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Influence of water chemistry and travel distance on bacteriophage PRD-1 transport in a sandy aquifer

William J. Blanford[a,*], Mark L. Brusseau[b,c], T.C. Jim Yeh[b,c], Charles P. Gerba[c], Ronald Harvey[d]

[a]*Department of Geology and Geophysics, Louisiana State University, Baton Rouge, LA 70803, USA*
[b]*Hydrology and Water Resources Department, University of Arizona, Tucson, AZ 85721, USA*
[c]*Soil, Water, and Environmental Science Department, University of Arizona, Tucson, AZ 85721, USA*
[d]*United States Geological Survey Boulder, CO 80303, USA*

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Abstract

Experiments were conducted to evaluate the impact of groundwater chemistry and travel distance on the transport and fate behavior of PRD-1, a bacteriophage employed as a surrogate tracer for pathogenic enteric viruses. The experiments were conducted in the unconfined aquifer at the United States Geological Survey Cape Cod Toxic-Substances Hydrology Research Site in Falmouth, Massachusetts. The transport behavior of bromide (Br^-) and PRD-1 were evaluated in a sewage-effluent contaminated zone and a shallower uncontaminated zone at this site. Several multilevel sampling devices located along a 13-m transect were used to collect vertically discrete samples to examine longitudinal and vertical variability of PRD-1 retardation and attenuation. The concentration of viable bacteriophage in the aqueous phase decreased greatly during the first few meters of transport. This decrease is attributed to a combination of colloid filtration (attachment) and inactivation. The removal was greater (10^{-12} relative recovery) and occurred within the first meter for the uncontaminated zone, whereas it was lesser (10^{-9} relative recovery) and occurred over 4 m in the contaminated zone. The lesser removal observed for the contaminated zone is attributed to the influence of sorbed and dissolved organic matter, phosphate, and other anions, which are present in higher concentrations in the contaminated zone, on PRD-1 attachment. After the initial decrease, the aqueous PRD-1 concentrations remained essentially constant in both zones for the remainder of the tests (total travel distances of 13 m), irrespective of variations in geochemical properties within and between the two zones. The viable, mobile PRD-1 particles traveled at nearly the rate of bromide, which was used as a non-reactive tracer. The results of this study indicate that a small fraction of viable virus particles may persist in the aqueous phase and travel significant distances in the subsurface environment.
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Keywords: Virus; Transport; Groundwater

1. Introduction

Enteric virus contamination of groundwater used for potable water is of concern (e.g., U.S.E.P.A., 2000). This concern has fomented interest in the transport and fate behavior of viruses in the subsurface environment. The

*Corresponding author. Tel.: +1 5206213244.
E-mail address: brusseau@ag.arizona.edu (M.L. Brusseau).

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factors controlling virus transport within the subsurface environment include advection, dispersion, attachment to and detachment from porous-medium surfaces, and inactivation. The results of previous work indicate that physical/chemical properties (e.g., porous-medium texture and composition, pH, temperature, total dissolved solids, ion composition, and dissolved organic matter) can influence the impact of these factors on the transport and fate of viruses. For example, attachment/detachment of viruses is sensitive to groundwater chemistry, especially hydrogen ion concentration (e.g., Goyal and Gerba, 1979; Shields and Farrah, 1987; Bales et al., 1991; Grant et al., 1993; Kinoshita et al., 1993; Loveland et al., 1996). In addition, ionic strength (Grant et al., 1993) and dissolved organic matter (DOM) (Sobsey et al., 1980; Gerba, 1984; Bales et al., 1993; Pieper et al., 1997; Ryan et al. 1999) have been shown to influence virus retention. Several field- scale virus transport studies have been conducted under well-characterized conditions, as reviewed by Schijven and Hassanizadeh (2000). Because of the hazards and costs associated with using human viruses in field experiments, bacteriophage such as PRD-1 are often employed as analogues to viruses of concern to human health.

Several biocolloid transport studies have been conducted at the United States Geological Survey (USGS) Cape Cod Toxic-Substances Hydrology Research Site in Falmouth, MA. A portion of the subsurface at this site is contaminated by a plume of treated sewage effluent. A zone of uncontaminated groundwater resides above the contaminant plume. Chemical properties such as pH, temperature, ionic composition, and sorbed/dissolved organic matter concentrations vary between the two zones. The results of a series of smaller-scale PRD-1 transport experiments conducted at the Cape Cod site show that retention and transport of PRD-1 is influenced by pH and sorbed/dissolved organic matter (Pieper et al., 1997; Ryan et al., 1999). Additionally, Bales et al. (1995) and Ryan et al. (1999) demonstrated that attached bacteriophage could be remobilized by changes in water chemistry (e.g., pH, surfactant concentration). Ryan et al. (1999) measured the surface-charge characteristics of PRD-1 and aquifer material from the contaminated and uncontaminated zones of the site. They found that zeta potentials of PRD-1 did not differ significantly in groundwater from the contaminated and uncontaminated zones. However, the aquifer material from the contaminated zone had fewer available electrostatic sorption sites, due primarily to higher concentrations of organic matter sorbed to the iron oxyhydroxides coating the grain surfaces.

The results of the aforementioned Cape Cod experiments provide insight into retention and transport of PRD-1 in an aquifer system composed of unconsolidated sediments, and the influence of water chemistry on these processes. The objective of the current study was to quantitatively investigate the transport behavior of PRD-1 over longer temporal and spatial scales than those associated with the prior studies. Several multi-level sampling devices located along a 13-m transect were used to collect vertically discrete samples to examine longitudinal and vertical variability of PRD-1 retardation and attenuation. Natural-gradient tests were conducted in both the contaminated and uncontaminated zones of the Cape Cod site to further examine the impact of water chemistry on PRD-1 transport.

2. Methods

2.1. Site description

The site has a shallow aquifer that varies in thickness between 90 and 100 m, and consists primarily of a sandy glacial outwash deposit. The water table is 3–7 m below land surface in the study area. The horizontal hydraulic conductivity of the sand and gravel zone is estimated at 60 to 120 m per day (Leblanc, 1984; Garabedian et al., 1988). The fine sand and sandy till is postulated to be one-tenth as conductive as the sand and gravel (Leblanc, 1984). The water table is unconfined and slopes south to southwest at about 1.5 m/km (Garabedian, 1988). The groundwater flows horizontally with a velocity in the range of 0.2–0.7 m/day in the sand and gravel. The porosity is reported to be between 0.2 and 0.4 (Leblanc, 1984).

Portions of the aquifer are contaminated by sewage emanating from sewage infiltration beds ~0.5 km up-gradient of the experiment study site (Leblanc, 1984; Harvey and Barber, 1992). In the vicinity of the study site, the sewage-effluent plume is overlain by up to 2 m of uncontaminated water originating primarily from infiltration of local precipitation. The sewage-effluent plume is characterized by elevated levels of pH (6.0 versus 5.6), total dissolved solids, chloride, bicarbonate, sulphate, phosphorous, ammonium, nitrate, and dissolved organic matter (DOM), and by lower levels of dissolved oxygen (Leblanc, 1984; Ryan et al., 1999).

The site is instrumented with an array of multi-level sampling (MLS) devices. Sampling ports protrude out of the side of each device in an up-gradient direction at approximately 25 cm intervals (Fig. 1). The sampling lines are polyethylene tubes with 0.47 cm inside diameter and 0.64 cm outside diameter, and the devices comprise PVC casing with a 3.17 cm diameter (Leblanc et al., 1991). Sampling ports located between elevations of 9.5 and 11.5 m above mean sea level are considered to reside in the sewage-contaminated zone, while ports located between elevations of 12–13.5 m are considered to reside in the uncontaminated zone. A more complete description of the site is found in Leblanc et al. (1984, 1991).

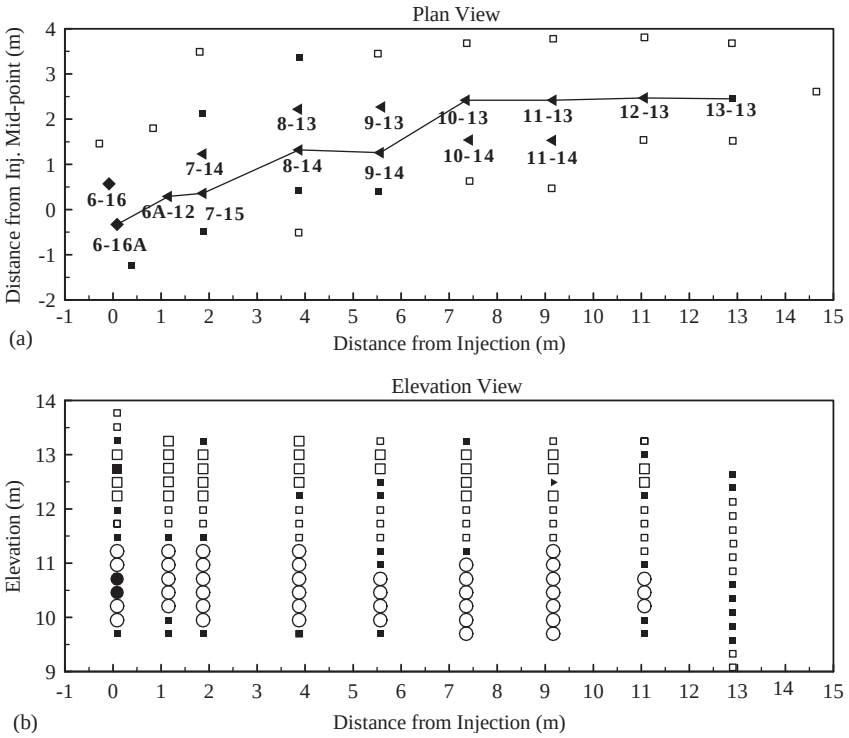

Fig. 1. Layout of multi-level sampling port at the field site. (a) plan view; (b) projection of ports along the cross-section line. Key describing tracer injection and recovery for (a): ♦ MLS where tracer solution was injected, ◄ MLS where a complete breakthrough curve was observed in at least one level, ■ MLS where incomplete breakthrough curves were observed, □ MLS where no tracer was observed, and—MLSs shown in the vertical cross-section. Key for (b): ● tracer injection ports for contaminated zone, ■ tracer injection ports for uncontaminated zone, ○ and □ sampling ports where complete breakthrough curves were observed, ■ sampling ports where incomplete breakthrough curves were observed, □ sampling ports where no tracer was observed, ► dysfunctional sampling port.

2.2. Materials

PRD-1 is an icosahedral (20 triangular faces) bacteriophage with an average diameter of 62 nm (Olsen et al., 1974). The isoelectric point, or point of zero net surface charge, is reported to be 4 (Bales et al., 1991; Dowd et al., 1998); it is slightly lower (3.2) in Cape Cod groundwater (Ryan et al., 1999). PRD-1 contains approximately 25 different proteins in its capsid (Bamford et al., 1995) and has an internal membrane, which may contribute to its observed stability in the environment (Harvey and Ryan, 2004). It shares a number of common traits with other viruses belonging to the Tectiviridae family of icosahedra-shaped, double-stranded DNA-containing bacteriophages and has been employed in recent subsurface injection and recovery studies at a dozen different sites in Ontario, Denmark, the Netherlands, and the US (Harvey and Ryan, 2004). PRD-1 was judged by Blanc and Nasser (1996) to be more suitable than another widely used enteric virus, MS-2, for determining the persistence of pathogenic viruses.

2.3. Experimental design

Groundwater samples were collected from all sample ports prior to the tests. Background levels of bromide and PRD-1 were both below their respective quantifiable detection limits (bromide 0.15 mg/L; PRD-1 detection level <0.25 plaque forming units per ml (PFU/ml)). Analysis of the vertical profile of pH, electrical conductivity, and temperature in MLS 6-16A shows differences in water chemistry between the sewage-contaminated and uncontaminated zones (Fig. 2). Such a condition enables us to investigate the collective influence of groundwater pH, ionic strength, and dissolved organic matter on bacteriophage transport and fate. Thus, two experiments were conducted, one in the zone of sewage-contaminated groundwater and the other in the zone of uncontaminated groundwater above the sewage plume.

For the experiment performed in the sewage-contaminated zone, approximately 600 l of groundwater were withdrawn with a peristaltic pump coupled to norprene tubing from two ports in MLS 6-16 and two in

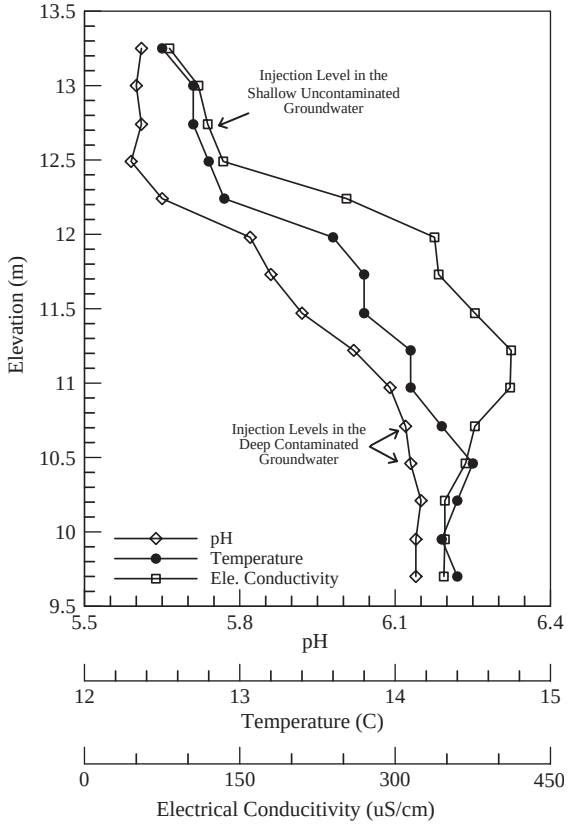

Fig. 2. Vertical profile of pH, temperature, and electrical conductivity from MLS 6-16A prior to test.

MLS 6-16A, both sets of which are located at elevations of 10.46 and 10.72 m (Fig. 1). Bacteriophage PRD-1 and potassium bromide (67 g) were mixed with the groundwater, and the resultant solution was injected through the same ports from which the groundwater was extracted. The injectate had an electrical conductivity of 530 μ-S/cm, pH of 6.14, a temperature of 15 °C, a bromide concentration of 75 mg/L, and a PRD-1 concentration of 10^{11} pfu/ml. The temperature of the solution was slightly greater than the natural groundwater temperature at the injection location (14.5 °C) (Fig. 2). Injection of the 600 l into the four injection ports was completed in 162 min.

For the experiment performed in the uncontaminated zone, approximately 300 l of groundwater were withdrawn from MLS 6-16 and 6-16A using the ports located at an elevation of 12.75 m (Fig. 1). The water was spiked with the potassium bromide salt and PRD-1 bacteriophage from the same source as used for the first experiment, providing concentrations of 75 mg/l for Br^- and 2.6×10^{14} PFU/ml for PRD-1. After cooling with blue ice, the solution had a conductivity of 335 μ-S/cm, pH of 5.5, and a temperature of 10.6 °C, similar to the ambient groundwater temperature. The solution was injected over a period of 140 min into the same ports from which the groundwater used to produce the injection solution was extracted. Samples collected from the injection ports after completion of injection showed that the temperature of the injected solution equaled the natural background level of 13 °C.

The sampling procedure for PRD-1 involved collecting 20 ml of sample in polystyrene vials containing approximately 10 mg of beef extract powder to preserve the bacteriophage. To further preserve viability, the vials were chilled to 4 °C. Bromide concentration, conductivity, and temperature were measured using another 50-ml sample. Periodically, 250-ml samples were collected for additional analysis. In total, a maximum of 560 ml was extracted per sample for the first 4 days of the study and 370 ml thereafter. To reduce potential for cross-contamination, MLS ports were flushed prior to sampling. For the first 4 days after injection, ports were flushed by extracting 250 ml prior to sample collection. This amount is approximately three times the dead volume for the deepest port sampled (9.7 m below sea-level). However, based on initial results, the flush volume was reduced to 100 ml to reduce the volume extracted for each sampling round. Preserved PRD-1 samples were shipped overnight to the University of Arizona and were analyzed within two days of receipt. Bromide concentrations were measured in the field using a bromide-specific electrode (Orion).

2.4. PRD-1 analysis

Aqueous samples were assayed for concentration of viable (infective) PRD-1 using plaque counting techniques previously described by Bales et al. (1991). Because of low potential for aggregation, each plaque was assumed to represent a discrete viral particle (Sharpe, 1965). The most precise counting results are obtained for plates with 30–150 plaques (ASTM, 1991). Because the bacteriophage concentrations were not known a priori, three parallel analyses were done for each sample, spanning 3–4 orders of magnitude in concentration.

To examine the accuracy of the analytical methods, multiple plaque assays were performed for a set of field samples. Five field samples with a range of concentrations were assayed 25 times each. No dilutions were made of these samples. The five average concentrations were 5.2, 30.4, 98.7, 294.2, and 450.2. The respective coefficients of variation were 0.30, 0.14, 0.06, 0.14 and 0.18. These results show that the most precise determination of PRD-1 concentrations is achieved with assays in which the PRD-1 plaque counts were near 100.

The vast majority of the field samples were assayed once at a particular concentration. Ten percent of the field samples were analyzed twice. Analytical accuracy

was measured by evaluating three subsets of 25 each from the samples that were analyzed twice. The subsets were chosen in such a way that the averages of the three 25 sample subsets were close to the counts (5.2, 30.4 and 98.7) determined from the previous plaque-assay reliability study. The averages of the three subsets of field samples were 5.2, 30.1 and 99.1. The average coefficients of variation of the replicate subsets were 0.26, 0.12, and 0.06. These levels of variation compare favorably with the results where individual samples were analyzed 25 times each.

2.5. Data analysis

The PRD-1 and bromide concentration data were analyzed by determining temporal moments. The zeroth temporal moment (t_0) is the area under the breakthrough curve, which reflects the mass of tracer traveling through the vicinity of the sampling port. The first temporal (t_1) moment, when divided by the zeroth moment, gives an average time for arrival of the mass under the curve. The average travel time is determined by subtracting one-half of the time span of the tracer injection from the normalized first temporal moment:

$$\bar{t}_1 = \frac{t_1}{t_0} - \frac{t_{\text{inj.pulse}}}{2}.$$

Longitudinal dispersivity (α_L) can be calculated from the second normalized temporal moment ($\bar{t}_2$) using:

$$\alpha_L = \frac{v}{2}\frac{\partial \bar{t}_2}{\partial \bar{t}_1},$$

where v is average linear pore-water velocity.

Tracer moments were analyzed only for "complete" breakthrough curves. For example, the data was not included if the sample with the maximum concentration of tracer for that breakthrough curve was among the first two or last two samples collected. In addition, breakthrough curves with multiple peaks of equal magnitude were not considered in the analysis of the peak travel times. The distance to each sampling location was computed using the mid-point between the injection ports as the zero-distance reference point. Given the predominance of horizontal flow in this aquifer, moment-analysis results are evaluated for several horizontal planes along the vertical direction. The locations of these planes are associated with the vertical positions of the ports of the multi-level sampling devices. Velocity calculations were made using the travel times as determined from the analysis of the entire breakthrough curve and the time of arrival of the observed peak concentration. Retardation factors for PRD-1 were calculated as the quotient of the computed bromide and PRD-1 travel times.

3. Results and discussion

3.1. PRD-1 inactivation

The inactivation rate of the bacteriophage PRD-1 in groundwater from the site was investigated under controlled conditions in the laboratory. Following the method of Yahya et al. (1993), 45 ml samples of groundwater from the same ports as those used for injection were collected in polypropylene tubes and spiked with 5 ml of solution containing PRD-1 to generate a concentration of 10^8 pfu/ml. These samples were then maintained for 75 days in a constant

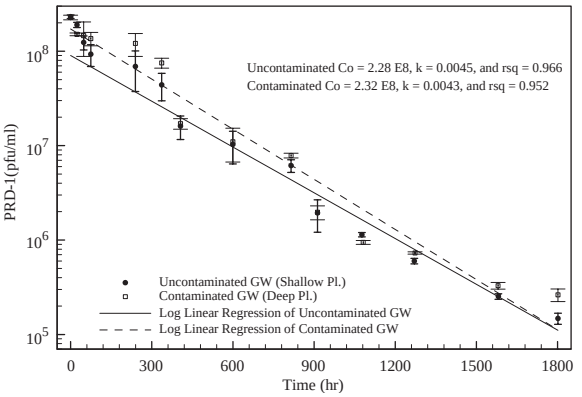

Fig. 3. PRD-1 inactivation in site groundwater (error bars represent results of triplicates).

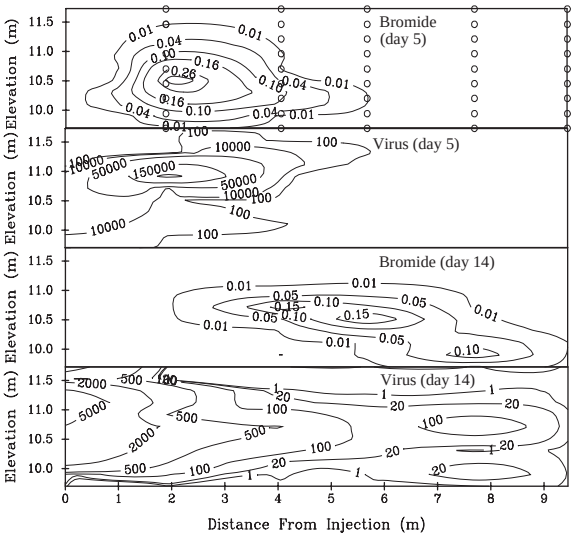

Fig. 4. Longitudinal cross-sections of the Bromide and PRD-1 plumes for the experiment conducted in the sewage-contaminated zone (circles on the bromide day 5 profile designate sampling locations, Bromide is shown in C/Co and PRD-1 is shown in pfu/ml).

temperature water bath of 13 °C, which is approximately midway between the temperatures of the contaminated and uncontaminated zones. The average and standard deviation of the measured PRD-1 concentrations as a function of time are shown in Fig. 3. The concentration of active viral particles (pfu/ml) declined over time for

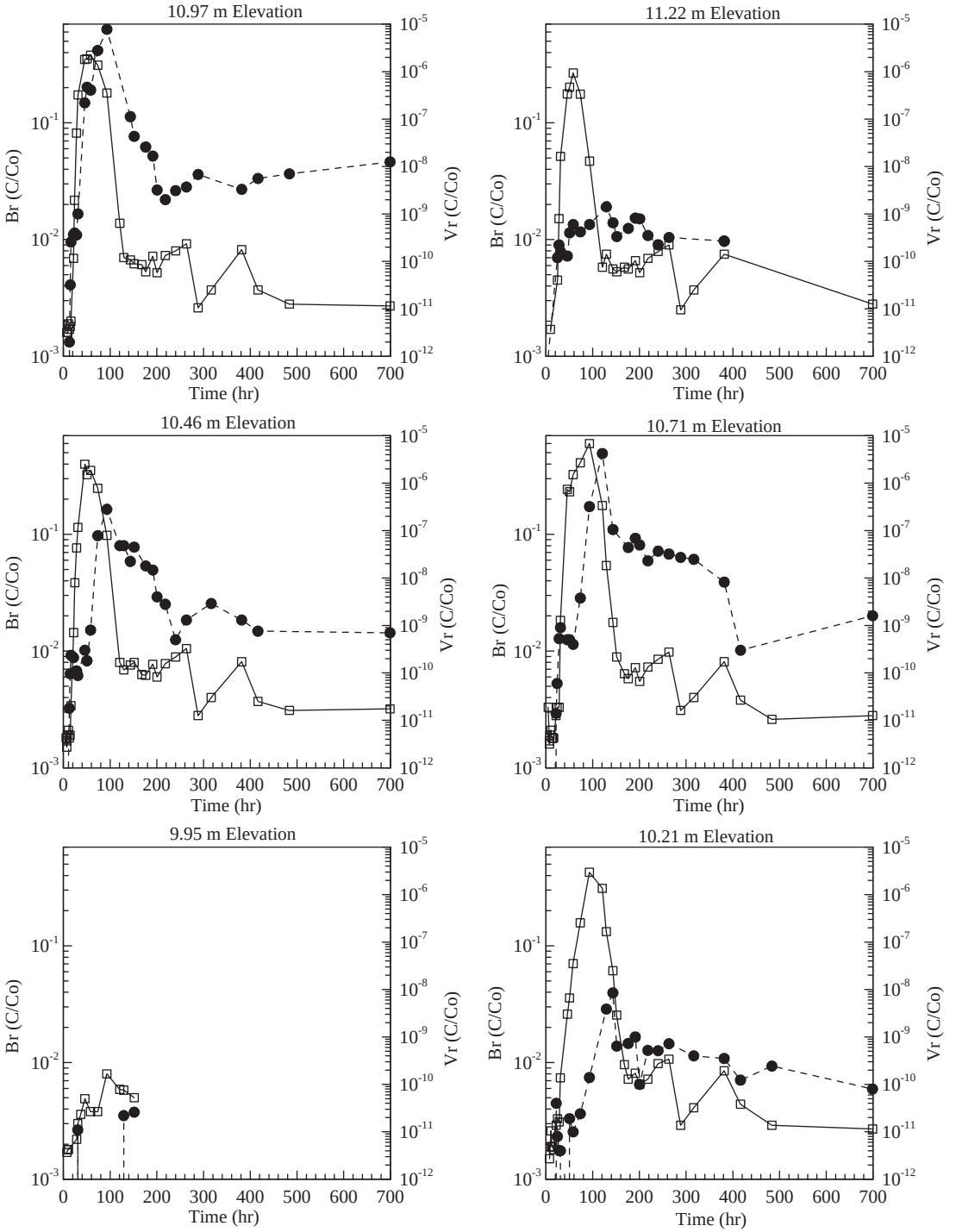

Fig. 5. Bromide and PRD-1 breakthrough curves for MLS 6A-12, sampling ports located in the sewage-contaminated zone (squares are Bromide and Circles are PRD-1).

both solutions. An exponential decay equation ($C = C_0 e^{-kt}$) provided a reasonable fit to the measured data ($r^2 \approx 0.96$). The first-order decay coefficients for the uncontaminated and contaminated groundwater experiments were 0.0045 and 0.0043 h^{-1}, respectively.

The slightly lower inactivation rate in the presence of contaminated water may be due to the higher concentration of dissolved organic matter. Ryan et al. (2002) showed that the inactivation rates of PRD-1 suspended in groundwater can be altered (reduced) in the presence of anionic surfactants, which are a major component of the dissolved organic carbon (DOC) present in the contaminated zone at the Cape Cod site. Schijven et al. (1999) reported inactivation rates for PRD-1 to be 34-fold lower in a peptone/saline solution than when suspended in native groundwater.

The aforementioned inactivation rate coefficients determined in this study (~0.046, when expressed as $\log_{10}$ per day) are slightly higher than the value (0.030) predicted by the equation of Harvey and Ryan (2004) describing PRD-1 inactivation in natural groundwater. The latter relationship represents an average of the collective observations for groundwaters from different geohydrologic settings by different investigators. Also, as noted in the latter reference, there is a possibility that the PRD-1 strains used in various studies are not structurally identical. The problem relating to the possibility of different PRD-1 strains should be ameliorated when this phage becomes available through the American Type Culture Collection (ATTC).

3.2. Bromide and PRD-1 transport behavior

The bromide and bacteriophage plumes were monitored for a distance of more than 13 m, which encompassed eight rows of MLS devices (Fig. 1). The plumes traveled in a south to southeast direction, coincident with the direction of the mean hydraulic gradient, and maintained a horizontal transverse width of roughly 2–3 m and a vertical thickness of about 1.5 m. The measured concentrations were used to create vertical cross-sectional contour maps of the bromide and PRD-1 plumes. For example, plumes for the experiment conducted in the sewage-effluent contaminated zone are presented in Fig. 4 for 5 and 14 days after injection. Inspection of Fig. 4 shows that bromide moved as a discrete pulse as would be expected. Conversely, PRD-1 exhibited behavior more consistent with a continuous-source injection, wherein concentrations in the vicinity of the injection zone remained high throughout the course of the experiment. Similar behavior was observed for the uncontaminated-zone study.

The general shape of the arrival waves of the PRD-1 and bromide breakthrough curves are similar for most sampling locations. Fig. 5 shows bromide and PRD-1

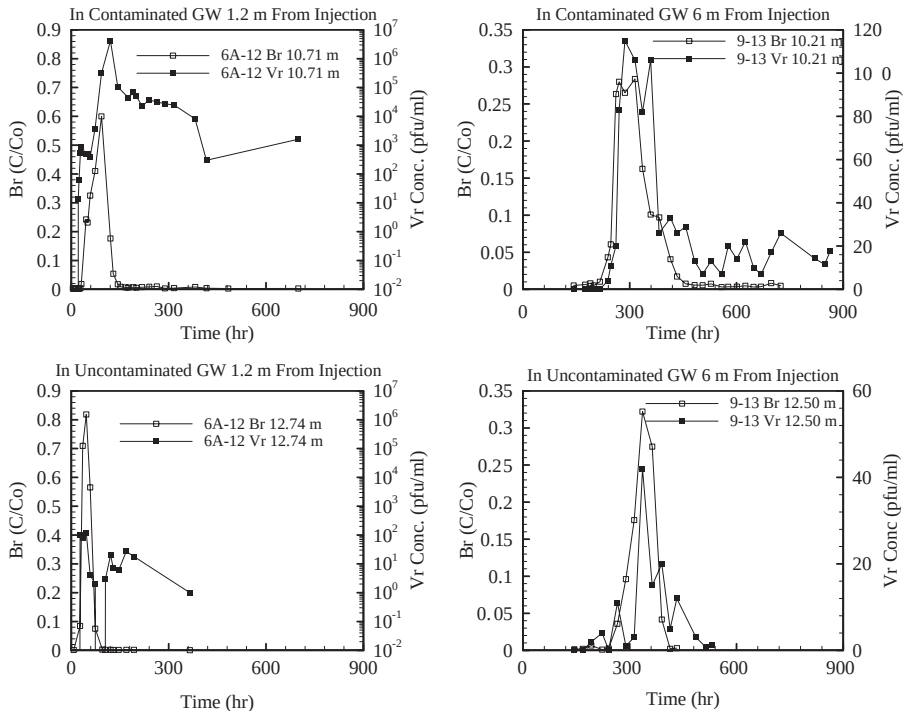

Fig. 6. Tailing of Bromide and PRD-1 breakthrough curves, comparison of behavior observed for sewage-contaminated and uncontaminated zones.

concentrations on a logarithmic scale versus time for port 6A-12 for the contaminated-zone experiment. The PRD-1 breakthrough curves obtained from the experiment conducted in the sewage-contaminated zone exhibited extended tailing compared to that of bromide for all sampling locations where complete breakthrough curves were observed. Examples of this behavior are provided in Fig. 6 for MLS 6A-12 and 9-13, which are 1.5 and 6 m downgradient from the injection zone, respectively. Conversely, extended tailing was not observed to such an extent for the PRD-1 breakthrough curves obtained for the uncontaminated-zone experiment. For example, less extended tailing occurs for the breakthrough curves measured at the sampling ports nearest the injection zone, such as 6A-12, and minimal tailing occurs for the breakthrough curves measured at more distant sampling locations, such as 9–13 (Fig. 6). The difference in the tailing behavior observed between the contaminated and uncontaminated zones is most likely related to differences in geochemical properties.

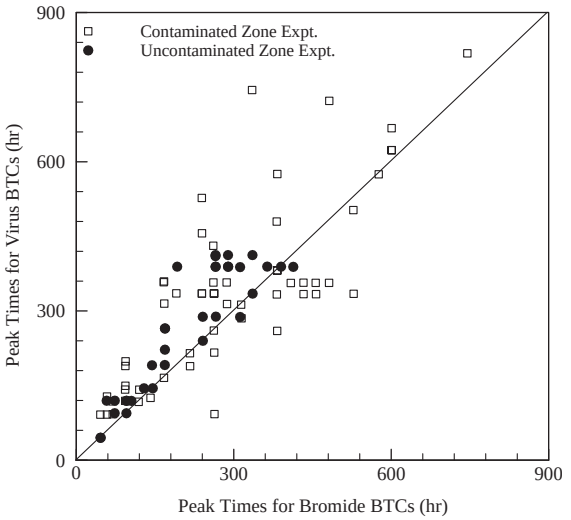

Fig. 7. Comparison between Bromide and PRD-1 travel times based on peak arrivals.

The times of first arrival for bromide and PRD-1 appear to be coincident for each of the six sampling locations shown in Fig. 5. Conversely, the PRD-1 peaks arrive after those of bromide. This is typical of the results for most of the breakthrough curves collected for both experiments, as illustrated in Fig. 7, where the bromide peak travel times are compared to those of PRD-1. Overall, the travel times calculated from the breakthrough-curve peaks are approximately 16% greater for PRD-1 as compared to those calculated for bromide.

The average bromide velocities determined from moment analysis of the complete breakthrough curves are 0.4 and 0.5 m/day for the contaminated and uncontaminated zones, respectively. The retardation factors determined by the quotient of the PRD-1 and bromide travel times for complete breakthrough curves are presented in Table 1. A relatively small degree of spatial variability is observed for each depth level (longitudinal) and between levels (vertical), likely

Table 1
PRD-1 retardation based on 1st temporal moments analysis of full breakthrough curves

Zone	Depth (m asl)	Num. of Obs. points	Retardation factor			
			Max.	Min.	Avg.	Std.
Contaminated	9.7	4	1.61	0.81	1.13	0.356
	9.95	10	1.38	0.32	1.04	0.293
	10.21	13	1.82	0.31	1.25	0.404
	10.46	10	1.90	1.11	1.48	0.308
	10.71	10	2.34	0.91	1.35	0.406
	10.97	10	1.29	0.84	1.09	0.142
	11.22	5	1.57	0.97	1.20	0.237
Avg.		8.9	1.70	0.75	1.22	0.306
C.O.V.		0.3	0.21	0.42	0.13	0.310
Uncontaminated	12.25	3	1.14	1.06	1.09	0.045
	12.5	6	1.37	1.00	1.11	0.149
	12.75	11	2.38	1.03	1.56	0.504
	13	9	2.72	0.99	1.62	0.659
Avg.		7.3	1.90	1.02	1.34	0.340
C.O.V.		0.5	0.40	0.03	0.21	0.853

reflecting subsurface heterogeneity of geochemical properties. Average retardation factors are 1.2 and 1.3 for the contaminated and uncontaminated zones, respectively. These values are slightly larger than those determined from the peak travel times, indicating the influence of tailing. These small retardation factors indicate minimal retention of the mobile PRD-1.

The calculated normalized second moments versus the normalized first moments are shown in Fig. 8 for the contaminated-zone experiment. The linear regressions, without forcing through the origin, provide reasonable fits (r^2 values greater than 0.94). Using the bromide data, the average dispersivities were calculated to be 0.27 and 0.33 m for the experiments conducted in the contaminated and uncontaminated zone, respectively. The values calculated using the PRD-1 data were 0.28 and 0.33 m, indicating no differences in dispersivities for the two tracers. Garabedian et al. (1991) reported a longitudinal dispersivity of 0.96 m for the site, which was computed for a larger bromide plume that was tracked for a much greater distance. The second moment increases linearly after 200–300 h (Fig. 8). By this time, the bromide plume had traveled between 3 and 4 m. This distance is approximately 10 to 15 times the computed

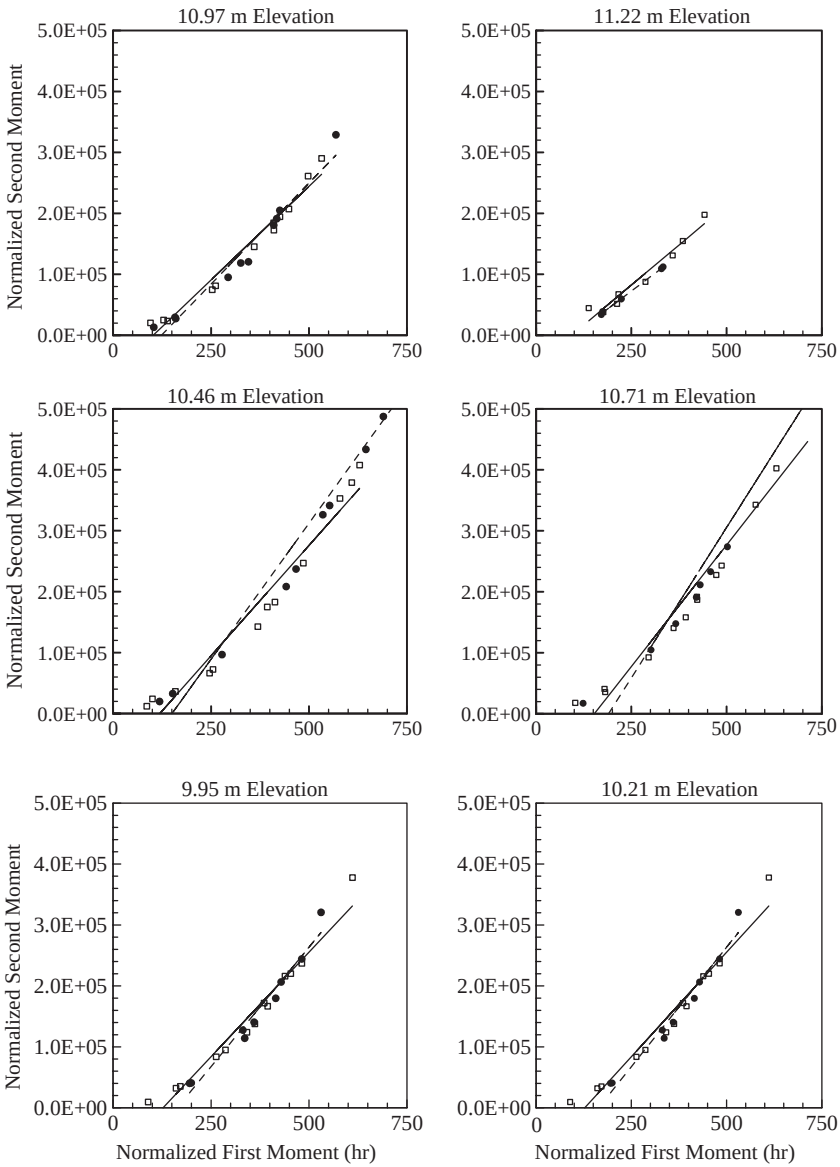

Fig. 8. Second temporal moments for the sewage-contaminated zone experiment (squares are Bromide, circles are PRD-1).

average dispersivity of 0.27 m for this plume. This is near the stability range of 27 dispersive lengths found by Garabedian et al. (1991) for this site.

3.3. Bromide and PRD-1 mass recoveries

The zeroth moments calculated for the complete bromide and PRD-1 breakthrough curves (BTCs) are plotted against distance from the injection zone in Figs. 9 and 10 for the contaminated- and uncontaminated-zone experiments, respectively. For both experiments, the major difference observed between the bromide and bacteriophage transport behavior is the much greater decrease in aqueous-phase concentrations observed for PRD-1. For example, the recovery of viable PRD-1 averaged 3.0×10^{-6} of the bromide recovery in the six monitoring levels of MLS 6a-12 located 1 m from the injection zone (Figs. 5 and 9). Final relative recoveries, based on samples collected at more distant MLS locations, were approximately 10^{-9} for the contaminated-zone experiment. Conversely, relative recoveries were approximately 10^{-12} for the uncontaminated-zone

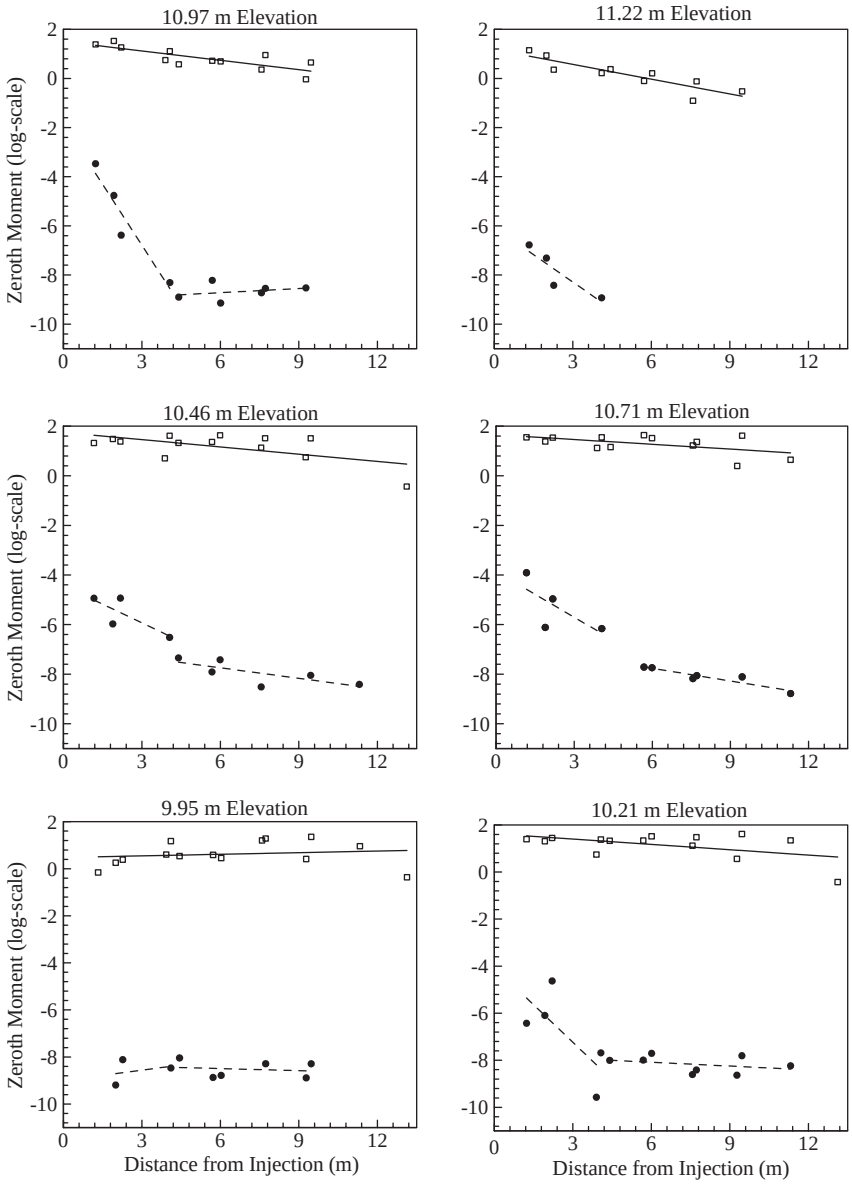

Fig. 9. Zeroth temporal moments versus distance for the sewage-contaminated zone experiment (squares are Bromide, circles are PRD-1).

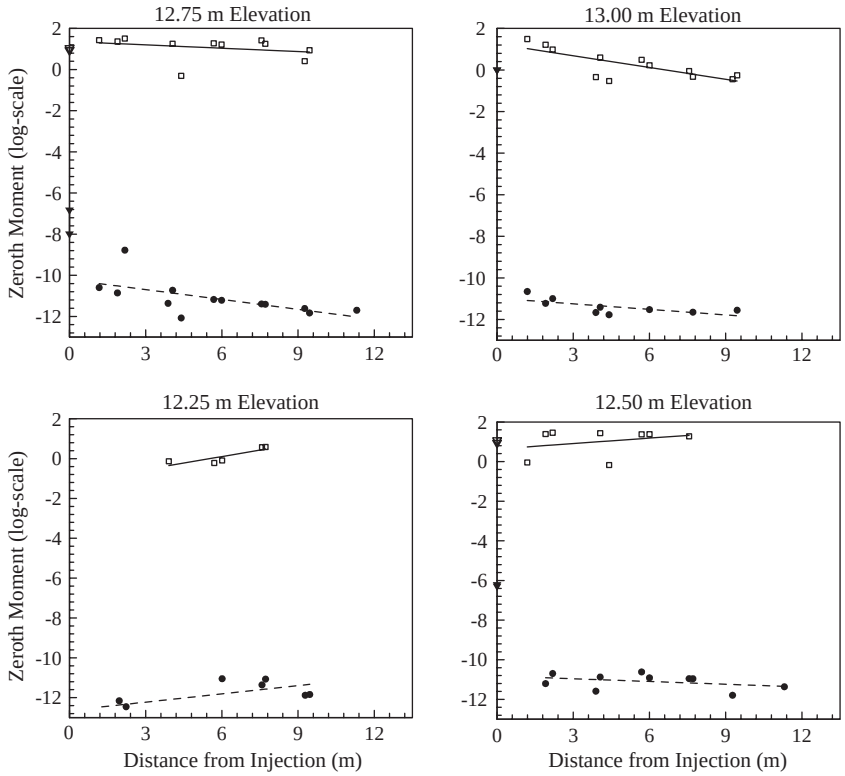

Fig. 10. Zeroth temporal moments versus distance for the uncontaminated zone experiment (squares are Bromide, circles are PRD-1, triangles are injection locations).

experiment. There does not appear to be significant variability of recoveries in the vertical dimension for either experiment (Figs. 9 and 10).

The results presented above indicate that, although PRD-1 traveled the entire 13-m transect, there was a tremendous decrease in viable PRD-1 concentrations in the aqueous phase. This decrease is attributed to a combination of colloid filtration (attachment) and inactivation. Estimates based on the results of the batch inactivation study indicate that approximately 20% and 65% of the initial decrease is due to inactivation for the uncontaminated and contaminated zones respectively, assuming direct translatability of the laboratory results. This suggests attachment contributed significantly to the mass removal. In addition, with inactivation contributing less than a single log decrease, it suggests that attachment was primarily responsible for the multiple-log removals observed. Based on prior research conducted at the Cape Cod site, this attachment has been considered to be effectively irreversible under prevailing conditions over the time scales typical to tracer tests (Pieper et al., 1997; Ryan et al., 1999). Additionally, it is possible that attached PRD-1 may be subject to inactivation, thus reducing the potential release of infective particles (Ryan et al., 2002).

The magnitude of PRD-1 removal was greater for the uncontaminated-zone experiment, as noted above. The greater magnitude of PRD-1 removal that occurred in the uncontaminated zone is more remarkable considering that the concentration of PRD-1 injected in the uncontaminated-zone experiment was more than 2500 times greater than that injected for the sewage-contaminated zone experiment. For the uncontaminated zone, the removal occurred within the first meter of transport. Conversely, the removal occurred over the first 4 m for the contaminated zone. The difference in recovery/removal observed for the two experiments is likely a result of the differences in geochemical properties of the two zones. Attachment of PRD-1 is expected to be reduced in the contaminated zone because of the higher concentrations of sorbed/dissolved organic matter, phosphate, and other anions, which can compete with PRD-1 for retention sites. Similar results were observed by Pieper et al. (1997) and Ryan et al. (1999) for the Cape Cod site.

The rates of change in the PRD-1 zeroth moments for both studies are minimal after the initial decreases occurred, and comparable to those observed for bromide (Figs. 9 and 10). This indicates that there was minimal loss of PRD-1 due to inactivation, retention, and other

processes after the first one and four meters of transport for the uncontaminated and contaminated zones, respectively. These results clearly show that a very small fraction of the injected PRD-1 population traveled 13 m and remained viable during the course of the studies (~70 days). It is hypothesized that this behavior may reflect intrapopulation variability, wherein a fraction of the population is more resistant to attachment. For example, intrapopulation variability of surface properties has been observed to influence the retention and transport of bacteria (Albinger et al., 1994; Baygent et al., 1998; Simoni et al., 1998; Bolster et al., 1999; Dong, 2002; Mailloux et al., 2003). While the concentrations of the mobile PRD-1 represent a minute fraction of the total mass injected, they are sufficient to pose a potential infectious threat to exposed receptors if they are representative of virus infective to humans (Regli et al., 1991).

4. Conclusions

The results of this study show that the concentration of the bacteriophage PRD-1 in the aqueous phase decreased greatly upon injection due to a combination of inactivation and attachment. The results also showed that this removal was greater for the uncontaminated zone. The lesser removal observed for the contaminated zone is attributed to higher concentrations of sorbed and dissolved organic matter, phosphate, and other anions present in the contaminated zone. After the initial decrease, the aqueous PRD-1 concentrations remained essentially constant in both zones for the remainder of the tests (total travel distances of 13 m), irrespective of variations in geochemical properties within and between the two zones. The results of this study suggest that a small fraction of viable virus particles may persist and travel significant distances in the subsurface, thus posing a potential infectious threat to down-gradient potable water supplies.

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