

# Microbial Transport and Fate in the Subsurface Environment: Introduction to the Special Section

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## Abstract

Microorganisms constitute an almost exclusive form of life in the earth's subsurface environment (not including caves), particularly at depths exceeding the soil horizon. While of broad interest to ecology and geology, scientific interest in the fate and transport of microorganisms, particularly those introduced through the anthropogenic environment, has focused on understanding the subsurface environment as a pathway for human pathogens and on optimizing the use of microbial organisms for remediation of potable groundwater. This special section, inspired by the 2014 Ninth International Symposium for Subsurface Microbiology, brings together recent efforts to better understand the spatiotemporal occurrence of anthropogenic microbial groundwater contamination and the fate and transport of microbes in the subsurface environment: in soils, deep unsaturated zones, and within aquifer systems. Work includes field reconnaissance, controlled laboratory studies to improve our understanding of specific fate and transport processes, and the development and application of improved mechanistic understanding of microbial fate and transport processes in the subsurface environment. The findings confirm and also challenge the limitations of our current understanding of highly complex microbial fate and transport processes across spatiotemporal scales in the subsurface environment; they also add to the increasing knowledge base to improve our ability to protect drinking water resources and perform in situ environmental remediation.

## Core Ideas

- Brings together recent research on microbial occurrence, fate, and transport in the subsurface.
- Papers include field reconnaissance, controlled laboratory studies, and mathematical models.
- Work adds to the knowledge base to protect water resources and perform environmental remediation.
- Findings identify critical knowledge gaps, conditions of increased risks, and model limitations.

**T**HE SUBSURFACE ENVIRONMENT is rich in microbial life forms, diversity, and intensity (Fredrickson and Fletcher, 2001; Pepper et al., 2014). An understanding of the transport and fate of microorganisms (viruses, bacteria, and protozoa) in the subsurface environment is needed for protection of public health at the interface between the environment and industrial, urban, and agricultural systems and for better understanding applications that utilize microbial systems. For example, the delivery or stimulation of beneficial microorganisms at specific locations in the subsurface can potentially be exploited in bioremediation and bioaugmentation (Mishra et al., 2001; Vidali, 2001) and microbially enhanced oil recovery (Banat, 1995). Conversely, pathogenic microorganisms pose a risk to human health through recreational exposure in surface water and contamination of drinking water and food supplies (Embrey and Runkle, 2006; WHO, 2011). Groundwater serves half of the global population as the primary source for drinking water and provides 43% of irrigation water including for fresh produce in many parts of the world with little or no additional treatment (WHO, 2011; Maupin et al., 2014; UNWWAP 2015). Surface water and wastewater with microbial contamination is frequently treated, incidentally or intentionally, to remove pathogens by passage through porous media during riverbank filtration, sand filtration, managed aquifer recharge, and infiltration (Chittaranjan et al., 2003; Kazner et al., 2012). The implicit assumption is that pathogens in groundwater resources can be sufficiently removed or inactivated during passage through the vadose zone and aquifer. However, indicator microorganisms as well as pathogens have frequently been found to occur in surveys of shallow and deep groundwater wells (WHO, 2011; Borchardt et al., 2007), and waterborne and foodborne disease outbreaks have been linked to microbial contamination of groundwater supplies (Steele and Odumeru, 2004; Craun et al., 2010; Borchardt et al., 2011; UNWWAP, 2015).

Considerable amounts of research have been directed toward the topic of microbial transport and fate in the environment to minimize risks or maximize benefits of microbial migration

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**Abbreviations:** CAFO, concentrated animal feeding operation; TMV, target microorganisms and viruses.

(Schijven and Hassanizadeh, 2000; Ginn et al., 2002; Harvey and Harms, 2002; Foppen and Schijven, 2005; Pang, 2009; Bradford et al., 2013). However, many gaps in knowledge still remain for understanding and predicting the occurrence and fate of microbes due to complex biogeochemical processes that exhibit multiscale spatial and/or temporal heterogeneity, the need to link results from surrogates (e.g., indicator microbes, colloids, and nanoparticles) to pathogens, and the large number of potential transport pathways. Furthermore, different models are required for various transport pathways, and there is currently not a consensus in the literature on the proper mechanistic framework to quantify microbial fate. The integration of extensive knowledge from a myriad of rapidly evolving disciplines is needed to overcome these gaps in knowledge. New paradigms, theories, and models must be developed at different scales and for various migration pathways to solve these pressing problems.

## Overview of the Special Section

In the fall of 2014, research scientists and regulatory agency personnel met at the Ninth International Symposium on Subsurface Microbiology in Pacific Grove, CA, organized by the International Society for Subsurface Microbiology and the National Water Research Institute. This collection of 15 papers was inspired by the symposium's exposition and discussion of recent research on microbial occurrence, fate and transport in the subsurface. For convenience, the editors have grouped these papers under four subject headings: (i) pathogen sources and occurrence, (ii) laboratory studies, (iii) field studies, and (iv) mathematical models. We point out, however, that many of these papers in fact highlight elements that pertain to multiple headings. Under each subject heading, we provide a brief introduction of the research topic and then a concise summary of the objective and contributions of papers in the special collection.

### Pathogen Sources and Occurrence

Land-applied animal manure and biopiles of slaughterhouse residuals are potentially important sources of microbial pathogens in the environment. Pathogens and indicators can be released from these sources to flowing water during precipitation and irrigation events. Overland and subsurface flow of contaminated effluent can adversely affect surface water and/or groundwater quality and human health through exposure to pathogens in recreational water, drinking water, and water used to irrigate fresh produce or grow shellfish. Two contributions to the special section examine pathogen sources and their release into the environment.

Blaustein et al. (2015) provide a critical review of the literature on processes, factors, mathematical models, and knowledge gaps related to microbial release and subsequent removal from manure and animal waste application areas. Aspects considered include animal sources and animal waste composition, waste aging and treatment effects, land application methods for animal waste, microbial properties, the role of suspended solids and reattachment in runoff, and environmental conditions such as temperature, vegetation, soil, and rainfall recurrence and intensity. Cumulative release of microbes from manure with total rainfall is a nonlinear process, with an initial fast stage that is followed by a slow stage. Release and removal processes are demonstrated

to depend on many physical, chemical, and biological factors. However, relatively little is still known about the influence of rainfall intensity, topography, soil properties, and scale size on microbial release or removal from application areas.

Michitsch et al. (2015) examine the transport and fate of indicator bacteria from biopiles (static compost piles) of slaughterhouse residuals. More than 90% of the indicator bacteria load followed large precipitation events, albeit only at a small fraction (<0.01%) of the original population in biopile effluent, suggesting a significant role for retention and inactivation in biopiles while significant external controls on water entering and leaving the biopile are effective and important measures to reduce risk for environmental impacts. Loads decreased exponentially with time. Enterobacteriaceae indicators remained low in conditions of higher soil water content and lower biopile temperatures, whereas the Enterococcaceae indicator appeared to regrow in these conditions.

### Laboratory Studies

Detailed research studies on the fate and transport of pathogens in the subsurface have been conducted largely at the laboratory scale using highly idealized systems and pathogen surrogates to better isolate the influence of specific processes and factors. Literature results demonstrate that the transport and fate of microbes and surrogates will be highly dependent on incompletely characterized interactions with diverse environmental surfaces that are coupled with a multitude of physical, chemical, and microbiological factors (Bradford et al., 2013). Regulatory constraints also require the use of pathogen surrogates in field studies, so it is critical to identify surrogates that provide a conservative estimate of pathogen transport and fate in risk assessment. Four contributions to the special section examine various aspects of microbe and surrogate transport and retention process at the laboratory scale.

In cleverly designed laboratory experiments, Lu et al. (2015) investigate the effect of swimming motility on the attachment of bacteria to porous media. Different strains of *Azotobacter vinelandii* cells and silica surfaces served as model systems for a comprehensive analysis of cell trajectories and deposition in a radial stagnation point flow cell and in a two-dimensional multiple-collector micromodel. Strong swimming strains exhibited reduced deposition on the upstream surface of collectors because horizontal and vertical movement limited their contact time with the surface.

Zhang et al. (2015) quantified colloid (300-nm carboxylate-modified latex microsphere) retention and release processes in micromodel experiments under various flow rates. Colloid retention was observed to decrease with an increase in flow rate, and release was observed when the flow rate was suddenly increased, for example, by a factor of 10. The measured breakthrough curves were successfully simulated using a pore-network model that included expressions for first-order colloid retention and release. The authors explain mechanisms of colloid retention in terms of the effects of surface roughness on the torque balance.

Burbery et al. (2015) conducted a series of saturated column experiments to examine the ability of calcareous coral sand, widely relied on for in situ sanitation on tropical low lying atolls, to retain *Escherichia coli* J6-2 and bacteriophage MS2. The coral sand proved highly effective at removing the *E. coli*

J6–2 (7.7 log units  $m^{-1}$ ), whereas the retention of MS2 could not be conclusively determined because the plastic apparatus used in the column studies induced inactivation of this phage.

Stevenson et al. (2015) examine the transport and retention behavior of human adenovirus, nanoparticles, and bacteriophages PRD1 and MS2 in fine granular limestone aquifer material taken from a borehole at a managed aquifer recharge site in Adelaide, Southern Australia. PRD1 was found to be a suitable surrogate for adenovirus to test managed aquifer recharge in an aquifer dominated by calcite material, however, not under high ionic strength or high pH conditions. These results suggest that indicators of a specific pathogen are not solely based on similar size, morphology and/or surface charge but may be dependent on site-specific conditions.

## Field Studies

Natural environments exhibit heterogeneity at multiple scales. In addition to heterogeneity that is commonly observed at the laboratory scale, field-scale studies also exhibit heterogeneity in soil properties and surface topography and temporal variability in temperature, water inputs, and pathogen sources. Limited field research with microbes and surrogates indicates that dominant migration pathways and fate processes will change with the spatial and temporal scales (Pang, 2009; Unc et al., 2012; Bradford et al., 2013). For example, intense rainfall events can generate runoff, preferential flow, and transients in solution chemistry and water saturation that can rapidly transport microbes in agricultural settings (Sterk et al., 2013; Wang et al., 2014). Pathogens and/or subpopulations that survive for extended periods of time have a greatly enhanced probability of being subject to such rapid-transport events (de Roda Husman et al., 2009). Most of these field-scale microbe transport and fate processes have received little research attention, and approaches to upscale laboratory information to the field-scale are almost nonexistent. Five contributions to the special section examine various aspects of microbe and surrogate transport, retention, and/or survival at the field scale.

Sidhu et al. (2015) studied the influence of pathogen type and aquifer characteristics (e.g., geochemistry and type of source water) on pathogen removal in four managed aquifer recharge settings representing unconfined and confined, calcareous and siliceous aquifer conditions. Bacterial pathogens and indicators (*Salmonella* 192 *enterica* serovar *Typhimurium*, *Escherichia coli*, and *Enterococcus fecalis*) had the shortest one log<sub>10</sub> removal time ( $T_{90} = <3$  d) followed by *Cryptosporidium* oocysts ( $T_{90} < 120$  d). Removal of the enteric viruses (Coxsackievirus, Adenovirus, and Rotavirus) was sensitive to the aquifer geochemistry ( $T_{90} = 18$  to  $>200$  d), and was less under anoxic conditions ( $T_{90} > 65$  d for Coxsackievirus, and  $> 200$  d for Adenovirus and Rotavirus). Adenovirus survived longer than other microorganisms examined and could be used as a conservative organism for pathogen decay in groundwater.

Harvey et al. (2015) investigated the removal of cyanobacteria (IS625), viruses (AS-1, MS2), carboxylated latex microspheres (1700 nm), and dissolved organic carbon (DOC) in the colmation layer during natural bank filtration. More than 99% of the microspheres, IU625, MS2, AS-1 and ~42% of the DOC were removed in the colmation layer at the bottom of a small lake

(upper 25 cm of sediments) at two test locations characterized by dissimilar seepage rates (1.7 versus 0.26  $m d^{-1}$ ). The colmation layer was responsible for rapid changes in the character of the DOC and was more effective (by 3 orders of magnitude) at removing microspheres than was the underlying 30 cm-long segment of sediment. A Lee-type seepage meter was critical to successfully performing the field studies.

Arnaud et al. (2015) monitored *E. coli* concentrations beneath a 12-m-thick vadose zone of coarse, heterogeneous glacial sediments, following surface application of liquid swine manure. *Escherichia coli* was detected on all 23 sample dates over a 5-mo period, with particularly elevated concentrations 1 wk after application and lasting for 5 wk. These findings indicate that fecal bacteria can rapidly migrate through a deep vadose zone due to incompletely characterized preferential flow pathways and/or remobilization processes.

Li et al. (2015) conducted a survey of microbiological groundwater quality in a region with intensive animal agriculture in California. Generic *E. coli* was detected in 24.2% (46/190) of CAFO monitoring wells, 4.2% (1/24) of concentrated animal feeding operation (CAFO) domestic wells, and 4.5% (9/200) of small supply wells. *Enterococcus* spp. was detected in 97.4% (185/190) of CAFO monitoring wells, 87.5% (21/24) of CAFO domestic wells, and 10.3% (21/200) of small supply wells. Concentrations of generic *E. coli* were not significantly associated to any factors, whereas concentrations of *Enterococcus* spp. were significantly associated to proximity to CAFOs, seasons, and concentrations of potassium in water. Important from a human health perspective, the majority of generic *E. coli* (63.6%) and *Enterococcus* spp. (86.1%) isolates from the surveys exhibited resistance to multiple (more than three) antibiotics, indicating widespread distribution of antibiotic resistant genes into drinking water via nonpathogenic bacteria.

Flynn et al. (2015) reappraise solute and microbial tracer tests to characterize the ability of peri-glacial sand and gravel aquifers to remove microbiological contaminants using a well characterized field site near Munich, Germany. The relative recovery of *E. coli*, the bacteriophage H40/1, and *Pseudomonas putida* varied strongly for transport distances of several tens of meters between injection and observation wells. These findings are explained in terms of filtration theory, microbe size and charge, and observations from fresh outcrops that demonstrated significant geochemical heterogeneity. Thin open framework gravel beds rapidly displace groundwater and pose significant microbial contamination risk, yet overlying finer-grained aquifer layers may provide significant attenuation from microbial pollution sources.

## Mathematical Models

Society has a pressing need to address many microbial transport issues at large spatial and temporal scales, including water treatment, bioremediation, and pathogen fate. Mathematical modeling tools can potentially help us understand and predict the complexities of microbial transport and survival for these scenarios under given assumptions and conditions (Schijven and Hassanizadeh, 2000; Ginn et al., 2002). However, there still is a critical need to improve our understanding and description of many fundamental microbial transport and fate processes, especially in independently determining model parameters for dynamic natural environments (Bradford et al., 2014; Molnar et

al., 2015). Furthermore, there is no consensus in the literature on the proper conceptual and/or mathematical framework to describe these processes under well-defined laboratory conditions, let alone at the field or watershed scales (see, e.g., Sadeghi and Arnold, 2002; Pachepsky et al., 2006; Drummond et al., 2014). These knowledge gaps have hampered the ability of the scientific community to develop predictive models and best management practices. Four modeling contributions to the special section provide an improved ability to simulate or increase our understanding of microbial fate in the environment.

Morales et al. (2015) simulated *E. coli* removal efficiencies in a conventional soil-based wastewater treatment system under environmental and operational conditions that might be expected under changing climatic conditions using HYDRUS 2D/3D software. The initial model parameters were calibrated to published experimental data. The simulated performance of the treatment system tended to increase in finer textured soils, for lower hydraulic loading and precipitation rates, in the presence of a biomat layer, and at higher subsurface temperatures.

Leij et al. (2015) determined a straightforward analytic solution, zero- and first-order time moments, and the setback distance for the problem of advective colloid transport with irreversible retention and Langmuirian blocking. *Escherichia coli* D21 g breakthrough curves and retention profiles in fine sand at four ionic strengths were well described by the model. Illustrative simulations demonstrated that blocking becomes more important for smaller retention capacity ( $S_m$ ) and for larger retention rate coefficient ( $k$ ), input concentration ( $C_0$ ), and pulse duration. Blocking tended to delay colloid arrival time at a particular location relative to a conservative tracer, and produced larger setback distances for smaller  $k$  and  $S_m/C_0$ .

Liao et al. (2015) evaluate the impact of bacteria sediment interactions in a continuous, watershed-scale model that was calibrated with measurements of fecal indicator bacteria concentrations in the water column and streambed sediment. The model performance under a “sediment-attached” scenario was essentially equivalent to the simpler “free-phase” scenario for this site-specific scenario. It is unclear whether the additional model complexity associated with bacteria sediment interactions are justified and/or when these interactions need to be considered.

Schijven et al. (2015) introduce the interactive computational tool QMRACatch to simulate concentrations of target microorganisms and viruses (TMVs) in catchment water resources, to assess infection risks from exposure, and to calculate the required treatment to meet health based water quality targets. Sources of TMVs include wastewater discharge into a river and fecal deposits on a floodplain. Travel times for a main river and a floodplain river were calculated using the Manning–Gauckler–Strickler formula, and concentrations of TMVs were subject to dilution and temperature dependent degradation. Applicability of QMRACatch is demonstrated by calibrating the tool for a study site at the River Danube near Vienna, Austria.

## Concluding Thoughts

We trust that readers of this special section will find that these papers make a valuable contribution to our ability to understand and model the highly complex processes of microbial occurrence, transport, and fate across spatiotemporal scales in the subsurface

environment. This information is needed to increase our knowledge base to enhance in situ environmental remediation, and to assess the risk to, and protection of, our water resources from microbial contamination. Findings from these studies also identify critical gaps in knowledge, conditions of increased risks, and limitations of models. Additional research is needed to overcome these challenges. Many opportunities exist to develop and/or use new scientific tools to quantify microbial transport and fate in the subsurface. For example, research is needed to quantitatively detect and monitor microbial fate, relate the behavior of surrogates to pathogens, incorporate laboratory findings into models, develop accurate/precise upscaling procedures, predict the influence of physicochemical conditions on model parameters, and obtain accurate model predictions at laboratory and field scales. These research endeavors should be focused on conditions that pose the greatest potential benefits and/or risks from microorganisms.

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