E. coli transport in soil columns: implications for reuse of treated wastewater in irrigation

E. Smith and S. Hegazy

Department of Construction Engineering, The American University in Cairo Cairo 11511, Egypt
(E-mail: *edsmith@aucegypt.edu*)

Abstract Reuse of treated wastewater in irrigation is gaining recognition as a vital element in the water resources management plan of developing countries, especially those situated in arid and semi-arid regions. An understanding of the transport of residual pollutants from treated wastewater, such as bacteria, in soil as a result of irrigation is critical to assessing health risks and the possible contamination of limited groundwater resources. In this work, retention of *E. coli* is evaluated for a soil that is irrigated by treated wastewater for growth of non-food crops near Egypt's Red Sea coast. In particular, the effects of soil organic fraction (SOF) and hydraulic loading rate (HLR) were investigated in laboratory soil columns. The matrix of experiments included three HLRs and three SOFs. The retention of bacteria by adsorption was observed at HLRs of 5 and 13 cm/h, with the magnitude of the adsorption increasing proportionally to the SOF. The impact of SOF was greater for the lower HLR. At the lowest HLR investigated (5 cm/h), filtration was also observed for the two higher SOFs (0.674 and 2.04 per cent). At a high HLR (66 cm/h) simulating flood irrigation, retention of bacteria was minimal regardless of the SOF. Since the bacterial solution is applied to a dry soil column to simulate field conditions, *E. coli* breakthrough after two pore volumes of throughput (vs. one) provided a meaningful comparison of bacterial retention as a function of HLR and SOF.
Keywords *E. coli*; soil columns; soil organic content; wastewater reuse

Water Science & Technology **Vol 54 No 11–12 pp 175–182** © IWA Publishing 2006

Introduction

Reuse of treated wastewater in irrigation is gaining recognition as a vital element in the water resources management plan of developing countries, especially those situated in arid and semi-arid regions. While several countries in the arid region of the Middle East and North Africa engage in wastewater treatment and reuse, only Israel and Tunisia, and to a certain extent Jordan, have truly integrated this practice into their water management and environmental protection strategies (UN-WHO, 1989; 2000). In Egypt, for instance, just over 20% of the total wastewater is treated, and less than 6% is reused (UN-FAO, 2000). Major concerns in reuse of treated wastewater in irrigation include potential threats to public health and contamination of groundwater resources. An understanding of the transport of residual pollutants from treated wastewater, such as bacteria, in soil as a result of irrigation is critical to assessing the actual risks associated with these concerns.

Investigations on the effects of sediment phase organic matter (SOM) and dissolved phase organic matter (DOM) on the transport of bacterial and viruses in soil water have produced mixed results (Powelson *et al.*, 1991; Johnson and Logan, 1996). In general, DOM may decrease microbial retention by altering the surface charge characteristics of organisms or via competition for adsorption sites, while SOM tends to increase adsorption (Johnson and Logan, 1996; Pieper *et al.*, 1997; Rogers and Logan, 2000). Modeling of bacterial transport has frequently utilized the advection-dispersion equation with retardation terms to account for filtration and adsorption. Studies with several soil types have indicated that bacterial retardation was due mostly to adsorption rather than straining, and

doi: 10.2166/wst.2006.851

that adsorption could be characterized according to simple linear isotherms (Tan *et al.*, 1992; Loveland *et al.*, 1996).

Describing microbial transport is complicated by the fact that the contaminant is a living organism that can manifest variable rates of growth in an aqueous environment, and that growth is sensitive to the numerous background characteristics of the water–soil environment in addition to the particulars of the organisms themselves (Franklin *et al.*, 2001; Maule *et al.*, 2001). Designing experiments to delineate the relative contributions of interacting phenomena and processes in such a complex system is an ongoing challenge. In this work, the transport of *E. coli* in laboratory soil columns is evaluated for a soil that is irrigated by effluent for growth of non-food crops near Egypt's Red Sea coast. The effluent derives from sewage from an industrial community that is treated using a series of waste stabilization ponds. In particular, the effects of soil organic fraction (SOF) and hydraulic loading rate (HLR) on coliform breakthrough curves were investigated.

Methods

Bacteria

An American Type Culture *Escherichia coli* Strain (ATCC 25922 – from the Naval American Marine Research Unit in Cairo) was obtained on a MacConkey culture plate. Two types of media were used. MacConkey Agar (#401670, Biolife, Rome) was prepared and sterilized as per the manufacturer's instructions. The test tubes were then sealed with parafilm after retrieval from the sterilization unit (autoclave) and stored at 4 °C in sealed containers for a maximum of 2 months. MacConkey Broth (#401675, Biolife, Rome) was used to grow cultures 24 hours prior to each experiment. A rigorous procedure was developed to prepare reproducible broth stock solution with concentration of approximately 2×10^9 CFU/100 mL. Buffer solutions were prepared to achieve equilibrium aqueous phase concentrations in the range observed at the site in the effluent from waste stabilization ponds (10^3 to 10^4 CFU/100 mL). The buffer solutions consisted of the bacterial stock solution and dilution water prepared by dissolving Hach BOD nutrient buffer pillows (Hach, USA) in distilled, de-ionized water. Stability with respect to the initial concentration was confirmed for up to about 72 hours; even so, a new buffer solution was prepared prior to each experiment.

All bacterial enumeration was performed using the standard membrane filter technique for fecal coliforms, Method 9222 D from *Standard Methods* (1998). The only deviation was the incubation of bacteria in a thermostatically controlled air incubator rather than a water bath. A half-filled water beaker was placed in the air incubator along with the plates to maintain a high relative humidity and avoid spurious results. A comparative experiment was conducted with a water bath; statistical analysis using the *f*-test demonstrated that the data belonged to the same population within a 95% confidence interval.

Soil

A field soil sample was collected from the project site for the laboratory studies. The soil is a slightly clayey, slightly loamy sandy soil collected from the upper 15 cm of an irrigated plot at the site. Some relevant soil properties are listed in Table 1. Prior to experimental investigations, the soil was sieved through a U.S. Standard No. 10 sieve to remove rocks and small stones. Tests for background levels of bacteria revealed a masking of fecal coliforms by numerous other unidentified soil bacteria. Therefore, the soil was sterilized, cooled, and placed in sterilized polyurethane containers until use. Two additional organic fractions were prepared from the base sample having an organic content of 0.67 ± 0.04 per cent. One lot with organic content less than this value were made ready by several washings with distilled water and gentle shaking. The coloration

Table 1 Properties of test soil

Parameter	Units	Value
Bulk density[a]	g/m^3	1.48 ± 0.039
Particle density[b]	g/m^3	2.48 ± 0.044
Porosity	%	40.3 ± 0.88
Volumetric water content	%	1.12 ± 0.01
Average organic fraction[c]	%	0.674 ± 0.04

[a]Average of five measurements from core sample of undisturbed top soil
[b]Calculations from gravimetric method outlined by Tindall and Kunkel (1999)
[c]Hach DR/2000 Method

of the aqueous phase, which diminished according to the number of washings, indicated the elution of organic matter from the sample. The remaining lot, or "high organic fraction," was obtained using uncomposted horse manure. Careful preparation and blending of the material was observed to eliminate extraneous microbial growth and produce a well homogenized soil for subsequent experiments. The manure was first oven dried at 105 °C for 3 hours, sieved through a U.S. Standard No. 10 sieve to remove larger particles and straw fibers, then autoclaved 3 times at 120 °C for 15 minutes in a glass container. Three samples were cultured on MacConkey agar at 37 °C, but no microbial growth was observed. Blending with soil was by a sterile glass rod rather than a mechanical blender so as to not affect the soil texture. Five samples of each soil lot were analyzed for their organic content with final values of soil organic fraction (SOF) 0.049, 0.674, and 2.04 per cent. Standard deviations ranged from 3 to 7 per cent of the respective values.

Soil column experiments

API drinking water grade PVC pipe (6.8 cm internal diameter) was used for the columns. To support the soil in the column, a perforated polyethylene disk (6 mm thick, 4 mm diameter openings) was mounted using circumferential PVC clips. A piece of 100 mesh stainless steel screen (150 μm openings) was placed on top of the disk to retain soil in each column. The assembly was tightly sealed in place by mounting another PVC clip on top. A polyurethane funnel was tightly fitted into the bottom of each column to feed effluent into test-tubes mounted in an autosample collector. A schematic of the assembly is given in Figure 1.

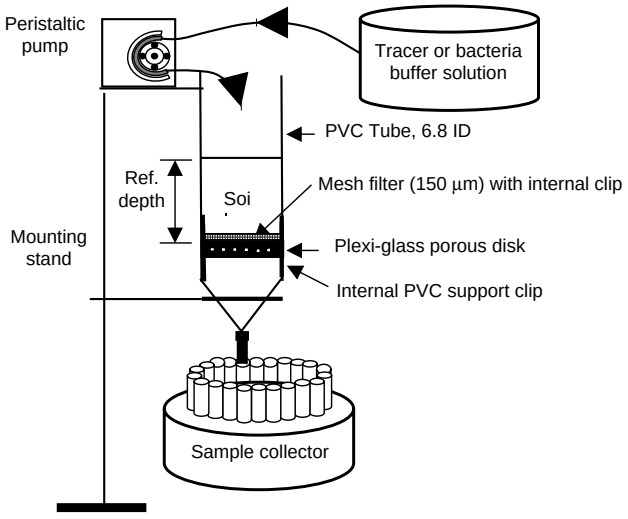

Figure 1 Soil column setup

Employing techniques described in the literature, caution was exercised in preparing the columns such that the column bulk density be equivalent to that of the field soil and to prevent soil layering and the creation of macropores (Abu-Ashour *et al.*, 1994; Powelson and Mills, 2001). A 5 cm soil column depth was considered sufficient to contain the primary bacterial accumulation. The mass of soil required to achieve the correct bulk density was carefully weighed and added incrementally to the (PVC) column. After each cm addition, the contents were lightly tamped with a piston of external diameter approximating the internal diameter of the column. This was accompanied by careful stirring with a sterilized pipette to maximize homogenization of the soil in the column. Once the entire amount of soil was placed, the piston was used to tamp to the desired height to achieve the desired bulk density. The volume of a 5 cm deep column, accounting for the clips, was 170.95 cm^3. To achieve the field bulk density of 1.48 g/cm^3, a soil mass of 253.05 g was used in each test column.

A 3 litre capped polyethylene bottle contained the working solution, which was conveyed using a variable-flow peristaltic pump with polyethylene tubing. The discharge from the supply tube was 3 cm above the top of the soil column which was covered with a stainless steel mesh screen that served to spread the flow over the column area better than when no cover was used; thus dead spaces near the flow entrance, channeling, and ponding of water in the top center of the soil column were minimized. A comprehensive sterilization procedure was carried out for every item in the column setup prior to each run. At the conclusion of each experiment, the inflow pump was stopped, the effluent feed funnel disconnected, and the column immediately placed on a measuring plate. The column and measuring plate were weighted together in order to obtain the steady state water content for each run.

For each of the three soil organic fractions, column experiments were conducted for three different hydraulic loading rates (HLR) used in Egyptian agricultural practice: (1) 5 cm/h (3 mL/min) simulating unsaturated flow at a relatively low HLR; (2) 13 cm/h (8 mL/min) corresponding to unsaturated flow at a higher delivery rate; and, (3) 66 cm/h (40 mL/min) simulating ponded flow or "flood" irrigation. Flood irrigation is still the most common used in Egypt. Schemes such as subsurface trickle irrigation are practiced to a limited degree, but were not addressed in this work. A tracer column experiment was performed for each HLR using bromophenol blue with "as is" soil (i.e. SOF = 0.674 per cent) as a baseline case and for characterizing dispersive transport. Bromophenol blue concentrations in effluent samples were measured by visible light spectroscopy using a DR 2000 spectrophotometer (Hach) and employing a four-point calibration curve.

Results and discussion

Water saturation measurement and calculations for the 12 column runs depicted in subsequent Figures 2–5 are presented in Table 2, keeping in mind that pore volume in the column (68.9 cm^3) corresponds to the porosity of the field soil (40.3 per cent) based on achieving the field bulk density in each experimental column. The results suggest that a fairly consistent packing and a hydraulic pattern were achieved for respective HLRs. Average volumetric water content was 23.48, 32.13, and 39.29 per cent, respectively, for HLRs of 5, 13, and 66 cm/h. In other words, the high loading rate indeed simulates ponded irrigation with essentially complete saturation of the soil column, while the other two HLRs result in proportional saturation levels.

While plate counts for determination of fecal coliform concentrations are more time and material intensive than photometric measurements, this technique precludes potential interferences or leaching of soil matter that may make anomalous contributions to turbidity. The multiple plate measurements (three) for each sample enable assessments of the

Table 2 Volumetric water content for column experiments

Run	Case	HLR (cm/h)	SOF (%)	Column + dry sand (g)	Column + wet sand (g)	Mass water (g)	Vol. water content (%)
1	Tracer	5	0.674	497.34	533.78	36.44	21.32
2	Tracer	13	0.674	496.69	551.73	55.04	32.20
3	Tracer	66	0.674	493.27	562.14	68.87	40.29
4	*E. coli*	5	0.049	495.81	536.79	40.99	23.98
5	*E. coli*	13	0.049	494.68	552.29	57.61	33.70
6	*E. coli*	66	0.049	495.56	562.61	67.05	39.22
7	*E. coli*	5	0.674	495.81	536.38	40.58	23.74
8	*E. coli*	13	0.674	494.68	546.90	52.22	30.54
9	*E. coli*	66	0.674	495.56	559.04	63.48	37.14
10	*E. coli*	5	2.04	495.81	538.36	42.55	24.89
11	*E. coli*	13	2.04	494.68	549.51	54.83	32.08
12	*E. coli*	66	2.04	494.56	564.77	69.21	40.49

precision of the data points and confirmation of the reproducibility of the procedure for preparing the buffer solution. For instance, the target initial concentration in the bacterial column runs was 3×10^4–4×10^4 CFU/100 mL. For the case of the soil "as is" (SOC = 0.67%), and HLR = 5 cm/h, the three plate counts from the buffer solution at the start and end of the experiment ranged from 32 to 35 and 30 to 37, respectively. When accounting for dilution, this resulted in an average C_o of 3.35×10^4 CFU/100 mL with a standard deviation of 504 CFU/100 mL.

Coliform breakthrough data as a function of SOF are presented for three HLRs in Figures 2–4. The designations "Low OF," "Normal OF," and "High OF" refer to the SOF per cent values 0.049, 0.674 ("as is" soil), and 2.04, respectively given previously. The solid curve in each of these figures is a best-fit model curve of the tracer data using CXTFIT, version 2.1 code (Toride *et al.*, 1999). As observed from the tracer curves, there is measurable dispersion in the columns, with dispersion coefficients being on the order of 10 cm^2/h. The dispersion is also observable in the bacterial breakthrough data.

When considering the different timescales, there is significant retention of *E. coli* in the soil for HLRs of 5 and 13 cm/h, with the retention greatest for the lower flow rate. This retardation is normally attributed to adsorption of bacteria to the soil, especially when the breakthrough curve follows the pattern of the tracer curve to a steady state effluent concentration of $C/C_o = 1$, but is simply displaced on the time or throughput scale. One major difference between the HLR = 5 cm/h (Figure 2) and HLR = 15 cm/h (Figure 3) cases is that for the lower rate, steady state is achieved at a breakthrough

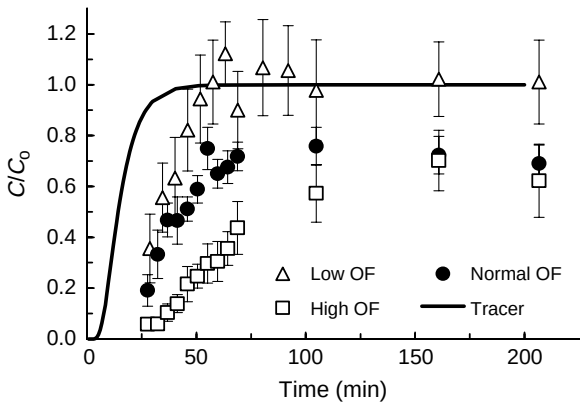

Figure 2 *E. coli* breakthrough as a function of soil organic fraction at HLR = 5 cm/h

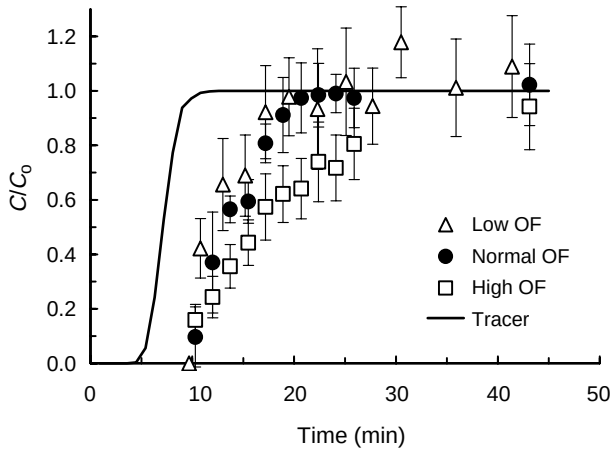

Figure 3 *E. coli* breakthrough as a function of soil organic fraction at HLR = 13 cm/h

concentration of $C/C_o = 0.6–0.7$ for the normal and high organic fractions. This is likely due to straining/filtration mechanism occuring at lower application rates. However, this appears to be tied to some extent to the SOF, since no straining is observed at the low fraction. Further investigations are required to determine the relationship of these mechanisms as well as possible effects of modifications to the pore/packing structure based on the methods used to modify the SOF.

Figures 2 and 3 indicate that *E. coli* retardation due to adsorption increases with an increase in SOF, a result consistent with bacterial transport studies in sediments (Pieper *et al.*, 1997; Rogers and Logan, 2000). The lower the HLR, the more pronounced the effect. In fact, Figure 4 illustrates that at very high loading rates the SOF does not impact transport, likely due to excessive pore velocity inhibiting sorptive attachment of bacteria to the soil.

Many investigations of bacteria transport in unsaturated soils establish a constant water saturation in columns prior to introduction of bacteria. However, this condition rarely exists in irrigation of well drained soils in Egypt. Therefore, in this work the buffer solution of *E. coli* was applied to dry soil to more closely simulate field conditions where the irrigated soil would contain, at most, only residual water content. For this reason it was normally not possible to obtain aqueous samples during the first pore volume (PV) of throughput, as given in Figure 5, in which the dimensionless throughput variable

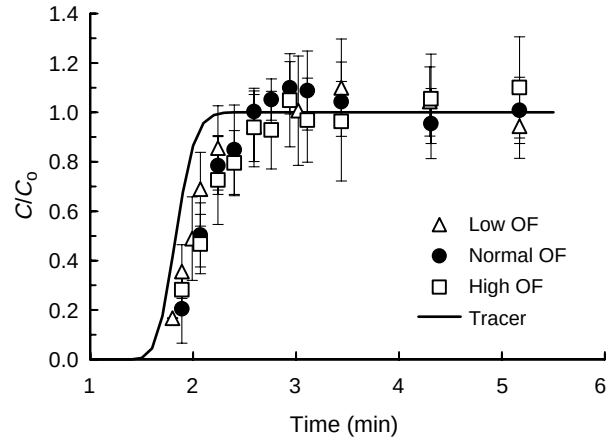

Figure 4 *E. coli* breakthrough as a function of soil organic fraction at HLR = 66 cm/h

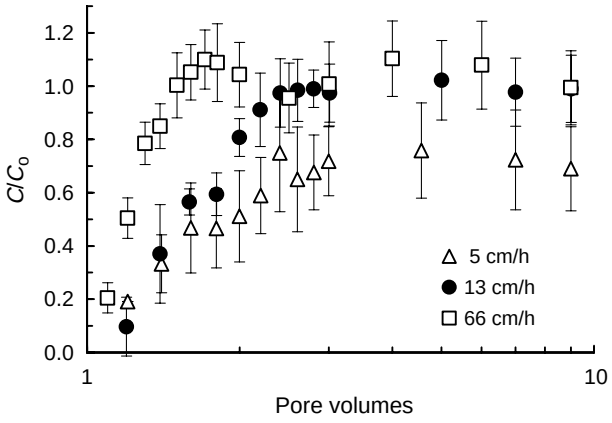

Figure 5 *E. coli* breakthrough as a function of HLR for "normal" organic fraction

illustrates more clearly the increased retention of bacteria at lower HLR. Figure 6 summarizes the findings by depicting the relative breakthrough concentration at 2 PVs of throughput, at which point the saturation condition in the column is assumed to be more stable than during application of the first PV to a dry column (Tindall and Kunkel, 1999). With data for additional HLRs, Figure 6 could yield the practical threshhold of HLR for a given soil; below which workers would have to be concerned about exposure to bacteria in the top layer of soil, and above which bacteria would rapidly leach through the soil and pose a potential pathway to groundwater contamination.

One of the next steps in this analysis is to model the *E. coli* breakthrough data using a code such as CXTFIT in order to quantify mass transport coefficients for adsorption and filtration. This will enable simulation of bacterial transport in field applications, enable identification of the most critical system parameters for engineering control, and facilitate development and assessment of guidelines for the resuse of treated wastewater for different soils under local conditions. First, however, additional data is required to characterize bacteria growth that occurs during the time scale of the event. Preliminary evidence suggests that this is considerable in certain cases and a function of soil properties including SOF (Badawy, 2005). Other soils and the impacts of variables such as ionic strength of the buffer solution on bacterial retention should also be investigated (Schäfer *et al.*, 1998).

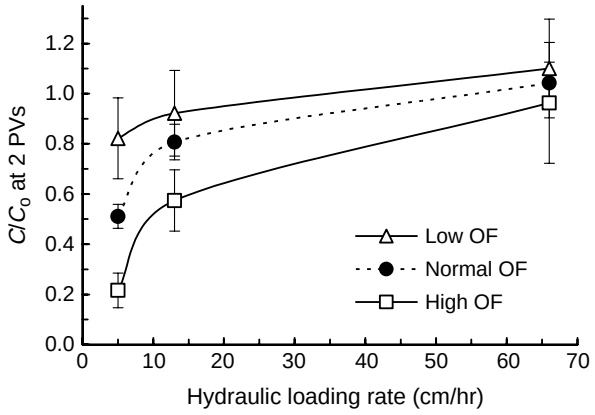

Figure 6 Effect of HLR on bacterial transport at three soil organic fractions

Conclusions

Transport of an *E. coli* strain in a sandy soil that is irrigated with effluent from treated sewage in an arid coastal environment was investigated in laboratory soil columns. The matrix of experiments included three HLRs and three SOFs.

1. The retention of bacteria by adsorption was observed at HLRs of 5 and 13 cm/h, with the magnitude of the adsorption increasing proportional to the SOF. The impact of SOF was greater for the lower HLR.
2. At the lowest HLR investigated (5 cm/h), straining/filtration was also observed for the two higher SOFs (0.674 and 2.04 per cent).
3. At a high HLR (66 cm/h) simulating flood irrigation, retention of bacteria was minimal regardless of the SOF.

References

Abu-Ashour, J., Joy, D.M., Lee, H., Whiteley, H.R. and Zelin, S. (1994). Transport of microorganisms through soil. *Wat. Air & Soil Poll.*, **74**, 141–158.

Badawy, A (2005). Unpublished data. The American University in Cairo.

Franklin, R.B., Garland, J.L., Bolster, C.H. and Mills, A.L. (2001). Impact of dilution on microbial community structure and functional potential: comparison of numerical simulations and batch culture experiments. *J. Applied Environ. Microbiol.*, **67**, 702–712.

Johnson, W.P. and Logan, B.E. (1996). Enhanced transport of bacteria in porous media by sediment-phase and aqueous-phase natural organic matter. *Wat. Sources Res.*, **30**, 923–931.

Loveland, J.P., Ryan, J.N., Amy, G.L. and Harvey, R.W. (1996). The reversibility of virus attachment to mineral surfaces. *J. Colloids and Surfaces*, **1**, 205–221.

Maule, A., Keevil, C.W., Walker, J.T. and James, B.W. (2001). Persistence and physiology of *Escherichia coli* O157:H7 in the environment. Unpublished paper. Center of Applied Microbiology and Research, Salisbury, UK.

Pieper, A.P., Ryan, J.N., Harvey, R.W., Amy, G.L., Illangasekare, T.H. and Metge, D.W. (1997). Transport and recovery of bacteriophases PRD1 in a sand and gravel aquifer:effect of sewage-derived organic matter. *Environ. Sci. Technol.*, **31**, 1163–1170.

Powelson, D.K. and Mills, A.L. (2001). Transport of *Escherichia coli* in sand columns with constant and changing water contents. *J. Environ. Qual.*, **30**, 238–245.

Powelson, D.K., Simpson, J.R. and Gerba, C.P. (1991). Effects of organic matter on virus transport in unsaturated flow. *J. Applied Environ. Microbiol.*, **57**, 2192–2196.

Rogers, B. and Logan, B.E. (2000). Bacterial transport in NAPL-contaminated porous media. *J. Environ. Engrg.*, ASCE, **126**, 657–666.

Schäfer, A., Ustohal, P., Harms, H., Stauffer, F., Dracos, T. and Zehnder, A.J.B. (1998). Transport of bacteria in unsaturated porous media. *J. Contam. Hydrol.*, **33**, 133–148.

Standard Methods for the Examination of Water and Wastewater (1998). 20th edn, APHA/AWWA/WEF, Washington, D.C., USA.

Tan, Y., Bond, W.J. and Griffin, D.M. (1992). Transport of bacteria during unsteady unsaturated soil water flow. *J. Soil Sci. Soc. Am.*, **56**, 1331–1340.

Tindall, J.A. and Kunkel, J.R. (1999). *Unsaturated Zone Hydrology for Scientists and Engineers*, Prentice-Hall, New Jersey.

Toride, N., Leij, F.J. and van Genuchten, M. Th (1999). *The CXTFIT Code for Estimating Transport Parameters from Laboratory or Field Tracer Experiments*. U.S. Salinity Laboratory, Report No. 137, U.S. Dept. of Agriculture, Riverside, CA.

United Nations Food and Agriculture Organization (UN-FAO) (2000). *World Water Statistics, AQUASTAT-2000*, FAO: Rome.

United Nations World Health Organization (UN-WHO) (1989). *Guidelines for the Safe Use of Wastewater and Excreta in Agriculture and Aquaculture: Measures for Public Health Protection*, WHO: Geneva.

United Nations World Health Organization (UN-WHO) (2000). *Reducing the Health Risks of Using Wastewater in Agriculture: Recommended Changes to WHO Guidelines*, WHO: Geneva.