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Saturated and unsaturated transport of cow manure-borne *Escherichia coli* through *in situ* clay loam lysimeters

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ABSTRACT

Despite relatively extensive laboratory trials, *in situ* research on transport of bacteria through layered soil is surprisingly lacking. We investigated the *in situ* transport of cow manure-borne *Escherichia coli* through a naturally layered clay loam soil profile, under saturated and unsaturated steady flows. Lysimeters (22 cm diameter and 50 cm height) were inserted vertically into the field soil. Water flow through the columns was controlled by a tension infiltrometer by imposing inlet matric suctions of 5 and 0 cm for the unsaturated and saturated conditions, respectively. When the steady-state flow was established, cow manure was applied on the lysimeters' surface at the rate of 10 Mg ha^{-1} (dry basis). Soil solution was sampled during leaching at two depths (20 and 40 cm) at 1, 2, 4, 6, 12, and 24 h after manure application. Concentrations of the bacteria in the influent (C_0) and soil solution (C) samples were determined. Bacterial filtration coefficients (λ_f) and relative adsorption indices (S_R) were calculated for each flow condition and soil depth. Flow condition, sampling depth and their interaction had significant effects ($P < 0.05$) on the C and C/C_0 values for all leaching times except at 24 h. At 24 h only the flow condition affected significantly the C and C/C_0. Flow condition and depth affected the λ_f and S_R. Maximum and minimum values of λ_f were calculated for the unsaturated condition/first depth and saturated condition/first depth combinations, respectively. The unsaturated λ_f was nearly 34% greater than the saturated λ_f. Although the topsoil had significant bacterial filtration capacity, equivalent filtration was measured for the deeper strata. Overall, variation of texture and structure along the *in situ* soil profile effectively altered the bacteria movement.

1. Introduction

Large amounts of manure are routinely applied to agricultural lands and pastures as a source of plant nutrients. Animal manures such as cow manure are improving the physical, chemical, nutritional and biological status of soils. However, their microbial and heavy metals contents may pose environmental and human health risks. Potentially pathogenic faecal microbes are carried by runoff or infiltration water, contaminating surface and groundwater resources. Strictly speaking, manuring may significantly increase bacterial transport through subsurface (e.g. Gerba and Smith, 2005) and thus increase the potential for faecal contamination of soils and waters. The transport time and distance depend on the rate at which bacteria are released from manure and soil dependent flow conditions such as the presence and extent of preferential pathways network and depth to groundwater (Abu-Ashour et al., 1994; Unc, 1999; Unc and Goss, 2004). Gerba et al. (1975) reported that faecal coliforms could travel from 0.6 m in a fine sandy loam to 830 m in a sand-gravel medium while the T4 bacteriophage travels up to 1600 m in limestone with continuous fractures.

Manures contain a huge number of microorganisms, some of which may be human pathogens (Kouznetsov et al., 2007). More than 150 different pathogens, associated with environmental and human health risks, have been found in livestock manures; *Campylobacter* sp., *Salmonella* sp., *Shigella* sp., *Listeria monocytogenes*, *Escherichia coli*, *Cryptosporidium parvum* and *Giardia lamblia*, account for more than 90% of the food and water-borne human diseases (USEPA, 2003). *E. coli*, a well-known faecal coliform, is consistently used as indicator microorganism for the risk of microbial contamination of groundwater resources due to its simple detection and its high numbers in agricultural wastes (Foppen and Schijven, 2006). *E. coli* is a gram-negative, mobile, aerobic or facultative anaerobic bacterium with cell diameter of 1–6 μm (Jawetz et al., 2001). The extensively applied cow manure is a significant source of pathogenic serotypes of *E. coli* (Jones, 1999).

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Transport and fate of bacteria are affected by cell properties, and the characteristics of the porous medium and transporting solution (Abu-Ashour et al., 1994; Becker et al., 2004; Beven and Germann, 1982; Huysman and Verstraete, 1993; Jewett et al., 1999; Lindqvist and Bengtsson, 1991; Mosaddeghi et al., 2009; Powelson and Mills, 1998). Governing soil characteristics include texture, with an accent on the clay content, structure, pore space, and pore size distribution, water content, organic matter content, solution ionic strength, pH and metal oxide coatings (Abu-Ashour et al., 1994; Fontes et al., 1991; Wan and Wilson, 1994). Transport of bacteria is mostly passive, determined by the presence of rapid water fluxes, although cell motility can play a role in transfer over short distances. Therefore, the path taken by water, infiltration or surface runoff, determines the transport direction of the manure bacteria (Unc and Goss, 2004). Several of these soil factors also affect the survival of faecal bacteria. Generally, water availability (i.e. soil water potential) overrides the impacts of other factors (Gerba and Bitton, 1984; Mubiru et al., 2000) and can obscure their significance (Unc and Goss, 2003). Tate (1978) found *E. coli* survival to be the highest in waterlogged organic soils. Hagedorn et al. (1978) observed the highest populations of *E. coli* after a rise in the water table following major rainfall events. Mubiru et al. (2000) linked lower mortality rates of *E. coli* O157:H7 to high soil matric potentials. Entry et al. (2000) correlated increased survival of faecal bacteria with increased soil water in grass buffer strips receiving swine waste. On the contrary, limited soil water availability reduces the survival rates of enteric bacteria in the manure-amended soils, although quantitative information on the issue is still lacking (Jamieson et al., 2002).

Generally transport fluxes in soil involve both vadose and saturated zones. Although the literature reports many field and laboratory experiments on the bacterial transport, most focus on the saturated zone transport (Jiang et al., 2007). Unsaturated transport is more complicated (Schafer et al., 1998) and has greater potential to remove bacteria from the flowing soil water (Chu et al., 2003; Lance and Gerba, 1984; Lenhart and Saiers, 2002; Mosaddeghi et al., 2009). Powelson and Mills (2001) found that unsaturated flows transported significantly fewer bacteria than saturated flows.

Due to such intrinsic variability in the transport of faecal bacteria transport mechanisms need to be studied at both lab and field scales. In early research, well-controlled bacteria transport studies were conducted in columns filled with either glass beads or homogenized sediments. Because homogenization and repacking processes destroy the natural soil structure (i.e. physical and chemical heterogeneity), results obtained with repacked cores may not accurately represent the transport behaviour of bacteria in the subsurface (Harvey et al., 1997). For example, Thiagarajan et al. (2007) revealed that tillage disrupts preferential flow pathways and attenuate bacterial transport in soil. It is believed that topsoil minimizes contaminant transport due to annual tillage-induced disturbance and thus greater pore tortuosity and discontinuity. Therefore, it is difficult to extrapolate results of laboratory experiments conducted on repacked soils to field conditions (Dong et al., 2002). Such extrapolation requires the consideration of additional factors, such as the effects of three-dimensional flow fields, soil layering, multiple scales of heterogeneity, interactions with native microbial communities, and temporal variations due to the transient nature of most environmental parameters. These factors usually confound the straightforward extension of laboratory results to field-scale predictions (Ginn et al., 2002; Jiang et al., 2005). Although controlled-system models are necessary to understand the mechanistic details of microbial transport, field tests are required to determine whether large-scale physicochemical or complex environmental stochastics may overwhelm mechanisms as described at laboratory scale. A limited number of *in situ* bacterial transport experiments have been reported in the literature. Unc and Goss (2003) used a rainfall simulator to study the *in situ* transport of manure-borne *E. coli* through the soil profile emphasizing the impact of preferential flow pathways through the continuous macropores along the soil profile.

Often when dealing with groundwater contamination issues, it is important to understand the local soil hydraulic properties. Thus, the development of *in situ* techniques to determine both the saturated and unsaturated hydraulic properties of soils has received increasing attention in the recent years, particularly for the challenging assessment of the role of preferential flow pathways (Angulo-Jaramillo et al., 2000) under various soil management practices. The tension infiltrometer was designed to measure the unsaturated flow of water into soil rapidly, accurately and easily; the extent of macropore flow is controlled by applying water to the soil at matric potentials less than 0 (Kirkham, 2005).

In bacterial transport experiments, a hanging tube or vacuum pump has been usually used to control the matric suction from the bottom of the column (Jewett et al., 1999; Mosaddeghi et al., 2009; Schafer et al., 1998). However, the hanging tube method may cause some post-leaching mixing while dispersion in the siphon and the fluctuation of pump suction is a matter of concern. Jiang et al. (2005, 2006, 2007) used the tension infiltrometer to conduct bacterial transport experiments in a lysimeter, in intact soil columns, and in sand columns, respectively. The virtues of this setup are the well-controlled water flow and soil matric suctions, continuously monitored inlet water potential, infiltration rate measurements, and reproducibility.

The objective of our study was to investigate the fate of cow manure-borne *E. coli* through an *in situ* soil profile, under unsaturated and saturated steady-state flow conditions. Water flow and matric potential in the soil surface were controlled using a tension infiltrometer.

2. Materials and methods

2.1. The study site and soil properties

We conducted the experiments in the autumn of 2007 on a clay loam soil at the Agricultural Research Centre (34°51′N, 48°32′E), 5 km north of Hamadan city, Hamadan province (western Iran). The region has a semi-arid climate, with long-term average annual precipitation of 328 mm most of which occurs from November to April. Mean annual temperature is 10 °C.

The soil is classified as Typic Haploxerepts (Soil Survey Staff, 2006) with a clay loam texture (CL) in both surface and subsurface layers. The subsurface layer had a greater clay content (Table 1). The surface layer (A_p, 0–30 cm) has a blocky structure with a high density of fine roots. The subsurface layer (30–75 cm) is a B horizon (B_1) with 5–10% carbonate content with strongly sub-angular blocky structure with fine and very fine blocks. Potato (*Solanum tuberosum*) and Alfalfa (*Medicago sativa*) have been cultivated at the site for the preceding few years with irregular rotations.

Disturbed and small undisturbed core samples taken from the surface and subsurface layers were used for routine soil characterization. Particle size distribution was determined using the hydrometer method. Particle density was measured using the pycnometer method. Bulk density (BD) was measured on the undisturbed soil cores of 5 cm diameter and 7.5 cm height. Total porosity was calculated from the BD and particle density. Mean weight diameter (MWD) of soil aggregates was determined using the wet sieving method. Saturated hydraulic conductivity (K_s) was measured using the constant-head procedure on the mentioned cores (Klute, 1986). Saturated water content (θ_s) and volumetric water content (θ_v) of the soil layers were determined by setting the