

Studying the transport pathways of viruses in aquifer

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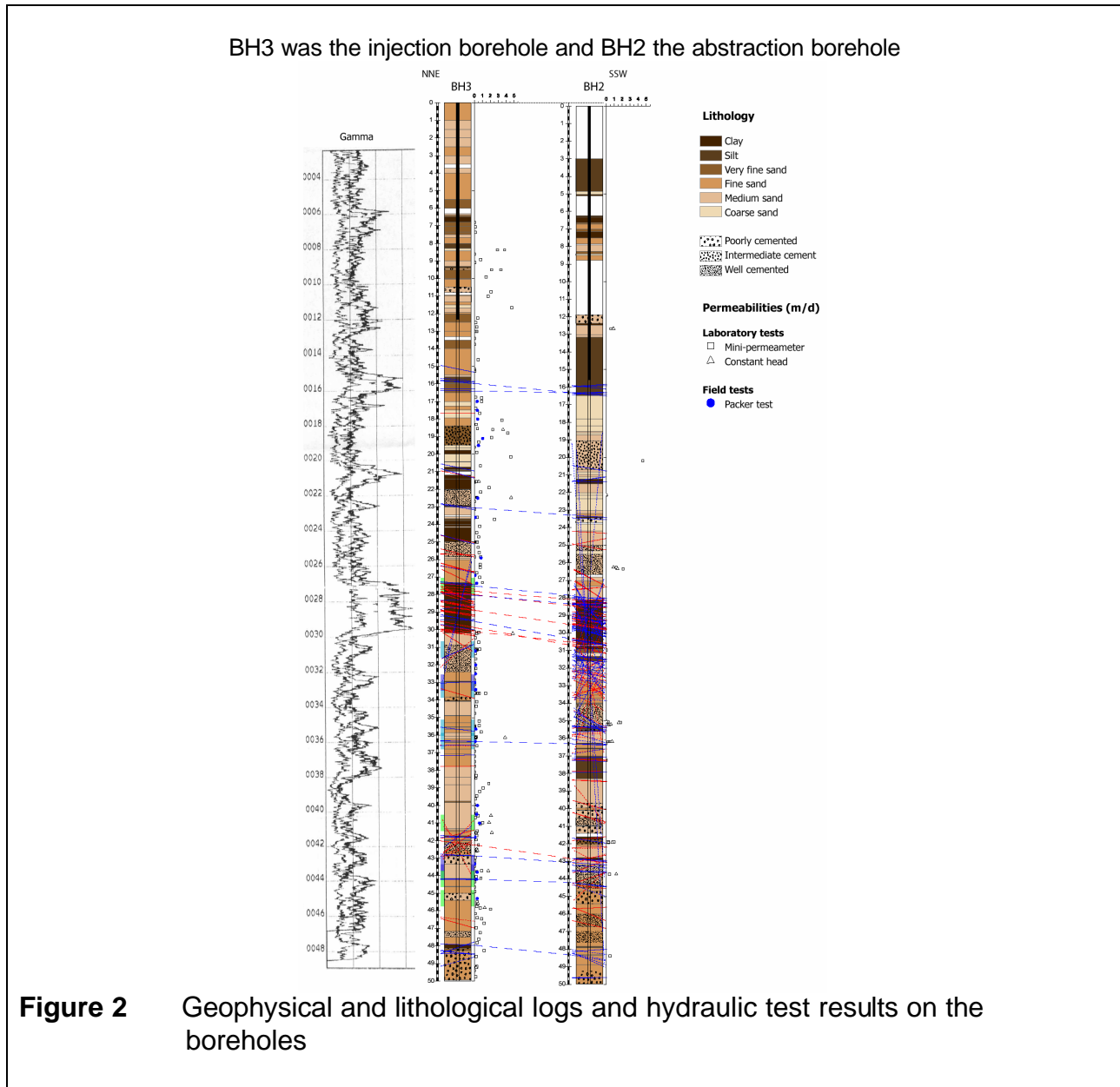
Introduction

Human enteric viruses have been found at depth in the Permo-Triassic Sandstone aquifer underlying the city of Nottingham in the UK (Powell et al, 2003). This finding was unexpected since the transission time from the source of contamination (assumed to be near surface) to depth is believed to be much greater than the expected survival time of the viruses. Consequently, a set of field and laboratory tests were conducted to characterise the transport of viruses in the sandstone. The Permo-Triassic Sandstone aquifers in the region are extensively used for water supply and underlie several other major cities, including Birmingham, Liverpool, and Manchester, and are described by Barker and Tellam, (2006). The sequence investigated comprises weakly- to well-cemented fluvial and aeolian sandstones, with occasional thin, usually decimetre scale, mudstones and palaeosols. The median pore sizes are typically 10-50 μm , much larger than the diameter of virus particles (often less than 100 nm). The sandstones are fractured, but modelling suggests that the regional scale permeability is close to that of the matrix (Hitchmough et al., 2007).

Methodology

The test site

The test boreholes (BH2 and BH3) are located on the University of Birmingham campus. Both are 50m in depth and approximately 15cm in diameter while the distance between boreholes is 7 m. The boreholes are cored to 50 m below ground level (mbgl). BH2 is cased to 15.6 metre below ground level (mbgl) and BH3 to 12.2 mbgl. The dashed line between the boreholes shows a possible correlation between planar features observed in each hole, based upon geometry alone (Figure 1). Open squares show the hydraulic conductivity based upon mini-permeameter tests on core, and the open triangles the results from falling head tests on core. The closed circles show the hydraulic conductivity determined from short-interval packer tests in each hole. Two superimposed natural gamma logs are presented for BH3. The gamma log for BH2 is not shown, but correlates well with that for BH3. The solid line in the centre of each hole represents that part of the hole that is cased. The field tests in 2004 and 2005 were conducted by injecting in BH2 and abstracting from BH3. However, cross-hole packer testing (Ferguson, 2006) indicated that the section of BH3 above 15 mbgl is effectively isolated hydraulically from BH2, and hence that pumping BH3 draws in water from levels unconnected to BH2. Thus for the new series of tracer tests, BH2 has been selected as the abstraction borehole. The cross-hole packer tests also showed that there is little hydraulic connection between the zones above 27 mbgl in each borehole and the zones below 37 mbgl in either hole.



The MS2 bacteriophage and flourescein dye

Bacteriophages are viruses that only attack specific bacteria, and they can be surrogate to human viruses. After a literature review the bacteriophage chosen to be used in the tracer experiment was MS2 phage on the basis of being most suitable surrogate for enteric viruses poliovirus and rotavirus, in relation to size, MS2 has small size 20-26 nm.

Fluorescent dyes have been used largely in ground water tracer experiments, flourescein was chosen in this experiment. It was injected to the borehole 24 hours before to inject the phages. Both flourescein and MS2 were injected into borehole (BH3) using a peristaltic pump and abstracted from the second, continuously pumped, borehole.

The abstraction borehole

The abstraction borehole (BH2) was instrumented with a main pump and sampling system that routes water from five predefined levels in the borehole to monitoring devices at the surface. The system employs a single Grundfos MP1 submersible pump to sample from each of five levels sequentially. This was achieved using a system of computer controlled solenoid valves that route the water samples through the pump via inlet and outlet manifolds. MS2 phages were collected at the surface in Argonide NanoCeram® virus filter traps. Each trap collected samples from a particular sampling level and was changed daily and taken to the laboratory for analysis. Thus, the virus sample represents an integrated viral throughput. In addition to sampling from the borehole at five levels, a sixth virus trap was connected to a sampling line from the main pump discharge. Effluent water from each of the virus traps was monitored for fluorescein using a logged Schnegg fluorimeter.

Laboratory work

Viruses were recovered from the traps by elution as described in US EPA (2001a) using 1.5% beef extract and 0.05 M of glycine, adjusted to pH 9.5 with sodium hydroxide (NaOH). The solution is passed through the filter trap slowly using a peristaltic pump, and the eluate adjusted to between pH 7.0 and 7.5 and filtered through a 0.45 µm sterilizing filter. The number of viruses were estimated by plaque assay using the Double Agar Layer (DAL) procedure (Adams 1959), according to the protocol set out in US EPA (2001b,c,d).

Pre-filter water were analysed in situ for pH, conductivity and alkalinity and pre-filter water samples collected to carry out chemical analysis and fluorescence.

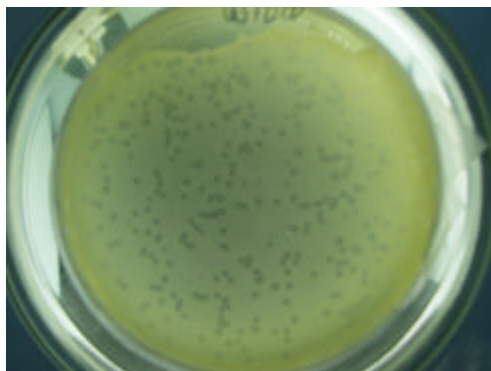
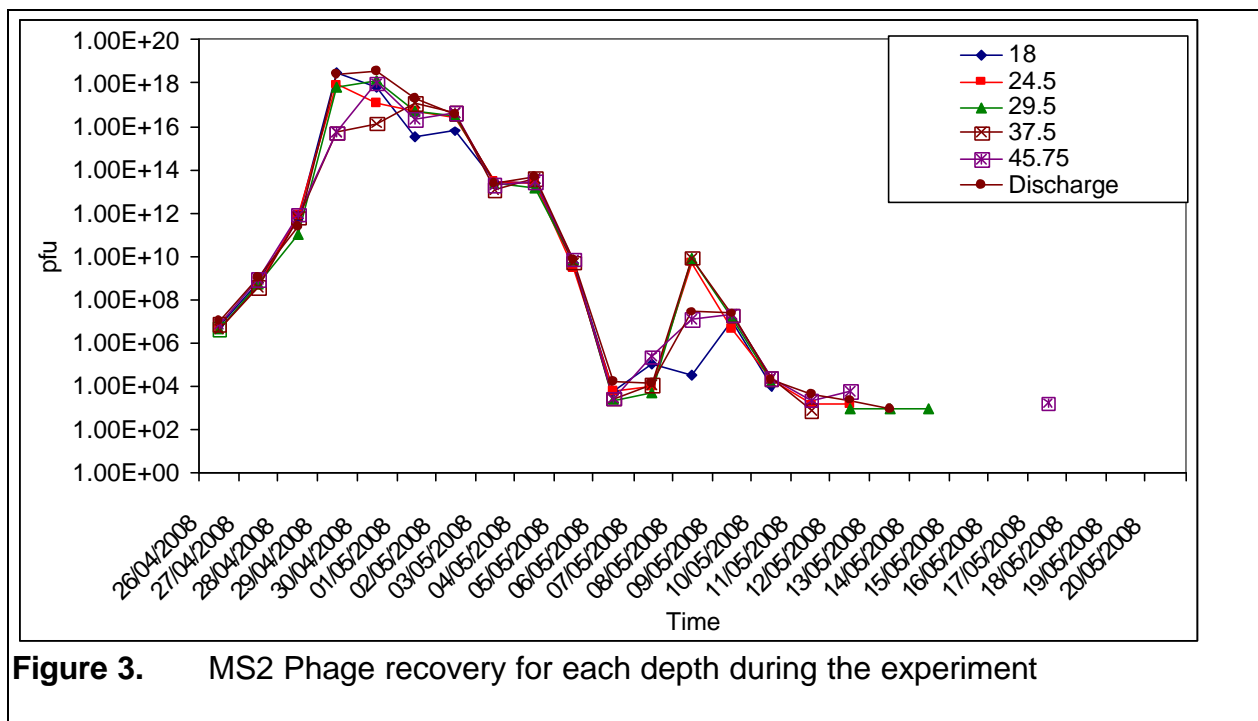


Figure 2. Double Agar Layer (DAL) plate showing bacteriophage plaques (clear patches or “holes”) in the opaque lawn of bacteria.

Results and future work

Preliminary results (Figure 3) show that MS2 phages move fast through the aquifer, through many pathways: virus recovery from the second borehole reaches a peak after four days and then declines slowly with a second, smaller peak appearing after twelve days, possibly corresponding to a slower

pathway of movement. This indicates that human virus could also move through the aquifers. Currently work is underway to characterise these pathways, and understand the main factors that have implications in this movement.



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