preglacial-lake origin. Hydrometer assays showed that the clay contained 40% silt and 60% clay sizes (Fig 3.1) (Wong and Enns 2005). The silt soil, also acquired locally, is non-plastic and contains 22% fine and 78% silt (Fig 3.1). The sand was medium grained, industrial grade silica sand with particles ranging from 0.85 mm to 0.18 mm in size (Fig 3.1) (Lukacs et al, 2007; Wong and Enns, 2005). The clay, silt and sand soils used are the same as those used in the *E. coli* sorption experimentation (Chapter 2). Key engineering properties and classifications of these soils are listed in Table 3.1.

3.2.2 Soil Column Preparation

The normally consolidated clay samples (NC-A through NC-D) were prepared from a slurry with a target moisture content slightly above the liquid limit. To set up each sample, the slurry was placed into a clear PVC (Polyvinyl chloride) cylinder with an inside diameter of 98 mm and length of about 200 mm. The compacted clay samples (CC-A through CC-C) were compacted in three lifts (i.e., three equal levels of soil, each of which are compacted) into similar cylinders at a moisture content of approximately 33% (which is slightly above the optimum moisture content of 31%) using standard Proctor effort (25 blows on each lift from a hammer weighing 2.5kg) (Bardet, 1997). The inside surface of the PVC cylinder was coated with lithium grease to prevent the sample from adhering to the cylinder wall and to prevent sample/cylinder wall interface flow during permeability testing. Geotextile (a non-woven synthetic fibre product used as a soil reinforcement agent) and filter paper were placed on the top and bottom surfaces of the samples and the PVC cylinders were clamped to a porous base and placed into the freeze-thaw apparatus as shown in Figure 3.2. The pores of the filter paper were 20 to 25 μm in diameter, much larger than the bacterial diameter of 0.8 μm. A surcharge of 4kPa was applied to the top of the samples during the freeze-thaw process.

The clay samples were then subjected to six freeze-thaw cycles consisting of two days of freezing and two days of thawing. These procedures followed those described in Eigenbrod (1996), using a freezing temperature of -20°C and a thawing temperature of approximately 22°C. The freezing and thawing were one-dimensional and open system (meaning temperature changes infiltrated from the top to the bottom of the column with insignificant lateral cooling or warming) with the use of glass fibre insulation preventing lateral freezing and thawing in the samples. The soil had free access to

liquid water at the bottom end (Figure 3.2). Each freeze-thaw cycle lasted four days (two days of freezing followed by two days of thawing). The soil column properties after the freeze-thaw process and prior to permeation with *E. coli* are shown in Table 3.2.

The compacted silt samples (S-A and S-B) were compacted at the optimum moisture content of 15.5% into similar PVC columns using standard Proctor effort. As with the clay samples, the inside wall of the PVC cylinders was coated with lithium grease prior to compaction of the silt sample. After compaction, the samples were subjected to freeze-thaw cycles in the same manner as described for the clay samples, except that each freeze-thaw cycle lasted only two days instead of four, as complete freezing and thawing were observed after this time. The silt samples expanded during the freeze-thaw cycles because of water uptake induced by the large suctions generated during freezing. After the three freeze-thaw cycles, the samples were virtually saturated.

As Table 3.2 indicates, the compacted silt samples had quite consistent dry densities and porosities after the completion of the freeze-thaw cycles.

Sand samples (Sn-A and Sn-B) were compacted at the optimum water content of approximately 9.5% using the same methods as used for the silt and clay columns. Freeze-thaw cycles were not performed on sand samples, as swelling and fracturing do not occur in coarse-grained soils as a result of freezing (Eigenbrod, 1996).

Table 3.1. Properties of soils used in experimentation

Soil Type	Liquid Limit[1] (W_L) (%)	Plastic Limit[2] (W_P) (%)	Plasticity Index[3] (I_P)	Optimum Water Content (W_{opt})[4] (%)	Maximum Dry Density (Dmax)[5] (g/cm^3)	Specific Gravity (G_s)[6]	USCS classification[7]
Clay	72	29	43	31	1.45	2.75	CH[8]
Silt		Non-Plastic		15.5	1.72	2.70	ML[9]
Sand		Non- Plastic		9.5	1.69	2.65	SP[10]

[1] The moisture content at the point of transition from the liquid to the plastic state

[2] The moisture content at the point of transition from the plastic to the semisolid state

[3] The difference between the liquid and plastic limit of a soil

[4] The moisture content at which the maximum dry unit weight is attained

[5] The dry density at the optimum water content

[6] The ratio of the unit weight of a given soil to the unit weight of water

[7] A two-letter soil classification system that classifies soils into one of 26 categories

[8] Clay of high plasticity ($W_L > 50\%$)

[9] Silt of low plasticity ($W_L < 50\%$)

[10] Poorly graded sand

3.2.3 *E. coli* Bacteria and Suspension Medium

The bacterium used in the experiments was a genetically modified, non-pathogenic *Escherichia coli* S-17-1λ strain containing a pJB29 green fluorescent protein plasmid, herein referred to as *E. coli* S17gfp. The plasmid includes a green fluorescence protein gene (*gfp*), an ampicillin resistance gene (β-lactamase) and a kanamycin resistance gene (*nptII*). These modifications allow for simple and effective assay to differentiate the target *E. coli* from other background bacteria.

The *E. coli* were suspended in a sterile Minimal Salt Medium (MSM) solution containing 1.25 mM KH_2PO_4, 0.40 mM $MgSO_4 \cdot 7H2O$, 0.02 mM $FeSO_4 \cdot 7H_2O$, 1.40 mM NH_4Cl, and 3.73 mM K_2HPO_4. The final salt concentration in this solution is approximately 999.2 ppm, and its pH is approximately 6.9.

The cell density of the *E. coli*-MSM cell suspension was approximately 1×10^7 CFU/mL. Independent survival experiments showed that the *E. coli* concentration in the cell suspension remained fairly constant over a one week period (Appendix 2). During longer experiments, cell suspension in the reservoir tank was replaced with freshly prepared cell suspension every six days. The indicator dye bromothymol blue was added to the stock solution at a concentration of 10 mg/L to facilitate visual tracking of the path of the stock solution. In addition, potassium bromide, a non-sorbing tracer compound, was added to the stock solution at a concentration of 2.1 mM in order to trace the breakthrough of the stock solution liquid through the soil columns.

3.2.4 Permeability Testing

After preparation of the sand columns and completion of the freeze-thaw cycles for the clay and silt columns, the specimens were set up for constant head permeability testing (Fig 3.3). Two soil columns were permeated simultaneously. The reservoir, tubing and valve systems, but not the soil columns, were sterilized by flushing with 25% bleach, followed by 85% ethanol, and then sterile MSM solution prior to test set-up in order to eliminate bacteria other than the target strain of *E. coli* from being introduced into the test. The filter paper and geotextile on top of each soil column was removed, and each PVC cylinder containing the specimen was placed between end plates which

were sealed tightly and clamped together at opposite ends. The soil columns were connected to the reservoir tank at the upper end and to a flask or autocollector at the outlet of the lower end of the column in order to collect the effluent.

The soil columns were permeated with tap water to establish baseline permeability values. After steady state flow with tap water was achieved, samples were permeated with sterile MSM solution for at least ten pore volumes, followed by permeation with the *E.* coli-MSM cell suspension. The baseline permeability values are shown in Table 3.2. The effluent flasks were emptied regularly; the weight of the effluent (from which the volume was calculated) and the corresponding time were recorded and permeability was calculated using Darcy's law (Das, 2006). The change in total hydraulic head across the sample (ΔH) shown in Figure 3.3 was maintained constant during the permeation stages. The hydraulic gradient applied across the columns (i.e., ΔH/L, where L is the length of the soil column) was approximately 10 to 15 cm/cm.

3.2.5 Microbiological Monitoring

To monitor the transport of the *E. coli* cells through the soil columns, cell densities of the *E. coli*-MSM cell suspension in the influent reservoir and the effluent from the soil columns were monitored regularly (at least once in every 24 hours, but more often at the beginning of permeation and during permeation of soils with high permeabilities). The cell counting procedure is as follows. LB Miller agar plates (EMD Biosciences, San Diego CA, USA) containing 50 μg/mL of kanamycin monosulphate and 100 μg/mL of ampicillin sodium salt were prepared, and 5μl of each cell dilution were dropped on the plates 6 times. The kanamycin monosulphate and ampicillin

sodium salt were used to prevent growth of background soil bacteria other than the target *E. coli.* Next, the plates were incubated at 37°C for 24 hours. In this time, the cells grew into colonies that were visualized, screened for green fluorescent protein (visualized with green fluorescence under UV light), and counted. The average number of CFU in the six drops was used to calculate the bacterial density in each sample.

Samples of the *E. coli* cell suspension were taken by releasing a valve in the tube directly above the soil columns, and filling a sterile 1.5mL eppendorf tube (Figure 3.3). This ensured that the sample to be counted represented exactly what was entering the column at the time of measurement.

In order to determine the *E. coli* cell density in the column effluent, samples were collected in sterile 1.5 mL tubes directly from the outlet at the bottom of the columns. The collected samples were diluted and plated as previously described in order to evaluate their cell concentration.

3.3.0 Results

3.3.1 Effects of Freezing and Thawing

The normally consolidated (NC) clay soil columns compressed during the freeze-thaw cycles because of the large suctions generated at the freezing front during freezing. These large suctions cause consolidation of the clay slurry and also result in the formation of shrinkage cracks and fissures. The cracks and fissures can increase the permeability of a fine grained soil by two or more orders of magnitude, with the greatest change in permeability occurring during the first three freeze-thaw cycles (Eigenbrod, 1996; Qian et al. 2002). The most significant compression of the clay

samples occurred during the first two freeze-thaw cycles. Compacted clay columns tended to expand slightly during the freeze-thaw process because of water uptake induced by the large suctions generated during freezing (Data not shown). As Table 3.2 indicates, the NC samples generally had lower dry densities and higher porosities than the compacted clay samples after the freeze-thaw cycles.

Table 3.2. Soil column properties at the beginning of *E. coli* permeation

Soil Type	Column Label	Height (cm)	Dry Density (g/cm^3)	Porosity	Baseline Permeability (cm/s)
Normally Consolidated Clay	NC-A	13.85	1.185	0.57	2.58E-05
	NC-B	14.05	1.180	0.57	1.05E-05
	NC-C	14.562	1.110	0.59	1.70E-05
	NC-D	16.8	1.080	0.59	1.40E-05
Compacted Clay	CC-A	7.04	1.475	0.44	4.59E-06
	CC-B	7.24	1.503	0.46	4.99E-06
	CC-C	8.865	1.352	0.40	2.81E-06
	CC-D	9.739	1.285	0.51	2.28E-06
Silt	S-A	13.5	1.640	0.39	6.33E-05
	S-B	13.4	1.644	0.39	5.61E-05
Sand	Sn-A	14.5	1.700	0.36	1.50E-03
	Sn-B	14.5	1.607	0.39	2.96E-03

3.3.2 Permeability of Soil Columns

The amount of flow in all graphed results is normalized to the volume of voids in the soil columns and is called "pore volumes of flow". One pore volume of flow corresponds to a flow volume equal to the volume of voids in the soil column.

The normally consolidated clay columns had higher permeabilities than the compacted clay columns (Fig 3.4), likely because they had less continuous flow paths and more dead end pores. The permeability of the silt columns was higher than the normally compacted clay columns, due to the relationship between big particle size and higher permeability. As expected, sand columns had much higher permeabilities than the fine-grained soil columns. The permeability of the clay and silt columns decreased significantly during testing. The sand may also have shown a decrease in permeability had the tests been ran for a longer period of time.

3.3.3 *E. coli* and Bromide Breakthrough

Breakthrough curves for the normally consolidated clay, compacted clay, silt and sand columns are shown in Figure 3.5. Relative cell concentration on the y axis of the breakthrough curves represents the cell concentration of *E. coli* in the column effluent (C) divided by the average concentration of *E. coli* in the influent (C_O) over the course of the experiment. Relative cell concentration is shown on a log scale as opposed to the typical natural scale used for breakthrough curves in order to effectively display the low *E. coli* cell densities in the clay column effluent.

Bromide breakthrough curves (Fig 3.6) indicate the breakthrough pattern of a non-sorbing tracer molecule, and were very similar for all the clay columns tested. Bromide was measured in the first samples taken from silt and clay columns (as little as 0.01 to 0.06 pore volumes), indicating preferential flow or diffusion in the sample. In sand samples, bromide was not measured until 0.7 pore volumes, indicating less

preferential flow or diffusion than in the fractured columns. Complete bromide breakthrough occurred between 2 to 3 pore volumes of flow.

A rapid initial breakthrough of *E. coli* occurred in the clay columns, as some *E. coli* were found in the first sample tested for each column (between 0.1 and 0.18 pore volumes). This suggests that *E. coli* follow preferential flow paths through the fractured clay soils. Full (100%) breakthrough of *E. coli* was not observed in any of the clay columns, even after as many as 55 pore volumes of flow. The compacted and normally consolidated clay column *E. coli* breakthrough curves were not significantly different between compacted and normally consolidated clay, and were somewhat variable, which can be expected due to the random fracture patterns developed during the freeze-thaw process. As shown in Figure 3.4, the CC columns tended to have lower permeabilities than the NC columns, but a trend of more rapid breakthrough in terms of pore volumes appears to be evident in Figure 3.6.

The silt columns had good repeatability and moderately fast complete *E. coli* breakthrough, showing 100% breakthrough after approximately 35 pore volumes of flow.

The sand columns showed a relatively fast complete *E. coli* breakthrough (at approximately three pore volumes), but full breakthrough was still delayed compared to the bromide breakthrough curves (approximately two pore volumes), indicating a relatively small amount of bacterial retardation inside of sand columns.

3.4.0 Analysis and Discussion

3.4.1 Permeability

As Figure 3.4 shows, permeability values of the normally consolidated and compacted clay columns with freeze-induced fractures are significantly greater than 10^{-7} cm/s, which is generally accepted as the maximum allowable value for a compacted clay liner (Qian et al, 2002). Likewise, Bardet (1997) predicts unfractured clay liner materials to be "practically impermeable", having a permeability ranging from 10^{-8} to 10^{-7} cm/s. Comparisons estimated unfractured permeabilities to the permeabilities of the test columns are shown in Table 3.3.

Table 3.3. Permeability ranges of clay and silt columns, compared to estimates by Bardet (1997) for intact samples.

Permeability	Normally Consolidated Clay (cm/s)	Compacted Clay (cm/s)	Compacted Silt (cm/s)
Lowest	2.36×10^{-6}	3.99×10^{-6}	5.86×10^{-6}
Highest	3.18×10^{-5}	9.26×10^{-6}	9.68×10^{-5}
Intact (Estimated from Bardet, 1997)	1×10^{-8}	1×10^{-8}	1×10^{-6}
Ratio $\frac{k_{fractured\ columns}}{k_{Bardet\ (1997)\ estimate}}$	250 to 3200 times faster	400 to 900 times faster	6 to 40 times faster

As Table 3.3 indicates, the freezing and thawing process increases the permeability of clay columns from 250 to 3200 times, and increases the permeability of silt columns by 6 to 40 times. This increase of permeability is caused by the formation of flow fracture paths (Eigenbrod, 1996).

The permeability of all clay columns decreased significantly over the testing period. The reduction in permeability is thought to be due to clogging of pores with *E. coli* and *E. coli* biofilms, which were visible on top of the columns and sometimes within pore fractions at the end of the tests. Closing of freeze-thaw fractures during permeation may also play a role in the permeability reduction observed, but is likely a lesser effect than the bioclogging (Lunsdorf et al, 2000; Eigenbrod, 1997).

Sand columns had good repeatability in terms of permeability during permeation, but all of the sand column permeabilities fell within the range of 10^{-2} to 10^{-4} cm/s, which are reasonable ranges given for sand by Bardet (1997).

3.4.2 *E. coli* and Bromide Breakthrough Characteristics

The early initial breakthrough seen in the *E. coli* breakthrough curves (Figure 3.5) illustrate that dispersion and preferential flow of *E. coli* cells was occurring. The delayed full breakthrough of these curves also indicates that the cells were strongly sorbed and/or filtered in all of the clay and silt columns tested. The strongest sorption/filtration occurred in the clay samples, as there was no complete breakthrough of *E. coli* cells in these columns (Figure 3.5). However, as many as 5×10^5 CFU/mL of *E. coli* were found to permeate through clay columns, indicating a possible public health risk from bacterial transport through freeze-fractured soil liners. High variability in the shapes and slopes of the breakthrough curves can be expected, as bacterial breakthrough models tend to be highly variable in unsterilized, heterogenous soils (Jordan et al, 2004).

The results of batch sorption tests with the same *E. coli* cells and MSM show that the *E. coli* cells are very strongly sorbed to the clay soil and less strongly sorbed to the silt soil (Chapter 2). This would indicate a larger *E. coli* cell retardation factor due to sorption for the clay soils, which is indicated by the breakthrough curves.

The sorptive capacity for each soil corresponding to each column's average influent concentration during permeation was determined from *E. coli* sorption tests performed on the soils (Chapter 2). The complete breakthrough time, at which cells are no longer retained by a soil column, can be estimated using equation 3.1, based on the assumption that full breakthrough will not occur until the sorption capacity of the soil in the column has been reached. This model ignores the filtering effect, which is strong in the clay columns, weak in the silt columns, and even weaker in the sand columns. It is calculated according to:

$$\text{Estimated Full Breakthrough Time} = \frac{(SC)(M_S)}{(\text{Flow rate})\,(\text{Influent cell concentration})} \quad \textbf{(Equation 3.1)}$$

Where,

SC = Sorptive capacity of soil at influent concentration (CFU/g soil)

M_S = Mass of solids of soil column (g)

Flow Rate = Flow rate of effluent out of column (mL/s)

Influent cell concentration = Cell concentration of influent entering soil column (CFU/mL)

Table 3.4 summarizes the complete breakthrough time for *E. coli* as either observed in the case of the sand and silt columns or projected using linear regression for the clay columns, compared to the estimated time according to equation 3.1.

Table 3.4. Full breakthrough times based on estimations from sorption data and observed or projected times from column testing.

Column Type	Sorptive Capacity at Influent Concentration (CFU/g dry soil)	*Estimated Full Breakthrough Time (Days)	Observed / Projected Full Breakthrough Time (Days)
Sand	3.12×10^6	0.00605	0.00876
Silt	7.76×10^7	3.14	6.00
Normally Consolidated Clay	1.04×10^9	118	5160
Compacted Clay	1.04×10^9	160	6380

* Based on equation 3.1

As Table 3.4 indicates, the observed or projected full breakthrough time is always longer than the estimated time, implying that in addition to sorption, filtration can also be a strong source of retention inside the columns. This finding is in agreement with the results of an extensive literature review on *E. coli* transport by Foppen and Schijven (2006), in which the authors report that when grain size decreases to 0.02 mm or below (clay sizes), straining is one of the most dominant transport retarding mechanisms. The sand columns (0% clay) showed moderate straining, the silt columns (about 5% clay) showed strong straining, and the two types of clay columns (about 78% clay) showed very strong straining. Another study by Foppen et al (2005) showed that straining in dead end pores was a strong method of retention inside of fine-grained sediment (ranging from 0.06-0.2mm) in a setup similar to this study, but was not as strong in

coarse grained sands, which helps to verify our results. Hence, modeling bacterial transport by estimating sorption rates is only useful for very coarse grained soils, in which filtration is a weak factor. The effect of freeze fracturing on the abundance and arrangements of dead end pores has not been studied, but freezing may reduce filtration by this mechanism by increasing the number of fractures in the soil.

Both the estimated and the observed full breakthrough times for the normally consolidated clay columns were moderately shorter than for the compacted clay columns, suggesting that compaction of soil can help to slow *E. coli* transport. This is likely accomplished by providing more sorption sites per area of volume (as seen by the higher dry density values) and by increasing filtration rates by reducing porosity (Table 3.2). Figure 3.5 indicates that compacted clay columns breakthrough faster than normally consolidated columns in terms of pore volumes of flow, but because they flow much slower, a longer breakthrough time is projected using Equation 3.1.

Further column testing using different concentrations of *E. coli,* different mixtures of soils, or different strains of bacteria or viruses may help to identify individual factors affecting the transport of bacteria through complex soil systems. In addition, permeating a pulse source of bacteria instead of a continuous source as in these experiments may help to more accurately quantify the number of bacteria by providing a more exact quantification of the number of cells entering the columns and the number exiting them. Performing scanning electron microscopy on samples of soil permeated with *E. coli* may also help to identify the specific role of biofilm growth inside of soil.

The results of this study show that clay liners with freeze-thaw fractures would allow the passage of the *E. coli* strain tested. Although complete breakthrough of *E. coli*

was not observed for the clay columns, the fact that the fecal bacterium could pass through fractures of the clay columns posts a concern on the effectiveness of clay barriers in geographic regions that are susceptible to freeze-thaw conditions.

3.5.0 Figures

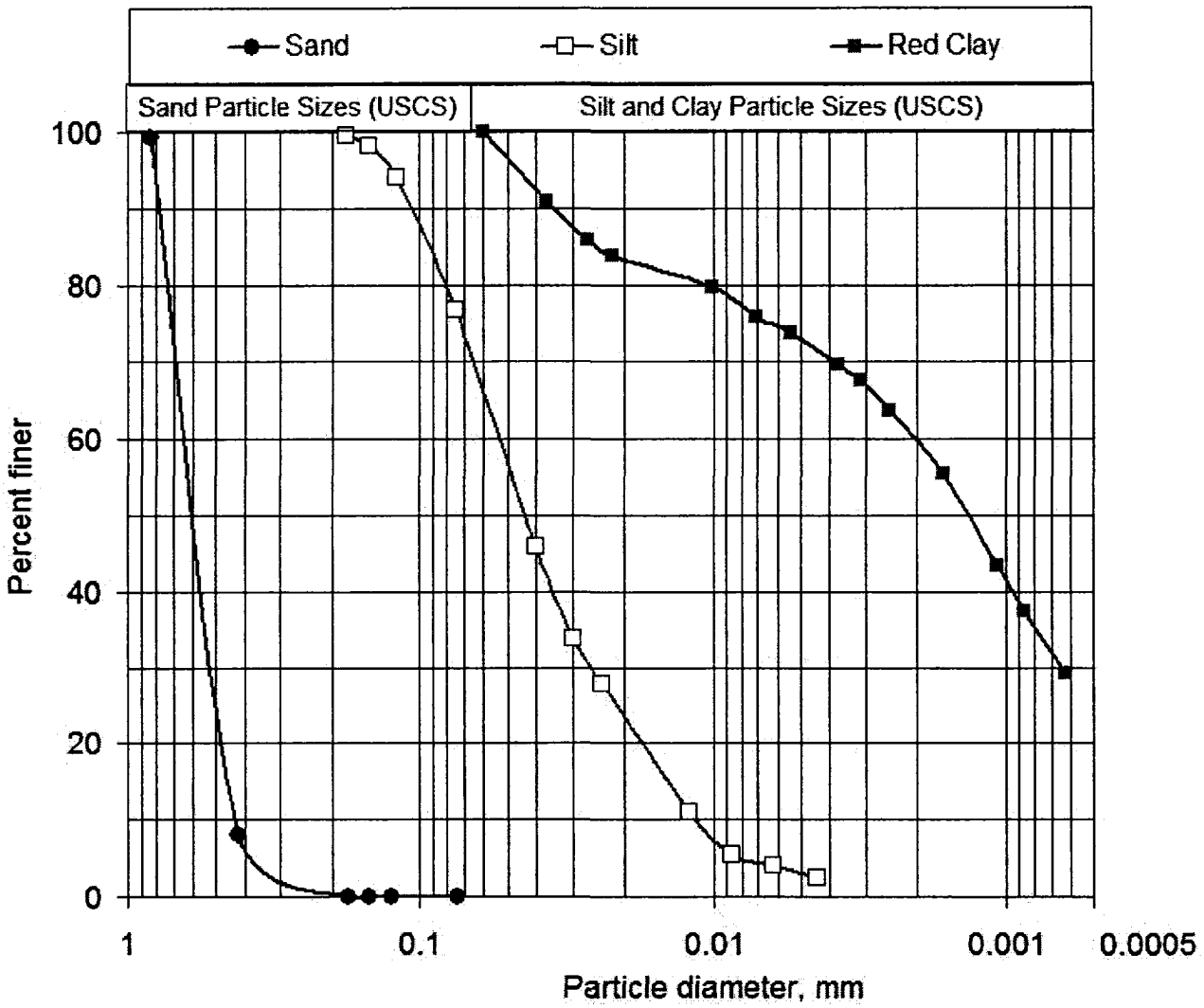

Figure 3.1. Particle size distribution for soils tested.

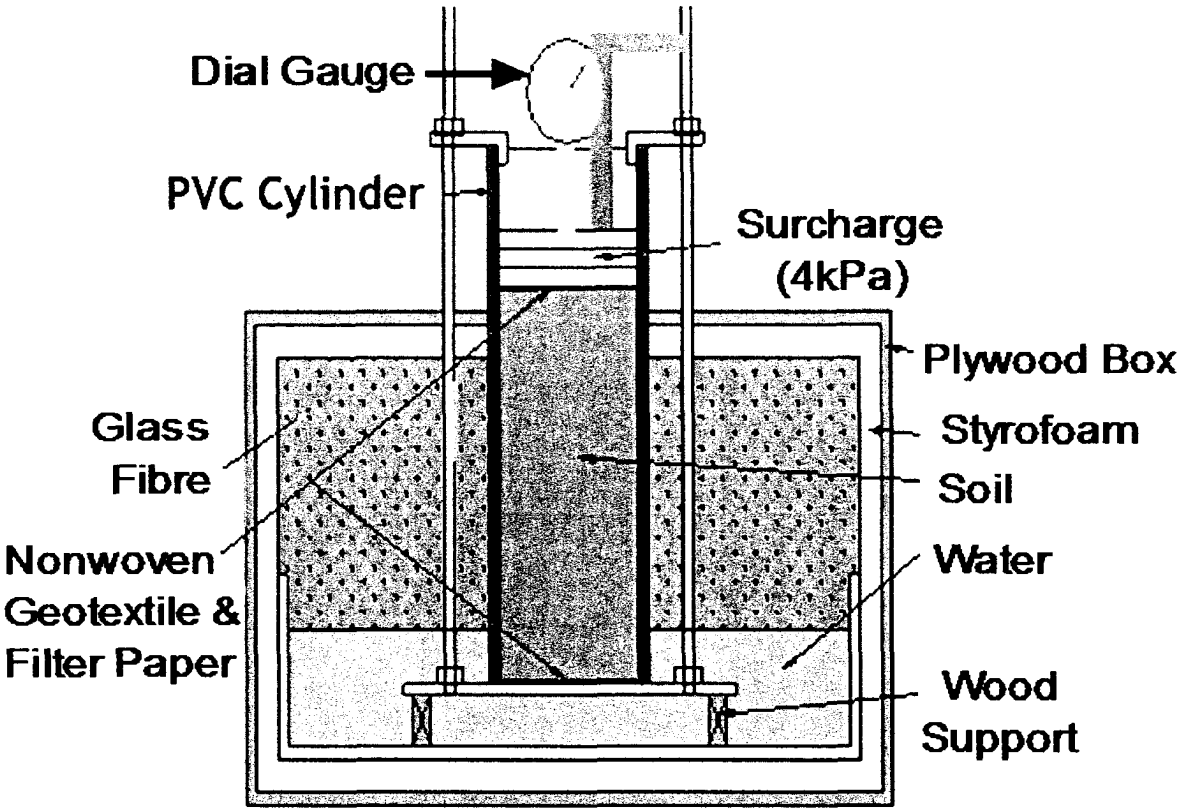

Figure 3.2. Apparatus used in freeze-thaw procedure

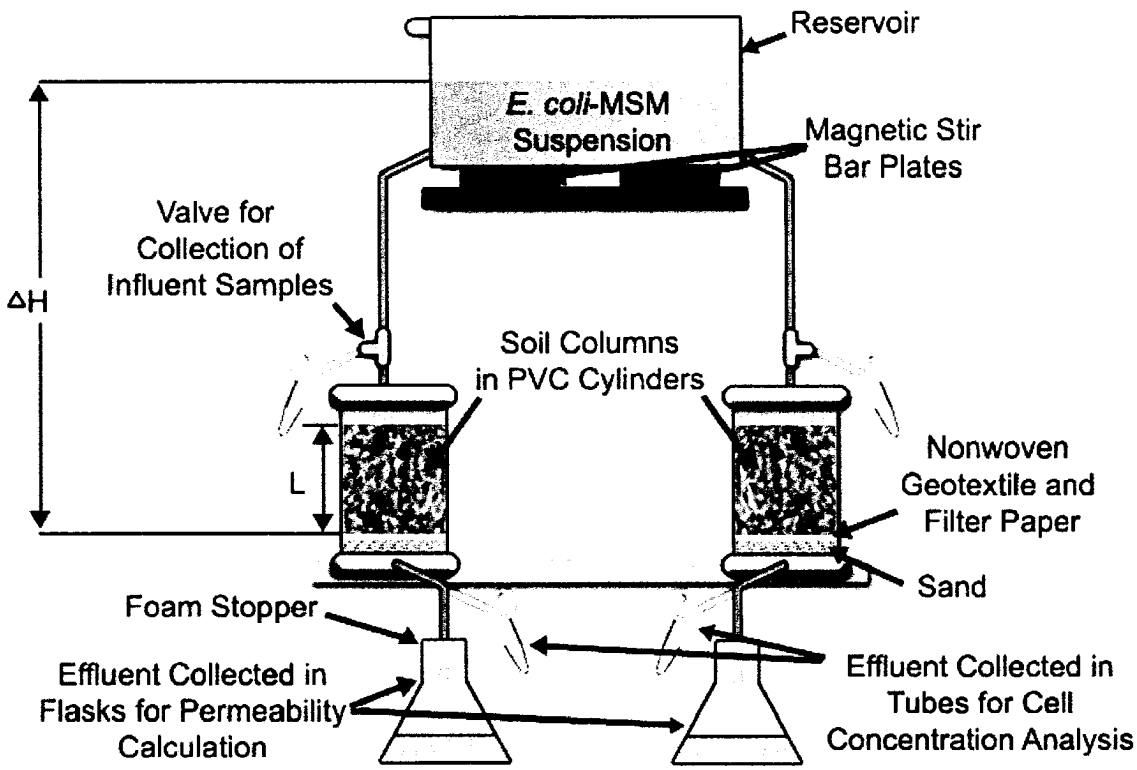

Figure 3.3. Schematic of the permeation setup

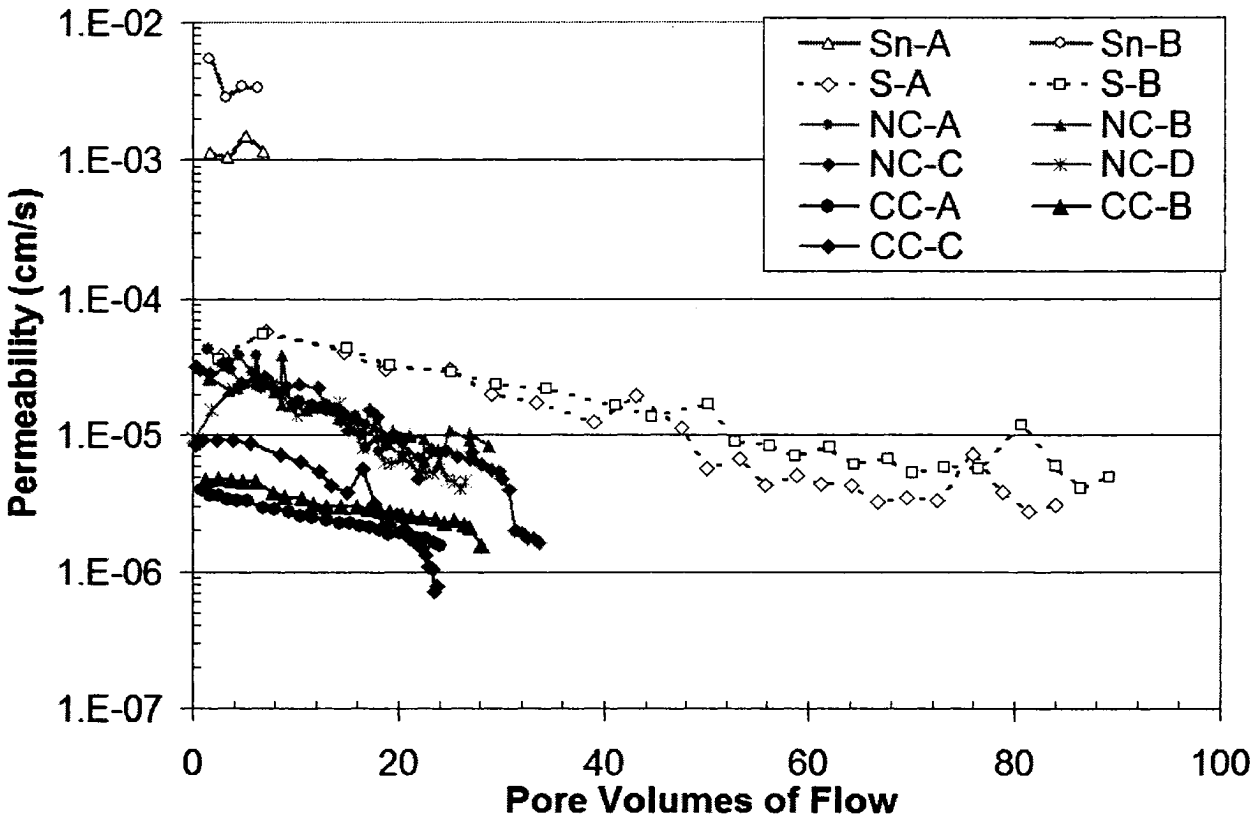

Figure 3.4. Permeability of normally consolidated clay (NC), compacted clay (CC), silt (S) and sand (Sn) soil columns with E. coli suspension.

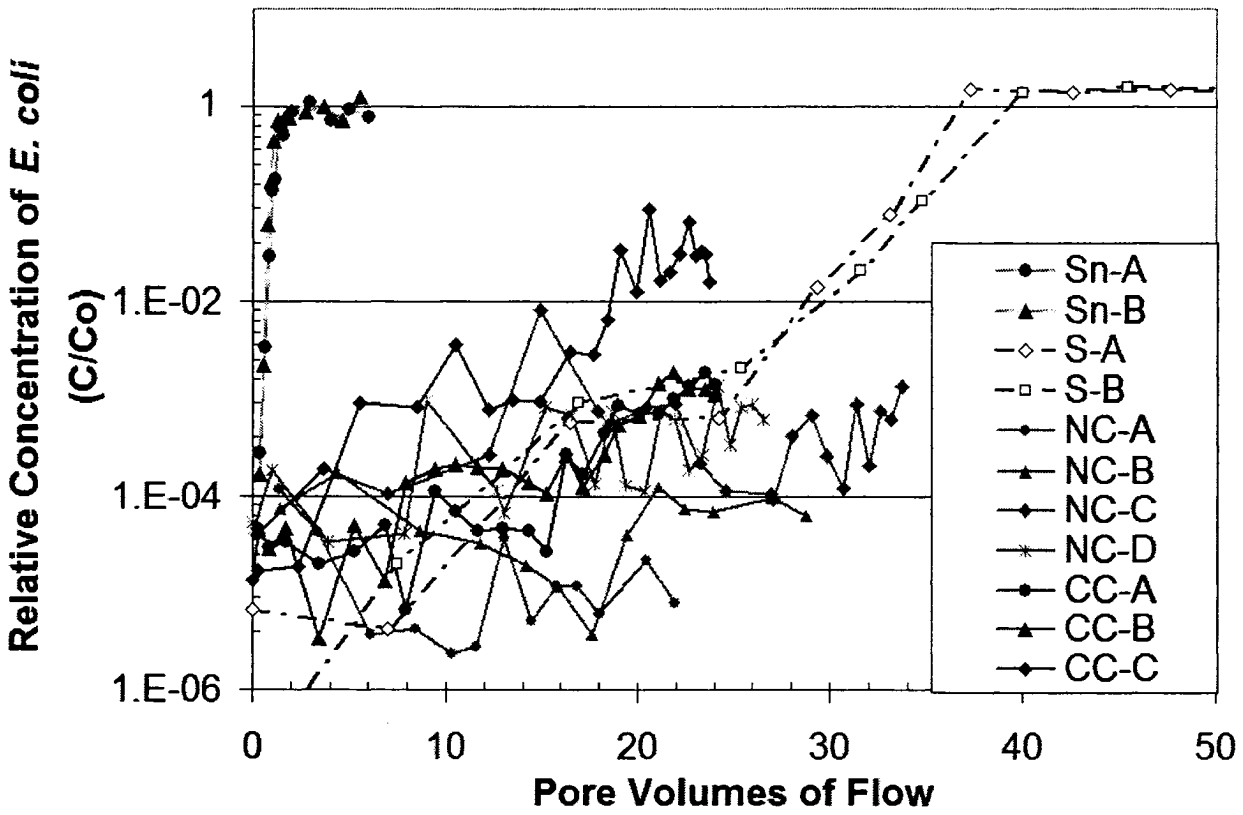

Figure 3.5. Breakthrough curves for the transport of E. coli through normally consolidated clay (NC), compacted clay (CC), silt (S) and sand (Sn) soil columns.

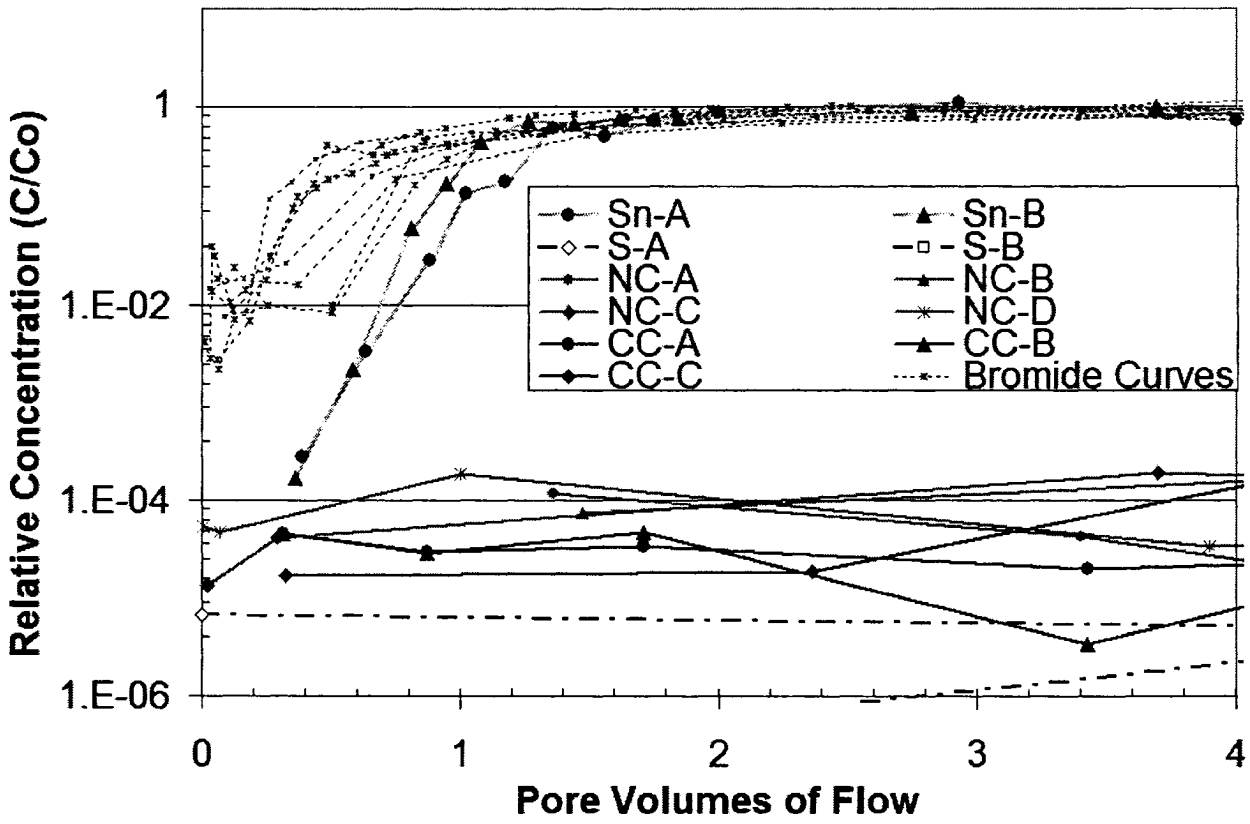

Figure 3.6. Breakthrough curves for the transport of E. coli and bromide tracer through normally consolidated clay (NC), compacted clay (CC), silt (S) and sand (Sn) soil columns.

Chapter 4: Conclusions and Recommendations

The experiments outlined in chapter 3 of this paper show that freeze-fracturing has serious implications for the transport of pathogenic bacteria through clay liners. The depth of frost penetration beneath contaminated waste is currently not monitored or considered in the design of the protective soil liners. Field studies of bacterial retention efficiency in relation to the depth of frost penetration into soil liners should be performed in order to determine if freeze-fracturing is an important factor in the failure of bacterial retention systems in nature. If a relationship between frost penetration and rates of groundwater contamination is found, then liner design requirements such as those outlined in Qian *et al* (2002) should be changed to ensure that soil barriers do not freeze during cold periods.

The diameter of the fractures inside the soil is an important factor in the determining the effectiveness of freeze-fractured soil barriers. The results of chapter 3 indicate that the diameter of the fractures in the clay and silt appears to be large enough to allow for the transport of *E. coli*. This means that the fractures tend to be at *least* 0.8 μM in diameter, but does not indicate a maximum fracture diameter. The actual maximum size of the fractures may be determined by permeating freeze-fractured soil columns with microspheres of various sizes. Sulfate (hydrophobic) microspheres such as those available from Duke Scientific Corporation (Palo Alto, CA, USA) are composed of plain polystyrene, can be ordered in various sizes from 0.04 to 60 μM in diameter, and can be detected by means of fluorescence. Columns of fractured soils could be permeated with several batches of increasingly large microspheres. The diameter of the first batch of microspheres that are completely retained in the soil columns could

indicate the maximum fracture diameter size in the soil because all of the microspheres would be filtered in pores smaller than the microsphere diameter. In addition to determining fracture size, these microsphere experiments would accurately quantify filtration rates inside of the soil columns, as they are non-sorbing and would only react with soil by means of physical filtration. Quantifying the filtration of microspheres with diameters similar to those of bacteria would help to more accurately model bacterial transport in the experiments performed in chapter 3.

The transport of viruses through freeze-fractured soil liners has not been investigated in this study, but is also an important health concern on sites of contaminated waste. Viruses will likely transport through freeze-fractured soil much more rapidly than bacteria due to their much smaller diameter. As well, viruses have been used to model fracturing in soils because they are less likely to enter the pores of clay soils than chemical tracers such as potassium bromide, and mainly follow fracture paths (McKay et al, 1993). Thus, permeation studies with viruses may reveal a potential danger to public health, and may also help to model bacterial transport through freeze-fractured soils.

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Appendix 1: Glossary of Soil Properties

A.1.0 Introduction to Soil Properties

Soils contain four major components: inorganic minerals, organic compounds, water, and air (Brady, 1984). The relative amounts of these four components heavily influence the properties of the soil. Because the inorganic mineral component of the soil is highly variable in size and chemical characteristics, it is typically used for soil classification purposes (Brady, 1984).

A.1.1 Inorganic Mineral Particle Size Classification

The particle size of inorganic components of the soil determines the broad classification of soil (Table A.1) (Hillel, 1982).

Table A.1: Soil classifications by particle size, according to the United Soil Classification System (USCS).

Particle Size	Class Name
4.75 – 76.2mm	Gravel
0.075 to 4.75mm	Sand
Less than 0.075mm	Fines (i.e., Silts and Clays)

After measuring the relative amounts of sand, silt, and clay in a soil, the soil texture can be determined using a soil texture triangle (Figure A.1) (Hillel, 1982). The texture of a soil is a useful for determining its ability to transport water. Soil texture is

often a determining factor of the effectiveness of soil barriers, such as those used on landfills and farms (Hillel, 1982).

A.1.2 Clay Mineral Classification

Clay particles are classified as particles less than 0.002mm in size. Clay particles are commonly platy in shape and highly plastic when moist (Brady, 1984). Because of their small size, clay particles have a very large specific surface area (10 to 1000 m^2/g compared to 1 m^2/g for silt and 0.1 m^2/g for sand) (Brady, 1984). In addition to size differences, clay also exhibits drastic mineralogical differences from sand and silt. Whereas sand and silt are composed mostly of quartz and other primary mineral particles, clay is largely composed of secondary mineral particles such as aluminum, which are much more physicochemically active (Hillel, 1982).

The most prevalent clay minerals are the layered aluminosilicates, which consist of crystals composed of two basic structural units. The first structural unit is a tetrahedron of oxygen atoms surrounding a central cation, usually aluminum or silicon, while the second structural unit is an octahedron of oxygen or hydroxyl groups surrounding a large cation such as aluminum or magnesium. The ratio of these two structural units is used to classify clay types. For example, kaolinite clay is classified as a clay in which one tetrahedral layer binds to each octahedral layer, while a montmorillonite clay has two tetrahedral layers binding to each octahedral layer (Hillel, 1982). The mineral composition of a clay sample determines the properties of the soil much more accurately than the particle size analysis because the layers tend to bind different cations, and different layer arrangements which result from cation binding have

varying capacities for water retention due to capillary force generated between layers (Hillel, 1982).

A.1.3 Organic Matter Content

Although particle size analysis determines the broad classification of soil, the properties of a given soil are affected by the presence of organic matter. Organic matter comprises from 2 to 6% of the weight of typical, well-drained mineral soils, but it has a strong influence on the soil's properties (Brady, 1984). Organic matter is the major source of phosphorus and sulfur and the sole source of nitrogen for the soil, all of which are essential for plant growth (Brady, 1984). Raw organic matter consists of partially decayed plant and animal residues, and is continually broken down by soil microorganisms. The more resistant products of microbial decomposition are collectively known as *humus*. Humus is a black or brown substance that usually forms colloids inside of soil, and has a very high water and nutrient ion holding capacity (Brady, 1984). The ability of humus to retain water and nutrient ions can aid in the survival and growth of bacteria by creating conditions in which they thrive.

A.1.4 Porosity

The spaces between soil particles comprise the pore space of the soil. The volume of the pore space depends largely on the arrangement and size of the soil particles (Hillel, 1982). All pores inside of soil are filled with either water or air. Hence, we can measure the pore space of a soil by the following equation:

$$V_f = V_a + V_w \qquad \textbf{(Equation A.1)}$$

Where,

V_f = Volume of pores

V_a = Volume of air

V_w = Volume of water

(Hillel, 1982)

Coarse soils such as sand tend to have larger pores than fine grained soils such as clay. However, because fine grained soils have many more pores than coarse grained soils, they tend to have a higher total pore volume despite their smaller individual pore size (Hillel, 1982).

The porosity of a soil is a measure of the volume of pores in relation to the total volume of the soil, and is measured by:

$f = V_f / V_t$ **(Equation A.2)**

Where,

f = Porosity

V_f = Volume of pores

V_t = Total volume of soil

(Hillel, 1982)

Geotechnical engineers sometimes prefer to express pore space in relation to the total volume of solids, rather than the total volume of soil. This parameter is known as the void ratio and is calculated by:

$e = V_f / (V_t - V_f)$ **(Equation A.3)**

Where,

e = Void ratio

V_f = Volume of pores

V_t = Total volume of soil

(Hillel, 1982)

In fine grained soils, some of the fluid in the pore space may be trapped in dead-end pores or sorbed to the surface of soil particles (Shackleford, 1993). This means that not all of the fluid in the pore space may be available for the transport of solutes. The volume of the trapped liquid can be ignored in solute transport studies, and the remaining porosity, also known as the effective porosity, is often used.

A.1.5 Densities and Water Content

The gravimetric water content of a soil (also referred to as "mass wetness") is typically expressed by the following equation:

$w = M_w / M_s$ **(Equation A.4)**

Where,

w = Water content (expressed either as a decimal or a percentage)

M_w = Mass of water

M_s = Mass of solids

The bulk density, or bulk unit weight, is the weight of the soil and water divided by the total volume of the soil and water. It is typically expressed by the following equation:

$\gamma = W_t / V_t$ **(Equation A.5)**

Where,

γ = Bulk unit weight

W_t = Total weight of soil and water

V_t = Total volume of soil and water

The dry unit weight, or dry density, relates the mass of solids to the total volume of the soil. By definition, it is also less than the bulk density. It is expressed by the following equation:

$\gamma_d = W_s / V_t$ **(Equation A.6)**

Where,

γ_d = Dry unit weight

W_s = Weight of solids

V_t = Total volume of soil and water

A.1.6 Cation Exchange Capacity

The cation exchange capacity (CEC) of a soil is a measure of the quantity of cations required to neutralize the negative charge of the clay particle surfaces per unit of mass (Hillel, 1982). Clay particles are negatively charged, and have an affinity for positively charged cations. Clay mineral types differ greatly in the number of exchange sites per unit area of particles and in their specific surface area (based on particle size), and hence differ greatly in their CEC (Hillel, 1982). For example, montmorillonite (small particle size) has a CEC of approximately 0.95 mEq/g, whereas kaolinite (relatively larger particle size) has a CEC of approximately 0.09 mEq/g (Hillel, 1982).

Soils with smaller particle sizes have more surface area per unit mass onto which cations may bind. For this reason, particle size influences the CEC of soils (Hillel, 1982).

A.1.7.0 Permeability

Permeability is a measure of water's ability to move through a soil, and is calculated using Darcy's law (Hillel, 1982):

$k = q/iA = qL/hA$ **(Equation A.7)**

Where,

k = permeability (hydraulic conductivity) (cm/s)

q = the rate of flow through the material (cm^3/s)

i = hydraulic gradient (h/L) (unitless)

A = total cross-sectional area of flow, perpendicular to the direction of flow (cm^2)

L = length over which total hydraulic head (h) is lost (e.g. the height of a column test sample) (cm)

h = the hydraulic head lost across length (L) (cm)

(Hillel, 1982).

Hence, the flow rate, thickness, cross-sectional area and position of the soil relative to the liquid source are incorporated into the calculation of permeability.

The value of permeability (k) varies widely for different soils. Table A.2 presents typical permeability values for saturated soils, but the permeability for unsaturated soils is lower and increases rapidly with the degree of saturation (Bardet, 1997).

Table A.2 Typical Values of Permeability of Saturated Soils

Soil Type	Permeability (cm/sec)
Gravel	Over 10^{-1}
Sandy Gravel, clean sand, fine sand	10^{-1} to 10^{-3}
Sand, dirty sand, silty sand	10^{-3} to 10^{-5}
Silt, silty clay	10^{-5} to 10^{-7}
Clay	10^{-7} to 10^{-8} or less

Water content plays an important role in soil permeability. When soil is saturated with water, water is able to flow through liquid-filled pores and hydraulic conductivity is high (Maier et al, 2000). As moisture content is reduced, pores become filled with air, and water has a decreased ability to flow through the system, so permeability is reduced (Maier et al, 2000). This means that the permeability at saturation theoretically determines the maximum flow rate of the soil, which will decrease as the soil dries (Johnson et al, 2004).

However, these measures of permeability depend on the system existing in ideal conditions where flow rate is directly proportional to moisture content. Theories and models for liquid flow through soils "have been validated, if at all, and only in highly artificial sets of laboratory produced conditions" (Hillel, 1982). Hydrologic heterogeneity is observed in real soils, where flow paths are created through fractures induced by either drying or freezing (Brady, 1984; Eigenbrod, 1996).

Active clay soils swell when exposed to water. The water molecules are adsorbed to the surfaces of the clay mineral particles (Brady, 1984). A reduction of the water content in swelled soils results in soil shrinkage, which creates cracks in the soil structure. These cracks act as preferential flow paths for water, and can drastically increase the permeability of a soil liner (Brady, 1984). Many types of clay such as kaolinite, chlorite and illite do not demonstrate swelling because they have a static crystal structure, but very fine soils such as montmorillonite experience intense swelling (Brady, 1984).

Under saturated conditions, a swelled sample of montmorillonite clay will have a very low permeability. However, at unsaturated conditions, it will have a very high permeability due to shrinkage (Brady, 1984). Hence, some sections of a soil field may swell more than others due to differences in water and mineral content, and quantifying permeability in the field is difficult.

A.1.7.1 Empirical Permeability Relationships for Granular Soils

There are two important correlations for predicting the permeability, *k*, of water through granular soils (Das, 2006). The first relationship, shown below, was proposed by Hazen:

$k = cD_{10}^2$ **(Equation A.8)**

where

k = Permeability (cm/sec)

c = Hazen's constant, ranging from 1 to 1.5

D_{10} = Particle size corresponding to 10% passing on a sieve analysis curve, also referred to as effective particle size

The second empirical relationship given to determine hydraulic conductivity in sandy soils is the Chapuis equation:

$k = 2.4622\ [D_{10}^2(e^3/(1+e))]^{0.7825}$ **(Equation A.9)**

Where,

k = Permeability (cm/sec)

D_{10} = Particle size corresponding to 10% passing on a sieve analysis curve, also referred to as effective particle size

e = Void ratio

A.1.8.0 - Breakthrough curves

The movement of a chemical or biological substance through a soil column saturated with liquid can be analyzed by plotting the data as breakthrough curves, which show the amount of the substance exiting the column (effluent) corresponding to the amount of liquid that has flowed through the column. The amount of flow is normalized to the volume of voids in the sample and is called "pore volumes of flow". One pore volume of flow corresponds to a flow volume equal to the volume of voids in the sample.

Under ideal flow conditions, new substances entering the column will displace the contents of the pores and will break through completely after 1 pore volume of flow (Figure A.2 - A). Dispersion flow results from the flow of liquid through flow paths, such as those created by drying shrinkage cracking or cyclic freezing and thawing (Figure A.2 - B). With dispersed flow, the substance will begin exiting the system before 1 pore volume of flow has been displaced, but will take longer to reach 100% breakthrough (the point at which the influent concentration is equal to the effluent concentration). If there are interactions (i.e. sorption and/or filtering) between the flowing substance and

the soil in the system, then the breakthrough curve will have a very gentle slope because the substance is retained inside of the system. Eventually, the substance will reach a saturation point within the soil column, and will exit the column with 100% breakthrough.

A.1.8.1 Use of Bromide Tracers

Potassium bromide (KBr) can be used as a non-sorbing tracer molecule. When permeated through a soil column, the breakthrough of bromide (Br^-) can be used to estimate transport coefficients such as diffusion (D_S), dispersion (D_h), and retardation, (R_d) (Foppen et al., 2005). Comparing the breakthrough curves of non-sorbing tracers such as bromide to particles which can become sorbed such as bacteria can help to determine sorption rates inside of soil columns.

A.1.9 Effects of Freezing and Thawing

Freezing and subsequent thawing of fine grained soils such as clay causes the soil to experience volume changes, loss in shear strength, and changes in hydraulic conductivity (Eigenbrod, 1996). The increase in permeability (often more than 100 times) is due to the formation of fissures and joints developed in conjunction with freeze-thaw consolidation (Eigenbrod, 1996). Although the rate of volume change is dependent on factors such as soil type, soil consistency, and rate of freezing, the rate of permeability change seems to be independent of these factors. In fact, no soil properties seemed to clearly correlate with their change in permeability as a result of the freeze process (Eigenbrod, 1996). The dramatic increase of hydraulic conductivity due

to cyclic freezing and thawing generally occurs within the first three freeze-thaw cycles, after which the hydraulic conductivity ceases to change (Fig A.3) (Qian et al, 2002).

A.3.0 Figures

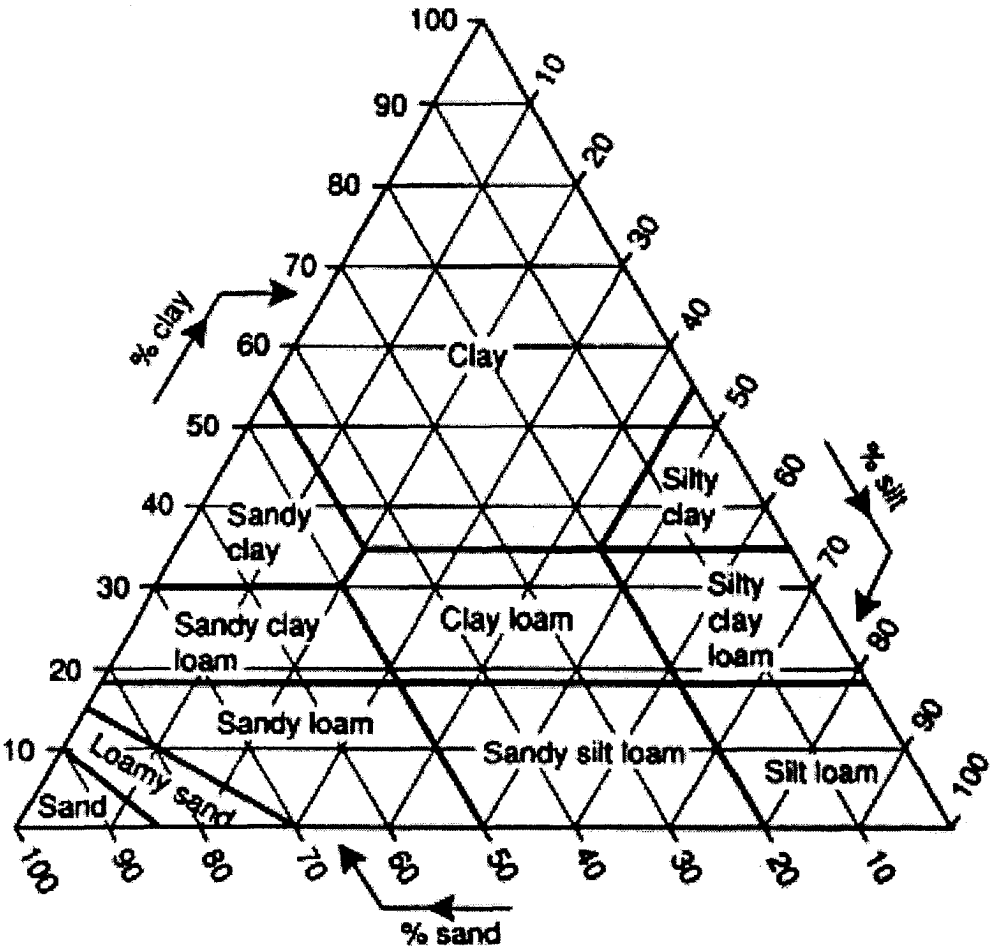

Figure A.1: Soil texture triangle (according to USDA classification)

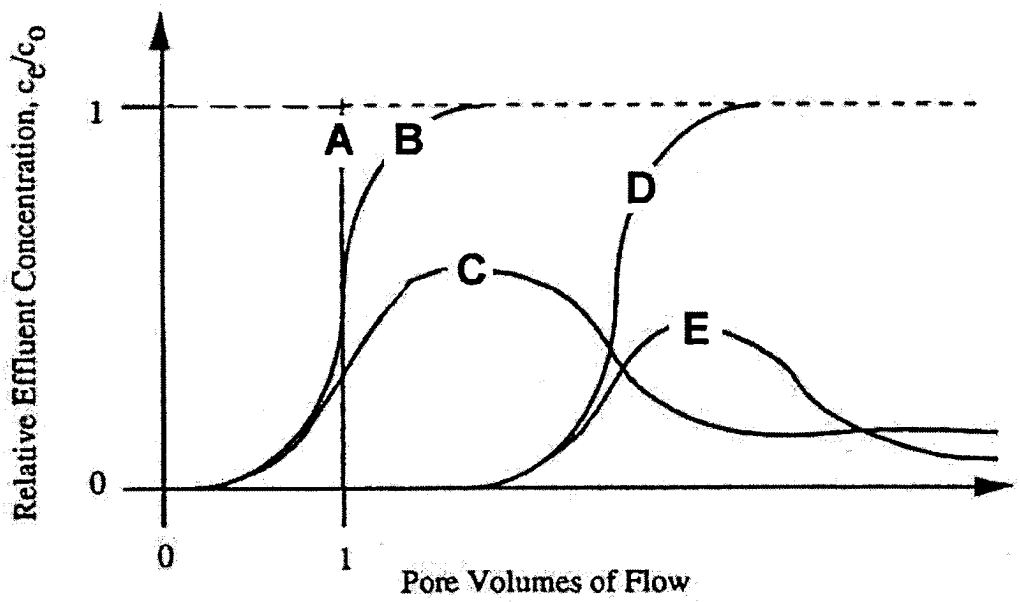

Figure A.2. Typical Breakthrough Curve Patterns (Modified from Shackelford, 1994).

Where,

A = Ideal plug flow

B = Dispersion

C = Dispersion and degradation

D = Dispersion and sorption

E = Dispersion, sorption and degradation

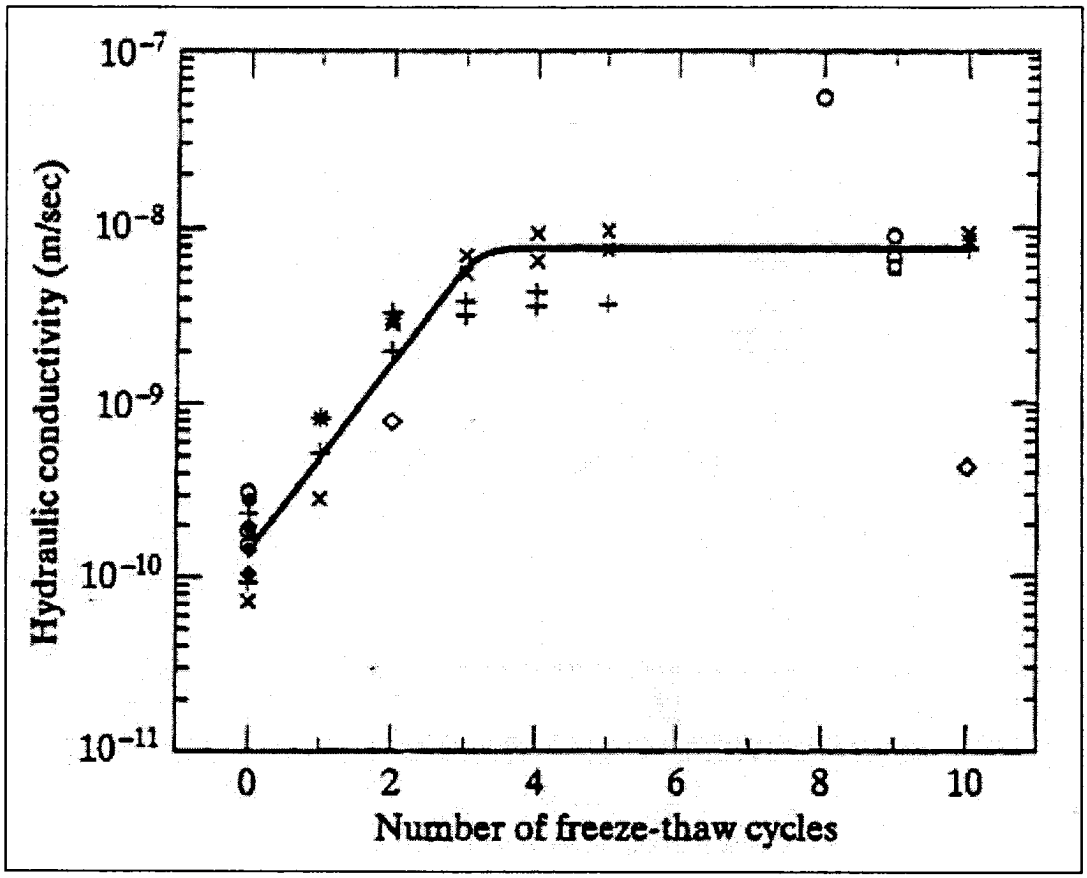

Figure A.3. Hydraulic conductivity measured on soils frozen and thawed in the laboratory and field (Qian et al., 2002)

Appendix 2: Additional Data

E. coli and Plasmid Survival Data

Cell count data collected during the experiments outlined in this thesis require that *E. coli* S-17-1λ are able to retain PJB29 plasmids over the course of experimentation. If some cells of *E. coli* began to lose this plasmid, they would no longer grow on nutrient agar plates containing the antibiotics Ampicillin and Kanamycin, and would not be included in cell counts. For this reason, experiments were ran in order to analyze the survival of the plasmid in *E. coli.*

Figure A.4 indicates the survival of *E. coli* and antibiotic resistant *E. coli* in MSM suspension medium. When total *E. coli* outnumber the antibiotic resistant *E. coli*, there has been a loss of the plasmid. As Figure A.4 indicates, the concentration of *E. coli* in MSM over a seven day period drops by about a factor of two. There is no significant difference between the total *E. coli* and the antibiotic resistant *E. coli* over the testing period, indicating good retention of the plasmid in MSM over a period of 7 days. During constant head permeability testing (Chapter 3), the MSM stock solution was refreshed every six days, so cell death and plasmid loss in the MSM were not significant factors affecting results. Each point in Figure A.4 represents the average of three replicates.

Figure A.5 indicates the survival of *E. coli* and antibiotic resistant *E. coli* in clay soil over a period of three weeks (21 days), and shows that the concentration of *E. coli* drops by a factor of about two or three over this time period. By the end of the testing period, there is still a difference of less than one order of magnitude between the total *E. coli* and the *E. coli* retaining plasmids, indicating a small but not very significant plasmid loss in the soil over the testing period. Typically, column experiments were ran for a

time period of approximately two weeks for the clay, 1.5 weeks for the silt and one day for the sand.

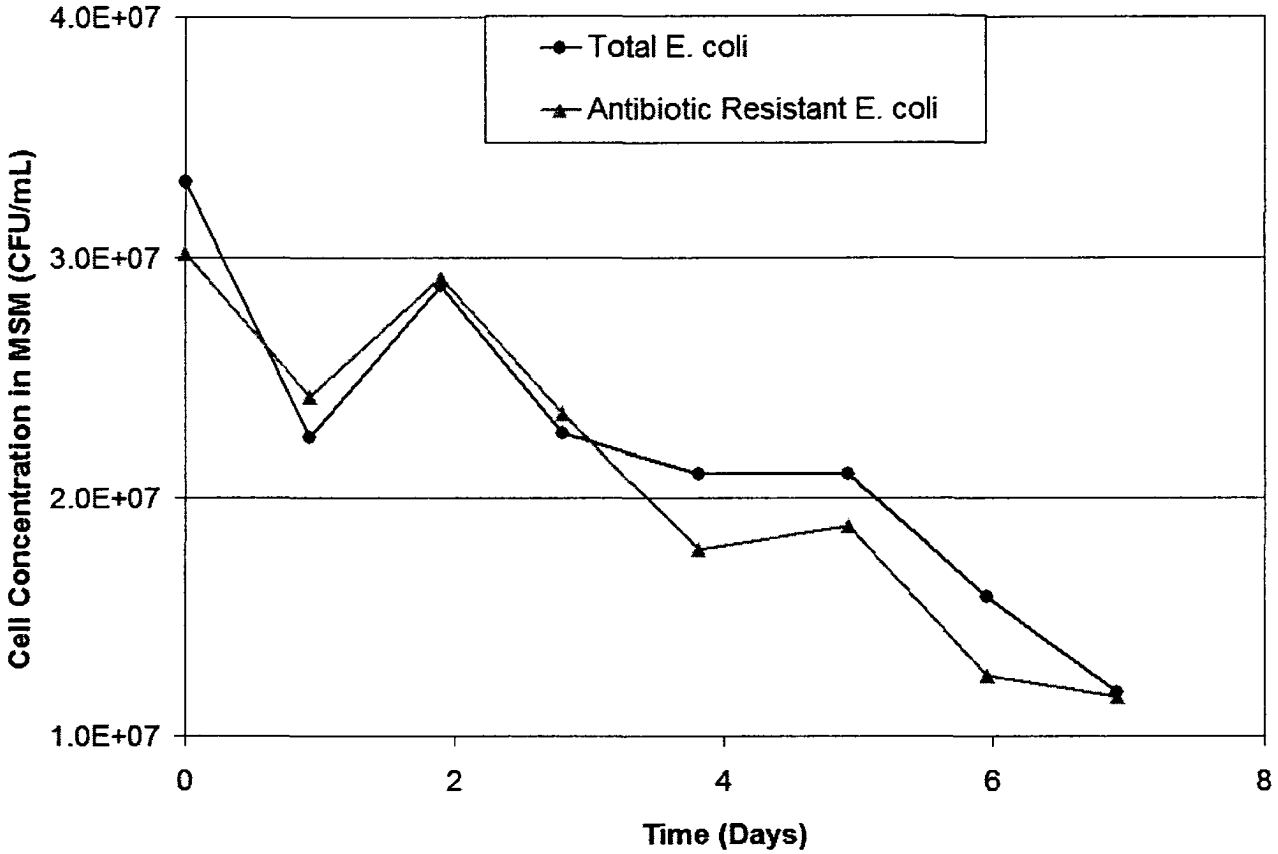

Figure A.4. Survival of PJB29 Plasmids inside *E. coli* S-17-1λ in MSM suspension medium.

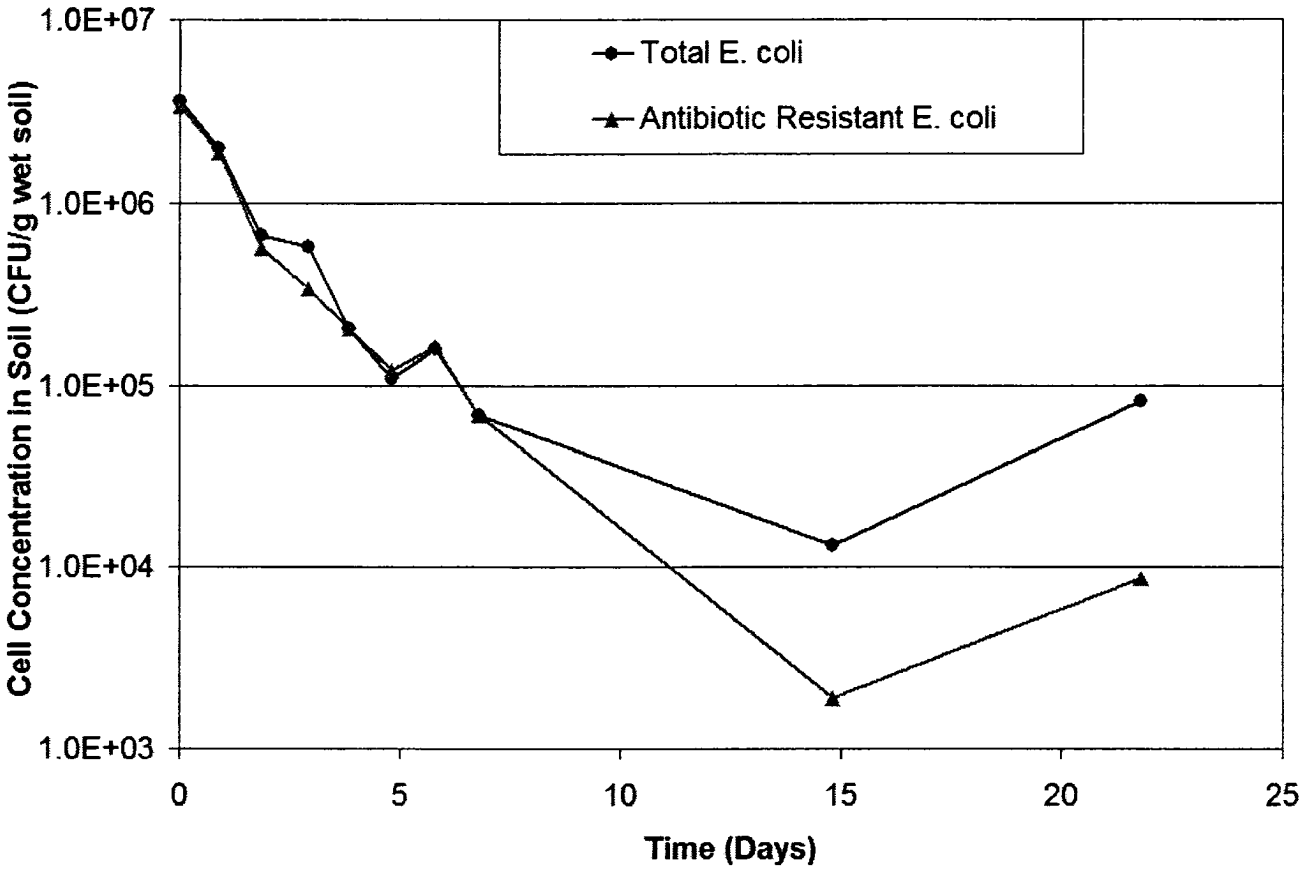

Figure A.5. Survival of PJB29 Plasmids inside *E. coli* S-17-1λ in clay soil suspension

Additional Figures

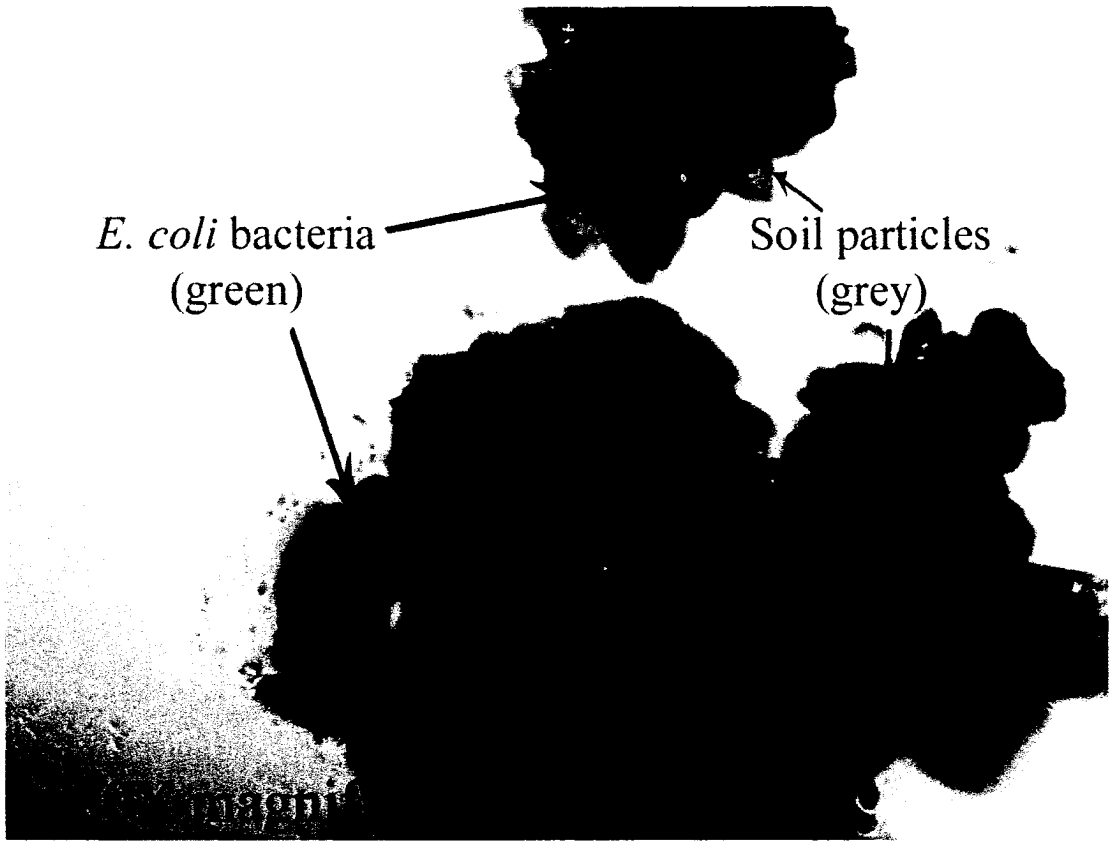

Figure A.6. Sorption of Fluorescently Labeled *E. coli* onto clay soil particles, as visualized by fluorescent microscopy

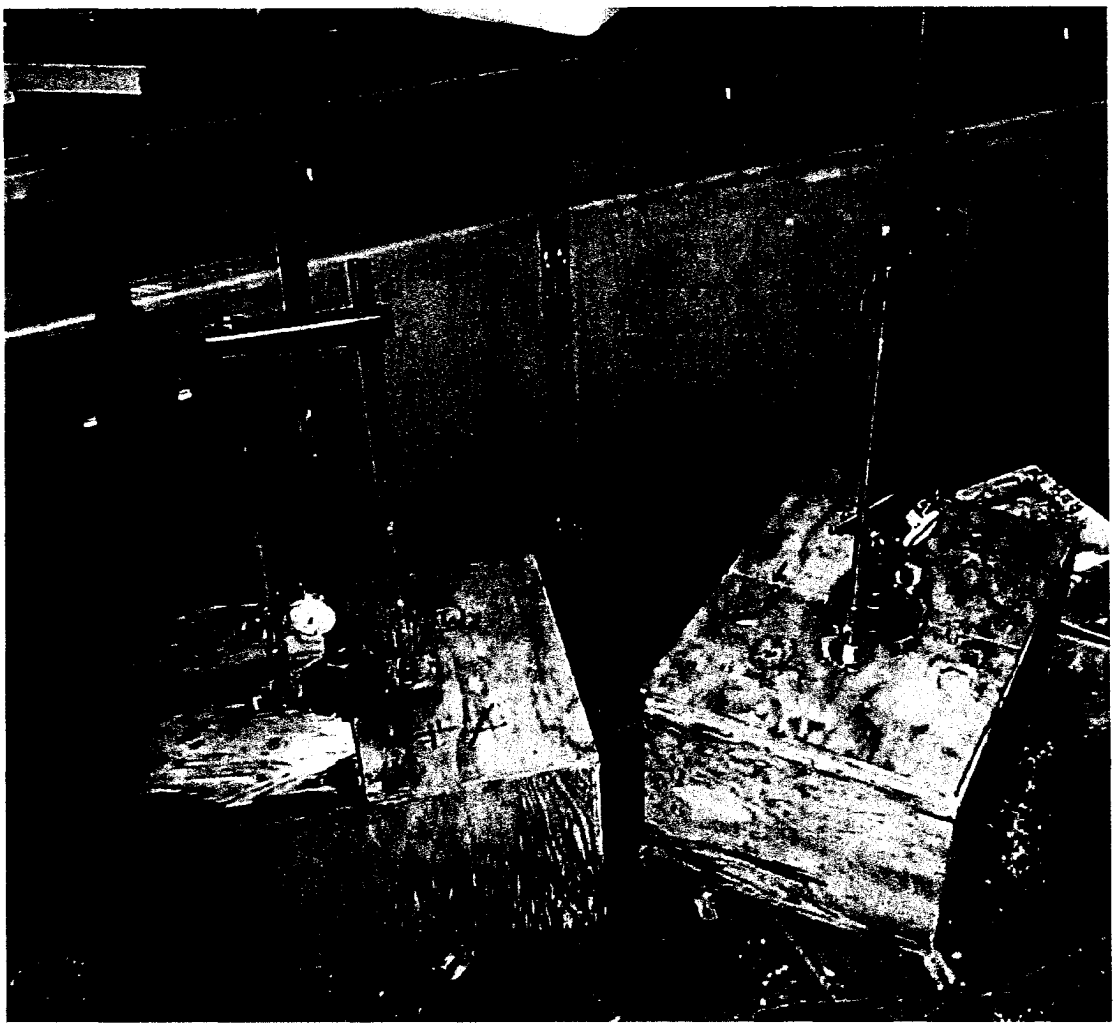

Figure A.7. Soil columns prepared for freezing in freeze-thaw apparatus

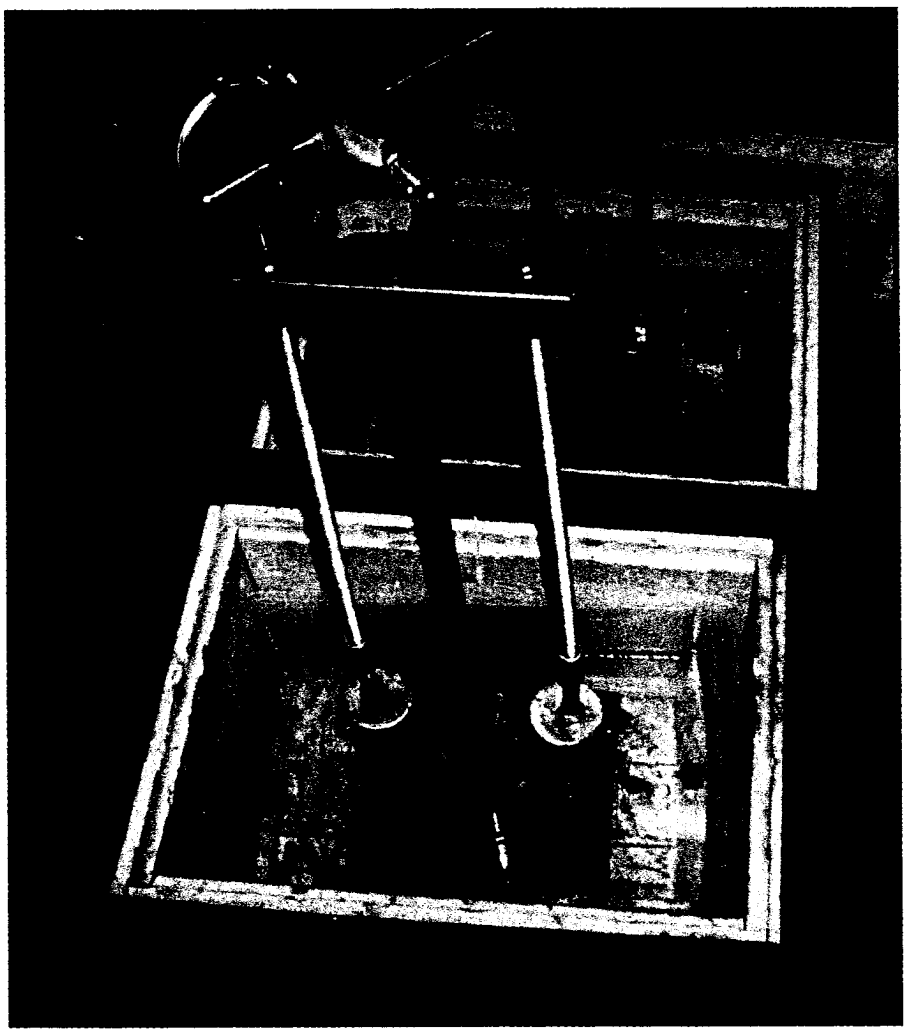

Figure A.8. Soil columns after thawing in freeze-thaw apparatus

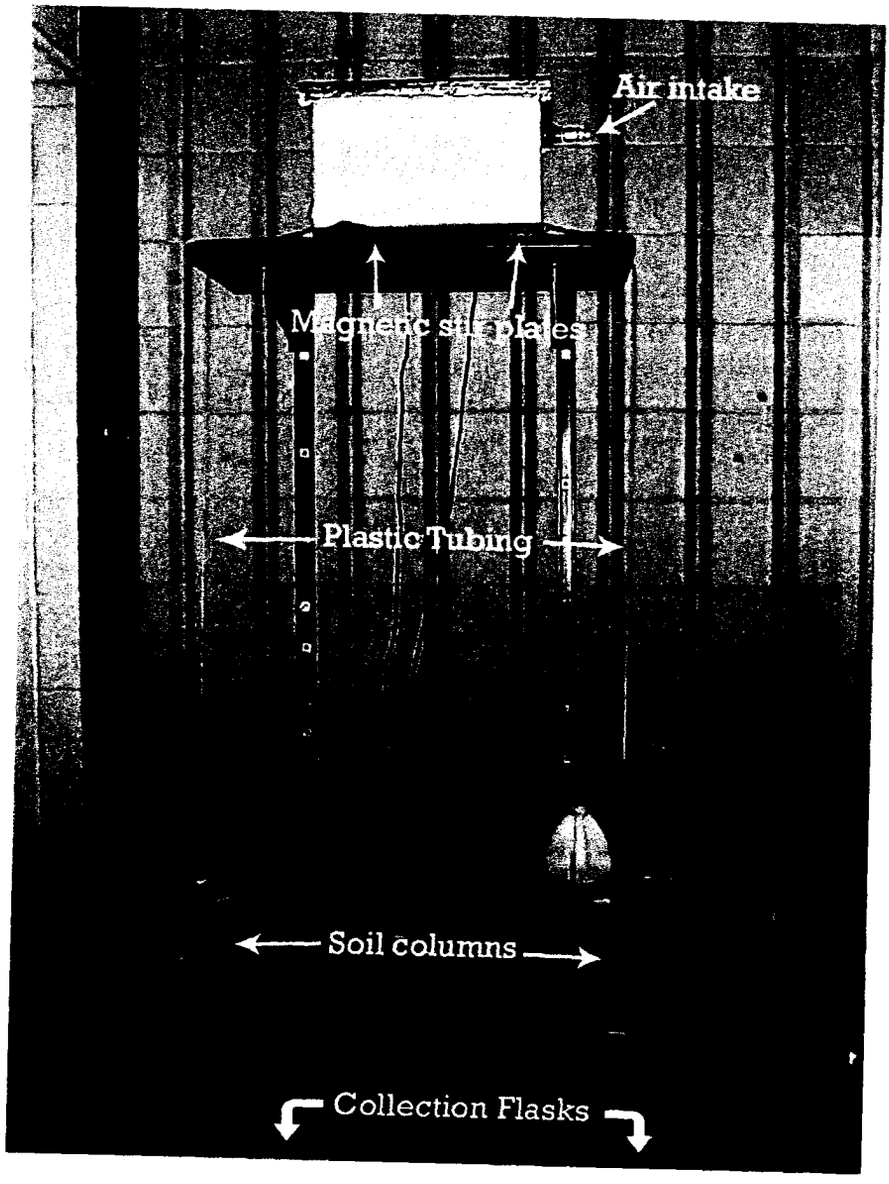

Figure A.9. Soil column Permeation Apparatus

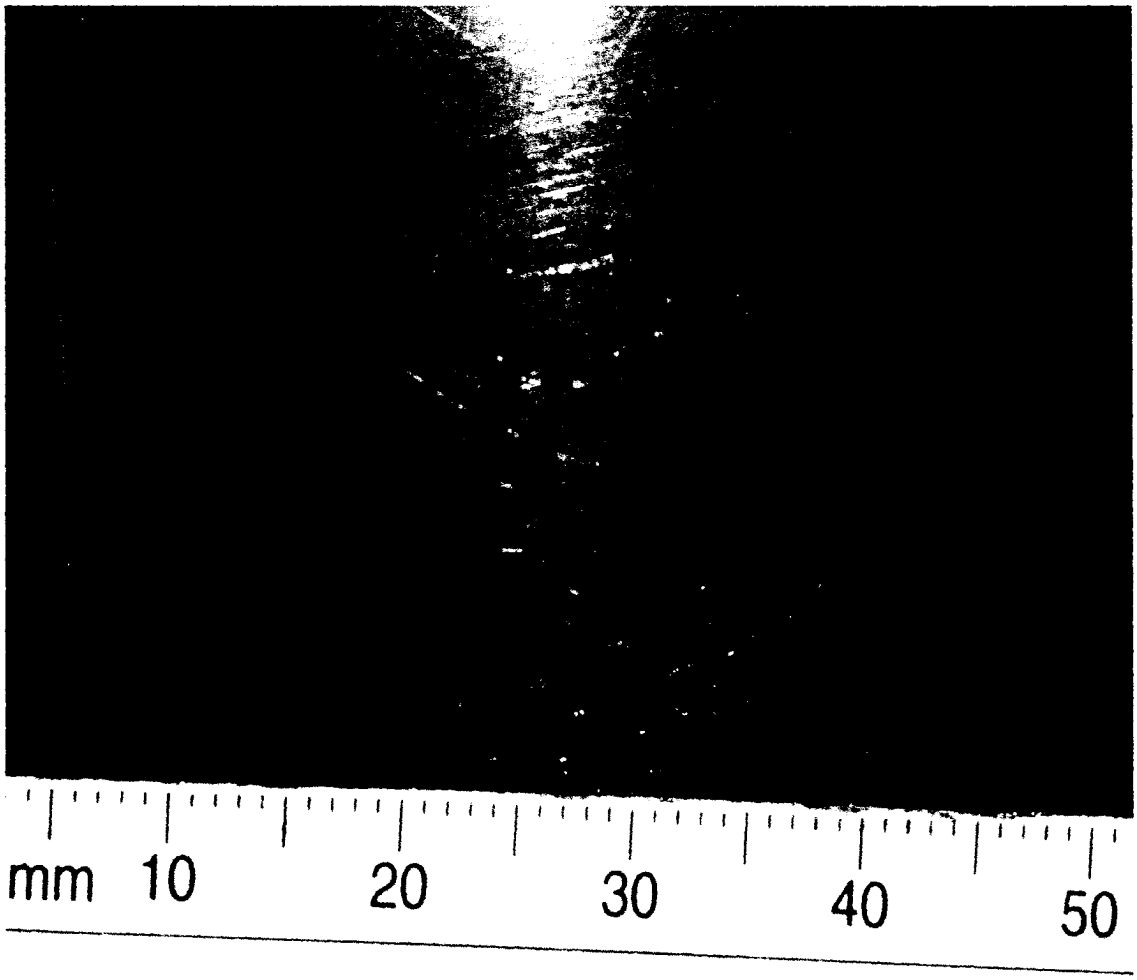

Figure A.10. Close-up view of a permeated soil column, showing texture that may be fractures induced by the freeze-thaw process