# Preferential Transport of *Escherichia coli* through Soil

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## Abstract

Contamination of water sources resulting from Escherichia coli 0157:H7 movement through the soil requires knowledge of the transport mechanisms. The objective of this study was to examine the effect of initial soil water content on the preferential transport of *E. coli* through the soil. The study was conducted on a heavy clay soil near Plenty, SK. *Escherichia coli* as well as conservative and adsorptive tracers were applied to treatments that were initially dry, partially wet, and initially wet. Relative breakthroughs of *E. coli* and $Cl^-$ were similar for all three treatments, showing a large proportion of the mass applied remained in the top 10 cm of soil, indicating transport through the soil matrix. Beyond this depth, *E. coli*: $Cl^-$ ratios remained consistent, suggesting transport along preferential pathways. The lack of differences between treatments may suggest that irrespective of the transport pathway, the end result is the same. This study is important for semi-arid areas where dry cracked soils can receive intense thunderstorms that provide enough water to transport *E. coli* to substantial depths.

## Introduction

Recent increases in the beef feedlot industry have led to issues surrounding disposal of animal wastes. Typically, beef manure is spread on the surface of agricultural soil and incorporated as a soil amendment. However, a large number of animals confined to a small area of land have meant that manure must be disposed of on increasing areas of soil. This may lead to spreading manure on land that is susceptible to water contamination from the animal manure. Along with the risks associated with an over-supply of nutrients that are susceptible to removal from the soil by leaching or overland flow, so too can pathogenic bacteria be transported to surface, ground and recreational water bodies. The primary reservoir for Escherichia coli 0157:H7 (*E. coli*) in the environment is cattle manure (Jones, 1999). Escherichia coli 0157:H7 is a virulent pathogen (Paton and Paton, 1998) and determining the pathways in which it is transported through soil is critical to avoid human health crises.

It has been previously reported that bacteria can be transported to substantial depths in field soil or through soil lysimeters (Vinten et al., 2002). In some studies it was reported that the soil acted as an effective filter (Fenlon et al., 2000) and in other cases it was found that bacteria had been transported to greater depths than would normally be attributed to matrix flow (McMurray et al., 1998). In these cases, preferential flow was implicated in the transport to depth. Despite the anecdotal accounts of preferential flow being responsible for greater transport of bacteria, very few studies have looked specifically at this transport phenomenon.

Preferential flow is an umbrella term for a number of flow phenomena, all of which are responsible for transport of water in a non-ideal fashion. All else being equal, preferential flow is a function of input flux density and initial soil water content and is exacerbated by soil pore size distribution and the presence of root channels, cracks or animal burrows (Smith, 1985). These transport pathways have long been known to provide expedient channels along which bacteria can be transported to substantial depths. However, the initial water content of the soil has not been studied as a factor in the preferential transport of bacteria. As well, heavy clay soils and their propensity for cracking have not been evaluated for their potential to allow transport of bacteria. Therefore, the objective of this study was to determine if initial water content had an effect on the transport of *E. coli* through a heavy clay soil.

## Materials and Methods

### *Site characteristics*

The study was conducted at a site near Plenty, Saskatchewan, Canada (legal land location: SW5-33-18-W3). The site is situated in the Dark Brown Soil Zone and the soil is classified as a Brown Chernozemic soil of the Regina Association and ranges from clay to heavy clay texture. The site was cropped under canary seed and was harvested by hand before the experiment. Annual precipitation and average annual air temperature for this site are 327 mm and 3°C, respectively.

### *Experimental design*

The study examined the effect of initial soil water content on the transport of *E. coli* through the soil. *Escherichia coli* and a suite of tracers were applied to the following treatments: (1) initially dry, (2) partially wet, and (3) initially wet. Each treatment occurred on a 1 m x 1 m plot and was arranged in a randomized complete block design and replicated three times.

### *Input Solutions*

### *Tracers*

Two tracers and *E. coli* were used to trace bacterial transport in this experiment. The tracers were conservative and adsorptive and were applied as part of the simulated rainfall solution. Chloride was used as a conservative tracer to determine the flow paths of the input water that did not interact with the surrounding soil. Chloride was applied as KCl (Panther Chemicals, Canada) at a rate of 2.5 g $L^{-1}$, to arrive at an input molarity of 0.03 M coinciding with the approximate molarity of beef manure. KeyAcid Blue dye (Keystone Aniline, IL) was added as an adsorptive tracer to visually determine the water flow path at the recommended rate of 4 g $L^{-1}$.

### *Cultivation of E. coli*

A wild-type *Escherichia coli* isolated from a beef feedlot in Lacombe, Alberta, Canada was used in this experiment. The *E. coli* isolate, known as Strain 8 was tagged with a

green fluorescing protein and is resistant to the antibiotic kanamycin (Kn). Strain 8 was cultured on 10 % trypticase soy agar (TSA) (Becton Dickinson, MD) infused with 50 _g $mL^{-1}$ kanamycin acid sulfate (Sigma, MO). Cells used in the experiment were harvested in the mid-logarithmic growth phase and were suspended in 100 mL of saline, which became part of the input solution.

*Field Methods*

The two categories of tracers were applied according to the end objective that was sought. Water tracers, consisting of the conservative and adsorptive tracers were applied uniformly through a spray nozzle over a 10,000 $cm^2$ area as simulated rainfall. A total of 15 L of distilled water and tracer mix was applied at a rate of 0.375 cm $min^{-1}$ to arrive at a total rainfall of 1.5 cm. Bacteria were applied to an inner area of 50 cm x 50 cm to avoid edge effects of the sample area. *E. coli* were added to 900 mL of distilled water to arrive at a 1 L input solution that was sprayed over the inner 2500 $cm^2$. Time of bacterial tracer application was dependent upon the treatment.

*Treatment Application*

The objective of the study was to examine the influence of initial soil water content on bacterial transport. Thus, the experiment began with each treatment at a different wetting state. At the outset of the experiment, Treatment 3 (pre-wet) had 4 cm of water applied over the entire 10,000 $cm^2$ and was allowed to redistribute for 48 h. After 48 h, 1.5 c of water and tracers were applied to all treatments. Bacterial tracers were applied to the inner 2500 $cm^2$, immediately followed by simulated rainfall for initially dry and pre-wet treatments. Bacterial tracers were applied for Treatment 2 (partially wet) in the same way as for initially dry and pre-wet, with the exception that application came after half of the simulated rainfall had been applied. Input subsamples were taken for both bacteria and water tracers during application. Tracers and rainfall were allowed to redistribute through the soil profile for 48 h.

*Sampling*

After 48 h the plots were excavated to determine the final location of *E. coli* and the tracers. Soil surrounding the inner 1 m x 1 m plot was excavated down to a depth of 50 cm, leaving the inner 50 cm x 50 cm x 50 cm block intact. A 50 cm x 50 cm grid was laid down over the surface of the soil to determine spatial locations of the samples. Dyed areas within each grid location were sampled along with 1-2 un-dyed locations for each depth. Samples were collected using a flame sterilized trowel and placed in a sterile 12 mL sample jar that were then transferred to a cooler. At the end of the day the coolers were placed in a 4º C fridge until analysis. Once a given depth was sampled, the top 10 cm was removed using hand trowels and the underlying surface was prepared to present a clean surface. Care was taken to avoid contact between dyed and un-dyed areas to prevent contamination. Once the surface was prepared, samples were taken in the same manner.

*Sample Analysis*

*Microbial*

All analyses performed on the soil samples used the same, limited amount of soil. Due to the limited quantities of soil, and to keep storage time to a minimum, soil was analysed for *E. coli* first. A 5 g subsample of soil was weighed out into a square dilution bottle containing 95 mL of sterile physiological saline and was vigourously shaken for 1 min. Aliquots of 1 mL, 0.1 mL, and 0.01mL were transferred to an empty sterile Petri dish. Tempered 10 % Kn-TSA was poured into the plates and allowed to set. Plates were grown for 36-48 h at 37º C, and *E. coli* were identified using a hand-held Ultra Violet light. Plate counts were adjusted for the soil gravimetric water content and are reported as colony forming units per gram of dry soil.

*Chloride*

Soil $Cl^-$ was extracted using 2:1 water: soil method. Chloride was measured using a SpectrAA 220 atomic absorption spectrometer (Varian, Canada) by reading the amount of silver that had not precipitated with $Cl^-$. Silver absorbance was measured using a Silver lamp emitting at 328.1 nm.

**Results and Discussion**

*Relative Breakthrough*

The relative breakthroughs of *E. coli* and $Cl^-$ for all three treatments are presented in Figures 1-3. All three treatments exhibited a similar pattern of breakthrough with a high concentration of tracers in the top 0-10 cm depth, along with a very slight breakthrough at lower depths. The partially wet treatment (Fig. 2) is a slight exception with low concentrations at all depths through the profile. It should also be noted that the breakthrough of $Cl^-$ was very similar to *E. coli* breakthrough for all depths in all treatments.

The high concentrations at shallow depths for all three treatments are indicative of matrix flow, whereby bacteria are transported through micropores and tortuous flow paths. This allows for close interaction between the bacteria and soil particles. Close interaction between *E. coli* and soil particles facilitates adsorption of the bacteria to the soil. This is reflected in the high concentrations at shallow depths, and the lower concentrations at deeper depths. Matrix flow transport is further supported by the relative breakthrough of $Cl^-$ in the profile. The concentration trend of *E. coli* is mirrored closely by the breakthrough of $Cl^-$ and suggests as well that the bacteria were transported in the initial depths through a discontinuous series of macropores.

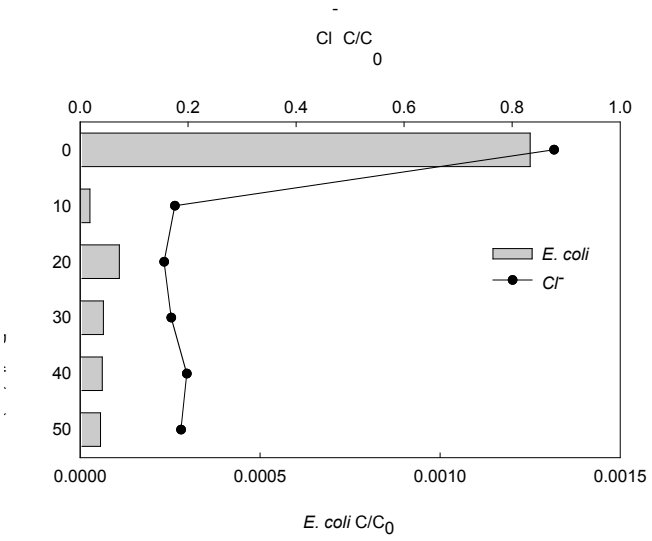


**Figure 1.** Relative breakthrough of *E. coli* and $Cl^-$ in the initially dry treatment.

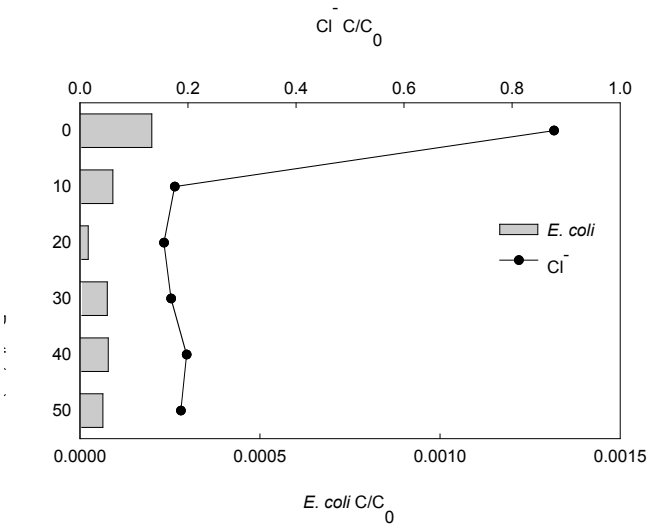


**Figure 2.** Relative breakthrough of *E. coli* and $Cl^-$ in the partially wet treatment.

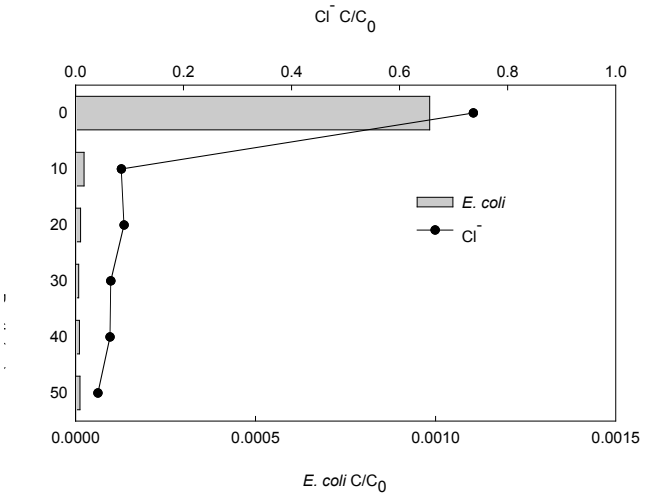


**Figure 3.** Relative breakthrough of *E. coli* and $Cl^-$ in the initially wet treatment.

E. coli: *$Cl^-$ ratios*

The ratio of *E. coli* concentration relative to $Cl^-$ concentration provides an indication of the mechanism through which *E. coli* were transported to depth and are given in Figures 4-6.

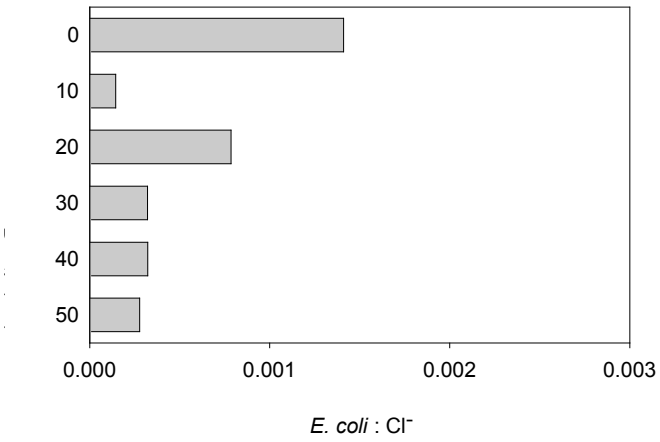


**Figure 4.** Concentration ratio of *E. coli*: $Cl^-$ for the initially dry treatment.

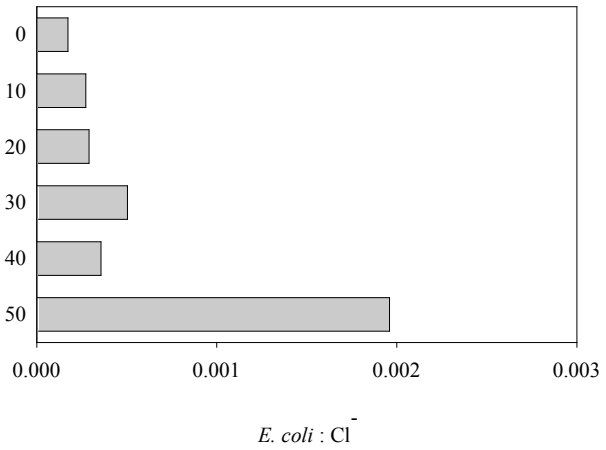


**Figure 5.** Concentration ratio of *E. coli*: $Cl^-$ for the partially wet treatment.

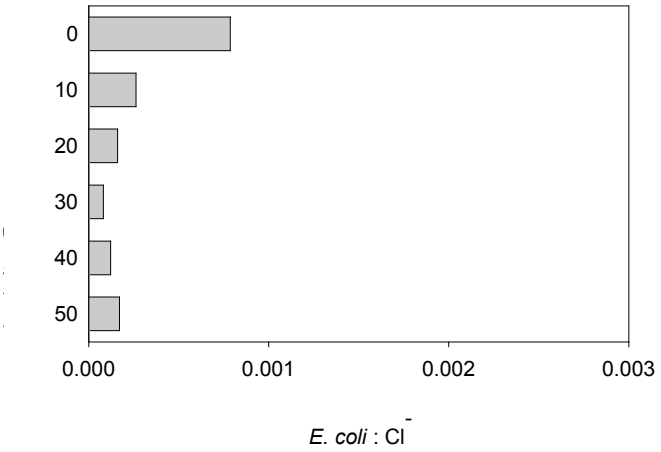


**Figure 6.** Concentration ratio of *E. coli*: $Cl^-$ for the initially wet treatment.

Relative breakthrough of *E. coli* gives an indication of where the bacteria were transported and in what concentrations. However, it does not provide much in the way of supplemental evidence as to how it was transported, especially to deeper depths. In order to elucidate the transport mechanisms of *E. coli*, the concentrations at each depth are considered relative to $Cl^-$ breakthrough and are given as a ratio. It is assumed that *E. coli* are adsorptive, and if in close enough proximity to soil particles, will adsorb to them. Chloride is assumed to be conservative and will not interact with the soil. Therefore, if the *E. coli* to $Cl^-$ ratio is consistent throughout the profile, it can be suggested that the

bacteria did not interact with the bulk soil matrix; a defining characteristic of preferential flow.

Transport through the soil matrix is evident in the top 10 cm of treatment 1 and 3 (Fig. 4 and 6). The increased ratio indicates that *E. coli* concentrations were higher suggesting interaction with the surrounding soil. A consistent ratio throughout the lower depths of the profile in all treatments is indicative of preferential transport. This suggests that *E. coli* were transported via pathways that did not allow the bacteria enough time or proximity to facilitate adsorption.

The preceding results suggest that *E. coli* were transported through the soil via a combination of mechanisms. In the top 10 cm of the profile, corresponding to the plough layer, *E. coli* were transported through the soil matrix in small pores and along tortuous paths. This led to interaction between the bacteria and soil particles and consequently to the retention of a large proportion of the *E. coli*. However, once past the top 10 cm, bacteria were funneled into active flow channels. There was very little difference between the treatments, suggesting that dry cracks and wet channels can both achieve the same result of transporting *E. coli* to substantial depths in the soil. This study has important implications for Saskatchewan where long periods of drought result in dry cracked soil. These large cracks can result in large preferential flow pathways through which bacteria can be transported when intense convective storms supply sufficient water.

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