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Geochemical influences on H40/1 bacteriophage inactivation in glaciofluvial sands

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Abstract Geochemical heterogeneities may cause spatial variations in virus inactivation rates resulting from interactions with minerals leading to differences in natural disinfection capacity within an aquifer. Column studies investigating the interaction of the bacteriophage H40/1 with natural sands sampled from the Kappelen test site (Kappelen), Bern, Switzerland indicated that inactivation rates are higher for adsorbed bacteriophages than for those suspended in groundwater. Moreover, breakthrough curves obtained from field-based tracer tests at Kappelen indicated that the adsorbed H40/1 is inactivated in-situ at comparable rates. Statistical analyses of mineralogical data failed to demonstrate significant spatial variations in aquifer composition either across the site or with depth. In contrast hydrochemical analyses of groundwater samples collected at Kappelen demonstrated that iron-reducing groundwater occurs below aerobic waters. Tracer breakthrough curves indicate that H40/1 survival is not affected by variable redox conditions. Investigation results suggest that spatial geochemical variability does not significantly affect H40/1s inactivation rate at Kappelen.

Keywords Bacteriophage · Groundwater · Geochemistry · Inactivation · Redox

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Introduction

Filtration by aquifer materials has historically been assumed to be an effective means of removing pathogenic microbiological contaminants (viruses, bacteria and protozoa) from groundwater. As a consequence of this assumption, regulations controlling the microbiological quality of groundwater for human consumption have often been less stringent than those for surface waters. (Macler and Merkle 2000). However, Craun (1986) estimated that untreated groundwater has been responsible for one third of water borne disease outbreaks in recent decades in the USA. Groundwater contamination by viruses has been a particular point of concern, in part due to their small size and their suspected greater mobility relative to protozoa and bacteria (Macler and Merkle 2000). Ryan and others (2002) noted that the fact that approximately 80% of water-borne disease outbreaks in the US where the causative agent was identified, were due to viruses prompted the United States Environmental Protection Agency (USEPA) to make viruses the focal point of the proposed groundwater disinfection rule. According to this rule, suppliers of water destined for public consumption would need to demonstrate natural disinfection if the water was not to be treated (Macler 1996). The USEPA defines natural disinfection as "Source water treatment via virus attenuation by natural subsurface processes such as virus inactivation, dispersion (dilution) and irreversible adsorption to aquifer framework solids" (USEPA 1992). Adsorption to sediments and inactivation are the two main processes by which infective viruses are attenuated in aquifers (Ryan and others 1999). Adsorption results from viruses interacting with solid surfaces and becoming attached. In an extensive review of viral attenuation processes, Schijven and Hassanizadeh (2000) noted that virus adsorption in groundwater systems is usually regarded as a kinetic process that results in reductions in virus concentration, relative to conservative tracers. Moreover, the authors noted that this process is strongly influenced by both the nature of the adsorbing surface and the chemistry of the water suspending the virus. However, studies by Bales and others (1991) have shown that adsorption may not necessarily be irreversible and that at least a portion of the viruses adsorbed onto mineral surfaces may be capable of subsequent desorption while remaining virulent. Moreover, Westwood and Sattar (1976) noted only one virus may be sufficient to cause illness. This suggests that

reversible adsorption processes, although reducing maximum virus concentrations, could be potentially more detrimental to public health than non-sorbing pathogens, since adsorbed virus could be released over a longer period and thus prolonging the risk of infection to those using a water supply.

Inactivation results in the loss of a virus' infective capacity. This loss is the result of the disruption of protein coating on the virus capsid (head) and associated nucleic acid degradation (Gerba 1984). The inactivation process in aquifers is controlled by both its physical environment (Yates and others 1987) and virus-specific physiochemical properties (Yamagishi and Ozeli 1972).

Virus inactivation may occur when viruses are both suspended in liquid and when adsorbed onto surfaces (Sobsey and others 1980). The enhancement/reduction of viral inactivation rates due to interactions with solids varies from one virus to another and depends on the strength of attachment (Hurst and others 1980).

Grant and others (1993) listed three alternative states affecting viral inactivation resulting from reversible viral interactions with solid surfaces:

1. Quasi-equilibrium adsorption (QEA): Virus inactivation rates on a surface are equal to those in the liquid.
2. Quasi-equilibrium adsorption reduced inactivation (QEARI): Virus inactivation rates on surfaces are lower than those in the liquid.
3. Quasi-equilibrium adsorption surface sink (QEASS): Virus inactivation rates on a surface are greater than those in a liquid.

Consequently, virus attenuation due to interactions with aquifer surfaces must be regarded as a complex process in which adsorption and inactivation can be inter-related. Furthermore, the dependence of virus adsorption processes on mineralogy and hydrochemistry imply that these parameters may indirectly influence viral inactivation by encouraging or discouraging virus adsorption to surfaces where inactivation rates may be different to those in suspension.

The contribution of viral inactivation due to interactions with aquifer materials is often difficult to determine. Indeed, Pedley and Howard (1997) noted that virus survival in groundwater systems remains poorly understood. Part of the reason for the lack of understanding concerning virus adsorption and inactivation in groundwater systems may relate to the various approaches used by different researchers to study this topic. Much research has been carried out into viral adsorption and inactivation with a variety of different techniques, particularly at the laboratory scale. These have included batch studies (Sobsey and others 1980; Gerba and others 1981; Sobsey and others 1986) and dynamic column experiments (Bales and others 1991; Loveland and others 1996; Penrod and others 1996). Harvey and others (1991) noted laboratory scale experiments allow controlled chemical and physical conditions to be imposed that may allow attenuation mechanisms to be confidently characterized.

More recently, field-scale experiments have investigated in-situ viral inactivation rates of adsorbed viruses in aquifers (Bales and others 1997, Schijven and others 2000, Ryan and others 2002). Investigations by Ryan and others (2002) in particular, have provided considerable insight into viral inactivation processes in natural porous deposits by integrating laboratory scale and field scale investigative techniques. Nonetheless, Harvey (1997) noted that the results of laboratory and field scale experiments investigating microbiological contaminant transport and attenuation often provide inconsistent results, due in part to heterogeneities in the deposits under investigation in the field.

The variable mineralogy and texture often encountered in natural deposits (Tucker 1981) can be considerably more complex than the compositionally and texturally uniform deposits often employed in many laboratory investigations (e.g. Bales and others 1991; Loveland and others 1996; Penrod and others 1996). The natural deposits making up many aquifers may have different compositions and/or textures that vary in space (Huggenberger and Aigner 1999; Kleinedam and others 1999). These different units may have variable virus adsorption characteristics, and thus by inference may inactivate viruses to different degrees. Furthermore, the minerals in contact with the groundwater may influence aquifer hydrochemistry (Stumm and Morgan 1996). This may result in differences in groundwater chemistry in different parts of the aquifer. Geochemical variations in mineralogy and/or hydrochemistry in aquifers may therefore either directly or indirectly influence virus inactivation rates. This may in turn result in variable disinfection rates in different parts on the same aquifer.

This study investigated virus inactivation in sand and gravel deposits at a site that forms part of a regionally important aquifer. Inactivation investigations have been carried out using laboratory-based and field-based methods. Laboratory studies examined viral adsorption and inactivation in the fine to very fine sand-sized fraction of samples of aquifer material to ascertain the degree to which suspended viruses are inactivated relative to those adsorbed on aquifer surfaces. Field-scale studies investigated viral transport and attenuation processes in the aquifer by means of tracer testing. These investigations were complimented by a program of geochemical site characterization, which examined the spatial variability of the mineralogy and hydrochemistry of that part of the aquifer underlying the site. The results of these studies were used to assess the potential degree to which virus inactivation may be influenced by compositional and textural aquifer heterogeneity, and the degree to which laboratory-based investigations could be used to further understand virus inactivation processes in the field.

Materials and methods

Field site setting and previous investigations

The Kappelen test site (Kappelen) is located approximately 15 km North West of the city of Bern, Switzerland. The site

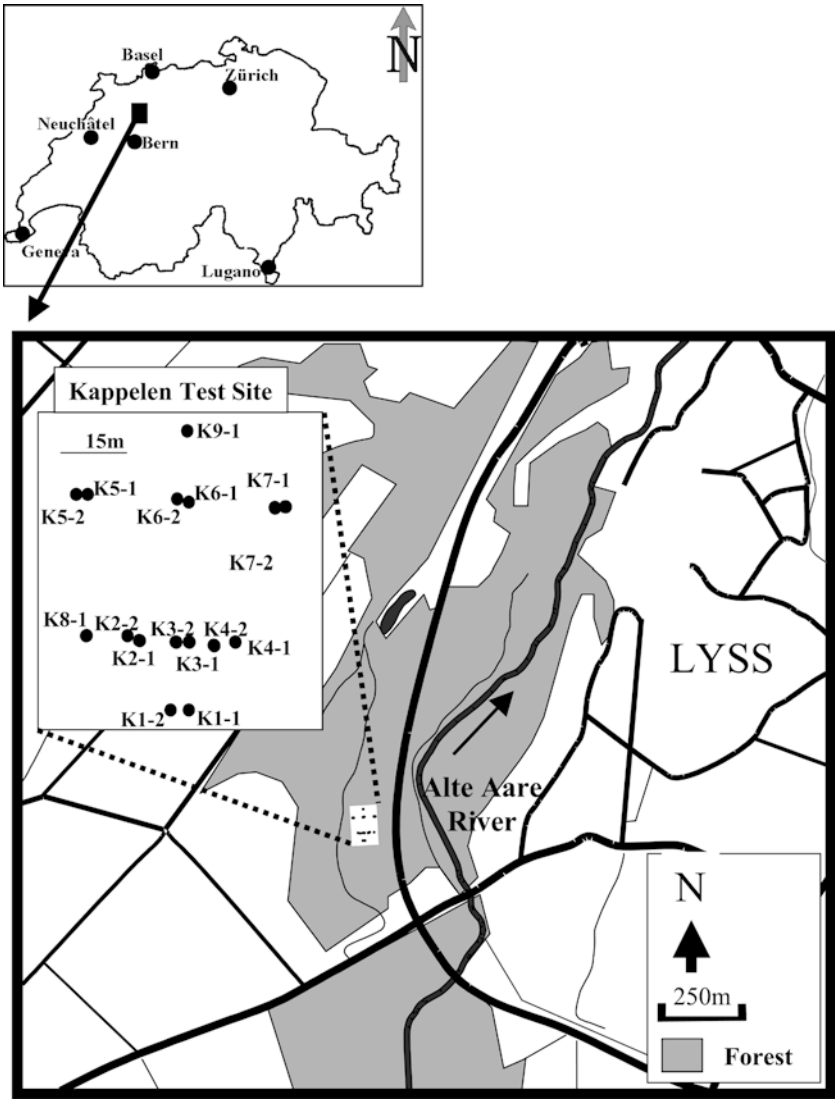

Fig. 1
Location map for the Kappelen test site, Canton Bern, Switzerland. *Inlay*: details of monitoring well locations

measures approximately 90x60 m^2 and is located in relatively flat wooded terrain that is bounded to the west by intensively cultivated agricultural land and to the east by a motorway and the town of Lyss (Fig. 1). A monitoring well network installed at the site consists of seven 4-inch diameter (100 mm) ID shallow/deep HDPE well pairs set in a roughly triangular array, and two additional deep wells, constructed with identical materials, to the north and west of the shallow/deep array. Wells set in the shallow part of the underlying aquifer (with the suffix –2) have 3 to 4 m long well screens set approximately from 4 to 8 m below ground surface (m BGS), while those set in the deep part (with the suffix –1) have a 3 to 5 m long well screen, screened at between 10 and 16 m BGS. Kennedy and others (2001) provide construction details of the wells.

Visual analysis of borehole cuttings from the nine locations drilled at Kappelen revealed that the site is underlain by approximately 16 m of unconsolidated polymineralic gravels with subordinate quantities of sand and silt. These gravels overlie a unit of fine-grained sands and silt/clay (Oyono 1996).

Specific capacity hydraulic testing of the monitoring wells by Kennedy and others (2001) provided estimates of the hydraulic conductivities of the gravels, assuming uniform contributions from all horizons set against the well screen. Using this approach, hydraulic conductivities in the lower part of the aquifer were estimated to be between 5×10^{-4} m/s and 1×10^{-2} m/s, while those in the upper part of the aquifer were estimated to be lower, at 1×10^{-4} m/s. However, grain size analysis of 33 sand and gravel samples collected from three borehole cores at K3–1, K7–1 and K8–1 indicated that the Kappelen deposits were highly heterogeneous. (Noseda 1999; Diomande 2000). Calculations based on these granulometric data have shown that although the sand and silt sized fractions of the samples investigated typically constitute no more that 20% of the sample mass, these materials contribute over 75% to the total surface area of the samples. Since available surface area controls the degree of adsorption to a surface by materials dissolved/suspended in adjacent liquid (Ross and Olivier 1964), these finer grained materials are thus believed to play an

important role in virus adsorption processes in the saturated deposits underlying the site.

Virus transport at Kappelen was previously studied by Kennedy and others (2001). The results of these investigations confirmed the groundwater flow direction indicated by hydraulic gradient data with tracing tests in both the shallow and deep parts of the aquifer. Tests involved injecting the bacteriophage (bacterial virus or phage) H40/1 into the groundwater along with the solute tracer, Uranine (Sodium Fluorescein) in either monitoring well K1–1, or monitoring well K1–2, and observing relative tracer responses in down-gradient observation wells. Both tracers were absent from the aquifer prior to injection. Tests completed between March 1997 and August 1997 investigated virus mass transport in the deeper part of the aquifer. Similarly, tests carried out between November 1997 and July 1998 studied virus behavior relative to Uranine in the shallower part of the aquifer.

Field hydrochemical sampling carried out in the framework of this earlier research in 1997 recognized that the regionally variable redox conditions, observed in groundwater samples collected from wells in the vicinity of the Kappelen test site (Wersin and others 2001), also affected the water present in the aquifer below the site (Kennedy and others 2001).

Mineralogical studies

An indication of the mineralogical composition of the Kappelen aquifer and its variability was obtained in a program of X-ray diffraction (XRD) analyses of the four grain fractions of 33 samples of aquifer material. Sand and gravel samples were collected from the cuttings of the three cored boreholes, at K3–1, K7–1 and K8–1 at approximately equal depth intervals. Due to their perceived importance in virus attenuation processes, investigations focused on the finer-grained fractions (diameter ($\varnothing$<4 mm) of the aquifer material. Four sand-sized and silt/clay-sized fractions were separated for each sample by passing them through DIN. ISO 3310/1 stainless steel sieves and rinsing in deionized water before drying at 40 °C overnight. The four grain size fractions analyzed for each sample were silt and clay ($\varnothing$<63 μm), very fine and fine sand (63 μm<$\varnothing$<250 μm), medium and coarse sand (250 μm<$\varnothing$<1000 μm) and very coarse sand/granular gravel (1000 μm<$\varnothing$<4000 μm). An X-ray diffraction (XRD) analysis of each fraction was carried out using a SCINTAG XRD 2000 diffractometer. Sample preparation and semi-quantitative analyses of the bulk mineralogy (volumetric percentage) followed the procedure described by Adatte and others (1996). Final composition quantification of the relative proportions of each mineral present, using external standards, generally provides an error varying between 5–10% for the phyllosilicates and 5% for other minerals. The results of these analyses permitted the dominant minerals in the sand and silt/clay sized fractions making up the deposits underlying Kappelen to be identified. Comparisons of results for various samples permitted the degree of spatial variation in mineralogy to be ascertained.

In addition to the mineralogical analyses, organic matter content was also investigated. The organic carbon content of eight aliquots of a composite sand sample was analyzed by Rock Eval 6 pyrolysis. This method has a detection limit of 0.1% organic carbon. Further details of this analytical method are contained in Disnar and others (2003).

Column experiments

Virus attenuation processes in Kappelen sands were investigated using one-dimensional column tests carried out under controlled chemical and hydrodynamic conditions. The marine phage H40/1 acted as the virus tracer for all column test experiments while Uranine was used as the solute tracer. Both Uranine and H40/1 were dissolved/suspended in a synthetic freshwater of fixed chemistry. H40/1 is a host-specific non-pathogenic B1 type marine bacterial virus (Siphoviridae) (Ackerman and DuBow 1997) hosted by the marine bacterium *Pseudoalteromonas gracilis*. Marine bacteriophages, such as H40/1 and their hosts are naturally absent from groundwater systems (Rossi and Käss 1997). Measurements by Rossi (1994) using transmission electron microscopy showed that H40/1 has a capsid (head) measuring 39 nm in diameter and a 46 nm long tail. Hydrophobicity measurements made using a contact angle goniometer microscope, in the framework of the current research program indicated that H40/1 was hydrophilic (contact angle: 52°±1°, n=6). Similarly, measurements of the electrophoretic mobility of this phage in the synthetic freshwater (SF) used in the experiments indicated that the H40/1 had a strong negative charge at the ambient experimental pH. (ζ-potential H40/1 (pH 7–8)=−23 mV±1 mV, n=6 for each pH).

A synthetic freshwater consisting of 8 mg/l of KCl, 62 mg/l of $MgSO_4.7H_20$, 294 mg/l of $CaCl_2.7H_20$, 21 mg/l of $NaHCO_3$ dissolved in Nanopure© water (Barnsted, Dubuque, IA) (resistivity-18.1 MΩ-cm) acted as the tracer solvent/suspending liquid and flush water for the column experiments. The water was buffered with 1.8 ml/l of 1 Molar Tris ($C_4H_{11}NO_3$, Fluka, Buchs, Switzerland) and adjusted with 1 N HCl to pH 8.0 (±0.1). Schijven and others (2000) indicated that concentrations of polyvalent cations can be critical in determining the degree of virus adsorption occurring under unfavorable deposition conditions. Consequently, the bivalent cation concentrations of the synthetic freshwater resembled those of groundwater samples collected from Kappelen, while the pH was approximately one unit higher than that observed at the site. A 100 ppb solution of Uranine, (Fluka, Buchs, Switzerland) acted as the solute tracer. Käss (1997) summarized studies indicating that although Uranine is pH sensitive and photodegrades in strong light, it undergoes little to no interaction with inorganic materials.

Before mixing with H40/1, the flush water and tracer reservoirs were agitated with a Teflon-coated magnetic stirrer under a vacuum of –70 mmHg for 15 min prior to all experiments to remove dissolved gases. At the start of an experiment, phage stock was diluted in saline buffer and 9 μL of the solution was added to the 100 mL-tracer reservoir containing Uranine. The tracer mixture was homogenized by magnetic stirring to give a stock concentration of approximately 400 plaque-forming units per mL (pfu/mL).

Tracer experiments involved injecting the tracer mixture into a 25 cm long x 1.8 cm diameter borosilicate glass column packed with saturated fine-to very fine-grained (63 μm<∅<250 μm) Kappelen sands, at a flow rate that resembled that observed in the field (4.1 ml/min±0.2 ml/min between experiments). Prior to each experiment, column was packed with sands in 1 cm increments poured into a degassed column of synthetic freshwater 3 cm deep or less. Packing the column matrix using a tap and fill method using a 1 cm diameter solid glass rod, reduced the possibility of grain bridging and the development of preferential flow paths. At least 15 pore volumes of tracer-free synthetic freshwater passed through the column prior to tracer injection, to permit chemical equilibration.
Tracer test experiments consisted of injecting a one pore volume pulse of the H40/1/ Uranine mixture into an actively pumping tracer-free system, followed by an additional eight pore volumes of tracer-free flush water. Following tracer injection/flushing, two pore volumes of 5 g/l of protein hydrolysate (Tryptone) mixed in SF with 100 μg/l Uranine were passed through the column to release H40/1 that was adsorbed to the aquifer sands yet still remained virulent. This flushing process was carried out either 30 minutes or 36 hours after the end of bacteriophage injection. Following Tryptone injection, the column was flushed with an additional six pore volumes of tracer-free SF before ending the experiment.
On-line fluorometers monitored solute tracer concentrations in column influent and effluent water at 10 second intervals and could detect Uranine at concentrations as low as 0.1 ppb (Schnegg and Bossy 2001). Regular on-line measurements of pH and conductivity during the experiments confirmed that hydrochemical conditions remained constant during all tracer tests. An automatic sampler continuously collected column effluent samples for bacteriophage analyses at 0.1 pore volume intervals. Column effluent samples were assayed for H40/1 content using the double layer technique (Rossi 1994) within three hours of sampling. Similarly, samples collected at regular intervals from the tracer reservoir were analyzed in a similar manner in order to ascertain the H40/1 concentration in the tracer source reservoir, and how it varied over the duration of the experiment. Due to logistical constraints, all experiments were carried out at room temperature (21–23°C). Both experiments, where Tryptone was injected 30 minutes after phage injection and 36 hours after phage injection, were repeated in triplicate to ensure experimental reproductability.

Field tracer testing

Tracer tests were carried out in the shallow and deep parts of the Kappelen aquifer in July 2001 and August 2001 respectively with a view to investigating virus transport and attenuation processes at the field scale. In both tests, Uranine acted as the solute tracer and the bacteriophage H40/1 was the virus tracer.
The test carried out in the shallow part of the aquifer involved injecting Uranine and H40/1 into K1–2 and monitoring tracer responses in K3–2 and K4–2 down gradient. The injection process involved gradually adding 15 liters of 5 g/liter Uranine mixed with 1.65×10^{13} pfu/l of H40/1, into an actively circulating system that pumped water from the base of the injection well to the ground surface before being re-injected at the top of the well screen. This approach had the benefit of not disturbing the static water level, and thus permitted tracer to leave the injection well under natural gradient conditions. Tracer injection was carried out over a 34 minute period, and circulated for 10 hours thereafter. Regular sampling and analysis of the injection well water permitted temporal Uranine and H40/1 concentrations changes in the injection well to be monitored. Samples of the original tracer mixture, kept in the field over the duration of a tracer test, were collected at 12 hour intervals to assess whether inactivation of suspended H40/1 was occurring in Kappelen groundwater.
Groundwater monitoring at K3–2 and K4–2 for Uranine and H40/1 content began 5.5 hours after the start of injection and continued until 165 hours after injection. On-line University of Neuchâtel Geomagnetism Group fluorometers (Schnegg and Bossy 2001) monitored Uranine concentrations at 4 minute intervals at both locations where well water was circulated using the same system as that employed in K1–2. Peristaltic pumps supplied aliquots of the circulating water to automatic samplers where water samples were collected for bacteriophage analysis at 20 minute intervals (Fig. 2).
All samples collected for bacteriophage analysis were refrigerated, and assayed within 24 hours of sampling using a two stage process. An initial stage qualitatively determined whether H40/1 was present in a sample or not. A subsequent stage permitted phage concentrations in samples to be accurately quantified using a series of successive dilutions, where necessary, until no more than 150–200 pfu/petri dish were present in each sample. H40/1 assays of all samples were carried out using the double layer technique. (Rossi 1994)
Tracer testing in the deep part of the aquifer involved injecting 75 g of Uranine and 6.3×10^{14} pfu of H40/1 in K1–1 over a 1 hour period following the same procedure as described for the shallow zone tracer test. Similarly, monitoring of tracer concentrations in groundwater samples was carried out at K2–1 and K3–1 in the same manner as that used at K3–2 and K4–2 during the shallow zone tracer test. Monitoring at both K2–1 and K3–1 continued for 72 hours after the start of injection.

Hydrochemical sampling

Two campaigns of hydrochemical sampling were carried out at Kappelen, in June 2001 and in February 2002. Sampling consisted of pumping groundwater at low flow rates (inducing less than 1 cm of drawdown) from the top and base of the screened interval of each well, while monitoring hydrochemical conditions of the discharge water at the well head. Well head hydrochemical parameters monitored (with measurement instruments in parenthesis) were temperature/electrical conductivity (WTW LF 318 electrical conductivity meter/thermometer), pH (Orion Research 407 ion analyzer/Sentix 60 pH electrode), redox potential (Orion research 407 ion analyzer/

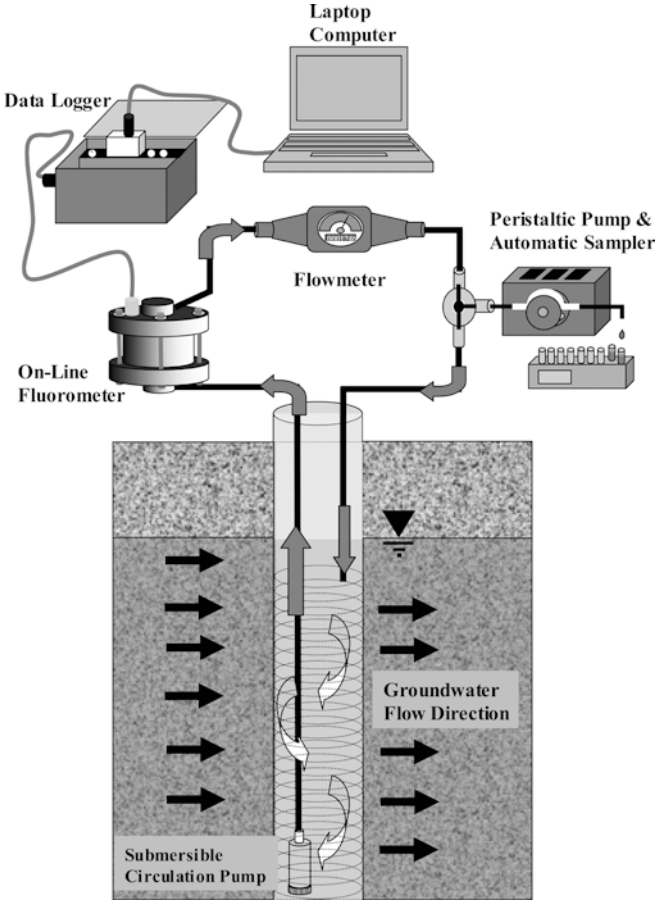

Fig. 2
Schematic diagram illustrating the operation of the sampling apparatus used for tracer testing. A submersible pump pumps well water through an on-line fluorometer at the well head, permitting Uranine concentrations to be measured. The circulating water (indicated by *dark grey arrows*) passes through a flowmeter before reaching a three-way valve. A portion of the water at the three way valve is pumped to an automatic sampler to permit bacteriophage analysis. Remaining water is returned to the well

Metrohm combined redox electrode) and dissolved oxygen (WTW oxi330 dissolved oxygen meter). Once the wellhead parameters stabilized, samples were collected for analyses. Analyses of groundwater samples using a Hanna instruments C-211 ion analyzer allowed concentrations of ferrous iron to be immediately determined at well head after sampling, while field-based hydrogen sulfide analysis was carried out during the February 2002 sampling event by degassing samples in the presence of compound sensitive paper (Hach, Ames-IA, USA). Anion and cation water samples were stored in two separate 250 mL HDPE bottles and analyzed in the laboratory using ion chromatography (DIONEX DX-120).

Acidification of major cation samples with 1 M HNO_3 ensured that no changes occurred in ammonium concentrations prior to analyses. Total organic carbon and dissolved organic carbon samples collected during the February 2002 campaign were stored in zero headspace 40 mL glass vials with 2 mL of 1 M H_2SO_4 prior to analysis by combustion. All samples were refrigerated to 5 °C immediately upon collection.

Results

Mineralogical/organic carbon analyses

Table 1 summarizes the results of the XRD analyses of the sand and silt/clay size fractions of the 33 samples collected from borehole cuttings at K3, K7 and K8. All samples are dominated by framework silicates (quartz, K feldspar and Na feldspar), carbonates (calcite and dolomite) and sheet silicates. The residual minerals presented in the table represent additional sheet silicates whose exact mineralogy could not be confidently identified by XRD (T. Adatte, pers comm). Significantly, iron-oxide minerals commonly associated with virus attenuation (Ryan and others 1999, Lukasik and others 2000) were not detected, except in one sample of silt/clay material where it made up no more than 1% of constituent minerals. This is consistent with microscopic observations of the aquifer material, where staining associated with these mineral types was not observed.

Fig. 3 graphically presents the relative compositions of the major minerals identified in each grain size fraction and compares the mineralogy of each grain size fraction in samples collected in the shallower part of the aquifer with that of samples obtained at depth. Similarly, Fig. 4 illustrates the variation in mineralogy for grain-size of each fraction by borehole.

Overall the XRD data for each mineral identified show that, although all grain size fractions contain the same minerals, the relative proportions of each mineral can vary from one fraction to the other. However, Mann-Whitney statistical comparisons of the mineralogy of each fraction in the upper and lower parts of the aquifer failed to find significant differences in the aquifer mineralogy between the two zones (at the α=1% confidence level). Similarly, Kuskall-Wallis analysis of variations in mineralogy from one borehole to another failed to reveal significant lateral variations in mineralogy.

Despite the similarity in mineralogy between sampling locations and with depth, it is possible that difference in the residual mineral fraction between samples collected from different parts of the aquifer existed. However, the lack of significant difference in the composition of identifiable minerals suggests that the source material was the same throughout the period in which the aquifer was deposited. Consequently, the composition of the residual mineral fraction is not anticipated to change either.

Organic carbon analysis failed to detect organic carbon in any of the samples analyzed.

Column tests

The results of H40/1 assays of column effluent samples plotted relative to source concentrations determined for the tracer reservoir permitted variations in bacteriophage concentration with time (breakthrough curve) for each experiment to be generated. Integration of the bacteriophage breakthrough curves generated prior to Tryptone flushing allowed the proportion of H40/1 that managed to flow through the column to be determined. Based on this figure, the H40/1 attenuation capacity of the Kappelen

Table 1
Summary of results of XRD mineralogical analyses

Size fraction	Calcite (%vol)	Quartz (%vol)	K feldspar (%vol)	Na feldspar (%vol)	Dolomite (%vol)	Sheet silicates (%vol)	Residual minerals[a] (%vol)
x<63 mm							
Mean	40.36	26.63	2.65	6.02	4.39	5.71	14.53
Maximum	54.43	35.87	12.47	12.12	16.20	9.82	48.02
Minimum	16.45	13.71	0.00	2.32	0.00	2.97	0.36
Standard deviation	9.72	5.33	2.14	2.24	4.02	1.68	12.03
No. of samples	33	33	33	33	33	33	32
<63mm<x<250 mm							
Mean	26.07	38.52	4.53	9.11	0.93	3.49	17.35
Maximum	37.24	47.50	10.53	18.43	5.89	5.94	62.64
Minimum	6.71	18.49	1.41	3.33	0.00	1.34	0.72
Standard deviation	6.76	6.38	2.33	3.51	1.20	1.17	13.30
No. of samples	33	33	33	33	33	33	33
<250mm<x<500 mm							
Mean	27.58	39.08	6.18	9.27	0.55	3.40	13.93
Maximum	51.74	53.51	15.52	21.80	2.02	6.01	63.57
Minimum	13.70	15.23	1.05	2.04	0.00	1.50	0.20
Standard deviation	8.01	8.36	4.49	4.31	0.49	1.09	13.23
No. of samples	33	33	33	33	33	33	33
500mm<x<2000 mm							
Mean	41.14	32.86	2.06	4.67	0.32	3.28	15.71
Maximum	60.31	43.88	19.46	12.88	1.99	6.74	59.95
Minimum	13.98	15.90	0.00	0.74	0.00	0.00	0.12
Standard deviation	10.14	6.55	3.25	2.77	0.47	1.56	15.35
No. of samples	34	34	34	34	34	34	34

[a]Residual minerals are those not identified by analyses. Regarded as undefined sheet silicates (T. Adatte Pers. Com). Goethite only detected in one sample

sands in the column could be determined. Integration of the H40/1 breakthrough curve generated by subsequent flushing using the Tryptone solution allowed the proportion of still virulent H40/1 that could be desorbed from the Kapelen sands by the solution to be determined.
Measurements of H40/1 concentration variation with time in the source tracer reservoir permitted inactivation rates of H40/1 suspended in SF at ambient experimental temperatures to be determined. These rates were used to correct virus recoveries calculated from column effluent assays, where inactivation rates of H40/1 suspended in liquid were shown to be significant.
Table 2 summarizes the results of the column testing. The results of viral assays indicated that H40/1 contents in both source and effluent samples individual analyses varied by ±25% of the average value measured. Data noise associated with this variability meant that inactivation rates of suspended H40/1 could not be calculated for short term experiments where Tryptone flushing was carried out 30 mins after bacteriophage injection. In contrast H40/1 contents in the tracer source reservoir had declined sufficiently after 36 hours to permit inactivation rates of between 0.27 and 0.34/day to be determined for phages suspended in liquid.
H40/1 recoveries calculated from the analysis of column effluent samples prior to Tryptone flushing ranged from 0.0 to 3.1% of the original mass injected. These data indicate that the Kappelen sands used in the column tests have a high capacity to attenuate H40/1. Nonetheless, despite the low recoveries observed almost all H40/1 injected were released (within a 20% margin of error) in subsequent flushing with the Tryptone half an hour after injection. Since assays of samples collected from the source reservoir indicated that suspended H40/1 concentrations did not decline significantly over the duration of these experiments, masses recovered based on effluent concentrations therefore indicate that adsorption was the dominant attenuation process removing virulent viruses and that neither the inactivation of suspended nor adsorbed phages was significant during the time frame of these experiments.
In contrast to the short term experiments, the masses of adsorbed viruses eluted by soaking the Kappelen sands in Tryptone after 36 hours range from 55.4 to 66.5% of the original mass injected, after suspended H40/1 inactivation rates are incorporated into the recovery calculations. Loveland and others (1996) demonstrated that virus adsorption to a solid surface can be reversible or irreversible but not both, implying that virus adsorption mechanisms do not change. Consequently, all of the viruses adsorbed to Kappelen sands, which could be released by Tryptone flushing during short-term experiments, should be released by the same process after longer time periods. This is not observed and thus suggests that another process, namely inactivation, is occurring on the surfaces of the Kappelen sands.
Calculated inactivation rates of H40/1 adsorbed on the Kappelen sands used in the column experiments ranged from 0.42 to 0.63/day, once the inactivation rate of suspended bacteriophages had been accounted for in calculations. These rates are greater than those determined for suspended H40/1. Consequently, the column experiments

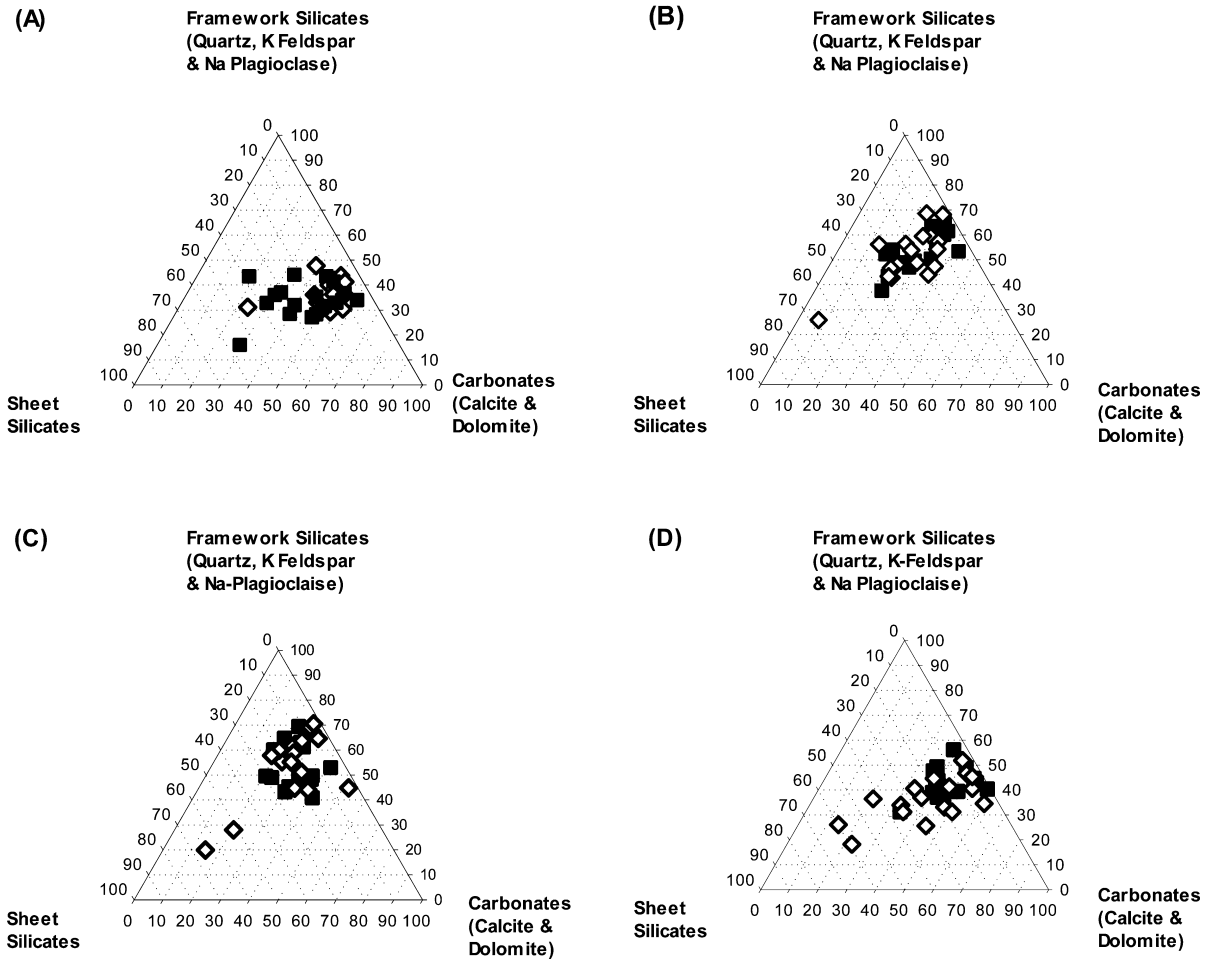

Fig. 3A–D
Trilinear plots of variation of mineralogy with grain size fraction Kappelen test site. **A** ∅<63 μm, **B** 63 μm<∅<250 μm, **C** 250 μm<∅<1000 μm, **D** 1000 μm<∅<4000 μm. *Hollow diamonds* denote samples from 8.5 to 16 m below ground surface (m BGS). *Black squares* denote samples from 3 to 8.5 m BGS

indicate that the Kappelen sands can preferentially inactivate H40/1 while it is adsorbed to Kappelen sands, relative to those in suspension (QEASS).

Field-based tracer test results

The results of H40/1 assays and Uranine analyses of groundwater samples collected during the July 2001 and August 2001 field-based tracer tests were used to generate breakthrough curves for observation wells K3–2 and K4–2 by dividing tracer concentrations by their respective masses injected. Table 3 summarizes the results of tracer tests carried out in July and August 2001 and compares them to results obtained during the previous phase of investigation carried out by Kennedy and others (2001). Both H40/1 and Uranine were detected in K3–2 and K4–2 following the shallow zone tracer test, as observed previously. However, differences exist in tracer first arrival times, peak concentration times and peak concentrations to various degrees, suggesting that the hydrodynamic regime may be different during the two tests. Nonetheless, it is noteworthy that relative recoveries of H40/1 with respect to Uranine in both cases are comparable, to within an order of magnitude.

Fig. 5A, B, present the breakthrough curves for H40/1 and Uranine observed in K3–2 during the July 2001 tracer test, along with the injection signal based on solute and particle concentrations in samples collected during tracer circulation. Fig. 5B presents the results of analyses of bacteriophage samples collected from the tracer reservoir at regular intervals throughout the duration of the July test. No apparent decline in concentration is apparent from the data suggesting that viral inactivation due to reactions with the Kappelen groundwater was not a significant process for the duration of this test.

In contrast to tracer tests carried out in the shallow part of the aquifer, neither the solute or bacteriophage tracer was detected in either K2–1 or K3–1 during the tracer test in the deep zone. This result is consistent with observations made by Kennedy and others (2001) who noted that tracer tests carried out during mid to late summer failed to demonstrate a connection between K1-1 and the observation wells K2–1 and K3–1. In contrast tests made by these authors in May 1997 detected the tracer at K2–1, further suggesting that the groundwater flow direction in the aquifer may vary temporally.

Since no solute or particle tracers were detected in the deep well tests during the August 2000 tracer test, Fig. 5D presents the breakthrough curve generated from K2–1

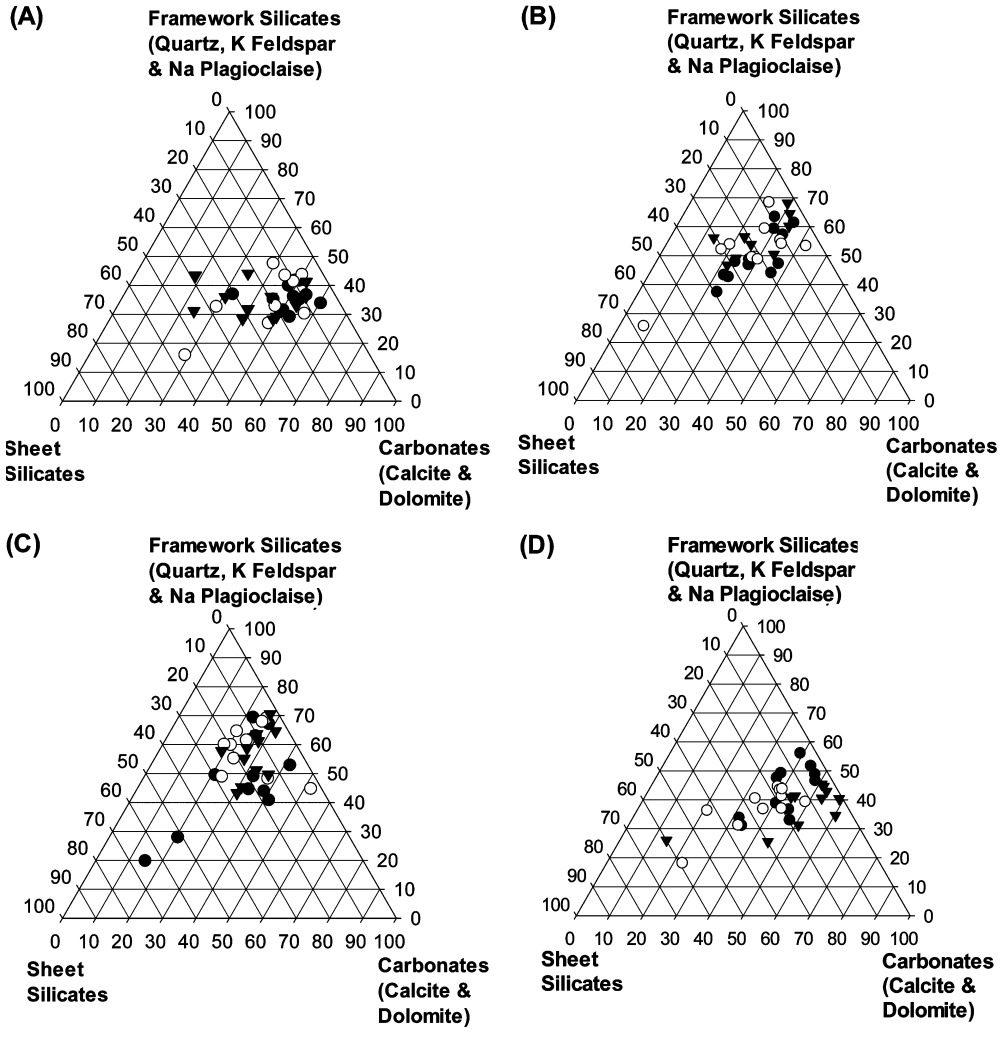

Fig. 4
Trilinear plots of variation of mineralogy with sampling location and grain size. **a** ∅<63 μm; **b** 63 μm<∅<250 μm; **c** 250 μm<∅<1000 μm; **d** 1000 μm<∅<4000 μm. *Solid circles* denote samples collected at K3–1; *Hollow circles* denote samples collected at K7–1; *Inverted triangles* denote samples collected at K8–1

Table 2
Summary table of results for column tests investigating H40/1 inactivation

Experiment	Recovery (%)	Recovery after extrusion (%)	Inactivation rate in liquid (1/day)	Inactivation rate due to interaction with sand (1/day)	Comment
Kappelen sand #1	0.4	91.1	n/c	n/c	Tryptone flush after 30 min
Kappelen sand #2	0.4	79.2	n/c	n/c	Tryptone flush after 30 min
Kappelen Sand #3	3.1	80.9	n/c	n/c	Tryptone flush after 30 min
Kappelen sand #4	0.0	66.5	0.28	0.56	Tryptone flush after 36 hrs
Kappelen sand #5	0.4	55.4	0.34	0.63	Tryptone Flush after 36 hrs
Kappelen sand #6	0.1	55.5	0.27	0.42	Tryptone flush after 36 hrs

Recovery is the mass of H40/1 recovered relative to the mass injected. n/c, Could not be calculated due to data noise. Inactivation rates assume first order decay. Recovery calculations using Tryptone flushing data incorporate inactivation rates in liquid. Margin of error in recovery calculations ±25%

from samples collected during the May 1997 tracer test to facilitate comparison of tracer responses in the shallow and deep parts of the aquifer. The breakthrough curve generated from data collected at K2–1 and that from K3–2 have a number of features in common. Concentrations of H40/1 in both curves peak before those of Uranine, but at substantially lower relative concentrations. H40/1 concentrations initially decline more sharply than Uranine after peaking, until they reach a point of inflection, after which bacteriophage levels decline much more gradually. Despite the noise observed in both cases, regression analysis shows the decline in H40/1 relative concentrations in both K2–1 and K3–2 for the last 100 hours of testing is statistically significant.

Field-based investigations of virus transport in dune sands carried out by Schijven and others (1999), using the bacteriophages MS-2 and PRD-1, obtained similarly shaped breakthrough curves to those obtained at Kappelen. These

Table 3
Summary table of Uranine and H40/1 tracer responses observe in K3–2 and K4–2 during shallow zone tracer testing in July 1998 and July 2001. Kappelen, Switzerland

		Uranine			H40/1			
Well	Date	First arrival (hrs)[a]	Peak conc. time (hrs)[a]	Peak C/M (1/L)	First arrival (hrs)[a]	Peak conc. time (hrs)[a]	Peak C/M (1/L)	Relative recovery[b] (%)
K3-2	July 2001	24.5	61.9	5.01×10^{-6}	19.2	30.8	3.45×10^{-9}	1.46×10^{-2}
	July 1998[c]	13	83	1.12×10^{-5}	12	27	1.10×10^{-8}	2.96×10^{-2}
K4-2	July 2001	13.8	41.8	4.11×10^{-6}	10.8	16.8	3.60×10^{-9}	6.30×10^{-3}
	July 1998[c]	14	89	1.60×10^{-8}	11	17	4.18×10^{-10}	7.61×10^{-2}

[a]Time since end of tracer injection
[b]Calculated according to Harvey and others (1991) at 160 hrs after injection
[c]Determined using data from Kennedy (2001)

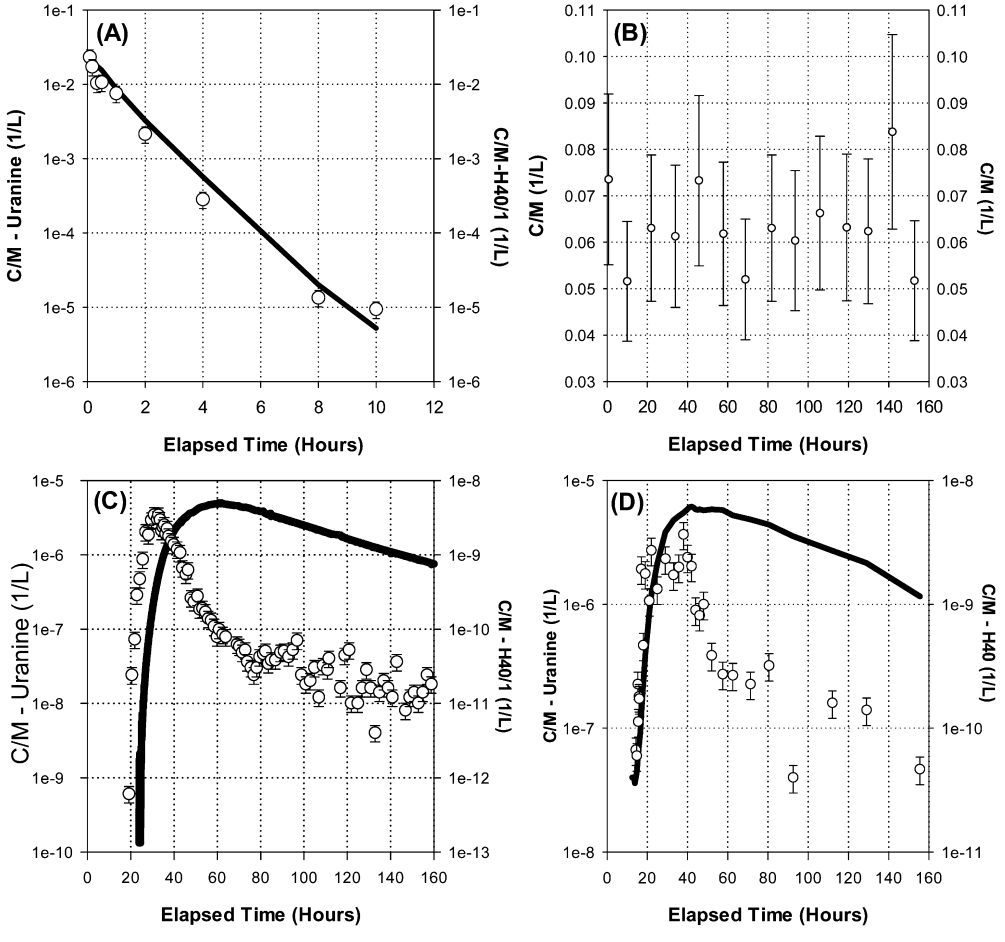

Fig. 5A–D
Plots of Uranine and H40/1 relative concentrations (C/M) with time. Solute concentrations are represented by a *solid line*. H40/1 concentrations are represented by *hollow circles*. **A** K1–2 injection well. July 2001 tracer test. **B** H40/1 variation in source reservoir. **C** Breakthrough curves observed at K3–2. July 2001. The origin of daily fluctuations in phage concentration remains undetermined. **D** Breakthrough curves observed at K2–1, May 1998. H40/1 errors: ±25% of observed value. Uranine errors incorporated into thickness of breakthrough curve line

authors attributed the differences between solute and bacteriophage breakthrough curves to three processes, adsorption, desorption and inactivation. Significantly, the authors noted that the slope of the later part of the tail of the breakthrough curve is dominated by the inactivation of adsorbed bacteriophages. More specifically, the rate of surface inactivation could be determined from the slope of a plot of the log of relative concentration with time. Applying this approach to the slopes of the tails of temporal C/Co the breakthrough curves obtained from the Kappelen tracer test data, surface inactivation rates of 0.33, 0.17 and 0.37/day were calculated from best fit lines of the tailing parts of the breakthrough curves from K3–2, K4–2 and K2–1 respectively. These data thus indicate that inactivation of adsorbed H40/1 occurs in both the shallow and deep parts of the aquifer.

Chemical Analyses

Table 4 and Table 5 summarize the results of hydrochemical analyses of organic carbon content in addition to well head and redox sensitive parameters in groundwater samples collected at Kappelen in June 2001 and January/

Table 4
Results of well head, redox indicator and total organic carbon analyses for groundwater samples collected during the June 2001 sampling campaign

Location	Depth (mBGS)	pH	Temp (°C)	0_2(mg/l)	Fe^{2+} (mg/l)	NH_4^+ (mg/l)	$N0_3^-$ (mg/l)	TOC (mg/l)	$S0_4^{2-}$(mg/l)
K1-1	10	7	10.7	0.06	0.93	0.28	0.67	1.33	38.44
K1-1	13	7.1	10.7	0.16	1.02	0.28	0.45	1.19	38.62
K1-2	5	7.25	12.7	3.38	0.04	0.02	6.22	0.94	35.25
K1-2	8	7.1	10.7	3.54	0.02	0.00	6.71	1.17	35.35
K2-1	10	6.85	10.6	0.05	0.79	0.08	0.22	1.12	40.97
K2-1	15	6.9	10.6	0.06	0.61	0.08	0.28	1.15	41.59
K2-2	5	7.2	10.7	1.80	0.02	0.00	10.10	1.03	34.67
K2-2	8	7.15	10.5	0.56	0.20	0.00	10.33	1.02	34.99
K3-1	10	7.25	10.8	0.06	0.63	0.19	0.52	1.12	39.02
K3-1	15	7.4	10.7	0.13	0.29	0.24	0.36	1.23	39.24
K3-2	5	7	10.7	3.90	0.00	0.00	7.55	1.15	33.79
K3-2	8	7.2	10.7	2.96	0.00	0.03	5.47	0.97	34.46
K4-1	15	7.3	10.6	0.11	0.01	0.06	0.96	0.97	37.35
K4-1	10	7.35	10.7	0.07	0.12	0.03	0.78	1.09	36.86
K4-2	4	7.15	10.7	2.04	0.03	0.02	6.00	0.91	34.98
K4-2	8	7.15	10.4	2.64	0.01	0.03	8.19	1.01	32.61
K5-1	10	6.75	11.1	0.11	1.71	0.64	0.40	0.82	38.66
K5-1	15	7.1	11	0.11	1.71	0.63	0.19	0.80	38.54
K5-2	5	6.9	11	0.33	0.30	0.02	0.17	0.91	39.06
K5-2	8	7	11	0.15	0.27	0.02	0.45	0.97	39.27
K6-1	15	6.7	11	0.11	1.22	0.58	0.61	0.82	38.22
K6-1	10	7	10.9	0.11	1.47	0.53	0.33	0.81	38.41
K6-2	5	7.2	10.4	2.78	0.28	0.00	12.03	0.96	30.37
K6-2	8	7.2	10.2	2.86	0.00	0.00	11.78	0.94	30.31
K7-1	15	7.2	10.9	0.11	0.84	0.58	0.20	0.84	37.83
K7-1	10	7.1	11	0.11	1.19	0.52	0.16	0.90	38.26
K7-2	5	7.3	10	3.14	0.00	0.00	10.07	0.96	32.11
K7-2	8	7.25	10.5	2.55	0.01	0.00	9.82	1.06	34.49
K9-1	12	7.5	11.2	0.11	1.48	0.68	0.37	0.84	37.35
K9-1	16	7.2	11	0.00	1.30	0.67	0.28	0.87	37.59

February 2002 respectively. The data demonstrate that there is not a significant difference in groundwater temperature or pH between wells screened in the upper part of the aquifer and those screened at depth. Moreover, the results of major ion analyses of samples collected in June 2001 differ little from those collected in January/February 2002. Overall, calcium and bicarbonate dominate the major ion hydrochemistry, and vary little between sampling events (Fig. 6a). Similarly, little difference is apparent in total and dissolved organic carbon contents (DOC/TOC) in waters samples collected from the various wells across the site.

In contrast to the major-ion hydrochemistry, the redox conditions in the various monitoring wells at Kappelen differ substantially with depth. Generally speaking, shallow wells have lower concentrations of species indicating reduced conditions such as ammonium and ferrous iron, while concentrations of dissolved oxygen and nitrate are higher than those in samples collected from deep wells. Superimposed on this depth-variable pattern is a trend towards more reducing conditions on the western side of the site compared to those on the eastern side. The hydrochemical section presented in Fig. 6b summarizes these phenomena for the two sampling events, using dissolved oxygen, ammonium and ferrous iron concentrations to illustrate the differences in redox conditions in different parts of the aquifer.

Temporally, the overall hydrochemical conditions at the site were slightly more reductive during the February 2002 sampling event. However, the results of the analyses of water samples collected by Kennedy and others (2001) in spring 1997 indicate little difference in the concentration of redox sensitive species to those observed during the two more recent sampling events. Consequently the variations are suspected to be a result of seasonal variations rather than forming part of a longer-term temporal trend.

It is noteworthy that testing of groundwater samples during the February 2002 water sampling event failed to detect hydrogen sulfide, despite the presence of sulfur in the water (as sulfate). This indicates that redox conditions at the site were insufficiently reductive to cause changes in the oxidation state of the inorganic sulfur present in the groundwater.

Discussion

The results of field-based and laboratory-based investigations have provided an insight into H40/1 inactivation processes in the deposits underlying Kappelen. Short-term column tests indicate that the Kappelen sand used in this study had a high H40/1 attenuation capacity. By flushing this sand with Tryptone, following the passage of the

Table 5
Results of well head, redox indicator and organic carbon analyses for groundwater samples collected during the January/February 2002 sampling campaign

Location	Depth (mBGS)[a]	EC (uS/cm)	pH	Temp (°C)	O_2 (mg/l)	Fe^{2+} (mg/l)	NH_4^+ (mg/l)	NO_3^- (mg/l)	TOC (mg/l)	DOC[b] (mg/l)	H_2S (mg/l)	$S0_4^{2-}$ (mg/l)
K1-1	10	446	7.17	11.8	0.50	0.53	0.52	0.03	0.84	0.68	<1	35.32
K1-1	13	449	7.22	12	0.40	0.84	1.68	0.03	0.68	0.52	<0.1	35.61
K1-2	5	448	7.2	11.5	0.60	0.31	0.30	0.07	0.86	0.70	<0.1	35.46
K1-2	8	452	7.33	11.8	0.40	0.23	0.29	0.03	0.79	0.63	<0.1	36.03
K2-1	10	454	7.17	11.7	0.46	0.53	042	000	n/m	n/m	<0.1	38.15
K2-1	15	449	7.24	11.7	0.26	n/m	0.00	n/m	n/m	n/m	n/m	n/m
K2-2	5	460	7.09	10.7	1.67	0.06	0.00	0.74	0.61	0.45	<01	36.63
K2-2	8	456	7.15	11.4	0.40	0.22	0.03	0.11	0.67	0.51	<01	36.74
K4-1	10	506	7.16	11.8	0.54	0.82	0.32	0.05	0.69	0.53	<01	31.87
K4-1	15	491	721	12.5	0.36	123	3.94	0.02	0.60	0.44	<01	35.36
K4-2	4	490	6.99	10.9	4.35	0.01	0.00	0.59	0.59	0.43	<01	31.37
K4-2	8	502	7.19	11.6	1.81	0.00	0.03	0.47	0.63	0.47	<01	33.48
K5-1	10	501	7.24	11.4	0.60	1.33	0.69	0.00	0.77	0.61	<01	38.81
K5-1	15	500	7.15	12.2	0.40	1.45	0.75	0.00	0.82	0.66	<01	38.88
K5-2	5	510	7.08	10.8	2.80	0.16	0.00	2.04	0.95	0.79	<01	35.85
K5-2	8	520	7.09	11.3	0.50	0.44	0.00	1.20	0.80	0.64	<01	35.37
K6-1	10	457	7.45	11.4	0.50	0.23	0.12	0.05	0.53	0.37	<01	37.65
K6-1	15	472	7.14	11.4	0.30	0.51	0.39	0.03	0.57	0.41	<01	37.05
K6-2	5	502	7.08	10.8	3.80	0.04	0.00	1.28	0.61	0.45	<01	3216
K6-2	8	505	7.3	11.8	1.60	0.01	0.00	0.71	0.59	0.43	<01	34.70
K7-1	10	507	6.9	11.3	0.30	0.84	0.32	0.10	0.71	0.55	<01	34.37
K7-1	15	502	7.17	11.5	0.26	0.79	0.82	0.01	0.61	0.45	<01	34.70
K7-2	5	515	7.07	11	3.07	0.00	0.00	0.65	0.62	0.46	<01	31.61
K7-2	8	493	717	11.9	1.54	n/a	000	0.55	0.55	0.39	<01	34.36
K9-1	12	495	73	11	0.40	1.39	0.84	0.00	0.81	0.65	<01	39.31
K9-1	16	507	742	11.3	0.40	0.40	1.34	0.02	0.79	0.63	<01	39.30
Alte Aare	n/a	385	8	7.1	10.70	n/m	0.83	6.12	1.83	1.67	<0 1	36.03

[a]mBGS metres below ground surface
[b]DOC corrected for filter effect. n/m, Not measured

bacteriophage, still-virulent H40/1 adsorbed to the sand could be released. Flushing 30 minutes after virus injection indicated that all adsorbed phages (with a 25% margin of error) were recovered in the column effluent. This result indicated that, in the short term, adsorption was the dominant attenuation mechanism removing H40/1 from suspension, and that inactivation was not significant. Flushing of the Kappelen sands with Tryptone 36 hours after bacteriophage injection demonstrated that H40/1 could be released from aquifer surfaces after prolonged periods. However, mass balance calculations indicated a deficit in the number of bacteriophages recovered, even when inactivation rates of suspended H40/1 were taken into account. These data suggest that H40/1's inactivation rate, while adsorbed to the Kappelen sands, was greater than in suspension.

The results of the study are consistent with those of Blanc and Nassar (1996) who reported accelerated inactivation of the bacteriophage MS-2 when it was adsorbed to loamy sand. In contrast, studies by Grant and others (1993) indicated that inactivation rates of the bacteriophage λ, were lower when suspended in liquid than while adsorbed to Ottawa sand. Rossi (1994) studied the inactivation of six different bacteriophages on three different clay minerals and observed that relative inactivation rates for suspended and adsorbed bacteriophages depended both on the bacteriophage type and the adsorbing mineral surface.

The results of statistical analyses of the mineralogy of the different grain size fractions of the samples collected from boreholes drilled at Kappelen suggest that although the relative abundance of different mineral types varies between samples, there was no evidence to suggest statistically significant differences in aquifer composition between boreholes, or between the shallower and deeper parts of the underlying aquifer.

Similarly, hydrochemical analyses of water samples collected from across the site failed to reveal differences in major-ion hydrochemistry, pH or organic carbon content in the groundwater either with depth or between sampling locations. In contrast, these analyses did display notable differences in redox potential between the shallower and deeper parts of the aquifer, and between the eastern and western sides of the site to a lesser degree.

The results of bacteriophage tracer testing in the shallower and deeper parts of the aquifer indicate that H40/1 inactivation is occurring while adsorbed to mineral surfaces in both parts of the aquifer. Inactivation rates at both levels are comparable, but are approximately twice those calculated in laboratory based column tests. Yates and others (1987) demonstrated the importance of temperature in accelerating virus inactivation processes. These differences are thus suspected to be a result of the higher temperature at which the laboratory experiments were carried out

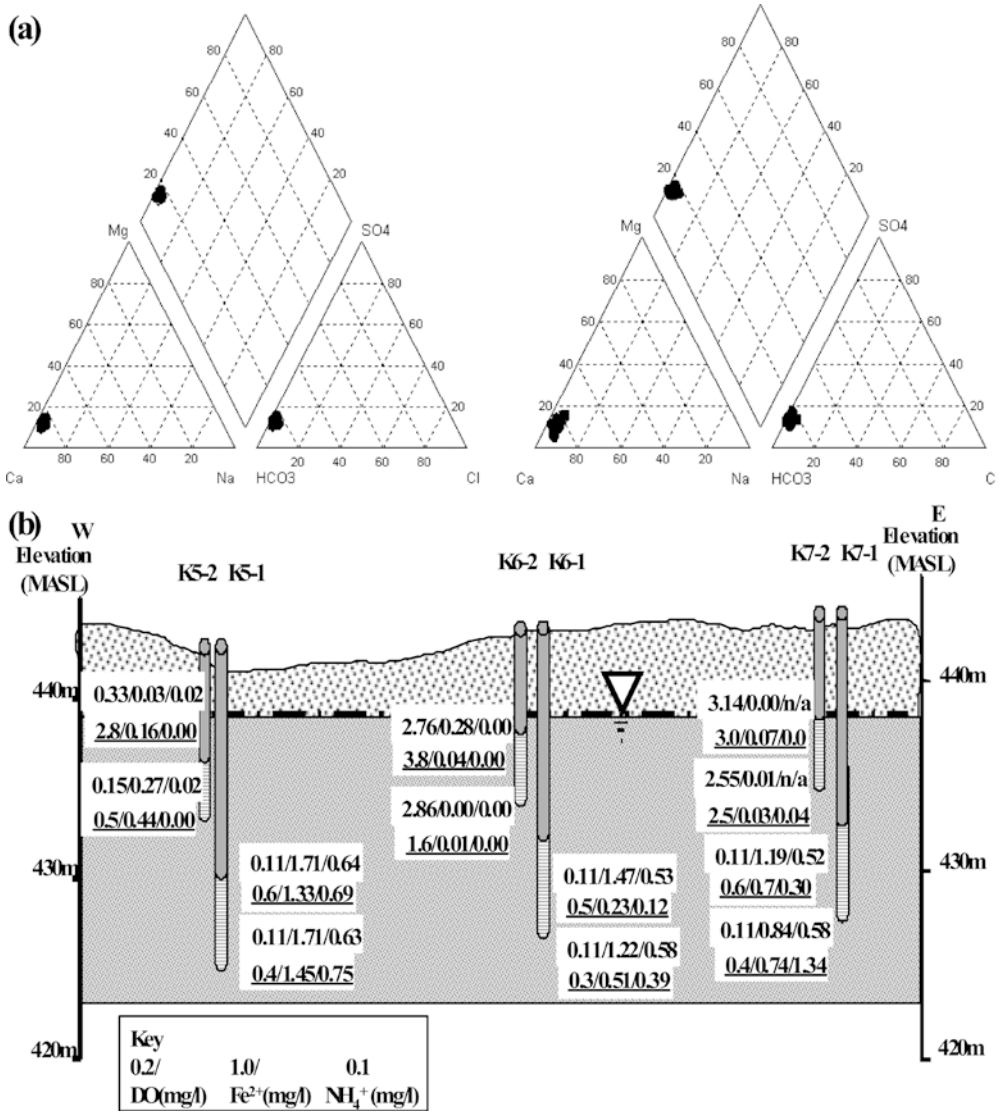

Fig. 6a,b
a Piper plots of major ion chemistry in groundwater samples from Kappelen. June 2001 - *Left*, January/February 2002 - *Right*. The similarity of analyses from shallow and deep parts of the aquifer means that water samples cannot be distinguished on the basis of major ion hydrochemistry. **b** East-west hydrochemical cross-section with representative redox indicators. June 2001 (*not underlined*), January/February 2002 - *underlined*

(21–23°C) relative to the ambient temperature of groundwater in the aquifer underlying Kappelen.
The comparable inactivation rates of adsorbed H40/1 in the upper and lower parts of the aquifer underlying Kappelen, coupled with the absence of mineralogical differences in the aquifer suggest that the differences in redox potential observed with depth did not significantly influence H40/1 inactivation rates.
The results of this study demonstrate the potential benefits of employing multiple investigative techniques in order to understand virus inactivation processes. Laboratory based column studies have been used to identify the importance of virus adsorption and inactivation of adsorbed viruses in the disinfection capacity of the deposits underlying Kappelen. Moreover, field-based studies have indicated similar processes operate in situ in the aquifer, albeit at lower rates. Mineralogical analyses have proved useful in removing potential ambiguities concerning the influence of spatial mineralogical variability in the aquifer on H40/1s inactivation rate. Similarly, hydrochemical analyses coupled with the results of the tracer tests have demonstrated that despite differences in redox potential of the water in the shallower and deeper parts of the aquifer underlying Kappelen, hydrochemical variations at the site do not appear to influence H40/1's inactivation rate.

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