Science of the Total Environment 443 (2013) 55–64

Contents lists available at SciVerse ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Transport of microbial tracers in clean and organically contaminated silica sand in laboratory columns compared with their transport in the field

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HIGHLIGHTS

- Organic carbon could lead to an increase in transport of microbial pathogens due to decreased removal.
- In the presence of increased organic carbon the removal of the microbial tracers *E. coli* and MS2 phage.
- MS2 phage showed a decreased removal in the presence of low concentrations of organic carbon (1% DOC).

ARTICLE INFO

Article history:
Received 4 November 2011
Received in revised form 7 September 2012
Accepted 22 September 2012
Available online 23 November 2012

Keywords:
Microbial transport
Sand
Aquifers
E. coli
Bacteriophage
Dissolved organic carbon (DOC)

ABSTRACT

Waste disposal on land and the consequent transport of bacterial and viral pathogens in soils and aquifers are of major concern worldwide. Pathogen transport can be enhanced in the presence of organic matter due to occupation of attachment sites in the aquifer materials thus preventing pathogen attachment leading to their faster transport for longer distances. Laboratory column studies were carried out to investigate the effect of organic matter, in the form of dissolved organic carbon (DOC), on the transport of *Escherichia coli* and MS2 phage in saturated clean silica sand. Transport rates of these microbial tracers were also studied in a contaminated field site. Laboratory column studies showed that low concentrations (0.17 mg L^{-1}) of DOC had little effect on *E. coli* J6-2 removal and slightly reduced the attachment of MS2 phage. After progressive conditioning of the column with DOC (1.7 mg L^{-1} and 17 mg L^{-1}), neither *E. coli* J6-2 nor MS2 phage showed any attachment and recovery rates increased dramatically (up to 100%). The results suggest that DOC can affect the transport rates of microbial contaminants. For *E. coli* J6-2 the predominant effect appeared to be an increase in the secondary energy minimum leading to an increase in *E. coli* attachment initially. However, after 17 mg L^{-1} DOC conditioning of the silica sand no attachment of *E. coli* was observed as the DOC took up attachment sites in the porous media. MS2 phage appeared to be affected predominantly by out-competition of binding sites in the clean silica sand and a steady reduction in attachment was observed as the DOC conditioning increased. Field study showed a high removal of both *E. coli* and MS2 phage, although *E. coli* was removed at a lower rate than MS2 phage. In the field it is likely that a combination of effects are seen as the aquifer material will be heterogeneous in its surface nanoscale properties, demonstrated by the differing removal of *E. coli* and MS2 phage compared to the laboratory scale experiments. This research demonstrates the importance of combining laboratory scale and field scale studies to fully understand removal of microbes in groundwater aquifers affected by organic matter (DOC).

1. Introduction

Worldwide, human populations rely heavily on groundwater as a drinking-water source; consequently, protection of these resources is vital. Groundwater contamination from activities on the land, including nutrient addition (fertilizer application, stock grazing) and subsurface discharge (septic tanks) can be detrimental to groundwater quality (Lerner and Harris, 2009; Close et al., 2008; McMahon et al., 2008; Pang et al., 2004; Morrison, 1981). Although high levels of removal of bacterial and viral contamination can be achieved in sandy aquifers, a variety of factors including the presence of organic matter and pH changes can increase contaminant transport rates and the distances contaminants can travel (Pang et al., 2005; Bales et al., 1997; DeFlaun et al., 1997).

The influence of organic matter has been studied previously as an important factor in increased transport of microbial contaminants in groundwater aquifers and laboratory column studies (Metge et al., 2010; Walshe et al., 2010; Wall et al., 2008). Many studies have investigated the effect of humic acid (Yang et al., 2012; Harvey et al., 2010;

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http://dx.doi.org/10.1016/j.scitotenv.2012.09.049

Foppen et al., 2008; Parent and Velegol, 2004), fulvic acid (Harvey et al., 2010), natural organic matter (NOM) (Dai and Hozalski, 2002) and dissolved organic carbon (DOC) (Metge et al., 2010; Walshe et al., 2010) on coated materials. There has been less research conducted on clean (un-coated) sand material which can constitute a large proportion of groundwater environments (Yang et al., 2012; Wall et al., 2008; Dai and Hozalski, 2002, 2003). A recent study highlighted the need for further investigation in this area of research including investigation into more than one type of microbial contaminant (Yang et al., 2012).

In New Zealand, many small coastal communities rely on shallow, coastal sand aquifers for their drinking-water supply. Generally shallow and unconfined, these aquifers are particularly vulnerable to microbial contamination from, for example, the septic tanks or small effluent treatment and irrigation systems that often serve these communities (DeBorde et al., 1998; Goyal et al., 1984).

Most of the information about microbial contamination of New Zealand's groundwater resources derives from studies on the alluvial gravel aquifer systems (Walshe et al., 2010; Wall et al., 2008; Sinton et al., 2000; Pang et al., 1998). These systems exhibit rapid rates of microbial transport and limited attenuation of both bacteria and viruses. Microbial transport in coastal sand aquifers in New Zealand has not been studied and information from other countries about these types of aquifers is sparse (Fuller et al., 2004; DeFlaun et al., 1997). Wall et al. (2008) investigated the transport and attenuation of *Escherichia coli* and F-RNA phage (MS2 phage) in saturated pumice sands in New Zealand, using both field studies and laboratory columns. At the field site, they found 99% removal of *E. coli* J6-2 and MS2 phage 2 m down gradient of an injection well. In the laboratory column experiments, Wall et al. (2008) recorded 90% removal of *E. coli* J6-2 and 65% removal of MS2 phage in clean pumice sand. However, removal rates decreased markedly, particularly for MS2 phage, with the progressive addition of ultrafiltered sewage (as a source of dissolved organic carbon (DOC)) to the column. This suggested that microbial attenuation studies in clean sand may underestimate transport distances in aquifers subject to organic matter accumulation, as would be found down gradient of a septic tank or effluent discharge system (Wall et al., 2008).

In this paper, we aimed to investigate the effect of contamination by conditioning columns with DOC and a field study, on the transport and attenuation of *E. coli* J6-2 and MS2 phage (as model organisms for predicting enteric virus transport) in sands. The study involved laboratory column experiments with unconditioned (no contamination present) silica sands and those that were progressively conditioned with DOC; and a field tracer experiment at a sewage-contaminated coastal sand site.

2. Materials and methods

2.1. Tracer characteristics, preparation and analysis

Potassium bromide (KBr) was used in each experiment as a conservative tracer (Fattal and Teltsch, 1982), and assayed using both a bromide (Br^-) specific ion electrode attached to an Orion 250A pH/ISE metre and high performance liquid chromatography (HPLC). The results for both methods of Br^- analysis were comparable (Sinton et al., 2010).

The bacterial tracer *E. coli* J6-2 (Sinton, 1980) is a non-pathogenic, lactose negative, nalidixic acid-resistant derivative of *E. coli* K-12. Cells were cultured in Brain Heart Infusion (BHI) broth (BBL, Sparks, MD, USA) at 37 °C, washed, resuspended in saline solution and stored at 4 °C prior to injection (less than 24 h). Enumeration was by membrane filtration (Millipore EZ-Pak™, 0.45 μm pore size) and incubation at 44.5 °C for 20 h (±4 h) on Chromocult® Coliform Agar ES media (Merck, Darmstadt, Germany). Bacterial counts were expressed as colony forming units (cfu) mL^{-1}. The detection limit for *E. coli* J6-2 by membrane filtration was 0.1 cfu mL^{-1} (10 mL maximum volume analysed).

MS2 phage (Havelaar et al., 1993; Goyal and Gerba, 1979) was used as a model organism for enteric viral transport. MS2 phages are icosahedral and approximately 26 nm in diameter. The host strain was *E. coli* HS (pFamp)R (Debartolomeis and Cabelli, 1991) which is resistant to ampicillin and streptomycin sulphate. Propagation of MS2 phage was as described in Wall et al. (2008), and analysis of water samples was by overlay pour plating of 1 mL volumes of serial dilutions (American Public Health Association (APHA), 1998). Counts were expressed as plaque forming units (pfu) mL^{-1}, and the detection limit was 0.1 pfu mL^{-1} as up to 10 mL of sample was analysed. Previous studies did not demonstrate any infectivity of *E. coli* J6-2 by MS2 phage or Br^- having any effect on the recovery of *E. coli* J6-2 or MS2 phage (Sinton et al., 2010).

To assess microbial tracer die-off during the column experiments, samples of the injection mix were held at similar temperatures (between 12 °C and 14 °C) to the column in the dark, on a rotary mixer to keep the microbial tracers mixed. Samples were taken and analysed for *E. coli* J6-2 and MS2 phage at 4-hourly intervals over the experimental period. During the field experiment, a sample of the injection mix was transported back to the laboratory and held in the dark over the experimental period at 12–14 °C and was analysed when field samples were analysed in the laboratory. *E. coli* J6-2 or MS2 phage showed little die off during the experiments (data not shown), with <1 log reduction in counts observed.

2.2. Column experiments

Laboratory column experiments were set up using clean silica sand (Grade F-50 Ottawa Sand, U.S. Silica Company, USA). The experiments were first conducted using clean (uncoated) silica sand as uncoated silica sand has been shown previously to offer little attachment to bacteria (Kim et al., 2008; Kinoshita et al., 1993). The DOC used in these experiments was ultrafiltered to remove particulate matter that could distort the effect of the dissolved organic matter in the attachment or enhanced transport of microbes through sand media.

2.2.1. Unconditioned sand experiments

A laboratory experiment was conducted to determine the relative transport and attenuation of *E. coli* J6-2 and MS2 phage in clean silica sand. Twin columns (500 mm length × 110 mm diameter) were set up, and fitted with a distribution plate at each end to ensure an even flow of water through the media. The plates were perforated with 2 mm diameter holes and covered with a 200 μM pore size mesh to prevent loss of sand particles. Each column was wet packed with clean silica sand (mean grain size 0.212–0.300 mm), using case percussion to minimise air entrapment. The bulk density of the sand was calculated as 1.526 g cm^{-3} for column 1 and 1.550 g cm^{-3} for column 2, and the calculated porosities were 0.326 and 0.328, respectively. The flow rates were set to 0.15 L h^{-1} for both columns (equating to a velocity of 1.2 m d^{-1}).

In each experiment, the column was supplied by a tank of de-aerated (boiled and cooled), filter-sterilised tap water (pH 6.9 ± 0.2). The water was prepared in this way to prevent degassing of the water through the column and to remove microbial contaminants, as the air and microbes in the water could block the column if not removed. The de-aerated, filter-sterilised tap water was pumped through each column from the bottom to the top, using a diaphragm pump (Chem-Tech, Novatech International Inc., USA) set to a flow rate of 0.15 L h^{-1}. To maintain the internal column temperature between 12 °C and 14 °C (typical groundwater temperature in New Zealand sand aquifers), both columns were fitted with external insulated coils through which cooled water was pumped. The internal