

## **WRRC (Arizona) Technical Report**

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**18 May 2001**

**Section 1 Title:** Field Studies of Virus Transport

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**Section 3 Congressional District for Each PI:** Arizona District 5

### **Section 4 Description Information**

#### **A. Problem and Research Objectives:**

Enteric virus contamination of groundwater used for potable water is of increasing concern (U.S.E.P.A., 2000). This concern has fomented interest in the transport and fate behavior of viruses in the subsurface environment. Accordingly, several bacteriophage transport studies have been conducted under well characterized conditions (see recent review by Schijven and Hassanizadeh 2000). Because of the hazards and costs associated with human viruses, the bacteriophage PRD-1 is often employed as an analogue to viruses of concern to human health (Gerba, 1984; Bales et al. 1993). The results of this previous work indicate that aquifer physical/chemical properties (e.g. permeability, aquifer heterogeneity, pH, temperature, total dissolved solids, and dissolved organic matter) influence the transport and fate of virus. Within the aquifer environment the factors controlling virus transport are attachment to and detachment from the porous medium surfaces, growth and inactivation, filtration, sedimentation, advection, and dispersion.

This study was initiated with the objective of isolating the influence of groundwater chemistry on the transport and fate of viruses under field conditions. The sewage plume at the Cape Cod site is higher in pH, ionic strength, and dissolved organic matter than the uncontaminated groundwater at the site. Experiments were conducted in both sewage-contaminated and uncontaminated zones to examine the impact of water chemistry on virus transport. The experiments were conducted at a larger scale than previous studies to provide additional information regarding transport behavior.

## **B. Methodology:**

### **Site Description**

Surficial strata at the site is associated with the last Pleistocene glacial retreat, which occurred approximately 14,000 years ago. Deposited in the top 9 to 15 meters is a section of outwash material consisting of well-sorted medium to coarse sand with some gravel (Leblanc, 1984). In the northern portion of the area, the sand and gravel overlie fine sand and silt lenses. To the south, the outwash material appears above fine sand, silt, and a dense sandy till. The till contains lenses composed of silt and clay, and others of sand and gravel. These unconsolidated sediments reside atop a crystalline granodiorite bedrock which generally slopes from west to east through the region.

The site has a shallow aquifer of thickness varying between 90 and 100 meters, the top of which is 3 to 7 meters below land surface in the study area (Leblanc et al. 1991). The horizontal hydraulic conductivity in the sand and gravel zone is estimated to be from 60 to 120 meters per day (Leblanc, 1984, Garabedian, 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 meter/km (Garbedian, 1988). The groundwater flows horizontally with a velocity in the range of 0.2 to 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 heavily contaminated by sewage disposal from Otis ANGB. The sewage effluent emanates from sewage infiltration beds up-gradient of the experimental station. In 1979, the contamination plume was 0.8 to 1.1 kilometers wide, 3 meters thick, and more than 3.4 km long (Leblanc, 1984). The body moves with the ambient groundwater flow in a south to southwest direction. It is overlain by 15 meters of uncontaminated water originating primarily from infiltration of local precipitation. Elevated levels of dissolved solids, boron, chloride, sodium, phosphorous, ammonium, nitrate, and detergents characterize the plume (Leblanc, 1984). In some locations, volatile organic compounds are also in the plume (Thurman et al., 1984). There background concentration of the bacteriophage used in this study, PRD-1, was below detection limits of 0.25 pfu/ml and the bromide was below 0.2 mg/L.

The site is instrumented with an array of multi-level sampling wells. Sampling ports protrude out the side of each well in an up-gradient direction at approximately 25 cm intervals. The sampling lines are polyethylene tubes with 0.47 cm inside diameter and 0.64 cm outside diameter and the wells are PVC casing with a 3.17 cm diameter (Leblanc et al., 1991). A more complete description of the site is found in Leblanc et al. (1991).

### **Experimental Design**

Groundwater samples were collected from all sample ports prior to the tests. Bromide and PRD-1 were both below their respective quantifiable detection limits (PRD-1 detection level <

0.25 PFU/ml, and bromide 0.15 mg/L). Analysis of the vertical profile of pH, electrical conductivity, and temperature in well 6-16A showed variation in water chemistry between the sewage-contaminated and uncontaminated zones (see Figure 1). In contrast, similarities in the bromide transport results for this study at the two experimental depths show that the study zone has a relatively uniform distribution of hydraulic properties. This condition enables the isolation of the relative influence of groundwater pH, ionic strength, and dissolved organic matter on the bacteriophage fate and transport.

For the first study (deep-plume experiment), approximately 600 liters was withdrawn by a peristaltic pump with noreprene tubing from two ports in well 6-16 and from two in well 6-16A (see Figure 2). The depths of the ports are 10.46 and 10.72 meters above mean sea-level (see Figure 2). The bacteriophage PRD-1 and potassium bromide were mixed into groundwater withdrawn from the injection location. The solution was injected through the same ports from which the groundwater was extracted. Sixty-seven grams of potassium bromide salt was dissolved into the water equating to a  $\text{Br}^-$  ion concentration of 75 mg/L. A solution containing the bacteriophage PRD-1 served as the viral tracer. The water at the time of injection had an electrical conductivity of 530  $\mu$ -siemens/cm, pH of 6.14, a temperature of 15EC, a bromide concentration of 75 mg/L, and a concentration of  $10^{11}$  PRD-1 plaque forming units per ml (pfu/ml). This temperature is slightly above the natural groundwater temperature at the injection location (14.5°C) (see Figure 1). Injection of the 600 liters into the four injection ports was completed in approximately 162 minutes.

For the second study (shallow-plume experiment), approximately 300 liters of groundwater was withdrawn from one port each from wells 6-16 and 6-16A at 12.75 meters above mean sea-level (see Figure 2). It was spiked with the potassium bromide salt, and PRD-1 bacteriophage from the same source as used for the first experiment. These led to concentrations of 75mg/L  $\text{Br}^-$  and  $2.6 \times 10^{14}$  PFU/ml PRD-1 at the time of injection. After cooling with blue ice, the injectate had a conductivity of 335  $\mu$ -siemens/cm, pH of 5.5, and a temperature of 10.6°C. Samples from the injection wells after completion of injection showed that the aquifer had mitigated the lower temperature of the injected solution to equal the natural level of 13EC. The solution was injected into the same ports from which the groundwater was extracted, and was completed in approximately 140 minutes.

Bromide concentration was measured in the field using both a bromide-specific electrode and an electrical conductivity detector. Samples for bacteriophage analysis were sent to off-site laboratories at the University of Arizona. To reduce potential for cross-contamination, sampling ports were flushed prior to sampling. For the first four days, 250 ml was extracted prior to sample collection. This amount is approximately three times the volume of water for the deepest port sampled (9.7m 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.

The sampling procedure for PRD-1 involved pouring 20 ml of groundwater into polystyrene vials containing approximately 10 milligrams 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 determined using another 50 ml. Also, 250 ml samples were intermittently drawn for analysis of microspheres. In total, at most 560 ml was extracted per sample for the first 4 days of the study and 370 ml afterward. Preserved PRD-1 samples were shipped overnight to the University of Arizona and were analyzed within two days of receipt.

### **PRD-1 Concentration Analysis**

The bacteriophage PRD-1 is an icosahedral (twenty triangular faces) lipid containing bacteriophage with an average diameter of 62 nm (Olsen et al., 1974). The lipid-containing protein cover of PRD-1 promotes hydrophobic behavior (Bales et al., 1993). Additionally, the protein coat contains amino and carboxyl groups that can lead to electrostatic sorption. For PRD-1, the point of zero net surface charge or isoelectric point was found by Dowd et al. (1998) to be a pH of 4.2. PRD-1 was judged by Blanc et al. (1996) to be more suitable than another widely used enteric virus analog tracer, MS-2, for determining the persistence of pathogenic viruses.

Bacteriophage concentration was determined by plaque counting techniques previously described by Bales et al. (1991). Because of low potential for aggregation, each PFU represents a discrete viral particle (Sharpe, 1965). Most precise counting results are for plates with 30 to 150 plaques  $\pm 20\%$  (ASTM, 1991). Because of only limited knowledge of potential bacteriophage concentration, normally three analyses were done covering 3 to 4 orders of magnitude of concentration.

In order to examine the accuracy of the analysis methods, multiple plaque assaying of field samples were performed. Five field samples with a range of concentrations were assayed 25 times each. No dilutions were made of these samples. The five average concentration 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 confident determination of PRD-1 concentrations are achieved with assays whose PRD-1 plaque counts were near 100.

Of the field samples, the vast majority were assayed once at a particular concentration. Ten percent of the field samples were analyzed twice. A measure of analytical accuracy can be made by evaluating three subsets of 25 each from the samples that were analyzed twice. The subsets were chosen where the average of the 25 samples were close to the counts 5.2, 30.4 and 98.7 from the previous plaque-assay reliability study. The averages of the three subsets of field

samples are 5.2, 30.1 and 99.1. The average coefficients of variation of the duplicates subsets are 0.26, 0.12, and 0.06. These levels of variation compare favorably with the results where individual samples were analyzed 25 times each.

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 of the viral plumes were collected in polypropylene tubes and spiked with 5ml of solution containing the PRD-1 to a concentration near  $10^8$  pfu/ml. These samples were then kept in a constant temperature water bath of  $13^\circ\text{C}$ , which is approximately midway between the temperatures of the shallow and deep injection (see Figure 1). The concentration of PRD-1 in the vials was periodically measured over the next 75 days. The concentration was determined twice at two different dilutions.

The concentration of active viral particles (pfu/ml) declined over time for both solutions. A log-linear regression of the results best approximated the decrease in the observed aqueous concentration of PRD-1 in both the shallow and deep groundwater samples. The fits for the exponential model are 0.900 and 0.960, respectively, for the shallow uncontaminated and the deep sewage-contaminated groundwater. The slope of the regressions indicate that the inactivation of virus in the groundwater in the shallow and deep experiments would decrease viral concentration by an order of magnitude every 554 and 565 hours, respectfully.

### **Data Analysis**

The time at which viruses reach a point can be estimated through analysis of the frontal portion of the virus breakthrough curve. Local aquifer physical properties can be determined from the transport of a non-reactive tracer. Bacteriophage in this sand and gravel aquifer will be exposed to similar flow conditions, but their movement is also influenced by chemical interactions within the aquifer. By comparing bromide and PRD-1 propagation patterns, the influence of physical and chemical properties on PRD-1 transport may be evaluated.

The movement of the virus and bromide groundwater plumes was determined from the trends in the calculated temporal moments of the breakthrough curves of these tracers at the monitoring points down-gradient of injection. In this analysis forward-difference versions of the temporal moment analysis equations were used. Specifically, trends in the zeroth temporal moment ( $t_0$ ) integrates the area under the breakthrough curve, which reflects the mass of tracer traveling through the vicinity of the sampling port.

Velocity calculations were made through comparing the average time of arrival or time of the peak observed concentration for all ports at a single depth with complete breakthrough curves with the distance from the midpoint of injection for those observation points. The velocity is computed as the inverse slope from linear regression analysis of the time versus distance data.

Overall retardation factors for the bacteriophage tracer were calculated by layer. This was accomplished through dividing the computed bromide velocity by that of the virus velocity. Secondary retardation analysis was performed through determining the existence of any trends with distance from observation in the ratio of the virus to the bromide average arrival time.

### **C. Principle Findings and Significance:**

Results indicate that during the two field experiments the vast majority of the bacteriophage were either lost due to inactivation or “irreversibly” lost from the fluid phase due to retention by the aquifer solids, and hence were not transported in viable form via groundwater during the time period of this study. The shallow and deep experiments vary in the amount of viral mass transported to the first downgradient sampler. For the deep experiment performed in the contaminated groundwater, the viral mass decreased to  $10^{-4.5}\%$  relative to the observed bromide mass between the injection well and first down-gradient sampler (1.15 m). For the same distance, the loss for the shallow plume was much greater with the ratio of bromide to viral mass decreasing to  $10^{-10.1}\%$ . The higher initial loss in the shallow plume is likely due to the lower levels of organic matter and dissolved anions and to a lesser extent the lower pH (5.7 versus 6.1) of that groundwater. Decreases in each of these factors favors greater electrostatic exchange of negatively charged viruses to the positively charged ferric oxyhydroxides and clay minerals which coat portions of the soil grains. Sorption in this manner is predominantly irreversible during short time spans unless groundwater chemistry is altered.

The results showed that the viral plume in the contaminated groundwater required a much larger volume of aquifer than the uncontaminated region of the same aquifer to reduce similar numbers ( $10^{-7}\% C/C_0$ ) of mobile virus. As shown in a smaller-scale study by Ryan et al. 1999, this could be attributed to the fewer sites on the positively charged ferric oxyhydroxides and clay mineral soil coatings being available for the negatively charged virus to electrostatically exchange due to competition for those sites by organic matter, phosphate, and other anions.

After the initial high rate of loss in viral mobile mass seen in the first 4.4 m of transport through the sewage contaminated groundwater and in the first 1 m of the uncontaminated groundwater, the remaining viral mobile mass levels decreased at far slower rates. These mobile virus traveled at nearly the same rate as bromide, and experienced rates of concentration decline consistent with hydraulic dilution effects. The velocity of unattached PRD-1 is within  $\pm 30\%$  of the  $\text{Br}^-$  velocities. Most of the differences in calculated travel times result from more extensive tailing of the PRD-1. The tailing in the breakthrough curves is most likely due to rate-limited desorption of previously adsorbed bacteriophage. The results of this study indicate that a small, but infectious fraction of viable virus particles can persist and travel significant distances in sedimentary aquifers, despite differences in water chemistry.

## REFERENCES

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**Section 5 Publication Information:**

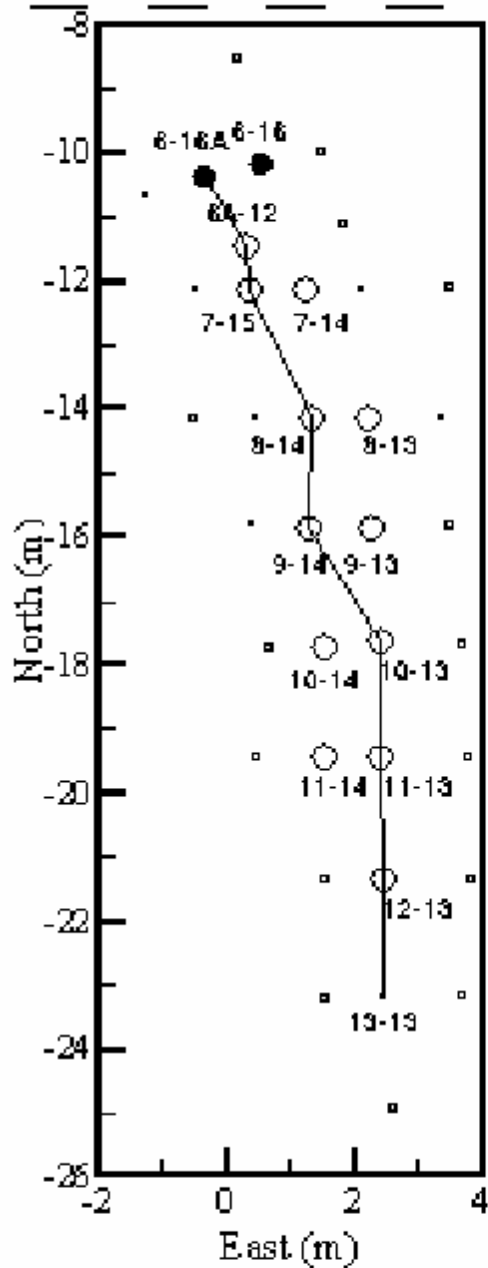
Blanford, William J.<sup>1</sup>, T. C. Jim Yeh<sup>1,2</sup>, Charles P. Gerba<sup>2</sup>, Roger C. Bales<sup>1</sup>, Ronald W. Havery<sup>3</sup>, Mark L. Brusseau<sup>1,2,\*</sup>. 2001. Influence of pH on Bacteriophage PRD-1 Transport in a Sandy Aquifer. Water Resources Research. Submitted.

**Section 6 Student Support:**

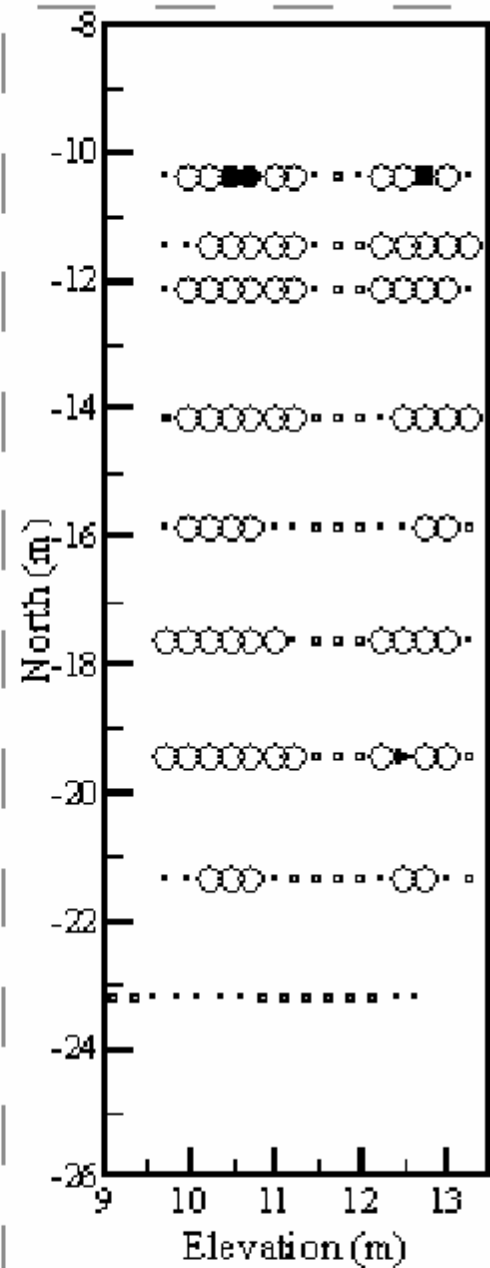
**Section 104 Base Grant**

PhD.: William J. Blanford

Figure 2 Location of Sample Ports Utilized in the Experiments



Plan View



Cross-section

- Deep Phase Injection Ports
- Shallow Injection Ports
- Complete Breakthrough Observed
- Incomplete Breakthrough Observed
- No Tracer Observed
- ◻ Directional Sampler
- ◂ Wellhead Cross-section

ELECTRICAL CONDUCTIVITY (US/GM)

