To the Graduate Council:

I am submitting herewith a thesis written by Andrew Boruff Kenst entitled "Virus Transport during Transient Unsaturated and Steady-State Saturated Flow Conditions in Memphis Aquifer Sand." I have examined the final paper copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Geology.

Ed Perfect, Major Professor

We have read this thesis and
recommend its acceptance:

Steve Wilhelm

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Vice Chancellor and Dean of
Graduate Studies

Virus Transport during Transient Unsaturated and Steady-State Saturated Flow Conditions in Memphis Aquifer Sand

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ABSTRACT

The overall goal of this research was to determine the effect of transient unsaturated flow conditions on the transport of a virus in aquifer material. It was hypothesized that viruses would be transported at the same rate and over the same distance as the migration of the wetting front and that virus retention during transient unsaturated flow would be similar to that during steady-state saturated flow. Virus transport during transient unsaturated horizontal flow was experimentally compared with its behavior under steady-state saturated vertical flow. In the transient flow experiment, virus (ϕX174) suspension was introduced into an initially air-dry repacked Memphis aquifer sand column with zero inflow head. In the steady-state saturated experiment, the sand was pre-saturated with water prior to virus injection at a constant inflow rate. Results obtained by sectioning the columns show that total (retained and free) virus concentrations decreased exponentially with the travel distance, and the decline was similar under transient unsaturated flow to steady-state saturated flow. Virus concentrations near the inlet in the Memphis aquifer sand columns were more than an order magnitude greater than the virus concentration of the influent solution in both experiments. A novel approach to quantifying virus retardation during transient unsaturated flow is proposed based on the centroids of the relative saturation and virus concentration versus relative distance functions. The results indicate similar virus retardation factors during transient unsaturated flow as compared to steady-state saturated flow. This may be a result of reduced air-water-solid interfaces as the wetting front moves through the soil during unsaturated transient flow.

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1.0 INTRODUCTION

Over 100 million people in the United States depend on underground sources for their potable water supply [*Atlas*, 1998]. Annually, nearly 50% of America's disease outbreaks are caused by contaminated groundwater [*Craun*, 1986]. The United States Environmental Protection Agency (USEPA) Science Advisory Board has recognized microbial contamination as one of the greatest challenges facing drinking water suppliers [*USEPA*, 1990]. While strict guidelines for treating surface waters for microbial contaminants exist, such regulations for controlling systems that use ground water are less stringent [*Macler and Merkle*, 2000].

Aquifers are subject to microbial contamination, both viral and bacterial, from a variety of sources, such as land application of human waste, broken sewer lines, and animal waste [*Woessner*, 2001]. Septic tank effluent is probably the greatest source of pathogenic viruses found in groundwater [*Rosen*, 2000].

The Memphis aquifer, composed of unconsolidated sand and overlain by loess, supplies much of the water used in Shelby County and west Tennessee. It is the main drinking water supply for over one-million residents in Shelby County alone [*Gentry*, 2002]. Much of the area east of Shelby County, along with many other parts of west Tennessee, relies on septic systems as the primary means of waste water disposal. Livestock production in the aquifer recharge area is another potential source of biological contaminants. Because of the large number of sources for biological contamination of underground water in west Tennessee, this study was designed to simulate viral contamination in order to determine the rate of virus transport through Memphis aquifer sand under initially unsaturated transient flow conditions and steady-state unsaturated flow conditions.

Bacteriophage will be used as analogs for pathogenic human viruses because they possess many of the same physical and chemical characteristics of viral human pathogens but are safer to work with than infectious viruses that typically pose contamination threats to subsurface water. Viruses are of greater concern than bacteria and protozoa because they are much smaller in size and may not be filtered out of soil water solutions

as easily as larger microorganisms [*Jin and Flury*, 2002]. Further concern involves virus survival time, which varies depending upon the pathogen of interest.

The ability of a porous medium to filter or disinfect biologically contaminated water depends on several factors thought to influence virus deactivation and/or adsorption, or "attachment" in colloid transport studies, to solid surfaces during water flow. These factors include soil water pH, soil solution ionic strength, metal oxides coating soil grains, and mass-fraction organic carbon of the soil. Virus characteristics, such as their isoelectric point (pH_{IEP}), and properties of their protein coat also affect their movement through soils and aquifer material. Hence, predicting groundwater vulnerability from viral contamination and modeling transport of these organisms is presently limited by an incomplete understanding of the factors that influence virus fate and transport.

1.1 LITERATURE REVIEW

1.1.1 Virus Characteristics

Viruses are parasites that require a host cell for replication and typically range in size from 20 to 100 nm [*Harrison*, 1985; *Powelson and Gerba*, 1995]. Those used in this study are termed bacteriophage, or phage, because they require a strain of bacteria as their host for replication. Virus characteristics, such as size, shape, and density are important for virus transport [*Jin and Flury*, 2002]. Net charge and hydrophobicity, which are determined by protein coat properties, also influence transport behavior.

Viruses are composed of either DNA or RNA surrounded by a protein coat (capsid) [*Harrison*, 1985]. The capsid is composed of protein subunits, and each of these subunits is a single folded peptide chain. Depending on the virus, it may have an envelope, composed of both glycoproteins and lipoproteins, which encloses the capsid.

Properties of the protein coat, either enveloped or unenveloped, greatly determine virus fate and transport through soils. Virus coats contain regions that are polar, non-polar, and ionic. The ionic regions have varying charges at different locations that are pH-dependent. Viruses are typically negatively charged in neutral solutions, but lowering the pH of the solution in which viruses are suspended causes surface proteins to shift which results in a net positive charge [*Reynolds and Pepper*, 2000]. Isoelectric

point is the term used to describe the solution pH necessary to cause this shift. When the solution pH is equal to a particular phage's pH_{IEP}, the net electric charge of the phage is zero [*Zhuang and Jin*, 2003]. Negatively charged viruses in neutral solutions tend to attract positively charged ions, which form an electrostatic double layer on the virus surface, which is an important factor controlling the magnitude and direction of the total interaction energy between the virus and sorbing surface [*Reynolds and Pepper*, 2000].

1.1.2 Virus Sorption and Inactivation Mechanisms

Developing an understanding of virus transport requires isolating the mechanisms responsible for virus sorption, transport and survival. It is debatable whether or not viruses are living organisms, so the term inactivation is used to describe a virus's inability to cause an infection, usually resulting from a decrease in virus concentration caused by the degradation of the protein coat and genetic material contained therein [*Gerba*, 1984]. Theories used to describe colloid behavior have also been applied to viruses, which are similar in size to colloids. There are numerous factors pertaining to viruses and the solid, air, and water phases of soils that influence virus fate and transport in the subsurface. Many of these factors vary according to the environment and the particular virus present in the system, so depending on a complex combination of factors, each system is unique. Difficulties in isolating the mechanisms responsible for virus fate and transport are due, in part, to the wide array of methods that have been developed to study viruses and their interactions with their surroundings [*Jin and Flury*, 2002]. The following describes some of the mechanisms that have been shown to influence virus survival, sorption, and consequently, transport in porous media.

Early work by *Gerba* [1984] reviewed the diffuse double-layer theory with respect to virus sorption, which explains why colloidal systems, overall, remain neutral even though individual colloids (i.e. viruses) may have a net charge. Colloidal systems are electrically stable because attractive van der Waals forces and repulsive diffuse double-layer interactions balance one another. *Chattopadhyay and Puls* [1999] concluded, however, that electrostatic interactions are less important and the dominating factor controlling sorption of virus particles is most likely the hydrophobicity of the virus and sorbing surface. The work of *Bales et al.* [1993] also stated that hydrophobic effects

could be much more important in controlling the sorption of MS2 bacteriophage to soil.

Virus sorption is typically thought to increase in the presence of cations, especially divalent cations, because they compress the electrostatic double layer around viruses [*Gerba*, 1984]. Generally, as the cation concentration of the soil solution increases, virus sorption to surfaces increases [*Bales et al.*, 1991; *Bitton et al.* 1976]. Solution pH is another important factor influencing virus sorption because it governs the overall net charge of viruses [*Bales et al., 1991*; *Goyal and Gerba*, 1979]. In low pH solutions, virus sorption is favored if the pH_{IEP} of the virus and sorbing surface are low. This is usually the case in natural systems. Temperature has an effect on virus sorption as well. *Bales et al.* [1991] observed that MS2 was more likely to sorb to solids at higher temperatures. However, increased temperature can lead to virus inactivation [*Straub et al.*, 1992; *Yahya et al.*, 1992; *Yates et al.*, 1985].

Organic matter has been shown to affect virus transport in a variety of ways. It can compete for adsorption sites in soils, and it sorbs to air-water interfaces [*Yates and Yates*, 1988; *Jin and Flury*, 2002]. Amassed organic matter at air-water interfaces may protect viruses from inactivation. Organic matter that blocks sorption sites on soil grains has been shown to increase virus transport [*Gerba et al.*, 1975; *Goyal and Gerba*, 1979; *Pieper et al.*, 1997]. *Gerba et al.* [1981] found that virus sorption increased with increased organic matter content for certain types of viruses even in soils with very low amounts of organic matter (0.27 to 4.2%). Lipid-containing phage were strongly sorbed to porous media coated with organic matter, and it was theorized that hydrophobic reactions were the cause of the increased sorption [*Bales et al.*, 1991]. Other work has shown that organic matter is relatively poor at sorbing certain types of viruses, such as poliovirus [Sobsey et al., 1980]. *Bitton et al.* [1986] also showed that organic materials had little effect on the sorption of viruses. The role of organic matter is one of the more ambiguous and least understood mechanisms influencing virus fate and transport. Clearly, the type of virus, as well as other soil physical and chemical factors, influence the role of organic matter. Virus adsorption to mineral solids, such as clay particles, can also help prevent virus inactivation. Protection of viruses by adsorption to solids does not hold true in the presence of many metal oxides, however. Iron and aluminum oxide

coatings on soil grains have been shown to increase virus sorption, thus reducing transport distances [*Chu et al.*, 2000]. Recent work by *Zhuang and Jin* [2003] showed similar effects for aluminum oxide coated grains. Phage sorption resulted from the strong attraction between the negatively charged phage and positively charged aluminum oxide. The effect of the ionic composition and strength of solution was also tested. As the concentration of divalent cations was increased, virus transport increased because the cations "screened" the phages' negative charge. Phage transport increased with increasing phosphate in solution, but the same effect was not observed in the presence of bicarbonate. The two bacteriophage used in the study, MS2 and ϕX174, also behaved differently in the presence of these two compounds, in that transport of MS2 was enhanced more than the transport of ϕX174.

1.1.3 Soil Water Content and Flow Conditions

The majority of virus transport studies have been laboratory column experiments conducted under steady-state saturated and unsaturated flow conditions. Table 1 is a summary of all virus work conducted under steady-state unsaturated flow through soil columns. Viruses tend to survive longer in moist media and can travel farther under saturated flow conditions [*Jin and Flury*, 2002; *Jin et al.*, 2000; *Lance and Gerba*, 1984; *Powelson et al.*, 1990; *Powelson and Gerba*, 1994]. In the work of *Lance and Gerba* [1984] sewage water containing poliovirus 1 was pumped through columns equipped

Table 1: Summary of column virus transport experiments performed under steady state unsaturated flow conditions [adapted from *Jin and Flury*, 2002].

Authors	Porous Media	Virus
Chu *et al.* (2001)	Accusand	MS2, ϕX174
Chu et al. (2003)	5 Soils	MS2, ϕX174
Jin *et al.* (2000)	Ottawa Sand	MS2, ϕX174
Jorgensen (1985)	Sand, Sandy Loam	Coxsackievirus B3, Adenovirus 1
Lance and Gerba (1984)	Sand	Poliovirus 1
Powelson *et al.* (1990)	Loamy Fine Sand	MS2
Powelson *et al.* (1991)	Loamy Fine Sand	MS2
Powelson and Gerba (1994)	Coarse Sand	MS2, PRD1, Poliovirus 1

with sampling ports along the column. Columns were filled with loamy sand and sampled under both unsaturated and saturated steady state conditions. Virus penetration in the columns was reduced under unsaturated conditions, which was attributed to thinner films of water surrounding soil grains. Thinner water films were thought to increase the likelihood of viruses contacting and sorbing to soil particles. Similar work was conducted by *Jorgensen* [1985] using sewage sludge containing coxsackievirus B3 and adenovirus 1. The virus solution was percolated through 30 cm steady-state unsaturated and saturated columns. One column was filled with sand and another with sandy loam. Effluent was assayed, and at the end of the experiment, soil samples from various depths of the columns were sampled for viruses after the columns had drained. No breakthrough of either virus was observed in the unsaturated soil and sand columns. Also, neither type of virus was detectable in eluent samples collected below 3.5 cm in the unsaturated columns. In the saturated columns, viruses were detected in nearly all the effluent samples collected from the sandy loam columns. None of the effluent samples collected from the saturated sand columns contained viruses, but viruses were detected at a depth of 14 cm in the sand column. Overall, transport was greatest in the saturated sandy loam column.

Powelson et al. [1990] studied the effect of water content on MS2 bacteriophage transport through steady-state saturated and unsaturated soil columns containing a loamy fine sand. Both effluent from the column and liquid extracted from sampling ports was assayed. Only 39% of viruses were accounted for in unsaturated flow conditions, which was attributed to inactivation. However, under saturated flow conditions, MS2 was highly recoverable and showed little loss to inactivation and adsorption.

The effect of organic matter on MS2 transport through steady state unsaturated soil columns was studied in the work of *Powelson et al.* [1991]. The same soil and sampling procedure was used as in the work of *Powelson et al.* [1990]. Virus concentration was greater in soil with higher organic matter concentration. It was concluded that organic matter competes with viruses for sorption sites at the air water interface, which reduced surface tension. This tension could possibly damage and

inactivate viruses, so a reduction in tension was thought to increase virus survival in soils containing high levels of organic matter.

Powelson and Gerba [1994] again found that unsaturated steady state flow conditions result in greater virus removal than saturated flow. Three viruses, MS2, PRD1, and poliovirus 1, were used in this study, and it was shown that virus type greatly influenced transport. Poliovirus was retarded more than PRD1 and MS2. Adsorption coefficients based on batch study data were used to calculate retardation, which was much higher than column retardation.

A comparison of the breakthrough curves for the conservative tracer bromide, MS2, and ϕX174 was conducted in a steady state column experiment by *Jin et al.* [2000]. Ottawa sand was used in vertical columns equipped with fraction collectors. One column was saturated, and the other was only partially saturated. It was made clear that virus breakthrough is reduced under partially saturated conditions compared to saturated conditions. It was also noted that virus characteristics, such as pH_{IEP}, also play a role in transport. The same amount of ϕX174 was recovered from both saturated and unsaturated columns, indicating that sorption was the dominant mechanism controlling virus retardation. Inability to recover MS2 from the unsaturated column was attributed to inactivation rather than adsorption. Possible mechanisms governing virus sorption and inactivation were also discussed, including sorption to solids, the role of the air-water interface (AWI), and film straining.

Chu et al. [2001] performed saturated and unsaturated steady state flow column experiments to study the effect of water content and metals on virus transport in which a pulse of virus was introduced to columns packed with Accusand containing iron oxide (reactive), as well as Accusand that was treated to remove metal oxides (inert). At the end of the experiments, beef extract was pumped through columns to desorb any virus attached to the sand. Mass balance analyses showed that ϕX174 sorption was largely reversible. MS2 mass recovery was low, indicating inactivation, and the degree of recovery was directly correlated to water content. Lower water content reduced recovery of MS2. At low water contents, reactive sand resulted in increased retention of both MS2 and ϕX174. In the inert sand, water content had an observable but minimal effect on the

retention of both viruses. A conceptual mathematical model was developed to test whether reactions at the AWI or solid-water interface (SWI) were responsible for virus removal in the reactive sand. Modeling indicated, as the measured results suggested, that the SWI was likely responsible for virus sorption at low water contents. Additionally, the role of the air-water-solid (AWS) interface was discussed, but deemed not present in these columns because of calculated thicknesses of water films surrounding soil particles and that the columns were saturated prior to establishing any unsaturated water content. It was noted, however, that more information concerning water distribution in the columns was needed before the role of AWS interfaces could be discussed further. The study concluded that not only water content, but also virus type and other factors, such as soil physicochemical properties, affect virus transport.

The previous work was furthered by *Chu et al.* [2003] who performed similar column studies using five different soils and the same bacteriophages as in *Chu et al.* [2001]. The soils used were more complex than the Accusand, with varying physicochemical properties such as metal oxides, organic matter (OM), and pH. The effect of water content in the five soils varied greatly and was attributed to various soil surface properties. It was concluded that water content may have a large or minimal effect depending on soil type. Metal oxides were again shown to greatly increase virus removal, and the authors proposed such soils be used as reactive barriers to help prevent virus movement in the subsurface. These last two studies illustrated that as flow regimes become more complex, a variety of transport mechanisms interact to determine the overall transport and retention of viruses in soils.

Theories used to describe colloid transport may also be applied to virus transport. *Crist et al.* [2004, 2005] observed colloid attenuation at AWS interfaces that are present between grains in partially saturated soil. *Wan and Tokunaga* [1997] observed film straining of colloids, which occurs as a result of particle size in relation to water film thickness surrounding soil grains. When the ratio of colloid diameter to water film thickness is less than unity, transport is more likely to occur. If however, this ratio is greater than unity, colloids become less mobile because of capillary forces that pull the colloid toward the grain surface.

To date, limited research has been conducted to examine virus movement through unsaturated media under transient-flow conditions. Only a few studies have investigated virus transport under unsaturated conditions, and all of these were for steady-state flow. Under such conditions, AWS interfaces are present throughout the medium, and water films tend to be thinner and more tortuous. AWS interfaces are thought to increase virus deactivation, while thinner water films increase the likelihood of viruses adsorbing to soil particles.

Steady-state flow rarely occurs in nature. Most flow in the vadose zone occurs under transient conditions, in which a zone of saturation forms as water advances into previously unsaturated soil. Under such conditions, water films are expanded and AWS interfaces are mostly restricted to the wetting front. Also, this work focused on virus distribution over time within columns as opposed to simply measuring column effluent, which results in breakthrough curves to describe virus transport.

1.2 GOAL

The overall goal of this research is to determine the effect of transient unsaturated flow conditions on the transport of a virus in aquifer material.

1.3 OBJECTIVES

The three main objectives of this study are to:

1. determine the extent and rate of transport of a bacteriophage under unsaturated, transient flow conditions in typical Memphis Aquifer sand,
2. determine the extent and rate of transport of bacteriophage under saturated, steady-state flow conditions in typical Memphis Aquifer sand,
3. develop methods to quantify bacteriophage retardation under transient flow conditions.

1.4 HYPOTHESES

1. Viruses will be transported at the same rate and over the same distance as the migration of the wetting front.
2. Virus retention during transient, unsaturated flow should be similar to that during saturated, steady-state flow.

2.0 MATERIALS AND METHODS

2.1 FIELD SITE DESCRIPTION

The Memphis aquifer in Shelby County is composed of unconsolidated sand, silt, and clay, with small amounts of lignite in some of the sand layers [*Brahana et al.* 1987]. Figure 1 shows an expanded cross section of the Memphis aquifer and surrounding strata. The aquifer sand dates back to the early to mid-tertiary Period and dips to the west in Shelby County. This layer is overlain by several fluvial deposit layers that fine up from sand and gravel to sandy and clayey silt. This layer is as deep as 18 meters in some of the present-day valleys and rivers in the area. There is a layer of loess overlying the alluvium, ranging in thickness from 20 meters near the Mississippi River Bluffs to 3 meters as it thins to the east.

Microbiological contamination is becoming a concern because of the large number of septic systems in the aquifer recharge zone. The upper Claiborne confining unit, a clay-rich aquitard that partially protects the lower Memphis aquifer, overlies most of the Memphis aquifer in Shelby County, but there are openings in the aquitard, called windows, that possibly allow recharge, along with contaminants, to enter the aquifer [*Gentry et al.*, 2002]. The aquifer outcrops in eastern Shelby County, which is another potential site for contaminant entry due to the aquifer's direct exposure to the surface. Down dip of the sand layer outcrop, there is a window in the Claiborne aquitard beneath Shelby Farms in the Eastern part of Shelby County. The combination of the exposed sand layer and window make this particular part of the county a likely point of entry for contaminants to the aquifer. Similar windows exist in several other parts of the upper Claiborne confining unit, which, over time, allow recharge of the Memphis Aquifer by lower quality water from overlying shallow, unconfined aquifers.

2.2 MEMPHIS AQUIFER SAND CHARACTERIZATION

A variety of methods were used to characterize the unconsolidated sediment used in this study. Memphis aquifer sand, collected from rotasonic cores from the up-dip area at Shelby Farms, was obtained by Dr. Randy Gentry of the Department of Civil and Environmental Engineering, University of Tennessee, for use in the bacteriophage batch adsorption and column studies. The sand is a composite sample collected over a depth

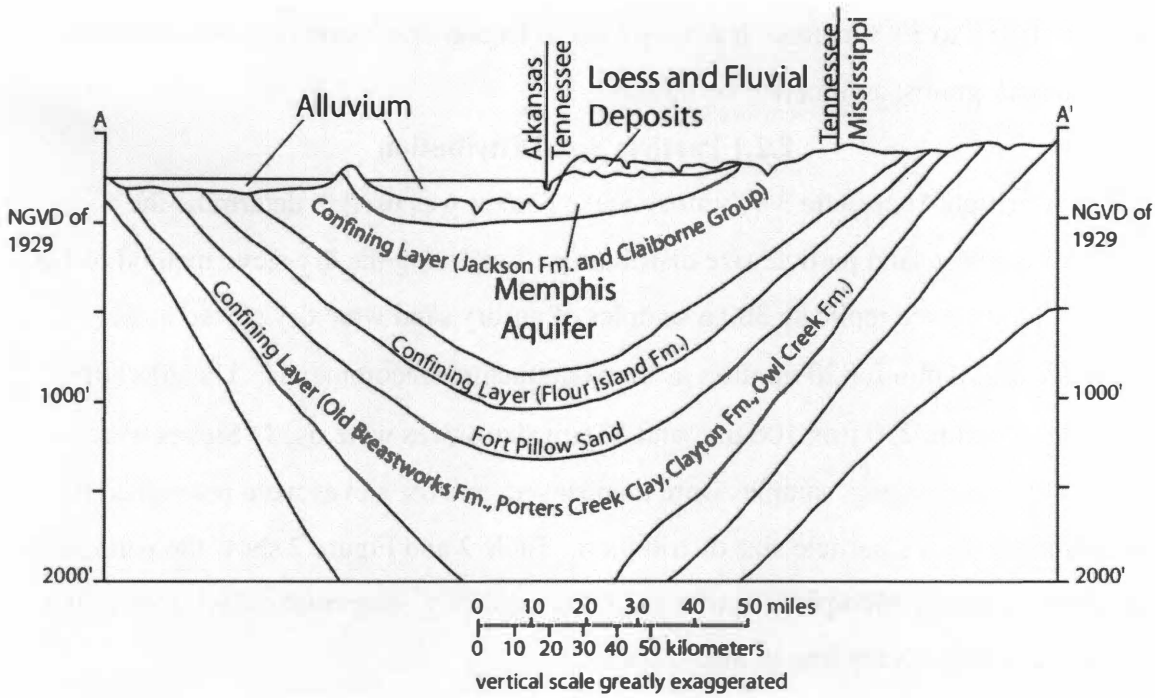


Figure 1: Expanded cross section of the Memphis aquifer and surrounding strata. The line A to A' strikes NNW to SSE [adapted from *Brahana et al.* 1987].

interval of 32.0 to 33.8 meters. It is very fine- to fine-grained sand that contains some clay and dark grains, assumed to be lignite.

2.2.1 Particle Size Distribution

A Fritsch Analysette 3 Vibratory Sieve Shaker was used to determine the Memphis Aquifer sand particle size distribution. Following the dry sieve method of *Gee and Or* [2002], three replicate 300 g samples of air dry sand were dry sieved at an amplitude of 2.0 mm for 20 minutes as the manufacturer recommends. The 2000 μm, 1000 μm, 500 μm, 250 μm, 106 μm, and 53 μm size sieves were used. Sieves were weighed prior to sieving, samples were then sieved, and the sieves were reweighed to determine the sand's particle size distribution. Table 2 and Figure 2 show the particle size distribution for Memphis Aquifer sand. Silt and clay comprised only 1.35% of the sample. The rest is very fine to medium sand.

2.2.2 Water Retention Curve

A tension table, similar to the tension plate method described by *Hillel* [1998] was used to measure the water retention curve for Memphis Aquifer sand. The table was filled with SPHERIGLASS solid glass microspheres (Potters Industries Inc., A-glass: product grade 2530) with a mean diameter of 71 μm. 90% of the glass beads used in the tension table were 100 microns or less in diameter, which is smaller than 98% of the Memphis Aquifer sand particles. This allowed for tension table measurements up to 70 cm of tension before the water column broke, and air entered the system. Experiments were performed in triplicate.

Table 2: Average and standard deviations of three replicate particle size distributions for Memphis aquifer sand.

Sieve size (μm)	% Less than (average of 3 reps)	Standard deviation
2000	99.991	0.008
1000	99.922	0.023
500	99.807	0.016
250	39.794	1.014
106	2.044	0.026
53	1.347	0.086

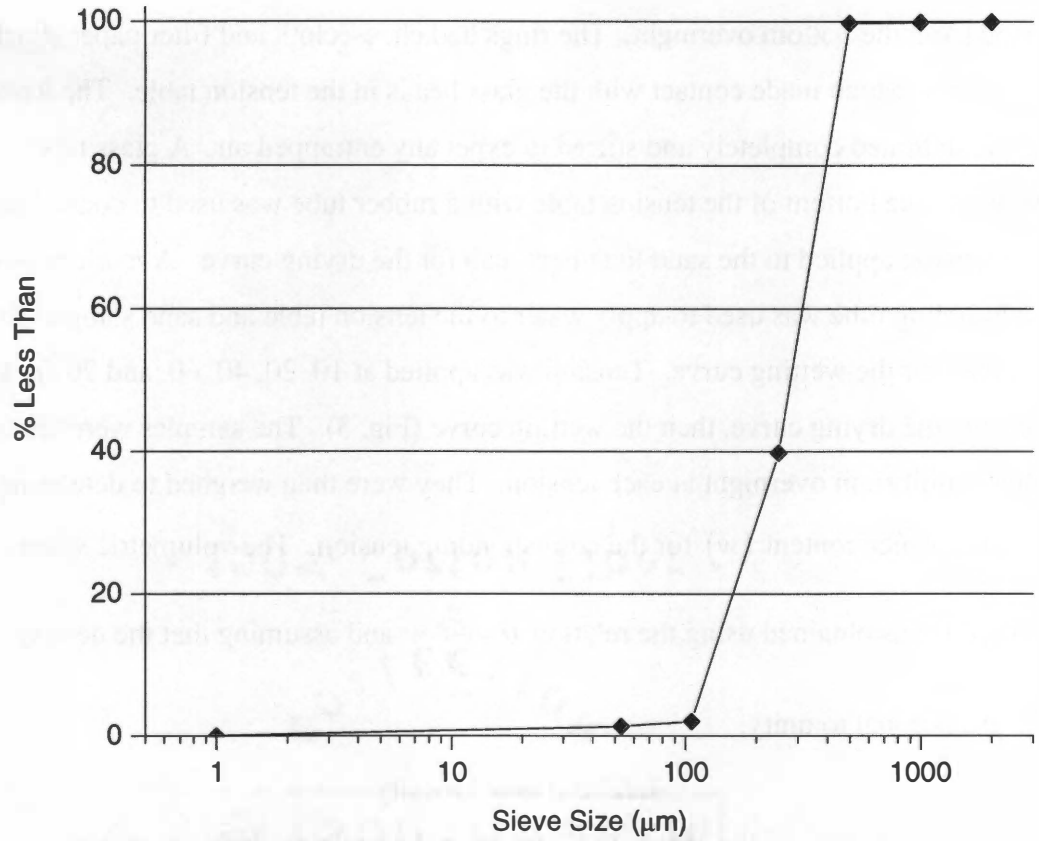


Figure 2: Average of three replicate particle size distributions for Memphis aquifer sand. Error bars were not included because the error between replications was too small to visually plot.

Sand was packed in stainless steel rings to a bulk density (ρ_b) of 1.56g/cm^3 and saturated from the bottom overnight. The rings had cheesecloth and filter paper attached to the bottom, which made contact with the glass beads in the tension table. The tension table was saturated completely and stirred to expel any entrapped air. A glass tube connected to the bottom of the tension table with a rubber tube was used to control the desired tension applied to the sand to collect data for the drying curve. A mariotte bottle with a bubbling tube was used to apply water to the tension table and sand samples to collect data for the wetting curve. Tension was applied at 10, 20, 40, 60, and 70 cm to collect first the drying curve, then the wetting curve (Fig. 3). The samples were allowed to reach equilibrium overnight at each tension. They were then weighed to determine the gravimetric water content (w) for the corresponding tension. The volumetric water content (θ) was obtained using the relation $\theta = \frac{\rho_b}{\rho_l} w$ and assuming that the density of water, ρ_l, is equal to unity.

2.2.3 Particle Density

Particle density of the Memphis Aquifer sand was determined using the pycnometer method as described by *Flint and Flint* (2002). This method is based on weight and the volume of water displaced inside a pycnometer, which is a small, stoppered glass container. The stopper has a capillary tube in it for excess water overflow and removal. Particle density (ρ_p) is calculated using the following equation:

$$\rho_p = [\rho_w (W_s - W_a)]/[(W_s - W_a) - (W_{sw} - W_w)] \quad [1]$$

where ρ_w is the density of water (g cm^{-3}) at the temperature observed, W_s is the weight of the pycnometer plus the weight of the soil sample corrected to oven-dry water content, W_a is the weight of the empty pycnometer, W_{sw} is the weight of the pycnometer filled with soil and water, and W_w is the weight of the pycnometer filled with water at the temperature observed. The mean particle density of three Memphis aquifer sand samples was 2.59 ($\pm$ 0.04).

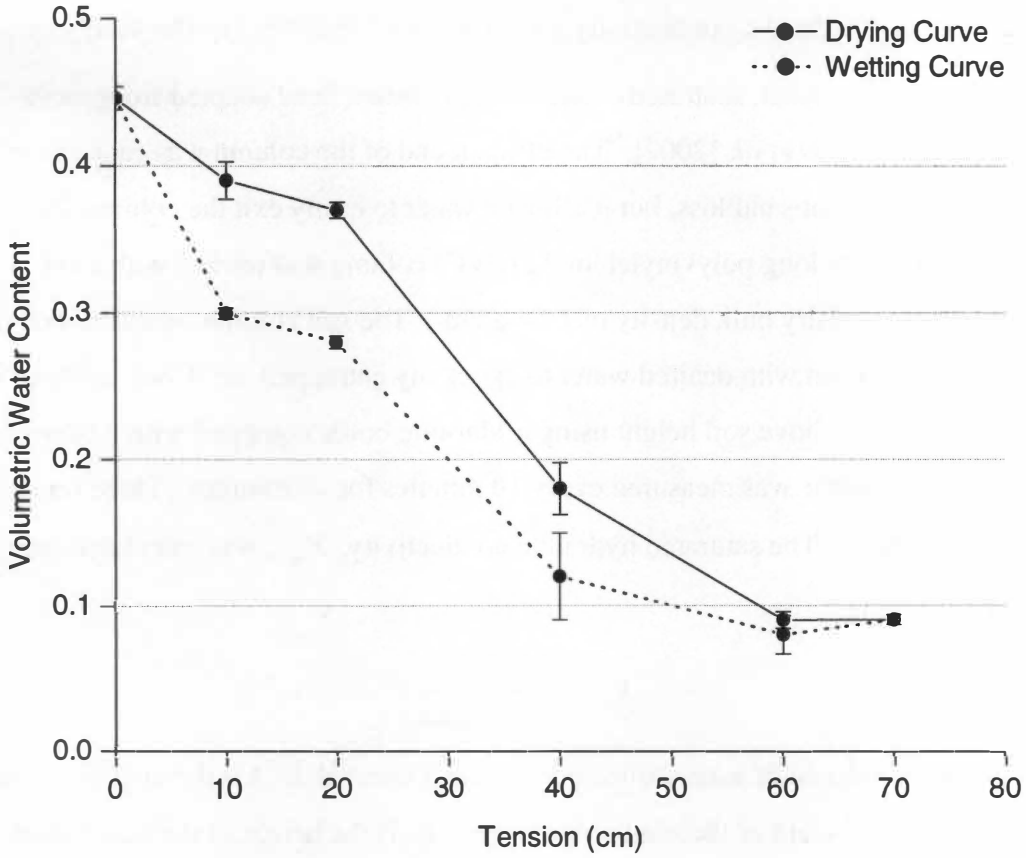


Figure 3: Average of three replicate water retention curve measurements with error bars indicating standard deviation.

2.2.4 Saturated Hydraulic Conductivity

Saturated hydraulic conductivity (K_{sat}) for the Memphis Aquifer sand was measured using a vertical, saturated column with constant head adapted from methods described by *Reynolds et al.* [2002]. The effluent end of the column was capped with cheesecloth to prevent sand loss, but it allowed water to easily exit the column for collection. A 30 cm long polyvinylchloride (PVC) column was packed with sand to a height of 20 cm and dry bulk density of 1.54 g/cm^3. The soil column was saturated over night from the bottom with deaired water to expel any entrapped air. Constant head was maintained at 8 cm above soil height using a Mariotte bottle equipped with a bubbling tube. Effluent volume was measured every 10 minutes for 70 minutes. Three replicate columns were used. The saturated hydraulic conductivity, K_{sat}, was calculated using the following equation,

$$K_{sat} = \left(\frac{V}{At}\right)\left(\frac{dz}{dh}\right) \qquad [2]$$

where V is the volume of water collected over time interval t, A is the area of the sand column, dz is the height of the sand column, and dh is the height of the water level with respect to the bottom of the column. The geometric average K_{sat} for the three replicates was 8.34×10^{-5} m/sec with a standard deviation factor of 1.11. The standard deviation factor was calculated by exponentiating the standard deviation of the natural logs of the three K_{sat} measurements.

2.2.5 Chemical Properties

Soil tests were performed on the Memphis aquifer sand by the Agricultural Extension Service of the University of Tennessee Institute of Agriculture. The Mehlich 1 extraction method was used to test for elements, such as calcium, magnesium, zinc, copper, iron, and manganese, which could influence virus transport [*Mehlich*, 1953]. Organic carbon was determined using the Walkley and Black method, which measures the amount of oxidizable organic carbon in the soil [*Walkley and Black*, 1934]. Soil water pH was also measured, using an electrometer to test the pH of a Memphis sand and deionized water slurry [Thomas, 1996]. Table 3 shows the results of these tests.

Table 3: Summary of chemical data for Memphis aquifer sand.

P (mg/kg)	K (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Fe (mg/kg)	Organic Matter (%)	Soluble Salts (PPM)	Soil Water pH
1.8	7.3	42.1	11.9	8.7	0.4	42	5.7

*PPM = Parts per Million

2.3 VIRUS CULTURE AND ENUMERATION

The agar layer assay method, described by *Adams* [1959], is considered the standard for virus culture and assay in experiments such as this. In general, a solution containing viruses is serially diluted and added to nutrient agar that contains host bacteria. The agar is then poured into a Petri dish that already contains a layer of agar. The bottom layer of agar provides nutrients to grow the host bacteria, which are used by viruses for replication. As the hosts replicate, an opaque lawn of bacteria develops on the originally clear agar. When viruses begin to replicate, clearings form in the bacterial lawn, indicating dead bacteria. These clearings are colonies of viruses, which are called plaques. Each plaque forms from a single virus particle referred to as a plaque forming unit (pfu). The plaques can be counted to determine the number of pfu's in the volume of solution that was applied to the Petri dish. Calculations can then be made to determine how many pfu's were in the original, undiluted volume of liquid. Acceptable error associated with this method is $\pm 20\%$ [*Chu et al.* 2001]. This method has been widely used by others studying bacteriophage movement through soils.

2.4 GROWTH MEDIA, BACTERIAL HOST, AND VIRUS PREPARATION

Bacteriophage ϕX-174, was used in this study because it is easy to culture and assay, is non-infectious, and relatively inexpensive. ϕX-174 ATCC 13706-B1 and its host *Escherichia coli* ATCC 13706 are currently available from the American Type Culture Collection (ATCC). ϕX-174 has been used to model a variety of enteric viruses including, Caliciviruses such as Norwalk and Norwalk-like viruses [*Newby et al.*, 2000; *Redman et al.*,1997]. It is a hydrophilic, single-stranded DNA phage ranging in diameter from 25 to 27 nm, and has a pH_{IEP} of 6.6 [*Ackermann and Dubow*, 1987].

Information regarding the growth and handling of ϕX-174 and its host bacteria are available at http://www.atcc.org. *E. coli* requires a growth medium (bottom agar) composed of 23 g of nutrient agar and 5 g of NaCl per 1L of distilled water. Only 6 g of nutrient agar was used to make the top agar. Both top and bottom agar were autoclaved for 20 minutes at 121°C and 103.4 kPa. A thin layer of bottom agar was then poured into petri dishes, which were allowed to cool, then placed in a cold room at 4°C until used for plaque assay.

Liquid broth for growing concentrated *E. coli* stock was made with 5 g of NaCl, 5 g of peptone, and 3 g of beef extract per 1 L of distilled water. It was autoclaved as well, and 8 mL of broth was pipetted into sterile polypropylene culture tubes to which *E. coli* were added. The tubes were incubated for 24 hours at 37°C to grow a high concentration stock of host, which was then placed in a cold room at 4°C until used. Host bacteria must be in log phase growth when used to culture viruses so that a sufficient amount of bacteria is produced for virus replication. This increases the chance of a virus contacting a bacterium, replicating, and producing a plaque so that an accurate plate count is achieved. To accomplish this, 150 mL of broth was pipetted into a sterile glass flask to which 2 mL of broth containing bacteria was transferred from one of the culture tubes in cold room storage. The flask was then incubated for 4 hours at 37°C prior to use for plaque assay.

A high concentration stock of ϕX174 was also necessary for use in the batch and column studies. Freeze dried bacteriophage and host arrive from ATCC in glass tubes. Viruses and bacteria are separately regenerated by mixing each with 1 mL of the broth used to grow *E. coli* 13706. Bacteria were regenerated first and grown into a high concentration stock and stored as described above. Next, bacteria were grown in a flask until log phase growth was achieved. The agar layer assay technique was used to expose viruses in the regenerated, undiluted virus stock in order to grow large numbers of pfu's. Petri dishes containing ϕX174 and its host bacteria, were next placed into an incubator at 37°C for 5 hours in order to grow a large number of viruses. When plaques formed on the plates, 2 mL of phosphate buffered saline solution (PBS) were added to each plate, the plates were rotated slowly by hand to help wash viruses into solution, and the plates

were placed in a cold room at 4°C for 24 hours. PBS has an ionic strength of 0.163 M and pH 7.5 and is composed of Na_2HPO_4 (20 mM), NaCl (100 mM), and KCl (3 mM). The next day, the plates were again each rotated slowly by hand, and the supernatant in each Petri dish was collected and passed through a 22 μm syringe filter to remove bacteria and cellular debris. The filtered supernatant at this point contained only ϕX174. This process was repeated until 500 mL of virus stock with a concentration of approximately $5x10^9$ pfu/mL was created. Viruses in PBS solution were stored at 4°C to prevent loss due to inactivation.

2.5 VIRUS BATCH AND COLUMN STUDIES

2.5.1 Virus Batch Tests

Batch experiments were conducted to determine the distribution coefficient (K_d) for ϕX174 bacteriophage when exposed to Memphis Aquifer sand. All the batch experiments were performed using PBS containing approximately 1×10^4, 1×10^5, 1×10^6, and 1×10^7 pfu/mL concentrations. This range of inputs was chosen because the virus concentration used for the column experiments was approximately 1×10^6 pfu/mL. It should be noted that the exact input concentrations varied among the three replicates performed for each of the virus experiments in this study, but input concentration was always measured, recorded, and used to accurately determine virus distribution in column experiments and, in the case of batch experiments, virus partitioning. Batch experiments were designed to be as similar as possible to the column flow experiments regarding time, temperature, and sampling protocol. BEX was used in the flow experiments to elute phage from the sand, so the partitioning of virus in BEX as well as the efficiency of BEX at desorbing viruses from Memphis Aquifer sand was also tested in these experiments. All experiments were conducted at room temperature (approximately 20°C), and virus samplings were performed in duplicate.

Five grams of Memphis Aquifer sand were added to sterile 15 mL polypropylene centrifuge tubes, each containing 4 mL of PBS with viruses at a concentration equal to one of the four concentrations mentioned above. Adding sand to the liquid insured complete saturation of the sand. The sample was allowed to equilibrate for 20 minutes then the liquid supernatant was sampled. This time was chosen because it is

approximately equal to the time viruses were exposed to the sand in the column experiments before the addition of BEX. The sampled supernatant was serially diluted in PBS, and added to a borosilicate glass tube containing 0.5 mL of high concentration *E. coli* stock and 4 mL of 0.6% top agar maintained at 40°C. The glass tube was then vortexed briefly and poured into a Petri dish that had been warmed to 37°C, which already contained 2.3% bottom agar. The plate was rocked to spread the 0.6% top agar, allowed to cool, then placed in an incubator. Plaques became visible after approximately 5 hours, at which point they were counted. Multiplying the number of plaques on a plate by the dilution factor yields the concentration of virus in the sampled volume of liquid. The number of viruses in the sampled PBS solution relative to the number of viruses initially input to each tube was determined by multiplying the number of pfu's on a plate by the volume of liquid in the tube. Next, the number of viruses sorbed to solids was calculated by difference, assuming there was no loss of viruses due to inactivation. This showed virus partitioning between the liquid and solid phases of the solution under saturated, zero flux conditions. An adsorption isotherm was constructed based on the results of the batch test for virus partitioning between solid and liquid phases of the soil in the presence of PBS.

The partitioning of ϕX174 in PBS was non-linear, so the data were fitted to a Langmuir isotherm [Jin, 2002] using non-linear regression analysis in the SAS software package. A K_d for viruses in PBS was then estimated from the slope of the modeled isotherms at low virus concentrations, which is equivalent to the product of the two parameters from the Langmuir model. Once K_d was determined, it was then possible to calculate a batch retardation coefficient (R) for viruses in the presence of PBS (R_{PBS}) using the following expression [*Clothier et al.*, 1988],

$$R = 1 + (\rho_b K_d / \theta_s) \qquad [3]$$

where θ_s is the saturated water content calculated using the expression $\theta_s = 1 - (\rho_b / \rho_s)$. Coefficient of variation (CV) was used to determine the precision among replicates in all experiments performed and was calculated by dividing the standard deviation of the replicates by the mean of the replicates.

After sampling the batch tubes containing PBS, 8 mL of Beef extract solution (BEX) was added to each sample. The samples were shaken by hand for 5 seconds to mix the BEX, sand, and PBS, then allowed to equilibrate for two hours at room temperature. Next, the tubes were shaken again, then sampled, serially diluted, plated, and counted as described previously. The number of viruses in the BEX and PBS solution relative to the number of viruses initially input to each tube was determined by multiplying the concentration of free viruses counted as plaques by the volume of liquid in the tube. Next, the number of viruses sorbed to solids was calculated by difference, assuming there was no loss of viruses due to inactivation.

An adsorption isotherm was constructed based on the results of the batch test for virus partitioning between solid and liquid phases of the soil in the presence of BEX. A linear model best described the partitioning of ϕX174 after the addition of BEX. There was no change in the partitioning of this virus in Memphis Aquifer sand as concentration changed, therefore the slope of the line corresponds to the K_d for virus in the presence of BEX [*Papiernik et al.*, 2002]. An R value for virus in the presence of PBS and BEX (R_{BEX}) was calculated from the resulting K_d using equation [3]. The efficiency of BEX at desorbing viruses from soil surfaces was also tested by comparing the number of viruses initially input in each sample tube to the number of viruses recovered using BEX.

2.5.2 Virus Transient Unsaturated Flow Experiments

A 20 cm long horizontal soil column with an inside diameter of 2.05 cm constructed of clear polyvinyl chloride (PVC) was used for the three replicate transient unsaturated flow experiments. The column was scored in 1 cm increments using a pipe cutter to make segmenting the column at the end of the experiment easier. The empty column was weighed, then packed with initially air-dry Memphis Aquifer sand using the standard tap and fill method, which ensured uniform ρ_b along the column [*Snyder and Kirkland*, 1979]. The ρ_b of the sand used in these measurements was 1.61 g/cm^3 ($\pm$ 0.003). Both ends of the column were equipped with a PVC fitting that contained a sintered stainless steel plate with an average pore size of 100 μm and rubber gasket to seal the tube and steel plate. This allowed PBS solution containing viruses to enter the

column and air to exit the other end of the column as water infiltrated. Teflon® tubing was used throughout the apparatus, except for the influent fitting which was PVC. The column was tested for virus interaction by slowly pouring 300 ml of PBS containing approximately 1×10^6 pfu/mL through an empty column and collecting the effluent. The concentration of effluent showed no measurable change from the influent concentration, indicating minimal interaction between the viruses and the materials used in the apparatus.

The PBS and virus solution was introduced to the column under zero pressure head from a Mariotte bottle equipped with a bubbling tube (Fig. 4). This ensured that the only mechanism controlling the transport of water along the column was the matric potential of the porous material (capillary action). PBS was allowed to infiltrate into the column a distance of 15 cm before the supply was stopped. This left at least four column segments completely dry to act as controls. The column was detached from the Mariotte bottle and sealed at the effluent end to minimize PBS and virus redistribution in the column.

Next, the column was segmented using a pipe cutter, and the soil in each segment was weighed to determine the water content. It was then washed into 15 mL polypropylene centrifuge tubes with BEX. Each sample was shaken by hand for five seconds to mix the BEX, sand, and PBS. The samples were then allowed to equilibrate for two hours at room temperature. The tubes were shaken again, vortexed briefly, then sampled, serially diluted, plated, and counted as described previously.

The relative water content (θ / θ_s) and relative virus concentration (C / C_o), where C is the virus concentration of the pore water in each column segment (pfu/ml) and C_o is the virus concentration of the input solution (pfu/mL), were measured in each segment and expressed as functions of $d/d_{max\ water}$ where d is distance and $d_{max\ water}$ is the maximum distance the wetting front moved. The $d_{max\ water}$ parameter corresponds to the distance at which the first dry column segment was measured in the transient unsaturated flow experiments and the maximum distance the water was pumped in the steady-state saturated flow experiments.

A novel approach was used to estimate R based on the ratio of the centroids for

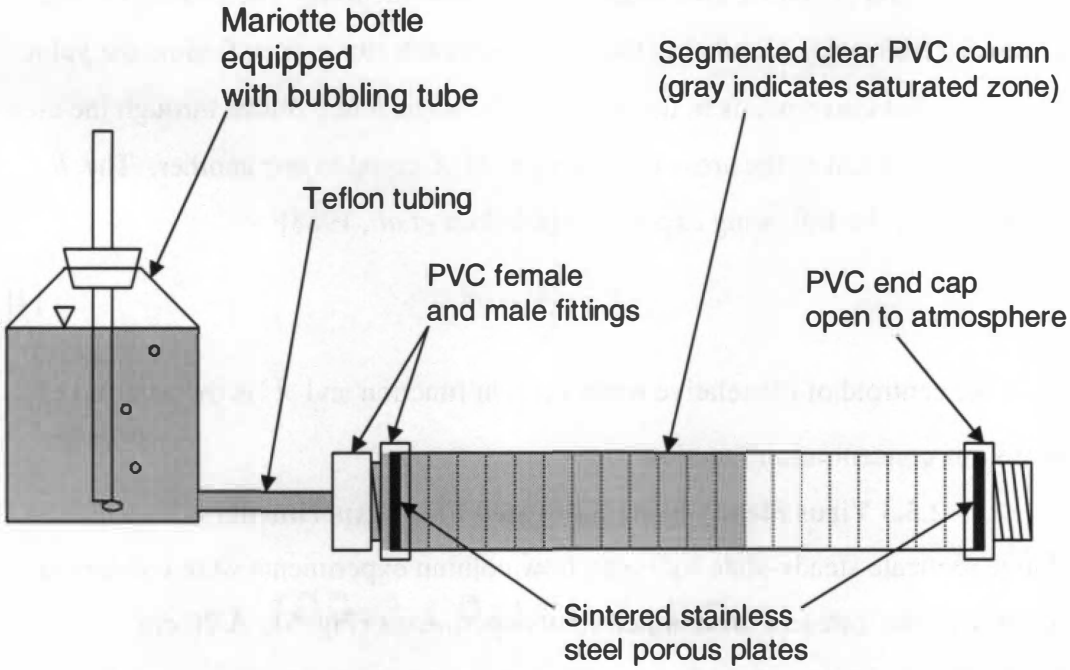


Figure 4: Transient unsaturated flow column experimental design.

the relative water content and relative virus concentration fuctions. The centroid of each function was determined by calculating the area under each curve, then finding the value of $d/d_{max\ water} = \lambda$ that corresponds to the position of a vertical line drawn through the area under each curve that makes the areas left and right of λ equal to one another. The R was calculated using the following expression [*Clothier et al.*, 1988]:

$$R = \frac{\lambda_w}{\lambda_v} \qquad [4]$$

where λ_w is the centroid of the relative water content function and λ_v is the centroid of the relative virus concentration function.

2.5.3 Virus Steady-State Saturated Flow Experiments

Three replicate steady-state saturated flow column experiments were conducted for comparison to the transient unsaturated flow experiments (Fig. 5). A 20 cm segmented PVC column was packed to the same ρ_b as the transient unsaturated flow columns. Next, the column was saturated from the bottom overnight with autoclaved, deaired PBS to expel any entrapped air in the column. The column was then positioned vertically, and the bottom of the column was attached to a Harvard Apparatus programmable PHD 2000 non-pulsing syringe pump, which injected PBS and virus solution at a constant flux of 0.054 cm/sec (Fig. 5). The duration of pumping was 12 minutes, which was approximately the same as the duration of the unsaturated transient flow experiments. At the end of the experiment, the column was segmented and sampled as described for the transient unsaturated flow experiments. The relative water content and relative virus concentration were measured in each segment and again expressed as functions of $d/d_{max\ water}$. The same analytical methods described in the previous section were used to calculate R for the steady-state saturated flow experiments, except that λ_w was calculated from the volume of water input to the column over the duration of the experiments.

2.5.4 Virus Mass Recovery

A mass recovery (or mass balance) of viruses input to the unsaturated transient flow columns and saturated steady state columns was calculated to determine total virus

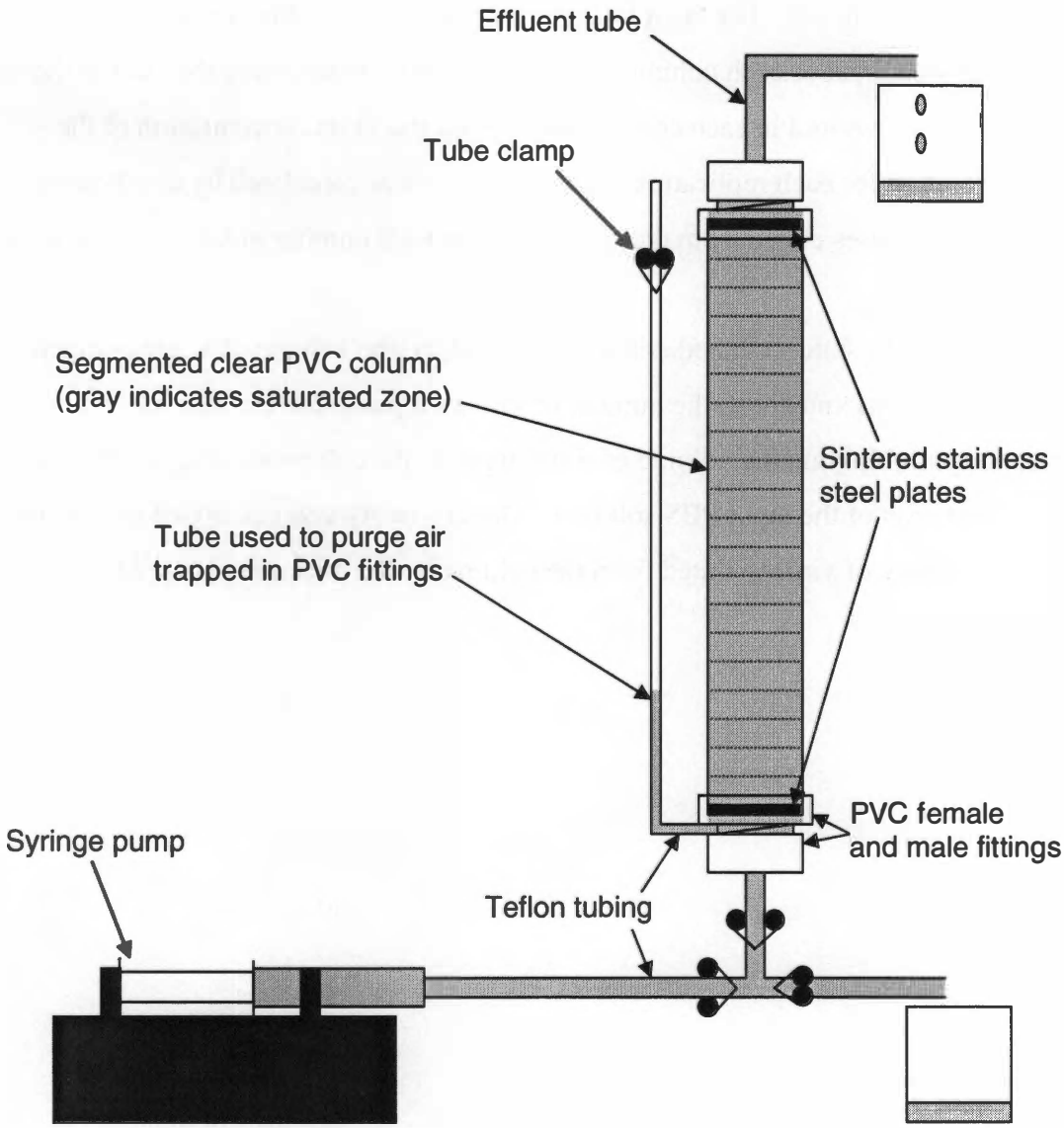


Figure 5: Steady-state saturated flow column experimental design.

recovery from the columns. For the transient unsaturated flow column experiments, the number of viruses input to each column was determined by multiplying the sum of the volume of water measured in each column segment by the virus concentration of the input PBS solution for each replication. Mass recovery was calculated by dividing the total number of viruses eluted from the column by the total number of viruses input to the column.

In the steady-state saturated column experiments, the volume of water pumped into the columns was known, so the number of viruses input to the column was determined by multiplying the volume of water input to the columns multiplied by the virus concentration of the input PBS solution. Mass recovery was calculated by dividing the total abundance of viruses eluted from the column by the total abundance of viruses input to the column.

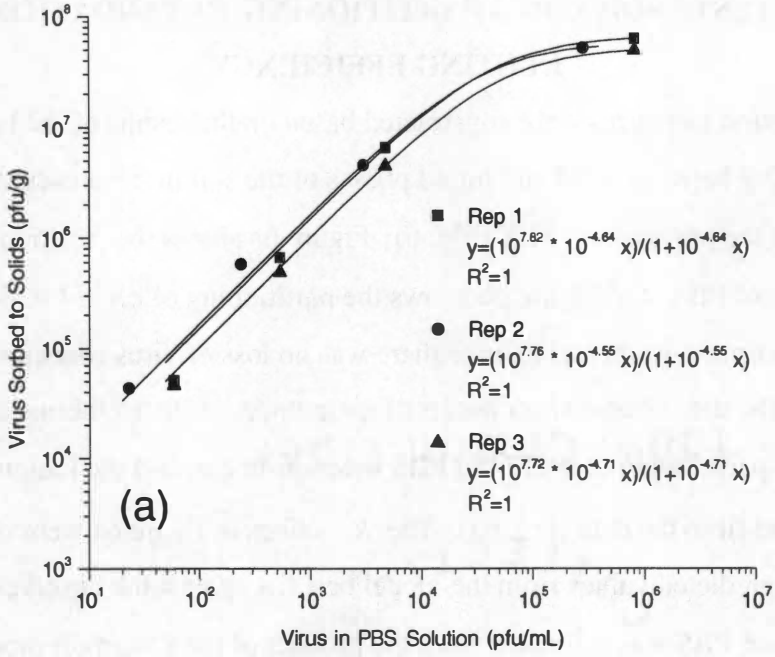
3.0 RESULTS

3.1 BATCH TESTS FOR VIRUS PARTITIONING, RETARDATION, AND BEX ELUTING EFFICIENCY

Adsorption isotherms were constructed based on the results of the batch tests for virus partitioning between solid and liquid phases of the soil in the presence of PBS solution and in the presence of BEX (Fig. 6). Figure 6a shows the partitioning of ϕX174 in the presence of PBS, and Figure 6b shows the partitioning of ϕX174 in the presence of BEX. The adsorption isotherms assume there was no loss of virus concentration due to inactivation. The data covered four orders of magnitude, so the isotherms are on a log-log scale. The partitioning of ϕX174 in PBS was non-linear, and the Langmuir isotherm provided a good fit to the data (Fig. 6a). The R^2 values in figure 6a were obtained by regressing the predicted values from the model best fits against the observed values. A K_d for viruses in PBS was estimated from the product of the Langmuir model parameters (Table 4).

A linear model best fitted the batch data for virus partitioning in the presence of BEX (Fig. 6b). The R^2 values for this model were all >0.99. There was no change in the partitioning of this virus in Memphis aquifer sand in the presence of BEX as concentration changed, so the slope of the fitted line is K_d [*Papiernik et al.*, 2002]. These values are summarized in Table 4. The K_d results from both types of batch experiments were quite reproducible among the three replications, as evidenced by the low CV values in Table 4.

The efficiency of BEX at eluting virus from the Memphis aquifer sand was tested, and Table 4 also contains the percent of virus recovered from batch tubes using BEX. Approximately 19% of ϕX174 remaining in the batch tubes after the batch adsorption sampling was recovered. Although low, the recovery data were consistent and reproducible among the three replications. Table 5 summarizes the R values calculated from the batch K_d results using equation 3.



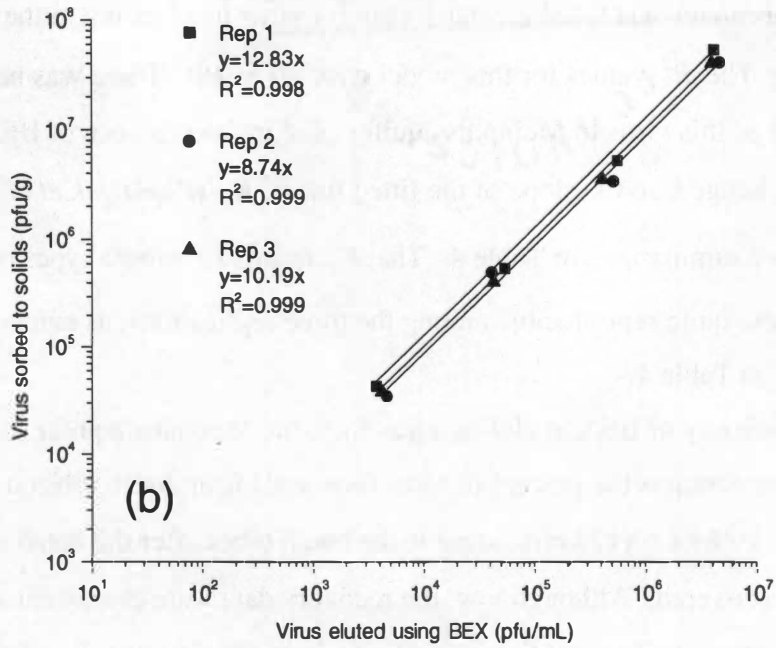


Figure 6: Partitioning of ϕX174 in PBS solution (a). Partitioning of ϕX174 in the presence of BEX (b).

Table 4: Summary of data from virus batch partitioning and percent recovery in PBS and BEX solutions.

	PBS	BEX	
Replication	**K_d (mL/g)**	**K_d (mL/g)**	**Recovery (%)**
1	1514	12.83	18.23
2	1658	8.74	19.08
3	1022	10.19	18.65
Average	1398	10.59	18.65
CV (%)	24	20.00	2.30

CV = Coefficient of variation = Standard deviation/mean

Table 5: Summary of batch, transient unsaturated flow and steady-state saturated flow experiment R values.

Replication	R_{PBS}	R_{BEX}	Transient Unsaturated Flow R	Steady-state Saturated R
1	6445	55.9	6.20	11.9
2	7057	38.4	6.33	5.80
3	4352	44.6	6.77	6.96
Average	1419	46.3	6.43	8.22
CV (%)	23.8	19.0	14.4	38.3

CV = Coefficient of variation = Standard deviation/mean

3.2 VIRUS TRANSIENT UNSATURATED FLOW EXPERIMENTS

The relative water content, θ / θ_s, and relative virus concentration, C / C_o, were measured in each of the transient unsaturated flow column segments and expressed as functions of the normalized distance, $d/d_{max\ water}$ (Figs. 7-9). Results were plotted on semi-log graphs, so the difference between θ / θ_s and virus C / C_o can readily be seen. Vertical lines indicate the centroids of both the θ / θ_s virus C / C_o functions. The centroids divide the area under each curve into two equal parts. The centroids' λ values are labeled. Equation 4 was used to estimate the retardation factor, R, based on the ratio of the centroids for these functions. The average R for transient unsaturated flow was 6.43 (CV = 14.4%), indicating that results for the transient flow experiments were highly reproducible (Table 5).

The data were also examined on linear plots, and it was clear that virus retardation and possible inactivation can be best described by an exponential decay function. Figure 10 shows data from all three replications of the transient unsaturated flow column experiments plotted on a linear scale and illustrates the exponential decay of viruses with $d/d_{max\ water}$. Viruses never traveled farther than $d/d_{max\ water}$ ~0.35 cm in any of the unsaturated transient flow column experiments. Most of the viruses were greatly retarded in the first few centimeters of the columns, and the virus C / C_o in the first two centimeters were typically greater than unity.

3.3 VIRUS STEADY-STATE SATURATED FLOW EXPERIMENTS

The relative water content and relative virus concentration were measured in each of the saturated steady state flow column segments and expressed as functions of $d/d_{max\ water}$ (Figs. 11-13). Again, results were plotted on semi-log graphs, so the difference between θ / θ_s and C / C_o could be readily seen. Vertical lines indicate the centroids of both the θ / θ_s and C / C_o functions. The centroids' λ values are labeled. Equation 4 was used to estimate the retardation factor, R, based on the ratio of the centroids for these functions. The average R for saturated steady state flow was 8.22 (CV = 38.3%), indicating that results for the steady state flow experiments were highly reproducible.

The steady state data were also examined on a linear plot, and it was clear that

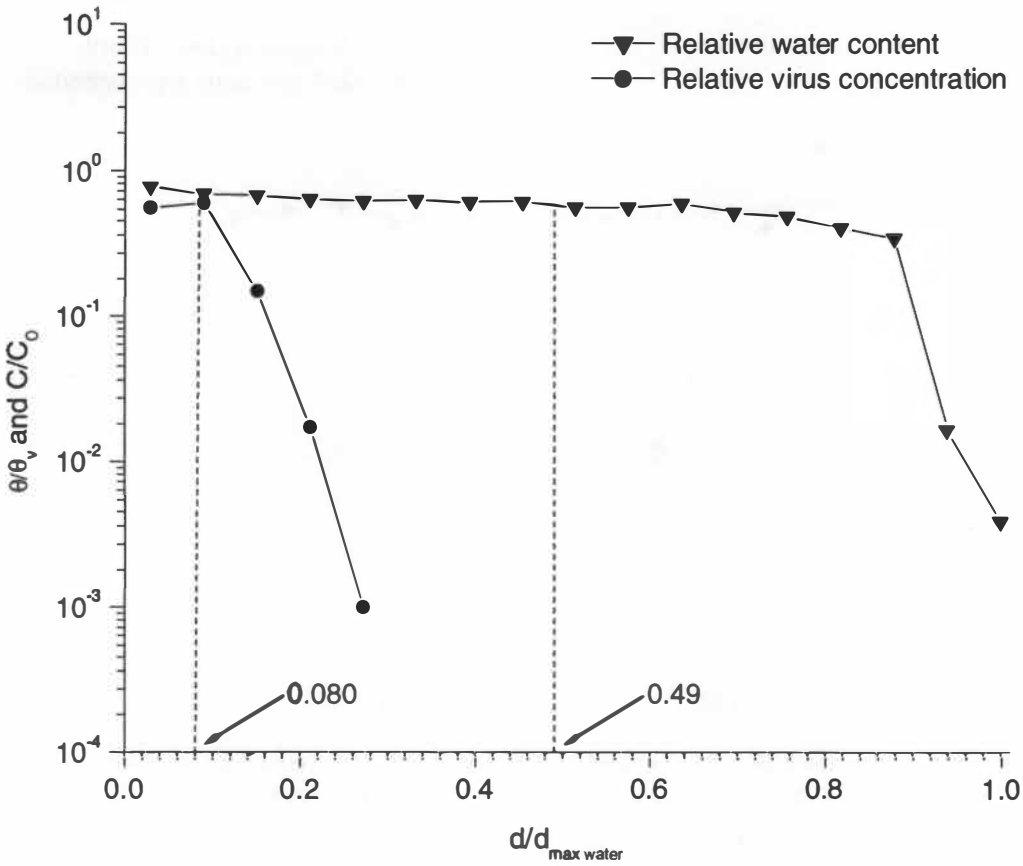


Figure 7: Semi-log plot of relative water content and relative virus concentration versus relative distance under transient unsaturated flow conditions in Memphis aquifer sand (replication 1). Vertical dashed lines indicate the centroids of the relative water content and virus concentration functions.

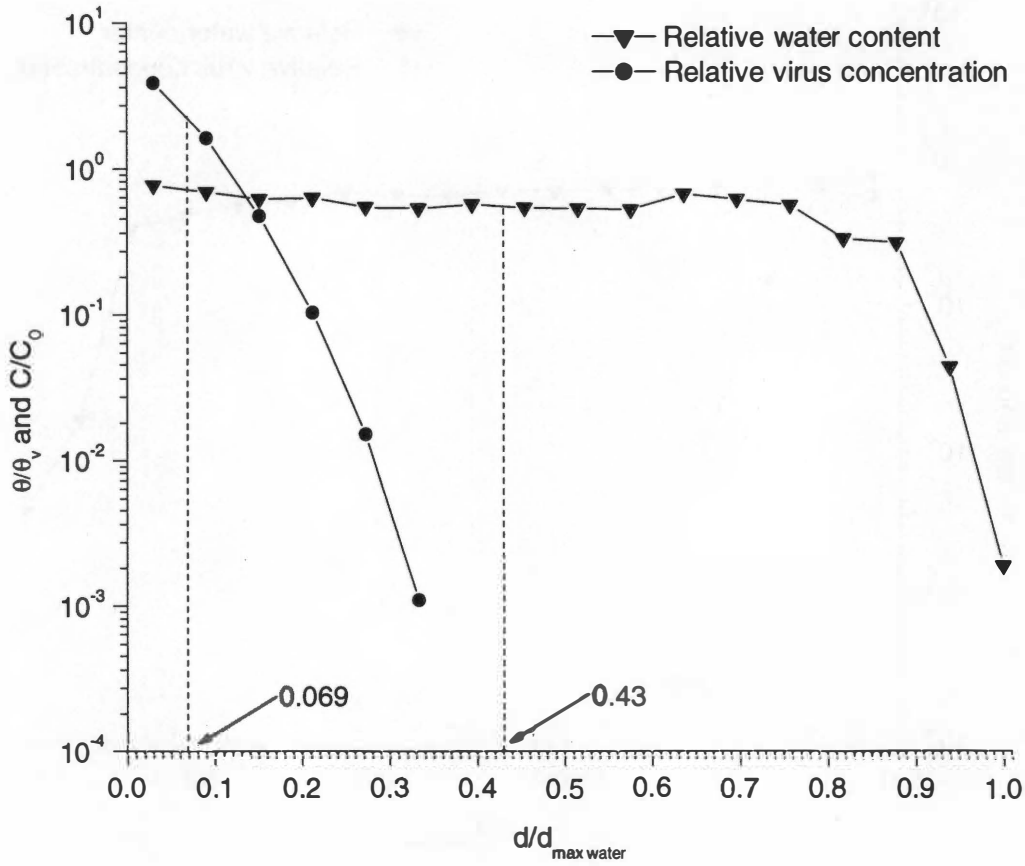


Figure 8: Semi-log plot of relative water content and relative virus concentration versus relative distance under transient unsaturated flow conditions in Memphis aquifer sand (replication 2). Vertical dashed lines indicate the centroids of the relative water content and virus concentration functions.

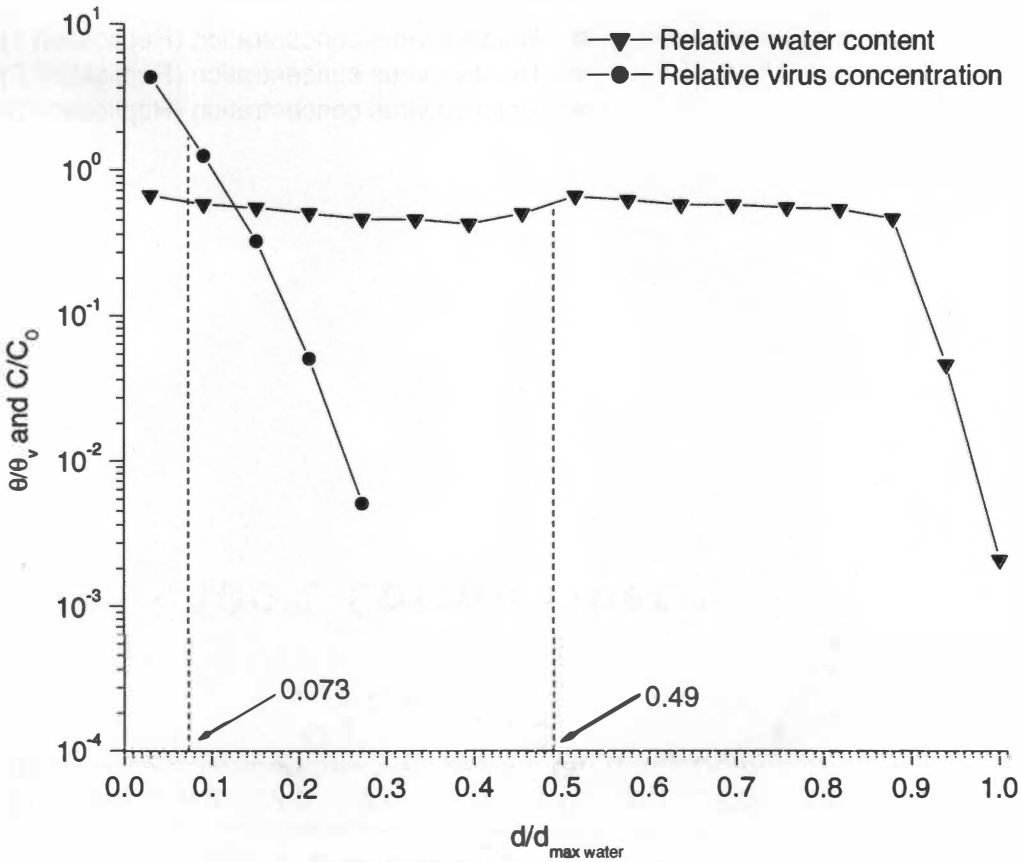


Figure 9: Semi-log plot of relative water content and relative virus concentration versus relative distance under transient unsaturated flow conditions in Memphis aquifer sand (replication 3). Vertical dashed lines indicate the centroids of the relative water content and virus concentration functions.

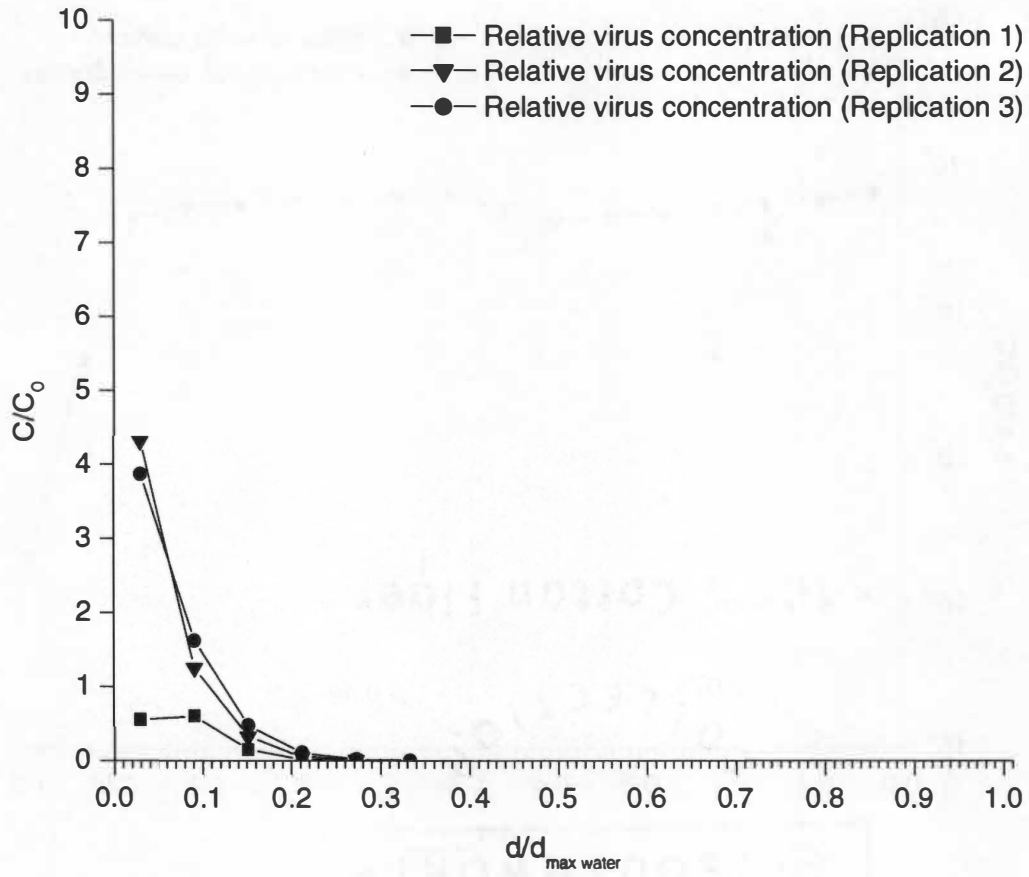


Figure 10: Arithmetic plot of relative water content and relative virus concentration versus relative distance under transient unsaturated flow conditions in Memphis aquifer sand, illustrating exponential decay of virus concentration (replications 1-3).

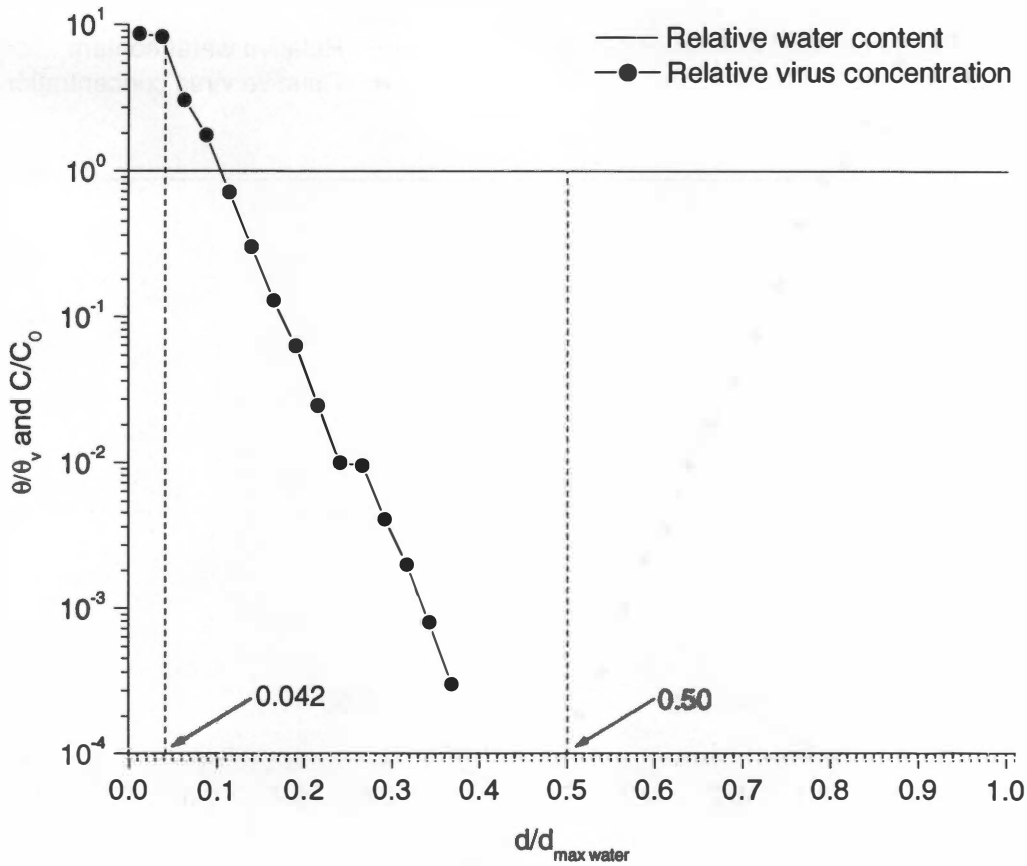


Figure 11: Semi-log plot of relative water content and relative virus concentration versus relative distance under steady-state saturated flow conditions in Memphis aquifer sand (replication 1). Vertical dashed lines indicate the centroids of the relative water content and virus concentration functions.

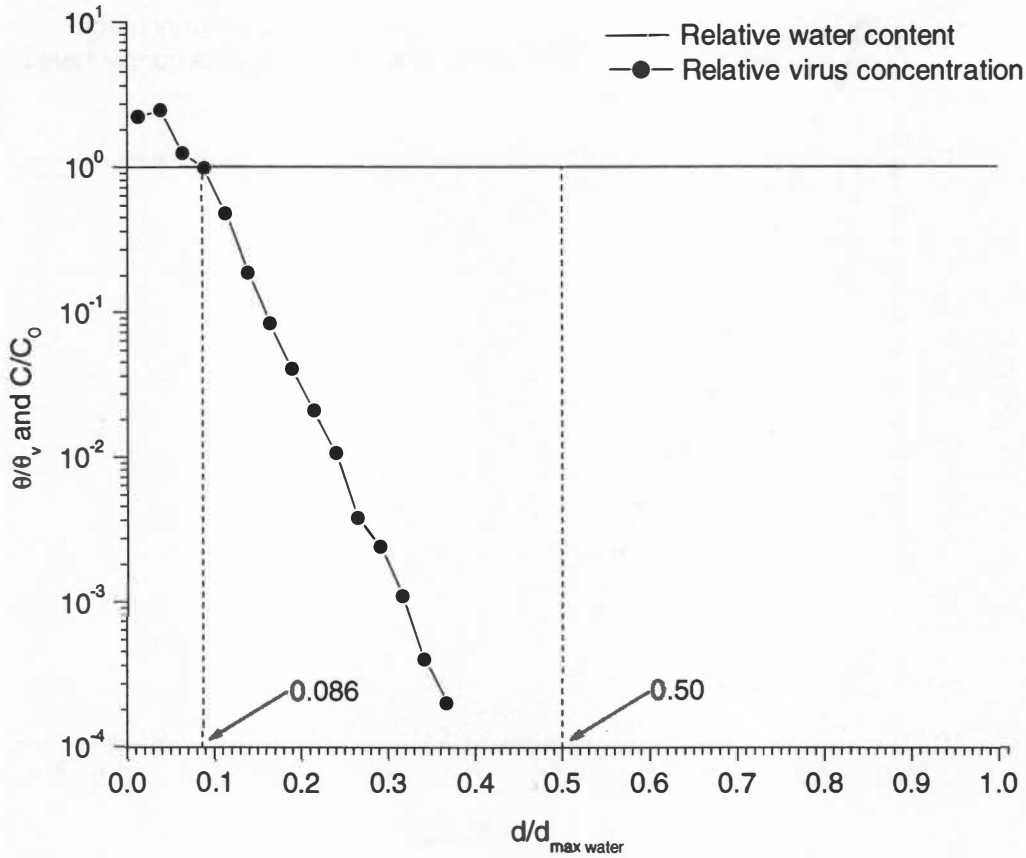


Figure 12: Semi-log plot of relative water content and relative virus concentration versus relative distance under steady-state saturated flow conditions in Memphis aquifer sand (replication 2). Vertical dashed lines indicate the centroids of the relative water content and virus concentration functions.

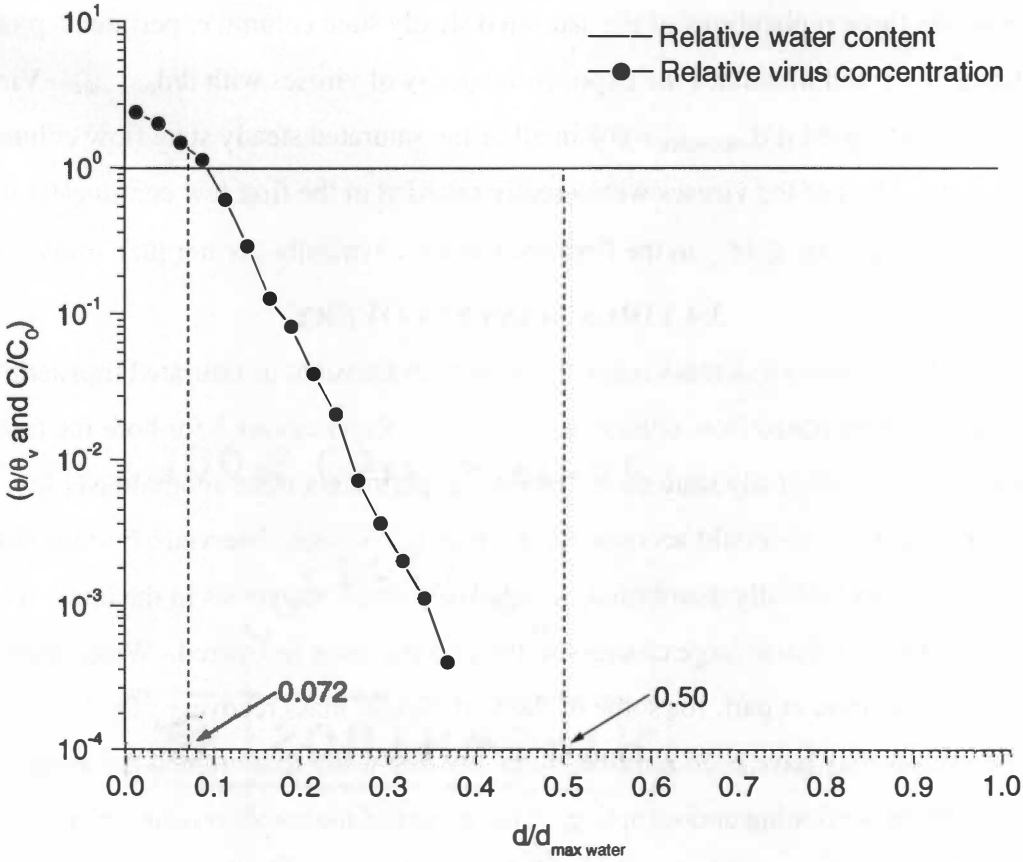


Figure 13: Semi-log plot of relative water content and relative virus concentration versus relative distance under steady-state saturated flow conditions in Memphis aquifer sand (replication 3). Vertical dashed lines indicate the centroids of the relative water content and virus concentration functions.

virus retardation and possible inactivation exhibited exponential decay. Figure 14 shows data from the three replications of the saturated steady state column experiments plotted on a linear scale and illustrates the exponential decay of viruses with $d/d_{max\ water}$. Viruses never traveled beyond $d/d_{max\ water} = 0.4$ in all of the saturated steady state flow column experiments. Most of the viruses were greatly retarded in the first few centimeters of the columns, and the virus C/C_o in the first few cm were typically greater than unity.

3.4 VIRUS MASS RECOVERY

Table 6 shows virus mass recovery from both transient unsaturated transient flow and steady-state saturated flow column experiments. Replications 1 for both the transient unsaturated flow and steady state saturated flow experiments were anomalously low and high, respectively. This could account for the high CV values observed. Furthermore, the data are logarithmically distributed, so relatively small variations in the amount of virus recovered will cause large changes in the percent mass recovered. Water drainage could be responsible, in part, for some of the variation in mass recovery of viruses because viruses may have been removed with any free water that drained from the columns during sectioning and sampling. This is part of the measurement error associated with this method, particularly in the case of coarse media like the sand used in this study.

Mass recovery from these column experiments ranged from 11.5-60.7%, and this range was similar to other column recoveries (36.2-166.2%) from another study using ϕX174 in columns filled with five different typical aquifer sands [Chu et al., 2003].

Table 6: Summary of virus mass recovery from transient unsaturated flow and steady-state saturated flow column experiments.

	% Mass Recovery	
Replication	**Transient Unsaturated**	**Steady-State Saturated**
1	11.5	56.9
2	60.7	19.2
3	52.4	20.2
Mean	41.5	32.1
CV (%)	63.4	66.9

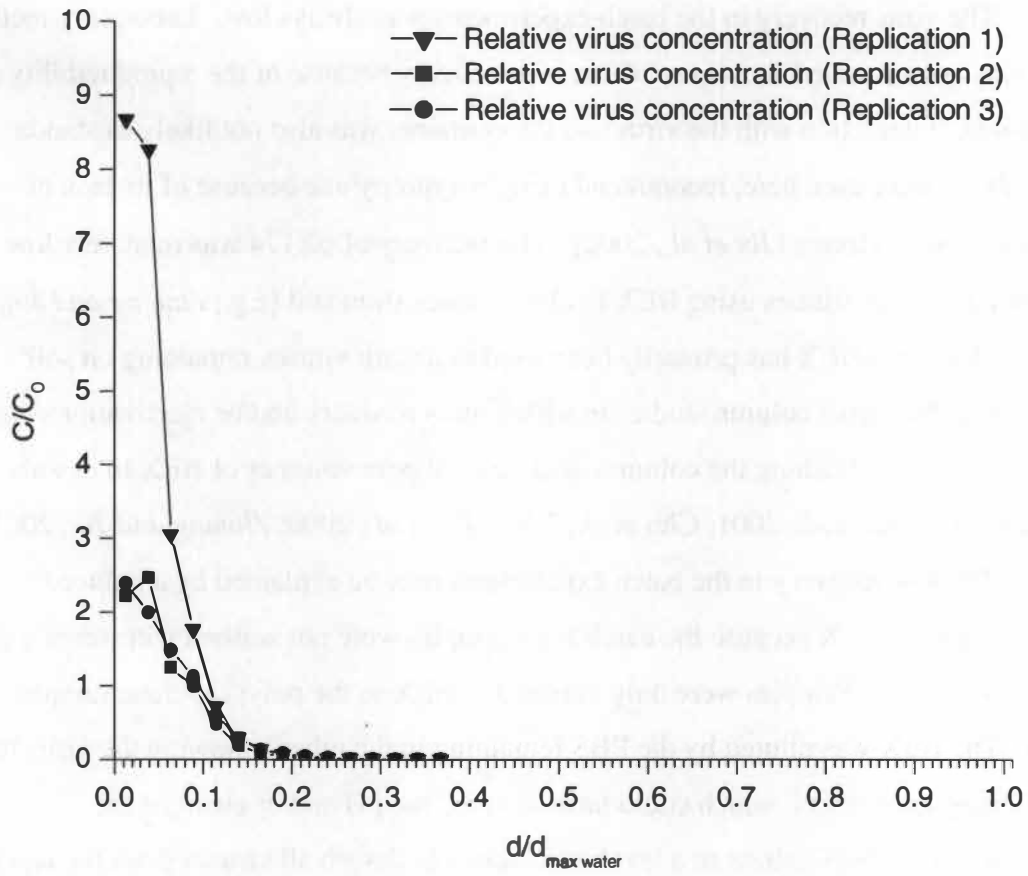


Figure 14: Arithmetic plot of relative water content and relative virus concentration versus relative distance under steady-state saturated flow conditions in Memphis aquifer sand, illustrating exponential decay of virus concentration (replications 1-3).

4.0 DISCUSSION

The virus recovery in the batch experiments was always low. Laboratory method error was not suspected as a cause of the low recovery because of the reproducibility of the results. Interaction with the virus and the container was also not likely as standard methods, as were used here, recommend using polypropylene because of its lack of interaction with viruses [*Jin et al.*, 2002]. The recovery of ϕX174 was relatively low compared to other studies using BEX to elute viruses from soil [e.g., *Zhuang and Jin*, 2003]. However, BEX has primarily been used to desorb viruses remaining on soil particles at the end of column studies in which mass recovery and/or inactivation data were collected by flushing the columns with several pore volumes of BEX to desorb viruses [e.g., *Chu et al.*, 2001; Chu et al., 2003; *Jin et al.*, 2000; *Zhuang and Jin*, 2003].

The low recovery in the batch experiments may be explained by a reduced effectiveness of BEX because the batch sand samples were not washed with several pore volumes of BEX. Samples were only exposed to BEX in the polypropylene sample tubes. The BEX was diluted by the PBS remaining in the tubes following the virus batch partitioning experiment, which could have lowered the pH and/or changed the concentration of beef extract to a level insufficient to desorb all viruses from the aquifer material, particularly from the silt and clay fractions. Virus mass recovery from the column experiments was higher than recovery from the batch experiments (compare Tables 4 and 6). Mass recovery from the transient unsaturated flow columns was generally higher than recovery from the steady-state saturated columns.

Throughout the column experiments in this study, viruses were constantly introduced into the columns for the duration of the experiments as a step change in C/C_o instead of as a pulse, which may explain the high C/C_o values near the influent end of all the columns. Viruses may have continued to fill available sorption sites on the Memphis aquifer sand near the influent end, and as the experiment progressed, the C of viruses in the free solution in segments nearest to the column inlet increased beyond C_o.

Table 5 is a summary of the R values for virus batch, transient unsaturated flow, and steady-state saturated flow experiments. The batch K_d in the presence of PBS and

subsequent calculated R_{PBS} values were two orders of magnitude greater than the batch R_{BEX} values, which indicates that ϕX174 was strongly sorbed to Memphis aquifer sand in the presence of PBS. Therefore, most of the viruses recovered in batch and column experiments using BEX were sorbed viruses. The R values from the steady-state saturated and transient unsaturated flow experiments were lower than the R_{BEX} possibly because under batch conditions there is no flow, which results in greater opportunity for viruses to interact with solid particles, causing a subsequent increase in sorbed virus. Modeling by Bales et al. [1991] resulted in a range of R values for MS2 (1.62-2.66) and PRD-1 (115-230) based on breakthrough data from steady-state saturated flow columns containing silica beads. Earlier work by Bales et al. [1989] resulted in R values for MS2 that indicated transport that was slightly faster than the water displacement in steady-state saturated flow columns. Poletika et al. [1995] noted that R values calculated from batch K_d values were much lower than R values fitted to field lysimeter data for MS2. Powelson and Gerba [1994] used three viruses to predicted R values based on batch data, which were an order of magnitude higher than R values determined from breakthrough curves for the same viruses. The results of these studies suggest that batch R values may not be useful for modeling virus transport in flowing systems. The large differences between batch and flow R values in this study support this assertion.

The range of steady-state saturated R values spans the range of transient unsaturated flow R values for the three replications. The fact that the R values for steady-state saturated flow were slightly higher than for unsaturated transient flow may have been a result of error associated with the virus assay method used. A two-tailed t-test indicated no significant difference between virus R values during transient unsaturated flow as compared to steady-state saturated flow. This may be a result of reduced air-water-solid interfaces as the wetting front moves through the soil during unsaturated transient flow as compared to steady state unsaturated flow. Most work has compared flow under steady-state unsaturated and saturated flow conditions. Viruses typically have greater R values, as evidenced by increased breakthrough, under steady-state saturated conditions as compared to steady-state saturated flow conditions

[Powelson and Gerba, 1994; Powelson et al., 1990]. Statistically similar R values between the two flow regimes used in this study indicate that transient unsaturated flow conditions can produce virus transport similar to steady-state saturated flow. In steady-state unsaturated flow it is possible that there are thinner water films surrounding the solid particles than in transient unsaturated flow, increasing the chance of viruses contacting and sorbing to solids. This phenomenon may not be present in the flow conditions used in this study.

The Young-Laplace equation describes the relationship between pore size and tension at AWS interfaces. Smaller pores produce greater tension, resulting in greater capillary pressure than larger pores. Viruses entrained in water drawn into small pores may have greater opportunity for contact with pore walls, resulting in greater retardation caused by electrostatic interactions between the virus and soil surfaces. This effect might be expected to play a significant role in virus transport in transient unsaturated flow, but this was not the case in this study.

Also, the moving wetting front, a macroscopic air-water interface in the column, may have a large surface area if the flux of water was driven by small pores, creating a microscale "fingering" effect. This air-water interface is absent in the saturated steady state columns, and flow under these conditions occurs preferentially through the larger pores, which may lead to a reduced opportunity for virus contact with pore walls. However, this did not appear to have a significant impact under the two flow conditions used in this study.

Whether or not water content influences virus transport depends greatly on soil properties. *Chu et al.* [2003] found that there was a profound difference in virus transport between saturated and unsaturated steady state flow columns packed with some aquifer sands, but other aquifer sands showed little difference in virus transport under the same flow conditions. The latter is consistent with the results of this study. Iron oxide, calcium, phosphorus, and organic matter, while present in relatively low concentrations, may have contributed to the strong sorption of viruses in these experiments.

There was a measurable amount of calcium, which in some studies has been shown to increase inactivation and sorption of viruses [*Yates et al.*, 1985; *Burge and*

Enkiri, 1978; *Goyal and Gerba*, 1979]. Calcium and other cations in the Memphis aquifer sand could have acted as an electrostatic bridge between the negatively charged aquifer material and virus. However, other soil properties are thought to have a greater influence on virus fate and transport in soils [*Gerba et al.*, 1981; *Moore et al*, 1981]. *Chu et al.* [2003] found that in some soils with high P and Ca contents there was no difference in inactivation and sorption of MS2 or ϕX174 in steady-state saturated and unsaturated flow columns.

At the experimental pH of 7.5, ϕX174 has a relatively weak negative electrical charge (compared to MS2) because of its pH_{IEP} of 6.6 [*Chu et al.*, 2003]. Therefore, there would be less repulsion of the virus from negatively charged soil surfaces. In sands with high metal oxide contents, the effect of metal oxides overwhelms this effect, however metal oxides were relatively low in the Memphis aquifer sand.

5.0 CONCLUSIONS AND RECOMMENDATIONS

Overall, the objectives of this study were met. A new laboratory apparatus and method were developed to examine virus transport during unsaturated transient flow in laboratory soil columns. This study described the extent and rate of bacteriophage ϕX174 transport under unsaturated transient flow and saturated steady state flow conditions in Memphis aquifer sand columns. Further, a novel approach to calculating R was used, and as a result of this approach, it was possible to make a direct comparison between virus R values in the two different flow regimes that were studied. This method could also possibly be applied to unsaturated steady state flow transport experiments as well.

It was originally hypothesized in this study that there would be greater virus transport in unsaturated transient flow conditions than in unsaturated steady state flow conditions because of the increased presence of air water interfaces in unsaturated steady state flow conditions. With this idea in mind, it was postulated that virus transport in unsaturated transient flow should be similar to transport in saturated steady state flow because of a similar lack of air-water interfaces, which have been cited as responsible for virus retardation and inactivation. This hypothesis was supported by the results of this study, and differences in the soil physical mechanisms controlling water flow in unsaturated transient flow columns and saturated steady state columns may have caused the observed similarity in the transport of bacteriophage ϕX174. It was also hypothesized that viruses should travel the same rate and distance as the moving wetting front in unsaturated transient flow conditions. This was not the case, as ϕX174 was retarded in both unsaturated transient flow and saturated steady state flow.

Some modifications to the methods used in this study may help acquire more accurate data concerning virus transport. Samples should be centrifuged after the addition of BEX but prior to sampling. Viruses may have adsorbed to the fine fraction of the aquifer material, which may have caused recovery greater than the amount of actual viruses in free solution in the column segments. Conversely, the fine fraction could have masked recovery if many viruses were sorbed to the fine fraction of the aquifer material. Since samples were not centrifuged and part of the fine fraction was sampled in these

experiments, there may have been many viruses sorbed to fine particles. Therefore, many of the plaques counted on the Petri dishes may have initially contained multiple pfu's instead of just one. An improved method of elution and sampling would help resolve this issue.

The batch recovery using BEX could be improved by using chromatograph tubes with an inlet and outlet to flush samples with BEX instead of merely exposing BEX to the sample in the polypropylene tube. This same method could then be applied to each column segment to remove viruses from the flow experiments, possibly increasing recovery and obtaining more accurate transport results.

Using a water washed and iron-oxide-removed fraction from the Memphis aquifer sand to determine the effect of the silt and clay fractions may elucidate one mechanism responsible for influencing virus transport. Virus sorption to silt and clay particles may have inhibited transport in these flow experiments. However, given the visible amount of fine particles in the effluent from the saturated steady state columns, colloids and fine particles may have helped facilitate virus transport under these conditions. Viruses may have sorbed to fine particles, which helps prevent virus inactivation, and the viruses may have been transported with the mobile fine fraction of the aquifer material. Treatment to remove iron, possibly responsible for sorbing viruses, may help elucidate the role iron plays in media with low iron content. Experiments with water washed sand, leaving the iron in the sample, may also help define the roles of iron and the fine fraction of the Memphis aquifer sand. Performing similar experiments using other viruses, such as MS2, and other porous media, such as the loess overlying the Memphis aquifer may also help determine the likelihood of viruses traveling through the vadose zone and entering aquifer systems.

More detailed information about the porous media used, such as that collected by *Chu et al.* [2003], would be useful. In particular, knowing the pH_{IEP} of both the Memphis aquifer sand and virus would help elucidate the role of electrostatic interactions between the sorbing surface and viruses. If the pH_{IEP} of the sand is low, sorption should be favored if solution pH is greater than the pH_{IEP} of the virus and Memphis aquifer sand.

Memphis aquifer sand was an untreated natural sample in these experiments, so it is conceivable that complex interactions between several mechanisms that govern virus transport and retention were responsible for the sorption of ϕX174. Not only are a variety of transport mechanisms at work in natural samples, but some mechanisms may be more dominant than others, and water content may not always be the major factor controlling the fate and transport of viruses. Virus survival time is a critical issue often overlooked. In this and many other studies, the viruses used as surrogates for human pathogens may not be as resilient in porous media as some pathogens. If a virus can survive in aquifer material for extended periods of time, it may be exposed to several transient flow events that may move it through the vadose zone over a longer period of time. Using natural samples with many physicochemical characteristics that may influence transport reduces the researcher's ability to define any single mechanism for transport. However, it is clear from this work that virus retardation under transient unsaturated flow is similar to that under steady-state saturated flow in Memphis aquifer sand. Further, convenient methods for determining R for flowing systems, such as those used here, may prove useful for future modeling work concerning virus transport.

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VITA

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