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Abstract

Transport of the bacteriophage PRD-1, bacteria, and latex microspheres was studied in a sandy aquifer under natural-gradient conditions. The field injection was carried out at the U.S. Geological Survey's Toxic Substances Hydrology research site on Cape Cod. The three colloids and a salt tracer (Br^-) moved along the same path. There was significant attenuation of the phage, with PRD-1 peak concentrations less than 0.001 percent of Br^- peaks 6 m from the source; but the low detection limit (one per ml) enabled tracking movement of the PRD-1 plume for 12 m downgradient over the 25-day experiment. Attenuation of phage was apparently due to retention on soil particles (adsorption). Attenuation of bacteria and microspheres was less, with peak concentrations 6 m from the source on the order of 10 and 0.4 percent of Br^- , respectively. Injection of a high-pH pulse of water 20 days into the experiment resulted in significant remobilization of retained phage, demonstrating that attached phage remained viable, and that PRD-1 attachment to and detachment from the sandy soil particles was highly pH dependent. Phage behavior in this experiment, i.e. attenuation at pH 5.7 and rapid resuspension at pH 6-8, was consistent with that observed previously in laboratory column studies. Results illustrate that biocolloids travel in a fairly narrow plume in sandy (relatively homogeneous) media, with virus concentrations dropping below detection limit several meters away from the source; bacteria concentrations above detection limits can persist over longer distances.

1. Introduction

Virus transport in sandy aquifer material has been observed to follow that of soluble salts in that virus are subject to dispersion in flowing ground water, and under certain chemical conditions are transported over distances of a few meters with little attenuation or retardation (Bales et al., 1989). Laboratory studies further show that pH (Kinoshita et al., 1993) and the amount of hydrophobic surface present in the porous media (Bales et al., 1993) are factors controlling transport. Virus survival is the other main determinant of transport (Yates et al., 1987), and depends on temperature (Yahya et al., 1993).

Knowing what controls virus transport in the field is of special concern for wellhead protection, i.e. designing proper protection distances between waste-disposal sites and drinking-water wells (Gerba et al., 1991). Because the few data available on virus transport in the field (e.g., Gerba and Bitton, 1984) were developed under conditions that were poorly characterized chemically, we still lack a sufficient understanding of how important chemical controls on virus transport are at the field scale. In addition, field-scale

experiments with the actual viruses that are of concern to human health are quite expensive to carry out and would require a high degree of control to mitigate possible health threats. This has led to use of bacteriophage as indicators of virus transport (Bales et al., 1993; Gerba, 1984).

The field study was undertaken to verify that the patterns of phage attenuation and transport observed in laboratory column studies reflect behavior in the field. These include slow attachment of phage to and detachment from soil particles, the strong pH dependence of attachment and detachment rates, and the overall reversibility of phage attachment to soil. The bacteriophage PRD-1, a virus that only infects and replicates in a strain of the bacteria *Salmonella typhimurium*, was chosen as a tracer because its size and surface characteristics mimic those of animal virus, and because it survives well in ground water. The coinjection of bacteria and microspheres with the phage enabled evaluation of these colloids as indicators of virus transport, and vice versa.

2. Methods

2.1 Site Description

The study was conducted at the U.S. Geological Survey's Toxic Substances Hydrology research site on Cape Cod, which is the location of several previous solute and biocolloid-transport studies (Harvey et al., 1989; Harvey and Garabedian, 1991; LeBlanc et al., 1991; Garabedian et al., 1991). The research area is on a broad sand and gravel outwash plain that was formed during the last Pleistocene glacial retreat (about 14,000 years ago) (Oldale, 1976). The top 30 to 50 m of sediment are a glacial outwash composed of stratified, well-sorted, medium to coarse sand with some gravel (LeBlanc, 1984).

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Estimates of the horizontal hydraulic conductivity range from 60 to 120 m dy⁻¹ (LeBlanc, 1984; Garabedian, 1987; Hess et al., 1992). Ground water in the unconsolidated sediments is unconfined in the area. The water table slopes toward the south, southwest at about 1.5 m per km (Garabedian, 1987). At the time of this experiment, the water table was around 13.73 m above sea level at the site. Estimated rates of horizontal ground-water velocity in the sand and gravel range from 0.2 to 0.7 m dy⁻¹ (LeBlanc, 1984), with porosity from 20 to 40 percent.

LeBlanc (1984) described the extent of contamination in the aquifer caused by sewage disposal at the Massachusetts Military Reservation, which is located north of the study area. In 1979, the plume of contaminated ground water was 0.8 to 1.1 kilometer wide, 3 m thick, and more than 3.4 km long. The plume moves in the direction of ambient flow to the south and southwest and is overlain by up to 15 m of uncontaminated ground water. The plume is characterized by elevated concentrations of dissolved solids, boron, chloride, sodium, phosphorus, ammonium, nitrate, detergents (LeBlanc, 1984) and, in some locations, volatile organic compounds (Thurman et al., 1984).

2.2 Field Methods

Ground water was collected by peristaltic pump from the injection depths (8.8 and 9.0 m below surface) from well 7-15 (Figure 1) and used as the suspending medium for the PRD-1 phage, fluorescently labeled bacteria, 0.7 μ m (diameter) carboxylated microspheres, and sodium bromide (NaBr). PRD-1 phage from the University of Arizona culture collection was purified and separated from cell debris by centrifugation at 80,000 \times g for 3 h. A morphologically diverse population of indigenous bacteria (0.2-1.6 μ m long) was concentrated from the aquifer using tangential-flow filtration and stained with the fluorochrome 4,6-diamidino-2-phenylindole (DAPI) at 5 μ M concentration for 24 h (Harvey et al., 1989). Concentrations of Br⁻, PRD-1, bacteria, and microspheres in the 97 liters of injectate were as noted in Table 1.

The injection was done on May 16, 1990 over a two-hr period. The injectate constituents were slowly added to the aquifer by gravity feed and monitored over a total horizontal distance of 12 m as they moved with the natural flow of ground water through rows of multilevel samplers spaced 2 m apart (Figure 1). The injection depths were \sim 2.3 and 2.5 m below the water table in the suboxic (zero to trace amounts of dissolved oxygen) zone of the contaminant plume, which lies between the unconfined zone of recharge water and the mildly reducing, Fe(II)-containing zone deeper in the plume (Kent, unpublished). The pH, temperature, and specific conductance at the points of injection were 5.7 \pm 0.1, 11.5 \pm 0.5°C, and 385 μ S cm⁻¹. Aquifer sediments sampled earlier near the test site had the following grain-size distribution: \sim 95 percent of the particulate mass was composed of grains smaller than 2.5 mm, \sim 80 percent smaller than 0.90 mm, \sim 60 percent smaller than 0.52 mm, \sim 30 percent smaller than 0.30 mm, and 10 percent smaller than 0.21 mm (Wolf, 1988).

Sampling was by means of a peristaltic pump using

multilevel samples previously installed at the field site (Smith et al., 1991). Bromide was measured in the field laboratory with a specific-ion electrode. Samples for phage analysis were collected in 30-ml vials and kept refrigerated at 4°C in the field laboratory. They were then shipped on ice by overnight delivery to the University of Arizona. Phage titers were determined using the plaque-counting procedures as described previously (Bales et al., 1991). Preparations for enumeration of DAPI-stained bacteria and fluorescent microspheres were made with 100 to 200 ml of sample. Where possible, enough fields were counted so that there were at least 350 stained bacteria enumerated, giving a counting precision of better than \pm 10 percent. Due to a greater degree of immobilization within the sediments, counting precision for the resulting lower numbers of microspheres was not as good, but was generally within \pm 20 percent. The DAPI-stained bacteria in these samples fluoresced under incident ultraviolet light (340 to 380 nm excitation) and were enumerated on black-polycarbonate membrane filters (0.2-mm pore size) using a Leitz microscope that was fitted for epifluorescence (Harvey et al., 1984). The microspheres (type YG, Polysciences, Warrington, PA) fluoresced at a different wavelength than the DAPI-stained bacteria and were enumerated under incident blue light (390-490 nm).

To further study the effects of pH and the reversibility of phage attachment, a second injection experiment was conducted to induce detachment. On June 5, 20 days after the injection, 100 liters of ground water were pumped into the carboy from well 7-15, as in the initial injection. The water pH was changed from the background value of 5.7 to 8.3 by adding phosphate buffer together with NaOH and Br⁻. This high-pH water was reinjected at the same sampling depths by gravity over 2 h.

3. Results

Samples were collected at five depths, 2-3 m below the water table for each sampling well. Thirteen wells were sampled, from row 8 to 13 (Figure 1), over a period of 23 days. Because the plumes of the bacteria, microspheres, virus, and Br⁻ showed the same pattern, only the Br⁻ and PRD-1 results are presented in detail. PRD-1 and Br⁻ results are shown on Figures 2-4 as breakthrough curves. PRD-1 concentrations as low as 1 pfu ml⁻¹ were detected. The plume center line generally passed between sampling wells in rows 8, 9, and 12; however, in rows 10, 11, and 13, wells 10-14, 11-14, and 13-13 appeared to fall on the center line. It was apparent that PRD-1 was attenuated relative to Br⁻ at all sampling points. This removal can be attributed to attachment to soil grains, and is enhanced by the low pH at the site (Kinoshita et al., 1993).

3.1 Br⁻ and PRD-1 Breakthrough Curves

The plume apparently passed between wells 8-14 and 8-15, with PRD-1 and Br⁻ moving at about the same velocity (Figure 2 shows 8-15). The plume arrived at 8-15 slightly later than at 8-14. The pulse at the depths of injection (2.26 and 2.50 m) had partially passed the well 8-15 when the samples were taken; the breakthrough curves at these depths

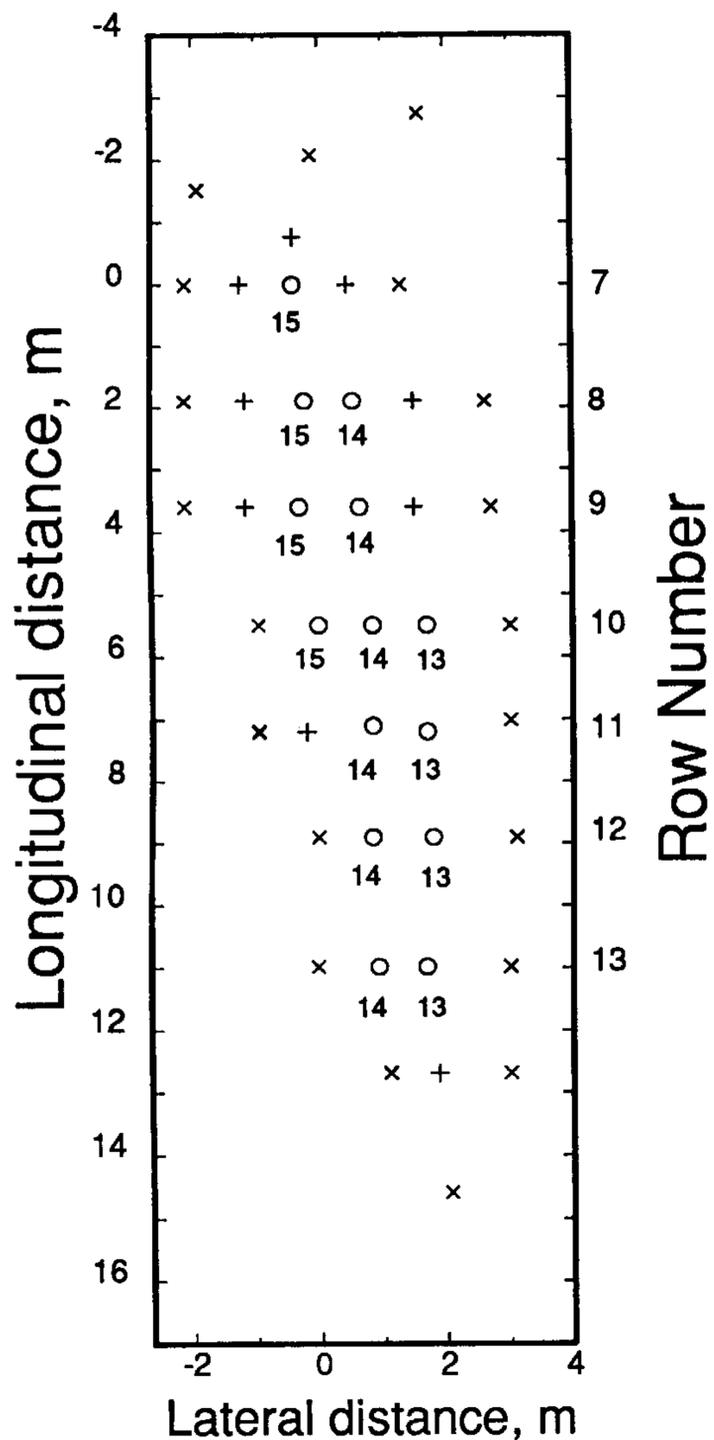


Fig. 1. Field-site arrangement. Each well had multilevel tubes for sampling. The direction of ground-water flow was from top to bottom. Symbols: \circ , well sampled; +, adjacent wells not sampled; \times , assumed zero concentration boundary. Wells sampled are also numbered. Complete designation for each well is by row and well number.

preceded those 0.25 m higher and lower, which were just above and below the plume's center. The incomplete breakthrough curves recorded at the injection depths in well 8-15 had Br^- peaks of 28 and 23 mg l^{-1} on the second day after injection; phage peaks were 19 and 26 pfu ml^{-1} , respectively.

At well 8-14, the Br^- concentration at all five sampling depths was around 0.4 to 0.6 mg l^{-1} , the background concentration. For PRD-1, a few nonzero concentrations were recorded at depths 1.98-2.74 m below the water table, with the highest being 99 and 50 pfu ml^{-1} on day 3. Either the main PRD-1 and Br^- plumes passed well 8-14 before the first sample was taken, or the main plume just missed the well.

Both Br^- and PRD-1 peaks at three of the five sampling depths of well 9-15 preceded those at well 9-14 by 2-3 days (Figures 2 and 3). At point 9-15 the 3.02-m depth and at 9-14 the 1.98-m depth failed to exhibit distinct peaks. Br^- concentrations were higher at well 9-14, but peak PRD-1 concentrations at 9-14 were not higher than that at well 9-15, and at two depths were distinctly lower. The lower PRD-1 levels at

9-14 could be attributed to the apparent longer travel time and thus greater phage adsorption. Complete Br^- breakthrough curves were recorded at most sampling depths of wells 9-14 and 9-15, with peak concentrations of 6-84 mg l^{-1} . PRD-1 breakthrough curves also showed complete peaks at most depths, with peak concentrations 0.6 to 38 pfu ml^{-1} on the 8th to 11th days. The PRD-1 plume appeared to have a slightly higher center (2.50 m) than did the plume for Br^- (2.74 m) in 9-14; but were at the same depth (2.50 m) in 9-15. There were no clear differences in Br^- and PRD-1 travel times.

Well 10-14 seemed to be located at the centers of both the Br^- and PRD-1 plumes (Figure 3). There were not significant differences in travel times of Br^- and PRD-1.

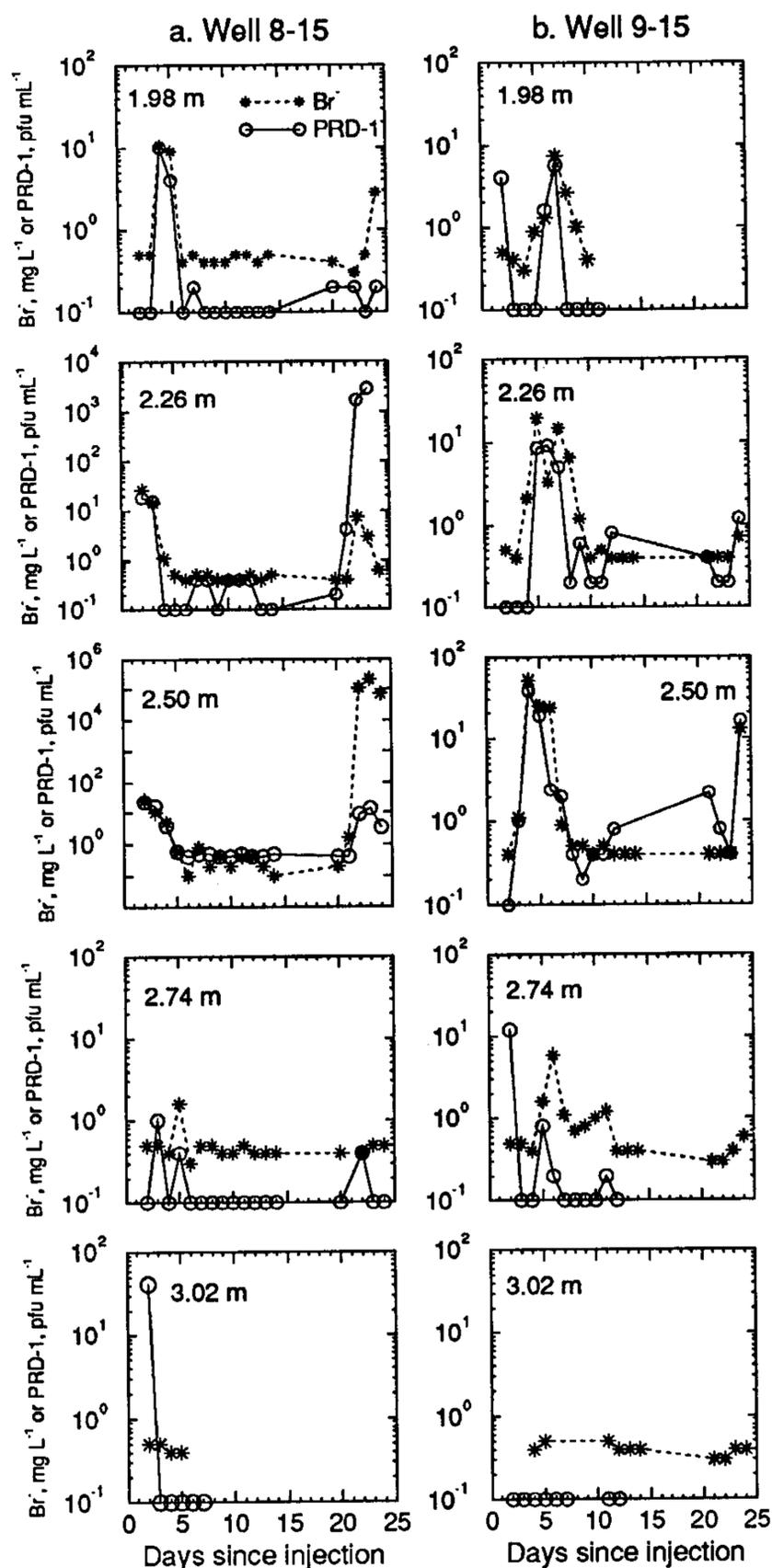


Fig. 2. Breakthrough curves for Br^- and PRD-1, rows 8 and 9. Samples below detection limit are plotted as 0.1. Depths are distance below water table. Note that depths 2.26 and 2.50 m of 8-15 have a different concentration scale than other locations.

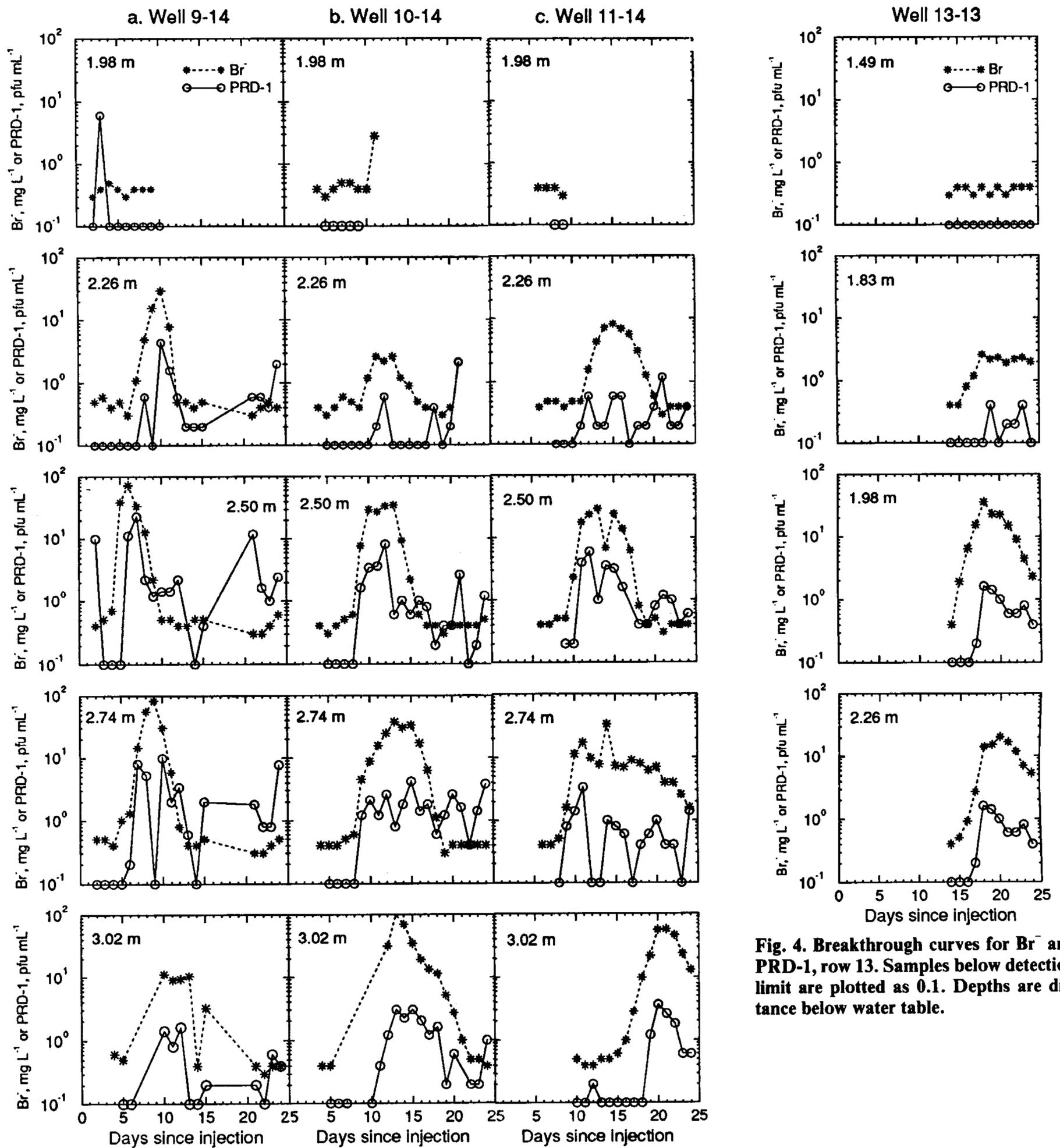


Fig. 3. Breakthrough curves for Br⁻ and PRD-1, rows 10 and 11. Samples below detection limit are plotted as 0.1. Depths are distance below water table.

Fig. 4. Breakthrough curves for Br⁻ and PRD-1, row 13. Samples below detection limit are plotted as 0.1. Depths are distance below water table.

Peaks at 10-14 were broad relative to 9-14 and 9-15, suggesting that flow came from the direction of both upgradient wells. Complete and broad Br⁻ breakthrough curves with short, flat plateaus were recorded at four sampling depths, with peak concentrations of up to 100 mg l⁻¹ around the 12th day. It is thus thought that the 2.74-m depth was near the plume center. PRD-1 breakthrough curves at the deeper four sampling depths were complete, with peak concentrations of 0.6, 8, 4.2, and 3.0 pfu ml⁻¹ on the 11th, 12th, 15th, and 14th days. The highest Br⁻ concentration occurred at a

lower sampling depth (3.02 m) than did PRD-1 (2.50 m) suggesting that the Br⁻ plume could be slightly lower than that for PRD-1. No significant PRD-1 and Br⁻ concentrations were recorded at well 10-13 or 10-15.

At well 11-14, complete and broad Br⁻ breakthrough curves were observed at three depths (2.26-2.74 m), with peak concentrations of 8-33 mg l⁻¹ on the 13th-15th days (Figure 3). No PRD-1 was found at the highest depth; low (0.2 to 1.2 pfu ml⁻¹), fluctuating concentrations were recorded at the 2.26-m depth. At 2.500 and 2.74 m depths,

Table 1. Injectate Concentrations and Relative Breakthrough of Constituents 6 m Downgradient from Point of Injection^a

Constituent	Injectate conc.	Avg. dia., μm	$\text{pH}_{i.e.p.}^c$	Max. C/C_0	Relative time to peak
Bromide	208 mg l^{-1}	—	—	1.7×10^{-1}	1.0
Bacteria	$3.5 \times 10^9 \text{ l}^{-1}$	0.60	na	1.2×10^{-2}	0.8-0.9
Microspheres	$3.8 \times 10^9 \text{ l}^{-1}$	0.74	~2.0	6×10^{-4}	1.5
PRD-1 phage ^b	$1.4 \times 10^{10} \text{ pfu l}^{-1}$	0.06	<4.0	5.7×10^{-7}	~1.0

^aWell 10-14, 2.5 m below the water table.

^bConcentration expressed in plaque-forming units (pfu) per l.

^cIsoelectric point.

the concentration values give a complete breakthrough curve; peak concentrations were 6 and 3.4 pfu ml^{-1} on the 12th and 11th days. At the deepest sampling depth, the PRD-1 breakthrough curve looked like that for Br^- at the same sampling depth, i.e., the tail was missing; the peak concentrations were 3.6 pfu ml^{-1} and 56 mg l^{-1} at the 20th day. The Br^- plume center was thus near the deepest sampling depth and lower than that of PRD-1. PRD-1 concentrations were in the same range at the three deepest depths, suggesting that a disperse plume passed this well, but with significantly different travel times over a 0.5 m depth interval. Overall, well 11-14 seemed to be near the plume centers of both Br^- and PRD-1. The PRD-1 plume may have lagged the Br^- plume by up to one day, but differences were small.

At well 12-13, two incomplete Br^- breakthrough curves were recorded at the 2.74 and 3.02 m sampling depths, with peak concentrations of 3.5 and 3.7 mg l^{-1} on the 20th and 24th days. No Br^- was found at the highest three sampling depths. Discontinuous and low-valued PRD-1 concentrations (0.2 to 1.0 pfu ml^{-1}) were recorded at the lower four depths. At well 12-14, no significant Br^- or PRD-1 concentrations were recorded at any of the five sampling depths. Both wells 12-14 and 12-15 were apparently off the plume centers of both PRD-1 and Br^- .

Three sampling depths of well 13-13 had Br^- and PRD-1 breakthrough curves at days 17-24 (Figure 4). Peak concentrations were 2-40 mg l^{-1} for Br^- and 1-3 pfu ml^{-1} for PRD-1. There were no obvious differences in the travel times of the Br^- and PRD-1. It thus appears that the 1.98 and 2.26 m sampling depths were close to the plume center.

3.2 Microsphere and Bacteria Transport

General features of the bacteria and microsphere breakthrough curves were similar to those of PRD-1, so only comparisons between the different colloids and Br^- are presented here. That is, bacteria and microsphere plumes followed the same general flow path as did PRD-1.

A comparison of peak heights and arrival times for Br^- , bacteria, microsphere, and phage breakthrough curves at well 10-14, the midpoint of the test (6 m downgradient from point of injection), are given in Table 1. The maximum concentration of Br^- at the 2.50-m level was ~17 percent of its concentration at the point of injection. Maximum dimensionless concentration of stained bacteria (avg. cell size 0.6 μm) was about 1.7 percent of the input value, and slightly preceded the peak in Br^- . The maximum dimensionless concentration of microspheres (0.7 μm) was less than 0.2 percent of the input value and was clearly retarded. The

arrival of the peak in PRD-1 (0.06 μm diameter) was coincident with Br^- with a concentration less than 0.001 percent of the input value.

Breakthrough curves for Br^- , bacteria, microspheres, and PRD-1 at well 13-13, the most distal sampling point in the test (12 m downgradient from point of injection) are shown in Figure 5. Again, the arrival times of peak concentrations for the phage and the conservative tracer were coincident. The bacterial peak appeared to occur within one day of the Br^- peak, but uncertainty remains because of a missing sample for day 18. The microsphere breakthrough curve was multip peaked and clearly retarded with respect to the bacteria, phage, and Br^- . Given the similar shapes of breakthrough curves, the apparent dispersion of both bacteria and PRD-1 appeared to be similar to that of Br^- .

3.3 Reversibility

Although not located directly on the plume center line, wells 8-15 and 9-15 recorded high desorption peaks of PRD-1 following injection of the high-pH water (Figure 2). The highest PRD-1 concentration was 10^6 pfu ml^{-1} , near that of the original injectate. This high concentration (2.50 m level) suggests that most phage removal occurred near the injection well, and that the buffered water efficiently reentrained the phage. The breakthrough curves on Figures 2-4 indicate that bacteriophage attachment to soil was reversible, and that increasing ground-water pH resulted in detachment. Measured pH's at well 8-15 on days 21, 23, and 24 were 6.4, 6.5, and 6.3 at 2.26 m, and were 5.6, 6.0, and 5.9 at 2.50 m. Measured pH's at well 9-15 on the same days were 6.3, 6.3, and 5.9 at 2.26 m, and were 5.9, 5.9, and 5.8 at 2.50 m. Thus, even these modest pH increases resulted in phage remobilization.

The lower PRD-1 concentrations in 9-15 as compared to 8-15 reflected both possible readsorption of PRD-1 between rows 8 and 9, and cessation of sampling before the peak reached row 9. Measured pH values at the sampling wells dropped to background levels as the second plume passed. The nonzero PRD-1 concentrations in 8-15, 9-14, and 9-15 show that some slow remobilization of phage occurred under steady-state conditions, prior to the high-pH pulse reaching the well.

4. Discussion

4.1 Pattern of Field Transport

In row 9 the highest Br^- concentrations observed were at the 2.50 and 2.74 m sampling depths, and at 2.50-3.02

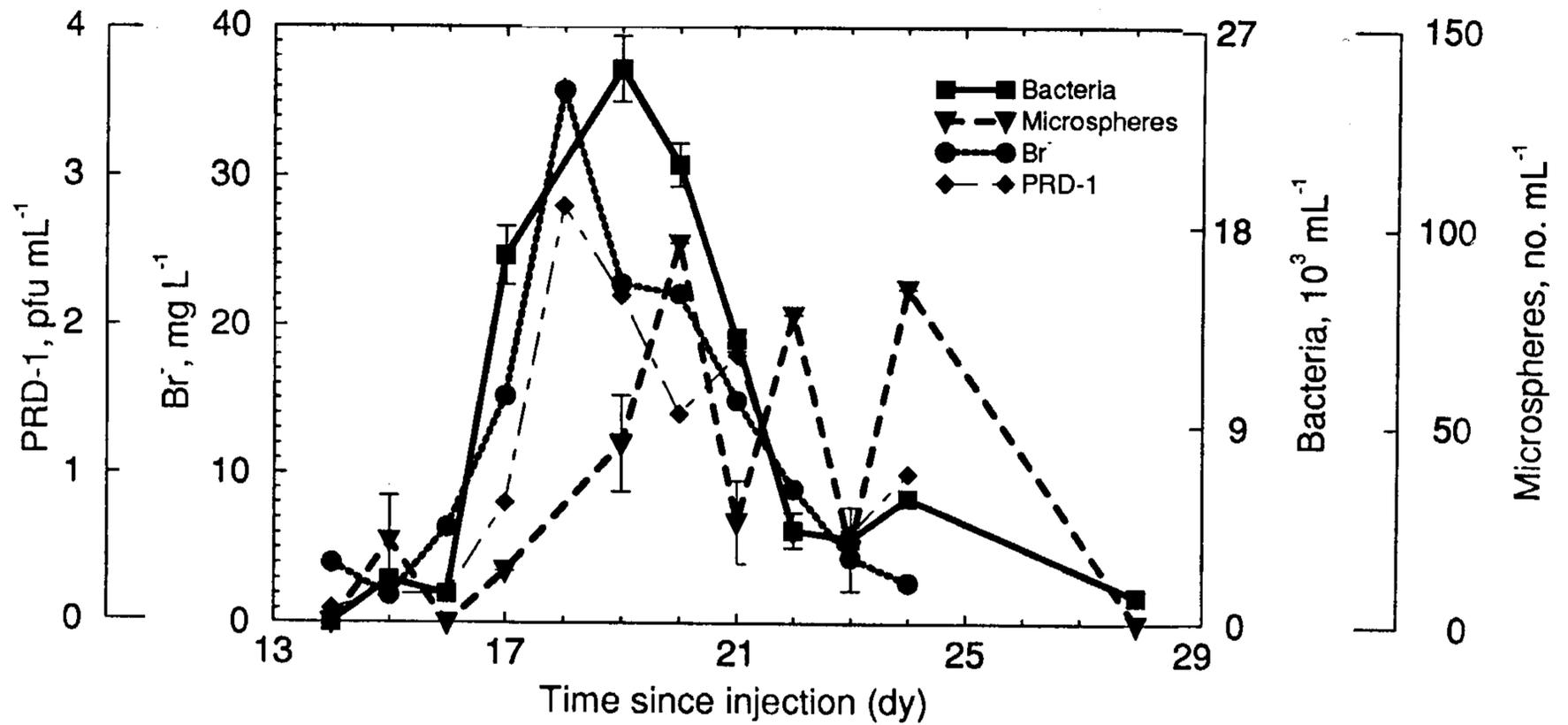


Fig. 5. Breakthrough curves for Br^- , PRD-1, bacteria, and microspheres at well 13-13, depth 1.98 m.

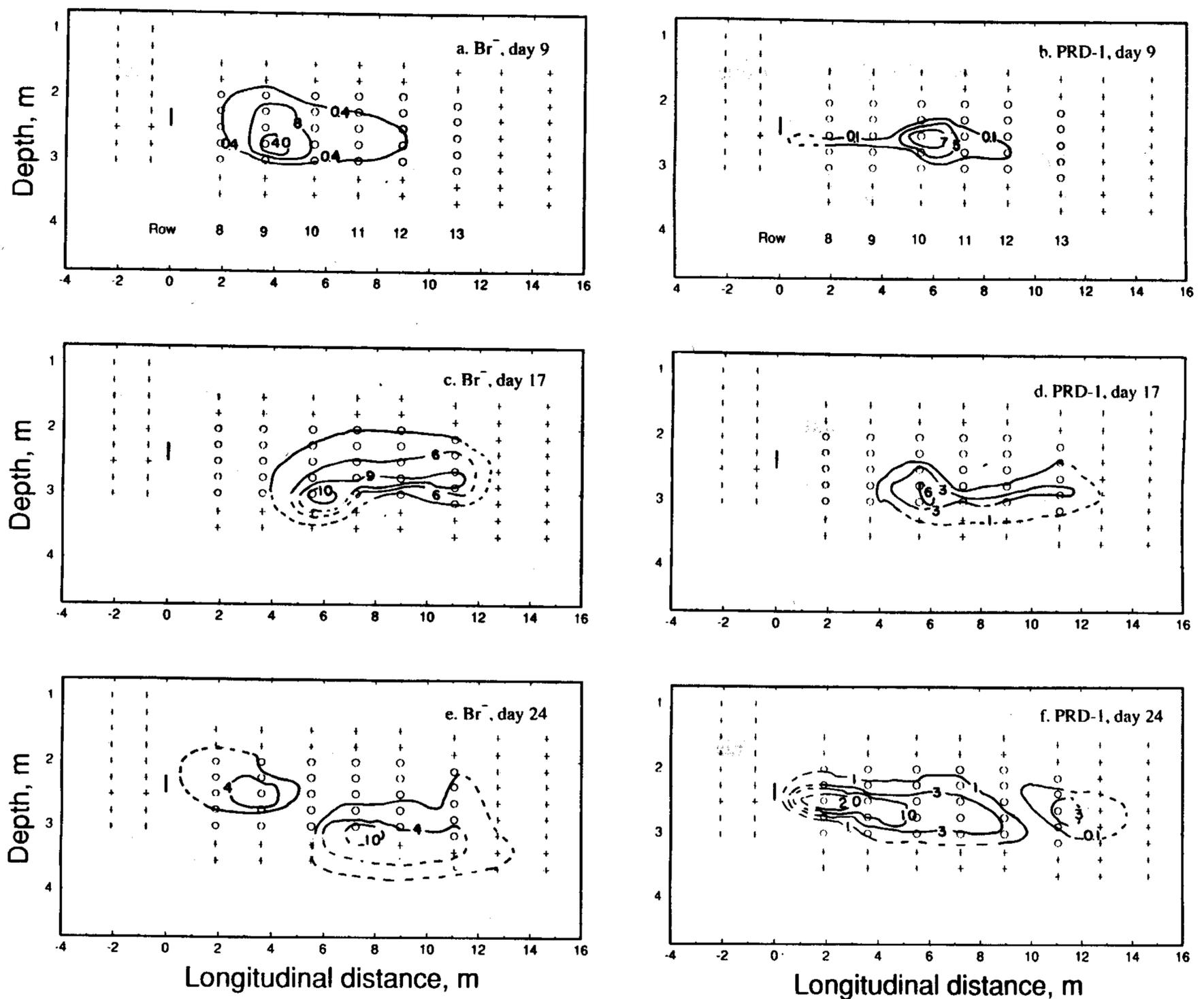


Fig. 6. Br^- and PRD-1 plumes in vertical cross sections at days 9, 17, and 24, based on apparent center-line concentrations. The direction of ground-water flow was from left to right.

depths in rows 10-11, suggesting that the plume dropped slightly from the 2.26-2.50 m injection depths. Peak Br^- concentrations at those same depths in rows 9-11 were about 41, 50, and 23 percent of the injectate concentration, respectively; these values suggest that the plume passed near these three sampling points. For PRD-1, the sampling depth with highest concentrations in rows 9-11 was 2.50 m, the injection depth, placing the PRD-1 somewhat above the Br^- plume. But concentrations were 38, 8, and 6 pfu ml^{-1} in rows 9-11, respectively. That is, PRD-1 concentrations were attenuated by a factor of 10^5 , versus 5-10 for Br^- . In the absence of sorption (retention on soil particles), PRD-1 should follow the Br^- plume. The apparent divergence of the two plumes suggests that PRD-1 attenuation was greater at the deeper depths, where pH was lower. This divergence can be seen by comparing vertical sections of the Br^- and PRD-1 plumes at three times, days 9, 17, and 24 (Figure 6). Contours were sketched by hand.

From the breakthrough curves (Figures 2-4), it is apparent that PRD-1 retention on the soil particles was important, since PRD-1 concentrations were attenuated several orders of magnitude over a short distance compared to Br^- . PRD-1 transport showed dispersion, since the breakthrough curves looked qualitatively like those for Br^- , which are affected only by dispersion and not retardation. Though only three wells were sampled at each row, Br^- values were, in general, consistent with classical dispersion, i.e., a symmetric bell-shape breakthrough curve (Figures 2-5). Day 24 was chosen for illustration as it shows both the furthest extent of the original injection and the movement of the second cloud from the injection of high-pH water. From these contours, it is apparent that the center of the Br^- plume traveled 7-10 m in 24 days. The Br^- plume appears to have exhibited significant longitudinal dispersion, but relatively less lateral dispersion. Dispersion in the vertical direction was more than in the horizontal, apparently due to a vertical pH gradient at the site (compare Figures 6-7).

The Br^- and PRD-1 plumes tracked each other in general direction of movement (e.g., Figure 7). The PRD-1 plume appeared to move slightly further than the Br^- ; this is due largely to the much greater sensitivity of the PRD-1 analysis, however. At the 2.50-m level, but not the 2.74- or 3.02-m levels, there was a second Br^- pulse along with the PRD-1 detachment pulse.

Further information is apparent from vertical profiles constructed along the apparent center lines of the plumes. Figure 6 shows profiles for day 17, which does not show the effect of the injection of high-pH water, and day 24, which does. From these figures it is apparent that on day 17, the PRD-1 and Br^- plumes had essentially the same horizontal extent. The centers of mass were about 6 m from the injection well. The Br^- plume center was one sampling depth below that of the PRD-1 on day 17, however.

From day 17 to 24, the center of the Br^- plume moved only about 2 m, whereas the apparent PRD-1 peak at row 13 on day 24 suggests that the PRD-1 plume center moved further. The disperse nature of the PRD-1 plume on day 17 and the appearance of the second plume preclude assigning a certain travel distance. Also, the Br^- plume continued to

drop vertically, whereas the PRD-1 plume continued horizontally or rose slightly. The second PRD-1 plume moved rapidly from the injection well and nearly overtook the first one. PRD-1 concentrations were actually higher in this detachment plume.

Patterns for the field-transport studies were consistent with laboratory results (Kinoshita et al., 1993). That is, PRD-1 was reversibly adsorbed at pH near 5.5, resulting in a small but detectable level of PRD-1 in downgradient wells. Attenuation was about a factor of 10^5 ; detachment, and thus retardation were small, with the small pulse that did break through following the Br^- fairly closely.

4.2 Comparison of Transport Behavior of the Three Colloids

Although there was a very clear order for the removal rates of the three colloids examined here (i.e., PRD-1 > stained-bacteria > microspheres), the nature of their interactions with grain surfaces within the aquifer sediments is less clear. A later arrival time for the 0.7 μm microspheres (Table 1) compared favorably to what was observed in an earlier, nearby natural-gradient experiment (Harvey et al., 1989) and suggests substantial reversibility in microsphere attachment within the experimental time frame. Enhanced retardation and dispersion relative to Br^- in the earlier experiment were thought to be due to the high reactive nature of the microsphere surface.

The order in which the peaks of conservative tracer, microorganisms, and microspheres appear at a given sampler downgradient from point of injection can be significantly influenced by the degree of physical heterogeneity within the Cape Cod aquifer (Harvey et al., 1993). For example, relative to that of Br^- , the peak in breakthrough for stained bacteria in small-scale tests has been observed to travel faster [e.g., at USGS well site F393 (Harvey et al., 1989)], slower at others [e.g., at 10-14 and a 2.26-m level in this experiment (Harvey et al., 1993)], and coincidentally at others with that of the conservative tracer that they were coinjected with (Harvey and Garabedian, 1991). However, near-coincident appearance with Br^- appeared to be most often the case for stained bacteria (data not shown) and the PRD-1 phage employed in this experiment (Figures 2-4).

Relative breakthrough for the 0.7- μm sized carboxylated microspheres was greater than that for the 0.06- μm sized phage, suggesting that the rate of immobilization was greater for the PRD-1 than for the microspheres. The smaller size of the phage would contribute to a higher frequency of surface contact, based upon its greater rate of diffusion relative to the microspheres [Rajagopalan and Tien, 1976]. But since the microspheres were retarded, they must also have had significant interactions with grain surfaces. The observations that microspheres were both retarded and present at downgradient observation points at higher concentrations than PRD-1 suggests that microsphere resuspension from soil particles was faster than for PRD-1. The in situ rate of PRD-1 inactivation, which would contribute to the apparent rate of loss during transport, was not known. In batch laboratory studies it was estimated to be only 10 percent per day (Yahya et al., 1993). Based upon the

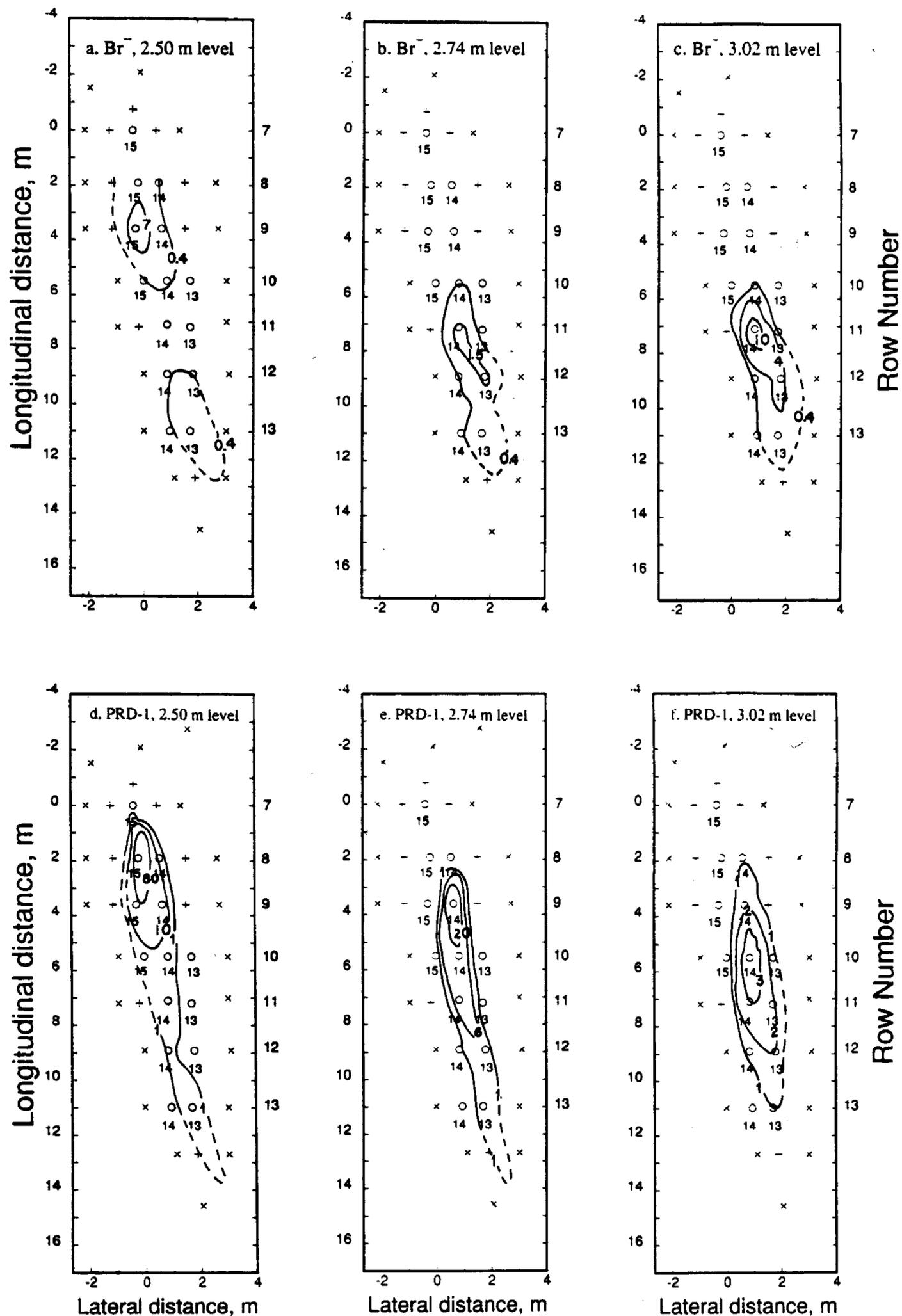


Fig. 7. Br^- and PRD-1 plume horizontal contours on day 24.

size distribution of the sediments, it is doubtful that straining would significantly contribute to the immobilization rate of either PRD-1 or the microspheres (Harvey et al., 1993). Thus, as in the earlier natural-gradient test at the site (Harvey et al., 1989), there was no clear relation between immobilization and retardation, at least for the three types of microspheres tested (neutral-, carbonyl-, and carboxyl-surfaces).

5. Conclusions

Field experiments reinforced the importance of pH as a controlling variable in bacteriophage sorption and desorption. Reversibility of phage attachment was also demonstrated in the field. Remobilization of phage was slow but still observable under constant chemical conditions. Increasing the pH caused rapid phage release into the flowing ground water. Under the conditions studied, there was no

significant phage movement ahead of the salt tracer. Greater sorption at lower pH apparently resulted in more bacteriophage attenuation, and an apparent vertical divergence of the virus and salt-tracer plumes. The three colloids and Br^- moved along the same path. Phage attenuation was significant, with peaks less than 0.001 percent of those for Br^- . Attenuation of bacteria and microspheres was less, with peak concentrations approximately 10 and 1 percent of those for Br^- , respectively. Thus, although all four tracers indicated the general pattern of plume movement, the attenuation of each was unique. Bacteria and PRD-1 had travel times near that for Br^- , whereas microspheres were apparently retarded (i.e., exhibited reversible sorption). Because of their comparable transport characteristics and good analytical sensitivity, bacteriophage have again been shown to be very good tracers of biocolloid plume movement. The relatively narrow plume and large degree of phage attenuation suggest that in sandy (relatively homogeneous) media, virus concentrations above detection limits would be confined to a few meters downgradient from the source; chemical perturbations can result in transport further from the source. Detectable concentrations of bacteria could persist for tens of meters downgradient.

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