Effect of pH on bacteriophage transport through sandy soils

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ABSTRACT


Effects of pH and hydrophobicity on attachment and detachment of PRD-1 and MS-2 in three different sandy soils were investigated in a series of laboratory-column experiments. Concentrations of the lipid-containing phage PRD-1 decreased 3–4 orders of magnitude during passage through the 10–15-cm-long columns. Attachment of the lipid-containing phage PRD-1 was insensitive to pH and was apparently controlled by hydrophobic interactions in soil media. The less-hydrophobic phage MS-2 acted conservatively; it was not removed in the columns at pH’s 5.7–8.0. The sticking efficiency (ζ) in a colloid-filtration model was between 0.1 and 1 for PRD-1, indicating a relatively high removal efficiency. Phage attachment was reversible, but detachment under steady-state conditions was slow. An increase in pH had a moderate effect on enhancing detachment. Still, these soils should continue to release phage to virus-free water for days to weeks following exposure to virus-containing water. In sandy soils with a mass-fraction organic carbon as low as a few hundredths of a percent, pH changes in the range 5.7–8.0 should have little effect on retention of more-hydrophobic virus (e.g., PRD-1), in that retardation will be dominated by hydrophobic effects. Sharp increases in pH should enhance detachment and transport of virus previously deposited on soil grains. A more hydrophilic virus (e.g., MS-2) will transport as a conservative tracer in low-carbon sandy soil.

1. INTRODUCTION

The occurrence of human enteric viruses in groundwater near-surface waste discharges and septic tanks has been well documented (Keswick and Gerba,
Such studies indicate that viruses can travel distances over 1 km under certain hydrological conditions (i.e. fractured and karst media). The fate of viruses in soil and groundwater is governed by attachment or release from immobile hydrological substrates and by inactivation (Yates et al., 1987). Three chemical variables — groundwater pH, ionic strength (I) and mass-fraction organic carbon of the soil (f_{oc}) — have been observed to control bacteriophage transport vs. attenuation in laboratory columns packed with silica beads (Bales et al., 1991, 1993). Lower pH, higher I and higher f_{oc} all enhance attachment and thus retard transport. Changes in pH and ionic strength also result in significant release of attached phage (Bales et al., 1991). Lance and Gerba (1984) reported greater poliovirus transport through laboratory soil columns with distilled water than with tap water; the pH ranged from 6.4 to 6.9. Gerba et al. (1981) found that attachment of some strains of virus were not sensitive to pH and mass-fraction organic carbon.

The research described in this paper is part of our ongoing studies of virus transport in porous media. The main objectives of this study were to examine the importance of pH to the attachment and detachment of viruses to sandy soils, and to compare these to prior model-surface results. We use bacteriophage as laboratory and field tracers of human-virus transport owing to the lack of infection risk, shorter assay times and commensurate lower cost (Gerba, 1985; Yates et al., 1985; Bales et al., 1989).

2. MATERIALS AND METHODS

Eight continuous-flow column experiments were conducted to obtain breakthrough curves of bacteriophage PRD-1 and MS-2 through sandy soil at pH's 5.7–8.2 (Table 1). Experiments were done at 7±3°C in order to minimize viral inactivation. Experiments consisted of pumping stock solutions of phage in a calcium phosphate buffer and phage-free buffer through 14.8 × 2.7- or 10.6 × 2.7-cm glass chromatography columns (Spectrum Medical Industries, Inc., Los Angeles, California, U.S.A.). Columns were packed by the tap-and-fill method (Snyder and Kirkland, 1979) and the weight of media recorded to estimate dry bulk density. Columns were packed with new soil for each experiment. Column fittings were Teflon®, and Teflon® tubing was used everywhere in the system except for a length of Tygon® tubing in the pump. In order to take column-inlet samples throughout an experiment without disrupting the flow, a parallel feed tubing was set up. Feed-reservoir bacteriophage titer remained constant during an experiment, within analytical uncertainty.
### TABLE 1

Experimental conditions and results

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<th>L (cm)</th>
<th>$u$ ($10^{-1}$ cm s$^{-1}$)</th>
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<th>$\theta$</th>
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2.1. **Bacteriophage**

Bacteriophage PRD-1 is an icosahedral (twenty triangular faces) lipid phage with an average diameter of 62 nm (Olsen et al., 1974). Its isoelectric point in calcium phosphate buffer (10^{-4} \text{ M} \text{ Ca}) is between pH 3 and 4 (Bales et al., 1991). Bacteriophage MS-2 is an icosahedral phage with an average diameter of 23 nm, with isoelectric point near pH 3 (Zerda, 1982). MS-2 is less hydrophobic than PRD-1, since its protein coat does not contain lipids. Both phage were enumerated by the plaque-forming method (pfu), as described previously (Bales et al., 1991). Because of a low potential for aggregation each pfu represents a physical viral particle (Sharpe, 1965). The precision of the phage assay in the range of plaques typically counted per sample (30–150) is ±20% (ASTM, 1991).

2.2. **Soils**

Soils used in this study came from three research sites, one in Borden, Ontario, Canada, one in Cambridge, Ontario, Canada, and one in Cape Cod, Massachusetts, U.S.A. The Borden site is located within the confines of the Canadian Forces Base, Borden, ~80 km northwest of Toronto, Ontario. The unconfined Borden aquifer is comprised of glacio-fluvial sand, which is common in the glaciated region of North America. The aquifer is locally very heterogeneous due to complex distributions of beds and lenses of fine-, medium- and coarse-grained sand. Thickness of the aquifer varies from 15 to 30 m, underlain by a clayey silt bed (MacFarlane et al., 1983). Groundwater velocity averages ~0.09 m day^{-1}; hydraulic conductivity averages 6.2 m day^{-1}, but varies over more than 2 orders of magnitude (Lemon et al., 1989). The dry bulk soil density is 1.81 g cm^{-3}, with a solids density of 2.71 g cm^{-3} and specific surface area of 0.8 m^{2} g^{-1} (Mackay et al., 1986). Organic-carbon content (0.03%) and cation-exchange capacity (0.5 meq g^{-1}) are low, with a clay-size fraction near zero (reported as 0–15%).

The Cambridge site is located near Cambridge, Ontario, at an agricultural-research station. The aquifer consists of glacio-lacustrine and outwash sand to a depth of 4–8 m, and is overlain by a silt till of low permeability (Robertson et al., 1991). Near the ground surface, the soil horizons exhibit slight variations in permeability, but otherwise the aquifer is relatively homogeneous (Shutter et al., 1993). The aquifer is comprised of clean medium-to-coarse sand having an average hydraulic conductivity of 2.6 m day^{-1}; groundwater velocity is similar to that at Borden. The aquifer materials are 95% sand; organic-carbon content is low, ~0.05%.

At the Cape Cod, Massachusetts, site, the top 30–50 m of sediment are a glacial outwash composed of stratified, well-sorted, medium-to-coarse sand
with some gravel. In the northern part of the study area, the sand and gravel overlie fine sand and silt. To the south, the outwash overlies fine sand, silt and dense sandy till. The till contains lenses of silt and clay, and sand and gravel (Garabedian et al., 1989). Groundwater velocity is 5–10 times that at Borden. Dry bulk density of the soil is reported to be 1.72 g cm$^{-3}$, and porosity 0.35 (Harvey and Garabedian, 1991). The total dissolved solids concentration was low ($\sim 39$ mg L$^{-1}$) because the aquifer is sand and gravel, composed predominantly of quartz and some feldspar derived from crystalline bedrock. The alkalinity is also low because of the absence of carbonate minerals in the aquifer. The pH of uncontaminated groundwater in the study area typically is $< 6.0$ (LeBlanc, 1984). The mass-fraction organic carbon is $<0.01\%$.

2.3. Experimental methods

To begin an experiment, soil gas was displaced with CO$_2$ and the column flooded from the bottom at a low flow rate to minimize air entrapment. The pump was then attached to the top of the column and buffer at the same pH as the stock solution passed through the column for $\sim 5–8$ pore volumes (PV) prior to the experiment. It was found that effluent pH became the same as that of the buffer in the input reservoir after this process.

During an experiment, solution was sent through the columns at 0.4–1 PV h$^{-1}$. Samples for replicate assays were collected for 4 min at least every hour. Flow rate was measured at the column outlet throughout the experiment. The fluctuations in flow rates for experiments 6 and 8 were $<12\%$, and for the other experiments $<6\%$. Switching from one pump channel to the other caused only a slight change. Measured flow rates were used to calculate the pore volumes of solution that had passed through the column. To verify the buffering, effluent pH was periodically checked.

As a check on bulk density measurements, column pore volume was calculated from breakthrough of a conservative tracer. A column was flooded with 0.1 M NaCl solution until the outlet conductivity reading became stable. Then 0.2 M NaCl was pumped into the column for $\sim 2$ PV, and finally switched back to 0.1 M NaCl. Outlet conductivity was monitored with a Wescan" model 213a conductivity detector (Wescan Instruments, Inc., Santa Clara, California, U.S.A.) connected to a strip-chart recorder. A salt-tracer experiment was done prior to each virus experiment. Beef extract (2.5% in weight) was used to flush attached phage out of the columns in four experiments.

3. RESULTS

Column-experiment results are presented as breakthrough curves in Figs. 1–5, with column outlet concentration ($C$) divided by inlet concentration ($C_i$)
plotted on a log scale to more easily see order-of-magnitude changes. The horizontal axis, pore volumes, is time since beginning the virus input divided by the residence time of water in the column. Horizontal dotted lines show the inlet phage concentration \( (C_0) \) and the detection limit of the phage assay (10 pfu mL\(^{-1}\)). The first vertical dotted line in Figs. 1-4 shows the point at which the input solution was switched to a phage-free buffer. The second vertical dotted line is the time at which the buffer was switched to one with a higher pH and/or beef extract added.

3.1. Borden soil

The breakthrough curves for experiments 1–3, with Borden soils, showed a steep rise in outlet phage concentration after \( \sim 1 \) PV of phage-containing solution had passed through the column, followed by a steady-state value (0.006–0.04% of \( C_0 \)) until the feed solution was switched to a phage-free solution. In experiments 1 and 2, \( C/C_0 \) declined to \( \sim 1-10\% \) of the previous steady-state value 1 PV after the feed was switched; there was no decline in experiment 3. Subsequent addition of beef extract, a high-ionic-strength protein solution, resulted in significant detachment, with outlet concentrations higher than the stock titer in all cases. The main purpose of adding the beef extract was to verify that the attached phage were still viable, and would remain viable upon detachment. In general, detachment rates were small relative to rates of phage collection by soil particles; experiments were not run long enough to get complete recovery of attached phage.

Buffer solution was sent through the columns at 60–150 min PV\(^{-1}\), which is \( \sim 1.4-2.7 \) m day\(^{-1}\). This velocity was 13–24 times that observed at the Borden site (mean of 0.09 m day\(^{-1}\)) (Lemon et al., 1989). The dry bulk densities of the soil packed in the column were 1.69–1.70 g cm\(^{-3}\), which is lower than the 1.81 ± 0.05 reported for 36 core samples (Mackay et al., 1986). Since the soil consisted of fine to coarse sand and the virus size was small, removal of phage by straining should be negligible.

In experiment 1 at pH 6.5 (Fig. 1), the steady-state \( C/C_0 \) was \( \sim 1.1 \) \( \times \) 10\(^{-4}\), with phage first detected after 1.1 PV had passed through the column. Upon switching the inlet feed to a phage-free buffer, \( C/C_0 \) dropped to \( \sim 0.7 \) \( \times \) 10\(^{-6}\). The attachment was slowly reversible with non-zero phage concentrations in the column outlet after switching to the phage-free buffer. The detachment rate decreased to below the detection limit by the time \( \sim 10 \) PV of phage-free buffer had passed.

In experiment 2 at pH 7.0 (Fig. 1), the steady-state \( C/C_0 \) was \( \sim 1.1 \) \( \times \) 10\(^{-4}\) — slightly higher than at pH 6.5. The phage were first detected after 0.9 PV had passed through the column. \( C/C_0 \) dropped to 2 \( \times \) 10\(^{-6}\) at 1.3 PV after switching to a phage-free buffer, then dropped to the detection limit (\( C/C_0 = \)
Fig. 1. Breakthrough curves of column experiment for PRD-1 through Borden soil: (a) pH 6.5 during attachment (att) and detachment (det) steps of experiment 1; (b) pH 7.0 during attachment and detachment steps of experiment 2; and (c) pH 7.6 during attachment and detachment steps of experiment 3. The second detachment step of each experiment involved addition of beef extract to the system (B.E.); pH-values given in parentheses. Vertical dotted lines indicate time at which experimental conditions were changed. Horizontal dotted line indicates detection limit.

$4 \cdot 10^{-8}$) 4.3 PV later. Addition of a pH-7.0 buffer containing beef extract resulted in a column-outlet pulse that was higher than the stock titer.
Fig. 2. Breakthrough curves of column experiment for PRD-1 through Cambridge soil at pH 7.0 during attachment (att) and detachment (det) steps of experiment 4. The second detachment step involved addition of beef extract. Dotted lines as in Fig. 1.

3.2. Cambridge soil

In experiment 3 at pH 7.6 (Fig. 1), the steady-state $C/C_0$ was $5.0 \cdot 10^{-4}$ — higher than at pH's 6.5 and 7.0. Phage in the column outlet were first detected after $\sim 0.9$ PV. The column-outlet concentration did not drop upon switching to a phage-free buffer, indicating that the detachment was faster than at pH's 6.5 and 7.0. Again, addition of beef extract to the pH-7.6 buffer resulted in a column-outlet pulse above the stock titer.

One experiment was conducted with Cambridge soil (Fig. 2), which is a sandy soil very similar to that from Borden. Dry bulk density of the soil packed into the column was 1.68 g cm$^{-3}$. Flow velocity was 2.5 m day$^{-1}$, which is $\sim 30$ times that observed at the Cambridge site (average 0.08 m day$^{-1}$) (Robertson et al., 1991). The initial sharp peak followed by a small decline in $C/C_0$ was caused by a drop in $C_0$ $\sim 1$ PV after the beginning of the experiment. The later $C_0$ is used for analysis, and the steady-state $C/C_0$ of $\sim 2.0 \cdot 10^{-4}$ was estimated from the later times. This value is slightly higher than that of the Borden soil ($C/C_0 = 1.1 \cdot 10^{-4}$). Phage were first detected after $\sim 0.6$ PV had passed through the column. $C/C_0$ dropped to $\sim 1.1 \cdot 10^{-5}$ upon switching to a phage-free buffer, a smaller decrease than observed with the Borden soil. Introducing the pH-7.0 buffer with beef extract raised the effluent phage concentration to a value 10 times that of the stock solution titer.

3.3. Cape Cod soil

The results for the Cape Cod soil are shown in Figs. 3-4. The dry bulk densities of the soil packed in the column were 1.68–1.71 g cm$^{-3}$, essentially the same as the reported value of 1.72 g cm$^{-3}$ (Harvey and Garabedian, 1991). Flow velocity was 2.0–2.6 m day$^{-1}$, which is $\sim 5$ times that in the field.
Fig. 3. Breakthrough curves of column experiment for PRD-1 and MS-2 through Cape Cod soil at pH 5.7; during attachment and detachment steps of: (a) PRD-1 and (b) MS-2 in experiment 5; and (c) PRD-1 and MS-2 in experiment 6. Dotted lines as in Fig. 1.

Fig. 4. Breakthrough curves of column experiment for PRD-1 and MS-2 through Cape Cod soil at pH 7.0: (a) PRD-1; and (b) MS-2. Dotted lines as in Fig. 1.
The breakthrough curves showed a steep rise after ~1 PV of water had passed. When switched to a virus-free solution, the steady-state attachment was followed by a slight (PRD-1) or steep (MS-2) decline of the detachment limb. The increase of pH caused a rapid detachment, but to different degrees for PRD-1 and MS-2. PRD-1 was attached to soil in larger amounts than was MS-2.

In experiment 5, the steady-state $C/C_0$-values for PRD-1 and MS-2 were $0.7 \cdot 10^{-3}$ and ~1.5, respectively (Fig. 3a and b). It is thought that the high MS-2 values were due to errors in the assay of the stock concentration ($C_0$), but the problem was detected too late to run additional assays. Both PRD-1 and MS-2 were first detected after ~0.7 PV. MS-2 concentration dropped to 1% of the steady-state value upon switching to a phage-free buffer; PRD-1 dropped only slightly following the switch. Upon increasing solution pH to 8.2, $C/C_0$ for PRD-1 rose to $4.2 \cdot 10^{-2}$, ~60 times the steady-state value. MS-2 did not respond to the increase of the pH from 5.7 to 8.2, probably because few were retained in the column.

Experiment 6 (Fig. 3c and d), was a replication of experiment 5, and exhibited the same trends. Steady-state $C/C_0$'s for PRD-1 and MS-2 were $0.4 \cdot 10^{-3}$ and 1.0, respectively. PRD-1 was first detected after 0.8 PV, vs. 0.6 PV for MS-2. Upon switching to a phage-free buffer, $C/C_0$ for PRD-1 dropped to $\leq 3 \cdot 10^{-5}$, which may represent a greater drop than in experiment 6. $C/C_0$ for MS-2 also declined, to ~1.5% of the previous inlet level. Raising the pH to 8.2 again resulted in higher column-outlet concentrations.

In experiment 7 at pH 7.0, steady-state $C/C_0$'s for PRD-1 and MS-2 were ~$0.3 \cdot 10^{-3}$ and ~0.8, respectively (Fig. 4). Trends for both breakthrough curves were qualitatively similar to those of experiments 5 and 6. There was little immediate change in $C/C_0$ for PRD-1 upon switching to phage-free buffer, and a rise to a $C/C_0$ ten times the previous value upon raising the pH to 8.2. MS-2 showed a more-pronounced drop following the switch to phage-free buffer, but was insensitive to the higher pH; again, little MS-2 was retained in the column.

In experiment 8, three different pH's of phage stock (pH 5.7, 7.0 and 8.2) were serially sent to the column, followed by a pH-8.2 phage-free buffer. The resulting breakthrough curves (Fig. 5) were consistent with previous experiments. The steady-state $C/C_0$ for PRD-1 increased slightly in going from pH 5.7 to 8.2; pH change and a phage-free buffer at pH 8.2 eluted the phage slowly. MS-2 acted conservatively in this range of pH's.
4. DISCUSSION

4.1. pH and hydrophobic effects

The rate of PRD-1 removal in Borden soil showed less retention at the higher pH, suggesting a weak electrostatic effect. The steady-state $C/C_0$ at pH 7.0 (1.1·10^{-4}) was only 57% greater than that at pH 6.5 (0.7·10^{-4}). This difference is less than that observed in the replicate experiments 5 and 6, and is not significant. At pH 7.6, $C/C_0$ was 5.0·10^{-4}, which is significantly greater than the pH 6.5 and 7.0 values. Both the soil and PRD-1 should be negatively charged at these natural-water pH's. It was previously observed that attachment of PRD-1 to silica beads was pH dependent, with more removal at pH 5.5 than at 7.0 (Bales et al., 1991). There was also a weak trend of decreasing concentration drop (increasing detachment rate) at higher pH upon switching to a phage-free buffer; ~2 orders of magnitude drop for pH lower than 7.0 and a slight drop for pH 7.6, also consistent with electrostatic effects.

But the large amount of phage attachment to soil at even the higher pH's and the relative insensitivity to pH suggests that hydrophobic effects were more important than were electrostatic effects. It has been observed that PRD-1 retention in columns of bonded-silica, i.e. partly hydrophobic surfaces, depends strongly on the mass-fraction of organic carbon on the beads (Bales et al., 1993). The fact that these soils have measurable organic-carbon contents (~0.03%) suggests that hydrophobic interactions dominated phage attachment. This is further supported by the fact that the surface of PRD-1 has lipid in its protein coat and is hydrophobic. Van Loosdrecht et al. (1987) found that electrostatic interactions were relatively unimportant for adhesion of hydrophobic bacteria to glass, but were dominant for hydrophilic bacteria.
On the other hand, the organic-carbon content of the Borden soil is relatively low. The Borden and Cambridge soils are \(\sim 60\%\) SiO\(_2\), and the remainder other minerals. Although the organic carbon content is low, it apparently offered enough hydrophobic surface to retain the relatively low phage loadings applied. A dry bulk density of 1.7 g cm\(^{-3}\) and a specific surface area of 0.8 m\(^2\) g\(^{-1}\) give an available surface area for adsorption of 1.4 \(\cdot\) 10\(^{17}\) nm\(^2\)/cm\(^3\) water. Using a cross-sectional area for PRD-1 of 3 \(\cdot\) 10\(^5\) nm\(^2\) and a stock solution concentration of 10\(^5\) pfu mL\(^{-1}\) gives a covered area of 3 \(\cdot\) 10\(^8\) nm\(^2\)/cm\(^3\) water which is far smaller. An organic-carbon content of 0.03\% (Borden soil) is sufficient for all of the surface to be organic coated assuming that molecules of 1,000–10,000 molecular weight are uniformly distributed over the surface of soil grains. A patchy coating would result in less than 100\% coverage.

The large attachment of PRD-1 in Cambridge soil at pH 7.0 suggests that hydrophobic interactions dominated in this soil material as well. Again, the organic-carbon content of the soil was low (0.05\%).

PRD-1 in Cape Cod soil also showed strong hydrophobic interactions, though the organic-carbon content is still lower (\(<\) 0.01\%). In this soil, attachment of PRD-1 was only weakly pH dependent. Also, PRD-1 removal was less in Borden soil than in Cape Cod soil (\(C/C_0\) at steady state at pH 7.0 of 1.1 \(\cdot\) 10\(^{-3}\) vs. 0.3 \(\cdot\) 10\(^{-3}\)). Borden soil had a more variable and thus possibly higher clay-size fraction (0–15 vs. \(<\) 1\%), offering more inorganic surface area and smaller average grain size as well as a higher organic-carbon content.

MS-2 in Cape Cod soil was apparently conservative, even at low pH. Apparently double-layer repulsion dominated for MS-2 in this medium, and the hydrophobic effect was less important. However, Bales et al. (1993) have reported the hydrophobic attachment of MS-2 to bonded (hydrophobic) silica beads with mass-fraction organic carbon of 0.005\%. Therefore, it is expected that hydrophobic interactions for MS-2 could dominate at higher organic-carbon contents. Since MS-2 is relatively hydrophilic, the hydrophobicity may be a minor effect in the Cape Cod and Borden soils.

4.2. Sticking efficiency

A transport and colloid-filtration model using a single-collector removal efficiency (\(\eta\)) and sticking efficiency (\(\alpha\)) to interpret the rate of bacteria removal in soil was applied to modeling bacterial movement at a field site on Cape Code by Harvey and Garabedian (1991) and to laboratory column experiments by Martin et al. (1992). A similar approach has been used to describe bacteriophage transport through laboratory columns packed with silica beads (Bales et al., 1991, 1993). In the steady-state filtration case, the
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change in concentration with distance can be expressed as:

\[ u \cdot \frac{dC}{dx} = -k_1 C \]  

(1)

where \( u \) is velocity and \( k_1 \) is a filtration coefficient, i.e. a pseudo-first-order rate coefficient for colloid removal. Using a single-collector model and assuming spherical collectors:

\[ k_1 = \frac{3}{2} \left[ (1 - \theta)/d \right] \eta \alpha C \]

where \( \theta \) is porosity; \( d \) is the collector diameter; \( \eta \) is the single-collector collision efficiency; and \( \alpha \) is the fraction of colloid-collector collisions that result in sticking (Bales et al., 1991). Virus-sized colloidal particles are transported from the moving fluid to collector surfaces by Brownian diffusion, with:

\[ \eta = 0.9 A_s^{1/3} \left[ \frac{k}{\mu d_p d_L} \right]^{2/3} \]

where \( \mu \) is water viscosity; \( k \) is the Boltzmann constant; \( T \) is temperature; \( A_s \) is a parameter that accounts for the effects of adjacent media grains on the flow about a collector; and \( d_p \) is the phage diameter (Tien and Payatakes, 1979; O'Melia, 1985). For a spherical collector, \( A_s \) has been given as:

\[ A_s = (1 - \epsilon^5)/(1 - 1.5\epsilon + 1.5\epsilon^5 - \epsilon^6) \]

where \( \epsilon = (1 - \theta)^{1/3} \). Average \( A_s \)-values for the Borden, Cambridge and Cape Cod soils were 90.7, 75.5 and 51.5, respectively.

Substitution of \( \eta \) into Eq. 1 for \( k_1 \) and integration gives:

\[ \log \left( \frac{C}{C_0} \right) = -\frac{1}{2.3032} \frac{31 - \theta}{d} \eta \alpha L \]

(2)

where \( L \) is column length.

The following values were used in estimating \( \eta \): \( k = 1.4 \cdot 10^{-16} \) g cm\(^{-2}\) s\(^{-1}\); \( T = 280 \) K; and \( \mu = 0.014 \) g cm s\(^{-1}\) (7°C). The viral diameters \( (d_p) \) are 6.2 \( \cdot \) 10\(^{-6}\) cm (PRD-I), 2.3 \( \cdot \) 10\(^{-6}\) cm (MS-2). The mean diameters of media are 0.038 cm (Borden soil), 0.103 cm (Cambridge soil) and 0.059 cm (Cape Cod soil). The average interstitial velocities and the estimated collision (\( \eta \)) and sticking (\( \alpha \)) efficiencies for each experiment are listed in Table 1. PRD-I, which exhibited 3–4 order-of-magnitude drops in concentration in the 10–15-cm columns, had \( \alpha \)-values in the range of 0.1–0.2 for Borden, near one for Cambridge and in the range 0.6–0.9 for Cape Cod. There was no apparent pH or \( f_{oc} \) dependence for \( \alpha \). The value of 1.11 for Cambridge is not statistically different from 1.0. For MS-2, some \( \alpha \)-values were below what could be measured in these experiments. However, we can say that the values are at most 1–3\% of those for PRD-I on the same soil.

Harvey and Garabedian (1991) report \( \alpha \)'s of 0.005–0.01 for bacteria (0.2–1.4 \( \mu \)m) transport in the Cape Cod field site, which are in the same range as those we estimated for MS-2 (Table 1). Values of 0.001–0.01 were also reported for phage and silica beads by Bales et al. (1991, 1993). But \( f_{oc} \) in the
experiments with silica beads was much lower, $\leq 5 \cdot 10^{-6}$. So the higher $z$-values in these soil experiments are consistent with those from model systems. That is, transport in soil with even low organic-carbon contents is less favorable than in silica beads.

5. CONCLUSIONS

In sandy soils with a low mass-fraction organic carbon ($< 0.1\%$), hydrophobic effects still exceeded electrostatic repulsion in causing retention of a lipid-containing phage, PRD-1, in the soils. On the other hand, transport of MS-2, which has no lipid in its structure and is less hydrophobic than PRD-1, was not retained on the lowest-organic content soil (from Cape Cod); it showed nearly conservative behavior. Apparently, higher organic-carbon contents are needed to retard MS-2 transport. Phage retention was relatively insensitive to pH over the range 5.7–8.0 for the soils studied. Phage attachment was reversible, but detachment under steady-state conditions was slow. An increase in pH had a moderate effect on enhancing detachment. The lack of pH dependence for attachment, apparent importance of hydrophobic effects and slow detachment were consistent with previous observations on model systems, i.e. silica beads with organic carbon added. Once exposed to virus-containing water, these soils should adsorb the phage and could subsequently release phage to virus-free water for several days to weeks.

The quite different behavior of these two phage demonstrates that no single virus will suffice to indicate virus transport or retardation in low-organic-carbon sandy soils. Lipid-containing virus, with a more hydrophobic outer coat, should be retained, but can detach and re-enter moving groundwater upon introduction of higher-pH water. Such detachment in response to chemical perturbations could result in quite high virus concentrations in the water. Less-hydrophobic (more hydrophilic) virus should move with little retardation, and generally be transported further than lipid-containing phage. The more-hydrophobic virus would be a more-continuous threat to drinking-water wells sited near virus sources; whereas more-hydrophilic virus could pose a more-severe problem due to higher concentrations immediately following perturbations in groundwater quality. Inactivation will also be important, but this is type and strain dependent, and not related to the ability of the virus to attach to surfaces.

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