SWITCH

Sixth Framework Programme (2002-2006)

018530 - SWITCH

Sustainable Water Management in the City of the Future

Integrated Project
Global Change and Ecosystems

D.3.2.3.1.c.i Field scale process descriptions of viral fate and transport and THM generation and attenuation: Preliminary results

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Organisation name of lead contractor for this deliverable: University of Birmingham, UK

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CONTENTS

Appendices

1. Introduction

This project comes under T3.2.3 – Aquifer Storage Recovery (ASR), Work Package 3.2 – Safe Water Reuse. It deals with the injection of waste water into aquifers, and in particular the potential threats to groundwaters posed by viruses and chlorination by-products. The work has links to Work Packages 2.3 and 5.3.

This report describes progress to February 2008 (i.e. up to month 25 of the SWITCH project), and is interim in nature, with some work having been completed and other work still underway. The main progress is summarized in the initial seven sections, much of the text being in bullet point format. To support and expand upon this information, twelve appendices are attached. Some of the appendices are completed reports on specific aspects of the work, and some contain partial interim reports. Where the work is too extensive to detail in one appendix (e.g. in the case of the hydraulic and geological data for the experimental site), a few example figures with accompanying explanations are provided.

2. Aims

In the early part of the project, the following specific aims were defined:

1. to determine the mechanisms controlling the natural attenuation of virus particles in an example matrix-flow–dominated aquifer
2. to use the results of Aim 1 to develop a method for evaluating the likely mobility of virus particles in a given system
3. to determine the mobility of trihalomethanes (THMs) in an example matrix-flow dominated aquifer, and to use the knowledge gained to suggest a means of assessing the importance of this hazard.

The example aquifer chosen is the Permo-Triassic sandstone aquifer underlying Birmingham, one of the SWITCH demonstration cities. The aquifer is a fluvial/aeolian redbed sequence, typical of many sandstones internationally (Tellam and Barker, 2006). The Permo-Triassic sandstones are the second most used aquifers in the UK.

3. Approach

Two approaches have been adopted in order to achieve Aims 1 and 2: field experimentation and field sampling and monitoring.

Field experimentation

Our previous work at the Birmingham University campus experimental borehole array has shown that under certain conditions virus particles are mobile in the groundwaters of the Birmingham urban sandstone aquifer (Joyce et al., 2007; Joyce et al., In Press, Appendix 12). This work indicated that the virus particles were only mobile in certain parts of the sandstone sequence, but was not able to indicate the exact pathways or the processes involved. Thus the intention of the field experiments in T3.2.3 is to isolate the pathways, and, having done so, to use further field or laboratory experimentation to determine the properties of the pathways which allow the virus particles to be transported (Aim 1): these may be hydraulic, chemical, or possibly a combination of the two. Knowledge of these properties will allow the development of a risk assessment procedure (Aim 2) based on knowledge of the distributions of chemical and hydraulic properties in the aquifer.

Field sampling and monitoring

Field sampling of abstraction wells and piezometers will provide data with which to test the risk assessment procedure (Aim 2). In addition, we hope to determine whether the apparent seasonal variation observed by Taylor et al. (2004) (see Joyce et al., 2007b, Appendix 12) can be confirmed, thus providing direct evidence of rapid virus transport under non-pumping conditions, adding support to the main evidence collected during the field experiments under necessarily artificial conditions (Aim 1).

Aim3, the determination of the mobility of THMs, will be undertaken by making use of the fact that in the UK, leakage rates from water supply systems, though now declining, have been high (up to at least 25%) for a considerable number of years. As mains water contains THMs, it should be possible to assess their mobility by examining the concentrations in urban groundwaters. Thus we will: collate existing Environment Agency data sets and data from our own previous regional surveys; sample abstraction wells in Birmingham; and sample piezometers in Nottingham and Birmingham to establish the vertical distribution of the THMs. These data will then allow a method for assessing the risk from THM mobility to be suggested.

4. Management

Three post-doctoral researchers work part-time on this project: Dr R. B. Greswell has designed and built field experimentation equipment, and supervised the field tests; Dr M. F. Aller has developed virus analytical methods, and undertaken sampling and analysis for inorganic and organic determinands; Dr V. Durand has been responsible for collating the large amount of existing data, developing interpretation methods, and analyzing test results. The virus work is supervised by two members of academic staff (Prof. J. H. Tellam and Mr M. S. Riley), and the THM work by an academic, Dr M. O. Rivett. Depending on the tasks involved, meetings of this team have occurred at up to weekly frequency. Contributions have been made by two further academic staff members: Dr J. C. Renshaw on microbiological aspects; and Prof. R. Mackay on design. The project comes under the auspices of the Learning Alliance set up for the four Birmingham University projects: it has had, in particular, links with the UK Environment Agency via Dr A. Hart.

A Surrey University researcher, under the guidance of Dr S. Pedley, will undertake work on human virus analysis of samples obtained by the Birmingham team. This sub-contract is expected to start in March 2008.

5. Progress

The work undertaken is listed below in outline. The appendices to this report give detail on the methods and results. Most work to date has concentrated on field experimentation of virus mobility; the investigation of THM transport will be undertaken in parallel with the field sampling for human viruses once the Surrey University sub-contract has been set up (point 7 below).

1. determined major features of the flow system in the two boreholes of the Birmingham University campus array planned for the virus experiments

    a. collated the geological and hydraulic data, and correlated across the site (Appendix 1)
    b. undertook geophysical logging and a set of single packer tests to deduce connectivity between the boreholes (Appendix 2.3)
    c. designed and built a new packer-mounted flow meter device (Appendix 2.2)
    d. used the packer flow meter to measure flow rates along the borehole profiles, and thus inferred the vertical variation in the inter-borehole flow system produced by abstraction (Appendix 2.2)

e. undertook chemical sampling of the well system, and analyzed for a wide range of organic and inorganic and fluorescence characteristics (Appendix 3)

2. developed for the first time in our labs the methods for analysis of phages to be used in the experiments

   a. plaque assay technique for PRD1 (Appendix 4)
   b. an epifluorescence technique (Appendix 5)

3. acquired and tested a new virus filter system (Appendix 6)

   a. during testing found that small concentrations of PRD1 had survived since the previous phage tracer tests, approximately 2 years previously (1.7 x 103 pfu/mL): comparison with previous experiments on the same site using phage suspensions in dialysis bags suggest that the presence of the rock, and the possible attachment to it, protects the virus against inactivation – an important result warranting further investigation

4. designed a new system to enable multiple depth sampling in a pumping well with the aim of identifying the inflow zones and therefore being able to determine the pathways of the phage all in one experiment (Appendix 7) [thus dispensing with the need for the very lengthy pumping test mentioned in the original proposal]

   a. initial modelling of the errors and precision of the system (will be reported at a later date)
   b. developed a simple model of the passage of solutes across the site using detailed geological and hydraulic data in order to aid with test design and interpretation (Appendix 8)

5. built the test system, and trialled it
   a. found that the sampling pumps purchased failed at the installed depths (Appendix 9)

6. partially redesigned the field system to avoid having to use 5 sampling pumps (Appendix 10)

   a. replace multiple sampling pumps with a single, more robust pump fitted with a switching manifold to enable sequential sampling from different depths
   b. because of problems with the suppliers of phage PRD1, we are currently developing methods for an alternative virus – MS2
   c. ran successful trial test of new system (Appendix 3)
   d. plan to commence full test at the start of April to run for approximately 4 weeks.

7. Monitoring of the variation of virus concentrations in time in the sandstone groundwaters involves the sub-contract of analyses of human viruses to Surrey University: now that funding has been cleared, the contract between Birmingham and Surrey universities is expected to be signed shortly, allowing work to commence soon afterwards. The sampling sites have been identified and researched (Appendix 11).

6. Products in Addition to this Report

1. MSc and BSc project reports

   a. H. Ferguson (MSc) [field hydraulic experiments, and virus sampling]
   b. D. Jefferies (MSc) [simple model of the well system]
   c. S. Dale-Lace (BSc) [includes chemical sampling at the array]
   d. B. Maundrell (BSc) [includes chemical sampling at the array]

2. Presentations, publications, and planned presentations (see Appendix 12)

    a. SWITCH – first and second scientific meetings (Birmingham, Jan 2007; Tel Aviv, Dec 2007)
    b. GQ07 – Groundwater Quality 2007 meeting, Fremantle, Australia: will be published in the IAH 'Red Book' series
    c. Nanoparticles and Nanomaterials Chemistry: environmental fate and behaviour of nanoparticles, Society of Experimental Biology Conference, Natural History Museum, London, Sept 2007
    d. Use of bacteriophage to study virus pathways in groundwater systems, 2nd International Conference on Environmental, Industrial and Applied Microbiology, 28 Nov – 1 Dec, Seville
    e. Assessing the hazard from viruses in waste-water recharge of urban sandstone aquifers, Poster, Geological Society of London, Bicentennial Meeting, London
    f. Determining the pathways of virus transport through a sandstone aquifer, Accepted for EGU Meeting, Vienna, 13-18 April, 2008
    g. J.H. Tellam, 2007. Urban groundwater quality sustainability: the case of Birmingham, England. Zbl. Geol. Paläont. Teil I, 71-86.

7. Plans

7.1 Virus Hazard

Task 1

Having completed a large number of preparatory tests, the first task for the period from month 25 will be the completion of the main phage tracer experiment, with attendant chemical sampling to define hydrochemical conditions and particulate sampling.

Task 2

This main experiment will provide a good idea of the pathway(s) taken by the viruses between the injection and recovery boreholes. However, it is likely that we will have to refine the location of the pathway further to target the later characterization work. This will be achieved by either repeating the main experiment but with flow in the opposite direction, or by targeted packered-interval tracer tests.

Task 3

Once the pathways have been located with sufficient precision, samples from the relevant rock unit(s) will be characterized hydraulically and geochemically in the laboratory in order to isolate what properties are necessary to define a pathway.

Task 4

The experimental work is necessarily based on forced gradient tests over a limited distance and on phage rather than human viruses. Although there is good evidence that the results are transferable, further confirmation will be sought by investigating the occurrence in time and space of human viruses in urban sandstone aquifer groundwaters. This work will involve sampling pumping wells and piezometers over a period of up to a year (partly sub-contracted through Surrey University). The samples obtained will also form the basis for assessing the issue of THM mobility and prevalence: the samples from both pumped wells and research piezometer nests for which extensive information exists will indicate both lateral and depth penetration of THM pollution.

Task 5

The results from Tasks 1-4, combined with the data already collected, will be used as the basis of an assessment method for determining the risk of virus migration in urban sandstone aquifers. If there is time, and virus transport occurs through matrix, we will test the predictions of the risk assessment by undertaking column experiments on samples predicted to be permeable to viruses and sparsely permeable to them.

7.2 THM

Task 6

The well and piezometer samples (Task 4), supplemented by data collected previously, will be used to develop an assessment of the issue of THM hazard in urban sandstone systems.

References

Dale-Lace, S., 2008. Can nano-pollutants be used to identify individual pollution sources in groundwater baseflow discharging in the River Tame?* Unpublished BSc Project Report, University of Birmingham, Birmingham.

Ferguson, H., 2006. Determining the hydraulic characteristics of the Wildmoor sandstone formation and evaluating the condition of the study site. Unpublished MSc Project Report, University of Birmingham, Birmingham.

Jefferies, D., 2007. Characterising virus flow paths in the Permo-Triassic Sandstone. Unpublished MSc Project Report, University of Birmingham, Birmingham.

Joyce, E., Charles, K., Rahman, H., Aller, M. F., Durand, V., Riley, M. S., Greswell, R. B., Renshaw, J. C., MACKAY, R., RIVETT, M. O., Hart, A., PEDLEY, S., & Tellam, J. H., In press. Assessing the hazard from viruses in wastewater recharge of urban sandstone aquifers. IAHS Red Book Series.

Joyce, E., Rueedi, J., Cronin, A. and Pedley ,S., Greswell, R.B., & Tellam, J.H., 2007. Fate and transport of phage and viruses in UK Sandstone aquifers Environment Agency of England and Wales Science Reports, SC030217/SR

Maundrell, B.G., 2008. Is identification of pollution sources in groundwater baseflow plausible using chemical signatures?* Unpublished BSc Project Report, University of Birmingham, Birmingham.

Taylor R.G., Cronin, A.A., Pedley, S., Barker, J., Atkinson, T., 2004. The implications of groundwater velocity variations on microbial transport and wellhead protection – review of field evidence. FEMS Microbiology Ecology, 49, 17–26.

Tellam, J.H., & Barker, R.D., 2006. Towards prediction of saturated zone pollutant movement in groundwaters in fractured permeable-matrix aquifers: the case of the UK Permo-Triassic Sandstones. In: Barker, R.D, &. Tellam, J.H. (eds), Fluid Flow and Solute Movement in Sandstones: The Onshore UK Permo-Triassic Red Bed Sequence. Geological Society Special Publication 263, 1-48

** note that both these projects used sampling of the campus borehole arrays to obtain less polluted groundwaters.*

Acknowledgements

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Appendix 1

Geological and Hydraulic Data Collation

A1.1 Introduction

The field and laboratory geological and hydraulic data for the University experimental site were collated by Ferguson (2006) and by the project staff. Correlations between the boreholes were examined in detail by project staff. Many of the results were summarized in a series of diagrams such as that shown in Figure 1 (see also Table 1), each of which shows some of the lithological, geophysical, and hydraulic property data available for Boreholes 3 and 2. These compilations have provided the primary data source for designing the field experiments, and will be used in interpreting the tracer tests.

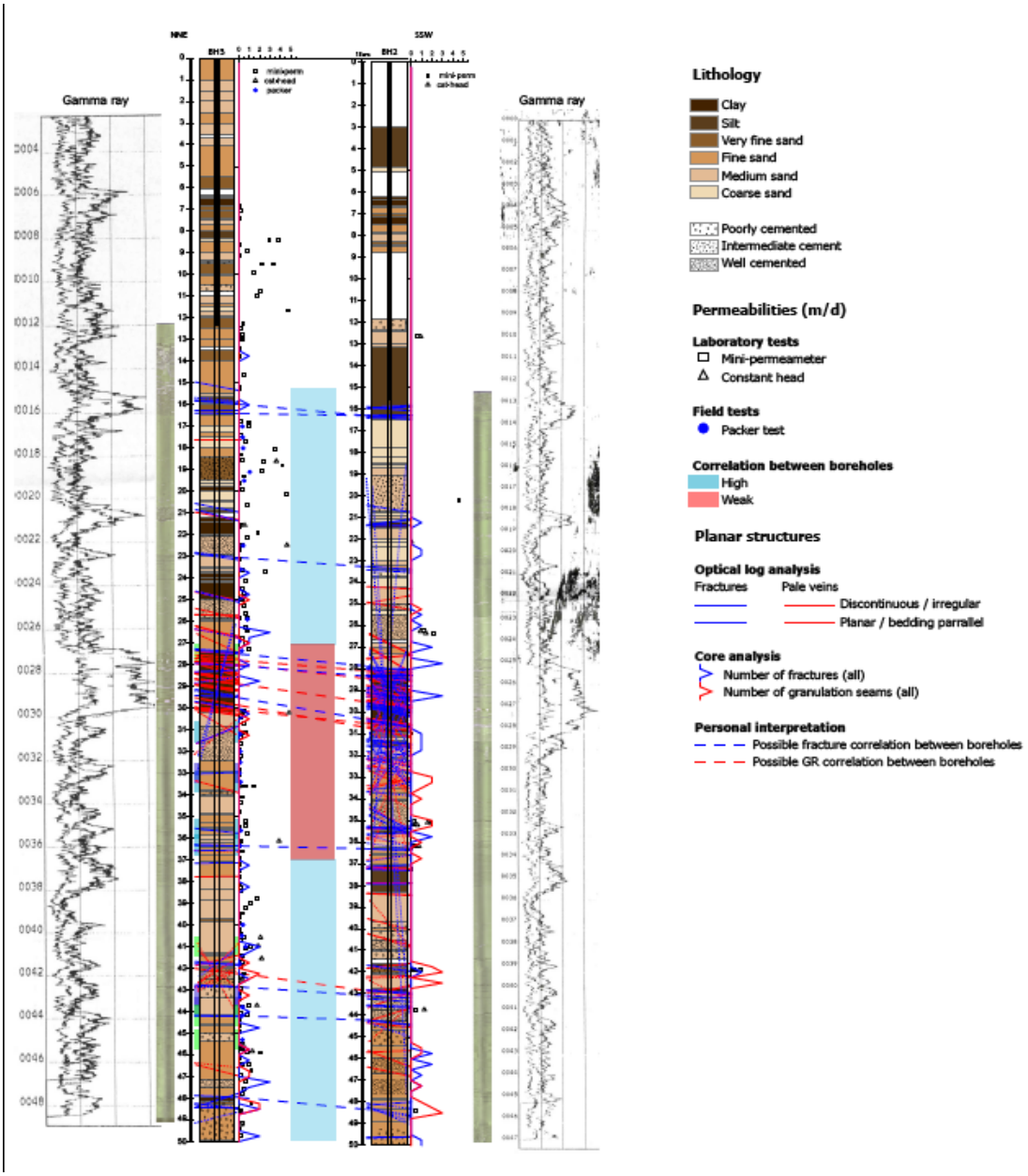


Figure 1: Compilation of hydrogeological data for boreholes 2 & 3.

A1.2 Description of the file sources for the borehole synthesis

The borehole synthesis, presented in correlations-BH2-BH3.pdf has been realized on the basis of many different data, described here in order to keep the trace of the different sources.

General characters

- depths in each borehole indicated in metres from the ground level
- BH3 ground level is 18.2 cm higher than BH2, and distance between BH3 and BH2 is 7.53 m, indicated by Julian Scott (hydro/Fracture Fill/Field Tests/Julians Disk/General/measurements.txt) checked by students and personal surveying
- Orientation of BH3-BH2 section : N020E (students and personal surveying, uncertainty of 2 degrees)

Lithologies

- hydro/Fracture Fill/geology/sedlogdata/Sed_log_data.xls: distinction of 6 granulometric ranges (clay, silt, very fine sand, fine sand, medium sand, coarse sand) and sometimes indications of well/poorly cemented
- particular cases:
    - intermediate ranges of sand (eg fine to medium grain): largest chosen
    - claystone, silty: claystone chosen
    - fine to coarse grain: medium chosen
    - just sandstone: medium chosen
    - sandy clay/claystone, sandy: silt chosen
    - claystone and siltstone: clay chosen
    - sandstone, silty: fine sand chosen
    - sandstone and claystone interbedded: clay chosen
- hydro/Fracture Fill/geology/petrography/ E82RG.ts.HXXX.doc (11 files on BH2, 9 files on BH3): comments on the cementation on some core samples, compared to the previous indications of "well/poorly" cemented

Planar structures

- hydro/Fracture Fill/Field Tests/Rgldip/BH2-3/optvdata.txt: planes measurements from optical logs, synthesized in planes-correlation.xls, with apparent dips between boreholes; the "pale veins" can be related to the granulation seams in certain cases;
- hydro/Fracture Fill/geology/frac.log.data/BH2-3.frac.log.xls: planes analysis on cores, without any dip directions; distinction between granulations seams, fractures and beddings; number of fractures and granulation seams per m depth kept in synth-nbfrac.xls

Permeabilities

- hydro/Fracture Fill/geology/misc tests and reps/all-miniperm-data.xls: mini-permeameter data on core samples, given in mD, converted in m/d and sorted in synth-K.xls;
- hydro/Fracture Fill/Field Tests/Appendix 2-Laboratory tests/Lab-tests/Lab tests results.xls: constant head data on core samples, given in m/d for the three boreholes, sorted in synth-K.xls;
- hydro/Virus/virus field work/rec' survey Jan 05/summary of packer test scan Jan 2005.xls: summary of packer tests permeabilities measurements in BH3, from different sources of data, especially the Richard and virus measurements, copied in synth-K.xls
- Julian Scott packer tests in hydro/Fracture Fill/Field Tests/Julians Disk/XX_XXm/results_date.xls analysed and synthesized in synth-K.xls, compared with other interpretations of the same data from Murtala Bashir (hydro/Fracture Fill/Field

Tests/Appendix 3-Packer tests/Packer test data/test X.xls) and (hydro/ Fracture Fill/Field Tests/Appendix 2-Laboratory tests/Lab tests/Lab test results.xls)

An example of the permeability data collated from previous studies available for the test site boreholes is shown in Figure 2.

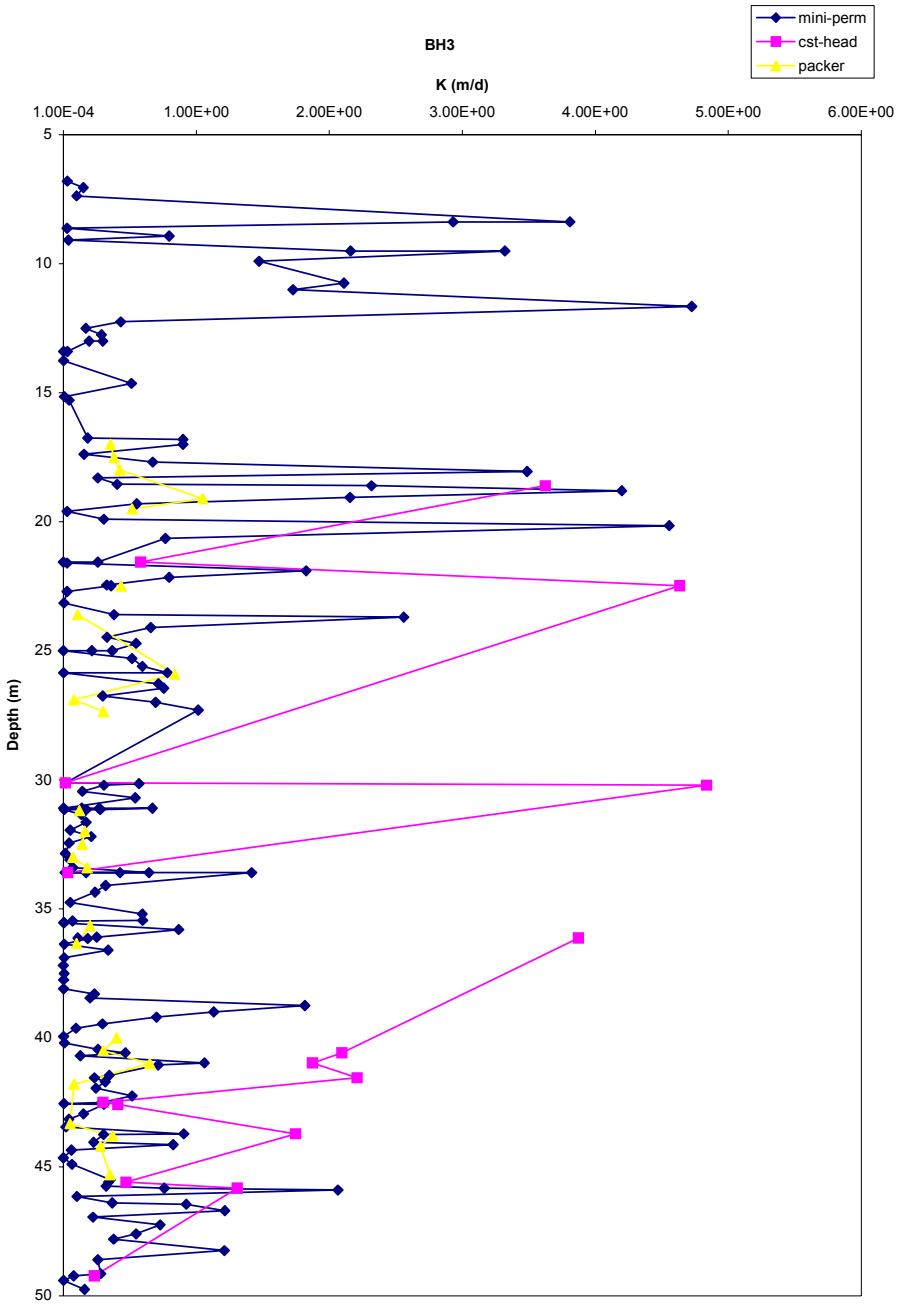


Figure 2: Example permeability data set for the experimental site (borehole 3)

Appendix 2

Field Hydraulic Tests

A2.1 Introduction

During the project, various hydraulic tests have been completed on the boreholes, including: (i) packer flow meter tests; (ii) step and constant yield pumping tests; and (iii) cross-borehole single packer testing over a range of intervals (Ferguson, 2006). (i) and (iii) are described below.

A2.2 Flow tests on BH2 and BH3, May 2007

Objective
The aim of this experiment is to quantify the flow rates along the depth of the boreholes, and to relate these flow rates to the known geology.
Under constant high pumping conditions at the top of the borehole, some local flow measurements are done at different depths, representing the local flows coming from the part below the measurement towards the pump above.

Experimental device
The experimental device is shown on Figure 1, and comprises:

- **submerged pump** at the top of the borehole: MP3 (Grundfos), from 90 l/min to 160 l/min, discharge not really precise→ needs a flow measurement at the surface

- **surface impeller flowmeter**: 100 pulses per min correspond to 10 l/min
- **borehole impeller flowmeter**: isolated impeller connected to a packer system to centralize the measurement; needs to be calibrated



- **pressure transducers to check the eventual head loss due to the packer**: one above the packer at a constant depth, and one at the impeller level, at the bottom of the packer (moves with the measurement depth)
- **registering system**: one computer (laptop) connected to the transducers box, reads the pressures with the "Keller-read30" software (address 3 corresponds to the one above the packer, and address 2 below the packer); one other computer is connected to the datalogger (DT500) that reads the pulses from

Both impeller flowmeters with the datataker software (address 1 is for the surface flowmeter, and address 3 for the groundwater one)

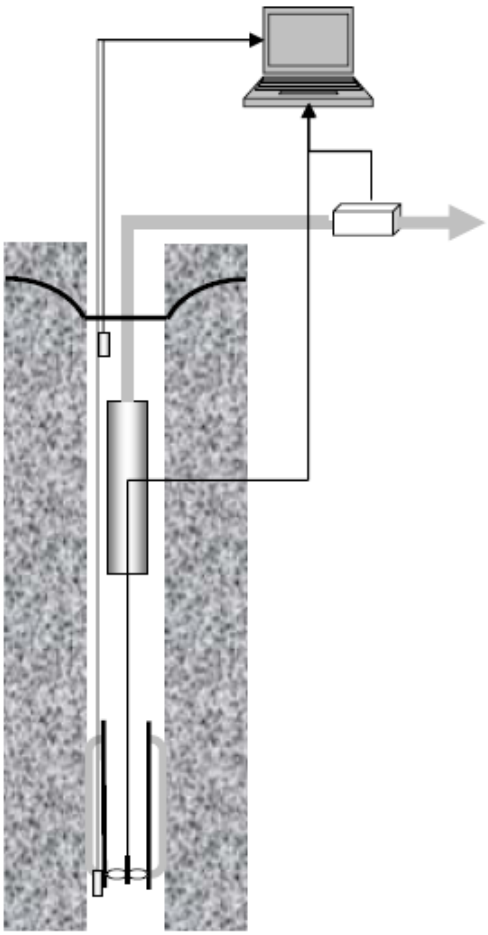


Figure 1: Scheme of the experimental device

Borehole impeller flowmeter calibration

Date: 17/05/07
Experimenters: Richard Greswell and Véronique Durand
File: flow-calibration.xls

The calibration of the borehole impeller flowmeter is done by comparing the surface flowmeter from the pump and the pulses from the borehole flowmeter. The pump has been placed just above the packer, both are inside the casing of the borehole BH2.
Depths from ground level:

- casing = 15.6 m
- top packer = 14.3 m
- bottom packer/impeller = 15.6 m (packer length = 1.3 m)
- bottom pump = 14 m (5 long pipes + 1 small)

Different discharge rates from the pump are tested, from 20 l/mn to 150 l/mn. Any higher rate would conduct to dry the pump at this depth. The pressures are pulses are registered together, at a trigger rate of 1 mn. Each measurement is done during approximately 5 mn, leading to five points. The average is calculated for each.
During the whole test, the pressure difference between the two transducers keeps constant (Figure 2), whenever the pump is running or not, which means that there is no head loss due to the packer system.

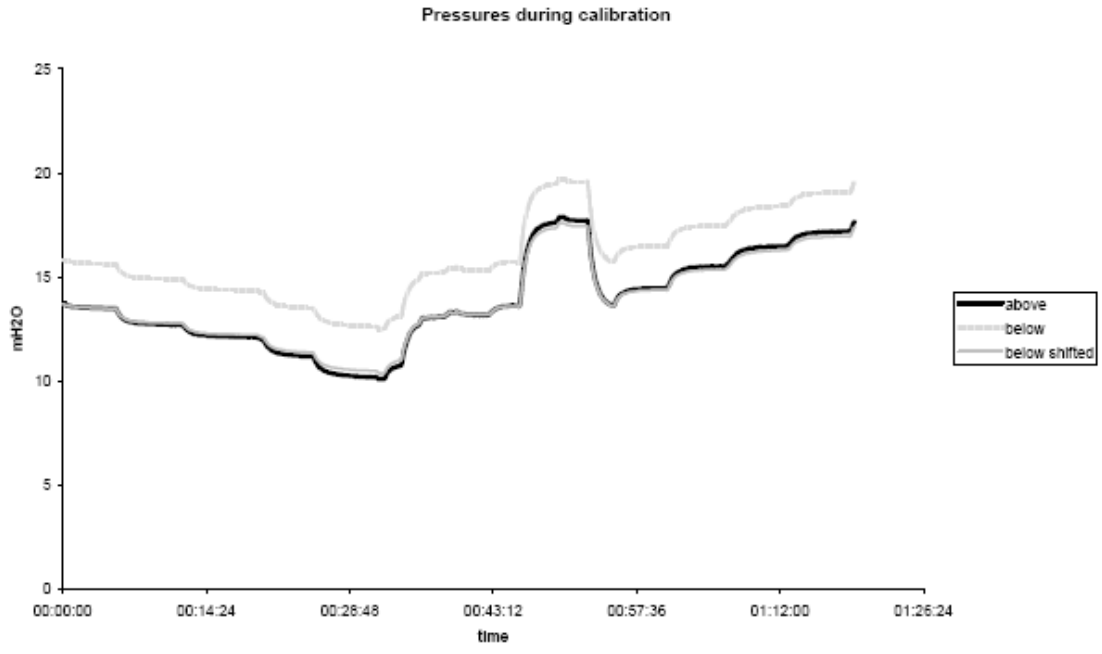


Figure 2: Pressures during the flowmeter calibration

The calibration result is good, the relationship between the counter and the discharge is linear (Figure 3):

Discharge (l/min) = 0.2421x flow meter rate (pulses/min), $R^2 = 0.994$

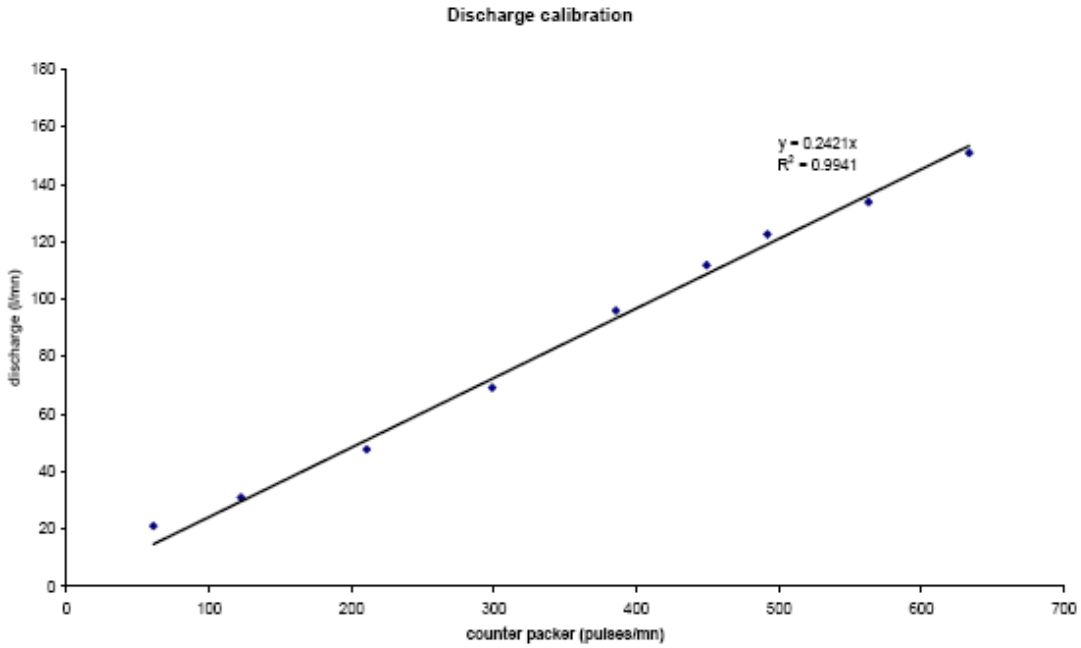


Figure 3: Calibration result

Flow tests on BH2 and BH3

Dates: 17/05/07 and 18/05/07 for BH2, 24/05/07 for BH3
Experimenters: Richard Gresswell, Véronique Durand and Dan Jefferies
Files: flow-tests-BH2-BH3.xls and pressure-flow-tests-BH2-BH3.xls

With a high constant discharge rate (160-170 l/min) at the top of the borehole, the borehole impeller flowmeter gives flow measurements at different depths down the same borehole. The flow is cumulative: it is higher towards the top (Figure 4), as the water comes from a bigger vertical zone. Nevertheless, the measurements allowed location of layers where the flow is low (Figure 5), related to low permeability lithologies (clay-rich).

As the pumping rate is not exactly constant, and changes from BH2 to BH3, the measured flows down the two boreholes are expressed in % of the pumping rate. As the casing in BH3 is less deep than in BH2 (12.3 m compared with 15.5 m), the relative flows in BH3 are systematically below the relative flows in BH2 (Figure 4).

The local flows are estimated by subtracting the successive flow measurements. They can be compared by dividing the difference in flow by the difference in elevation (Figure 5).

The highest flows are observed at the top and at the bottom of the boreholes, with a relative high level around 40 m bgl.

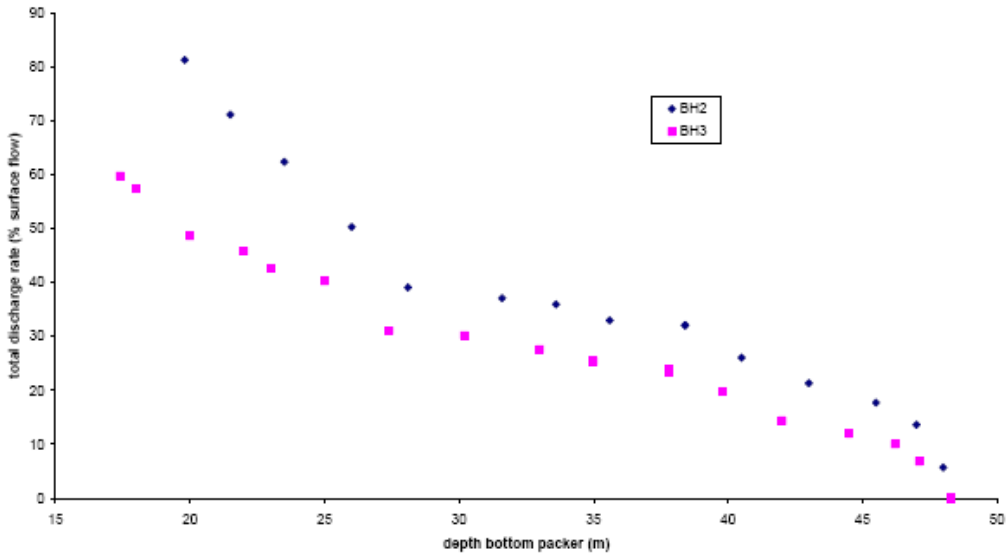


Figure 4: Results of the total discharge for the two boreholes

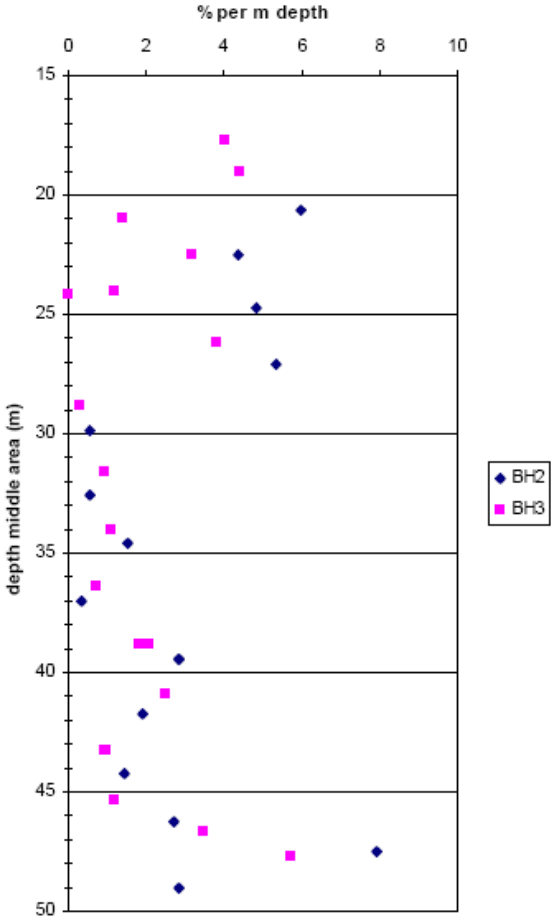


Figure 5: Results of the local discharge for the two boreholes

Conclusion

The chosen device gives some good results. It is possible to quantify the local flows coming through the different layers. The influence of fractures and granulation seams is nevertheless difficult to observe. The measured flows will be compared to the locations of virus breakthrough during the phage tracer experiment.

A.2.3 Note on Correcting Flows from Packer Flow Meter Measurements

The following may be required if the head loss across the flow meter is significant.

It is assumed that the formation can be divided neatly into non-leaky, confined horizons characterised by a hydraulic resistance and that the flow meter is located at an aquitard. The development is for a two layered system, but it will probably generalise easily.

Under steady state pumping conditions **without** the flow meter in place, define:

Q the rate at which the borehole is pumped.
Q_A the discharge from the aquifer below the flow meter location.
Q_B the discharge from the aquifer above the flow meter location.
h the head in the borehole
R_A the hydraulic resistance of the formation below the flow meter location
R_B the hydraulic resistance of the formation above the flow meter location

Then

$$H_A - h = R_A Q_A \tag{A2.3.1}$$

$$H_B - h = R_B (Q - Q_A) \tag{A2.3.2}$$

Under steady state pumping conditions **with** the flow meter in place, define:

Q' the rate at which the borehole is pumped.
Q'_A the discharge from the aquifer below the flow meter location.
Q'_B the discharge from the aquifer above the flow meter location.
$\Delta h(Q'_A)$ the head loss across the flow meter as a function of discharge through it.

Then

$$H_A - h' + \Delta h(Q'_A) = R_A Q'_A \tag{A2.3.4}$$

$$H_B - h' = R_B (Q - Q'_A) \tag{A2.3.5}$$

Subtracting equation (A.2.2) from equation (A.2.1) and rearranging gives

$$Q_A = \frac{R_B}{R_A + R_B} Q + \frac{H_A - H_B}{R_A + R_B} \tag{A2.3.6}$$

and subtracting equation (A.2.4) from equation (A.2.3) and rearranging gives:

$$Q'_A = \frac{R_B}{R_A + R_B} Q' + \frac{H_A - H_B}{R_A + R_B} - \frac{\Delta h(Q'_A)}{R_A + R_B} \quad \text{(A.2.7)}$$

From equation (A.2.5) and equation (A.2.6)

$$\boxed{Q_A = Q'_A + \frac{R_B}{R_A + R_B}(Q - Q') + \frac{\Delta h(Q'_A)}{R_A + R_B}} \quad \text{(A.2.8)}$$

Comments

- Confidence in the flow measurement will be increased if $Q = Q'$.
- $\Delta h(Q'_A)$ can be measured with pressure transducers above and below the packer or can be determined more generally in the laboratory.
- $\frac{1}{R_A + R_B}$ has to be determined in the field.
- As an alternative, using two flow meters in parallel should reduce the head loss by a factor of 4.

A.2.4 Testing of Head Losses across the Flow Meter [report in progress]

The experimental layout for testing of the packer flow meter is shown in Figur 6. The data collected are shown in Figure 7, and the relationship between measured head losses and flow in Figur 8.

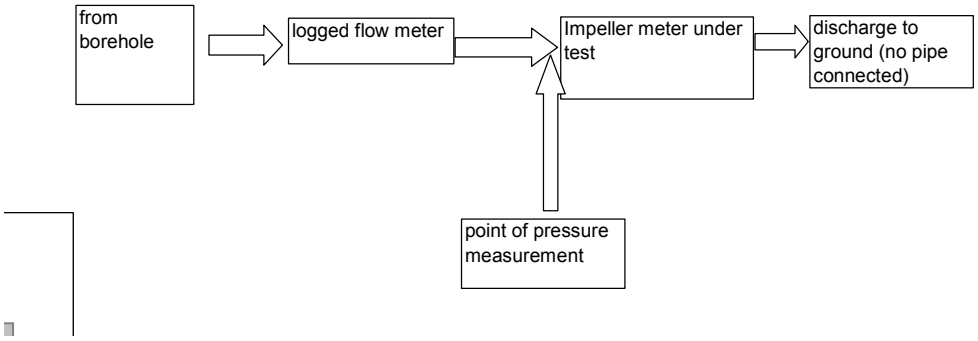


Figure 6: Experimental layout for measurement of head losses across the packer flow meter.

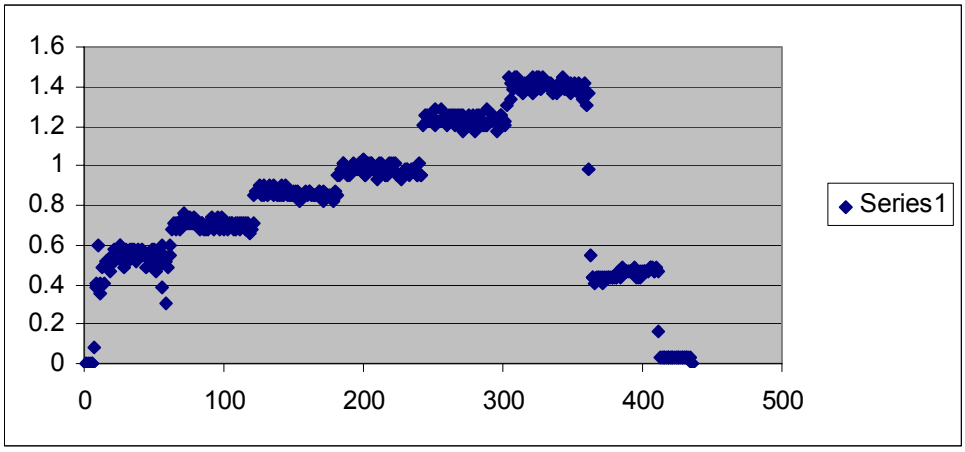


Figure 7: Data collected [head loss (m) against time (seconds)].

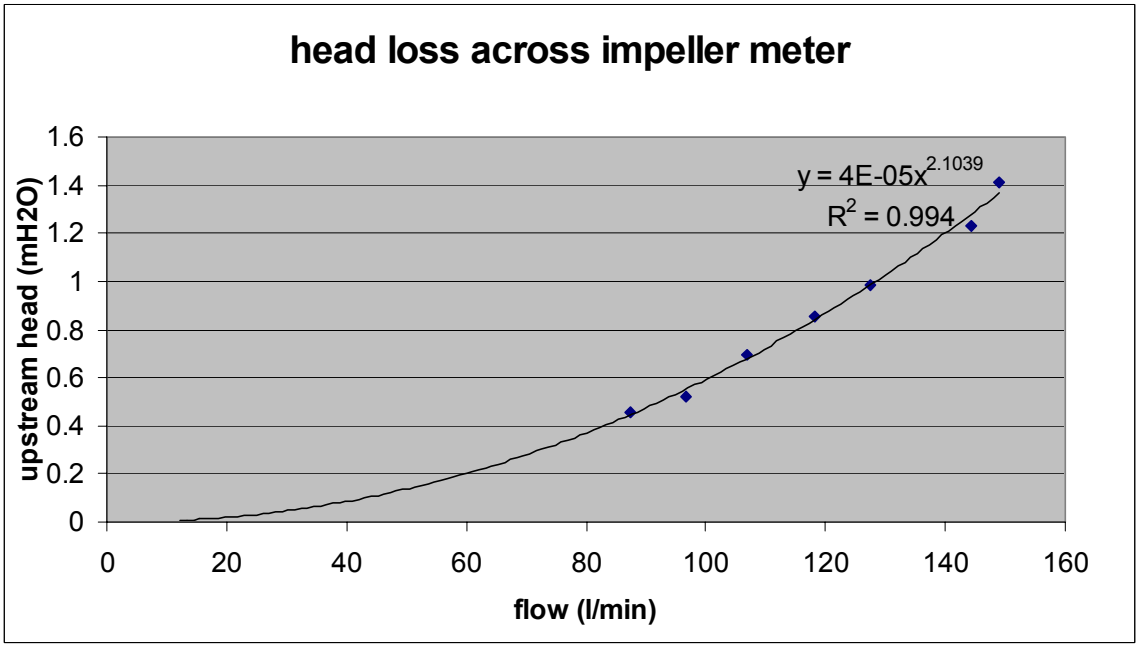


Figure 8: Measured head loss against flow rate [downstream head = 0]

A.2.5 Cross-borehole Packer Testing

Field hydraulic testing has also been carried out by Ferguson (2006). This included a detailed study of head distributions within the unpumped system and cross-borehole packer testing which revealed that the central part of the borehole sequence has a very low vertical connectivity (Figure 9). The work has also indicated the importance of an upper aquifer unit at around the level of the base of the casing in the boreholes. All these results have further developed our conceptual model of the system, and will be used in the interpretation of the phage tracer experiments.

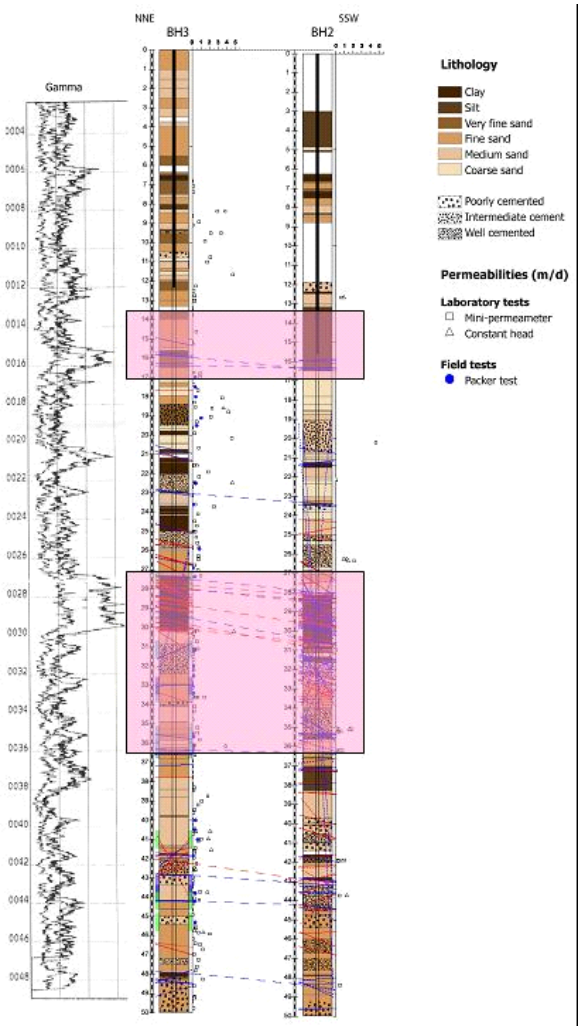


Figure 9: Results of cross-borehole packer testing (Ferguson, 2006). Horizons with low vertical permeability highlighted.

Appendix 3

Chemical Sampling of the Borehole Array

A3.1 Sampling Rounds

Samples from the boreholes were collected at three times:

- 14th November, 2007, the week before the student fieldweek; samples were collected from boreholes 2 and 3 by pumping.
- 14th December, 2007; 5 samples from different sampling pumps located at different depths and one from the discharge line were collected from borehole 2. Water had been pumped for 24 hours before samples were collected.
- 5th February, 2008; boreholes 1, 2, 3 and the Environment Agency open borehole on the University campus were sampled manually using a depth sampler.

Due to different systems of sampling, the depth and 'catchment' of each sample was different. Table 1 shows the sampling depth, in each borehole for each sampling time.

Table 1 Sampling depth for each borehole and time

Time	Depth (m)	Borehole
November	16.20	B2 and B3
December	16.20	B2
	18.00	
	24.50	
	29.50	
	37.50	
	45.75	
February	25	B1, B2 and B3
	16	OB

A3.2 pH and conductivity

Water pH values in boreholes are showed in Tables 2 and 3 and Figure 1. Borehole 3 had the lowest value (6.5) of pH and the open borehole (8.1) the highest. Data indicated similar pH values in November and February in boreholes 2 and 3 although the December pH data were higher: the December samples were collected after 4 days of borehole pumping. Also similar values were recorded for boreholes 1 to 3 in February. pH decreased slightly with depth in sampling carried out in December in borehole 2 (Table 4).

Table 2 pH in boreholes

Date	B1	B2	B3	OB
Nov		6.7	6.5	
Dec		7.6*		
Feb	6.9	6.8	6.9	8.1

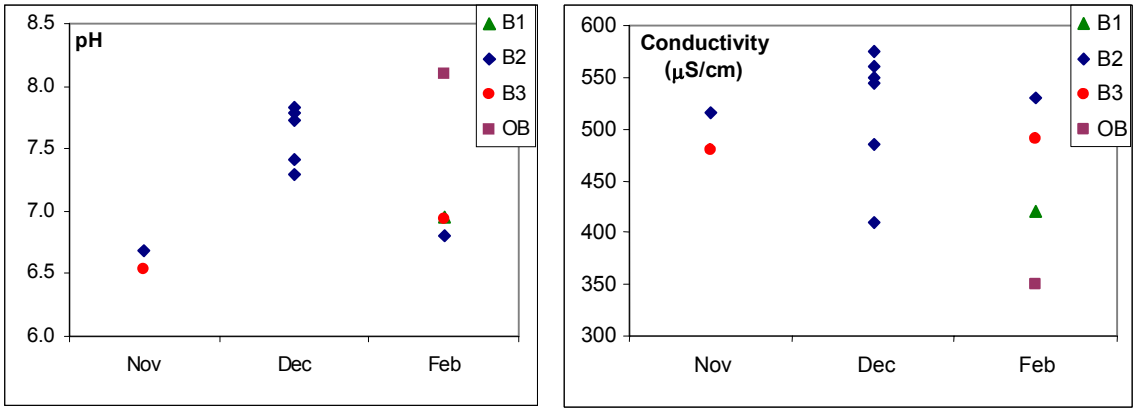
* Average of all depths

Table 3 Conductivity (µS/cm) in boreholes

Date	B1	B2	B3	OB
Nov		515.6	480	
Dec		510.2*		
Feb	420	530	490	350

* Average of all depths

Figure 1 pH and conductivity in boreholes across time

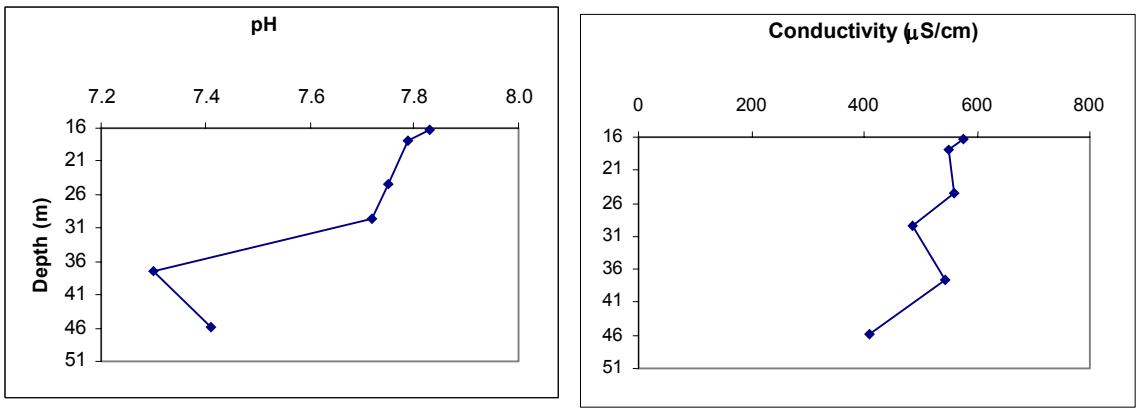


Borehole 2 (530 µS/cm) had the highest conductivity (Table 3 and Figure 1) while conductivity in the open borehole (350 µS/cm) was the lowest. There were no changes in conductivity in boreholes 2 and 3 between November and December. Slight conductivity changes with depth were found in the December sampling of borehole 2 (Table 4)

Table 4 pH and conductivity values in borehole 2

Pump	Depth (m)	pH	Conductivity (µS/cm)
Discharge	16.20	7.83	575
1	18.00	7.79	550
2	24.50	7.75	561
3	29.50	7.72	485
4	37.50	7.30	545
5	45.75	7.41	410

Figure 2 pH and conductivity in borehole 2



A3.3 Total Organic Carbon and Fluorescence

High levels of total organic carbon (TOC) were found in all boreholes sampled (Table 5 and Figure 3). Higher concentrations of TOC with more than 8 mg/l were found in borehole 1 and 2, the lowest TOC was found in November in borehole 1. TOC concentrations were constant during all months in borehole 2 but borehole 3 had a wide variation in TOC concentrations.

Fluorescence Excitation-Emission Matrix (FEEM) carried out on the February samples are shown in Figure 4. In this figure, borehole 1 and the open borehole have greater fluorescence signatures than the samples from boreholes 2 and 3. However, all samples have lower than expected fluorescence than expected for their TOC concentrations, and thus the TOC source is unusually poorly fluorescent. Possible candidates include sugars, alcohols, glycol, and possibly fluorescein degradation products.

Table 7 shows values of TOC, TC, and IC from borehole 2 at different depths. High levels of TOC were found at all depths, but at 29.5 m the value was very high (17.74 mg/l). Figure 6 shows the FEEM images for each depth.

Table 5 TOC (mg/l)

Date	B1	B2	B3	OB
Nov		8.93	4.13	
Dec		8.99*		
Feb	8.87	8.16	7.09	6.34

* Average of all depths

Table 6 IC (mg/l)

Date	B1	B2	B3	OB
Nov		52.2	39.83	
Dec		46.2*		
Feb	42.82	47.37	44.02	33.57

* Average of all depths

Figure 3 TOC, IC in boreholes

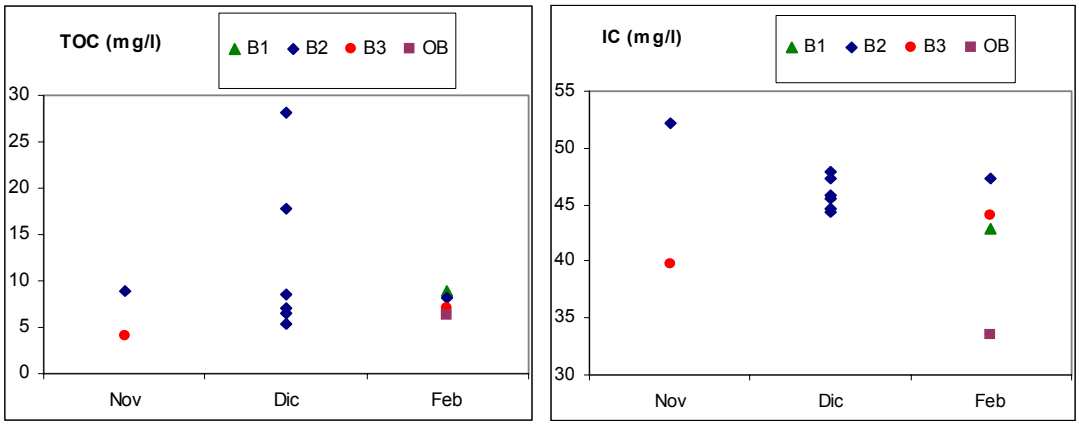
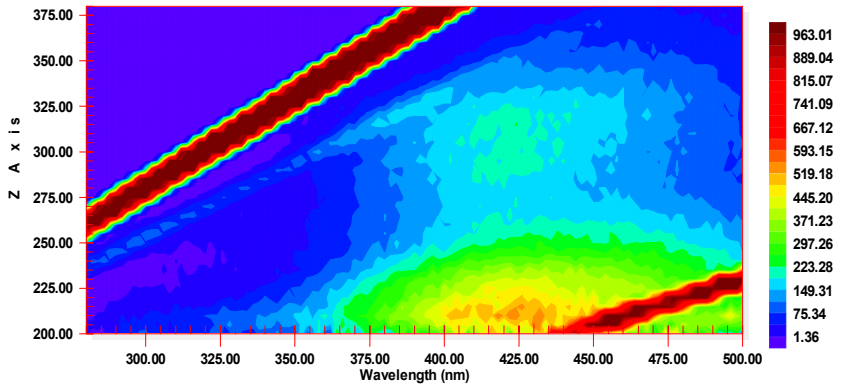
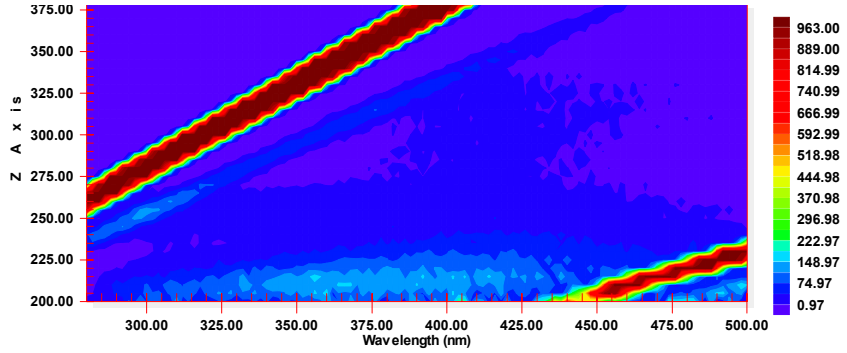


Figure 4. Borehole FEEM images

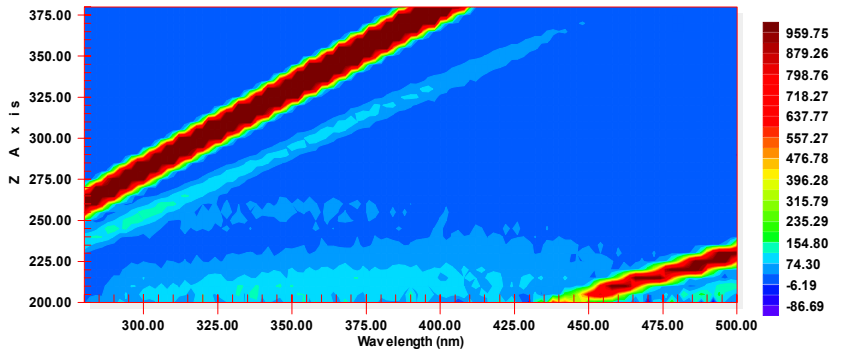
B1



B2



B3



Open Borehole

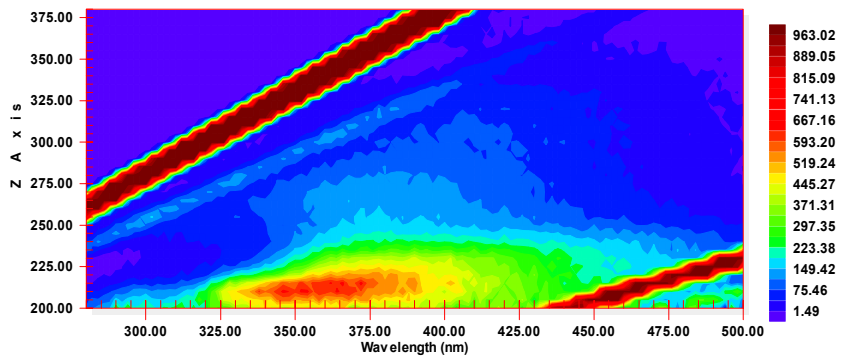


Table 7 TOC, TC and IC in borehole 2

Pump	Depth (m)	TOC (mg/l)	TC (mg/l	IC (mg/l)
Discharge	16.20	28.14	72.84	44.70
1	18.00	6.96	52.83	45.87
2	24.50	5.41	52.76	47.35
3	29.50	17.74	62.16	44.42
4	37.50	8.47	53.96	45.50
5	45.75	6.39	54.24	47.86

Figure 5 TOC in borehole 2

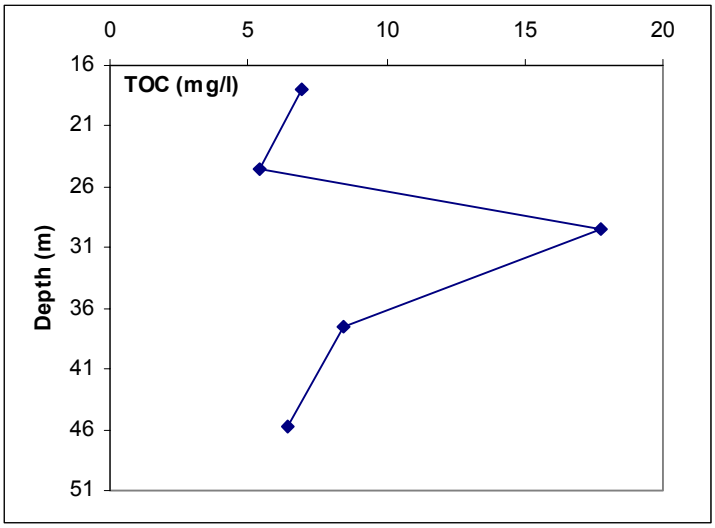
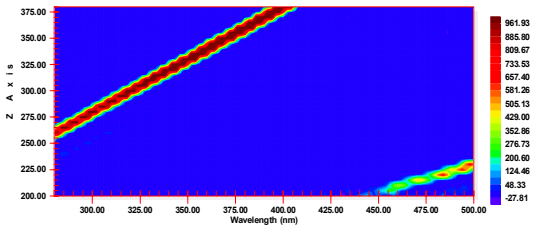
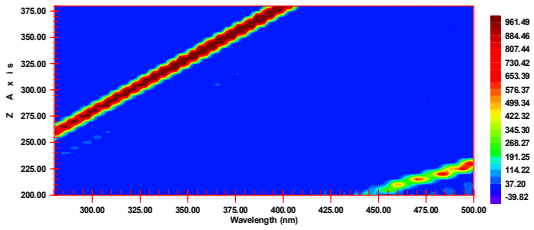


Figure 6 Borehole 2 FEEM images

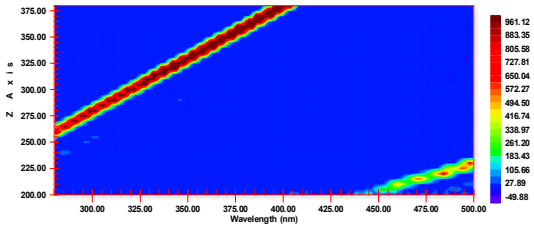
16.2 m Discharge



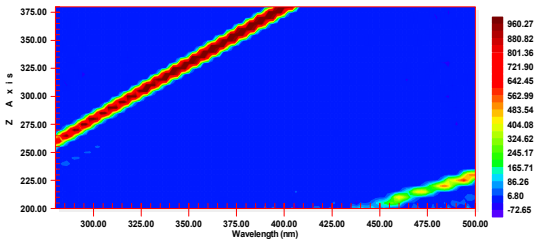
18.0 m



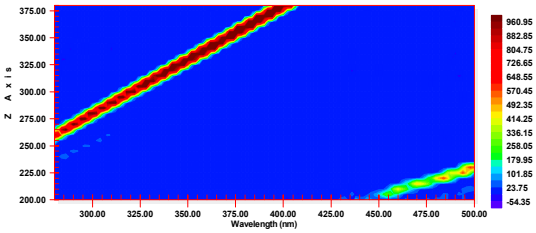
21.5 m



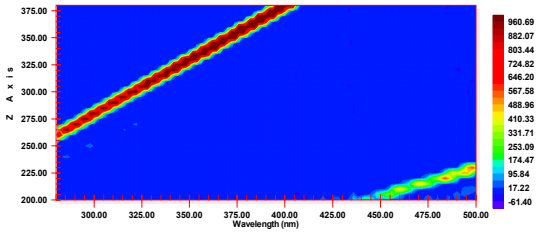
29.5 m



37.5 m



45.75 m



A3.4 Anions

Chloride, nitrite, nitrate, bromide, phosphate, and sulphate were determined using ion chromatography (Dionex). Anion concentrations are shown in Table 8 and Figure 7. Table 9 and Figure 8 list the concentrations of anions for borehole 2 at each depth in the December sampling.

Table 8 Anions concentration (mg/l)

Chloride	**(mg/l)**			
Date	**B1**	**B2**	**B3**	**OB**
Nov		24.3	19.1	
Dec		17.8*		
Feb	12.6	11.4	11.1	15.8

Nitrite	**(mg/l)**			
Date	**B1**	**B2**	**B3**	**OB**
Nov		1.16	0.05	
Dec		0.10*		
Feb	0.78	0.35	0.35	0.00

Bromide	**(mg/l)**			
Date	**B1**	**B2**	**B3**	**OB**
Nov		0.23	0.10	
Dec		0.09*		
Feb	0.57	0.23	0.18	0.00

Nitrate	**(mg/l)**			
Date	**B1**	**B2**	**B3**	**OB**
Nov		17.4	17.9	
Dec		17.8*		
Feb	9.6	15.4	18.6	5.7

Phosphate	**(mg/l)**			
Date	**B1**	**B2**	**B3**	**OB**
Nov		34.3	24.3	
Dec		11.5*		
Feb	34.4	14.1	10.9	5.1

Sulphate	**(mg/l)**			
Date	**B1**	**B2**	**B3**	**OB**
Nov		53.5	27.5	
Dec		40.6*		
Feb	19.4	45.4	31.5	24.7

* Average of concentration

Figure 7 Anion concentrations in all four boreholes

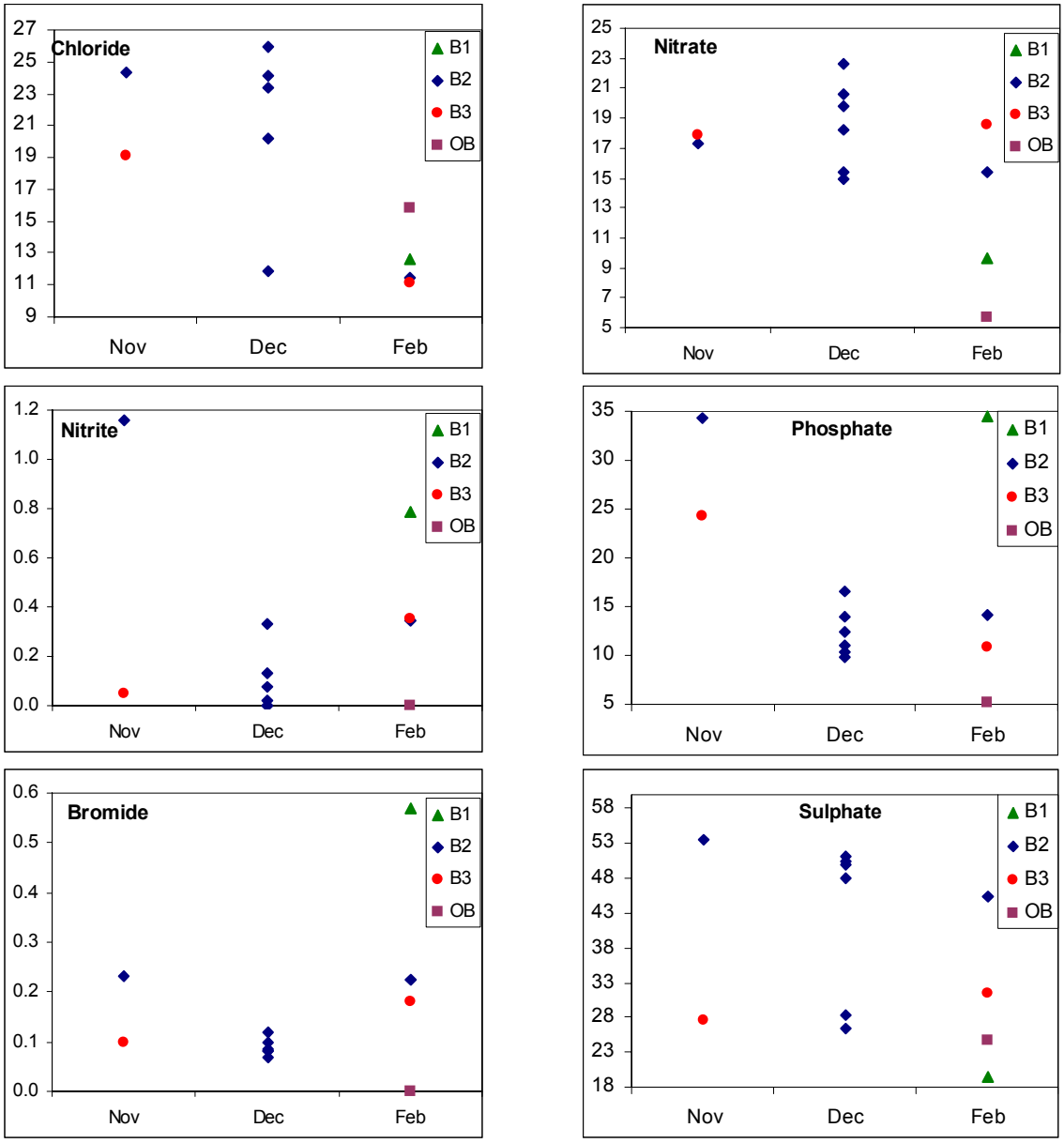
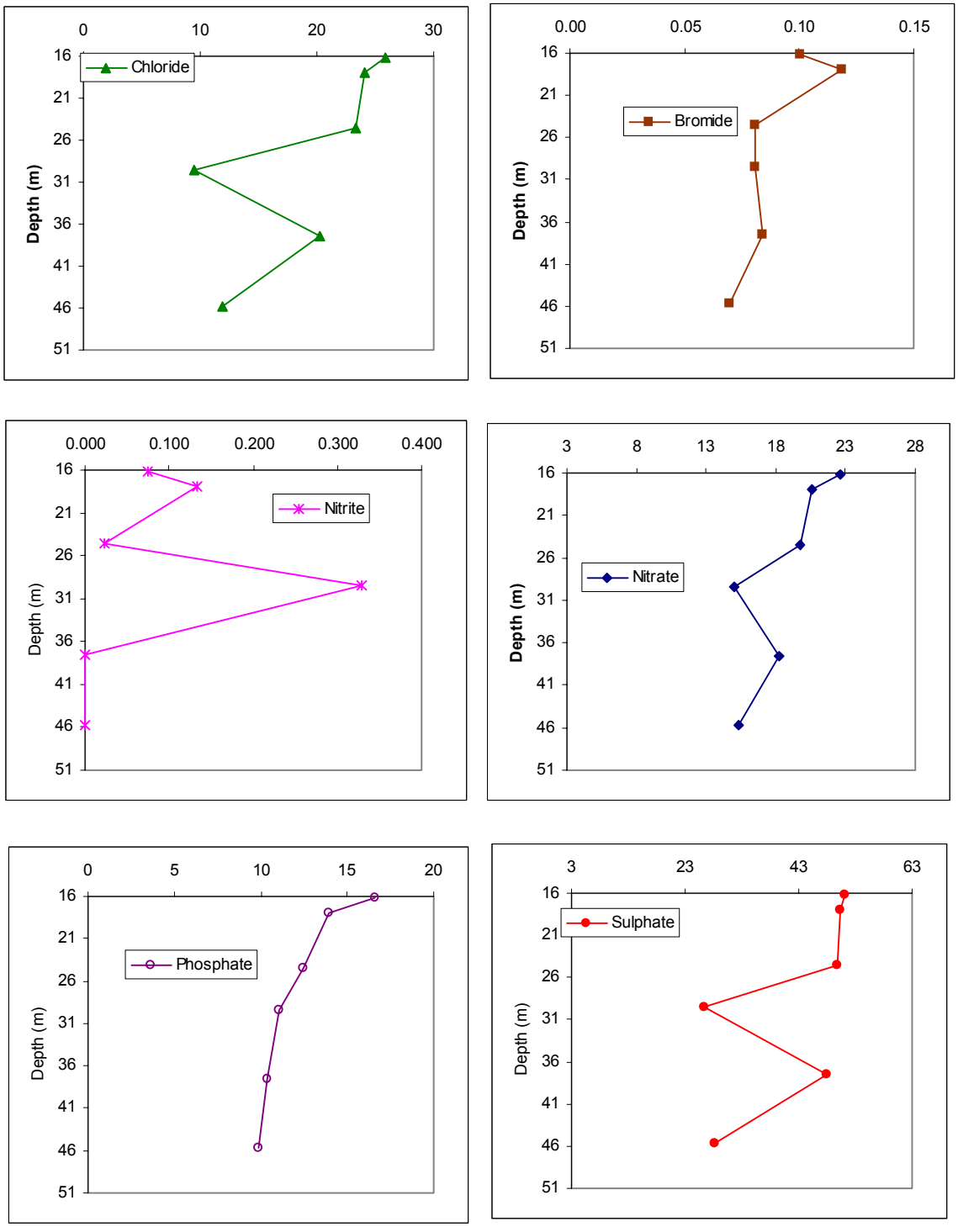


Table 9 Anion concentrations in borehole 2 (mg/l)

Pump	Depth (m)	Chloride	Nitrite	Bromide	Nitrate	Phosphate	Sulphate
Discharge	16.20	25.93	0.08	0.10	22.67	16.58	51.09
1	18.00	24.15	0.13	0.12	20.60	13.93	50.31
2	24.50	23.35	0.02	0.08	19.75	12.45	49.89
3	29.50	9.46	0.33	0.08	14.97	11.09	26.50
4	37.50	20.22	0.00	0.08	18.23	10.37	48.07
5	45.75	11.92	0.00	0.07	15.36	9.86	28.34

Figure 8 Anion concentrations in borehole 2



A3.5 Cations

Lithium, sodium, potassium, magnesium, and ammonium were determined using ion chromatography (Dionex Ion Pac CS 12A). Concentrations are shown in Table 10 and Figure 9. Ammonium was not found in any of the samples. Table 11 and Figure 10 show cation concentrations in borehole 2 for the December round of sampling.

Table 10 Cation concentrations

Lithium	**(mg/l)**		
Date	**B1**	**B2**	**B3**
Nov		0.94	1.69
Dec		0.95*	
Feb	0.98	0.99	1.74

Sodium	**(mg/l)**		
Date	**B1**	**B2**	**B3**
Nov		29.3	30.9
Dec		16.3*	
Feb	17.4	16.2	15.2

Potassium	**(mg/l)**		
Date	**B1**	**B2**	**B3**
Nov		13.7	11.8
Dec		11.7*	
Feb	14.0	12.4	11.5

Magnesium	**(mg/l)**		
Date	**B1**	**B2**	**B3**
Nov		37.6	25.0
Dec		23.4*	
Feb	24.0	32.3	31.5

Na^++K^+	**(mg/l)**		
Date	**B1**	**B2**	**B3**
Nov		43.1	42.7
Dec		28.0*	
Feb	31.4	28.6	26.7

* Average of concentration

Figure 9 Cation concentrations in all four boreholes

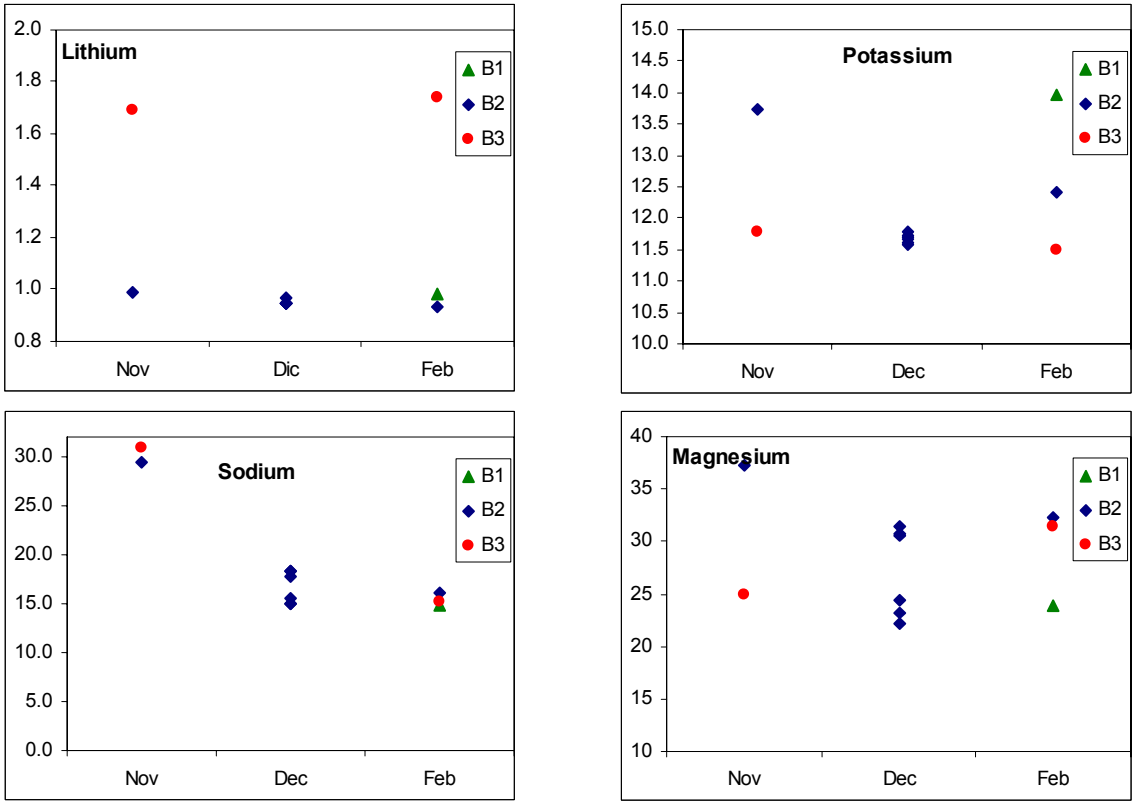
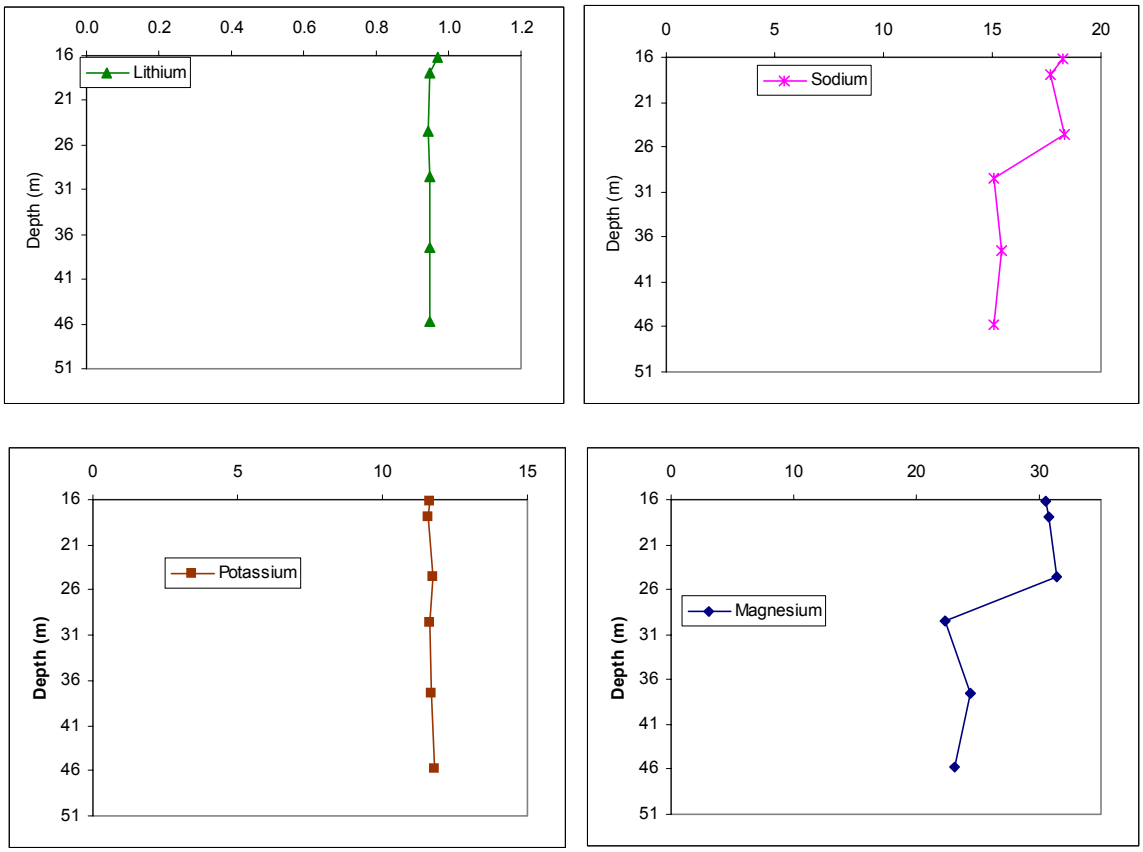


Table 11 Cation concentrations in borehole 2

Pump	Depth (m)	Lithium	Sodium	Potassium	Magnesium
Discharge	16.20	0.97	18.27	11.65	30.55
1	18.00	0.95	17.69	11.58	30.74
2	24.50	0.94	18.35	11.73	31.35
3	29.50	0.95	15.06	11.60	22.25
4	37.50	0.95	15.45	11.69	24.39
5	45.75	0.95	15.05	11.77	23.13

Figure 10 Cation concentrations in borehole 2



A3.6 Conclusion

Chemical analyses of the borehole samples indicate that water has large amounts of TOC of uncertain source. Most other determinands show considerable variation both with depth (borehole 2) and time. Other analyses including volatile organic compounds and heavy metals will be carried out also.

Appendix 4

Development of Plaque Assay Method for PRD1

A4.1 INTRODUCTION

This document describes work carried out in the laboratory to develop and test the procedures for the sampling and analysis of phages prior to the tracer experiments, including the testing of the virus traps.

A4.2 GROWING BACTERIOPHAGES

A4.2.1 Introduction

Growing phages is necessary in order to obtain sufficient virus mass to develop the virus analysis methods, to complete the testing of the virus traps, and to carry out the field tracer testing.

Bacteriophages and their host bacteria were supplied by NCIMB (www.**ncimb**.co.uk/) or ATCC (http://www.lgcpromochem-atcc.com/). The phage vial titre is approximately 10^8 to 10^{11} pfu/ml (plaque forming unit per ml). Phage and bacteria vials were stored at 4 to 5 ˚C.

A4.2.2 Growth of host bacteria culture

The procedure used was as follows: open the vial following the instructions given by the bacteria supplier; add 0.5ml of specific bacteria broth to pellet (Tryptone Soya Broth (TSB) for PRD1); heat broth to 20˚ C and mixed gently; transfer rehydrated pellet to 5ml of broth and mix; add 1ml of broth plus bacteria to ml of broth and culture at 37 ˚C in a shaking incubator overnight. All material and broth used to growth bacteria were autoclaved before use.

A4.2.3 Log phase test

In order to determine the 'log phase' of bacteria growth, that is the optimum condition for inoculating phages, an optical density test was undertaken for 24 hours. Optical density (OD) is a technique to estimate the total number of bacterial cells present in a broth culture. A spectrophotometer is used to measure the light intensity transferred through a liquid. If microbial cells are present, the light will be scattered, thus reducing the amount that is transferred through the suspension. The OD produced by a culture will depend on the concentration of cells present, the strain of microbes present, the growth conditions, and the wavelength of the light being transmitted. OD measurements were made at 600nm, that is the appropriate wavelength for the *E. coli* PRD1 host. 5ml of overnight bacteria culture were inoculated into 50ml of autoclaved broth, and 50ml of autoclaved broth with no bacteria were used in the OD test. Table 1 and Figure 1 show the results of OD measurements for *E. coli* specific PRD1 host.

Table 1 OD values vs. time

Time (h)	OD *E. coli*	Control	Difference
0	0.105	0.036	0.069
1	0.227	0.034	0.193
2	0.614	0.035	0.579
3	1.071	0.036	1.035
4	1.518	0.039	1.479
5	1.574	0.038	1.536
7	1.600	0.039	1.561
24	1.600	0.038	1.562

From these results, the best time to do the phage inoculation was determined to be between 3 and 4 hours after bacteria inoculation, with an optimum around 3 and half hours.

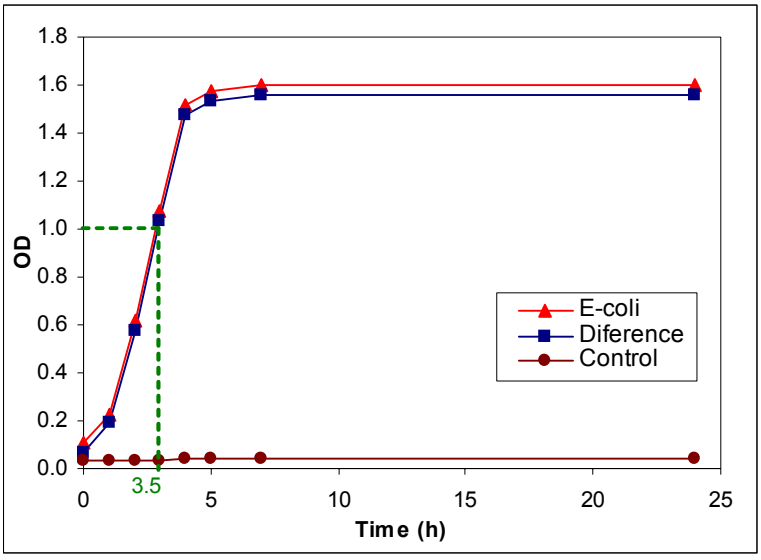


Figure 1 OD vs. time for PRD1 host

A4.2.4 Growing phages from vials

Open the vial following the instructions given with the phage. Add 0.5ml of specific bacteria broth to phage stock (Tryptone Soya Broth (TSB) for PRD1); heat broth to 20˚ C and mix gently. Transfer rehydrated stock to 5ml of broth and mix. All material and broth used to growth bacteria autoclaved before use.

Due to high phage concentration, it is essential to do serial decimal dilutions of the initial phage culture. A minimum of four different dilutions are necessary; in the initial work, six serial decimal dilutions were carried out using the following protocol:

1. 9ml of autoclaved broth were added to six sterile dilution tubes and labelled as 10, 10^2, 10^3, 10^4, 10^5, 10^6.
2. 1ml of initial phage dilution was added to the tube labelled "10". Tube was capped and vortexed for 5 second on a medium–high setting
3. 1ml of the well mixed 10 dilution was added to tube labelled "10^2". Tube was capped and vortexed for 5 seconds until well mixed.
4. The same technique was repeated to create dilutions 10^3, 10^4, 10^5, 10^6.

Tubes were caped and stored in a fridge until use.

A4.2.5 Growing phages in liquid culture

A4.2.5.1 Introduction

This protocol describes how to grow phage in liquid culture. The description is for PRD1, but the procedure can used to grow any phage provided the appropriate host bacteria and bacteria broth are used.

A4.2.5.2 DAY 1 Growth overnight of host culture (1)

Materials

1. 50ml of Tryptone Soya Broth (TSB), autoclaved and stored at 37 ˚C
2. Tryptone Soya Broth
3. 5ml *E. coli* bacteria
4. 5ml pipette
5. autoclaved 5 ml tips
6. shaker incubator

Method

Inoculate with 5 ml of *E. coli* bacteria into 50ml of TSB. Cover the flask, label and place in the shaker incubator at 37 ˚C and leave overnight.
Prepare, for the next day, 50ml of TSB following the procedure as specified on bottle of media. Autoclave the broth and store in the fridge.

A4.2.5.3 DAY 2 (4 hours host bacteria and phage inoculation)

Materials

1. 50ml of Tryptone Soya Broth (TSB), prepared the day before and heated to 37˚ C
2. 5ml overnight *E. coli* (1)
3. 5ml pipette
4. autoclaved 5 ml tips
5. shaker incubator
6. 5 ml of PRD1 phage (used one serial dilution between 10^2 to 10^4) heated to 37˚ C
7. Tryptone Soya Broth

Method

Inoculate with 5 ml of *E. coli* overnight culture (1) into 50ml of TSB, covered and label and place in the shaker incubator at 37C for 3.5 hours (do not leave more than 4 hours)
Keep the overnight culture (1) in the fridge.

After 3.5 hours inoculate the 50 ml flask host bacteria with 5 ml of PRD1; carefully seal the flask, label and place in the shaker incubator at 37˚ C overnight.
Bacteria phage ratio used in this protocol is 1/1 (phage/bacteria) but other studies have used ratios between ½ and 1/20 (phage/bacteria).
Prepare, for the next day, 50ml and 300ml of TSB following the procedure as specified on bottle of media. Autoclave the broth and store in the fridge.

A4.2.5.4 DAY 3 (overnight culture and 300ml phage culture)

Materials

1. 300ml and 50ml of Tryptone Soya Broth (TSB), prepared the day before and heated to 37˚ C
2. 35ml overnight *E. coli* (1)
3. 50ml of PRD1 (made day2)
4. 5ml pipette
5. autoclaved 5 ml tips
6. shaker incubator
7. Tryptone Soya Broth

Method
New overnight culture (2): Inoculate with 5 ml of *E. coli* overnight culture (1) into 50ml of TSB, cover and label and place in the shaker incubator at 37C and leave overnight.
Take 30 ml of the *E. coli* overnight culture (1) and inoculate it into 300ml of TSB, cover and label and place in the shaker incubator at 37C and leave overnight.
Discard the overnight culture (1) remaining.
After 3.5 hours inoculate the 300 ml bacteria with 35 ml of PRD1 culture grown in day 2, securely closing the flask, label and place in the shaker incubator at 37C overnight. Place the rest of the PRD1 culture in the fridge. Follow this procedure until the required phage volume is obtained.

A4.2.6 Purification of phages

Purification is the separation of the bacteria host from the phages and is the last stage in the growing procedure. Phages needed to be purified before use.

Materials
1. NaCl
2. 5000 – 6000 rpm centrifuge
3. Sterile centrifuge tube
4. Polyethylene glycol 6000 (PEG6000)
5. Phage buffer (phosphate buffer/tris buffer)

Method

Add 58.4 g/l NaCl (1M) to liquid phage culture and dissolve gently. Put in ice or in fridge for at least one hour. Centrifuge for 10 minutes at 5000m rpm and retain supernatant. Add PEG for a final concentration of 10% to supernatant. Put in the fridge and leave overnight or longer (experience indicates that 2 days is the optimum time for PRD1).
Centrifuge at 6000 rpm for 25minutes. Discard the supernatant and invert the tube on paper towels for a few minutes. Resuspend the pellet in a phage buffer solution and store at 4˚C.

A4.3 BACTERIOPHAGE ENUMERATION

A4.3.1 Introduction

There are several different techniques to enumerate phages, including cytometry, plaque assay, epifluorescence, electron microscopy, quantitative PCR (polymerase chain reaction). Some of them are expensive and others have cumbersome protocols and/or require special technology. Due to the large number of samples generated in a tracer experiment, a low cost method is important: however, the method must also have high reproducibility, be simple and rapid, and have a very low detection limit. Plaque assay and epifluorescence are two techniques that have all of these qualities, and in addition plaque assay allows assessment of viable viruses.

A4.3.2 Plaque Assay: Double Agar Layer (DAL)

The double agar layer (DAL) method (Adams, 1959) is the most common method used today to enumerate phages. The protocol of this method is described below for PRD1 [Methods 1601 and 1602, USEPA (2001a, b)], but by changing the agar and broth, it can be used for other bacteriophages.

Materials

1. Tryptone Soya Broth (TSB), autoclaved and stored at 37 ˚C
2. Tryptone Soya Agar (TSA),
3. 0.5% Tryptone Soya Broth (Soft agar)
4. Petri dish
5. Water bath
6. Dilution tubes
7. Phage decimal dilution
8. *E. coli* host bacteria in log-phase
9. incubator

Method

Bottom agar
Following the procedure given on media bottle, prepare TSA, autoclave, and pour into the bottom of a Petri dish approximately 10ml.
Prepare the broth and soft agar and autoclave.

Decimal dilution
Prepare a set of decimal serial dilutions of the water samples. A minimum of four different dilutions are necessary. See Section 2.4.

Preparation of Petri dish
Place 5ml of soft agar in dilution tubes and place them in a 45 to 48 ˚C water bath. Each sample is analysed in triplicate. Add 100 µl of *E. coli* host bacteria in log-phase. Immediately add 500 µl of sample. Mix gently by rolling the tube in the palm of the hand. Pour tube contents into the bottom agar plates. Leave for some minutes to cool after the top agar hardens. Close the plates and place inverted in an incubator at 37 ˚C, and incubate for 16 to 24 hours.

Blanks and positive controls
To ensure the quality of determination, method blanks and positive controls are used.
Method blanks allow the investigation of contamination. No phages are used in this method and it is done following the same preparation method as described above except that 500 µl of sample are replaced by 500 µl of reagent water.
Positive controls allow investigation of whether the stock of phage suspensions, host bacteria, broths, and agar are all performing properly. Positive controls are undertaken by replacing the 500 µl of sample with 500 µl of phage culture at a concentration of approximately 20 pfu/ml.

Plate quantification
After 16 hours of incubation the lyses in the plate are visible (Figure 1). The lyses or plaque forming units are counted using a colony counter. The desired range is 0 to 300 pfu per plate for male-specific phages and 0 to 100 pfu per plate for somatic phages. If counts exceed the upper range recorded, the result is recorded as “too numerous to count”.
Use the equation:
Undiluted spiking suspension pfu/ml=$(pfu_1+ pfu_2+.... pfu_n)/(v_1+ v_2+.... v_n)$
where:
pfu = number of plaque forming units per plates of all countable sample dilutions
V = volume of undiluted sample in all plates with countable plaques

N = number of counts.

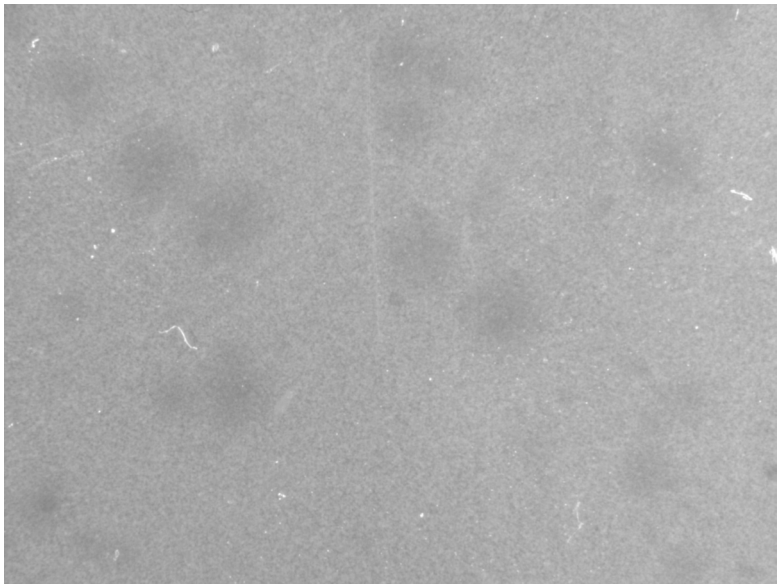


Figure 2 PRD1 lyses

References

Adams, M. H. (1959). Bacteriophages. New York, INTERSGIENCE PUBLISHERS.

USEPA (2001a). Method 1601: Male-specific (F+) and Somatic Coliphage in Water by Two-step Enrichment Procedure. EPA, EPA.

USEPA (2001b). Method 1602: Male-specific (F+) and Somatic Coliphage in Water by Single Agar Layer (SAL) Procedure. EPA, EPA.

USEPA (2001c). USEPA Manual of methods for virology. Chapter 14 Apr11 2001.

Manual of Practical workshop on virus ecology methods. Virus ecology Workshop, Plymouth 2006. http://viruses.bluemicrobe.com/workshoponvirusecologyme.htm

Rossi, P. (1994). Advances in biological tracer techniques for hydrology and hydrogeology using bacteriophages. Optimization of the methods and investigation of the behavior of bacterial viruses in surface waters and in porous and fractured aquifers. PhD Thesis, Faculty of Sciences. Neufchatel, University of Neufchatel.

Appendix 5

Development of Epifluorescence Method for PRD1

A5.1 Epifluorescence

The protocol described in this report was developed by Hennes and Suttle (Suttle 1995). Epifluorescence is only used with groundwater and all samples are processed on the day of sample collection.

Material

1. 0.02 µm pore size Anodisc 25 membrane filter (Whatman)
2. 0.45 µm pore size cellulose nitrate membrane
3. Microscopy slides and glass covers
4. Vacuum pump
5. Filter forces
6. 10 cm plastic Petri dishes
7. Filter paper
8. Epifluorescence microscope with the appropriate filter

Reagents

1. 2mM sodium cyanide (NaCN)
2. Yo-Pro-1
3. 0.3% NaCl (wt/vol)
4. Tween 80
5. Glycerol

Method

Prepare 50 µM Yo-Pro-1 solution by dilution of Yo-Pro-1 stock solution in 2mM sodium cyanide (NaCN) aqueous solution. Dispense 80 µl drops of 50 µM Yo-Pro onto the bottom of a Petri dish. A single Petri dish can be used for three samples. Soak a filter paper with 3 ml of 0.3% NaCl and place it in the lid of a Petri dish.

Add 0.5-1% (final concentration) Twen 80 to water samples to dislodge bacteria and viruses from particles. Dilute water samples using deionised-distilled water (100 □l of sample in 700 □l of deionised water).

Filter each sample using a pressure of 15 kPa. Use a remoistened 45 µm pore size cellulose nitrate membrane as a backing filter. Place an Anodisc membrane over the backing membrane before filtering. Remove the Anodisc membrane from the vacuum system and lay sample side up on drops of the staining solution. Petri dishes are then covered, with the filter soaked in NaCl in the lid, and incubated in the dark at room temperature for 2 days.

After two days wash the filters twice by filtering with 800 µl of deionised-distilled water.

Transfer the damp membranes to glass slides immediately, and cover with a drop of spectrophotometry-grade glycerol and a cover slip. Sometimes samples where analyzed immediately: where this was not possible, they were stored at -20 ˚C

Epifluorescence microscopy with a blue pack appropriate for Yo-Pro-1 was used to quantify the viruses. There are two ways to count the viruses: the first uses a reticular with grid divided into squares of known area; the second uses a camera to obtain an image of a field, which can then be counted manually or analyzed with image analysis computer software. Figure 1 shows a microscope image.

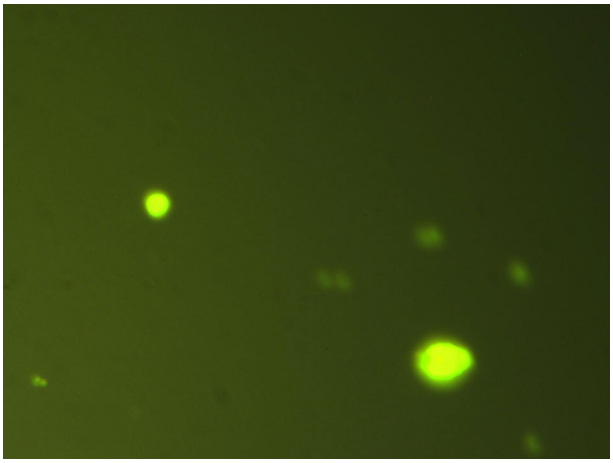


Figure 1 PRD1 Epifluorescence image.

References

Suttle, K. P. H. a. C. A. (1995). "Direct counts of viruses in natural waters and laboratory cultures by epifluorescence microscopy." Limnology Oceanography 40(6): 1050-1055.

Appendix 6

Designing a New Filter System

A.6.1 Introduction

To recover the phage from the pumped water, it was decided to use a filter system rather than taking discrete samples, as this would lower detection limits. Because a relatively large amount of water needed to be passed through the filters, the 'Biocap' filters which we have used previously (Joyce et al., 2007) would not be appropriate, being normally used for medical purposes where flows are small. We therefore investigated other types of filter, and identified a manufacturer of filters designed for use in environmental sampling: a new product, the manufacturers claimed that these 'Nanoceram' filters had ~ 90% efficiency for recovery of viruses, including MS2. In order to be sure that the filters would be appropriate, we have tested their hydraulic performance in our system (in case they became clogged too rapidly) and their ability to recover PRD1, the virus which we intended to use at least initially. The following reports summarize these tests and the results: in summary the filters performed very well, and in agreement with the manufacturer's claims.

A.6.2 Investigation of the viability of filters as 'virus traps' when undergoing particle build-up during the sampling of a pumped well.

Introduction

A series of experiments have been conducted to examine the efficacy of 'Nanoceram' filters for the sampling of bacteriophage that will be used in tracer experiments at the University of Birmingham. This report documents the change of permeability undergone by a filter placed in a sampling line from a pumped borehole over a period of approximately 6 hours. The purpose of the experiment was to determine if filter clogging was problematic at particle loadings likely to be present during the proposed tracer test.

The method

The test was conducted in borehole two, which will be that pumped during the tracer test. A Grundfos MP3 pump was placed at approximately 17m bgl, Rest water level before pumping was approximately 4.0m bgl. A 12v sampling pump (Wasp P3) was placed at 20m bgl and connected to the surface with a 15mm id polythene discharge pipe. Power for the pump was provided by two 12v battery chargers, though these subsequently proved inadequate due to the large current (8A) required by the pump which resulted in a voltage drop to the pump.
At the surface the discharge from the sampling pump was fed to the inlet of the filter holder. Immediately before the inlet was placed a Keller digital pressure transducer that was logged by PC such that the head across the filter could be monitored with time, the assumption being that any loss of permeability of the filter due to clogging would result in an increase in back pressure. It was also assumed that the pressure at the discharge side of the filter trap was equal to 1 bar (atmospheric pressure) so no compensation was made for the actual barometric pressure or any slight differences between the level of the virus trap and the point of discharge, nor any pressure resulting from pipe losses in the discharge line.

The voltage supplied to the sampling pump was monitored with digital voltmeter.

Discharge from the sampling pump was to have been measured and logged automatically, however, technical problems meant that this was subsequently done manually using a measuring cylinder and stopwatch.

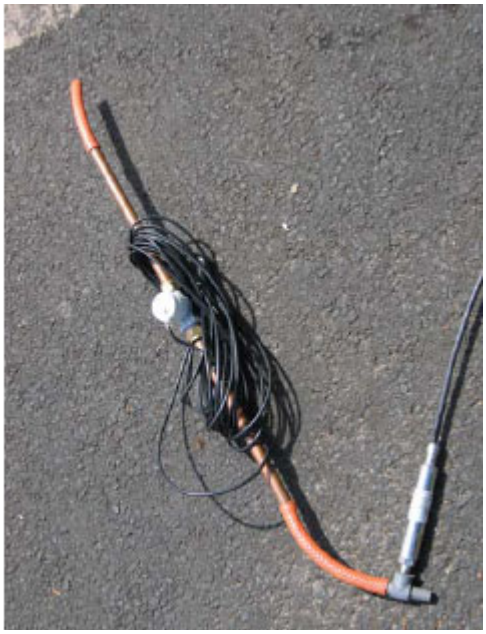
The experiment

Date: 21/05/07
Experimenter: Richard Greswell

File: to write

The MP3 pump was turned on and set to maximum discharge (~160 l/min) approximately 1 hour before the sampling began. The purpose of this was twofold: firstly it was assumed that the majority of particles commonly present in the discharge would, as seen in previous experiments, reduce to minimal levels after this time, and secondly, further drawdown would be negligible such that the lift of the sampling pump would be constant.

After one hour, the depth to water in the pumped well was approximately 13m bgl (a drawdown of ~9 m)



When the sampling pump was switched on it became immediately apparent that the discharge from the filter was very low. The pump voltage was only 10v which indicated that the power supply was failing to provide enough current. To increase the discharge from the sampling pump, the speed of the MP3 was reduced to its lowest setting (lowering discharge to ~92 l/min) which subsequently reduced the drawdown in the borehole to approximately 6.3m. With the depth to groundwater now approximately 10.3, the discharge from the filter visibly increased and so these conditions were chosen to conduct the experiment proper.

As the power supply was overloaded it was deemed unsafe to continue the experiment unattended and so pumping took place over a two day period. On the first day the sampling pump ran for approximately 2.8 hours. On the second day the experiment continued, but a further decline in supply voltage was apparent with only 9v available. In consequence the discharge was also lower. After 2 hours the experiment was briefly halted before a car battery was added to the power supply in an attempt to boost the available current. The addition of the car battery raised the voltage to 12v. Pumping continued at a much higher rate for another 1.1 hours before the experiment was halted. Discharges were measured at the beginning and the end of each period of pumping

Results

Fig. 1 shows the pressure recorded by the transducer just upstream of the virus trap over the whole of the experimental period. The graph shows three distinct phases resulting from the different pump supply voltages described above during which the pressure readings remain relatively stable. Table 1 summarises the conditions during each phase of the experiment.

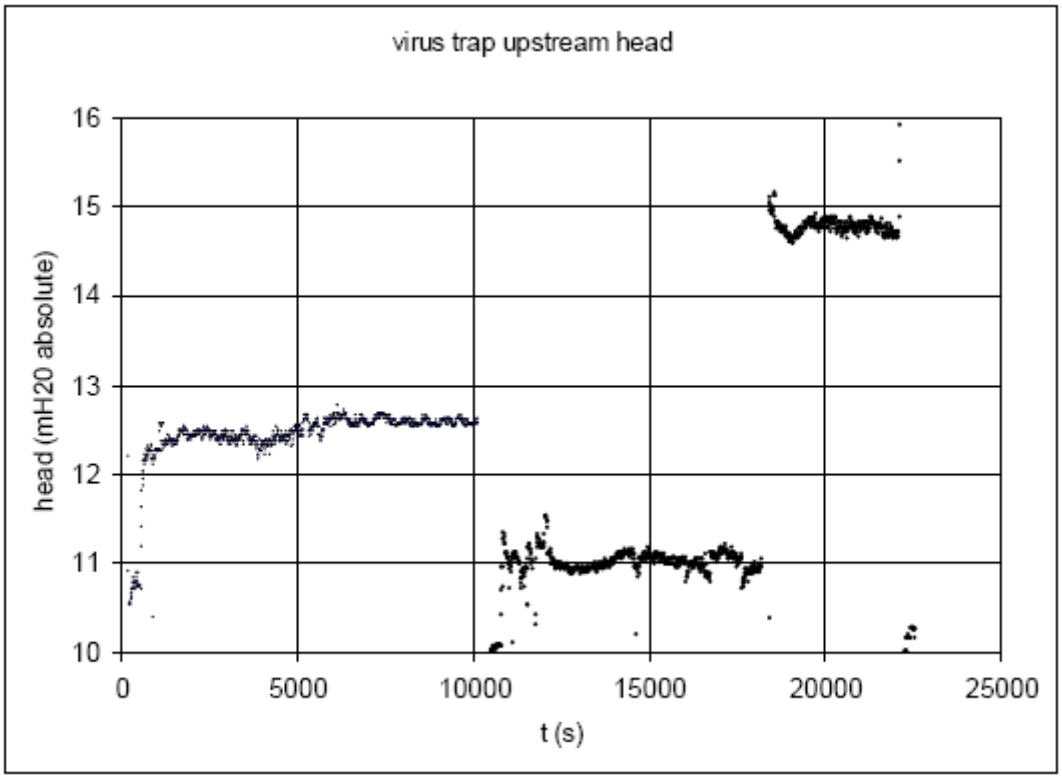


Figure 1: Head upstream of virus trap as a function of time during the testing.

Phase	Supply (v)	Q (start) (l/min)	Q (end) (l/min)	Ave Head (mH20)	Duration (hrs)	Volume discharged (l)
1	10	2.4	2.5	2.5	2.8	403
2	9.3	1.65	1.6	1.0	2.0	198
3	12.26	4.0	4.0	4.8	1.1	264

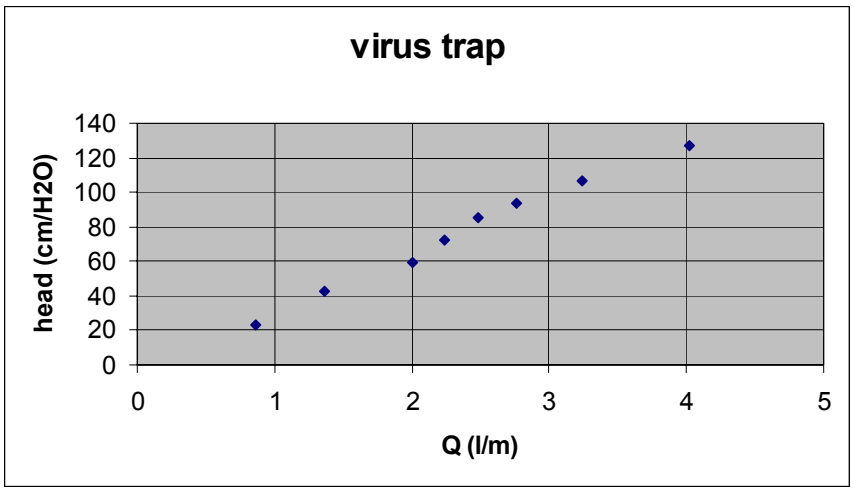
Table 1. Flow and pressure recorded during each phase of the experiment. The total volume passed through the filter was approximately 865 litres.

Data Collected

Virus trap permeability experiment
(note: trap in filter holder)

volume	sample time (sec)	Q (l/m)	head (cm/H2O)	
430	30	0.86	23	
680	30	1.36	42.5	
500	15	2	59.8	
560	15	2.24	72	
620	15	2.48	85	
690	15	2.76	93.6	
810	15	3.24	106.6	
1005	15	4.02	127.3	<see note*

* at this Q, the head due to the filter holder alone (filter removed) is about 5cm H2O



Conclusions

- The stable pressure readings indicate that for bh2 over the period of pumping, the filter trap does not retain sufficient particles to noticeably reduce the permeability
- The pump discharge is strongly controlled by head and, due to the depths to water seen on site, this will be an important factor to consider in the field experiment.
- This experiment was influenced by the lack of a suitable power supply but when such is available, the discharge at a total head (depth to water plus back pressure from trap) of ~ 15m H20 will be approximately 4 l/min.
- Under the above conditions, it is reasonable to assume we may successfully use the trap over a period of say 12 hours for continuous sampling without seeing a deleterious change in permeability. If this were to be translated into practice, approximately 2900 litres of water would be passed through the filter. However, it should be remembered that in the proposed method each sampling pump will only run intermittently so in any 12 hour period 2900/*n* litres will be sampled, where *n* represents the number of pumps utilised.

A.6.3 Trap efficiency experiments

A.6.3.1 Traps

Nano-Ceram® sterile filter cartridges manufactured for the American company Argonide Advance Filtration technologies, were selected to use in the tracer experiments. These filters are approved by the US Environment Protection Agency for use in virus sampling.

A.6.3.2 Experiment 1

PRD1 phages after purification with an initial concentration of 3.55 10^7 pfu/ml were diluted 100 times in sterile water by adding 50ml and 30ml of phage culture to 4950 and 2970ml of sterile water respectively. A sample of both dilutions was collected and labelled as D1 and D3. Both dilutions D1 and D3 were passed through the trap, using a peristaltic pump, and a sample of the filtrate generated was collected and labelled D2 and D4.

Immediately after passing the dilutions through the trap, the trap was eluted using 2000 ml of beef extract following the instructions given in USEPA (2001c). The eluate generated was collected and labelled as E1, E2, E3. Finally 2000 ml of sterile water was passed through the trap in the direction opposite to that when sampling, and a sample of the filtrate was collected and labelled as D5: this step is not including in the USEPA protocol.

Samples were immediately analysed using the double agar technique. Results are presented in Tables 1 and 2.

Table 1 Phage concentrations in each dilution. Experiment 1

Sample name	Type of filtrate	Volume Sample (ml)	Phage pfu/ml	Total phage pfu
D1	Phage initial	5000	6.00E+05	3.00E+09
D3	Phage initial	3000	4.95E+05	1.49E+09
D2	Phage after filter	4500	3.99E+05	1.80E+09
D4	Phage after filter	2500	3.77E+05	9.44E+08
E1	Eluate	1000	1.70E+06	1.70E+09
E2	Eluate	1700	4.78E+05	8.13E+08
E3	Eluate	200	6.55E+04	1.31E+07
D5	Water after filter	2000	2.98E+04	5.96E+07

Table 2 Phage retention. Experiment 1

% of phage	
Retain	38.9
Eluate	56.3
Water	1.3

A.6.3.3 Experiment 2

1ml of purified PRD1 suspension was diluted in two 5 litre samples of deionised-distilled water: the initial dilutions where labelled D1 and D2. Using a peristaltic pump between 100 and 120 rpm, the two dilutions were passed through a single filter. 9800ml of filtrate was collected in two conical flasks (D3 and D4) each containing 4900 ml. Filters were eluted using 2000ml beef extract and

glycine (USEPA 2001c), and 2000 ml of eluate were collected and labelled E1. The results are given in Tables 3 and 4.

Table 3 Phage concentrations in each dilution. Experiment 2

Sample name	Type of filtrate	Volume Sample (ml)	Phage pfu/ml	Total phage pfu
D1	Phage initial	5000	3.86E+02	1.93E+06
D2	Phage initial	5000	5.25E+02	2.62E+06
D3	Phage after filter	4900	2.70E+00	1.32E+04
D4	Phage after filter	4900	0.00E+00	0.00E+00
E1	Eluate	2000	5.13E+02	4.10E+06

Table 4 Phage retention experiment 2

% of phage	
Retain	100
Eluate	90

A.6.3.4 Experiment 3

A third virus trap experiment was carried out. In this experiment the filter was placed in the borehole discharge main and left for 24 hours. After this time the filter was taken to the laboratory where it was eluted using the method of USEPA (2001c). Samples of water from the borehole and from eluate were enumerated using the DAL method. Surprisingly, the unfiltered samples contained significant numbers of viruses (25 pfu/ml) which must have survived from the previous experiments undertaken at the site ~ 2 years previously (Joyce et al., 2007): no viruses were seen in the blank samples. The total phage recovery was estimated by comparing the recovery from the filters with the unfiltered sample results. Calculated in this way, recovery was apparently only 14%: it is likely that the single unfiltered water sample was unrepresentative of the period of sampling. Subsequent analysis of the borehole water has not yielded measurable amounts of virus, and hence it is concluded that the pumping undertaken during experiment 3 and subsequently has removed all the viruses from the system.

A.6.3.5 Conclusions

The relatively poor results in the first experiment were probably due to inexperience in performing this kind of experiment: in subsequent experiments, more appropriate glassware was purchased, and the filter cartridge used had a bleed facility. The figures obtained in the second experiment agree with published results (www.**argonide**.com/), and it is concluded that the filter system works well.

The results from the third experiment were unexpected, and demonstrate that viable phage survival is rather longer than is predicted from laboratory studies previously undertaken (Pedley, S., pers comm.).

References

Adams, M. H. (1959). Bacteriophages. New York, INTERSGIENCE PUBLISHERS.

USEPA (2001a). Method 1601: Male-specific (F+) and Somatic Coliphage in Water by Two-step Enrichment Procedure. EPA, EPA.

USEPA (2001b). Method 1602: Male-specific (F+) and Somatic Coliphage in Water by Single Agar Layer (SAL) Procedure. EPA, EPA.

USEPA (2001c). USEPA Manual of methods for virology. Chapter 14 Apr11 2001.

Manual of Practical workshop on virus ecology methods. http://viruses.bluemicrobe.com/workshoponvirusecologyme.htm

Rossi, P. (1994). Advances in biological tracer techniques for hydrology and hydrogeology using bacteriophages. Optimization of the methods and investigation of the behavior of bacterial viruses in surface waters and in porous and fractured aquifers. PhD Thesis, Faculty of Sciences. Neufchatel, University of Neufchatel.

Suttle, K. P. H. a. C. A. (1995). "Direct counts of viruses in natural waters and laboratory cultures by epifluorescence microscopy." Limnology Oceanography 40(6): 1050-1055.

Details of Filter Manufacturer and Supplier

http://www.argonide.com/cartridges.html

http://www.premium-water-filters.com/biological-samplers.htm

UK supplier:

http://www2.m-techmicro.com/

Appendix 7

Design of Field Experimentt

Designs Considered

Several designs for the field experiments to determine the virus pathways between two of the boreholes were considered, including the use of packered intervals, the use of single packers, and a series of suspended virus traps (Figure 1).

Design Finally Built and Tested

It was eventually decided to use the following system:

1. main pump in the recovery well located with its intake in the casing
2. five sampling pumps suspended below the main pump intake, each pump being used in turn
3. injection in the adjacent borehole, with a pump abstracting and discharging within the borehole to mix the tracer

The design, the Birmingham University Multiple Pumping System (BUMPS) is illustrated in Figure 2. Following testing (Appendix 9), this design was modified (Appendix 10), though the principle remained the same.

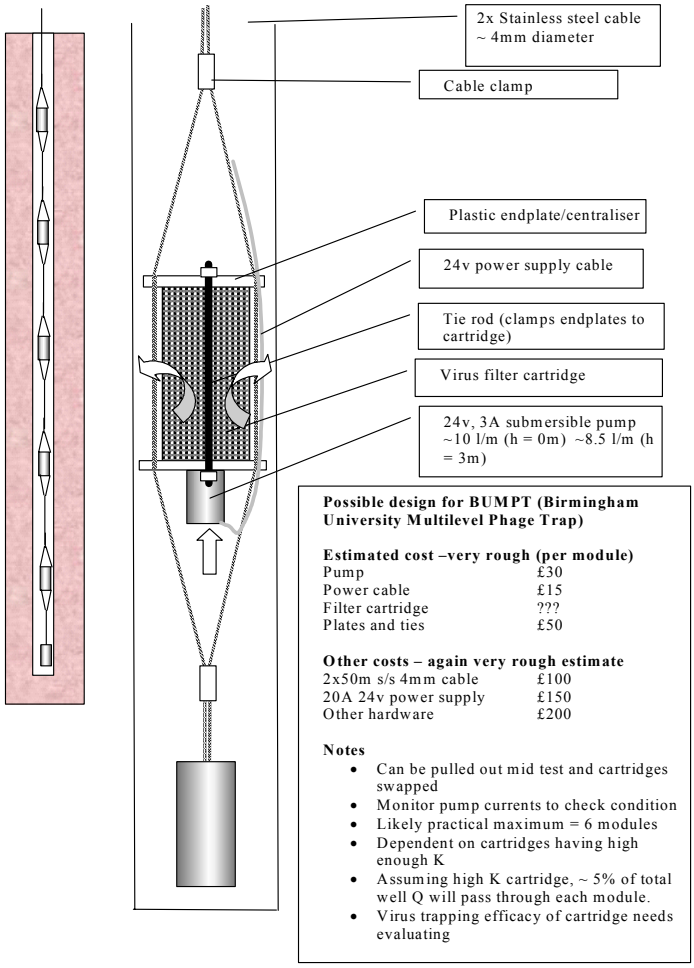


Figure 1: Example of one of the experimental rigs initially considered (Birmingham University Multilevel Phage Trap – BUMPT).

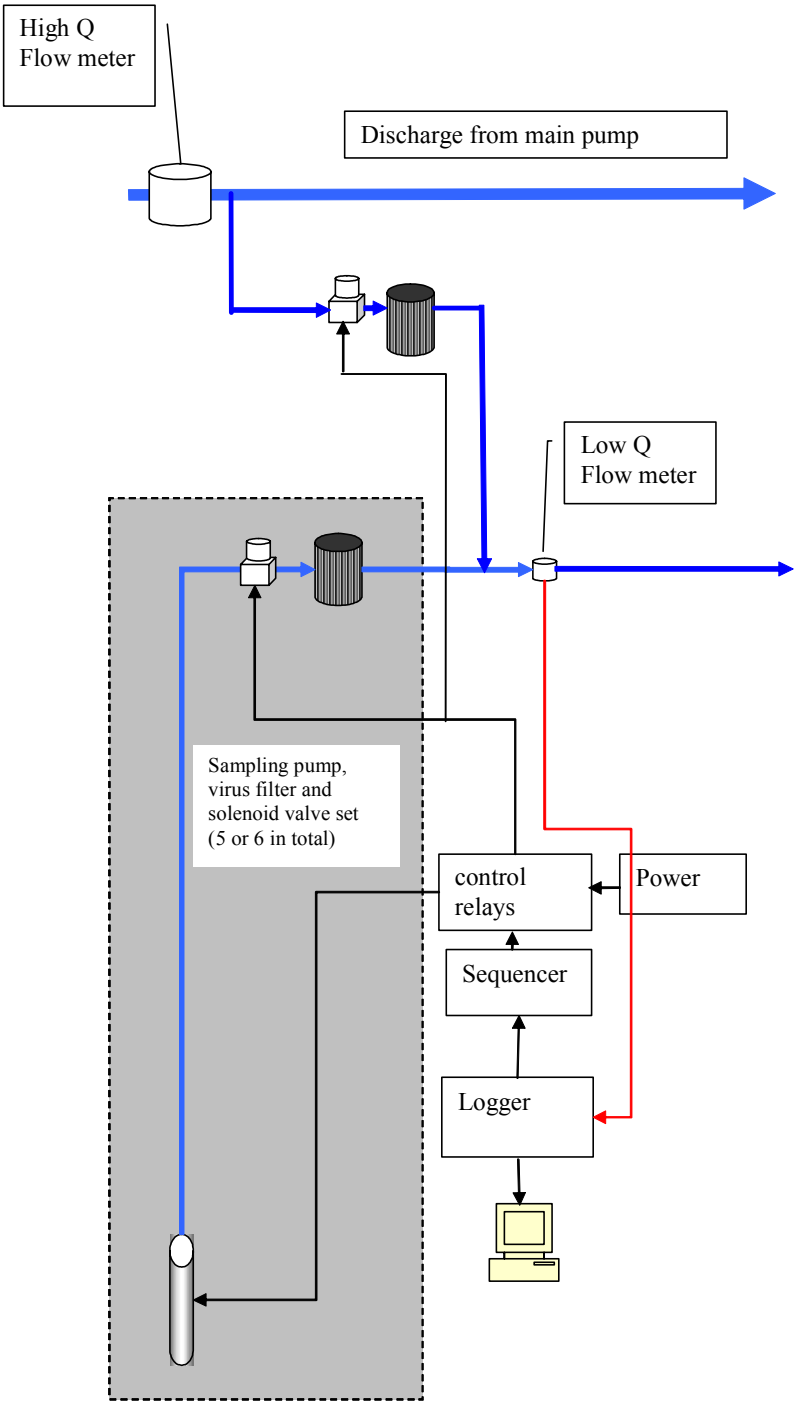


Figure 2: The experimental rig initially built and tested (Birmingham University Multilevel Pumping System – BUMPS – mark 1).

Calculation of viral mass inflows in BUMPS from the mass retained in each trap

Define the following:

C_n the viral concentration in the borehole at the nth sampling intake [ML^{-3}]
M_n the cumulative viral mass that has entered the nth section of the borehole from the bottom through the borehole walls in time Δt [M]
Q_n the constant discharge due to pumping at the top of the nth section of the borehole [L^3T^{-1}]
Q_n^s the sampling discharge in the nth virus trap [L^3T^{-1}]
f_n the fraction of the total time Δt for which the nth level is sampled [1]
α the efficiency of each virus trap [1]

The up-hole discharge of viral mass in the borehole at the depth of the nth sampling pipe is C_nQ_n, which must be equal to the mass inflow below that depth. Hence

$$Q_nC_n\Delta t = \sum_{i=1}^{n} M_i \tag{A.7.1}$$

The viral mass caught in the nth trap in time Δt is

$$M_{T_n} = \alpha f_n Q_n^s C_n \Delta t \tag{A.7.2}$$

Hence the cumulative viral mass inflow below the nth sampling level is given by

$$\sum_{i=1}^{n} M_i = \frac{M_{T_n} Q_n}{\alpha f_n Q_n^s} \tag{A.7.3}$$

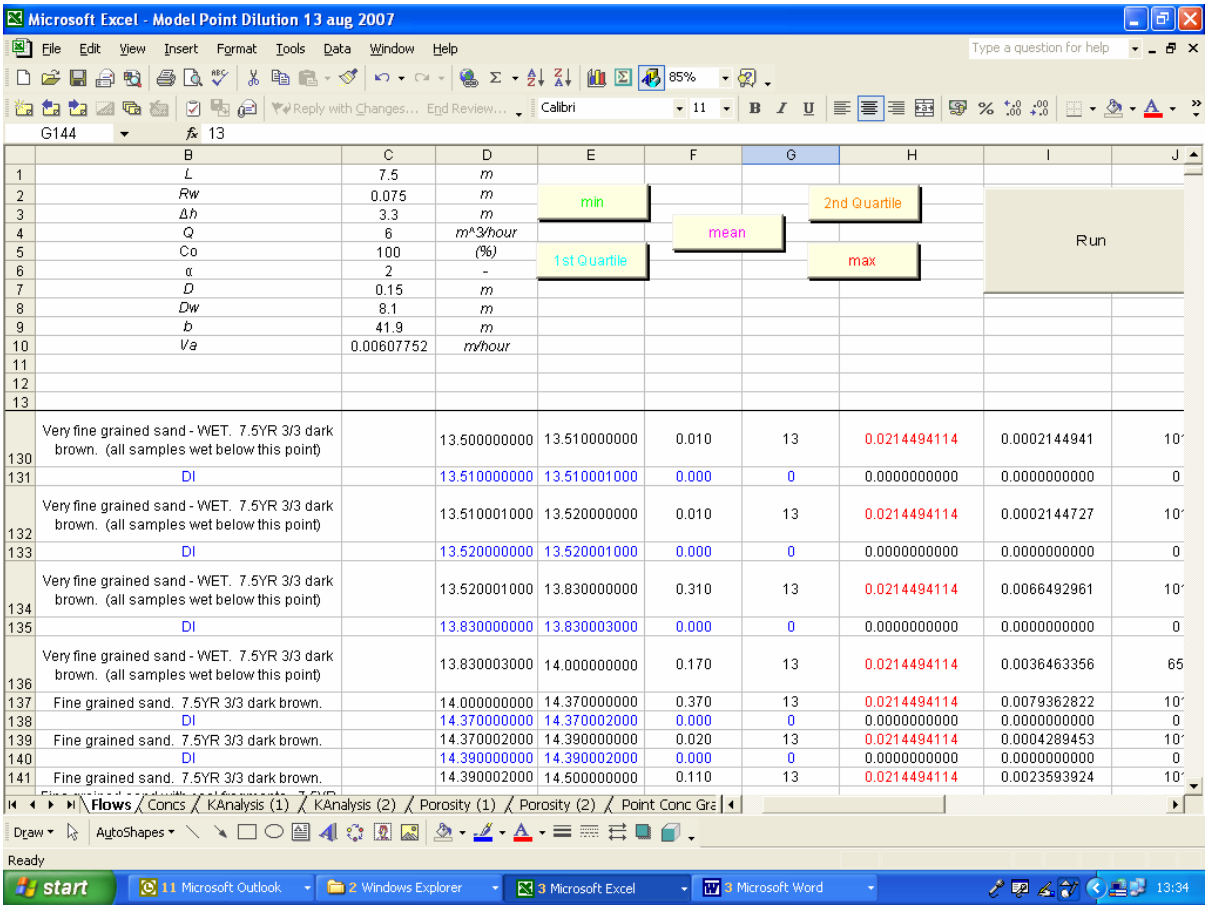
from which the inflow at each level can be determined.

Appendix 8

A Simple Tool for Aid in Design and Analysis of the Multi-Level Tracer Experiment

A Simple Tool for Aid in Design and Analysis of the Multi-Level Tracer Experiment

A spreadsheet model of solute movement between two of the boreholes was developed during an MSc student project (Jefferies, 2007) in order to provide a further tool for planning the tracer experiments and possibly aiding in the interpretation of the tracer results. The model contains a detailed description of the hydrogeological properties of the system inferred from the detailed geological, geophysical, and property measurement data sets collected previously. It has been tested against some of the field hydraulic test results. The Figure 1 shows a screen shot of the input page of the model, and some output.



	B	C	D	E	F	G	H	I	J
130	Very fine grained sand - WET. 7.5YR 3/3 dark brown. (all samples wet below this point)		13.500000000	13.510000000	0.010	13	0.0214494114	0.0002144941	10
131	DI		13.510000000	13.510001000	0.000	0	0.0000000000	0.0000000000	0
132	Very fine grained sand - WET. 7.5YR 3/3 dark brown. (all samples wet below this point)		13.510001000	13.520000000	0.010	13	0.0214494114	0.0002144727	10
133	DI		13.520000000	13.520001000	0.000	0	0.0000000000	0.0000000000	0
134	Very fine grained sand - WET. 7.5YR 3/3 dark brown. (all samples wet below this point)		13.520001000	13.830000000	0.310	13	0.0214494114	0.0066492961	10
135	DI		13.830000000	13.830003000	0.000	0	0.0000000000	0.0000000000	0
136	Very fine grained sand - WET. 7.5YR 3/3 dark brown. (all samples wet below this point)		13.830003000	14.000000000	0.170	13	0.0214494114	0.0036463356	65
137	Fine grained sand. 7.5YR 3/3 dark brown.		14.000000000	14.370000000	0.370	13	0.0214494114	0.0079362822	10
138	DI		14.370000000	14.370002000	0.000	0	0.0000000000	0.0000000000	0
139	Fine grained sand. 7.5YR 3/3 dark brown.		14.370002000	14.390000000	0.020	13	0.0214494114	0.0004289453	10
140	DI		14.390000000	14.390002000	0.000	0	0.0000000000	0.0000000000	0
141	Fine grained sand. 7.5YR 3/3 dark brown.		14.390002000	14.500000000	0.110	13	0.0214494114	0.0023593924	10

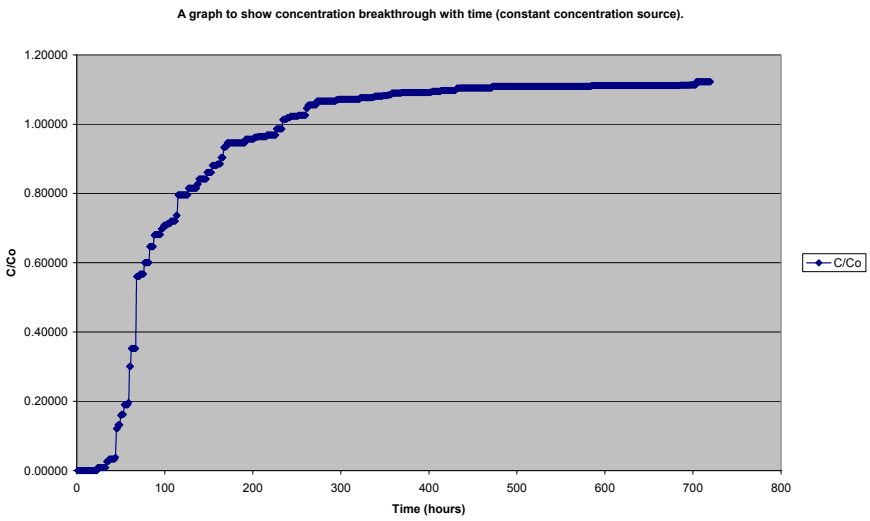


Figure 1: Screen shot and example output for borehole model developed by Jefferies (2007)

Reference

Jefferies, D., 2007. Characterising virus flow paths in the Permo-Triassic Sandstone. Unpublished MSc Project Report, University of Birmingham, Birmingham.

Appendix 9

Results from the First Run of BUMPS Mark 1

Results from the First Run of BUMPS Mark 1

The first run of the full BUMP mk 1 system showed that the system worked well in almost all respects. Some data collected on background fluorescein concentrations are shown in Figure 1. The plot is of uncalibrated fluorescein concentration against time: each sampling pump is pumped for twenty minutes in turn, and the plot shows the concentrations for all pumps. The oscillation in concentrations is the result of two of the sampled intervals having lower concentrations and three having higher concentrations. Some chemical data from this experiment are reported in Appendix 3.

Despite the system working well up until this point, after around one day the sampling pumps failed. As a result the system was modified, replacing the sampling pumps with a much higher quality pump connected to a switching system to allow it to sample from different depths. The new system is described in Appendix 10.

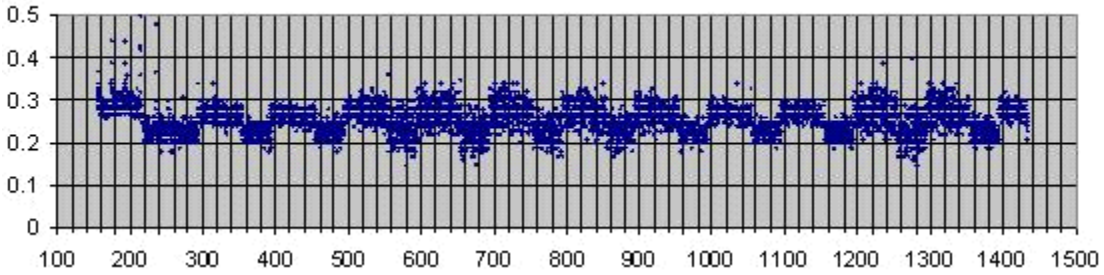


Figure 1: Uncalibrated fluorescein concentrations during the first test plotted against time (minutes). The fluorescein is present as background concentrations from previous tests. The plot shows the concentrations from all 5 levels sampled, with each sampling pump sampling for 20 minutes at a time.

Appendix 10

Modified Test System

Modified Test System

As a result of the pump failures in the initial run of BUMPS mk1 (Appendix 9), the test system was modified (BUMPS mk2), replacing the sampling pumps with one much higher quality pump and a switching system (Figure 1). This switching system allows the sampling pump to pump in turn from pipes going down to the same depths as the sampling pumps in the mk1 version. Thus the sampling pump in mk2 is continuously abstracting water, at any one time the water coming from one of 5 possible depths. The opportunity has also been taken to make a couple of other modifications, so that the system now includes the following:

1. fluorescein detection at 5 levels plus the main pump discharge
2. virus traps attached to all 5 discharge lines, plus the main pump discharge line
3. particle counter/sizer attached to discharge lines on the 5 levels (not on the main discharge line)
4. facility for chemical sampling avoiding the filters on all discharge lines
5. flow rate measurement of main and sampling pumps
6. head measurement in both boreholes
7. mixing of tracer in the injection borehole
8. facility for sampling of the injection borehole

Figure 2 shows more detail of the measurement systems for the experiment.

Brief trials with the new system suggest that the arrangement works well. Example output are given in Figure 3: the same differences in the background concentrations of fluorescein are seen in this test as for the BUMPS mk1 test (Appendix 9)

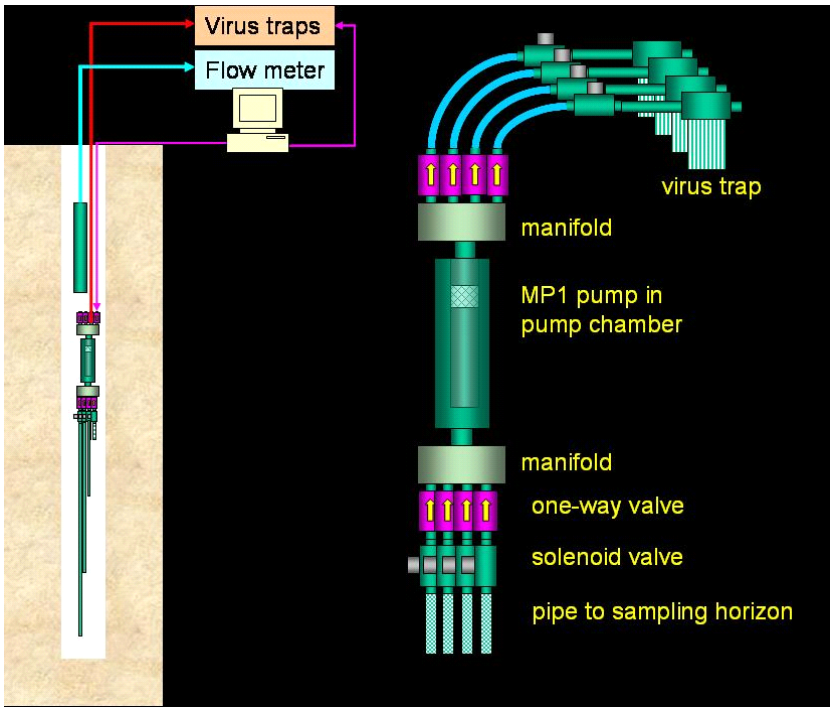


Figure 1: The modified test system, BUMPS mk2.

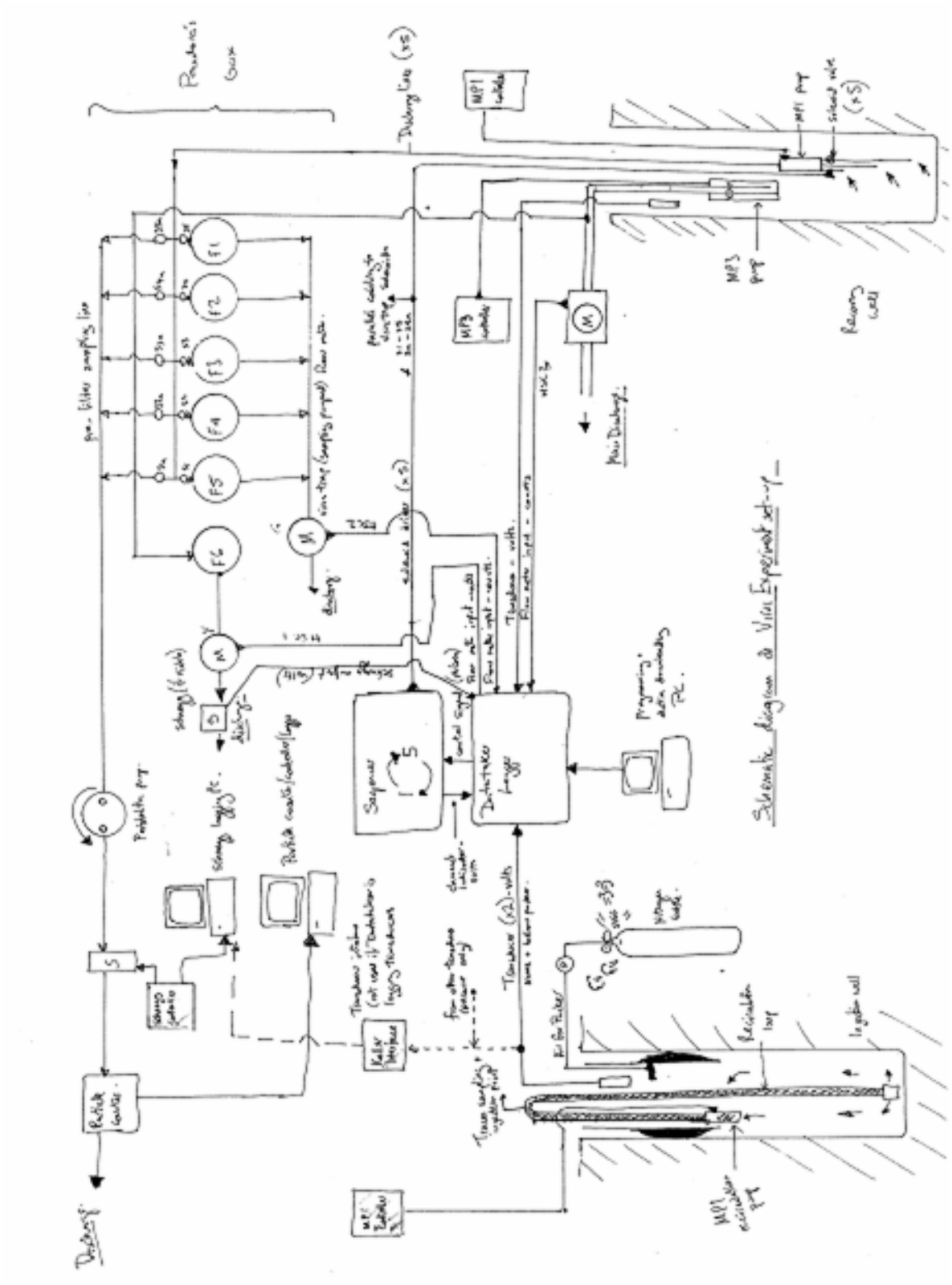


Figure 2: Working sketch of BUMPS mk2

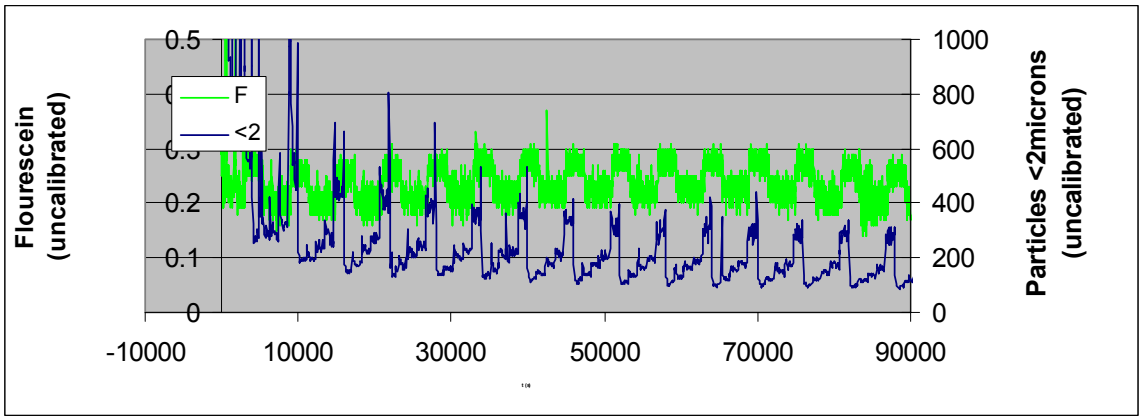


Figure 3: Some results of the monitoring during the first run of BUMPS mk2. The x-axis is time in seconds.

Appendix 11

Monitoring of Human Viruses in Groundwaters

Monitoring of Human Viruses in Groundwaters

Monitoring of human viruses will commence shortly. Preparatory work has centred on examining records from previous work in Birmingham and Nottingham, the latter having a very similar hydrogeological setting to Birmingham. The results are summarized in Table 1. It is clear from this table that the most appropriate location to monitor in detail will be Piezometer Nest #1 at Old Basford, Nottingham. Work has also begun on sampling protocols for the piezometer.

Water will also be sampled from wells in Birmingham, and analyzed for viruses and THMs. A collation of the site information is available.

Table 1: Summary of virus analysis records from Birmingham and Nottingham piezometers

Piezometer Microbiology Results	Comments
Piezometer Nest #1: Old Basford, Nottingham	
1. Viable unidentified enteroviruses to 47m	Possible
a. Norovirus PCR to 39m (Mar, no Coxsackie)	Seasonal variation
b. Coxsackie B4 PCR to 39m (Jun, no norovirus)	
2. TTC and FS to 47m	
Piezometer Nest #2: Meadows, Nottingham	
1. No enteroviruses detected	No Enterovirus
2. Coliphage detected at 2 intervals - 2/3 and 1/3 visits.	
3. Bacteria rather more common	
a. SRC to 50m (base)	
b. TTC & FS near surface only (<20 m)	
Sampling intervals are not separated by bentonite seals	
Piezometer Nest #3: ?Daybrook Hospital, Nottingham	
1. Enteroviruses - 1/2 samples @ 46m, none elsewhere	Too sparse to use; no seasonal variation
2. Coliphage - 1/2 samples @ 12 m, none elsewhere	
3. TTC - 1/3 @ 4/10 levels	
4. FS - 1/3 @ 3/10 levels	
5. SRC - 1/1 @ 2/10 levels	
Piezometer Nest #4: Bromford (confined), Birmingham	
1. Enteroviruses - none	No Enterovirus
2. Coliphage - 1/1 @ 90m	
3. TTC - detects at most levels (to 3/3)	
4. FS - detects at most levels (1/2)	
5. SRC - 1/1 @ 90m	
Piezometer Nest #5: Witton, Birmingham	
1. Enteroviruses - none detected	No Enterovirus
2. Coliphage - 1/1, 1/2 @ 35 & 47m	
3. TTC - detects at 9/11 levels, deepest 60m (lowest piezometer)	
4. FS - detects at 10/11 levels, deepest 60m (lowest piezometer)	
5. SRC - detects at 6/11 levels, deepest 60m (lowest piezometer)	

In general, large changes in inorganic concentrations with time - e.g. 10-35mg/L NO3, 10-50mg/L Cl, -25 to +50 mg/L SO4 in #1.

Appendix 12

Presentations and Publications

Aller et al. (2007) SWITCH Meeting, Tel Aviv, Israel

Transport pathways for viruses in a sandstone aquifer

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Abstract

Following the discovery of viable human enteric viruses at depth in a sandstone aquifer in the U.K., a set of tracer experiments was conducted between March 2004 and August 2005 at a test site in a similar sandstone aquifer, using bacteriophages as human virus surrogates. Initial tests showed a range of bacteriophages (PRD1, φX174, H40/1 and MS2) to be transported between two 50 m boreholes, but attempts to identify the transport pathway(s) by tracer tests in packered horizons between boreholes proved unsuccessful. A new inter-borehole tracer test with injection at one borehole and abstraction from a pumping borehole has now been designed with novel instrumentation to identify the lithological horizons transporting viruses. In addition to the detailed descriptions of core available, the array of boreholes has been subjected to extensive geophysical logging (resistivity, natural gamma, optical televiewer) and hydraulic testing (constant discharge tests, single-hole and cross-hole packer tests). On the basis of these data a detailed conceptualisation of the site has been constructed, with a number of hydraulically significant low permeability horizons clearly identified. The new instrumentation allows the viruses and a conservative tracer to be sampled at different horizons in the abstraction borehole while the hole is being pumped. Viruses are enumerated by plaque assay and epifluorescence microscopy. To quantify the virus inflow at each horizon, a purpose-built packer flow meter was used to determine the up-hole discharge of water in the borehole at the sampling depths. A preliminary test without the injection of viruses was conducted to test the experimental equipment and to assess the degree of contamination of the site from previous experiments. The fluorescent dye used previously was found only at concentrations marginally above background, but PRD1 bacteriophages were found at concentrations in the order of 10^3 ml^{-1}, indicating that these viruses survive longer (more than two years) under field conditions than predicted by previous laboratory experiments. Following the results of the preliminary trial, modifications are being made to the instrumentation in preparation for the forthcoming full scale tracer test.

Keywords: virus, bacteriophage, groundwater, sandstone, epifluorescence,

Introduction

The spread of viral infection through groundwater continues to be a subject of concern. Maunula et al. (2005) report that groundwater was the method of transmission in 15 out of 18 identified outbreaks of norovirus in Finland between 1998 and 2003. Many cases of waterborne enteric viral disease occur through faecal contamination, and so the reuse of treated sewage in artificial recharge schemes in cities requires that a risk assessment of the virus hazard be undertaken. Although many outbreaks occur through transmission through shallow aquifers, 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 transit 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 was 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 (Fig. 1). 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).

Figure 1: An outcrop of the sandstone showing both sedimentary structures and fractures

Field and laboratory column studies were carried out between March 2004 and August 2005 using bacteriophages as human enteric virus surrogates (Joyce et al., 2007). These studies were followed by further detailed laboratory investigations on the survival of enteric viruses in groundwater and the effect of natural colloids on the migration of viruses in sandstone (Pedley et al., 2007; Joyce et al., in press). The field studies were carried out at the University of Birmingham campus between two boreholes in the sandstone and consisted of a set of inter-borehole tracer tests using the bacteriophages PRD1, φX174, H40/1 and MS2 with a fluorescent dye as tracers. Although each virus was transported from one borehole to the other, attempts to identify the principal transmission pathways, by targeting fractures and high permeability horizons for detailed testing, proved unsuccessful. The aim of the present study is to identify these pathways by a more systematic study of the boreholes and to attempt to relate them to characteristic features in the geological sequence. To achieve this, a set of tracer tests has been designed with novel instrumentation allowing six horizons to be investigated simultaneously. This paper describes the experimental design and results from preliminary testing.

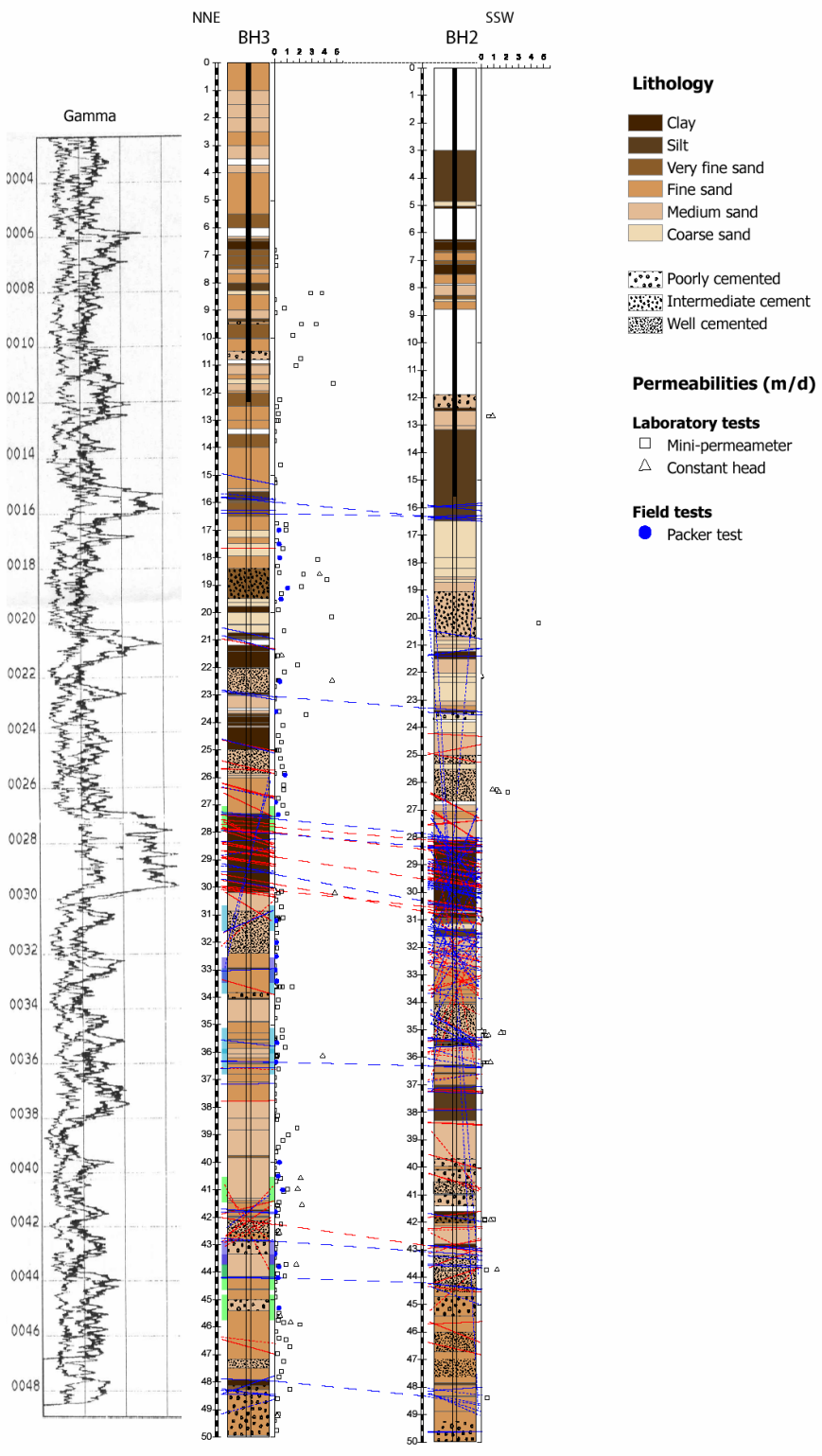


Figure 2: Synthesis of the geophysical and lithological logs and hydraulic test results on the test boreholes

The test site

The test boreholes (BH2 and BH3) are located on the University of Birmingham campus. Both are cored to 50 mbgl. BH2 is cased to 15.6 mbgl and BH3 to 12.2 mbgl. Figure 2 shows a summary of the lithological description of the cores from both boreholes and the planar features

observed in optical televiewer logs. The dashed line between the boreholes shows a possible correlation between planar features observed in each hole, based upon geometry alone. 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 close 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.

New tracer test design

As in the previous tracer tests, bacteriophages and a fluorescent dye (fluorescein) will be injected into one borehole and abstracted from the second, continuously pumped, borehole. In addition to the main pump (Grundfos MP3), the abstraction borehole (BH2) will be instrumented with a sampling system that routes water from five predefined levels in the borehole to monitoring devices at the surface. Following unsatisfactory trials with multiple sampling pumps, the present system employs a single Grundfos MP1 submersible pump to sample from each of five levels sequentially. This is achieved using a system of computer controlled solenoid valves that route the water samples through the pump via inlet and outlet manifolds. Viruses are collected at the surface in Argonide NanoCeram® virus filter traps. Each trap collects samples from a particular sampling level and is 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 is connected to a sampling line from the main pump discharge. Effluent water from each of the virus traps is monitored for fluorescein using a logged Schnegg fluorimeter.

Since the sampled borehole is being continually pumped, the water sampled from each level represents a mixture of the water from lower levels. The relationship between the number of viruses flowing into one section of the borehole is related to the number of viruses recovered from the traps by Equation (1).

$$M_n(t) = \frac{Q_n}{\alpha Q_n^s} m_n(t) - \left(\frac{Q_{n-1}}{\alpha Q_{n-1}^s} - 1 \right) m_{(n-1)}(t) \qquad (1)$$

where

$M_n(t)$ is the cumulative number of viruses at time t that has entered the nth section of the borehole from the bottom.
$m_n(t)$ is the cumulative number of viruses at time t that is recovered from the nth virus trap.
Q_n is the constant discharge due to pumping at the top of the nth section of the borehole.
Q_n^s is the constant sampling pump discharge in the nth virus trap.
α is the efficiency of each virus trap.

The use of Equation (1) depends on knowing the up-hole discharge at each sampling level, which is not simple to achieve during the tracer test. However, since the discharge during the tracer

tests is at a constant rate, the up-hole discharges are measured before and after the test at the same discharge rate. A preliminary survey has been conducted in each borehole to help identify the sampling locations for the full tracer test. This was achieved using a spinner flow meter installed in a purpose built packer system, which ensures that the entire upflow at the measurement depth contributes to the measurement, whilst maintaining very small head losses across the device.

Viruses are recovered from the traps by elution as described in US EPA (2001a) using 1.5% beef extract and 0.05M 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 0.45µm sterilizing filter. The number of viruses is 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). Initial tests indicate the efficiency of the virus trap and elution process in recovering influent viruses (α) to be high at approximately 91%.

Hourly spot measurements of total virus concentrations in the water discharged from the borehole will be made using an automatic sampler, with laboratory analysis by epifluorescence microscopy. This technique estimates the phage abundance using a DNA cyanine based nucleic acid stain (YO-PRO-1). The stain is prepared as described in Hennes and Suttle (1995). YO-PRO-1 is diluted to 50 µM in an aqueous solution of 2mM NaCN (sodium cyanide), and 80 µl of stain are placed in the bottom of a Petri dish. 100 µl water samples are diluted with 700 µl of deionised water. Diluted samples are filtered using a 0.02 µm pore size Al_2O_3 Anodisc 25 membrane filter (Whatman) with a premoistened backing filter (pore size 0.45µm). The Anodisc membrane samples are placed side up on the YO-PRO-1 drops. The Petri dishes are placed in the dark for 2 days at room temperature. The filter is then washed twice with 800µl of sterile deionised water. The membranes are transferred to a glass slide, immediately covered with a drop of spectrophotometry-grade glycerol and a cover slip. A Zeiss Axioplan 2 epifluorescence microscope with blue filter at magnification of 1000 time magnification is used to view the viruses.

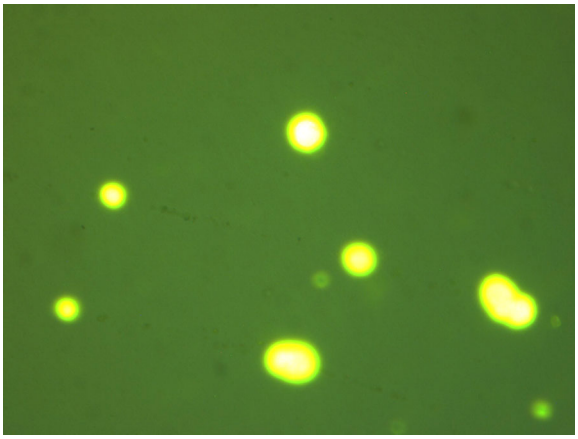


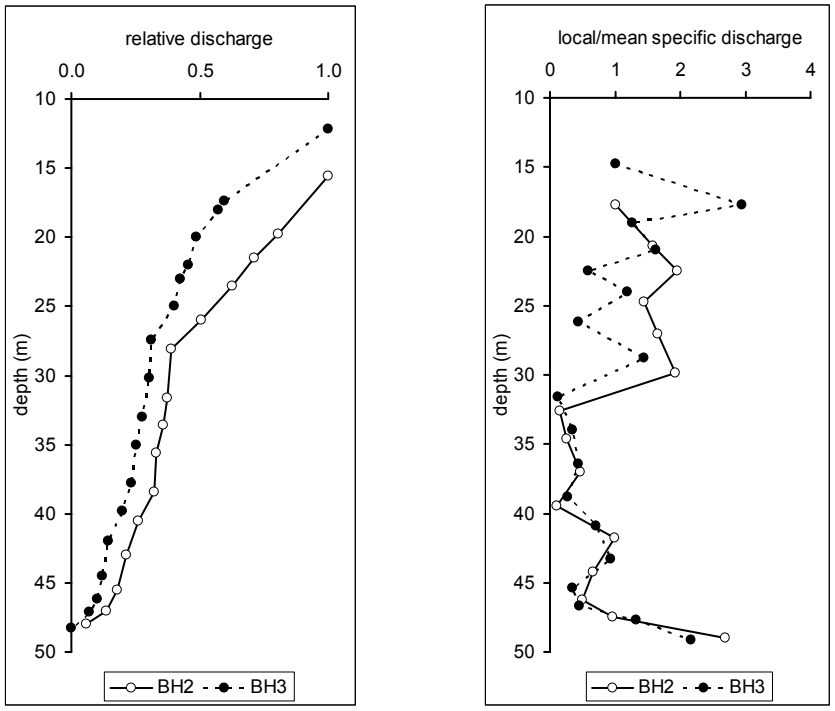
Figure 4: Epifluorescence image of PRD1 bacteriophages

Results to date

The results of the borehole discharge survey are shown in Figure 5. Apart from determining the up-hole discharges (Figure 5a), this test gives the inflow profile up each borehole (Figure 5b). From the results of the geophysical logging and the hydraulic testing, including the up-hole discharge tests, the subsurface sampling levels in the first full tracer test will be at 18, 21.5, 29.5, 37.5 and 45.75 mbgl.

During 2004 and 2005, microcosm studies in which bacteriophages were suspended in dialysis bags in a borehole approximately 90 m from the test site, indicated a PRD1 inactivation rate in groundwater of approximately 0.024 day^{-1} (Joyce et al., 2007). This rate would suggest a PRD1 concentration in September 2007 of at most 1.2×10^{-8} times that injected in August 2005. However, during the 2007 tests, concentrations of approximately 1.7×10^{3} pfu/ml were recovered from the pumped borehole, representing 2.6×10^{-6} times the injected concentration. This high concentration occurs despite the existence of natural horizontal and vertical head gradients in the aquifer tending to remove the viruses from the vicinity of the borehole, and the fact that all three holes at the site had been pumped during hydraulic testing (without virus assays). It appears, therefore, that although PRD1 does not migrate readily through a large proportion of the aquifer thickness, the presence of the rock (and the possible attachment to its surface) protects the virus against inactivation. Thus, although transport pathways through the sandstone appear to be rare, this in itself does not provide the effective protection previously expected.

Further tests with injections of fluorescent dye are planned in the remainder of 2007, with a full tracer test with injection of PRD1 scheduled for early 2008.



(a) (b)

Figure 5: (a) Discharge as a function of depth in BH2 and BH3 expressed as a fraction of the total discharge (b) Specific discharge in the aquifer as a function of depth in BH2 and BH3 expressed as a fraction of the mean specific discharge

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Joyce et al. (2007): GQ2007, Fremantle, Australia

Assessing the hazard from viruses in wastewater recharge of urban sandstone aquifers

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Abstract Increasing water demand in urban areas is focussing attention on the possibilities of the reuse of urban waste-waters, waters that often contain human and animal (including avian) viruses. In urban red-bed sandstone aquifers from the UK, which are predominantly matrix flow systems, evidence from well and piezometer monitoring shows that viable human viruses can be transported to depths of at least 80 m. The aim of the studies described here is therefore to determine the processes controlling the virus transport as a basis for risk assessment. Laboratory column experiments show that virus breakthrough is severely attenuated in synthetic groundwater solutions, some viruses remaining effectively irreversibly attached to the rock: attenuation capacity is only slowly reduced as more viruses are eluted. However, addition to the virus solutions of silica colloids, which when injected by themselves are also severely attenuated, results in breakthrough of the injected virus particles and release of previously attached virus particles. Forced-gradient tracer field experiments suggest that (severely attenuated) virus breakthrough occurs, but only through specific pathways. Current fieldwork is aimed at determining the location and hence the hydraulic and geochemical characteristics of these pathways. It appears, therefore, that virus attenuation is reduced by the presence of other colloidal matter, low ionic strength, and continuous virus loading, and that conditions for transport occur only in specific pathways. Future laboratory work will be aimed at further quantifying these processes and relating them to the petrographic and geochemical properties of the various sandstone (hydro)lithofacies which the field experiments indicate are important. This will provide the understanding necessary for a process-based risk assessment procedure.

Key words virus; phage; sandstone; artificial recharge; waste-water

INTRODUCTION

Increasing water demand in urban areas is focussing attention on the possibilities of the reuse of urban waste-waters. Urban waste-waters will often contain both human and animal (including avian) viruses, with concentrations up to at least 3,500 enterovirus particles per litre or 10^7 Norovirus particles per litre in the most polluted waters. If waste-waters are used in, for example, artificial recharge, it is therefore necessary that a risk assessment of virus hazard be undertaken. A particular issue is that only a few virus particles are needed to cause infection, unlike the case for most bacteria (e.g. Sair et al, 2002): this means that, as with highly toxic chemicals, even severe attenuation may not be sufficient to remove the hazard.

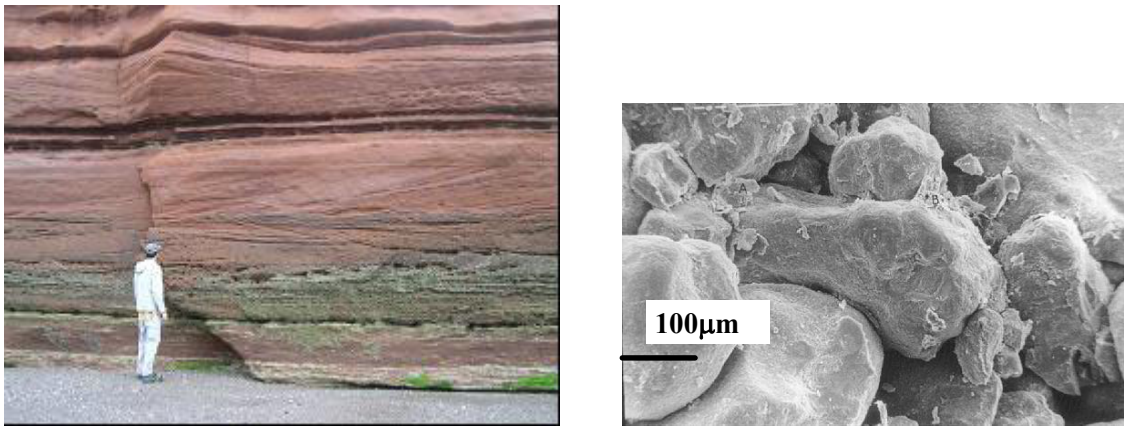
Although a risk assessment procedure could be based on empirical data acquired by undertaking extensive sampling of existing wells, a process-based procedure would be more flexible and would avoid reliance on mixed wellwater samples. In this paper we describe the progress made towards understanding the processes involved in virus movement in example continental red-bed sandstone aquifers.

THE AQUIFERS INVESTIGATED

The aquifers investigated are located within the UK Permo-Triassic Sandstone sequence (e.g. Barker and Tellam, 2006). This sequence comprises weakly to well-cemented fluvial and aeolian sandstones, with occasional thin, usually decimetre scale, mudstones and palaeosols (Fig.

1). The sandstones are typically dominated by quartz clasts, but also contain feldspars, micas, and lithic clasts. Carbonate cementation is common, though may be removed in the upper few tens of metres. Both detrital and authigenic clays are present, including smectites. Haematite coats all grains giving rise to the red colour of the rock. Matrix permeability is very variable, but averages around 1 m/d (e.g. Allen et al., 1997), with porosities averaging ~0.25. The sandstones are fractured (e.g. Hitchmough et al., 2007), and locally fracturing increases permeability. However, modelling suggests that the regional scale permeability is close to that of the matrix. Cation exchange capacities are typically 1-2 meq/100g (e.g. Carlyle et al., 2004), though occasionally up to 20 meq/100g. Median pore sizes are typically 10-50 μm, much larger than the diameter of virus particles (often <100 nm).

The UK Permo-Triassic sandstones are extensively used for water supply and underlie several major cities, including Birmingham, Nottingham, Liverpool, and Manchester. In some places the sandstones are overlain by Quaternary sands, tills, clays, and peat, but elsewhere (e.g. in Nottingham) they are exposed at surface. Water quality is usually good, though the urban aquifers often have high NO_3 and Cl concentrations (up to ~ 100 mg/L), and locally have elevated metal and chlorinated solvent concentrations (e.g. Shepherd et al., 2006; Tellam, 2007), the latter varying greatly from well to well (Tellam and Thomas, 2002).



(a) (b)

Fig. 1 (a) An outcrop of the sandstone showing both sedimentary structures and fractures. (b) An electron micrograph of an example 'clean' sandstone.

EVIDENCE OF VIRUS TRANSPORT

Reconnaissance sampling by Powell *et al.* (2000) of a small number of UK urban sandstone aquifer abstraction boreholes using glasswool traps showed that viable human viruses were present, albeit at small concentrations (1 to 1000 plaque forming units (pfu) /L) in most of the wells sampled (Table 1). Given that the wells are 100 – 200 m deep and cased over the upper few tens of metres, this result was not expected, it being usually assumed that the viruses would be rapidly attenuated by attachment to the rock, breakdown, and predation. Subsequent work on specially-installed multilevel piezometers by Powell *et al*. (2003) showed that viable human enteric viruses were found to depths of at least 80m below ground level (Table 1). Again, concentrations are usually low and, not unexpectedly, less frequent. Concentrations varied considerably over the 1-2 years of the sampling.

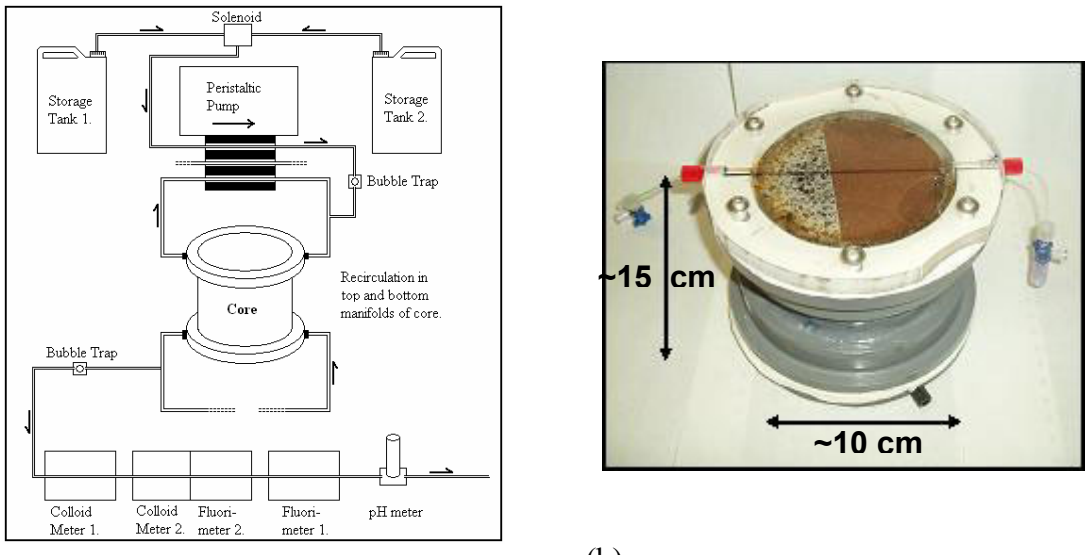
Table 1 Field detection of human enteric (Ent) viruses and coliphage (Coli) at sites in Nottingham (N) and Birmingham (B). Well indicates a pumped well sample (Powell et al., 2000); Sites 1 to 5 are multilevel piezometers (Powell et al., 2003).

Depth (m)	Well 8	Well 10	Well 17	Well B1	Well U2	Site 1	Site 2	Site 3	Site 4	Site 5
City:	**N**	**N**	**N**	**B**	**B**	**N**	**N**	**N**	**B**	**B**
Pumped	Ent	Ent+Rot	Ent	Rot						
0-9										
10-19						Ent		Coli		
20-29						Ent				
30-39						Coli+Ent	Coli			Coli
40-49						Coli+Ent	Coli	Ent		Coli
50-59										
60-69										
70-79										
80-89						Ent			Coli	

These results demonstrate that viruses can penetrate a predominantly matrix flow aquifer system to some depth, and still remain viable. It also shows that the concentrations involved are low, implying severe, though not sufficient, attenuation. Our subsequent work has been aimed at determining the mechanisms for virus transport, and hence the likely pathways through the sandstones: in particular we are interested in determining the relative importance of geochemical and hydraulic factors. Two approaches have been adopted: laboratory investigation and field experimentation. The results are summarized in the following sections.

LABORATORY INVESTIGATIONS

Experiments to determine the attenuation of viruses by interaction with the rock matrix have been carried out using 8 - 24 cm long intact sandstone cores taken from an experimental site on the University of Birmingham campus. Virus and bacteriophage suspensions made up in particle-free synthetic groundwater were passed through the columns maintained at *in situ* groundwater temperature (12°C) at rates equivalent to field groundwater velocities (Fig. 2). The eluant was collected and analyzed by plaque assay. Fluorescein was used to indicate un-reacting solute breakthrough.



(a) (b)

Fig. 2 (a) Column experiment layout. (b) Mounting of a half core (dark) showing upper transparent-topped manifold through which fluid was re-circulated to maintain mixing.

Fig. 3 shows typical breakthrough curves. In each case, a 'top hat' injection of tracer was used. It is clear that although breakthrough is recorded, severe attenuation occurs, implying that over field-scales attenuation would be effectively complete. Repeated application of virus suspensions, even though followed each time by extensive flushing with virus-free water, resulted in less attenuation (Fig. 5 below).

Real groundwater systems contain colloidal material which would be expected to interact with viruses. Previous work on the colloid concentrations within the sandstone groundwaters suggested around 10^{11} particles/L, mainly silicate in origin (Stagg *et al.*, 1997). To determine the effect of colloid/virus interaction, we have experimented with 100 nm diameter silica colloid suspensions.

Experiments were initially undertaken on the passage of virus-free silica colloid suspensions through intact sandstone columns of lengths from 7 to 12 cm. It was found that the colloids were almost unattenuated when made up in zero ionic strength solutions, but were almost completely removed when made up in synthetic groundwater (I~0.01)(Fig. 4). Over this ionic strength range, colloid ζ potentials change from ~-50mV to ~-25mV, though even in synthetic groundwater solutions, colloid suspensions are stable over much longer time spans than the experiments: the sandstone surfaces have a ζ potential of ~-30mV at the pH of the experiments. Long term experiments suggest the colloid retention capacity may be > 2 kg / m^3 rock (equivalent to <1% total rock surface area). The attachment is reversible in low ionic strength solutions.

Despite the severe attenuation of silica colloids, column experiments using combined phage and silica colloid suspensions made up in synthetic groundwater resulted not only in breakthrough of the injected phage, but also release of the phage which had not been eluted by extensive flushing following previous experiments (Fig. 5). The mechanisms involved are, as yet, unknown.

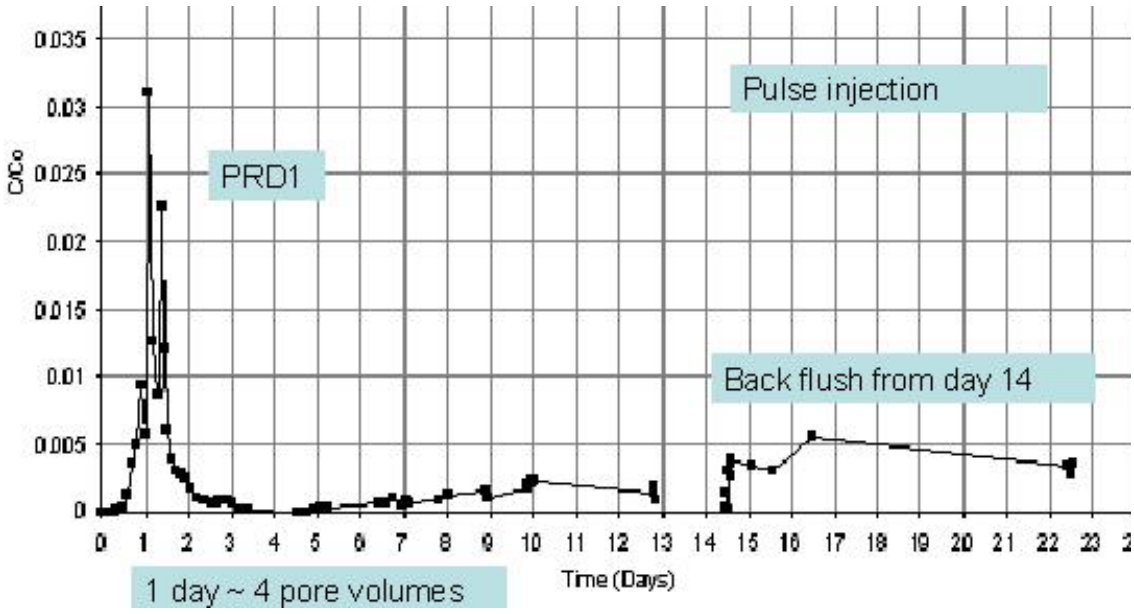


Fig. 3 Example virus (PRD1) breakthrough curve from pulsed, 'top-hat' injection of approximately a quarter of a day.

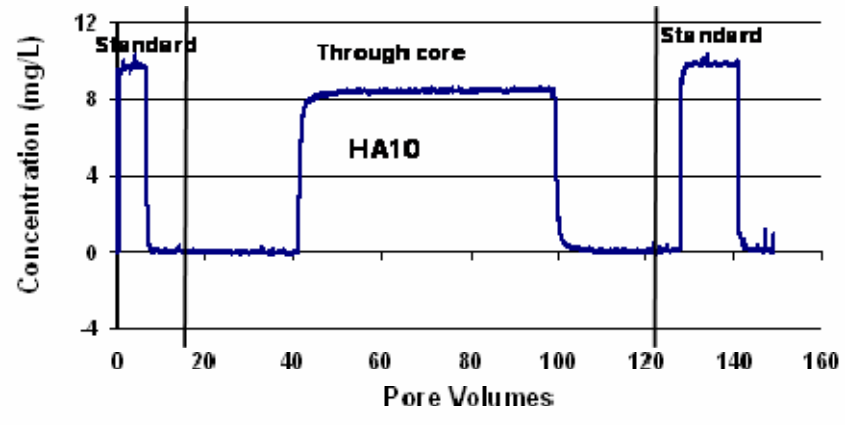


Fig. 4 Example 100nm diameter silica colloid breakthrough curve. Comparison with fluorescein (not shown) indicates little retardation.

FIELD INVESTIGATIONS

To investigate the movement of viruses in field systems, forced gradient tracer tests have been carried out using the University of Birmingham campus field experiment facility (Joyce et al., 2007). The first series of experiments involved the injection of phage suspensions into 1 metre packered intervals, and abstracting from the same unit, also packered off, in a borehole 7 m away. Although the un-reacting tracer (fluorescein) was detected in all experiments, phage did not breakthrough, even when the interval tested contained a fracture. However, when the whole saturated depth of the borehole was tested, breakthrough of phage occurred at about the same time as breakthrough of the inert tracer (Fig. 6). The implication is that the phage transport is limited to certain pathways rather than occurring uniformly, but the experiments provide no indication of the location or nature of the pathways.

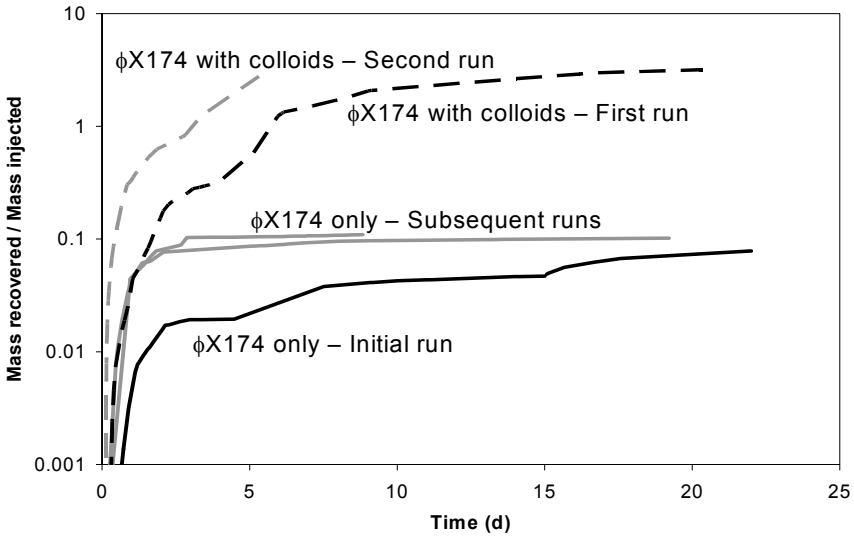


Fig. 5 Eluted mass (as indicated by pfu) as a function of time for 5 experiments on one column using the phage ϕX174.

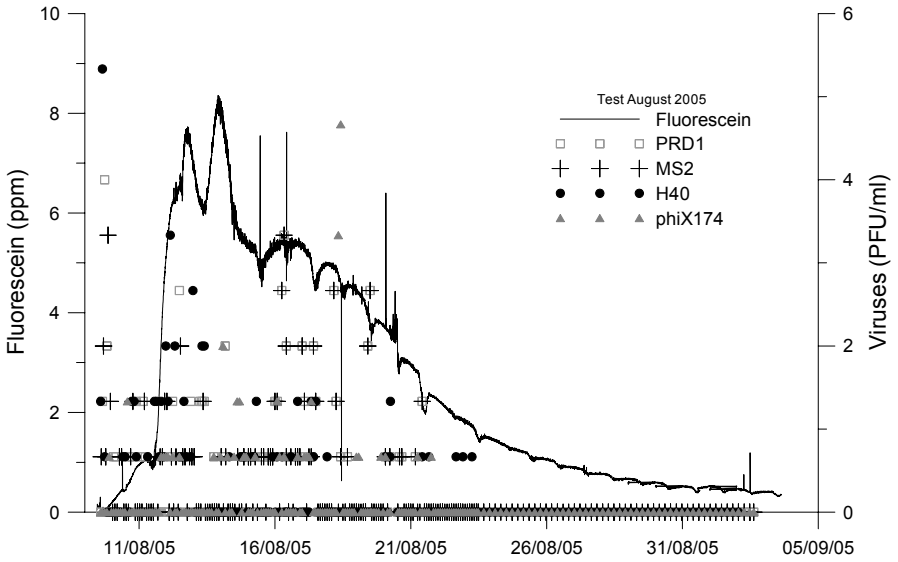
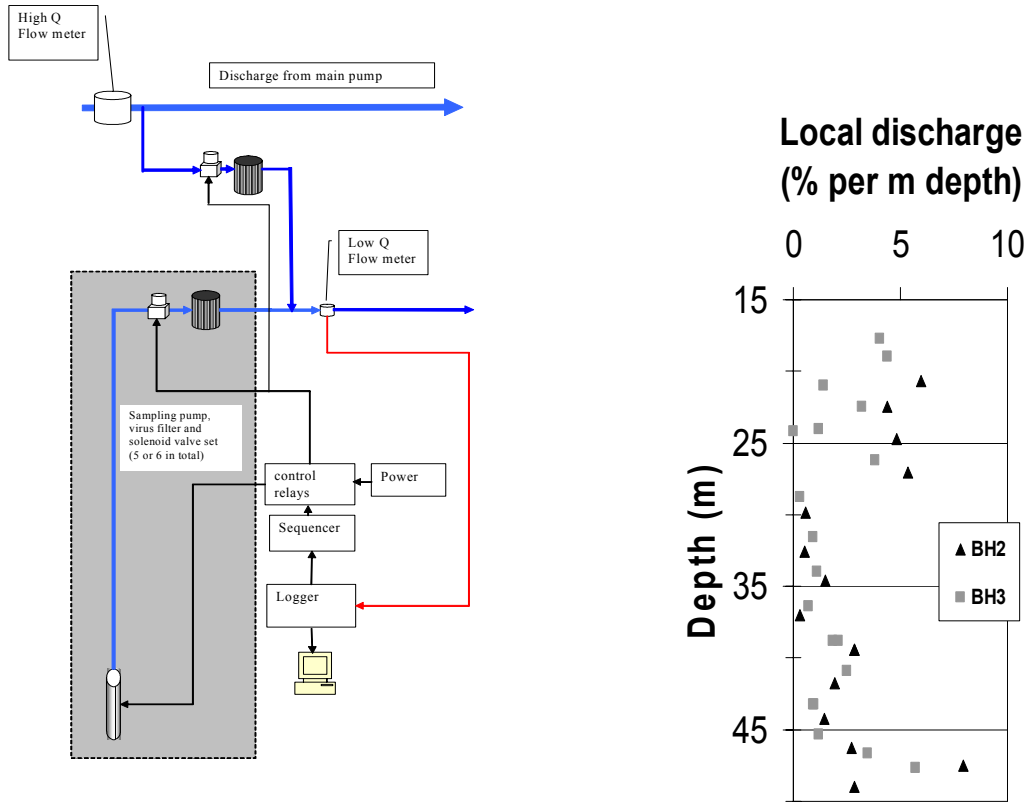


Fig. 6 Breakthrough of four phages and fluorescein at a pumping borehole following injection over the whole saturated depth of a second borehole 7 m distant.

The next phase of the experimentation is currently underway. As in the previous tests, a range of somatic phage (PRD1, ΦX174, H40/1) and a male-specific (F^+) phage (MS2) will be used. However, the pump in the recovery borehole will be located just below the water level, and a series of sampling lines at different depths will feed water samples through special, charged-filter virus traps (Nanoceram)(Fig. 7a): the latter will be eluted and phage enumerated by plaque assay and epifluorescence microscopy. The flow rates in the recovery borehole will be measured by an impellor device (Fig. 7b), allowing the variation in phage influx with depth to be calculated.



(a) (b)

Fig. 7 (a) Test design to determine virus pathways between boreholes. (b) Preliminary results indicating borehole inflow variation with depth during pumping.

CONCLUSIONS

Piezometer sampling, well sampling, and forced-gradient tracer testing in red-bed sandstones from the UK indicate that it should not be assumed, even in predominantly matrix flow systems, that viruses are immobile. This has implications especially for urban aquifers, particularly in the context of waste-water re-use.

Laboratory experimentation indicates that low ionic strength, previous exposure to viruses, and, especially, increased concentrations of other types of colloidal particle are important factors in promoting virus mobility: in other circumstances, pH is also likely to be of importance.

As yet, field evidence on the role of fracture flow on virus movement in the sandstones considered here is sparse. Undoubtedly, given a large enough fracture, enhanced virus mobility will occur. But often the fractures in the sandstone are of small aperture, contain some filling material, are often of limited lateral extent, and may well not be of significantly greater permeability than the host rock.

Field monitoring evidence (Powell et al., 2003) suggests sporadic occurrence of viruses both in space and time which is consistent with fracture flow: however, it is also consistent with variations in source strength and type, both of viruses and other particles. By undertaking experiments to identify the pathways taken by viruses between boreholes, we aim to determine the relative importance of these factors.

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Nanoparticles Meeting, London, September 2007

Presentation Abstract

Colloid-Enhanced Virus Mobility in Sandstone Groundwaters?

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Recent work has indicated that viable human viruses can penetrate to depths exceeding 80m in sandstone groundwater systems. One possible mechanism enhancing virus mobility is attachment to mobile colloidal particles, and this process has been investigated in a series of laboratory experiments. Field sampling indicates that the dominant colloid particle composition in sandstone groundwaters is silica, with wellwater samples yielding around 10^{11} particles/L. Accordingly, we have undertaken intact [continental red-bed] sandstone column experiments using 100 nm diameter mono-disperse silica spheres (pzc = 3.5-4). In water approaching zero ionic strength, the colloids passed through the sandstone almost un-retarded, but at ionic strengths equivalent to field values (I = 0.001M), no breakthrough was detected. This is consistent with measured ζ potentials of ~ -25-30 mV for the sandstones and ~-50 mV for the colloids in pure water, and ~-30 mV in synthetic groundwater. Similar experiments on viruses showed significant attenuation in synthetic groundwater, but breakthroughs were nevertheless still observed. Because of the lack of colloid breakthrough, enhanced virus mobility was not expected in the presence of the colloids in synthetic groundwater experiments involving both colloids and viruses. However, when mixed colloid and virus suspensions were passed through the columns, not only was the virus mobility enhanced, previously attached viruses were also released. Although work continues on the mechanism, and on field experimentation, the results suggest colloid-virus interactions may be important in virus migration, and therefore of direct relevance to public health issues in, for example, urban aquifers.

J.H. Tellam, 2007. Urban groundwater quality sustainability: the case of Birmingham, England. Zbl. Geol. Paläont. Teil I, 71-86.

Urban groundwater quality sustainability: the case of Birmingham, England

John H. Tellam

Abstract: Sustainability of urban groundwater quality in deep sandstone systems is discussed in the context of Birmingham, a long-industrialized city. Previous studies suggest sewage and de-icing salt diffuse pollution by inorganics and microbes, and metals and chlorinated solvent point source pollution. The latter is mainly associated with the industrial areas of the city. Here interception of wandering plumes/plume fragments results in a highly heterogeneous distribution of well-water quality. The large volume of the aquifer, the significant amount of good quality recharge, including piped water leakage, and the depth of the wells have rendered the median wellwater quality relatively good. It is concluded tentatively that, overall, sustainability is possible, especially if various measures are implemented, though may be locally impracticable. New chemicals (e.g. nanoparticles) may pose new problems.

1. Introduction

With rapidly rising urban populations worldwide (e.g. FOSTER et al. 1999), urban groundwater sustainability, in terms of both quantity and quality, is a major concern globally (e.g. TELLAM et al. 2006). Here the case of Birmingham, a heavily industrialized city for as long as any in the world, is taken as an example for urban areas overlying thick sandstone aquifers.

2. The hydrogeology of the Birmingham aquifer

Birmingham, a city presently of ~1 million inhabitants, grew rapidly during the industrial revolution of the 18th century from a market town to a major world producer, of metal goods in particular. A large part of the city is underlain by a Triassic Sandstone aquifer, confined to the southeast by Triassic mudstone (Figure 1). The sandstones, underlain by effectively impermeable Carboniferous deposits, are fluvio-aeolian redbeds, typically carbonate-cemented feldspar and lithic clast -containing quartz-dominated arenites, with both detrital and authigenic clays. Quaternary glacio-fluvial deposits, 0 to ~30m thick and including clays, sands, and peat, overlie the Triassic: 'made ground' (depressions in the ground surface infilled by, e.g., building waste) is present in some areas (KNIPE et al. 1992).

Although varying over a range of several orders of magnitude, the sandstones have a representative intergranular permeability of around 2 m/d: porosity is typically ~ 0.25, specific yield 0.15. The aquifer thus has a slow response time. Fracturing affects regional permeability only to a small degree, but can affect flow on a small scale considerably (TELLAM & BARKER 2006). The lower permeability Quaternary deposits locally reduce recharge rates, as does the urban cover (THOMAS & TELLAM 2006).

Around 1900, Birmingham built reservoirs ~120 km away in the Elan Valley, Wales, and piped the water to the city (UPTON 1993). A few public water supply wells survived until ~1920, but the main use for the aquifer since 1900 has been for industrial supplies. Increasingly heavy abstraction caused water levels to fall to a minimum in ~1960, since which time reduction in heavy industry activity has resulted in falling abstraction and rising water levels (Figure 2) (KNIPE et al. 1992).

The groundwater flow system in the city (Figure 1) is controlled largely by: the river Tame, the main natural discharge point; the low permeability Birmingham Fault; and abstraction. Flow velocities distant from wells are typically 10s m/y. Modelling suggests that reduction in

recharge due to increased 'impermeablization' of the catchment has been compensated for by increased piped water leakage (Table 1).

3. Water quality

3.1 Introduction

The main sources of information on groundwater quality are four well sampling surveys undertaken since 1980, with a maximum of ~ 80 wells per survey. Most data therefore represent wellwater chemistry rather than groundwater chemistry. TELLAM & THOMAS (2002) show that for an average Birmingham well of 120 m depth, 400 m^3/d pumping rate, and cased to 35 m, time to steady-state breakthrough of a diffuse pollutant released at ground surface is around 350 years: as the average age of wells in Birmingham is 60 years, the sampled waters are expected often to represent mixtures of polluted and relatively unpolluted groundwaters.

Only data from the unconfined part of the aquifer will be considered here. Overall wellwater quality is reasonable (Figure 3), with median concentrations below potable water standards. In the following sections an assessment is made of the contributions of various types of pollutant sources: urban runoff, chemical spills/leaks, made ground leaching, surface water infiltration, and sewer and piped water leakage.

3.2 Urban runoff

Industrial sites cover only a few % of the aquifer (Figure 2). Despite occasional post- dry weather flushes of poor quality discharges to surface water courses, much urban runoff from non-industrial surfaces appears to be of low ionic strength, and although some contains higher than natural metal concentrations, these concentrations are not high enough to be of major concern (HARRIS, unpub data; Table 2). Using the event mean concentration approach with data from both Birmingham and from the literature, it is not surprising that the maximum pollutant loading for inorganic species such as nitrate is low, with concentrations typically less than a few mg/L (THOMAS & TELLAM 2006). Organic data are few, but calculations suggest concentrations of some compounds at the water table will be low (THOMAS & TELLAM 2006).

Although most runoff waters are of good quality, during winter inorganic loads can increase considerably due to the spreading of rock salt for de-icing purposes (Table 2, maximum gully pot analysis). If all the salt applied were to enter the aquifer, the overall steady-state concentration would reach 250-300mg Cl/L (JENKINS 1995).

3.3 Spills and leaks other than from sewers and water supply pipelines

There is a correlation between wellwater quality and industry type, at least in the cases of total dissolved solids, total metals, and boron (a flux used in metal working): all are higher in metal working sites as might be expected (FORD & TELLAM 1994). At all sites where they have been used, chlorinated volatile organic compounds (VOCs) have been found in the groundwaters (RIVETT et al. 1990), an observation subsequently made in other cities (*e.g.* LERNER & TELLAM, 1992). Hydrocarbon VOC concentrations are generally very low (SHEPHERD et al. 2006). Both inorganic and organic concentration distributions are 'spotty' (Figure 4), consistent with point sources: in many cases metal concentrations are relatively low, but in a few cases they reach mg/L levels (Figure 3). Indeed, the two depth profile studies available also suggest the presence of plumes. In the first study, porewaters were extracted from a core at a site in west Birmingham which had been a metal working site for most of its history since its founding in about 1810. The chemical profile obtained indicates the presence of a Cr plume with concentrations up to 14 mg/L, about 5 m thick, with very sharp boundaries unrelated to lithology (Figure 4): the abstraction well at the same site occasionally yielded water with detectable Cr indicating either dilution in the well, or that the plume had not been captured. Colloid compositions also indicate the influence of industry, with Cr, Ni, Au, Hg, and Ce having been identified using EDX (STAGG et al. 1997): as these elements were below detection limits in bulk solution, either there is a very low concentration source or the source has been much

diluted. The second depth profile study, using piezometers, showed the presence of a less well defined and quite complex plume with two distinct parts (Taylor et al., 2006). It is unknown whether either plume in these two studies is now detached from its source.

3.4 Made ground

There is little direct evidence of made ground influences on the groundwater chemistry. However, as plaster is likely to be a major component of demolition wastes which in turn comprise a major component of fill material, it is suspected that sulphate concentrations will be increased by the made ground. SHEPHERD et al. (2006) show that leaching of metal-contaminated land does not give rise to excessive groundwater concentrations, a result in keeping with work by CZEREWKO et al. (2002) in the neighbouring city of Wolverhampton.

3.5 Surface water bodies

Birmingham presently has around 180 km of canals. The relative losses from infiltration and flow through the numerous locks is not known precisely. The source of the water for the system includes groundwater pumped from old mineshafts present to the west of the aquifer. Water quality data are few, but those available suggest that major ion concentrations are not presently particularly high. However, metal concentrations in the canal sediments are known to have been elevated in places (HoC 2000), and historical evidence suggests gross pollution in the past (UPTON 1993). It is possible that organic-rich colmation sediments may provide some protection for the underlying aquifer (or *vice versa*) as has been suggested for rivers (SMITH 2005).

The river Tame is currently effluent, though may have been influent at least locally in the past. Plumes of chlorinated solvent and inorganic pollutants (Al, F, Cl) have been mapped discharging into the river, though at rates small in comparison with the river flows (ELLIS & RIVETT 2006).

3.4 Sewage-related pollution

The related problems of water supply and sewage removal were tackled by the Birmingham Corporation in the second half of the nineteenth century, spurred on by two reports by Robert Rawlinson (in 1849 and 1871) (UPTON 1993). Thus until at least 1900, much sewage drained into city waterways and/or infiltrated, or was collected and spread outside the then city boundaries. As the numerous private wells were replaced by piped municipal supplies from ~1870, many of the wells would have been used as drains. The present-day sewerage system, some of which dates from the late 1800s, is one with both separate (foul, storm) and combined sewers.

Nitrate concentrations are relatively high across the aquifer (Figure 4), nitrate being one of the few determinands which has a median concentration close to the UK water standards. The few data available suggest NO_3 penetration to at least 50m below ground level (e.g. Figure 5; TAYLOR et al. 2006). Although there are several potential sources of N in the aquifer, including landfill, industrial waste discharge/spillage, and garden/parkland fertilizers, the high concentrations and almost ubiquitous presence (e.g. Figure 4) suggest that much comes from sewage sources (including animals - ALVES & TELLAM 2002) (see also POWELL et al. 2003). This conclusion is supported by high Cl concentrations and the presence in Birmingham and the hydrogeologically similar Nottingham aquifer of human viruses and faecally-associated bacteria in wells and piezometers to at least 50m depth (POWELL et al. 2000; 2003; TAYLOR et al. 2006). Although data are limited, viable human viruses were found in 5 out of 7 wells sampled, suggesting present-day sewage pollution is common. However, given the greater sources in the past and the relatively long residence times expected in this aquifer, much NO_3 may come from historic pollution: industrial sites, and therefore the sampling sites, are located predominantly in the older parts of the city.

3.5 Piped water (mains water) leakage

Leakage rates from water mains supply ~20% current recharge (Table 1). Although of good quality, trihalomethanes have been detected in small concentrations.

3.6 Time trends

Time series data are sparse, those available showing various trends as might be expected in a complex flow system with periodic point source events (FORD & TELLAM 1994). Comparing the datasets from the JACKSON & LLOYD (1983) and FORD & TELLAM (1994) surveys, the only significant trend present is a drop in pH with time (FORD et al. 1992): this decrease seems to have slowed over the following decade (SHEPHERD et al. 2006). Comparing post-1990 surveys, RIVETT et al. (2005) found that chlorinated VOC concentrations continue to increase. The evidence indicates, therefore, that some pollutants have stabilized, whereas others have not, again as expected from a dynamic system with continuously changing point source inputs, but an only slowly changing diffuse load.

3.7 Summary

The principal features of the Birmingham system relevant to the present discussion are: (i) diffuse sewage-associated NO_3 and Cl pollution, some being recent, but much historical; (ii) diffuse Cl pollution resulting from de-icing salt applications; (iii) point source pollution, giving rise to wandering plumes and plume fragments, mainly from industry and therefore clumped in certain parts of the city, and defined by increased concentrations of major ion and chlorinated VOCs and/or metals, but not hydrocarbon VOCs; (iv) some indication of increase in organic contamination with time, but less clear trends in other pollutants.

4. Is the aquifer a sustainable source of usable groundwater?

4.1 Introduction

Even after over 200 years of overlying urban and industrial land use, the water quality of the Birmingham aquifer is such that a significant quantity of usable groundwater can still be abstracted. To assess if this will continue, the importance of the three main potential ameliorating processes will first be considered: these processes are (i) natural attenuation; (ii) recovery of pollutants by abstraction and discharge to the Tame; and (iii) dilution. An integration is attempted in the final sub-section.

4.2 Natural attenuation

The aquifer has a modest natural attenuation capacity, with a low organic carbon fraction (typically 0.05% or less), moderate cation exchange capacity (typically a few meq/100g), low concentrations of mineral reductants, and probably limited concentrations of active mineral oxidants (TELLAM & BARKER 2006). pH is buffered at slightly alkaline, though may be <5 in the upper 10m or so of the aquifer where carbonates have been stripped. Some sorption of metals occurs, though possibly not as much as might be expected from the ubiquitous cover of iron oxides: sorption of organics is limited compared with many aquifers. The generally oxidizing conditions mean that degradation of NO_3 and the chlorinated VOCs, in particular, are not favoured generally (FORD & TELLAM 1994; SHEPHERD et al. 2006). The aerobic conditions do, however, favour removal of hydrocarbon VOCs (SHEPHERD et al. 2006).

Some by-pass flow appears to occur within the aquifer, as indicated by modelling studies which in some cases over-predict times to breakthrough from ground surface [TELLAM & THOMAS 2002 (chlorinated VOCs); BUTCHER et al. 2006 (NO_3)]. Experimental studies on human viruses indicate that survival beyond 2 years is very unlikely in groundwaters (J.SELLWOOD, Pers Comm, 2006; JOYCE et al. In Press), and yet penetration to depths of > 50 m has been recorded as discussed above. It is clear that for some contaminants even the limited attenuation capacity of the sandstone cannot be assumed to act with complete efficiency, at least over short distances.

4.4 Recovery of pollutants by abstraction and discharge to the Tame

RIVETT et al. (2005) calculate that the amount of chlorinated solvent removed per year in well abstraction water was ~1800 kg (a few drums) in the late 1980s, declining to 230 kg by the late 1990s, largely due to reduced demand for water. Discharge to the Tame is less well constrained, and estimates range from 22 to 200 kg/y (ELLIS & RIVETT 2006)(very low compared with the river discharge rates). These masses are likely to be much less than the total NAPL mass. Similar calculations have yet to be undertaken for inorganic compounds, but again total masses are expected to be limited.

4.5 Dilution

Although dispersive dilution will also occur (and be enhanced by varying abstraction patterns), the most efficient dilution will be within wells. The extent of dilution will depend on local hydrogeology and plume properties. The maximum dilution factors could be high for a thin, narrow plume travelling through a low permeability interval and entering the well from a limited radial arc. Diffuse pollution, even of modest thickness, entering a well radially at an average discharge rate would only be diluted by a factor of a few tens at most.

Taking the estimates of THOMAS & TELLAM (2006), the proportion of recharge entering the aquifer through industrial sites each year is very approximately 0.015% of the water stored in the aquifer, and hence a large dilution factor (6500) is available *if* it can be mobilized. Industrial sites are clumped rather than spread across the aquifer (Figure 2), and therefore locally less dilution capacity is available. The corollary of this is that in the significant parts of the city with little industry, including upflow of the industrial sites, greater dilution factors may be achievable, possibly even reducing the impacts of isolated sources, e.g. petrol stations and dry cleaning facilities, located in the residential areas.

4.6 Prognosis and possible treatments

It seems likely that in-well dilution is the most important process *governing* wellwater concentrations, though some natural attenuation and discharge from the system also act to remediate the aquifer. Diffuse pollution, particularly NO_3, has penetrated to at least 50m in places (Figure 4), and although the possible maximum dilution factors are hence relatively modest, the dilution necessary to lower wellwater concentrations (Figure 3) below health limits is also modest. If much of the NO_3 pollution is historical, then it would be expected that the concentrations will gradually improve: this may not be the case for Cl where significant loads come from de-icing salts. Point source pollution gives rise to greater maximum concentrations, but because plumes are smaller, dilution factors potentially can be much greater. Reduction in heavy industry and tighter regulation may mean lower loadings in future, but the presence now of NAPLs in the aquifer suggests that improvement in quality will be long term in some areas.

Modelling, which would be useful in estimating dilution, is at an early stage: major problems, even with stochastic approaches, include the inadequate present understanding of solute transport processes (TELLAM & BARKER 2006), irrevocably lost source and abstraction data, and incomplete hydraulic data. At present it is unclear whether empirical monitoring data may not provide the most useful predictions!

It is concluded, tentatively in advance of further modelling, that the system overall, if not always locally, is sustainable as a whole. Table 3 lists some possible actions, of a range of viability, which may enhance dilution, supplement natural attenuation by 'artificial attenuation', and reduce loadings. Future arrivals of new chemicals mean that vigilance will always be needed, as chlorinated VOCs have clearly demonstrated. For example, what problems will the infiltration of pharmaceuticals or manufactured nanoparticles have for indigenous bacterial populations, and what new transport mechanisms will have to be considered?

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Figure Captions

1	Outline geological map (with generalized groundwater flow directions) (left) and section (right) of Birmingham. City covers entire area shown on map.
2	Abstractions and hydrographs (left), and land cover map (THOMAS & TELLAM 2006). The map is based in part on Ordnance Survey data: © Crown copyright OS. All rights reserved.
3	Concentration frequency plots for the (industrial) wellwaters of the unconfined zone for the surveys of FORD & TELLAM (1994) and RIVETT et al. (1990). All samples included (number ~ 140; 61 wells).
4	The distribution of wellwater concentrations in the Tame Valley (left). Smallest circles generally indicate detection limits, and maximum concentrations can be estimated using Figure 3. Survey of FORD & TELLAM (1994) [after TELLAM & THOMAS (2002)]. Cr and NO_3 porewater concentrations below an industrial site in west Birmingham (right): note perched water level above clay immediately overlying the sandstone.

Table Captions

1	Water balance for unconfined + confined aquifer (KNIPE et al. 1992)
2	Example analysis summaries for urban runoff waters and rainfall sampled on the University campus (mg/L)(HARRIS, In Preparation).
3	Possible management instruments (*: already planned by Severn Trent Water)

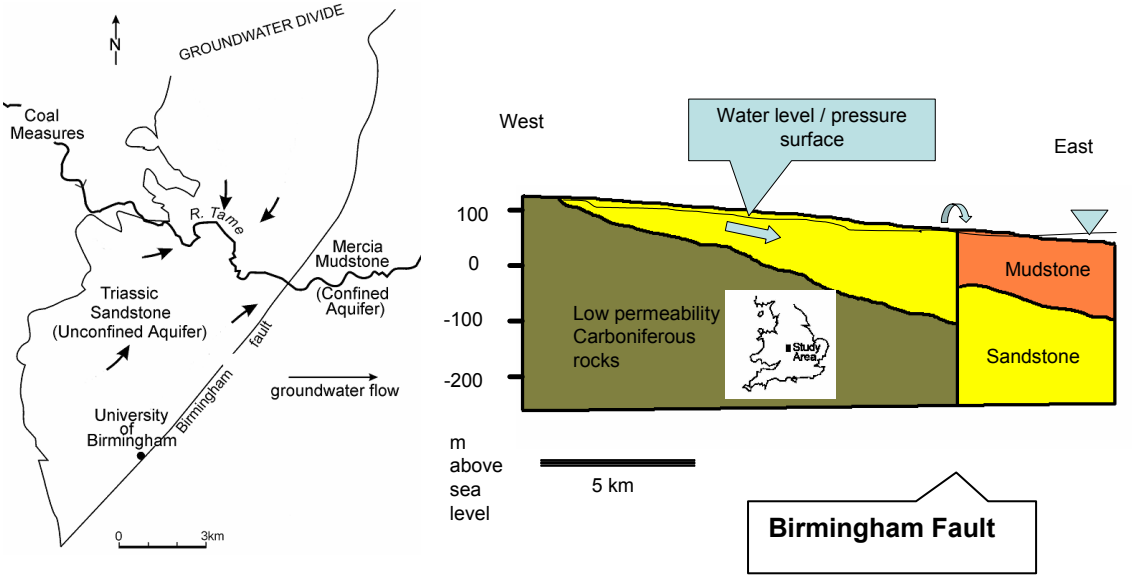

Fig. 1: Outline geological map (with generalized groundwater flow directions) (left) and section (right) of Birmingham. City covers entire area shown on map.

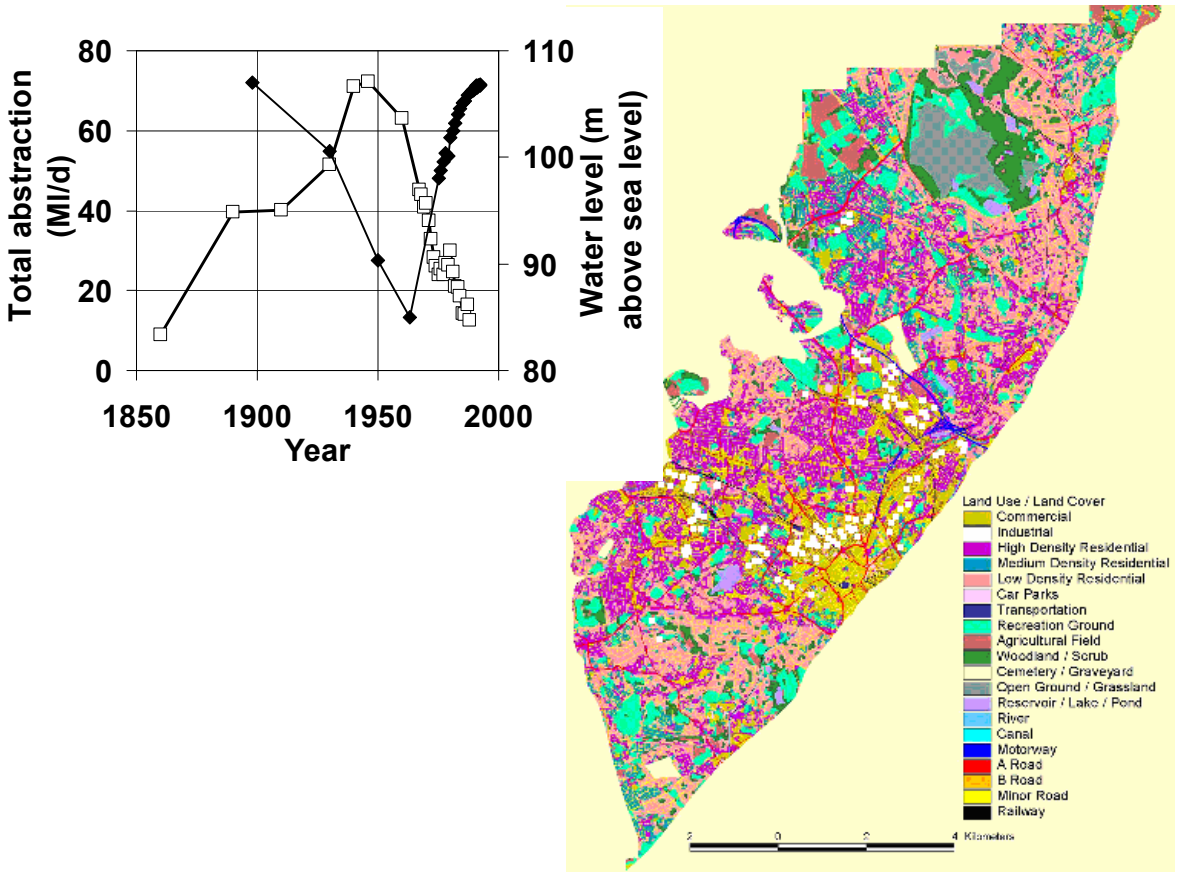

Fig. 2: Abstractions and hydrographs (left), and land cover map (THOMAS & TELLAM 2006). The map is based in part on Ordnance Survey data: © Crown copyright OS. All rights reserved.

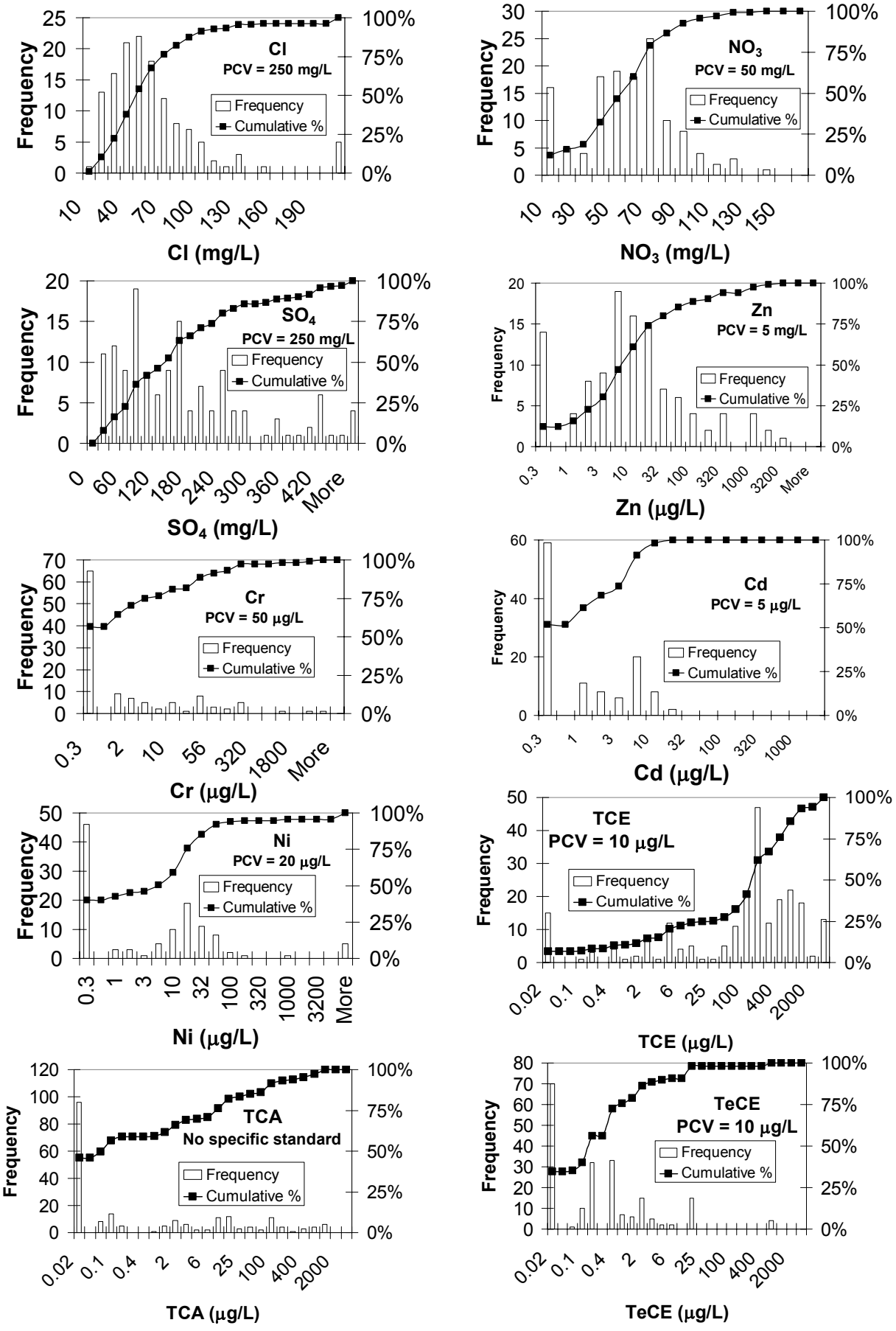

Fig. 3: Concentration frequency plots for the (industrial) wellwaters of the unconfined zone for the surveys of FORD & TELLAM (1994) and RIVETT et al. (1990).

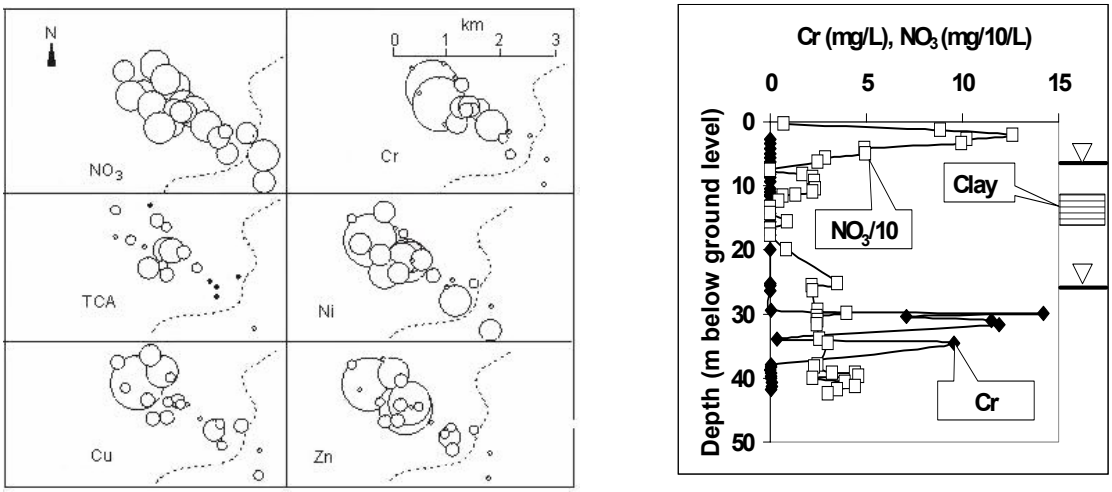

Fig. 4: Left: distribution of wellwater concentrations in Tame Valley. Smallest circles generally indicate detection limits, and maximum concentrations can be estimated using Figure 3. Survey of FORD & TELLAM (1994) [after TELLAM & THOMAS (2002)]. Right: Cr and NO_3 porewater concentrations below an industrial site in west Birmingham: note perched watertable above clay immediately overlying the sandstone.

Table 1: Water balance for unconfined + confined aquifer (KNIPE et al. 1992)

(ML/d)[mm/y]	1900	1945	1965	1989
Recharge {% mains leakage}	41.3 [83.7]{6}	40.6 [82.3]{16}	39.9 [80.9]{20}	39.7 [80.5]{21}
Surface water infiltration	-9.6 [-19.5]	16.3 [33.1]	19.5 [39.5]	-10.1 [-20.5]
Abstraction	-39.6 [-80.3]	-71.1 [-144.2]	-63.0 [-127.8]	-16.4 [-33.3]
Increase in storage	-7.9 [-16.0]	-14.2 [-28.8]	-3.7 [-7.5]	13.2 [26.8]

Table 2: Example analysis summaries for urban runoff waters and rainfall sampled on the University campus (mg/L)(HARRIS, In Preparation).

Statistic	**Ca**	**Mg**	**Na**	**K**	**Zn**	**Fe**	**NH_4**	**NO_3**	**PO_4**	**Cl**	**SO_4**
Gully pots											
Average	28	1.4	750	3.4	0.03	0.75	1.9	1.8	0.24	1838	
Median	17	0.7	8.2	1.6	0.01	0.05	0.73	1.5	0.15	20	
Min	1.8	0.08	0.14	<0.8	<0.01	<0.01	0.09	<0.1	0.01	<1.0	
Max	185	16.3	43290	185	2.01	12.6	13.0	9.19	3.66	99390	
Footpaths											
Average	13.0	0.46	22	2.85	0.01	0.08	0.09	5.5	0.08	29	14.0
Median	9.6	0.29	5.1	1.30	<0.01	0.04	<0.1	5.5	<0.1	3.9	6.47
Min	0.39	0.05	1.47	<0.5	<0.01	<0.01	<0.1	0.33	<0.1	1.36	4.23
Max	68.7	1.94	186	35.2	0.09	0.66	0.6	12.4	1.06	380	85.2
Roof Runoff											
Average	7.66	0.54	2.79	1.41	0.08	0.03				5.79	
Rainfall											
Average	0.64	0.06	0.87	0.56	0.05	0.03				0.91	

Table 3: Possible management instruments (*: already planned by Severn Trent Water)

Principle	**Approach**	**Comment** (£ = particularly expensive; L = legislatively difficult)
Dilute	Avoid plumes	Allow plumes to discharge to surface water, making use of attenuation in aquifer and hyporheic zone. Will increase volume of polluted aquifer. £, L
	Pump to river	Make use of river dilution & reaction, recovering downstream*
	Deepen wells	Increase in dilution, but > probability of encountering another plume?
	Inject waste water	Effects of organics, particulates, microbes, and trihalomethanes especially. Effects on redox systems. By-passing of unsaturated zone.
	Blending	£
Treat	At well-head	£
	Downhole	Reactive sand pack: new wells only? (£)
Source	Cut off supply	Liners below industrial sites, onsite waste treatment, limit development to one part of the city, label chemicals used, register of chemicals / processes, rolling sewer replacement programme. £, L
	Remove	All industrial sites to abstract own groundwater; groundwater pollution credit system (*cf.* carbon credit system). L

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ASSESSING THE HAZARD FROM VIRUSES IN WASTE-WATER RECHARGE OF URBAN SANDSTONE AQUIFERS

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Increasing water demand in cities is focussing attention on the possibilities of the reuse of waste-waters.These will often contain viruses, human and avian. Use of waste-water in, for example, artificial recharge therefore requires that a risk assessment of virus hazard be undertaken. Even in predominantly matrix flow aquifers such as the UK continental red-bed sandstones, which are the subject of the work described here, viable human viruses have been found to depths of at least 40 m. A previous tracer test showed bacteriophages frequently used as surrogates for human viruses to be transported between two 50 m boreholes. However, attempts to identify the transport pathway(s) by inter-borehole tracer tests in packered horizons, selected from analysis of cores and geophysical logs, proved unsuccessful. New tracer tests will now be conducted to identify these pathways directly. A range of somatic bacteriophages (PRD1, ΦX174, H40/1) and a male-specific phage (MS2) will be injected with a fluorescent tracer. The pumped recovery borehole will be sampled at discrete depths coincident with low permeability horizons. Samples will be pumped through a fluorimeter and a set of virus filter traps at the surface that will be eluted and replaced regularly, and the phages enumerated by plaque assay and epifluorescence microscopy. The up-hole flow rate at the sampling depths will be measured by an impeller device, allowing the variation in phage influx with depth to be calculated. Further tests focussed on specific horizons may be conducted subsequently.

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Use of bacteriophages to study potential human virus pathways in sandstone aquifers

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Due to increased water demand in cities, reuse of urban waste-water has been seen as a management option to be considered . But urban waste-water will often contain viruses, which may be mammalian, including human, or avian. Use of waste-water in, for example, artificial recharge therefore requires that a risk assessment of virus hazard be undertaken. Studies in different countries have shown the presence of viable human viruses in groundwater, indicating that they can remain active following passage across the soil. In urban red-bed sandstone aquifers from the UK, which are predominantly matrix flow systems, evidence from well and piezometer monitoring shows that viable human viruses can be transported to depths of at least 80 m. The main objective of this study is to find the lateral pathways of the viruses through this sandstone system. This is being attempted by undertaking borehole-to-borehole tracer tests using bacteriophages as surrogates for human viruses together with fluorescein as an unreactive tracer. A range of somatic phages (PRD1, ΦX174, H40/1) and a male-specific (F^+) phage (MS2) will be used. They will be injected in one borehole and recovered in a second, 7 m distant. The intake of the main pump will be located in the cased section at the top of the recovery well, and sampling pumps will be used to sample virus and fluorescein concentrations at five different depths. The up-hole flow rate at the sampling depths will be measured by an impeller device, allowing the variation in phage influx with depth to be calculated. Samples will be pumped through a fluorimeter and a set of virus filter traps at the surface. The virus filter traps will be eluted using a mixture of beef extract and glycine, and the eluate analyzed using the double agar layer method and epifluorescence microscopy. The pathways identified will then be investigated with targeted tracer tests. In addition, laboratory column experiments will be undertaken once the virus-permeable units are identified in order to determine the transport mechanisms.

Key words virus; phage; epifluorescence , double agar layer, artificial recharge; waste-water