

Transport and deposition of *Escherichia coli* O157:H7 and *Enterococcus faecalis* in three Italian soils

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Abstract

A series of column experiments was conducted under saturated flow conditions to investigate the factors affecting *Escherichia coli* O157:H7 and *Enterococcus faecalis* transport in three Italian soils. Breakthrough curves (BTC) differed according to the bacteria species and soils. *E. coli* moved faster through the columns, most likely due to size exclusion phenomena, which constrained the cells to more conductive flow domains and large pore networks. Macroporosity seems to be a key factor for identifying the vulnerability of groundwater resources to microbiological contamination.

Key Words

Bacterial transport, soil columns, saturated flow, macroporosity.

Introduction

Increasing livestock densities have compounded the environmental contamination problems associated with disposal of the resulting manure. A lot of effort has been made in Italy to control nitrate water pollution, but little is known about the potential microbiological contamination risks coming from manure distribution. Indeed manure and biosolids contain many pathogenic microorganisms, such as *Cryptosporidium*, *Giardia*, *Escherichia coli* O157:H7, *Enterococcus faecalis*, *Salmonella*, and some viruses (e.g., rotavirus). Microbial contamination of groundwater is a serious problem that can result in major outbreaks of waterborne diseases. Predicting the transport and fate of pathogenic microorganisms is therefore required to delineate areas vulnerable to microbiological contamination and protect groundwater resources. The objective of this work was to investigate the transport and deposition behavior of *E. coli* O157:H7 and *Enterococcus faecalis* in three Italian soils.

Methods

Breakthrough (BTC) experiments were carried out on nine undisturbed soil cores (internal diameter 6 cm, length 15 cm) collected in three soils (three replicates per soil) located in NE Italy within two densely populated livestock areas. Soils were clay loam (S1), sandy clay loam with 30% of gravel (diameter of 2-10 mm) (S2) and sandy loam (S3). Prior to BTC experiments, cores were sterilized by tyndallization (10 min at 75 °C for 3 days) and then subjected to capillary saturation with sterilized deionized water. A mixed bacterial suspension was prepared at concentrations of approximately 10⁷ CFU /mL of *E. coli* O157:H7 and 10⁸ CFU/mL of *Enterococcus faecalis*. Br⁻ (500 mg/L) was added to the suspension as conservative tracer. BTC experiments were conducted under saturated flow conditions pumping 3 pore volumes (PVs) of suspension and then 2 PVs of sterilized water. Bacteria effluent concentrations were measured by membrane filtration, serial dilution and plate counting in mFC agar. Deposition of the bacteria at five depths along the columns was measured at the end of the experiments. Adsorption of bacteria cells to the soils and inactivation rate in the liquid were measured in batch experiments. To evaluate the potential effect of soil structure on bacteria transport, soil porosity distribution (0.007 µm-3 mm) and specific surface were measured by mercury intrusion and N₂ adsorption at 77 K, applying the BET equation, respectively.

Results

BTCs differed according to bacteria species and soils. *E. coli* relative concentration in the effluent was higher than *E. faecalis* (Figures 1-2): on average a peak of 0.72 was observed at 3 PVs in S1 while in S2 a first peak of 0.94 at 0.7 PVs was followed by a second one at 2.3 PVs (Figure 1). *E. coli* breakthrough was slightly accelerated relative to Br⁻, most likely due to size exclusion phenomena which constrained the cells to more conductive flow domains and large pore networks, which were physically accessible (Bradford *et al.* 2006). Slower mobility was observed for *E. faecalis*, especially in S1 where the relative concentration increased steadily and slowly up to 0.063 at 4.3 PV (Figure 2), suggesting the blocking of favorable

attachment sites (Tong *et al.* 2005) and/or filling of straining sites (Bradford *et al.* 2006). No cell breakthrough was instead observed in S3 for either bacteria species, which were transported in the soil column only up to a depth of 7.5 cm from the top surface. Bacteria recovery in the leachate was higher for *E. coli*, with more than 30% and 70% in S1 and S2, respectively, while recoveries of *E. faecalis* were 5% and 40%. The higher transport in S2 could be associated to the presence of a relevant macropore system (diameter range 200-3000 μm), that according to intrusion analysis represented 13% of the overall porosity.

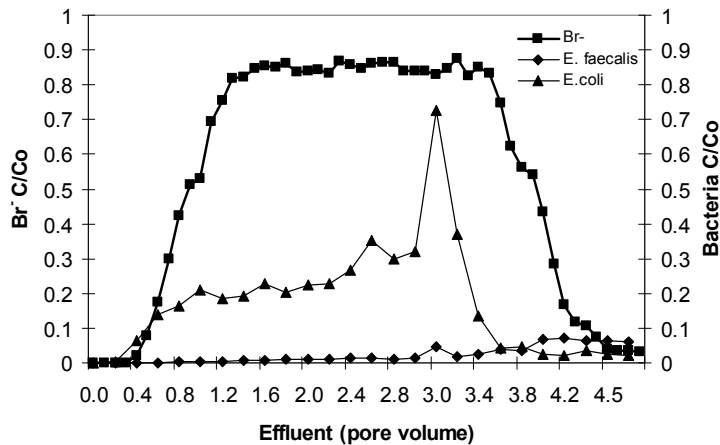


Figure 1. Effluent concentration curves for Br-, *E. coli* and *E. faecalis* in the clay loam soil (S1).

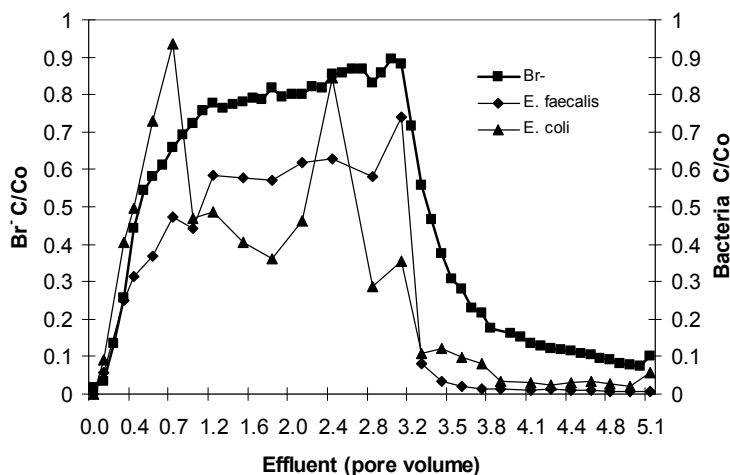


Figure 2. Effluent concentration curves for Br-, *E. coli* and *E. faecalis* in the sandy clay loam (S2).

Conclusion

Column experiments have demonstrated that the interaction between bacteria species and soil type can affect microbiological transport. Macroporosity appears to be a key factor for identifying the vulnerability of groundwater resources to microbiological contamination. Further experiments are needed to confirm this evidence at larger scales.

References

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