FINAL REPORT

FIELD EXPERIMENTS AND MODELING OF VIRAL TRANSPORT IN GROUNDWATER

Volume 1

FIELD EXPERIMENTS AND MODELING

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ABSTRACT

Ground water withdrawals in the United States make up roughly one-fifth of the total water use in the United States. For this reason, it is one of our most important resources which must be protected from contamination. The occurrence of human enteric viruses in ground water, however, has been well documented. Viral contamination of ground water from septic tank, sewer line, or waste disposal site failures has lead to increased efforts to quantify the transport of viruses through the ground water.

Ground water transport models can be used to predict the transport of viruses. However, if current public domain virus transport models are to be used for this purpose, they need to be verified under operating field conditions. To evaluate these models it is useful to use data from a "worst case scenario" for contaminant transport; a sand and gravel aquifer under a forced gradient.

Field studies were conducted to trace large-scale (34 m) and small-scale (10 m) virus transport under a forced gradient through the sand and gravel aquifer at the ground water research site and the Texas A&M Research Farm. Field instrumentation was developed to inject and monitor the virus tracer. Virus transport was monitored under natural and forced gradient conditions using MS-2 and PRD-1 bacteriophage as virus tracers and bromide as a conservative tracer. Simulations of the virus transport at the research site were also completed using the VIRALT virus transport model and parameters taken from past studies at the site.

Results indicate virus and bromide transport in the ground water in the both the large and small-scale field tests. However, comparison of results for the two large-scale and the two small-scale tests were not consistent. These differences can be attributed to the heterogeneity of the

sand and gravel aquifer at the site; different injection methods, and different sampling methods. The VIRALT simulations of virus and bromide transport at the site compared favorably with the field data.

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CHAPTER I

INTRODUCTION

Ground water withdrawals in the United States make up roughly one-fifth of the total water use in the United States (Linsley et al., 1992). For this reason, it is one of our most important resources which must be protected from contamination. The occurrence of human enteric viruses in ground water, however, has been well documented (Keswick and Gerba, 1980). Viral contamination of ground water from septic tank, sewer line, or waste disposal site failures has lead to increased efforts to quantify the transport of viruses through the ground water (Kinoshita et al., 1993; Maguire et al., 1993; Powelson et al., 1993; Bales et al., 1995).

Research Objectives

The overall goal of this research project was to conduct field studies of virus transport using bacteriophage tracers injected into a sand and gravel aquifer under natural and forced gradient conditions. To reach this goal, the specific objectives of this research were (1) developing the instrumentation to inject virus tracers and a conservative tracer into a sandy aquifer, (2) monitoring and analysis of virus and conservative tracer transport through a sandy aquifer under natural and forced gradient conditions, (3) analysis of the heterogeneity of the aquifer at the research site, and (4) comparing field tracer data with model simulations.

Literature Review

Viruses are colloidal size (20-250 nm) obligate noncellular parasites which enlist other cells for self-replication (Matthess and Pekdeger, 1985). The cell in which the virus infects and

replicates is called a host. Because of the virus's dependence on a host cell structure and metabolic components, they cannot reproduce or multiply outside of a specific host (Brock , 1994).

Forced-gradient experiments using conservative tracers such as bromide and chloride are well documented (Yeh et al., 1995; Mackay et al., 1994; Molz et al., 1988). These studies describes large-scale forced gradient experiments and show that the concentration breakthrough of the conservative tracer at the withdrawal well can be reasonably predicted if sufficient information is available on the hydraulic conductivity of the aquifer.

The transport of viruses in ground water and the use of bacteriophage as tracers was first evaluated by Wimpenny et al. (1972). This study found that a lambda-like bacteriophage proved excellent as a river marker because (1) detection was easy, sensitive, and rapid, (2) the bacteriophage was highly selective for its host organism, (3) no problems with naturally occurring bacteriophage were observed, (4) the bacteriophage showed no significant drop in viability over one week in laboratory experiments, and (5) the bacteriophage are entirely inert to all organisms except the host organism, thus overcoming public health and pollution problems associated with their use.

Snowdon and Oliver (1989) discuss the use of bacteriophage as indicators of human enteric viruses such as poliovirus, coxsackievirus, Hepatitus A. and others. They conclude that coliphage (bacteriophage which infect *E. coli* bacteria) could be used for accurate prediction of human enteric viruses. Two of the most common bacteriophage used as ground water tracers are MS-2 and PRD-1. They also note that further work is needed to establish sampling and sample-handling techniques that lead to accurate estimation of bacteriophage levels present at the time of sampling.

Many factors affect virus survival and transport in ground water. Viruses are removed from the water phase by fixation onto soil particles. Therefore, the amount of surface area present in the porous media is one factor which controls transport (Bales, et al., 1993). Laboratory studies have also shown that pH is a dominant factor controlling virus transport. MS-2 was not removed from 100-150 mm long columns at pH's 5.7-8.0 (Kinoshita, et al., 1993). Studies by Yahya et al., (1993) indicated ground water temperature as a dominant factor in virus transport. Increased ground water temperature lowers virus survival rates. Therefore, virus concentration down-gradient from the point of release declines more rapidly at higher temperatures. Other factors that have been shown to affect virus survival and/or transport include soil organics, soil permeability, cation-exchange capacity, virus type, and sunlight (Keswick and Gerba, 1980).

Bales et al., (1995) examined the pattern of attenuation and transport of bacteriophage under a natural gradient in a sandy aquifer in field studies in Cape Cod, Massachusetts. This study indicated significant attenuation of the bacteriophage and a fairly narrow plume with virus concentration dropping below the detection limit of one plaque forming unit (PFU)/ml several meters from the source when acted on by a natural gradient. Results also verified that the attenuation of the bacteriophage was largely dependent on the pH of the aquifer. The soils at this site, however, were chemically poorly characterized because of contamination from sewage disposal (LeBlanc, 1984). Further experimentation is needed to evaluate virus transport under forced gradient conditions in a relatively uncontaminated aquifer.

The characteristics of the ground water research site at the Texas A&M Research Farm have been determined during past field studies. The aquifer at the research site is an alluvial, heterogeneous, unconfined system having a saturated thickness of approximately 12.2 m. The

surface layer at the research site is a Ships clay unit approximately 6.7 meters deep. Beneath the clay surface is a sand and gravel aquifer that extends to a depth of approximately 21.3 meters. The aquifer grades from fine sand at the top to a coarse sand mixed with gravel at the bottom. The water table is typically located in the aquifer at a depth of approximately 9.1 meters. The aquifer is underlain by an impermeable shale formation. The hydraulic conductivity values calculated for the aquifer range from 55 m/day to 95 m/day, with an average hydraulic of 85 m/day (Wrobleski, 1996). The aquifer at the site is also relatively uncontaminated by chemical and biological pollutants (Munster et al., 1996).

Overview

The results of this study are presented in the chapters which follow. Chapter II includes a discussion of the sample collection, handling, storage, and transport of virus samples and describes in-depth the methodology used for the tracer tests. In Chapter III, the results of three large-scale virus tracer tests, two small-scale tracer tests, an aquifer heterogeneity test, and simulations of virus transport using the VIRALT (VIRAL Transport) model are presented and discussed. Conclusions and areas of future work relating to this study are presented in Chapter IV.

CHAPTER II

GROUND WATER SAMPLING PROCEDURES FOR VIRUS TRACER TESTS IN A SANDY AQUIFER USING MS-2 AND PRD-1 BACTERIOPHAGE

Introduction

Viral contamination of ground water from a septic tank, sewer line, or waste disposal site failures has lead to increased efforts to quantify the transport of viruses through the ground water (Craun, G.F., 1990; Keswick and Gerba, 1980). These efforts include the use of bacterial viruses (bacteriophage) in tracer tests to simulate the transport of enteric viruses through ground water. Because of the potential consequences of a viral outbreak from ground water sources, the sampling and analytical techniques required to detect or substantiate the presence of small numbers of viruses in large quantities of ground water is a complex and precise process. The objectives of this paper are 1) to describe the sampling methods used to obtain ground water samples used for viral analysis in the virus transport project at the Texas A&M University Research Farm; and, 2) to generalize these techniques to be utilized for ground water sampling in a sandy aquifer under various field conditions.

Background

Viruses

Viruses are colloidal size (20-250 nm) obligate noncellular parasites which enlist other cells for self-replication (Matthess and Pekdeger, 1985). The cell in which the virus infects and replicates is called a host. Because of the virus's dependence on a host cell structure and

metabolic components, they cannot reproduce or multiply outside of a specific host (Brock , 1994).

Viruses are removed from the water phase by fixation onto soil particles. Therefore, the amount of surface area present in the porous media is one factor which controls transport (Bales, et al., 1993). Laboratory studies have also shown that pH (Kinoshita, et al., 1993) and temperature (Yahya et al., 1993) are dominant factors controlling virus transport.

One way to classify viruses is by the hosts which they infect. The three basic types of viruses are plant viruses, animal viruses, and bacterial viruses (bacteriophage). The use of viruses such as polio virus, Hepatitis A virus, and other enteroviruses in field scale experiments to determine their transport through ground water would require a high degree of control to mitigate possible health threats. This has led to the use of bacteriophage as indicators of virus transport (Bales et al., 1993, Gerba, 1984). Bacteriophage have been proven to be totally benign and non-toxic to humans and animals (Joklik, 1988). The bacteriophages MS-2 and PRD-1 have been utilized in field studies because of: 1) the adsorption to soils and overall survival kinetics which resemble human pathogenic viruses (Powelson et al., 1990; Powelson et al., 1993; Bales et al., 1991; Stetler, 1984); 2) low economic cost; 3) relative ease of production; and, 4) benign nature which overcomes some of the regulatory issues concerning the release of pathogenic animal viruses into the environment.

Factors Affecting Sample Collection

Obtaining water samples for viral analysis is not a simple task. Several factors associated with the well structure affect the validity of the data obtained from the sample. These factors include the possible contamination by microbes growing within the well casing; different

temperatures in the well which may cause increased or decreased viral activity; any viable viruses present in a non-sterile sampling container; casual infection of the water with airborne or skinborne microorganisms arising from dirty conditions or poor sampling techniques; and, erratic microbial composition of the ground water because of spurious microbial events such as the initial turbulence of starting up the pump (Cullimore, 1993). Any or all of these factors could seriously affect the reliability of the results obtained from the ground water sample.

The specific problems associated with sampling for viruses have been described to be one or more of the following: (1) small size of the virus particle, (2) low concentrations of viruses in the ground water, (3) instability of the virus particle, (4) quality of water, and (5) assay procedures. Of these factors, instability of the virus particle and quality of water can be maintained during sampling and transport by controlling the temperature and pH of the ground water sample. Dealing with the small size of the virus particle, low concentrations of viruses in ground water, and assay procedures is related to detection methods used in the laboratory (The Incidence, Monitoring, and Treatment of Viruses in Water Supply Systems - A State of the Art Review, 1983).

Field Study Description

Virus tracer studies have been completed in an uncontaminated sandy aquifer at the Texas A&M University Research Farm in College Station, Texas. The bacteriophages MS-2 and PRD-1 were used as surrogates to study the transport of hazardous human viruses through the ground water (Vogel et al., 1996).

The aquifer at the research site is an alluvial, heterogeneous, unconfined system having a saturated thickness of approximately 12.2 m. The surface layer at the research site is a Ships clay

unit approximately 6.7 meters deep. Beneath the clay surface is a sand and gravel aquifer that extends to a depth of approximately 21.3 meters. The aquifer grades from fine sand at the top to a coarse sand mixed with gravel at the bottom. The water table is typically located in the aquifer at a depth of approximately 9.1 meters. The aquifer is underlain by an impermeable shale formation. The hydraulic conductivity values calculated for the aquifer range from 55 m/day to 95 m/day, with an average hydraulic of 85 m/day (Wrobleski, 1996).

During the field studies, large-scale (113 m) and small-scale (12 m) forced gradient tests were completed. A total of ten wellnests, two fully screened monitoring wells, two injection wells, and a pumping well are installed at the site. Two of the wellnests located directly up-gradient from the pumping well were monitored during the forced gradient tests. Each wellnest has four monitoring wells which are constructed of 50.8 mm flush threaded polyvinylchloride (PVC) well casing with 152.4 mm long well screens. The short well screens are located at different depths to provide discrete sampling points within the aquifer. During the small-scale virus transport study, two monitoring wells which are located 2 m and 7 m up-gradient from the pumping well were monitored. These wells are screened the entire length of the aquifer. A 203.2 mm diameter pumping well with a 150.8 mm diameter submersible pump rated at 760 liters per minute is installed. Two 50.8 mm diameter injection wells screened throughout the saturated zone which are located 12 m and 113 m up-gradient from the pumping well were used for the small-scale and large-scale tests, respectively.

Discussion

Ground Water Collection Methods

Various methods are currently available to collect ground water samples for viral analysis. Ground water samples may also be obtained from vertical water wells by the use of

bailers. The bailers are lowered to the desired sample depth and manually retrieved to obtain the samples. Bailers allow passage of water freely through them during descent into the well and capture a volume of water when retrieved. Another simple method is to insert a flexible tube directly into the well and pump the water by peristaltic or flap valve pump. If the water table is more than approximately nine meters below ground level, however, this method cannot be used. This is because the head pressures which are generated cannot be overcome by the pump on the surface. Another method utilizes tubing attached to a submersible pump which fits inside the monitoring well. The pump is lowered to the desired depth and the sample retrieved. These pumps can be dedicated to the well and are available for AC or DC power sources. Other studies have also utilized multi-level samplers to obtain samples from discrete sections of the aquifer (Bales, 1995). These samplers utilize "packer" systems which seal off discrete sections of the well and take a sample from that discrete level.

Questions have arisen about the use of dedicated equipment such as multi-level samplers or submerged pumps for obtaining ground water samples for viral analysis. Virus particles may sorb and desorb to dedicated equipment. A study was conducted by inducing an artificial MS-2 breakthrough curve to a known volume of ground water in a 500 gallon stainless steel tank under a controlled temperature (21°C). For the upward slope of the artificial breakthrough curve, pre-determined volumes of concentrated MS-2 solution were added to known volume of ground water, taking into account virus die-off. For the downward slope, ground water containing no viruses was added to bring the virus concentration in the tank down quickly. Samples were collected every twelve hours for 4.5 days by three methods: 1) a submerged. dedicated pump with Tygon tubing; 2) a bailer; and, 3) directly from the tank. Before each sample was collected,

fifteen gallons of the tank water was run through the pump and the bailers to simulate purging of a monitoring well in the field.

The results of this study are shown in Figure 2-1. Analysis of this data using a paired *t* test and confidence interval of α=0.05 shows no significant difference between taking samples using a bailer, pump, or grab sample. This would indicate that similar results can be obtained using all three sampling techniques. In fact, no bacteriophage was detected in any of the samples occurring after the peak. This does not exactly match to the predicted MS-2 concentrations in the downward slope of the curve, but does indicate that sample contamination from virus desorption after the peak was not a problem during this test.

Type of Monitoring Well

Monitoring wells can be classified by the length of the well screen. For the field study completed at the Texas A&M Research Farm, wells with short six-inch screens and wells fully screened throughout the saturated zone of the aquifer were both used. Sampling from a well with a short well screen allows the sample to be obtained from a definite defined area of the aquifer. When sampling from this type of well, it is desirable to purge the well to be sure it contains fresh water from the aquifer. This is because important environmental conditions such as temperature and pH may differ in or near the well from the conditions in the surrounding aquifer. The recommended amount of water to be removed from the well may range from one well volume to ten well volumes, depending on many factors including the characteristics of the well, the hydrogeologic nature of the aquifer, the type of sampling equipment being used, and the parameters being sampled (Handbook for Sampling and Sample Preservation of Water and

Wastewater, 1982). For the field study at the Texas A&M Research Farm, three well volumes were removed before each sample was collected from the monitoring wells with short six inch screens. When sampling from flow-through wells constructed with screens which fully or nearly fully penetrate the aquifer, however, purging requirements are dependent on the type of sample to be obtained. If three or more well volumes are heavily pumped or bailed prior to sampling, the sample obtained will be a composite sample representative of the most permeable zones of the aquifer penetrated by the well. A discrete sample may also be obtained from this sort of well by lowering the sampler or pump carefully to the desired sample depth and taking a grab sample or pumping at a very slow rate. This sample will be minimally disturbed and representative of the immediate proximity of where the sample was taken. (Handbook for Suggested Practices of the of the Design and Installation of Ground-Water Monitoring Wells, 1991). Both of these methods were used during the virus transport studies which utilized the fully-screened monitoring wells at the Texas A&M Research Farm.

Sample Preservation Techniques

Virus survival is largely dependent on pH, temperature, and exposure to radiation (Kinoshita, et al., 1993; Yahya et al., 1993). Ground water temperature and pH were monitored in the field to detect changes which could influence virus survival and transport. The wells were purged if necessary and two 100 ml samples obtained. One sample was used for viral analysis and was immediately sealed in a plastic bag. Using the duplicate sample from the same well, the water temperature and pH were measured in the field. Storage of the samples at 4°C in a temperature controlled, covered container while in the field was necessary to control the effects

of heat and solar radiation on virus survival. This was accomplished in the field by placing the samples in a cooler filled with ice. Excess water from bailing was disposed of off site.

Sample container size to be used is largely dependent on the type of assay procedure used for viral analysis. The procedures used for the field studies at Texas A&M required a sample volume of <100 ml, so 125 ml sample bottles were used. If larger samples are desired to increase sensitivity of analysis, filter systems can be used concentrate the samples (Gerba, 1984). Amber polypropylene bottles are best suited for viral sample collection. These bottles minimize radiation effects and are easily sterilized for reuse. As long as the samples are maintained at 4°C and quickly analyzed, head space in the sample bottle is not critical.

Analytical labs equipped to analyze virus samples can also be located a substantial distance from the field site. Temperature and travel time are the controlling factors when transporting the samples to lab to be analyzed. If it necessary to transport the sample large distances, the use of overnight mail and "blue" ice packs minimizes the effect of these factors on the virus sample. Storage of samples to be used for viral analysis should be minimized. If storage is necessary refrigerate the sample at 4°C. Freezing of the sample could kill most of the viable viruses present in the sample.

Sterilization Techniques

When dealing with viruses the best policy is to dispose of any materials which come into contact with the viruses immediately after use. If this is not economically feasible, equipment and supplies should be sterilized before each use. In the field study, polypropylene sample bottles were autoclaved using standard autoclaving procedure (15 psi. 121°C, 20 minutes).

Autoclaving will completely destroy all microorganisms present (Brock, et.al, 1994). The bailers and sampling pumps were disinfected using ethyl alcohol (Texas A&M University Safety Manual, 1996). For this procedure, the bailers or pumps were repeatedly rinsed with ethyl alcohol and distilled water. The bailers were then allowed to sit for at least 20 minutes for evaporation of the ethyl alcohol. After rinsing once again with distilled water, the bailers were wrapped in aluminum foil to maintain sterility. Submersible pumps should be dedicated if possible, thus reducing the risk of drying out the seals by the ethyl alcohol from frequent rinsing. Other disinfectants effective against viruses also are available, including phenols, formaldehyde, quaternary ammonium compounds, chlorine, and iodine. When using these disinfectants, however, caution must be exercised so disinfectant residue is not left on the equipment.

Conclusions

Methods of ground water sample collection have been explored and many lessons learned while completing virus tracer studies at the Texas A&M Research Farm near College Station, Texas. These methods combine ground water sampling procedures for chemical and bacterial analysis with factors specific to viral analysis. Ground water samples obtained for viral analysis must be dealt with in an expedient and careful manner in insure the integrity of the results. All sampling equipment must be properly sterilized for each sample. While collecting the sample the technician must be sure to control the temperature and exposure to radiation which could affect the survival of viruses in the sample. The aquifer temperature, pH, and water level are also monitored to detect changes which could affect virus survival in the aquifer. As increased concern is put towards possible threats from viral contamination in ground water, proper sampling techniques will help enable us to accurately determine the presence of these viruses.

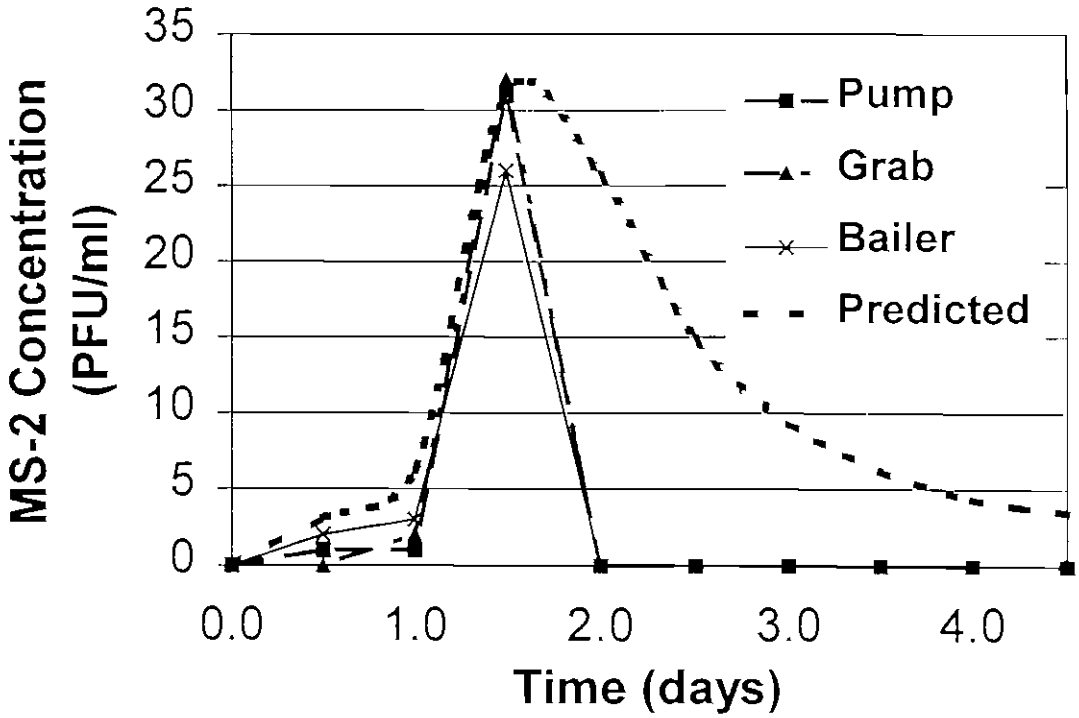

Figure 2-1. Results from sorption-desorption study comparing three types of ground water sampling methods.

CHAPTER III

FIELD STUDIES OF VIRUS TRANSPORT THROUGH A HETEROGENEOUS SANDY AQUIFER

Introduction

Concern for viral contamination of ground water from septic tank, sewer line, or waste disposal site failures has lead to increased efforts to quantify the transport of viruses through the ground water (Kinoshita et al., 1993; Maguire et al., 1993; Powelson et al., 1993: Bales et al., 1995). The Safe Drinking Water Act Amendments of 1986 requires the Environmental Protection Agency (EPA) to make known the disinfecting requirements for ground water used as drinking water. A variance to this rule may be granted if the ground water system in question is free of viral contamination. Groundwater transport models can be used as screening tools to determine the appropriateness of granting a variance. In the case of viruses, if there is no virus transport from a septic system or sewer system to the water supply well, the variance can be granted.

If the public domain virus transport models CANVAS (Composite Analytical Numerical Viral And solute transport Simulation) (1993) and VIRALT (VIRAL Transport) (1990) are to be used for granting variances, they need to be verified under field conditions. To evaluate these models it is useful to use data from a "worst case scenario" for contaminant transport, a sandy aquifer under a forced gradient.

The transport of viruses in ground water and the use of bacteriophage as tracers was first evaluated by Wimpenny et al. (1972). This study found that a lambda-like bacteriophage proved excellent as a river marker because (1) detection was easy, sensitive, and rapid, (2) the bacteriophage was highly selective for its host organism, (3) no problems with naturally

occurring bacteriophage were observed, (4) the bacteriophage showed no significant drop in viability over one week in laboratory experiments, and (5) the bacteriophage are entirely inert to all organisms except the host organism, thus overcoming public health and pollution problems associated with their use.

Snowdon and Oliver (1989) discuss the use of bacteriophage as indicators of human enteric viruses such as poliovirus, coxsackievirus, Hepatitis A, and others. They conclude that coliphage (bacteriophage which infect *E. coli* bacteria) could be used for accurate prediction of human enteric viruses. Two of the most common coliphage used as ground water tracers are MS-2 and PRD-1.

Many factors affect virus survival and transport in ground water. Viruses are removed from the water phase by fixation onto soil particles. Therefore, the amount of surface area present in the porous media is one factor which controls transport (Bales, et al., 1993). Laboratory studies have also shown that pH is a dominant factor controlling virus transport. MS-2 was not removed from 100-150 mm long columns at pH's 5.7-8.0 (Kinoshita, et al., 1993). Yahya et al., (1993) indicates temperature as a dominant factor in virus transport. Increased ground water temperature lowers virus survival rates. Therefore, virus concentration down-gradient from the point of release declines more rapidly at higher temperatures. Other factors that have been shown to affect virus survival and/or transport include soil organics, soil permeability, cation-exchange capacity, virus type, and sunlight (Keswick and Gerba, 1980).

Bales et.al., (1995) examined the pattern of attenuation and transport of bacteriophage under a natural gradient in a sandy aquifer in field studies in Cape Cod, Massachusetts. This study indicated significant attenuation of the bacteriophage and a fairly narrow plume with virus concentration dropping below the detection limit of one PFU/ml several meters from the source

when acted on by a natural gradient. Results also verified that the attenuation of the bacteriophage was largely dependent on the pH of the aquifer. The soils at this site, however, were chemically poorly characterized because of contamination from sewage disposal (LeBlanc, 1984). Further experimentation was needed to evaluate virus transport under forced gradient conditions in a relatively uncontaminated aquifer.

For this study, the bacteriophages MS-2 and PRD-1, viruses which only infect and replicate in a specific strains of bacteria, (Joklik, 1988) were used as surrogates to study the transport of hazardous human viruses through the ground water. Bromide was used as a comparative conservative tracer. The study was conducted at an uncontaminated ground water research site at the Texas A&M Research Farm near College Station, Texas (Munster et al., 1996).

The objectives for this project were; (1) development of the instrumentation to inject virus tracers and a conservative tracer into a sandy aquifer, (2) monitoring and analysis of virus and conservative tracer transport through a sandy aquifer under natural and forced gradient conditions, (3) analysis of the heterogeneity of the aquifer at the research site, and (4) comparing field tracer data with model simulations.

Materials and Methods

Research Site

A ten acre research site on the Brazos river flood plain at the Texas A&M University Research Farm near College Station. TX has been instrumented for ground water research (Munster, et al., 1996). A total of ten wellnests, two fully screened monitoring wells, two injection wells, and a pumping well (Figure 3-1 and Figure 3-3) were installed at the site. Each wellnest had four monitoring wells which are constructed of 50.8 mm flush threaded

polyvinylchloride (PVC) well casing with 152.4 mm long well screens. The short well screens were located at different depths to provide discrete sampling points within the aquifer (Figure 3-2). In addition to the wellnests, two monitoring wells were located 2 m and 7 m up-gradient from the pumping well. These wells were screened the entire length of the aquifer. A 203.2 mm diameter pumping well with a 150.8 mm diameter submersible pump rated at 760 liters per minute was installed. Two 50.8 mm diameter injection wells screened throughout the saturated zone were also located 12 m and 113 m up-gradient from the pumping well.

The aquifer at the research site is an alluvial, heterogeneous, unconfined system having a saturated thickness of approximately 12.2 m. The surface layer at the research site is a Ships clay unit approximately 6.7 meters deep. Beneath the clay surface is a sand and gravel aquifer that extends to a depth of approximately 21.3 meters. In general, the aquifer grades from fine sand at the top to a coarse sand mixed with gravel at the bottom (Figure 3-4). However, because of the heterogeneity of the aquifer material at the site, many preferential flowpaths are present which can affect the transport of contaminants. The water table was typically located in the aquifer at a depth of approximately 9.1 meters. The aquifer is underlain by an impermeable shale formation. A direct hydrologic connection has been shown between the river stage and angle of flow in the aquifer. The hydraulic conductivity values calculated for the aquifer range from 55 m/day to 95 m/day, with an average hydraulic of 85 m/day (Wrobleski, 1996).

Virus Tracers

Two types of bacteriophage, MS-2 and PRD-1 were used as virus tracers. The characteristics of these two bacteriophage are shown in Table 3-1 (Joklik, 1988). Previous studies have shown that these bacteriophages have adsorption to soils and overall survival kinetics which could be considered similar to pathogenic human viruses (Gerba, 1984; Snowdon

and Oliver, 1989). Therefore, the use of these bacteriophages is a conservative model for virus transport in ground water (Bales et al., 1993).

Field Methods

Three large scale and two small scale virus transport field studies were conducted. Viral suspensions mixed with ground water from the site were injected into the aquifer under natural gradient and forced gradient conditions at a depth of 17.7-18.3 m and monitored in the down-gradient wells. Forced gradients were created by the site pumping well.

A double packer system (Figure 3-5) was used to inject the virus solution into a discrete section of the injection well screen these tests. The packer system consists of inflatable bladders which partition off a 400 mm section of the well and the tracer is injected between the bladders. A 500 liter tank containing the virus solution was located over the well casing. The injection solution was allowed to flow by gravity into the well. The flow rate was checked periodically and adjusted by an in-line valve.

Table 3-1 Characteristics of bacteriophages used as tracers for the virus transport study.

Bacteriophage	Diameter (nm)	Common Host	Die-off Rate @ 21°C (log PFU/day)[1]	Pathogenic Virus Simulated
MS-2	24	*E. coli 15597*	0.2	polio, Hepatitis A
PRD-1	63	S. typhimurim LT-2	0.15	animal viruses

[1] (Dowd and Pillai. 1996)

Sampling Protocol

After injection, the down-gradient wells and the pumping well were sampled periodically. In the field, groundwater samples were analyzed for pH and temperature. The water level in each of the monitoring wells and the pumping well was measured before sampling. Nearby wellnests

and the water table well at the research site were also monitored less frequently to detect bacteriophage movement not directly in-line with the pumping well.

To obtain a representative ground water sample, the wells were purged three well volumes using PVC bailers or submersible pumps. Purged well water was placed in buckets and dumped off site. Two, 100 ml ground water samples were collected in sterile polypropylene containers from each well. The pH and temperature of the duplicate sample was measured in the field. The bottles were sterilized by rinsing with distilled water and autoclaving at 121°C and 15 psi for 20 minutes. The bailers were sterilized by rinsing with distilled water and ethyl alcohol. The samples used for viral analysis were transported overnight packed in "blue ice" by courier mail to the Texas A&M Research Center in El Paso, Texas.

Virus Detection

The ground water samples transported to El Paso were assayed for bacteriophages. For detection of the virus, one ml sub-samples are aseptically removed, diluted serially in 0.85% NaCl and plated for plaque enumeration using the agar overlay method (Adams, 1959). MS-2 and PRD-1 concentrations were determined using *E. coli* ATCC 15597 and *S. typhimurium* LT-2 as host bacteria, respectively.

To increase the sensitivity of analysis, the samples from the forced gradient tests were also analyzed using a protocol modified from Sobsey, et al. (1990). For detection in the forced gradient virus tracer test samples, magnesium chloride was added to the 100 ml water sample to achieve a final concentration of 0.05M. The sample was filtered through a 47 mm diameter cellulose acetate filter (0.45 micron pore size). This filter effectively traps most bacteria and the viruses that were retained due to the presence of $MgCl_2$. Next, 3.5 ml of a 1.5% beef extract-0.05 M glycine buffer was added to the filter and the filtrate was collected to elute the viruses off

the filter. From this filtrate, 1.0 ml and 0.1 ml aliquots were assayed for the presence of viruses. The number of plaque forming units on the assay plates was used to calculate the number of plaque forming units in the original 100 ml sample.

Natural Gradient Field Study

To characterize the large-scale transport of viruses under a natural gradient in the sandy aquifer at the research site, a virus tracer field study was started on November 16, 1995. A total of 190 liters of site ground water was mixed in the field with 1 liter of MS-2 bacteriophage solution at a concentration of was 4.6×10^{11} PFU/ml. This solution was injected at a depth of approximately 18.3 meters below the surface over a 32 hour period. The MS-2 injection concentration from tank samples collected during injection was 2.4×10^{9} PFU/ml. Sampling was conducted every other day for a period of 32 days. A total of 75 samples were obtained from the C2, V1, and B2 wellnests and C-WT well during the natural gradient virus tracer test.

Large-Scale Forced Gradient Field Study #1

To characterize the large-scale transport of viruses toward the on-site pumping well under a forced ground water gradient in the sandy aquifer at the research site, a virus tracer field study was started on January 13, 1996. The pumping well was started on this date and continually pumped approximately 700 liters per minute to create the forced gradient. Previous tests have shown that the pumping well needed to run for five days prior to injection to stabilize forced gradient conditions (Wrobleski, 1996). On January 20, a total of 190 liters of site ground water was mixed in the field with 3 liters of MS-2 and PRD-1 bacteriophage solution at a concentration of 3.2×10^{11} PFU/ml and 6.4×10^{9} PFU/ml, respectively. This solution was injected at a depth of approximately 18.3 meters below the surface over a 32 hour period. The MS-2 and PRD-1 injection concentration from tank samples collected during injection was 5×10^{9} PFU/ml and

$1x10^8$ PFU/ml, respectively. Sampling was conducted every other day for a period of 30 days. A total of 155 samples were obtained from the C2 and V1 wellnests, and pumping well during this test.

Large-Scale Forced Gradient Field Study #2

To further characterize the large-scale transport of viruses under a forced ground water gradient in the sandy aquifer at the research site, an additional virus tracer field study was started on July 17, 1996. The pumping well was started on this date and continually pumped approximately 700 liters per minute to create forced gradient conditions. On July 24, fifteen liters of deionized water was mixed in the field with 2 liters of MS-2 bacteriophage solution at a concentration $8.5x10^{11}$ PFU/ml. Deionized water was used for this injection due to laboratory studies which showed that deionized water inhibits adsorption of the viruses and would facilitate the movement of the bacteriophage out of the injection well and into the aquifer (Reference). This solution was injected at a depth of approximately 18.3 meters below the surface over a 20 minute period. The MS-2 injection concentration from tank samples collected during injection was $1.0x10^{10}$ PFU/ml. On July 25, 95 liters of site ground water was mixed in the field with 19,237.5 g of KBr. This solution was injected at a depth of approximately 18.3 meters below the surface over a 16 hour period. The Br injection concentration from tank samples collected during injection was $1.36x10^5$ mg/L. Sampling was conducted every six hours for a period of 28 days. A total of 203 samples were obtained from the C2 and V1 wellnests, and the pumping well during the second large-scale forced gradient virus tracer test.

Small-Scale Forced Gradient Field Study #1

Virus tracers were injected in the injection well 12 m from the pumping well to characterize the small-scale transport of viruses under a forced ground water gradient. The first

small-scale virus tracer field study was started on July 31, 1996. This test was run simultaneously with the second large-scale field study, for the forced gradient was already established. A total of 238 liters of site ground water was mixed in the field with 1 liter of MS-2 solution with a concentration of 6.0 x 10^{10} PFU/mL, 1 liter of PRD-1 solution with a concentration of 8.3 x 10^{9} PFU/mL, and 19.0 kg of KBr. This solution was injected at a depth of approximately 18.3 meters below the surface over a 42 hour period. The MS-2 and PRD-1 injection concentration from tank samples collected during injection was $2.5x10^{8}$ PFU/ml and $3.5x10^{7}$ PFU/ml, respectively. The bromide injection concentration from tank samples was 12,800 mg/L. Sampling was conducted every two hours for a period of 104 hours. A total of 73 samples were obtained from the P1 and P2 wells during the first small-scale forced gradient virus tracer test. Samples were collected by purging three well volumes using a submersible pump. The pump was located at 13.7 m below the surface. The samples were collected in this manner so as to be representative of the most permeable level of the aquifer (Handbook of Suggested Practices for the Design and Installation of Ground-Water Monitoring Wells, 1991).

Small-Scale Forced Gradient Field Study #2

To further characterize the small-scale transport of viruses under a forced ground water gradient in the sandy aquifer at the research site, a second virus tracer field study was undertaken on August 28, 1996. The forced gradient was already established from the preceding tests. A total of 15 liters of distilled water was mixed in the field with 1 liter of MS-2 solution with a concentration of 3.6 x 10^{10} PFU/mL, 1 liter of PRD-1 solution with a concentration of 5.8 x 10^{9} PFU/mL, and 2.67 kg of KBr. This solution was injected at a depth of approximately 17.7 meters below the surface over a 42 hour period. The MS-2 and PRD-1 injection concentration from tank samples collected during injection was $2.1x10^{9}$ PFU/ml and $3.4x10^{8}$ PFU/ml,

respectively. The bromide injection concentration from tank samples was 105,600 mg/L. Sampling was conducted every two hours for a period of 104 hours. A total of 137 samples were obtained from the P1-WT (P1), P2-WT (P2), and P-WT (pumping) wells during the second small-scale forced gradient virus tracer test. Samples were collected from the P2 well without purging except to empty the line using a submersible pump. The pump was located at 17.7 m below the surface. Samples were collected from the P1 well by steadily lowering a bailer to the desired level, allowing the ball to settle in the bailer, and steadily retrieving the bailer and the sample. The samples were collected in this manner so as to be representative sample of the level where the pump or bailer was located (Handbook of Suggested Practices for the Design and Installation of Ground-Water Monitoring Wells, 1991).

Aquifer Heterogeneity Test

Previous studies have documented the heterogeneity of the aquifer at the research site (Wrobleski, 1996; Chakka, 1996). The need arose, however, to further characterize the field site vertically in the saturated zone in the vicinity of the small-scale test to be sure that the ground water samples being analyzed were collected from the tracer plume. On September 29, 1996, ninety-six liters of site ground water was mixed with 17.9 kg of KBr. The solution was injected at a depth of approximately 17.7 m below the surface over a 17 hour period. The bromide injection concentration from tank samples was approximately 125.000 mg/L. Samples were collected every 0.6 m in the P2-WT well before injection, 21 hours after injection, and 96 hours after using a portable submersible pump. These samples were collected by slowly lowering the pump into the well so as to collect the sample from relatively undisturbed areas of the aquifer as described in Handbook of Suggested Practices for the Design and Installation of Ground-Water

Monitoring Wells, 1991. The electrical conductivity for each sample taken after injection was then compared to the background electrical conductivity at that level.

VIRALT Simulations

Public domain models are available which are capable of simulating the transport of viruses in ground water under forced gradient conditions. VIRALT was the first two-dimensional virus transport code. The code was developed to delineate groundwater pathlines and well capture zones and to compute viral concentrations in pumping wells (VIRALT, 1990). It utilizes the viral transport equations of Corapcioglu and Haridas, (1984, 1985).

One small-scale simulation and one large-scale simulation were completed and the results compared with field data. Input parameters were derived from field measurements and results of past studies at the field site (Alden, 1995, Chakka, 1996, Wrobleski, 1996). The input parameters used for these simulations are described in Tables 3-2 and 3-3.

Table 3- 2. Input parameters for the VIRALT model used for simulation of large-scale transport of MS-2.

Number of monitoring wells	2
Transmissivity	900 m^2/day
Natural Hydraulic gradient	0.0023
Aquifer porosity	0.35
Aquifer thickness	12 m
Boundary type and location	no boundary
Initial boundary concentration in aquifer	0 PFU/ml
Pumping rate	700 L/min
Type of viral transport analysis	transient
Longitudinal dispersivity	10 m
Molecular diffusion coefficient	0 m^2/day
Ambient ground water temperature	non-temp dependent
First-order decay rate	0.46/day
Retardation factor	3.2
Boundary condition type	prescribed concentration transient

Table 3- 3. Input parameters for the VIRALT model used for simulation of small-scale transport of bromide.

Number of monitoring wells	2
Transmissivity	900 m^2/day
Natural Hydraulic gradient	0.0023
Aquifer porosity	0.35
Aquifer thickness	10 m
Boundary type and location	no boundary
Initial boundary concentration in aquifer	0 PFU/ml
Pumping rate	700 L/min
Type of viral transport analysis	transient
Longitudinal dispersivity	1 m
Molecular diffusion coefficient	0 m^2/day
Ambient ground water temperature	non-temp dependent
First-order decay rate	0.46/day
Retardation factor	1.0
Boundary condition type	prescribed concentration transient

Results

Natural Gradient Test

Ground water samples were collected at the C2 wellnest, 34.1 m from the injection well, every other day for a period of 32 days after injection of the MS-2 bacteriophage. The 152 mm long well screens of the C2 wellnest were located at 9.8 meters, 12.8 meters, 15.5 meters, and 18.3 meters below the surface. The V1 and B2 wellnest were sampled less frequently to detect any virus tracer not moving directly in-line with the pumping well. A summary of the field measured ground water parameters for the samples obtained during the natural gradient test is shown in Table 3-4.

Table 3- 4. Field measured ground water parameters at the C2 wellnest during the natural gradient virus tracer test.

Well Name	Sample Depth (m)	Distance from Injection (m)	Average Temperature / st. dev. (°C)	Average pH / st. dev.
C2-2	9.8	34.1	18.6 / 1.5	6.70 / 0.12
C2-3A	12.8	34.1	18.8 / 1.0	6.75 / 0.11
C2-3B	15.5	34.1	18.9 / 1.3	6.80 / 0.13
C2-4	18.3	34.1	18.9 / 1.3	6.95 / 0.12

The breakthrough curve for the C2 wellnest (34 m down-gradient) in the natural gradient test is shown in Figure 3-6 and summarized in Table 3-5. An average hydraulic gradient of 0.002 m/m was calculated from water level measurements during the test. Background testing showed no detectable levels of MS-2 in the aquifer. The breakthrough curve peaked at 5.5 PFU/ml in the 18.9 m well approximately 16 days after injection. This decrease in concentration can be attributed to adsorption to soil, dilution, dispersion, and/or virus die-off. The other wells in the C2 wellnests showed lower peaks 24-26 days after injection. Detectable levels of MS-2 bacteriophage were not found in the V1 wellnest or any of the other wells at the site. Water level measurements taken along with the samples showed the ground water to be traveling in the general direction from the injection well to the C2 wellnest.

Table 3- 5. Injection concentration and breakthrough of MS-2 bacteriophage tracer 34 m down-gradient from point of injection in the natural gradient virus tracer test.

Constituent	Injection Concentration (PFU/ml)	Maximum Concentration (PFU/ml)	Maximum C/C_0	Time to Peak
MS-2	2.4×10^{9} PFU/ml	5.5	1.0×10^{-9}	16 days

Large-Scale Forced Gradient Test #1

For the first large-scale forced gradient test, samples were collected at 12.8 meters, 15.5 meters, and 18.3 meters below the surface at the C2 wellnest, 34.1 meters from the injection

well. The 9.8 meter deep well at the C2 wellnest was not sampled because the water table had dropped below the level where it could be sampled with a bailer. Samples were also collected at 10.7 meters, 13.4 meters, 16.2 meters, 18.9 meters below the surface at the V1 wellnest, 74.4 meters from the injection well. The pumping well was also sampled. These samples were collected every day for a period of 30 days after injection of the MS-2 and PRD-1 bacteriophage. A summary of the field measured ground water parameters is shown in Table 3-6.

Table 3- 6. Field measured ground water parameters at the C2 wellnest during the first forced gradient virus tracer test.

Well Name	Sample Depth (m)	Distance from Injection (m)	Average Temperature / standard deviation (°C)	Average pH / standard deviation
C2-3A	12.8	34.1	19.8 / 1.0	6.79 / 0.10
C2-3B	15.5	34.1	19.9 / 1.0	6.82 / 0.09
C2-4	18.3	34.1	19.7 / 1.1	6.95 / 0.10

The MS-2 breakthrough curves for the C2 wellnest in the first large-scale forced gradient test are shown in Figure 3-7 and summarized in Table 3-7. An average hydraulic gradient of 0.006 m/m between the injection well and the C2 wellnest (34 m down-gradient) and 0.008 m/m between the C2 wellnest and the V1 wellnest (74 m down-gradient) were calculated from water level measurements during the test. Background testing indicated no detectable levels of MS-2 or PRD-1 in the aquifer. The breakthrough curve peaked at 662 PFU/ml at 18.3 m depth, 17 days after injection. The 15.5 m depth peaked at 150 PFU/ml, also on the seventeenth day. A peak of 11.6 PFU/ml was found at the 12.8 m depth. Detectable levels of MS-2 bacteriophage were not found in the V1 wellnest (74 m down-gradient) or the pumping well (113 m down-gradient). The virus tracer in this study appears to exhibit little dispersion, showing a narrow peak at the wellnest 34 m down-gradient.

Table 3- 7. Injection concentration and breakthrough of MS-2 bacteriophage tracer 34 m down-gradient from point of injection in the first forced gradient virus tracer test.

Constituent	Injection Concentration (PFU/ml)	Maximum Concentration (PFU/ml)	Maximum C/C_0	Time to Peak (days)
MS-2	5.0×10^{9}	662	1.3×10^{-7}	17
PRD-1	1.0×10^{8}	0	0	--

No PRD-1 was detected in the C2 wellnest. Since these two bacteriophage were injected at the same time, PRD-1 was likely not detected because of different adsorption characteristics of the larger diameter of the PRD-1 bacteriophage (63 nm) compared to the MS-2 bacteriophage (24 nm).

Large-Scale Forced Gradient Test #2

Samples were collected at 12.8 meters, 15.5 meters, 18.3 meters below the surface at the C2 wellnest, 34.1 meters from the injection well. The pumping well was also sampled. These samples were collected every six hours for a period of 28 days after injection. A summary of the field measured ground water parameters is shown in Table 3-8.

Table 3- 8. Field measured ground water parameters at the C2 wellnest during the second large-scale forced gradient virus tracer test.

Well Name	Sample Depth (m)	Distance from Injection (m)	Average Temperature / standard deviation (°C)	Average pH / standard deviation
C2-3A	12.8	34.1	22.6 / 0.6	6.79 / 0.10
C2-3B	15.5	34.1	22.5 / 0.5	6.82 / 0.09
C2-4	18.3	34.1	22.5 / 0.5	6.95 / 0.10

The bromide breakthrough curves for the C2 wellnest in the second large-scale forced gradient test are shown in Figure 3-8 and summarized in Table 3-9. An average hydraulic gradient of 0.006 m/m between the injection well and the C2 wellnest (34 m down-gradient) was

calculated from water level measurements during the test. Background testing of Br in the aquifer showed levels below the detection limit of 0.622 mg/L. There were also no detectable background levels of MS-2 in the aquifer. The breakthrough curve peaked at 5.24 mg/L at the 12.5 m depth 20 days after injection. At the 15.5 m depth, a peak of 2.47 mg/L was detected on the 22nd day. No significant levels of bromide were detected at the 18.3 m depth. Detectable levels of bromide were not found in the V1 wellnest (74 m down-gradient) or the pumping well (113 m down-gradient). The bromide tracer appears to exhibit an upward movement in the aquifer during this test. This result, however, is contradicted by the vertical hydraulic gradient calculated from water level readings. Because of this, this movement is likely caused by preferential flowpaths with lower hydraulic conductivity in the aquifer.

No bacteriophage (MS-2) was detected at any depth in the C2 or V1 wellnest. This can be attributed to dispersion, diffusion, adsorption to soil, and/or virus die-off. The heterogeneity of the aquifer described earlier could have also had a significant effect on virus transport.

Table 3- 9. Injection concentrations and breakthrough of the bromide and MS-2 bacteriophage tracers 34 m down-gradient from point of injection in the second large-scale forced gradient tracer test.

Constituent	Injection Concentration	Maximum Concentration	Maximum C/C_0	Time to Peak (days)
Bromide	136,000 mg/L	5.24 mg/L	3.9×10^{-5}	20
MS-2	1.0×10^{10} PFU/ml	0 PFU/ml	0	--

Small-Scale Forced Gradient Test #1

Samples were collected from the P1 well and the P2 well by purging three well volumes using a submersible pump which was located at 13.7 m below the surface. These samples were collected every two hours for 104 hours after injection of MS-2 and PRD-1 bacteriophage and bromide. A summary of the field measured ground water parameters is shown in Table 3-10.

Table 3- 10. Field measured ground water parameters of the P1-WT and P2-WT well for the first small-scale forced gradient virus tracer test.

Well Name	Sample Depth (m)	Distance from Injection (m)	Average Temperature / standard deviation (°C)	Average pH / standard deviation
P1-WT	composite	5.0	22.9 / 0.9	6.68 / 0.13
P2-WT	composite	10.0	22.7 / 0.7	6.66 / 0.13

The MS-2 and Br breakthrough curves for the P2-WT well in the first small-scale forced gradient test are shown in Figure 3-9 and summarized in Table 3-11. An average hydraulic gradient of 0.036 m/m between the injection well and the P2-WT well (10 m down-gradient) was calculated from water table measurements during the test. Background testing showed no MS-2 or bromide in the aquifer. Background testing of Br in the aquifer showed levels below the detection limit of 0.622 mg/L. The MS-2 breakthrough curve in the P2-WT well shows a peak of 1.87 PFU/ml occurring 44 hours after injection. Bromide peaked at 3.59 mg/L in this well at 50 hours after injection.

Detectable levels of MS-2 bacteriophage or were not found in the P1-WT well (5 m down-gradient). PRD-1 and bromide was also not detected above background levels in the P1-WT well.

Table 3- 11. Injection concentrations and breakthrough of the bromide and MS-2 bacteriophage tracers 10 m down-gradient from point of injection in the first small-scale forced gradient tracer test.

Constituent	Injection Concentration	Maximum Concentration	Maximum C/C_0	Peak Time (hours)	Relative Peak Time
Bromide	12,800 mg/L	3.59 mg/L	2.8×10^{-4}	50	1.0
MS-2	2.48×10^{8} PFU/ml	1.87 PFU/ml	7.5×10^{-9}	44	0.88
PRD-1	3.45×10^{7} PFU/ml	0.00 PFU/ml	0	--	--

Small-Scale Forced Gradient Test #2

Virus tracers were injected in the injection well located 12 m from the pumping well at a rate of 1700 ml/min for 10 minutes on August 28, 1996. Samples were collected from 17.7 m below the surface from the P1 well and the P2 well without purging except to empty the line when using the submersible pump. Samples were also collected from the pumping well. These samples were collected every two hours for 108 hours after injection of MS-2 and PRD-1 bacteriophage and bromide. A summary of the field measured ground water parameters is shown in Table 3-12.

Table 3- 12. Field measured ground water parameters of the P1-WT, P2-WT, and P-WT wells for the second small-scale forced gradient virus tracer test.

Well Name	Sample Depth (m)	Distance from Injection (m)	Average Temperature / standard deviation (°C)	Average pH / standard deviation
P1-WT	12.8	34.1	19.8 / 1.0	6.79 / 0.10
P2-WT	15.5	34.1	19.9 / 1.0	6.82 / 0.09
P-WT	18.3	34.1	19.7 / 1.1	6.95 / 0.10

The tracer breakthrough curves for the second small-scale forced gradient test are shown in Figure 3-10, 3-11, and 3-12 and summarized in Table 3-13. An average hydraulic gradient of 0.036 m/m between the injection well and the P2-WT well (10 m down-gradient) was calculated from water level measurements during the test. Background testing showed no detectable levels of MS-2 or PRD-1 in the aquifer. Background testing of Br in the aquifer showed levels below the detection limit of 0.622 mg/L.

Table 3- 13. Injection concentrations and breakthrough of bromide, MS-2, and PRD-1 tracers 5, 10 and 12 m down-gradient from point of injection in the second small-scale forced gradient tracer test.

Constituent	Injection Concentration (PFU/ml)	Distance down-gradient (m)	Maximum Conc. (PFU/ml)	Max. C/C_0	Time to Peak (hours)	Relative Time to Peak [a]
Bromide	12,800 mg/L	5.0	1.08 mg/L	1.0×10^{-5}	40	1.0
		10.0	0.9 mg/L	8.5×10^{-6}	66	1.0
		12.0	1.69 mg/L	1.6×10^{-5}	30	1.0
MS-2	2.48×10^{8} PFU/ml	5.0	6.2	6.2×10^{-9}	6	0.96 [b]
		10.0	66	3.1×10^{-8}	6	0.24 [c]
		12.0	0	0	--	--
PRD-1	3.45×10^{7} PFU/ml	5.0	0	0	--	--
		10.0	18.4	8.4×10^{-8}	6	0.24 [c]
		12.0	0	0	--	--

[a] Relative to bromide at same distance down-gradient
[b] compared to time = 12.5 hrs (5/12 of 12 m breakthrough peak time)
[c] compared to time = 25 hrs (10/12 of 12 m breakthrough peak time)

The MS-2 breakthrough curve in the P1-WT well (5 m down-gradient) shows a peak of 20 PFU/ml occurring 62 hours after injection. A more reliable peak of 6.2 PFU/ml occurs at 12 hours. This peak is more reliable because more that one consecutive detection occurred around this time. In the P2-WT well (10 m down-gradient), the MS-2 peaked is 66 PFU/ml at 6 hours after injection. These varied detection levels and times in the different wells are a result of the sampling methods used and heterogeneity of the aquifer. The sampling methods used for this test obtained a sample from a discrete section of the aquifer. If preferential flowpaths present in the heterogeneous aquifer did not pass through the sampling point, the tracer may not have been detected. In addition, because the experiment was conducted in close proximity to the pumping well where a larger gradient is present, these flowpaths are likely to be well established. MS-2 was not detected in the pumping well. This is partly because of the dilution effect of water entering the pumping well from 360 degrees. This result is also consistent with past studies in

electrical conductivity for each sample taken after injection was then compared to the background electrical conductivity at that level. The difference between these values are shown in Figure 3-14. The corresponding bromide concentrations as calculated from the linear regression relationship from Figure 3-13 are also shown. Similar patterns of different magnitudes were indicated at 21 and 92 hours after injection. The region from 16.8 m to 18.6 m showed a consistent electrical conductivity. The largest peaks were located at 11.9 m and 14.3 m, which indicate there may be some upward movement of the bromide tracer to these levels. This would also agree with the upward movement of bromide detected in the second large-scale forced gradient experiment.

VIRALT Simulations

Simulations were run using the VIRALT virus transport model. MS-2 bacteriophage transport was simulated for the first large-scale forced gradient test and bromide transport to the pumping well was simulated for the second small-scale forced gradient test. The results of these simulations along with the field data from these tests are shown in Figures 3-15 and 3-16, respectively.

The breakthrough curve shown in Figure 3-15 simulates MS-2 transport for the first large-scale study. It shows a peak of 643 PFU/ml occurring 17.6 days after injection at the C2 wellnest 34 m down-gradient. The field study detected a peak of 662 PFU/ml occurring 17 days after injection as shown in Figure 3-15.

The VIRALT simulation of the small-scale bromide test resulted in a bromide peak of 1.92 mg/L in the pumping well 12 m down-gradient 34.8 hours after injection, as shown in Figure 3-16. A peak of 1.69 mg/L occurred 29.5 hours after injection in the pumping well during the field test.

which wells installed with drilling mud, of which the pumping well was, have shown little virus movement into the wells, presumably from virus sorption to the drilling mud (Bales, 1996).

PRD-1 bacteriophage was not detected in the P1-WT well. In the P2-WT well, PRD-1 bacteriophage peaked at 18.4 PFU/ml six hours after injection. PRD-1 was not detected in the pumping well. Once again, the PRD-1 was not detected because of sampling methods and aquifer heterogeneity and was not detected in the pumping well because of dilution and adsorption.

In the P1-WT well, bromide peaked at 1.08 mg/L forty hours after injection. Because this was only a single detection point, however, it is not substantial to say this is the peak of a breakthrough curve for the bromide. The P2-WT well showed one detection point above background of 0.9 mg/L occurring at 66 hours after injection. The pumping well gave the best breakthrough curve for bromide. A peak of 1.69 mg/L was detected at 30 hours. Because the pumping well gave the best breakthrough curve for bromide transport through the aquifer, a time proportional to this value was used when calculating the relative time to peak in Table 3-13 for the 5 m and 10 m down-gradient wells.

Aquifer Heterogeneity Test

Laboratory studies were conducted to determine the relationship between bromide concentration and electrical conductivity in ground water from the research site. Bromide standards from 0 to 200 mg/L were prepared using site ground water and potassium bromide salt and a linear relationship was found (Figure 3-13).

Potassium bromide was injected on September 29, 1996 into the injection well located 12 m up-gradient from the pumping well. Samples were collected every two feet in the well before injection, 21 hours after injection, and 96 hours after injection from the P2-WT well. The

Discussion

Large-Scale Forced Gradient Tests

Results of the large-scale natural gradient and two large-scale forced gradient tests indicate that the MS-2 virus is capable of traveling at least 34 m in the sand and gravel floodplain aquifer at the Texas A&M Research Farm. The relative peak breakthrough concentration in the first forced gradient test was two logs greater than the relative peak breakthrough for the natural gradient test. The decrease in concentration is due to sorption to soil, dilution, dispersion, and/or virus die-off. Both tests, however, peaked at approximately the same time after injection. These results are explained by the presence of preferential pathways in heterogeneous aquifer. Aquifer heterogeneity and different flowpaths are indicated by the "double peak" found in the natural gradient test in Figure 3-6. As a result of these preferential flowpaths, the virus plume may not have passed through the sampling point.

The PRD-1 bacteriophage was not detected when used in the first forced gradient virus tracer test. Because all other factors were the same for both MS-2 and PRD-1 bacteriophage, the explanation for this is related to different adsorption characteristics of the larger diameter of the PRD-1 bacteriophage (63 nm) compared to the MS-2 bacteriophage (24 nm).

MS-2 bacteriophage was not detected during the second large-scale forced gradient test. One significant factor to account for no detection was that this injection was a slug injection which took only 20 minutes to complete, while the first forced-gradient test was a trickle injection lasting 32 hours. Bromide was detected during this test, peaking 20 days after injection at the shallowest depth. This depth at which the peak was detected was unexpected since the injection point coincided with the deepest sampling depth. The gradient indicated by water level readings in the wellnests also indicated a downward gradient in the aquifer. The upward

movement of the bromide, therefore, must be a result of zones of higher hydraulic conductivity within the aquifer which caused the bromide to migrate to the shallower depth.

Small-Scale Forced Gradient Tests

The small-scale forced gradient tests reinforced the notion that the heterogeneity of the aquifer and depth of sample collection affects the concentration of tracers recovered. During the first small-scale forced gradient test, samples were collected from a 13.7 m depth in the monitoring well by purging three well volume using submersible pump. This method should give a composite sample representative of the most porous area of the aquifer. In this test however, this did not appear to occur. The injection was a trickle injection lasting 42 hours, and MS-2 and bromide was detected in the monitoring well 10 m down-gradient between 40 and 48 hours after the beginning of the injection.

The second small-scale forced gradient test was a slug injection which lasted only 10 minutes. In the 10 m down-gradient well, PRD-1 and MS-2 peaked after six hours. The most consistent breakthrough curve for MS-2 showed a peak after twelve hours in the P1-WT well 5 m down-gradient. No breakthrough curve for bromide was indicated in the 5 m or 10 m down-gradient wells, but a bromide breakthrough curve was indicated in the pumping well, 12 m from the point of injection. Bromide in the pumping well peaked 30 hours after injection. For relative bromide peak comparisons in this test, ratios of this time were used in the 5 m and 10 m wells.

For both tests, the varied detection levels and peak times in the wells down-gradient wells are a result of the length of injection, sampling methods used and heterogeneity of the aquifer. The natural gradient test, first large-scale test, and first small-scale test were trickle injections where a relatively large volume was injected over a long time period. The second large-scale test and the second small-scale test, however, were pulse injections where a smaller, more

concentrated amount was injected quickly. In the first test, the proposed sample was to be a composite sample of the entire aquifer. The sample, however, appears to be a discrete sample from the 13.7 m depth where the submersible pump was set in the monitoring well. The sampling methods used for the second test obtained a sample from a discrete section of the aquifer at the same depth as the injection (17.7 m). The tracer may not have been detected, however, if preferential flowpaths present in the heterogeneous aquifer caused the tracer to not pass through the sampling point. In addition, because the experiment was conducted in close proximity to the pumping well where a larger gradient is present, these flowpaths are likely to have been well established.

Heterogeneity Comparisons

An aquifer heterogeneity test which was conducted by tracing a concentrated injection of potassium bromide solution using electrical conductivity as an indicator. Laboratory exercises using site ground water showed a linear relationship between bromide concentration and electrical conductivity with an R^2 value of 0.95. Field exercises showed much variability in the aquifer (Figure 3-14). Similar patterns of different magnitudes were indicated at 21 and 92 hours after injection. The region from 16.8 m to 18.6 m, which includes the injection depth of 18.3 m, showed a consistent electrical conductivity. The largest peaks were located at 11.9 m and 14.3 m, which indicate there may be some upward movement of the bromide tracer to these levels. This could have been a result of paths of higher conductivity or clay lenses within the aquifer.

VIRALT Comparisons

The VIRALT model was used to predict virus and bromide breakthrough peak times and concentrations. For the large-scale transport of MS-2, the model predicted a peak on the same day and within 20 PFU/ml (2.9%) of the concentration detected in the field studies when using

parameters taken from past studies (Wrobleski, 1996; Chakka, 1996). The distribution of the virus with respect to time was much narrower for the field data, however, indicating that there may be less dispersion in the field than predicted by the model.

For small-scale bromide transport to the pumping well, the model predicted a peak within 5.5 hours and within 0.23 mg/L (14%) of the concentration detected in the field studies. The simulated concentration approaching the peak concentration matched the field breakthrough curve very well. However, the model curve continued upward for an additional 5.5 hours after the field data had peaked.

Conclusions

The field research indicated that MS-2 bacteriophage is capable of traveling at least 34 m in the heterogeneous sandy aquifer. The natural gradient showed a peak MS-2 concentration that was less than 1 percent of the detected forced gradient peak. The bacteriophage also showed much attenuation in the forced gradient condition, with peaks less than 1 percent than those of Br. The large-scale experiment also showed much less dispersion than predicted by conventional transport equations. PRD-1 was not detected in the monitoring wells 34 m down-gradient. The small-scale tests reinforced the importance of aquifer heterogeneity on sampling results. The two tests indicated peaks in the same wells at different times even though the same gradient was present. This is a result of sampling location. Heterogeneity tests indicate that flowpaths from the injection zone may transport the tracer in an upward direction. After calibration the EPA virus model VIRALT predicted the approximate peak time and peak concentrations within 14 percent for MS-2 and bromide.

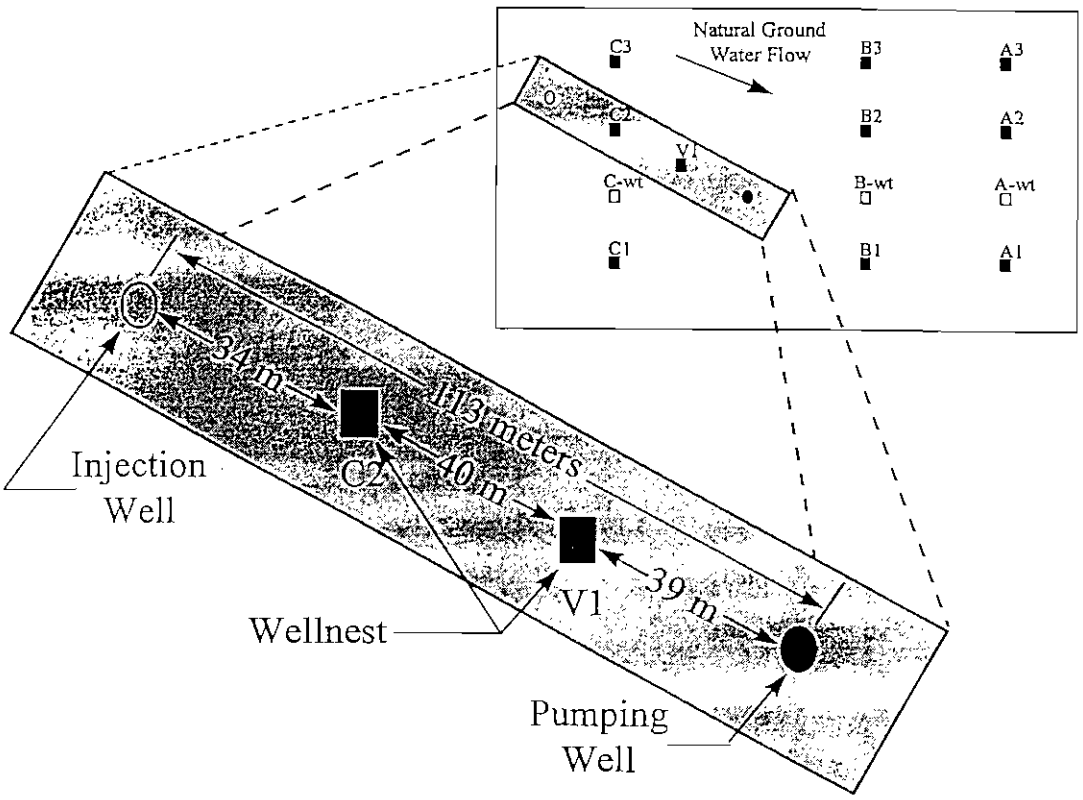

Figure 3- 1. Site layout for the large-scale virus tracer injection study.

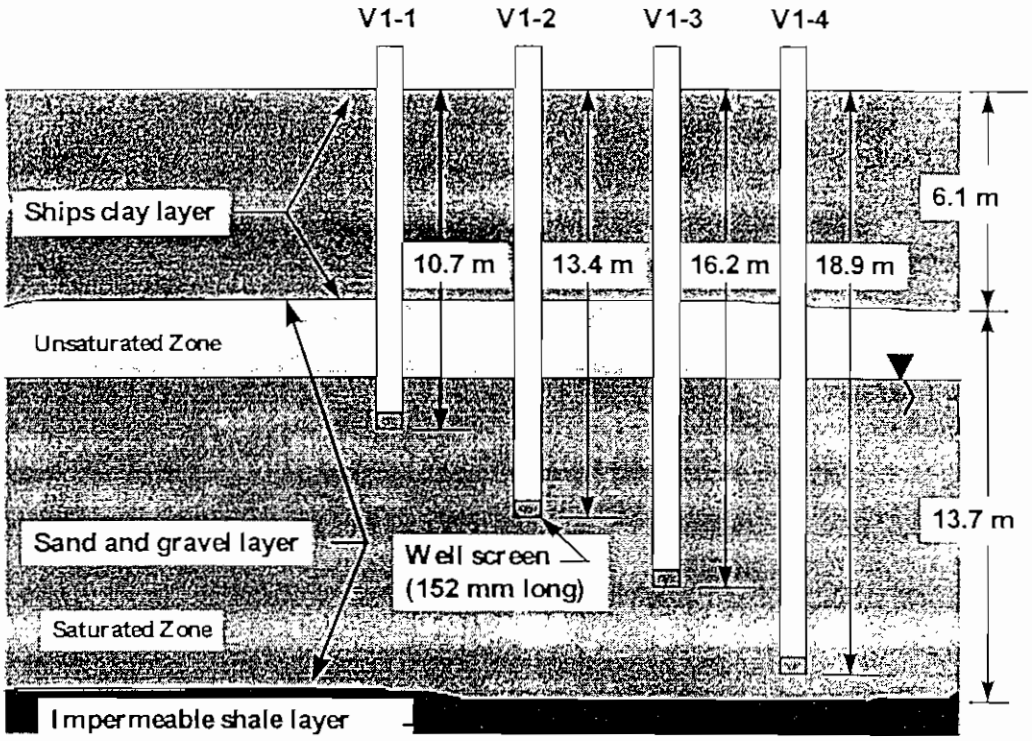

Figure 3- 2. Well nest design with soil profile..

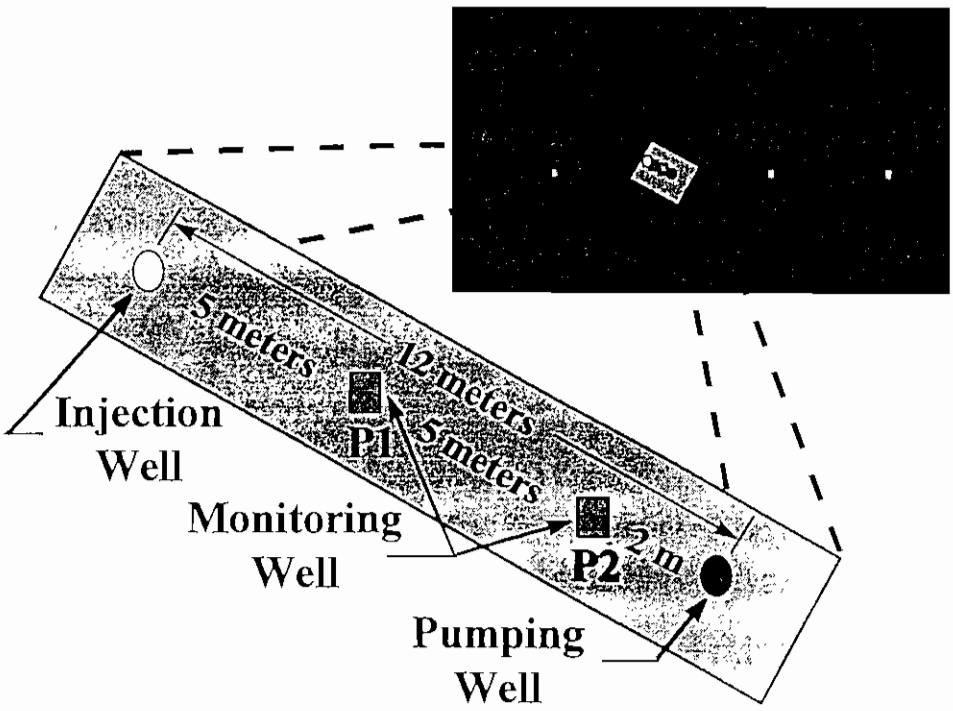

Figure 3- 3. Site Layout for the small scale virus tracer injection study.

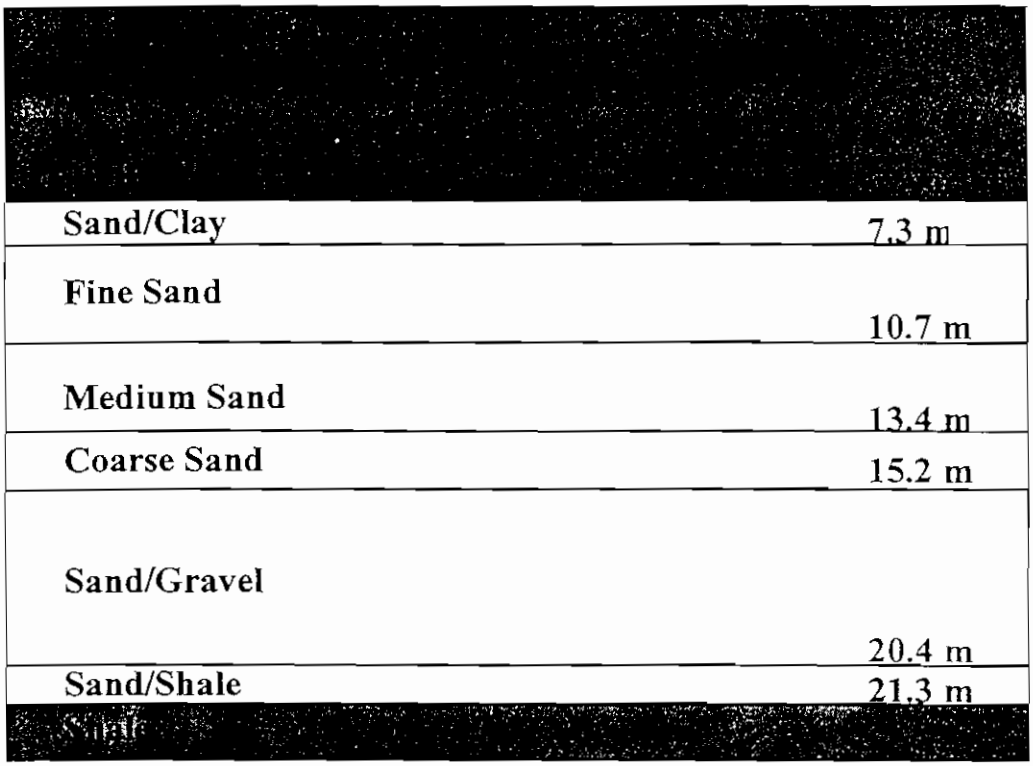

Figure 3- 4. Aquifer characteristics of the ground water research site at the Texas A&M Research Farm.

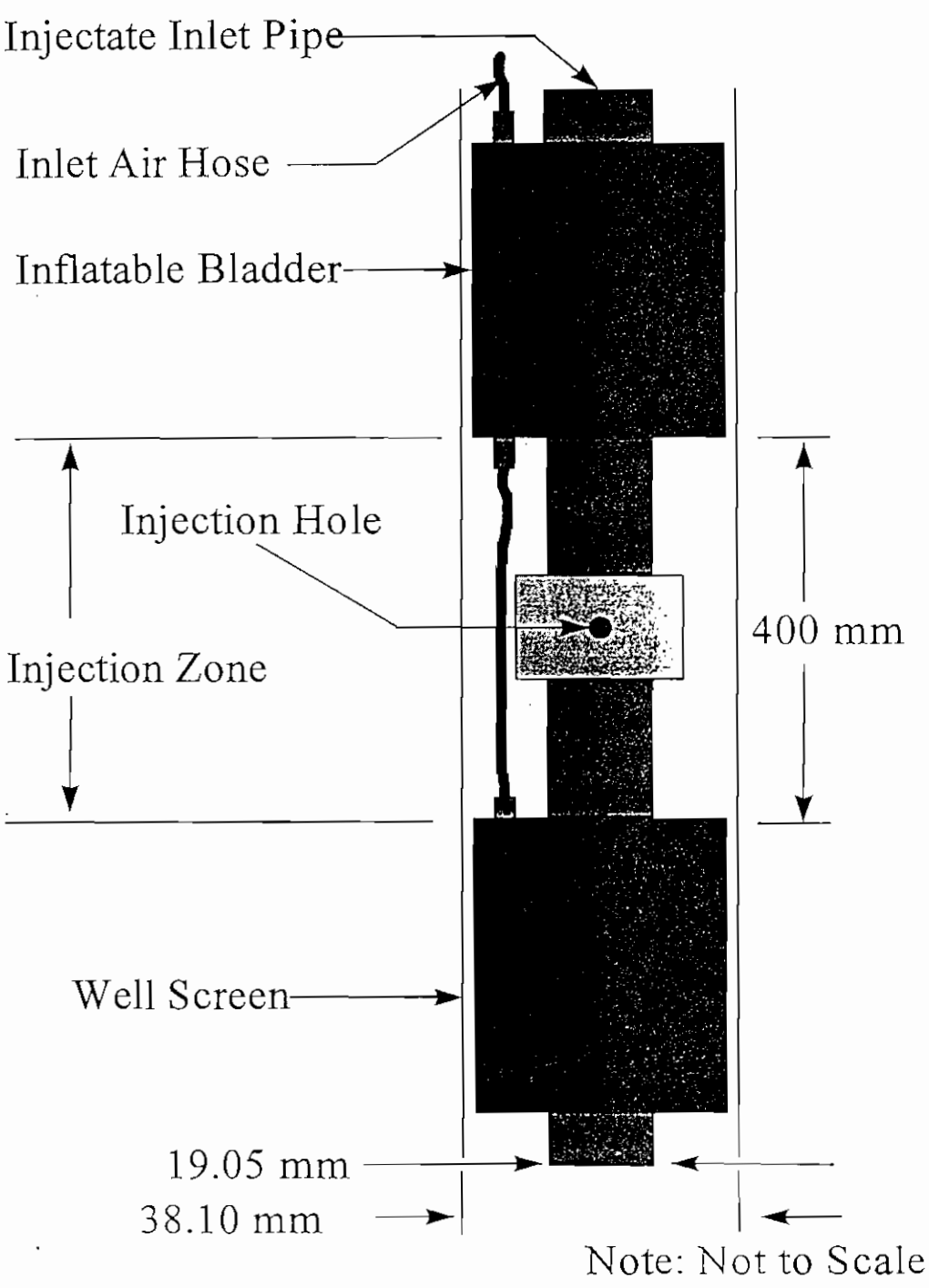

Figure 3-5. "Double packer" system used for virus tracer injection.

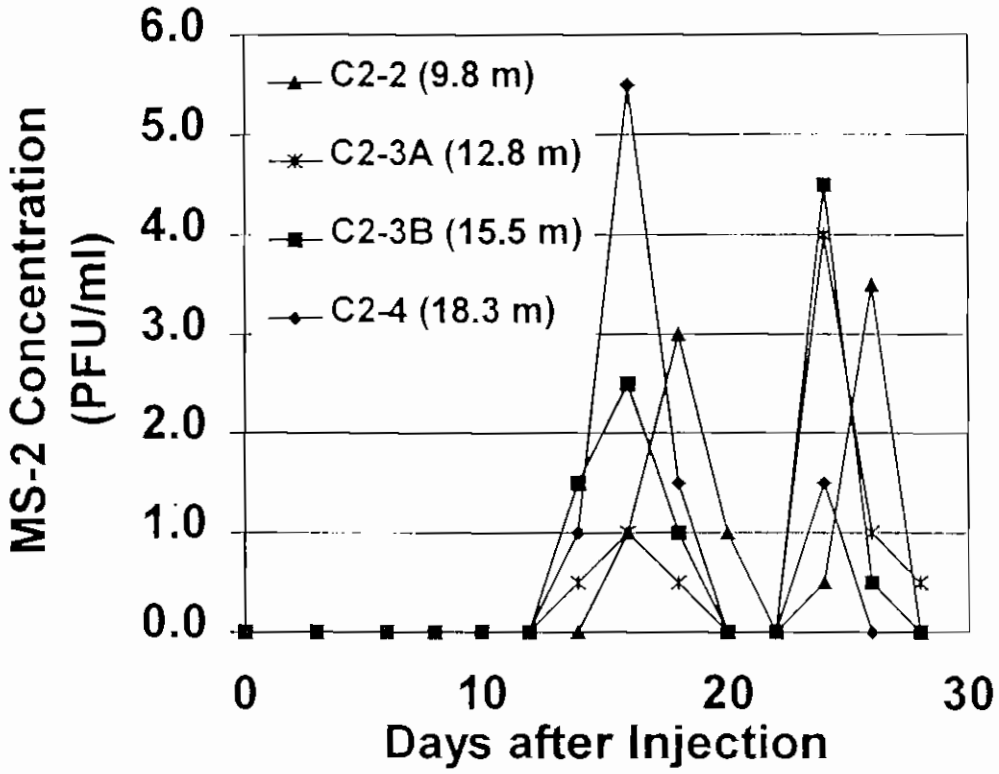

Figure 3-6. Breakthrough curve for MS-2 in the C2 wellnest for the natural gradient virus tracer test.

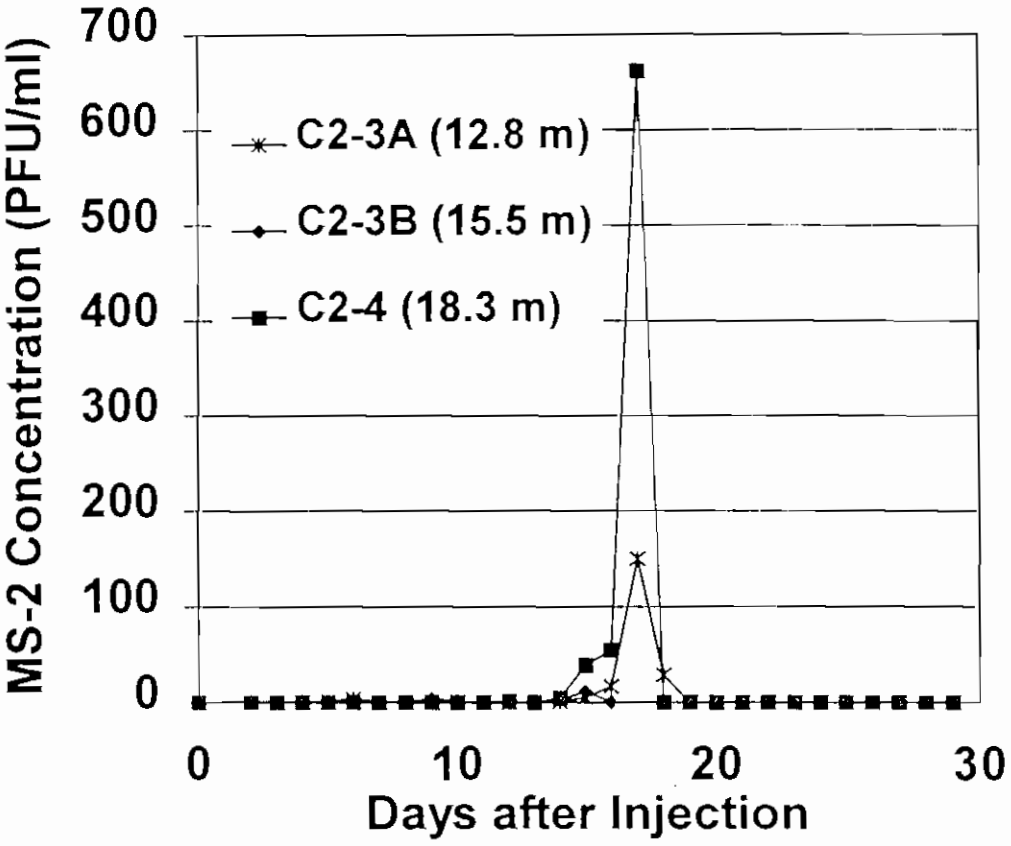

Figure 3-7. Breakthrough curve for MS-2 in the C2 wellnest (34 m down-gradient) for the first large-scale forced gradient virus tracer test.

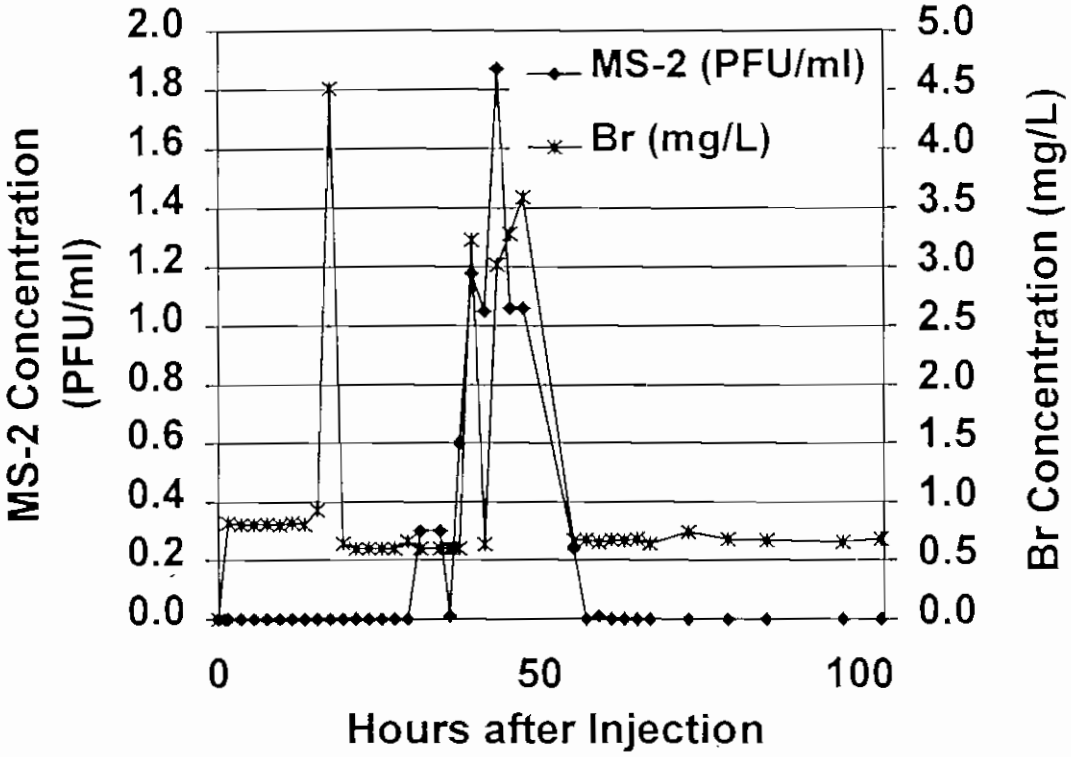

Figure 3-9. Breakthrough curve for MS-2 in the P2-WT well (10 m down-gradient) for the first small-scale forced gradient virus tracer test.

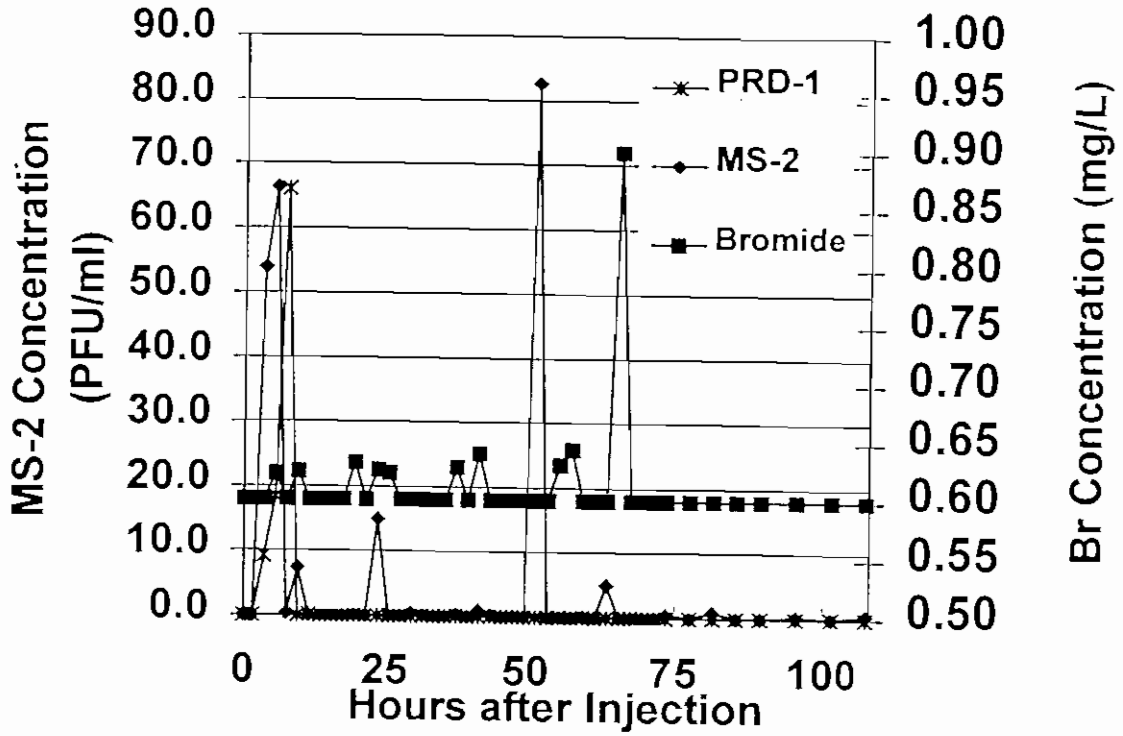

Figure 3-10. Breakthrough curve for MS-2, PRD-1, and bromide in the P2-WT well (10 m down-gradient) for the second small-scale forced gradient virus tracer test.

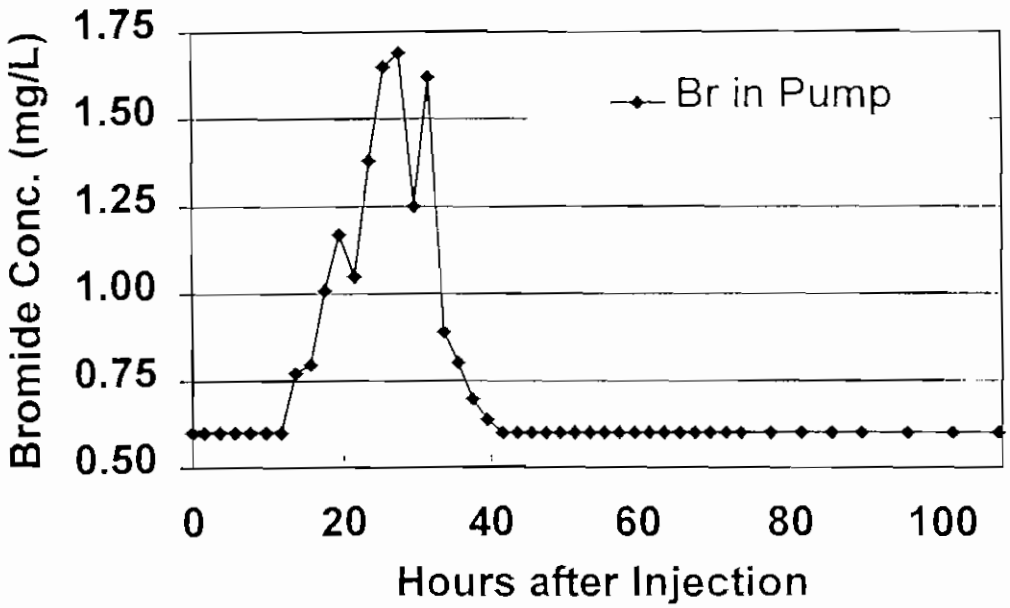

Figure 3-12. Breakthrough curve for bromide in the pumping well (P-WT, 12 m down-gradient) for the second small-scale forced gradient virus tracer test.

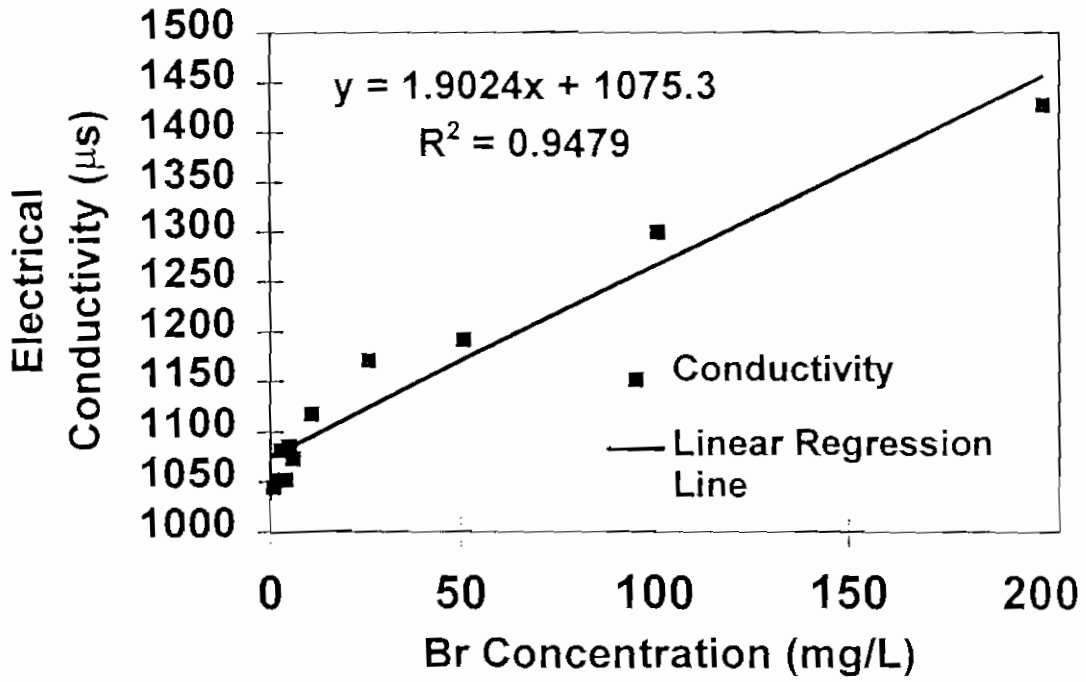

Figure 3-13. Plot of bromide concentration in the ground water research site versus electrical conductivity with a linear regression line also shown.

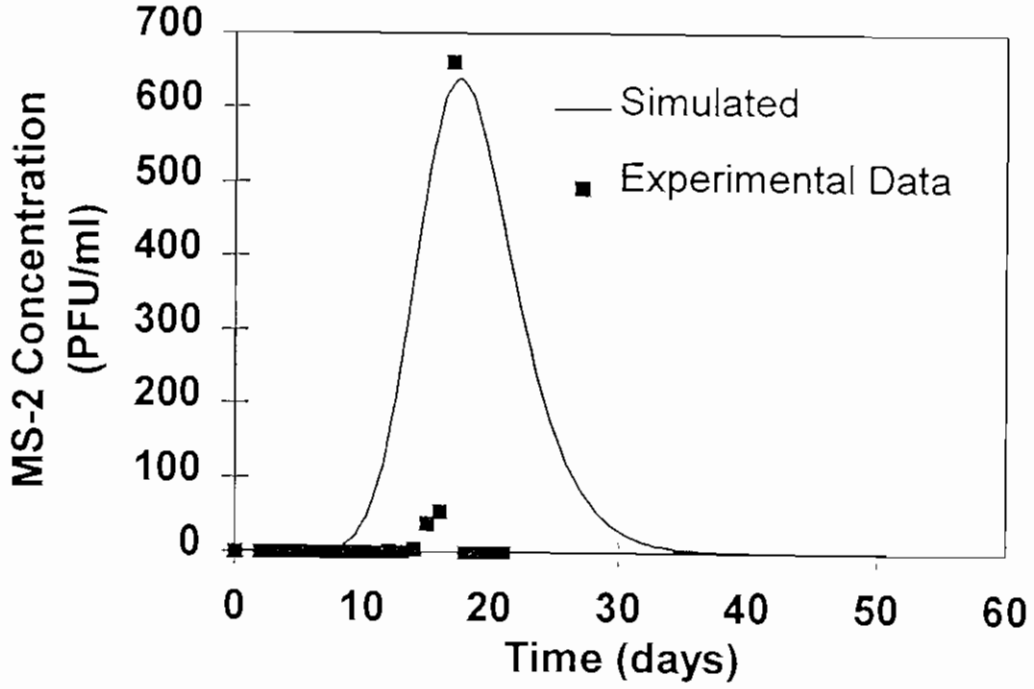

Figure 3-15. VIRALT simulation of large-scale MS-2 transport under a forced gradient to the C2 wellnest 34 m down-gradient. Experimental data from first large-scale forced gradient virus tracer test is also shown.

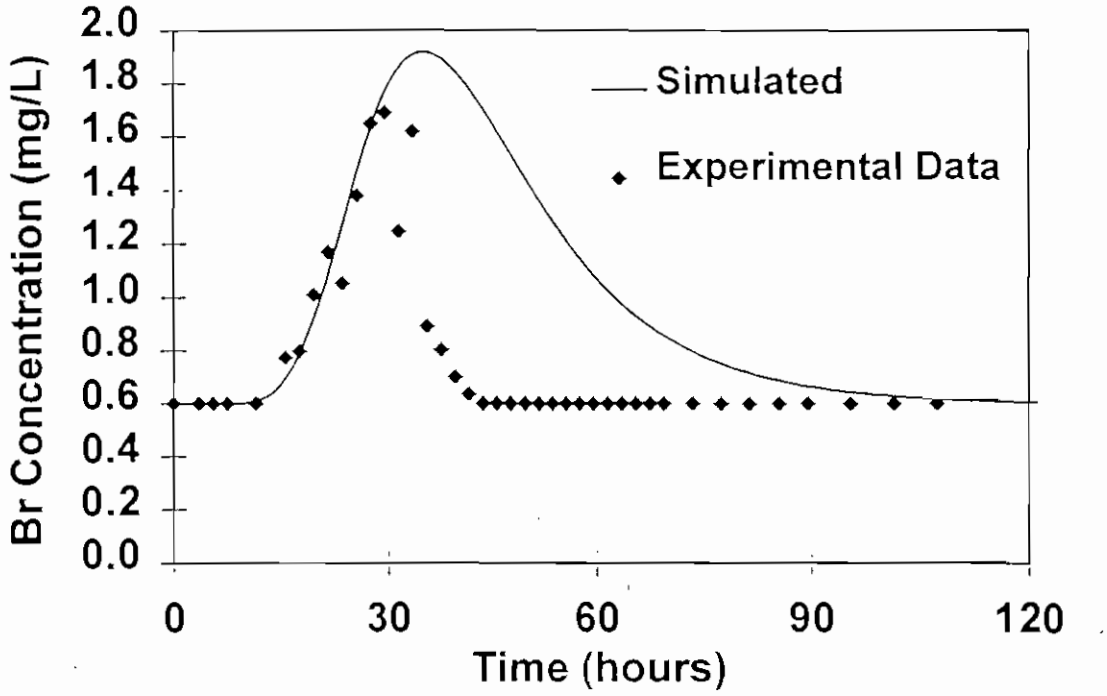

Figure 3-16. VIRALT simulation of small-scale bromide transport under a forced gradient to the pumping well 12 m down-gradient. Experimental data from second small-scale forced gradient virus tracer test is also shown.

CHAPTER IV

CONCLUSIONS

Summary

During this research five virus tracer tests were completed. Methods of ground water sample collection have been explored and many lessons learned while completing virus tracer studies at the Texas A&M Research Farm near College Station, Texas. These methods combine ground water sampling procedures for chemical and bacterial analysis with factors specific to viral analysis. Ground water samples obtained for viral analysis must be dealt with in an expedient and careful manner to insure the integrity of the results. All sampling equipment must be properly sterilized for each sample. If the temperature and exposure to radiation is not controlled during collecting of the sample, the survival of viruses in the sample could be affected. The aquifer temperature, pH, dissolved oxygen, and water level should also be monitored to detect changes which could affect virus survival in the aquifer. As increased concern is put towards possible threats from viral contamination in ground water, proper sampling techniques will help enable us to accurately determine the presence of these viruses.

These studies indicated that MS-2 bacteriophage is capable of traveling at least 34 m in this heterogeneous sandy aquifer. The natural gradient showed a peak MS-2 concentration less than 1 percent of the detected forced gradient peak. The bacteriophage also showed much attenuation in the forced gradient condition, with peaks less than 1 percent of those of bromide. The large-scale experiment also showed much less dispersion than predicted by the VIRALT model. PRD-1 was not detected in the monitoring wells 34 m down-gradient. The small-scale tests reinforced the importance of sampling methods and aquifer heterogeneity on

results. These tests indicated peaks in the same wells at different times for each test, even though the same gradient was present. This difference is because the samples from each test were obtained from a different depth in the aquifer. Heterogeneity tests indicate that flowpaths from the injection zone may transport the tracer in an upward direction. Using values obtained from past studies at the site, the EPA virus model VIRALT predicted the approximate peak time and peak concentrations with 2.9 percent for MS-2 and 14 percent for bromide.

Future Research

Because this study was the first tracer study at the Texas A&M Research Farm site, much was discovered and new questions have arisen which should be addressed in future tracer studies at the site. Further characterization of aquifer in the vicinity both the small-scale and the large-scale experiments is needed so that the location of the virus plumes can be more readily estimated for sampling purposes. Also, a more elaborate sampling network should be put in place so that virus transport not directly in line between the injection well and the pumping well can be measured. This network should include wells which are closer to the injection well than the C2 wellnest, located in-line with the current wells and offset to each side. Fully-screened wells with multi-level samplers would work the best for these wells as they would not take up as much space in the field and would give discrete samples from various levels in the aquifer.

Once a better well network has been installed, multiple tracer tests with a conservative tracer such as bromide should be performed to characterize the contaminant

transport characteristics of the aquifer. This will allow for better planning of the sampling intervals and locations needed to show the transport of the virus.

The final problem which should be dealt with in future studies is sample status at all times between sampling and analysis. With the procedures used for this project, the parameters which influence virus survival (temperature, pH, dissolved oxygen, exposure to solar radiation) were controlled at all times except during transport from College Station to the analysis lab in El Paso, Texas. Because the temperature of the samples may have changed during transport, survival of the viruses may have been compromised. A better alternative would be to have sample analysis as close to the research site as possible so that these parameters do not change.

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APPENDIX A

WATER TABLE ELEVATIONS DURING VIRUS TRACER TESTS

Table A-1. Water table elevations (m) and average hydraulic gradients during the natural gradient virus tracer test.

	C2-2	C2-3A	C2-3B	C2-4	V1-1	V1-2	V1-3	V1-4	I-WT	P-WT
11/19/95	58.96	58.96	58.95	58.91	58.89	58.84	58.86	58.85	59.01	
11/22/95	58.96	58.95	58.94	58.91						58.74
11/24/95	58.92	58.93	58.92	58.88						58.72
11/26/95	58.93	58.93	58.93	58.89	58.84	58.81	58.82	58.77		58.71
11/28/95	58.91	58.91	58.90	58.87	58.83	58.80	58.80	58.78		58.71
11/30/95	58.89	58.89	58.88	58.85	58.81	58.79	58.79	58.77		58.69
12/4/95	58.86	58.87	58.86	58.83	58.78	58.75	58.76	58.75		58.66
12/6/95	58.85	58.86	58.85	58.82	58.77	58.74	58.75	58.74		58.65
12/8/95	58.84	58.84	58.83	58.81	58.75	58.75	58.73	58.71		58.64
12/10/95	58.83	58.82	58.79	58.77	58.73	58.73	58.73	58.70		58.63
12/12/95	58.82	58.82	58.81	58.78	58.72	58.72	58.71	58.69		58.62
12/14/95	58.81	58.80	58.80	58.77	58.71	58.71	58.70	58.68	58.85	58.61
12/16/95	58.79	58.79	58.78	58.76	58.70	58.70	58.68	58.67		
12/18/95	58.78	58.77	58.77	58.75	58.68	58.69	58.67	58.66		58.59

Average Hydraulic Gradient	
IWT - C2	0.0018
C2 - V1	0.0023
V1 - PWT	0.0024
IWT - PWT	0.0022

Table A-2. Water table elevations (m) and average hydraulic gradients during the first large-scale forced-gradient virus tracer test.

	C2-2	C2-3A	C2-3B	C2-4	V1-1	V1-2	V1-3	V1-4	I-WT	P-WT
1/15/96	58.53	58.52	58.50	58.41	58.32	58.32	58.21	58.04	58.65	56.58
1/16/96	58.51	58.50	58.48	58.39	58.28	58.28	58.18	58.01	58.64	56.54
1/20/96	58.46	58.45	58.43	58.34	58.20	58.20	58.10	57.93	55.95	56.43
1/22/96	58.45	58.44	58.42	58.33	58.17	58.18	58.09	57.91		56.42
1/23/96	58.44	58.43	58.41	58.32	58.17	58.17	58.09	57.90		56.41
1/24/96	58.43	58.42	58.41	58.31	58.16	58.16	58.38	57.89		
1/25/96	58.42	58.41	58.40	58.31	58.15	58.15	58.36	57.88		
1/26/96	58.41	58.41	58.39	58.30	58.14	58.14	58.04	57.87		56.45
1/27/96	58.41	58.40	58.38	58.28	58.14	58.14	58.04	57.86		56.34
1/28/96	58.40	58.39	58.38	58.28	58.13	58.13	58.03	57.86		56.38
1/29/96	58.39	58.38	58.37	58.27	58.21	58.21	58.02	57.86		56.46
1/30 96	58.39	58.37	58.35	58.25	58.11	58.11	58.01	57.84		56.39
1/31/96	58.37	58.36	58.35	58.25	58.10	58.10	58.01	57.84		
2/1/96	58.35	58.34	58.33	58.23	58.09	58.09	57.99	57.82		
2/2/96	58.34	58.33	58.32	58.22	58.08	58.08	57.98	57.81		
2/3/96	58.33	58.32	58.30	58.20	58.07	58.07	57.97	57.80		
2/4/96	58.32	58.29	58.28	58.20	58.06	58.06	57.96	57.79	58.49	56.33
2/5/96	58.32	58.29	58.28	58.20	58.06	58.06	57.96	57.79		56.32
2/6/96	58.31	58.30	58.28	58.18	58.03	58.04	57.94	57.77		56.33
2/7/96	58.30	58.29	58.27	58.18	58.03	58.03	57.94	57.77		56.39
2/8/96	58.29	58.28	58.26	58.16	58.02	58.02	57.92	57.75	58.45	56.29
2/9/96	58.27	58.27	58.25	58.15	58.02	58.02	57.92	57.75	58.44	56.27
2/10/96	58.21	58.20	58.19	58.09	57.96	57.96	57.86	57.69	58.37	56.32
2/11/96	58.25	58.24	58.23	58.13	57.99	57.99	57.89	57.71	58.41	56.26
2/12/96	58.23	58.22	58.21	58.11	57.99	57.99	57.88	57.71	58.39	56.26
2/13/96	58.22	58.21	58.20	58.10	57.96	57.96	57.86	57.69	58.39	56.25
2/15/96	58.19	58.18	58.16	58.06	57.93	57.93	57.82	57.65		56.11

Average Hydraulic Gradient	
IWT - C2	0.006
C2 - V1	0.008
V1 - PWT	0.042
IWT - PWT	0.019

Table A-3. Water table elevations (m) and average hydraulic gradients during the second large-scale forced-gradient virus tracer test.

	C2-2	C2-3A	C2-3B	C2-4	V1-1	V1-2	V1-3	V1-4	P-WT
4/25/96				57.85				57.49	56.05
4/27/96	57.92	57.90	57.89		57.69	57.69	57.59	57.42	55.96
4/28/96	57.91	57.88	57.88		57.67	57.67	57.56	57.40	55.93
5/1/96	58.02	58.00	57.99		57.81	57.81	57.70	57.53	57.19
5/2/96	57.94	57.92	57.90		57.72	57.72	57.62	57.44	56.29
5/3/96	57.88	57.90	57.88		57.68	57.68	57.58	57.41	56.06
5/4/96	57.88	57.87	57.85		57.65	57.64	57.55	57.38	55.95
5/5/96	57.85	57.84	57.83	57.73	57.62	57.62	57.52	57.35	55.92
5/6/96	57.84	57.82	57.81	57.72	57.58	57.58	57.48	57.32	55.89
5/7/96	57.82	57.81	57.79	57.70	57.57	57.57	57.47	57.31	55.88
5/8/96	dry	57.78	57.77	57.68	57.55	57.54	57.45	57.29	55.85
5/9/96	dry	57.76	57.74	57.65	57.51	57.51	57.43	57.27	55.83
5/10/96	dry	57.75	57.73	57.63	57.51	57.51	57.42	57.25	55.82
5/11/96	dry	57.71	57.70	57.60	57.47	57.47	57.38	57.21	55.78
5/12/96	dry	57.70	57.68	57.59	57.47	57.47	57.37	57.20	55.78
5/13/96	dry	57.68	57.67	57.58	57.46	57.46	57.36	57.20	55.77
5/14/96	dry	57.67	57.66	57.57	57.44	57.45	57.35	57.17	55.75
5/15/96	dry	57.66	57.64	57.56	57.42	57.42	57.32	57.15	55.73
5/16/96	dry	57.65	57.63	57.54	57.41	57.41	57.31	57.14	55.72
5/17/96	dry	57.64	57.63	57.53	57.40	57.40	57.30	57.13	55.70
5/18/96	dry	57.62	57.61	57.51	57.39	57.39	57.29	57.12	55.69
5/19/96	dry	57.61	57.59	57.50	57.37	57.37	57.27	57.10	55.68
5/21/96	dry	57.47	57.56	57.57	57.34	57.33	57.24	57.06	55.64
5/22/96	dry			57.45				57.06	55.62
5/23/96	dry	57.54	57.53	57.44	57.31	57.31	57.21	57.04	55.79
5/26/96	dry	57.54	57.53	57.47	57.33	57.34	57.29	57.20	55.86
5/30/96	dry	57.53	57.53	57.50	57.35	57.36	57.36	57.36	55.93

Average Hydraulic Gradient	
C2 - V1	0.007
V1 - PWT	0.040
C2 - PWT	0.023

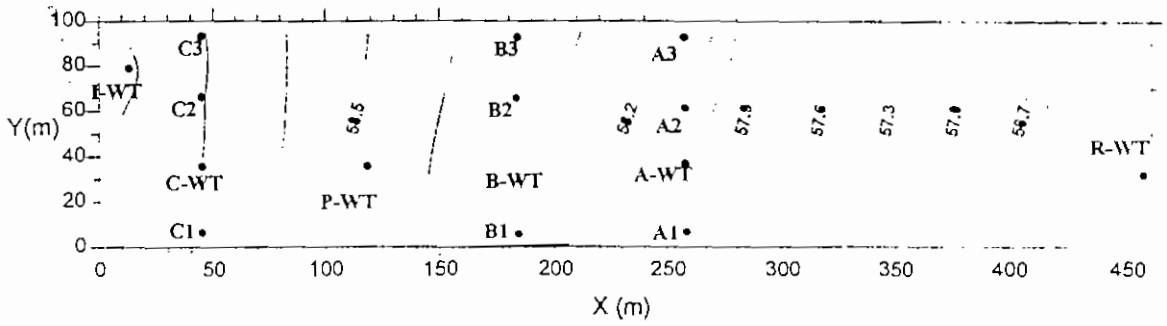

Figure A-1. Example ground water contour map for the natural gradient virus tracer test.

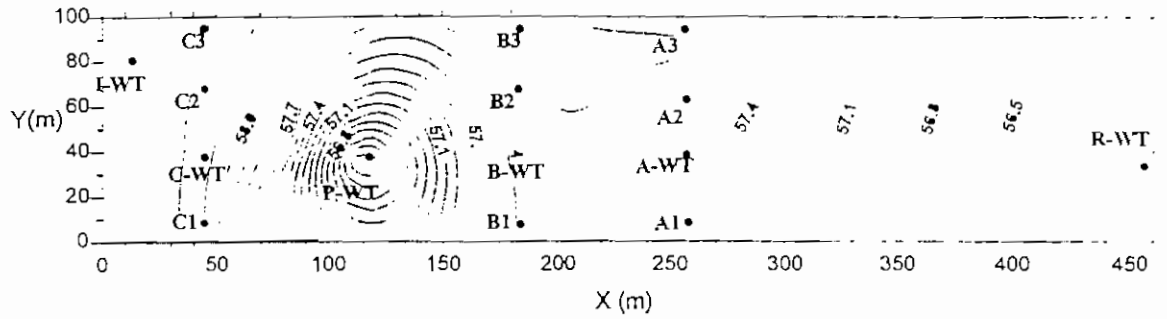

Figure A-2. Example ground water contour map for first large-scale forced gradient virus tracer test.

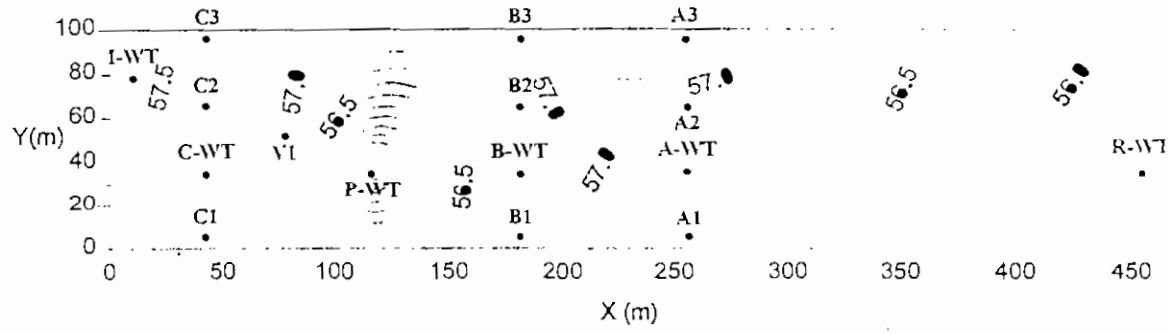

Figure A-3. Example ground water contour map for third large-scale forced gradient virus tracer test.

APPENDIX B

GROUND WATER TEMPERATURES DURING VIRUS TRACER TESTS

Table B-1. Ground water temperature (°C) during the natural gradient virus tracer test.

	C2-2	C2-3A	C2-3B	C2-4	V1-1	V1-2	V1-3	V1-4
11/22/95	19.0	19.0	19.5	19.0				
11/24/95	18.5	19.0	19.0	18.5				
11/26/95	19.0	19.0	20.0	19.0	18.0	18.5	18.5	19.5
11/28/95	17.5	17.5	18.0	18.0	17.5	17.0	17.5	18.0
11/30/95	17.5	18.0	18.0	18.5				
12/4/95	17.5	18.0	18.0	18.5				
12/6/95	19.0	19.0	19.0	19.0				
12/8/95	18.5	18.5	18.5	19.0				
12/10/95	17.0	17.5	17.0	17.0				
12/14/95	21.5	21.0	21.5	21.0				
12/18/95	19.0	19.5	20.0	19.5				

Table B-2. Ground water temperature (°C) during the first forced-gradient virus tracer test.

	C2-2	C2-3A	C2-3B	C2-4	V1-1	V1-2	V1-3	V1-4	Injection	Pump
1/22/96	20.5	20.5	20.5	20.5						
1/23/96	21.0	21.5	21.0	21.5						
1/24/96	20.0	20.0	20.5	20.5				20.5		
1/25/96	20.5	20.5	21.0	20.0				19.5		
1/26/96	19.0	21.0	20.0	20.0	19.5	19.0	19.0	19.5		19.5
1/27/96	20.0	20.0	20.0	19.5	20.0	20.0		19.5		
1/28/96	19.5	19.0	19.5	20.0	19.5	19.5	19.5	19.0		19.0
1/29/96	21.0	21.0	21.0	21.0	21.0	21.0	20.5	21.0		21.0
1/30/96	21.0	21.0	21.0	21.0	21.5	21.0	21.5	21.0		21.5
1/31 96	17.5	17.5	17.5	18.0						
2/1/96	18.0	19.0	19.0	18.5						
2/2/96	18.5	19.0	19.0	17.5						
2/3/96	19.5	19.5	19.0	19.0						
2/4/96	19.5	19.5	19.5	19.0						
2/5/96	19.5	19.0	19.5	19.5	19.0	19.0	19.0	19.5		18.5
2/6/96	20.0	20.0	20.0	20.0	20.0	19.5		19.5		20.0
2/7/96	21.0	21.0	21.0	21.0	21.0	21.0	21.0	21.0		20.5
2/8/96					21.0	21.5	21.0	21.0		21.0
2/9/96	21.0	21.0	21.0	21.0	21.0	21.0	21.0	21.0		21.0
2/10/96					21.0	21.0	21.0	21.0		21.0
2/11/96	20.5	20.5	20.5	20.5	20.5	20.0	20.0	20.0	20.0	
2/12/96					21.0	21.0	21.0	21.0		21.0
2/13/96	21.0	21.0	21.0	21.0	21.0	20.0	20.0	20.5		21.0
2/15/96	21.0	21.0	21.0	20.0	20.0			20.5	21.0	21.0

Table B-3. Ground water temperature (°C) during the second forced-gradient virus tracer test.

	V1-1	V1-2	V1-3	V1-4	Pumping
4/27/96	21.5	21.5	21.0	21.0	21.0
4/28/96	21.5	21.5	21.0	21.0	21.0
4/29/96	21.5	21.0	21.0	21.0	21.5
4/30/96	21.0	21.0	21.0	21.0	
5/1/96	22.0	22.0	22.0	22.0	21.5
5/2/96	21.5	21.0	21.0	21.0	21.0
5/3/96	22.0	22.0	22.0	22.0	21.5
5/4/96	22.0	22.0	22.0	22.0	22.0
5/5/96	22.0	22.0	22.0	22.0	22.0
5/6/96	21.5	21.0	21.0		21.5
5/7/96	21.5	21.5	21.5	21.0	21.5
5/8/96	22.0	21.5	21.0	21.0	21.5
5/9/96	22.0	22.0	22.5	22.5	22.0
5/10/96	22.0	22.0	22.0	22.0	22.0
5/11/96	22.0	22.0	22.0	22.0	22.0
5/12/96	22.0	22.0	22.0	22.5	22.0
5/13/96	22.0	21.5	21.5	21.5	22.0
5/14/96	22.0	21.0	21.5	22.0	21.5
5/15/96	21.0	21.0	21.0	21.0	21.5
5/16/96	21.5	21.5	21.0	21.0	21.0
5/17/96	22.0	22.0	21.5	22.0	21.5
5/18/96	21.5	21.0	21.0	21.0	21.0
5/19/96	22.0	21.0	21.0	21.0	21.0
5/21/96	21.5	21.0	21.0	21.0	
5/22/96				21.0	
5/23/96	22.5	22.0	22.0	22.0	
5/26/96	22.0	22.0	22.0	22.0	
5/30/96	20.0	20.0	20.0	19.5	

Table B-4. Ground water temperature (°C) during the third large-scale forced-gradient virus tracer test.

		C2-3A	C2-3B	C2-4			C2-3A	C2-3B	C2-4
7/26/96		22.8	22.8	22.8	8/7/96	7:00 AM	22.8	22.8	22.2
7/27/96		21.7	22.2	22.2	8/7/96	1:00 PM	23.3	22.8	22.8
7/28/96		22.2	22.8	22.2	8/7/96	7:00 PM			22.2
7/29/96		22.2	22.2	22.2	8/8/96	1:00 AM	22.2	22.2	22.2
7/30/96		22.8	22.2	22.2	8/8/96	7:00 AM	22.8	22.8	22.2
7/31/96		22.8	22.8	22.8	8/8/96	1:00 PM	23.3	23.3	22.8
8/1/96	1:00 PM	23.9	22.8	23.9	8/8/96	7:00 PM	22.2	22.2	22.2
8/1/96	7:00 PM	23.9	23.9	23.3	8/9/96	1:00 AM	22.2	22.2	22.2
8/2/96	1:00 AM	22.2	22.2	22.2	8/9/96	7:00 AM	22.2	22.2	22.2
8/2/96	7:00 AM	22.2	22.8	22.8	8/9/96	1:00 PM	23.3	23.3	23.9
8/2/96	1:00 PM	24.4	22.2	22.2	8/9/96	7:00 PM	22.8	22.2	22.8
8/2/96	7:00 PM	22.2	22.2	22.8	8/10/96	1:00 AM	21.7	22.2	21.7
8/3/96	1:00 AM	22.2	22.2	22.2	8/10/96	7:00 AM			
8/3/96	7:00 AM	21.7	21.7	21.7	8/10/96	1:00 PM	23.3	23.9	23.3
8/3/96	1:00 PM	23.3	22.2	22.2	8/10/96	7:00 PM	22.8	22.2	22.2
8/3/96	7:00 PM	23.3	22.8	22.2	8/11/96	1:00 AM	22.2	21.7	21.7
8/4/96	1:00 AM				8/11/96	7:00 AM	21.7	21.7	21.1
8/4/96	7:00 AM	22.2	22.2	22.2	8/11/96	1:00 PM	22.2	22.8	22.2
8/4/96	1:00 PM	23.3	23.3	23.9	8/11/96	7:00 PM	22.2	22.2	22.2
8/4/96	7:00 PM	22.2	22.2	22.8	8/12/96	7:00 AM			
8/5/96	1:00 AM	22.2	22.2	22.2	8/12/96	7:00 PM	22.2	22.8	22.8
8/5/96	7:00 AM	21.7	22.2	22.2	8/13/96	7:00 AM	22.2	22.8	22.2
8/5/96	1:00 PM	23.3	23.3	23.3	8/13/96	7:00 PM	22.2	22.2	22.2
8/5/96	7:00 PM	23.3	23.3	22.8	8/14/96	1:00 AM	21.7	22.2	21.7
8/6/96	1:00 AM	22.2	22.2	22.2	8/14/96	7:00 AM	22.2	22.2	22.2
8/6/96	7:00 AM	22.8	22.2	22.2	8/15/96	7:00 AM	22.2	22.8	22.2
8/6/96	1:00 PM	22.2	23.3	22.8	8/16/96	7:00 AM		22.2	22.2
8/6/96	7:00 PM	23.3	23.3	22.2	8/18/96	7:00 PM			22.8
8/7/96	1:00 AM	22.2	22.2	22.2	8/19/96	7:00 PM	22.2	22.2	22.8

Table B-5. Ground water temperature (°C) during the first small-scale forced-gradient virus tracer test.

		P1-WT	P2-WT			P1-WT	P2-WT
7/31/96	8:00 AM	22.2	22.2	8/2/96	12:00 AM	22.2	22.2
7/31/96	10:00 AM	22.2	22.2	8/2/96	2:00 AM	22.2	22.2
7/31/96	12:00 PM	22.8	23.3	8/2/96	4:00 AM	22.2	22.2
7/31/96	2:00 PM	23.3	22.8	8/2/96	6:00 AM		22.2
7/31/96	4:00 PM	23.3	23.3	8/2/96	8:00 AM	22.8	22.8
7/31/96	6:00 PM	23.3	22.8	8/2/96	10:00 AM		
7/31/96	8:00 PM	22.2	22.2	8/2/96	12:00 PM		23.3
7/31/96	10:00 PM	22.2	22.2	8/2/96	2:00 PM	23.3	23.3
8/1/96	12:00 AM	22.2	22.2	8/2/96	4:00 PM		22.8
8/1/96	2:00 AM	22.8	22.2	8/2/96	6:00 PM		22.8
8/1/96	4:00 AM	22.2	22.2	8/2/96	8:00 PM	22.8	22.8
8/1/96	6:00 AM	22.2	21.7	8/2/96	10:00 PM		22.2
8/1/96	8:00 AM		22.2	8/3/96	12:00 AM		22.8
8/1/96	10:00 AM	23.9	23.3	8/3/96	2:00 AM	22.2	22.2
8/1/96	12:00 PM	23.9	25.0	8/3/96	8:00 AM	22.2	22.2
8/1/96	2:00 PM	23.9	22.8	8/3/96	2:00 PM	23.9	23.3
8/1/96	4:00 PM	24.4		8/3/96	8:00 PM	22.8	22.8
8/1/96	6:00 PM	24.4	22.8	8/3/96	2:00 AM		
8/1/96	8:00 PM	23.3	22.2	8/4/96	8:00 AM		23.3
8/1/96	10:00 PM	22.8	22.2	8/4/96	2:00 PM	23.3	23.3

Table B-6. Ground water temperature (°C) during the second small-scale forced-gradient virus tracer test.

		P1-WT	P2-WT			P1-WT	P2-WT
8/28/96	10:00 PM	22.2	22.8	8/30/96	6:00 PM	22.2	22.2
8/29/96	12:00 AM	22.2	22.2	8/30/96	8:00 PM	22.2	22.2
8/29/96	2:00 AM	22.2	22.2	8/30/96	10:00 PM	21.7	22.2
8/29/96	4:00 AM	22.8	22.2	8/31/96	12:00 AM	22.2	22.2
8/29/96	6:00 AM	23.3	23.3	8/31/96	2:00 AM	22.2	22.2
8/29/96	8:00 AM	22.8	23.3	8/31/96	4:00 AM	22.2	22.2
8/29/96	10:00 AM	24.4	24.4	8/31/96	6:00 AM	22.8	22.8
8/29/96	12:00 PM	23.3	23.3	8/31/96	8:00 AM	22.8	23.1
8/29/96	2:00 PM	23.9	23.3	8/31/96	10:00 AM	23.3	23.3
8/29/96	4:00 PM	22.2	22.8	8/31/96	12:00 PM	23.1	23.3
8/29/96	6:00 PM	22.2	22.2	8/31/96	2:00 PM	23.3	24.4
8/29/96	8:00 PM	22.2	22.2	8/31/96	4:00 PM	23.6	24.4
8/29/96	10:00 PM	22.2	22.2	8/31/96	6:00 PM	23.9	23.9
8/30/96	12:00 AM	22.2	22.2	8/31/96	10:00 PM	22.2	22.2
8/30/96	2:00 AM	22.2	22.2	9/1/96	2:00 AM	22.2	22.2
8/30/96	4:00 AM	22.2	22.2	9/1/96	6:00 AM	22.2	22.2
8/30/96	6:00 AM	22.2	22.2	9/1/96	10:00 AM	23.3	23.3
8/30/96	8:00 AM	22.2	22.2	9/1/96	2:00 PM	23.3	24.4
8/30/96	10:00 AM	23.3	23.3	9/1/96	8:00 PM	22.8	22.8
8/30/96	12:00 PM	24.4	22.8	9/2/96	2:00 AM	22.2	22.2
8/30/96	2:00 PM	22.8	23.6	9/2/96	8:00 AM	22.2	22.2
8/30/96	4:00 PM	22.5	22.8				

APPENDIX C

GROUND WATER pH DURING VIRUS TRACER TESTS

Table C-1. Ground water pH during the natural gradient virus tracer test.

	C2-2	C2-3A	C2-3B	C2-4	V1-1	V1-2	V1-3	V1-4
11/22/95	6.84	6.85	6.93	6.99				
11/24/95	6.52	6.56	6.59	6.80				
11/26/95	6.85	6.86	6.98	7.20	6.67	6.76	6.77	6.85
11/28/95	6.83	6.90	6.92	7.00	6.80	6.76	6.77	6.95
11/30/95	6.66	6.72	6.73	6.86				
12/4/95	6.52	6.59	6.61	6.80				
12/6/95	6.76	6.77	6.87	7.00				
12/8/95	6.70	6.74	6.81	6.94				
12/10/95	6.63	6.71	6.74	6.88				
12/14/95	6.66	6.80	6.77	6.98				

Table C-2. Ground water pH during the first forced-gradient virus tracer test.

	C2-2	C2-3A	C2-3B	C2-4	V1-1	V1-2	V1-3	V1-4	Injection	Pump
1/22/96	6.68	6.78	6.85	6.96						
1/23/96	6.63	6.74	6.83	6.99						
1/24/96	6.84	6.87	6.86	6.87				6.96		
1/25/96	6.80	6.91	6.97	7.04				6.96		
1/26/96	6.75	6.81	6.71	6.94	6.73	6.81	6.91	6.86		6.87
1/27/96	6.78	6.82	6.91	7.03	6.87	6.85		7.00		
1/28/96	6.68	6.81	6.83	6.99	6.74	6.82	6.84	6.87		6.91
1/29/96	6.79	6.81	6.84	7.04	6.80	6.88	6.98	6.93		6.91
1/30/96	6.91	6.97	7.01	7.14	6.93	7.01	7.04	7.13		6.96
1/31/96	6.47	6.65	6.63	6.78						
2/1/96	6.63	6.62	6.66	6.75						
2/2/96	6.75	6.70	6.81	6.91						
2/3/96	6.70	6.81	6.80	6.91						
2/4/96	6.70	6.80	6.83	6.91						
2/5/96	6.68	6.76	6.71	6.90	6.69	6.80	6.84	6.76		6.64
2/6/96	6.76	6.82	6.83	6.98	6.84	6.97		6.97		6.89
2/7/96	6.73	6.86	6.87	6.96	6.83	6.88	6.84	6.92		6.90
2/8/96					6.68	6.78	6.85	6.94	6.85	6.85
2/9/96	6.70	6.88	6.90	6.99	6.85	6.86	6.88	6.95		6.85
2/10/96					6.80	6.83	6.90	6.96		6.88
2/11/96	6.83	6.91	6.95	7.04	6.76	6.85	6.85	6.91	6.91	6.90
2/12/96					6.78	6.89	6.88	6.89		6.94
2/13/96	6.83	6.83	6.98	6.93	6.98	6.94	7.01	6.91		6.97
2/15/96	6.65	6.82	6.73	6.99	6.79			6.93	6.92	6.89

Table C-3. Ground water pH during the second forced-gradient virus tracer test.

	V1-1	V1-2	V1-3	V1-4	Pumping
4/27/96	6.79	6.85	6.84	6.92	6.84
4/28/96	6.90	6.92	6.98	7.02	6.91
4/29/96	6.80	6.85	6.86	6.93	6.82
4/30/96	6.88	6.76	6.74	6.82	
5/1/96	6.93	6.96	7.11	7.05	7.11
5/2/96	6.64	6.76	6.88	6.85	6.74
5/3/96	6.75	6.83	6.88	6.95	6.80
5/4/96	6.83	6.92	6.96	7.03	6.94
5/5/96	6.84	6.92	6.95	7.00	6.87
5/6/96	6.82	6.92	6.91		6.84
5/7/96	6.80	6.90	6.91	6.98	6.83
5/8/96	6.70	6.81	6.86	6.94	6.78
5/9/96	6.83	6.93	6.94	7.00	6.87
5/10/96	6.79	6.90	6.91	6.98	6.86
5/11/96	6.80	6.85	6.90	6.91	6.86
5/12/96	6.94	6.96	6.99	7.04	6.97
5/13/96	6.91	6.92	6.99	7.06	7.01
5/14/96	6.94	6.96	7.09	7.04	7.05
5/15/96	6.83	6.86	6.94	7.02	6.85
5/16/96	6.84	6.92	7.02	7.07	6.85
5/17/96	6.92	7.10	7.13	7.16	7.07
5/18/96	6.92	7.02	7.01	7.01	6.96
5/19/96	6.84	6.87	6.95	7.03	6.94

Table C-4. Ground water pH during the third large-scale forced-gradient virus tracer test.

		C2-3A	C2-3B	C2-4			C2-3A	C2-3B	C2-4
7/26/96		7.10	7.08	7.17	8/8/96	1:00 AM	6.74	6.71	6.76
7/27/96		6.98	7.07	7.12	8/8/96	7:00 AM	6.74	6.81	6.76
7/28/96		6.94	6.95	7.16	8/8/96	1:00 PM	6.83	6.77	6.79
7/29/96		6.98	7.01	7.09	8/8/96	7:00 PM	6.80	6.68	6.83
7/30/96		6.95	6.98	7.05	8/9/96	1:00 AM	6.57	6.62	6.73
7/31/96		6.98	7.01	7.09	8/9/96	7:00 AM	6.75	6.69	6.73
8/1/96	1:00 PM	6.84	6.94	6.86	8/9/96	1:00 PM	6.85	6.78	6.91
8/1/96	7:00 PM	6.85	6.92	6.89	8/9/96	7:00 PM	6.70	6.63	6.82
8/2/96	1:00 AM	6.78	6.80	6.84	8/10/96	1:00 AM	6.63	6.62	6.72
8/2/96	7:00 AM	6.58	6.72	6.82	8/10/96	7:00 AM			
8/2/96	1:00 PM	6.83	6.73	6.82	8/10/96	1:00 PM	6.79	6.77	6.86
8/2/96	7:00 PM			6.64	8/10/96	7:00 PM	6.68	6.64	6.67
8/3/96	1:00 AM	6.58	6.70	6.75	8/11/96	1:00 AM	6.62	6.63	6.75
8/3/96	7:00 AM	6.65	6.71	6.78	8/11/96	7:00 AM	6.71	6.67	6.70
8/3/96	1:00 PM	6.72	6.83	6.87	8/11/96	1:00 PM	6.85	6.83	7.04
8/3/96	7:00 PM	6.67	6.73	6.71	8/11/96	7:00 PM	6.68	6.70	6.78
8/4/96	1:00 AM				8/12/96	1:00 AM			
8/4/96	7:00 AM	6.76	6.71	6.71	8/12/96	7:00 AM			
8/4/96	1:00 PM	6.63	6.72	6.82	8/12/96	1:00 PM	6.76	6.82	6.86
8/4/96	7:00 PM	6.66	6.69	6.73	8/12/96	7:00 PM			
8/5/96	1:00 AM	6.61	6.53	6.70	8/13/96	1:00 AM			
8/5/96	7:00 AM	6.85	6.70	6.67	8/13/96	7:00 AM	6.70	6.80	6.81
8/5/96	1:00 PM	6.73	6.64	6.76	8/13/96	1:00 PM			
8/5/96	7:00 PM	6.73	6.70	6.76	8/13/96	7:00 PM	6.74	6.83	6.88
8/6/96	1:00 AM	6.49	6.60	6.66	8/14/96	1:00 AM	6.67	6.75	6.77
8/6/96	7:00 AM	6.56	6.62	6.63	8/14/96	7:00 AM	6.68	6.74	6.71
8/6/96	1:00 PM	6.85	6.68	6.94	8/15/96	1:00 PM	6.70	6.74	6.82
8/6/96	7:00 PM	6.88	6.72	6.84	8/16/96	7:00 PM		6.79	6.77
8/7/96	1:00 AM	6.52	6.61	6.69	8/17/96	1:00 AM			
8/7/96	7:00 AM	6.89	6.84	6.89	8/18/96	7:00 AM			6.72
8/7/96	1:00 PM	6.80	6.77	6.74	8/19/96	1:00 PM	6.62	6.59	6.74
8/7/96	7:00 PM			6.77	8/21/96	7:00 PM		6.69	6.76

Table C-5. Ground water pH during the first small-scale forced-gradient virus tracer test.

		P1-WT	P2-WT			P1-WT	P2-WT
7/31/96	8:00 AM	6.79	6.76	8/2/96	12:00 AM	6.52	6.51
7/31/96	10:00 AM	6.78	6.86	8/2/96	2:00 AM	6.51	6.65
7/31/96	12:00 PM	6.90	6.86	8/2/96	4:00 AM	6.55	6.56
7/31/96	2:00 PM	6.95	6.93	8/2/96	6:00 AM		6.49
7/31/96	4:00 PM	6.75	6.80	8/2/96	8:00 AM	6.78	6.82
7/31/96	6:00 PM	6.77	6.72	8/2/96	10:00 AM		
7/31/96	8:00 PM	6.68	6.66	8/2/96	12:00 PM		6.80
7/31/96	10:00 PM	6.51	6.46	8/2/96	2:00 PM	6.67	6.79
8/1/96	12:00 AM	6.51	6.54	8/2/96	4:00 PM		6.61
8/1/96	2:00 AM	6.55	6.62	8/2/96	6:00 PM		6.64
8/1/96	4:00 AM	6.53	6.51	8/2/96	8:00 PM	6.69	6.52
8/1/96	6:00 AM	6.50	6.55	8/2/96	10:00 PM		6.50
8/1/96	8:00 AM		6.64	8/3/96	12:00 AM		6.52
8/1/96	10:00 AM	6.89	6.76	8/3/96	2:00 AM	6.73	6.64
8/1/96	12:00 PM	6.76	6.69	8/3/96	8:00 AM	6.71	6.69
8/1/96	2:00 PM	6.83	6.78	8/3/96	2:00 PM	6.76	6.78
8/1/96	4:00 PM	6.83		8/3/96	8:00 PM	6.48	6.52
8/1/96	6:00 PM	6.67	6.80	8/3/96	2:00 AM		
8/1/96	8:00 PM	6.59	6.50	8/4/96	8:00 AM		6.71
8/1/96	10:00 PM	6.61	6.49	8/4/96	2:00 PM	6.64	6.69

Table C-6. Ground water pH during the second small-scale forced-gradient virus tracer test.

		P1-WT	P2-WT			P1-WT	P2-WT
8/28/96	10:00 PM	6.59	6.60	8/30/96	6:00 PM	6.70	6.72
8/29/96	12:00 AM	6.60	6.64	8/30/96	8:00 PM	6.65	6.70
8/29/96	2:00 AM	6.59	6.59	8/30/96	10:00 PM	6.60	6.67
8/29/96	4:00 AM	6.58	6.67	8/31/96	12:00 AM	6.56	6.57
8/29/96	6:00 AM	6.61	6.63	8/31/96	2:00 AM	6.65	6.62
8/29/96	8:00 AM	6.60	6.61	8/31/96	4:00 AM	6.57	6.60
8/29/96	10:00 AM	6.68	6.68	8/31/96	6:00 AM	6.63	6.70
8/29/96	12:00 PM	6.69	6.67	8/31/96	8:00 AM	6.69	6.76
8/29/96	2:00 PM	6.51	6.50	8/31/96	10:00 AM	6.71	6.71
8/29/96	4:00 PM	6.56	6.60	8/31/96	12:00 PM	6.74	6.75
8/29/96	6:00 PM	6.69	6.69	8/31/96	2:00 PM	6.78	6.78
8/29/96	8:00 PM	6.61	6.65	8/31/96	4:00 PM	6.78	6.86
8/29/96	10:00 PM	6.63	6.59	8/31/96	6:00 PM	6.72	6.78
8/30/96	12:00 AM	6.63	6.59	8/31/96	10:00 PM		
8/30/96	2:00 AM	6.63	6.65	9/1/96	2:00 AM	6.58	6.62
8/30/96	4:00 AM	6.56	6.64	9/1/96	6:00 AM	6.60	6.66
8/30/96	6:00 AM	6.56	6.60	9/1/96	10:00 AM	6.57	6.54
8/30/96	8:00 AM	6.60	6.59	9/1/96	2:00 PM	6.79	6.84
8/30/96	10:00 AM	6.74	6.75	9/1/96	8:00 PM	6.80	6.65
8/30/96	12:00 PM	6.60	6.61	9/2/96	2:00 AM	6.57	6.61
8/30/96	2:00 PM	6.70	6.82	9/2/96	8:00 AM	6.61	6.49
8/30/96	4:00 PM	6.65	6.62				

APPENDIX D

OXIDATION REDUCTION POTENTIAL DATA

Table D-1. Ground water oxidation-reduction potential (ORP) during the natural-gradient virus tracer test.

	C2-2	C2-3A	C2-3B	C2-4	V1-1	V1-2	V1-3	V1-4	I-WT	B2-2	B2-3	B2-4
11/20/95												
11/22/95	-12	-11	-16	-30								
11/24/95	-17	-15	-17	-28								
11/26/95	-15	-17	-16	-34	2	-1	-1	-5				
11/28/95	-7	-12	-13	-18	-3	-13	-9	-12				
11/30/95	-20	-17	-20	-33								
12/4/95	-11	-11	-13	-21								
12/6/95	-24	-15	-11	-12								
12/8/95	-12	-12	-14	-20								
12/10/95	-5	-6	-9	-12								
12/12/95												
12/14/95	0	-6	-4	-15								
12/16/95												
12/18/95												
12/20/95												
12/27/95	-23	-22	-29	-31	-21	-22	-25	-28	-21	-17	0.24	-28

Table D-2. Ground water oxidation-reduction potential (ORP) during the first forced-gradient virus tracer test.

	C2-2	C2-3A	C2-3B	C2-4	V1-1	V1-2	V1-3	V1-4	I-WT	P-WT
1/15/96										
1/16/96										
1/20/96										
1/22/96	-3	-8	-10	-17						
1/23/96	-4	-8	-12	-19						
1/24/96	-13	-15	-13	-16				-21		
1/25/96										
1/26/96	-13	-5	-5	-4	-5	-9	-6	-12		-10
1/27/96	-24	-26	-34	-40	-23	-20		-33		
1/28/96	-10	-14	-15	-23	-9	-13	-14	-16		-19
1/29/96	-8	-7	-8	-18	-9	-16	-15	-12		-10
1/30/96	-18	-18	-21	-28	-13	-20	-19	-21		-20
1/31/96	-5	-10	-9	-17						
2/1/96	-5	-12	-10	-15						
2/2/96	-4	-2	-8	-11						
2/3/96	-1	-5	-4	-10						
2/4/96	-2	-6	-8	-9						
2/5/96	-8	-12	-10	-19	-5	-11	-13	-9		-10
2/6/96	-17	-19	-18	-26	-17	-23		-24		-19
2/7/96	-15	-16	-19	-20	-15	-18	-19	-20		-17
2/8/96					-10	-14	-13	-18	-9	-9
2/9/96	-4	-8	-7	-14	-5	-9	-10	-11		-7
2/10/96					-8	-11	-12	-13		-9
2/11/96	-16	-20	-21	-27	-14	-19	-19	-23		-18
2/12/96					-6	-8	-13	-13		-12
2/13/96	-13	-16	-17	-24	-18	-17	-22	-15		
2/15/96	-4	-10	-12	-18	-5			-11	-14	-9

Table D-2. Ground water oxidation-reduction potential (ORP) during the first forced-gradient virus tracer test.

	V1-1	V1-2	V1-3	V1-4	P-WT
4/25/96					
4/27/96	-3	-7	-9	-12	-10
4/28/96	-11	-14	-16	-17	-14
4/29/96	-5	-8	-10	-12	-6
4/30/96	-6	-2	-4	0	
5/1/96	-2	-17	-22	-25	-10
5/2/96	-8	-13	-20	-17	-16
5/3/96	-4	-12	-17	-15	-13
5/4/96	-9	-14	-22	-21	-15
5/5/96	-4	-9	-10	-12	-9
5/6/96	-6	-12	-18		-8
5/7/96	-5	-11	-19	-21	-10
5/8/96	1	-8	-13	-12	-7
5/9/96	-9	-11	-17	-20	-12
5/10/96	-3	-6	-7	-9	-5
5/11/96	-8	-7	-9	-11	-3
5/12/96	-9	-11	-13	-17	-9
5/13/96	-10	-14	-15	-9	-11
5/14/96	-13	-15	-16	-13	-22
5/15/96	-8	-6	-7	-7	-12
5/16/96	8	9	6	2	2
5/17/96	-5	-10	-13	-11	-8
5/18/96	-10	-7	-10	-5	-16
5/19/96	-7	-4	-7	-3	-14

APPENDIX E
TRACER CONCENTRATION DATA

Table E-1. MS-2 concentrations (PFU/ml) during the natural gradient virus tracer test. Injection concentration = 2.4 x 10⁹ PFU/ml. Minimum Detection Limit = 1 PFU/ml. Analysis completed at the Texas A&M Research Center in El Paso, Texas.

	C2-2	C2-3A	C2-3B	C2-4
11/19/95	0.0	0.0	0.0	0.0
11/22/95	0.0	0.0	0.0	0.0
11/24/95	0.0	0.0	0.0	0.0
11/26/95	0.0	0.0	0.0	0.0
11/28/95	0.0	0.0	0.0	0.0
11/30/95	0.0	0.5	1.5	1.0
12/2/95	1.0	1.0	2.5	5.5
12/4/95	3.0	0.5	1.0	1.5
12/6/95	1.0	0.0	0.0	0.0
12/8/95	0.0	0.0	0.0	0.0
12/10/95	0.5	4.0	4.5	1.5
12/12/95	3.5	1.0	0.5	0.0
12/14/95	0.0	0.5	0.0	0.0

Table E-2. MS-2 concentrations (PFU/ml) during the first large-scale forced-gradient virus tracer test. Injection concentration = 5.0 x 10⁹ PFU/ml. Minimum Detection Limit = 0.01 PFU/ml. Analysis completed at the Texas A&M Research Center in El Paso, Texas.

	C2-3A	C2-3B	C2-4
1/20/96	0.0	0.0	0.0
1/22/96	0.4	1.4	0.0
1/23/96	0.0	0.7	0.0
1/24/96	0.0	1.1	0.0
1/25/96	1.7	0.7	0.0
1/26/96	4.2	0.2	0.0
1/27/96	0.8	0.1	0.0
1/28/96	0.5	0.9	0.0
1/29/96	0.7	3.9	0.0
1/30/96	0.9	1.1	0.0
1/31/96	0.0	0.0	0.0
2/1/96	0.1	0.7	1.5
2/2/96	0.0	0.3	0.0
2/3/96	0.5	2.4	4.6
2/4/96	5.4	11.6	39.0
2/5/96	16.9	0.8	55.0
2/6/96	150.3	0.0	661.7
2/7/96	28.5	0.0	0.0
2/9/96	0.1	0.0	0.0
2/11/96	0.1	0.0	0.0
2/13/96	0.0	0.0	0.0

Table E-3. PRD-1 concentrations (PFU/ml) during the first large-scale forced-gradient virus tracer test. Injection concentration = 1.0 x 10⁸ PFU/mL Minimum Detection Limit = 1 PFU/ml. Analysis completed at the Texas A&M Research Center in El Paso, Texas.

	C2-3A	C2-3B	C2-4
1/20/96	0.0	0.0	0.0
1/22/96	0.0	0.0	0.0
1/23/96	0.0	0.0	0.0
1/24/96	0.0	0.0	0.0
1/25/96	0.0	0.0	0.0
1/26/96	0.0	0.0	0.0
1/27/96	0.0	0.0	0.0
1/28/96	0.0	0.0	0.0
1/29/96	0.0	0.0	0.0
1/30/96	0.0	0.0	0.0
1 31/96	0.0	0.0	0.0
2/1/96	0.0	0.0	0.0
2/2/96	0.0	0.0	0.0
2/3/96	0.0	0.0	0.0
2/4/96	0.0	0.0	0.0
2/5/96	0.0	0.0	0.0
2/6/96	0.0	0.0	0.0
2/7/96	0.0	0.0	0.0
2/9/96	0.0	0.0	0.0
2/11/96	0.0	0.0	0.0
2/13/96	0.0	0.0	0.0

Table E-4. PRD-1 concentrations (PFU/ml) during the second large-scale forced-gradient virus tracer test. Injection concentration = 1.0 x 10^4 PFU/mL. Minimum Detection Limit = 1 PFU/mL. Analysis completed at the Texas A&M Research Center in El Paso, Texas.

	V1-1	V1-2	V1-3	V1-4	Pumping
4/25/96	0	0	0	0	0
4/27/96	0	0	0	0	0
4/28/96	0	0	0	0	0
4/29/96	0	0	0	0	0
4/30/96	0	0	0	0	0
5/1/96	0	0	0	0	0
5/2/96	0	0	0	0	0
5/3/96	0	0	0	0	0
5/4/96	0	0	0	0	0
5/5/96	0	0	0	0	0
5/6/96	0	0	0	0	0
5/7/96	0	0	0	0	0
5/8/96	0	0	0	0	0
5/9/96	0	0	0	0	0
5/10/96	0	0	0	0	0
5/11/96	0	0	0	0	0
5/12/96	0	0	0	0	0
5/13/96	0	0	0	0	0
5/14/96	0	0	0	0	0
5/15/96	0	0	0	0	0
5/16/96	0	0	0	0	0
5/17/96	0	0	0	0	0
5/18/96	0	0	0	0	0
5/19/96	0	0	0	0	0
5/21/96	0	0	0	0	0
5/22/96	0	0	0	0	0
5/23/96	0	0	0	0	0
5/26/96	0	0	0	0	0
5/30/96	0	0	0	0	0

Table E-5. ΦX-174 concentrations (PFU/ml) during the second large-scale forced-gradient virus tracer test. Injection concentration = 5.0 x 10^6 PFU/mL. Minimum Detection Limit = 1 PFU/mL. Analysis completed at the Texas A&M Research Center in El Paso, Texas.

	V1-1	V1-2	V1-3	V1-4	Pumping
4/25/96	0	0	0	0	0
4/27/96	0	0	0	0	0
4/28/96	0	0	0	0	0
4/29/96	0	0	0	0	0
4/30/96	0	0	0	0	0
5/1/96	0	0	0	0	0
5/2/96	0	0	0	0	0
5/3/96	0	0	0	0	0
5/4/96	0	0	0	0	0
5/5/96	0	0	0	0	0
5/6/96	0	0	0	0	0
5/7/96	0	0	0	0	0
5/8/96	0	0	0	0	0
5/9/96	0	0	0	0	0
5/10/96	0	0	0	0	0
5/11/96	0	0	0	0	0
5/12/96	0	0	0	0	0
5/13/96	0	0	0	0	0
5/14/96	0	0	0	0	0
5/15/96	0	0	0	0	0
5/16/96	0	0	0	0	0
5/17/96	0	0	0	0	0
5/18/96	0	0	0	0	0
5/19/96	0	0	0	0	0
5/21/96	0	0	0	0	0
5/22/96	0	0	0	0	0
5/23/96	0	0	0	0	0
5/26/96	0	0	0	0	0
5/30/96	0	0	0	0	0

Table E-6. MS-2 concentrations (PFU/ml) during the third large-scale forced-gradient virus tracer test. Injection concentration = 1.0×10^{10} PFU/mL. Minimum Detection Limit = 0.01 PFU/mL. Analysis completed at the Texas A&M Research Center in El Paso, Texas.

		C2-3A	C2-3B	C2-4
8/1/96	2:00 PM			
8/1/96	3:00 PM			
8/2/96	2:00 PM			
8/2/96	7:00 AM			
8/2/96	1:00 PM			
8/2/96	5:00 PM			0
8/2/96	7:00 PM			0
8/3/96	1:00 AM	0	0	0
8/3/96	7:00 AM	0	0	0
8/3/96	1:00 PM	0	0	0
8/3/96	7:00 PM	0	0	0
8/4/96	7:00 AM	0	0	0
8/4/96	1:00 PM	0	0	0
8/4/96	7:00 PM	0	0	0
8/5/96	1:00 AM	0	0	0
8/5/96	7:00 AM	0	0	0
8/5/96	1:00 PM	0	0	0
8/5/96	7:00 PM	0	0	0
8/6/96	1:00 AM	0	0	0
8/6/96	7:00 AM	0	0	0
8/6/96	1:00 PM	0	0	0
8/6/96	7:00 PM	0	0	0
8/7/96	7:00 AM	0		0
8/7/96	1:00 PM	0	0	0
8/7/96	7:00 PM	0		0
8/8/96	1:00 AM	0	0	0
8/8/96	7:00 AM	0	0	0
8/8/96	1:00 PM	0	0	0
8/8/96	7:00 PM	0	0	0
8/9/96	1:00 AM	0	0	0
8/9/96	7:00 AM	0	0	0
8/9/96	7:00 PM	0	0	0
8/10/96	1:00 AM	0	0	0
8/10/96	1:00 PM	0	0	0
8/10/96	7:00 PM	0	0	0
8/11/96	1:00 AM			0
8/11/96	1:00 PM	0	0	0
8/11/96	7:00 PM	0	0	0
8/12/96	7:00 PM	0	0	0
8/13/96	7:00 AM	0	0	0
8/14/96	7:00 AM	0	0	0
8/15/96	7:00 AM		0	0
8/16/96	7:00 AM		0	0
8/19/96	7:00 PM	0	0	0
8/20/96	7:00 PM		0	0
8/21/96	7:00 PM	0		0

Table E-7. Bromide concentrations (mg/L) during the third large-scale forced-gradient virus tracer test. Injection concentration = 136,000 mg/L. Minimum Detection Limit = 0.6 mg/L. Analysis completed at the Frank Hernandez Environmental Laboratory in El Paso, Texas.

		C2-3A	C2-3B	C2-4
8/1/96	2:00 PM			
8/1/96	3:00 PM			
8/2/96	2:00 PM			
8/2/96	7:00 AM			
8/2/96	1:00 PM			
8/2/96	5:00 PM			0.75
8/2/96	7:00 PM			0.75
8/3/96	1:00 AM	0.69	<MDL	0.76
8/3/96	7:00 AM	0.74	<MDL	0.70
8/3/96	1:00 PM	1.15	<MDL	0.74
8/3/96	7:00 PM	0.99	1.00	0.82
8/4/96	7:00 AM	0.77	1.08	0.68
8/4/96	1:00 PM	1.02	0.88	<MDL
8/4/96	7:00 PM	0.68	0.63	0.74
8/5/96	1:00 AM	0.92	0.63	
8/5/96	7:00 AM	1.17	<MDL	0.69
8/5/96	1:00 PM	1.07	<MDL	0.71
8/5/96	7:00 PM	0.79	0.65	0.63
8/6/96	1:00 AM	0.75	<MDL	0.70
8/6/96	7:00 AM	1.10	0.64	0.74
8/6/96	1:00 PM	0.65	0.84	0.79
8/6/96	7:00 PM	0.86	<MDL	0.63
8/7/96	7:00 AM			0.73
8/7/96	1:00 PM	0.92	<MDL	0.63
8/7/96	7:00 PM			0.72
8/8/96	1:00 AM	2.56	<MDL	<MDL
8/8/96	7:00 AM	1.21	<MDL	
8/8/96	1:00 PM	0.91	<MDL	0.75
8/8/96	7:00 PM	1.22	<MDL	0.73
8/9/96	1:00 AM	1.01	<MDL	0.72
8/9/96	7:00 AM	0.86	<MDL	0.71
8/9/96	7:00 PM	0.87	<MDL	0.76
8/10/96	1:00 AM	0.90	<MDL	<MDL
8/10/96	1:00 PM	1.44	<MDL	<MDL
8/10/96	7:00 PM	0.83	<MDL	<MDL
8/11/96	1:00 AM			<MDL
8/11/96	1:00 PM	0.97	<MDL	<MDL
8/11/96	7:00 PM	1.01	<MDL	<MDL
8/12/96	7:00 PM	<MDL	<MDL	0.67
8/13/96	7:00 AM	3.69	<MDL	0.84
8/14/96	7:00 AM	5.24	<MDL	0.62
8/15/96	7:00 AM		2.27	<MDL
8/16/96	7:00 AM		2.47	<MDL
8/19/96	7:00 PM	4.58	<MDL	0.65
8/20/96	7:00 PM		<MDL	<MDL
8/21/96	7:00 PM	4.28		<MDL

Table E-10. ΦX-174 concentrations (PFU/ml) during the first small-scale forced-gradient virus tracer test. Injection concentration = 2.1 x 10⁴ PFU/ml. Minimum Detection Limit = 1 PFU/ml. Analysis completed at the Texas A&M Research Center in El Paso, Texas.

		P1-WT	P2-WT			P1-WT	P2-WT
7/31/96	8:00 AM	0	0	8/1/96	8:00 PM	0	0
7/31/96	10:00 AM	0	0	8/1/96	10:00 PM	0	0
7/31/96	12:00 PM	0	0	8/2/96	12:00 AM	0	0
7/31/96	2:00 PM	0	0	8/2/96	2:00 AM	0	0
7/31/96	4:00 PM	0	0	8/2/96	4:00 AM	0	0
7/31/96	6:00 PM	0	0	8/2/96	6:00 AM	0	0
7/31/96	8:00 PM	0	0	8/2/96	2:00 PM	0	0
7/31/96	10:00 PM	0	0	8/2/96	4:00 PM	0	0
8/1/96	12:00 AM	0	0	8/2/96	6:00 PM	0	0
8/1/96	2:00 AM	0	0	8/2/96	8:00 PM	0	0
8/1/96	4:00 AM	0	0	8/2/96	10:00 PM	0	0
8/1/96	6:00 AM	0	0	8/3/96	12:00 AM	0	0
8/1/96	8:00 AM	0	0	8/3/96	2:00 AM	0	0
8/1/96	10:00 AM	0	0	8/3/96	8:00 AM	0	0
8/1/96	12:00 PM	0	0	8/3/96	2:00 PM	0	0
8/1/96	2:00 PM	0	0	8/3/96	8:00 PM	0	0
8/1/96	5:00 PM	0	0	8/4/96	8:00 AM	0	0
8/1/96	6:30 PM	0	0	8/4/96	2:00 PM	0	0

Table E-11. Bromide concentrations (mg/L) during the first small-scale forced-gradient virus tracer test. Injection concentration = 12,800. Minimum Detection Limit = 0.6 mg/L. Analysis completed at the Frank Hernandez Environmental Laboratory in El Paso, Texas.

		P1-WT	P2-WT			P1-WT	P2-WT
7/31/96	8:00 AM	1.66	0.823	8/1/96	8:00 PM	<MDL	<MDL
7/31/96	10:00 AM	0.847	0.807	8/1/96	10:00 PM	<MDL	3.23
7/31/96	12:00 PM	0.757	0.804	8/2/96	12:00 AM	0.624	0.638
7/31/96	2:00 PM	0.756	0.809	8/2/96	2:00 AM	0.623	3.02
7/31/96	4:00 PM	0.717	0.798	8/2/96	4:00 AM	0.624	3.28
7/31/96	6:00 PM	0.744	0.821	8/2/96	6:00 AM		3.59
7/31/96	8:00 PM	0.736	0.804	8/2/96	2:00 PM		0.67
7/31/96	10:00 PM	0.744	0.928	8/2/96	4:00 PM		0.677
8/1/96	12:00 AM	0.789	4.51	8/2/96	6:00 PM		0.653
8/1/96	2:00 AM	0.772	0.643	8/2/96	8:00 PM		0.675
8/1/96	4:00 AM	0.784	<MDL	8/2/96	10:00 PM		0.672
8/1/96	6:00 AM	<MDL	<MDL	8/3/96	12:00 AM		0.683
8/1/96	8:00 AM		<MDL	8/3/96	2:00 AM		0.645
8/1/96	10:00 AM	<MDL	<MDL	8/3/96	8:00 AM		0.739
8/1/96	12:00 PM	<MDL	0.657	8/3/96	2:00 PM		0.68
8/1/96	2:00 PM	<MDL	<MDL	8/3/96	8:00 PM		0.67
8/1/96	5:00 PM	<MDL	<MDL	8/4/96	8:00 AM		0.651
8/1/96	6:30 PM	<MDL	<MDL	8/4/96	2:00 PM	0.657	0.678

Table E-12. MS-2 concentrations (PFU/ml) during the second small-scale forced-gradient virus tracer test. Injection concentration = 2.1 x 10' PFU/ml. Minimum Detection Limit = 0.01 PFU/ml. Analysis completed at the Texas A&M Research Center in El Paso, Texas.

		P1-WT	P2-WT	Pumping
8/28/96	10:00 PM	0.46	0	0
8/29/96	12:00 AM	1.50	54.00	0
8/29/96	2:00 AM	0	66.25	0
8/29/96	4:00 AM	0	0.32	0
8/29/96	6:00 AM	4.50	7.40	0
8/29/96	8:00 AM	6.40	0	0
8/29/96	10:00 AM	0	0.28	0
8/29/96	12:00 PM	0	0	0
8/29/96	2:00 PM	0	0	0
8/29/96	4:00 PM	0	0.13	0
8/29/96	6:00 PM	0	0	0
8/29/96	8:00 PM	0	15.01	0
8/29/96	10:00 PM	0	0	0
8/30/96	12:00 AM	0	0	0
8/30/96	2:00 AM	0	0.50	0
8/30/96	4:00 AM	0	0.10	0
8/30/96	6:00 AM	0	0	0
8/30/96	8:00 AM	0	0	0
8/30/96	10:00 AM	1.88	0.25	0
8/30/96	12:00 PM	0	0	0
8/30/96	2:00 PM	0	0.95	0
8/30/96	4:00 PM	0	0.25	0
8/30/96	6:00 PM	0	0	0
8/30/96	8:00 PM	0	0.05	0
8/30/96	10:00 PM	0	0	0
8/31/96	12:00 AM	0	82.58	0
8/31/96	2:00 AM	0	0	0
8/31/96	4:00 AM	0	0	0
8/31/96	6:00 AM	0	0	0
8/31/96	8:00 AM	0	0.05	0
8/31/96	10:00 AM	20.00	0	0
8/31/96	12:00 PM	0	5.01	0
8/31/96	2:00 PM	0	0	0
8/31/96	4:00 PM	0	0	0
8/31/96	6:00 PM	0	0	0
8/31/96	8:00 PM	0.38	0	0
8/31/96	10:00 PM	0	0.50	0
9/1/96	2:00 AM	0	0	0
9/1/96	6:00 AM	0	1	0
9/1/96	10:00 AM	0	0	0
9/1/96	2:00 PM	0	0	0
9/1/96	8:00 PM	0	0.28	0
9/2/96	2:00 AM	0	0	0
9/2/96	8:00 AM	0	0.50	0

Table E-13. PRD-1 concentrations (PFU/ml) during the second small-scale forced-gradient virus tracer test. Injection concentration = 3.4 x 10^8 PFU/ml. Minimum Detection Limit = 1 PFU/ml. Analysis completed at the Texas A&M Research Center in El Paso, Texas.

		P1-WT	P2-WT	Pumping
8/28/96	10:00 PM	0	0	0
8/29/96	12:00 AM	0	9	0
8/29/96	2:00 AM	0	18	0
8/29/96	4:00 AM	0	66	0
8/29/96	6:00 AM	0	0	0
8/29/96	8:00 AM	0	0	0
8/29/96	10:00 AM	0	0	0
8/29/96	12:00 PM	0	0	0
8/29/96	2:00 PM	0	0	0
8/29/96	4:00 PM	0	0	0
8/29/96	6:00 PM	0	0	0
8/29/96	8:00 PM	0	0	0
8/29/96	10:00 PM	0	0	0
8/30/96	12:00 AM	0	0	0
8/30/96	2:00 AM	0	0	0
8/30/96	4:00 AM	0	0	0
8/30/96	6:00 AM	0	0	0
8/30/96	8:00 AM	0	0	0
8/30/96	10:00 AM	0	0	0
8/30/96	12:00 PM	0	0	0
8/30/96	2:00 PM	0	0	0
8/30/96	4:00 PM	0	0	0
8/30/96	6:00 PM	0	0	0
8/30/96	8:00 PM	0	0	0
8/30/96	10:00 PM	0	0	0
8/31/96	12:00 AM	0	0	0
8/31/96	2:00 AM	0	0	0
8/31/96	4:00 AM	0	0	0
8/31/96	6:00 AM	0	0	0
8/31/96	8:00 AM	0	0	0
8/31/96	10:00 AM	0	0	0
8/31/96	12:00 PM	0	0	0
8/31/96	2:00 PM	0	0	0
8/31/96	4:00 PM	0	0	0
8/31/96	6:00 PM	0	0	0
8/31/96	8:00 PM	0	0	0
8/31/96	10:00 PM	0	0	0
9/1/96	2:00 AM	0	0	0
9/1/96	6:00 AM	0	0	0
9/1/96	10:00 AM	0	0	0
9/1/96	2:00 PM	0	0	0
9/1/96	8:00 PM	0	0	0
9/2/96	2:00 AM	0	0	0
9/2/96	8:00 AM	0	0	0

Table E-14. Bromide concentrations (mg/L) during the first small-scale forced-gradient virus tracer test. Injection concentration = 105,600. Minimum Detection Limit = 0.6 mg/L. Analysis completed at the Frank Hernandez Environmental Laboratory in El Paso, Texas.

		P1-WT	P2-WT	Pumping
8/28/96	10:00 PM	0.943	<MDL	
8/29/96	12:00 AM	0.752	<MDL	<MDL
8/29/96	2:00 AM	<MDL	0.622	<MDL
8/29/96	4:00 AM	0.697	<MDL	<MDL
8/29/96	6:00 AM	0.719	0.624	
8/29/96	8:00 AM	<MDL	<MDL	<MDL
8/29/96	10:00 AM	0.735	<MDL	
8/29/96	12:00 PM	<MDL	<MDL	0.772
8/29/96	2:00 PM	0.627	<MDL	0.797
8/29/96	4:00 PM	0.643	0.632	1.01
8/29/96	6:00 PM	<MDL	<MDL	1.17
8/29/96	8:00 PM	<MDL	0.626	1.05
8/29/96	10:00 PM	0.707	0.623	1.38
8/30/96	12:00 AM	0.639	<MDL	1.65
8/30/96	2:00 AM	0.664	<MDL	1.69
8/30/96	4:00 AM	<MDL	<MDL	1.25
8/30/96	6:00 AM	<MDL	<MDL	1.62
8/30/96	8:00 AM	<MDL	<MDL	0.892
8/30/96	10:00 AM	0.638	0.628	0.802
8/30/96	12:00 PM	1.08	<MDL	0.697
8/30/96	2:00 PM	0.642	0.64	0.638
8/30/96	4:00 PM	0.679	<MDL	<MDL
8/30/96	6:00 PM	<MDL	<MDL	<MDL
8/30/96	8:00 PM	0.637	<MDL	<MDL
8/30/96	10:00 PM	<MDL	<MDL	<MDL
8/31/96	12:00 AM	<MDL	<MDL	<MDL
8/31/96	2:00 AM	0.775	<MDL	<MDL
8/31/96	4:00 AM	<MDL	0.631	<MDL
8/31/96	6:00 AM	0.634	0.644	<MDL
8/31/96	8:00 AM	<MDL	<MDL	<MDL
8/31/96	10:00 AM	<MDL	<MDL	<MDL
8/31/96	12:00 PM	<MDL	<MDL	<MDL
8/31/96	2:00 PM	<MDL	0.9	<MDL
8/31/96	4:00 PM	<MDL	<MDL	<MDL
8/31/96	6:00 PM	<MDL	<MDL	<MDL
8/31/96	8:00 PM	<MDL	<MDL	
8/31/96	10:00 PM	<MDL	<MDL	<MDL
9/1/96	2:00 AM	<MDL	<MDL	<MDL
9/1/96	6:00 AM	<MDL	<MDL	<MDL
9/1/96	10:00 AM	0.712	<MDL	<MDL
9/1/96	2:00 PM	0.629	<MDL	<MDL
9/1/96	8:00 PM	<MDL	<MDL	<MDL
9/2/96	2:00 AM	<MDL	<MDL	<MDL
9/2/96	8:00 AM	<MDL	<MDL	<MDL

APPENDIX F

AQUIFER HETEROGENEITY TEST DATA

Table F-1. Electrical conductivity (μS) of laboratory prepared bromide solutions mixed in site distilled water.

Br Concentration (mg/L)	Electrical Conductivity (μS)
0.0	14.34
1.0	17.46
2.0	19.68
3.0	23.9
4.0	31.5
5.0	35.7
10.0	38.0
25.0	56.1
50.0	91.3
100.0	177.0

Table F-2. Electrical conductivity (μS) of laboratory prepared bromide solutions mixed in site ground water.

Br Concentration (mg/L)	Electrical Conductivity (μS)
0.6	1045
1.6	1052
2.6	1082
3.6	1053
4.6	1086
5.6	1074
10.6	1118
25.6	1171
50.6	1192
100.6	1300
200.6	1429

Table F-3. Electrical conductivity (μS) of field samples at various depths and various times after injection in the P2-WT well (10 m down-gradient) during the aquifer heterogeneity test.

Depth (m)	Electrical Conductivity (time = 0 hours) (μS)	Electrical Conductivity (time = 21 hours) (μS)	Electrical Conductivity (time = 92 hours) (μS)
11.9	1138	1220	1187
12.8	1129	1138	1097
13.7	1124	1084	1106
14.3	1094	1142	1143
14.9	1108	1128	1142
15.5	1106	1112	1124
16.2	1108	1122	1128
16.8	1103	1113	1136
17.4	1102	1122	1136
18.0	1102	1113	1131
18.6	1101	1117	1137
19.2	1106	1113	1124
19.8	1106	1114	1124
20.4	1104	1114	1133
21.0	1115	1120	1136

APPENDIX G

SAMPLING METHODOLOGY DATA

Table G-1. MS-2 concentration of tank samples comparing three sampling methods.

Days after injection	Bailer (PFU/ml)	Grab (PFU/ml)	Pump (PFU/ml)
0.0	0	0	0
0.5	2	0	1
1.0	3	2	1
1.5	26	32	31
2.0	0	0	0
2.5	0	0	0
3.0	0	0	0
3.5	0	0	0
4.0	0	0	0
4.5	0	0	0

APPENDIX H

OVERVIEW OF VIRALT
(from VIRALT, 1990)

Code Purpose

The primary objective of the VIRALT model is to provide technical staff of utilities, EPA, and State and local agencies with a screening tool for estimating concentrations of viruses in ground water withdrawn from pumping wells that are located in the vicinity of viral contaminant sources. VIRALT is a modular, semi-analytical and numerical code that simulates the subsurface transport and fate of viruses in both the unsaturated and saturated zones. The code also delineates ground water pathlines and well capture zones, and computes viral concentrations in extracted ground water.

VIRALT provides both steady-state and transient transport analyses taking into account the major physical processes of: advection and dispersion of viral particles along ground water pathlines, adsorption, and inactivation or die-off. The code can handle contaminant sources of various shapes; both areal and line sources can be accommodated. Typically, areal sources may be used to represent leaky septic tanks, cesspools or surface and subsurface disposal of waste water. Line sources may be used to represent leaky sewer lines or pipes.

Model Overview

The VIRALT model can be divided conceptually into two major sections. The computational module section contains the FORTRAN programs and subroutines that

perform ground water flow and viral transport computations. All of the "number crunching" is performed by these computational modules. The remaining portion of the VIRALT model is the user-interface. The interface provides an efficient mechanism for data entry as well as the viewing of model results. The composition of, and the relationship between, these two portions of the model is discussed in the next two sections.

Computational Modules

The VIRALT model contains two key computational modules for unsaturated- and saturated-zone modeling, respectively. Each module contains ground water flow and viral transport models. The steady ground water flow fields in both zones are determined semi-analytically. The semi-analytical solutions for ground water flow were adapted from HydroGeoLogic (1989) and Blandford and Huyakorn (1990) for the unsaturated and saturated zones, respectively. Following the ground water flow computation, the transport models are used to simulate the fate or viruses in each zone. The unsaturated-zone and saturated-zone groung-water flow and viral transport modules may be run in sequence or independently.

Preprocessor

VIRALT comes complete with a user-friendly preprocessor to assist the user in entering or editing input data for the computational modules. Parameter values are entered through a series of input screens that appear on the monitor. Each input screen has one or more associated help screens that define the input parameters and guide the user in the selection of appropriate modeling options. The user is prompted only for

those parameter values that are required for a given application. Where possible, checks are made to insure the reasonableness of input parameters and to screen out typographical errors.

Postprocessor

The postprocessor of the VIRALT code is a plotting routine named VTGRAF. When the flow and transport computations have been completed, the VTGRAF module may be invoked to plot the well capture zones and viral concentration values at the pumping well(s) on the monitor. The capture zones for multiple wells will be plotted in different colors if a color monitor is used. The contaminant source is also depicted. For each pumping well, a plot of the breakthrough curve of viral concentration versus time is also provided by VTGRAF.

Assumptions and Limitations

VIRALT employs two sets of assumptions for ground water flow and viral transport analyses. These assumptions are discussed below together with model limitations.

Unsaturated Ground water Flow Assumptions

The flow of water (fluid phase) in the unsaturated zone is assumed to be vertically downward (one-dimensional) from the bottom of the viral source to the water table. The flow is also considered to be at steady-state, isothermal, and governed by Darcy's Law. Boundary conditions (leakage rates) that vary in time may be input to the code, but the flow field is assumed to adjust instantaneously from an existing steady-state condition to one indicative of the new boundary condition. The ground water is

assumed to be homogeneous and slightly compressible, and the effects of hysteresis in the soil constitutive relations are neglected.

Although up to 10 soil layers of variable hydraulic properties may be specified, the soil composing each layer is assumed to be a uniform and incompressible porous medium that does not contain fractures or macro pores.

Unsaturated Zone Transport Assumptions

The transport of viruses in the unsaturated zone is assumed to be governed by the one-dimensional advection-dispersion equation. One of the bases of this equation is that the diffusive/dispersive transport in the porous media is governed by Fick's Law. The hydrodynamic dispersion coefficient is defined as the sum of the coefficients of mechanical dispersion and molecular diffusion. Additional assumptions are as follows:

- Fluid properties are independent of concentrations of contaminants
- Sorption of viruses can be described by a linear Freundlich equilibrium isotherm
- Viral die-off may be described as a first-order degradation rate
- Each soil layer is a uniform porous medium

Saturated Ground water Flow Assumptions

Capture zones delineated using the semi-analytical flow module of VIRALT are valid for fully penetrating wells screened in aquifers that are essentially homogeneous. Ground water flow must be two-dimensional in an areal x-y plane, and the aquifer may be confined or unconfined. A steady state ground water flow field is assumed.

If a stream or a barrier is present, the boundary is assumed to be linear and fully

penetrating. The latter assumption is often violated in cases where stream boundaries exist. The effect of a partially penetrating stream may be an important one and each application should be examined on a site-by-site basis. In general, the greater the depth and breadth of the stream in relation to the aquifer thickness, the more valid the fully penetrating stream assumption. Also, stream boundary partial penetration effects decrease as the distance from the stream to the well increases. The stream and the aquifer are assumed to be in perfect hydraulic connection; the effects of a "clogging layer" between the stream bed and the aquifer are not considered.

If, in actuality, the stream is partially penetrating and/or there is a clogging layer of fine grained material that lines the stream bed, the capture zone predicted by the flow module of VIRALT will be smaller than the "true" capture zone. The amount of error incurred will be dependent upon the degree to which the above assumptions are violated.

Saturated Zone Transport Assumptions

The transport of viruses in the aquifer (saturated zone) is assumed to be governed by the one-dimensional advection-dispersion along ground water flow pathlines that intercept a single contaminant source and one or more pumping wells. The ground water flow field is assumed to be at steady state, and the effects of dispersion of viral particles in the vertical direction and the horizontal transverse direction (perpendicular to the ground water flow path) are assumed negligible. The longitudinal (along the flow path) dispersion accounted for by the code is represented as the sum of the coefficients of mechanical dispersion and molecular diffusion. The

adsorption and inactivation of viruses is described by a linear equilibrium isotherm and a first-order decay or inactivation rate constant, respectively. Finally, the concentrations of virus specified as boundary conditions are assumed to be valid over the entire depth of the aquifer.

General Model Limitations

Due to the modeling assumptions listed in the previous four sections, the VIRALT code is subject to the following limitations:

- The code considers only single-porosity or granular porous media. Flow and transport in fractures and macropores of the subsurface system are not taken into account.
- The code does not take into account kinetic sorption, nonlinear sorption, and reversible viral capture and desorption mechanisms which, in some instances, may be important.
- The code cannot handle transient flow situations or situations where the effects of vertical flow components in the saturated zone are important.
- The code does not account for dilution of viral concentration due to transverse and vertical dispersion.
- Although multiple wells can be accommodated, the code handles only one contaminant source in each simulation run.

APPENDIX I

VIRALT INPUT AND OUTPUT DATA

Table I-1. Raw input data for large-scale MS-2 bacteriophage tracer simulation using VIRALT.

```
2
Viral Transport MS-2
  0   1   1   0   0   1   0   1   0   0
0.000000      125.000     0.000000      50.0000
0.350000      12.0000     75.0000     0.100000E-01
0.230000E-02  360.000           0        0  0.000000
 3   0    60.00
 1    0.12   47.20   25.00   1  20
  0.15    1.95
 2    0.12   81.30   25.00   1  20
  0.15    1.95
 3  1036.00  121.60   25.00   1  20
  0.30   181.60
 0.000   10.000   3.300   0.460   25.000   1.720   0.000
 4   0
 58.6000
  9.50   24.50
  9.50   25.50
 10.50   24.50
 10.50   25.50
-1   1
 4
0.000000     0.500000E+10
 1.33000     0.500000E+10
0.100000     0.000000
 58.6000     0.000000
0.000000
 60.00
 0
 0
```

Table I-2. Raw input data for small-scale bromide tracer simulation using VIRALT.

```
2
Bromide Tracer Test
  0   1   1   0   0   1   0   1   0   0
 0.000000      30.0000     0.000000      30.0000
 0.350000      12.0000      75.0000     0.100000E-01
 0.230000E-02   360.000           0        0  0.000000
  1   0     7.00
  1  1036.00    22.00    15.00   1  20
   0.10   181.60
  0.000    1.000    1.000    0.000   17.000    1.720    0.000
 4   0
 5.49000
  9.99    15.01
  9.99    14.99
 10.01    15.01
 10.01    14.99
-1   1
 4
 0.000000      12800.0
 0.500000      12800.0
 0.100000E-01  0.000000
 5.49000      0.000000
 0.000000
   7.00
 0
 0
```

Table I-3. Output data for large-scale MS-2 tracer simulation using VIRALT.

Days	MS-2 (PFU/ml)	Days	MS-2 (PFU/ml)	Days	MS-2 (PFU/ml)	Days	MS-2 (PFU/ml)
0	0	0.12	0.00	0.23	0.00	0.35	0.00
0.47	0.00	0.59	0.00	0.70	0.00	0.82	0.00
0.94	0.00	1.06	0.00	1.17	0.00	1.29	0.00
1.41	0.00	1.52	0.00	1.64	0.00	1.76	0.00
1.88	0.00	1.99	0.00	2.11	0.00	2.23	0.00
2.34	0.00	2.46	0.00	2.58	0.00	2.70	0.00
2.81	0.00	2.93	0.00	3.05	0.00	3.16	0.00
3.28	0.00	3.40	0.00	3.52	0.00	3.63	0.01
3.75	0.01	3.87	0.02	3.99	0.02	4.10	0.02
4.22	0.03	4.34	0.03	4.45	0.04	4.57	0.04
4.69	0.05	4.81	0.07	4.92	0.10	5.04	0.12
5.16	0.15	5.27	0.18	5.39	0.20	5.51	0.23
5.63	0.26	5.74	0.28	5.86	0.31	5.98	0.43
6.09	0.56	6.21	0.69	6.33	0.82	6.45	0.95
6.56	1.08	6.68	1.21	6.80	1.34	6.92	1.47
7.03	1.62	7.15	2.08	7.27	2.57	7.38	3.06
7.50	3.55	7.62	4.04	7.74	4.53	7.85	5.02
7.97	5.51	8.09	6.00	8.20	6.55	8.32	7.93
8.44	9.41	8.56	10.88	8.67	12.36	8.79	13.84
8.91	15.31	9.02	16.79	9.14	18.27	9.26	19.74
9.38	21.38	9.49	24.72	9.61	28.29	9.73	31.87
9.85	35.44	9.96	39.02	10.08	42.60	10.20	46.17
10.31	49.75	10.43	53.32	10.55	57.18	10.67	63.76
10.78	70.76	10.90	77.76	11.02	84.76	11.13	91.76
11.25	98.76	11.37	105.8	11.49	112.8	11.60	119.8
11.72	127.1	11.84	137.7	11.95	148.7	12.07	159.8
12.19	170.9	12.31	181.9	12.42	193	12.54	204.1
12.66	215.1	12.77	226.2	12.89	237.6	13.01	251.2
13.13	265.2	13.24	279.2	13.36	293.2	13.48	307.2
13.60	321.2	13.71	335.2	13.83	349.2	13.95	363.2
14.06	377.2	14.18	391.0	14.30	404.8	14.42	418.5
14.53	432.3	14.65	446.1	14.77	459.8	14.88	473.6
15.00	487.4	15.12	501.1	15.24	514.4	15.35	524.8
15.47	534.5	15.59	544.2	15.70	553.9	15.82	563.6
15.94	573.2	16.06	582.9	16.17	592.6	16.29	602.3
16.41	611.1	16.53	615.3	16.64	618.6	16.76	621.8
16.88	624.9	16.99	628.1	17.11	631.2	17.23	634.4
17.35	637.5	17.46	640.6	17.58	642.9	17.70	640.6
17.81	637.6	17.93	634.3	18.05	631.0	18.17	627.7
18.28	624.4	18.40	621.1	18.52	617.8	18.63	614.5
18.75	610.6	18.87	603.5	18.99	596.0	19.10	588.3
19.22	580.5	19.34	572.8	19.46	565.1	19.57	557.3
19.69	549.6	19.81	541.8	19.92	533.8	20.04	524.4
20.16	514.8	20.28	505.0	20.39	495.3	20.51	485.6
20.63	475.9	20.74	466.1	20.86	456.4	20.98	446.7
21.10	436.9	21.21	427.1	21.33	417.3	21.45	407.5
21.56	397.8	21.68	388.0	21.80	378.2	21.92	368.4
22.03	358.6	22.15	348.8	22.27	339.2	22.39	330.3

Table I-3. (continued)

Days	MS-2 (PFU/ml)	Days	MS-2 (PFU/ml)	Days	MS-2 (PFU/ml)	Days	MS-2 (PFU/ml)
22.50	321.5	22.62	312.8	22.74	304.1	22.85	295.5
22.97	286.8	23.09	278.1	23.21	269.4	23.32	260.7
23.44	252.3	23.56	244.9	23.67	237.6	23.79	230.5
23.91	223.4	24.03	216.3	24.14	209.2	24.26	202.1
24.38	195.0	24.49	187.9	24.61	181.1	24.73	175.3
24.85	169.7	24.96	164.3	25.08	158.8	25.20	153.3
25.32	147.9	25.43	142.4	25.55	136.9	25.67	131.5
25.78	126.3	25.90	122.0	26.02	117.8	26.14	113.8
26.25	109.8	26.37	105.8	26.49	101.8	26.60	97.73
26.72	93.72	26.84	89.7	26.96	85.93	27.07	82.85
27.19	79.89	27.31	77.02	27.42	74.17	27.54	71.32
27.66	68.47	27.78	65.62	27.89	62.77	28.01	59.92
28.13	57.27	28.25	55.13	28.36	53.08	28.48	51.10
28.60	49.13	28.71	47.17	28.83	45.21	28.95	43.24
29.07	41.28	29.18	39.31	29.30	37.50	29.42	36.05
29.53	34.67	29.65	33.33	29.77	32.00	29.89	30.68
30.00	29.36	30.12	28.04	30.24	26.72	30.35	25.40
30.47	24.19	30.59	23.22	30.71	22.31	30.82	21.42
30.94	20.55	31.06	19.68	31.18	18.80	31.29	17.93
31.41	17.06	31.53	16.19	31.64	15.40	31.76	14.77
31.88	14.17	32.00	13.59	32.11	13.03	32.23	12.46
32.35	11.90	32.46	11.33	32.58	10.77	32.70	10.20
32.82	9.69	32.93	9.29	33.05	8.90	33.17	8.53
33.28	8.17	33.40	7.81	33.52	7.45	33.64	7.08
33.75	6.72	33.87	6.36	33.99	6.04	34.11	5.78
34.22	5.54	34.34	5.30	34.46	5.07	34.57	4.85
34.69	4.62	34.81	4.39	34.93	4.16	35.04	3.93
35.16	3.73	35.28	3.57	35.39	3.42	35.51	3.27
35.63	3.13	35.75	2.98	35.86	2.84	35.98	2.70
36.10	2.55	36.21	2.41	36.33	2.29	36.45	2.19
36.57	2.09	36.68	2.00	36.80	1.91	36.92	1.82
37.04	1.73	37.15	1.65	37.27	1.56	37.39	1.47
37.50	1.39	37.62	1.33	37.74	1.27	37.86	1.21
37.97	1.16	38.09	1.11	38.21	1.05	38.32	1.00
38.44	0.94	38.56	0.89	38.68	0.84	38.79	0.80
38.91	0.77	39.03	0.73	39.14	0.70	39.26	0.67
39.38	0.63	39.50	0.60	39.61	0.57	39.73	0.53
39.85	0.51	39.97	0.48	40.08	0.46	40.20	0.44
40.32	0.42	40.43	0.40	40.55	0.38	40.67	0.36
40.79	0.34	40.90	0.32	41.02	0.30	41.14	0.29
41.25	0.28	41.37	0.26	41.49	0.25	41.61	0.24
41.72	0.23	41.84	0.21	41.96	0.20	42.07	0.19
42.19	0.18	42.31	0.17	42.43	0.16	42.54	0.16
42.66	0.15	42.78	0.14	42.90	0.13	43.01	0.13
43.13	0.12	43.25	0.11	43.36	0.11	43.48	0.10
43.60	0.10	43.72	0.09	43.83	0.09	43.95	0.08
44.07	0.08	44.18	0.08	44.30	0.07	44.42	0.07
44.54	0.06	44.65	0.06	44.77	0.06	44.89	0.05

Table I-3. (continued)

Days	MS-2 (PFU/ml)	Days	MS-2 (PFU/ml)	Days	MS-2 (PFU/ml)	Days	MS-2 (PFU/ml)
45.00	0.05	45.12	0.05	45.24	0.05	45.36	0.04
45.47	0.04	45.59	0.04	45.71	0.04	45.83	0.04
45.94	0.03	46.06	0.03	46.18	0.03	46.29	0.03
46.41	0.03	46.53	0.03	46.65	0.02	46.76	0.02
46.88	0.02	47.00	0.02	47.11	0.02	47.23	0.02
47.35	0.02	47.47	0.02	47.58	0.02	47.70	0.02
47.82	0.01	47.93	0.01	48.05	0.01	48.17	0.01
48.29	0.01	48.40	0.01	48.52	0.01	48.64	0.01
48.76	0.01	48.87	0.01	48.99	0.01	49.11	0.01
49.22	0.01	49.34	0.01	49.46	0.01	49.58	0.01
49.69	0.01	49.81	0.01	49.93	0.01	50.04	0.01
50.16	0.00	50.28	0.00	50.40	0.00	50.51	0.00
50.63	0.00	50.75	0.00	50.86	0.00	50.98	0.00
51.10	0.00	51.22	0.00	51.33	0.00	51.45	0.00
51.57	0.00	51.69	0.00	51.80	0.00	51.92	0.00
52.04	0.00	52.15	0.00	52.27	0.00	52.39	0.00
52.51	0.00	52.62	0.00	52.74	0.00	52.86	0.00
52.97	0.00	53.09	0.00	53.21	0.00	53.33	0.00
53.44	0.00	53.56	0.00	53.68	0.00	53.79	0.00
53.91	0.00	54.03	0.00	54.15	0.00	54.26	0.00
54.38	0.00	54.50	0.00	54.62	0.00	54.73	0.00
54.85	0.00	54.97	0.00	55.08	0.00	55.20	0.00
55.32	0.00	55.44	0.00	55.55	0.00	55.67	0.00
55.79	0.00	55.90	0.00	56.02	0.00	56.14	0.00
56.26	0.00	56.37	0.00	56.49	0.00	56.61	0.00
56.72	0.00	56.84	0.00	56.96	0.00	57.08	0.00
57.19	0.00	57.31	0.00	57.43	0.00	57.55	0.00
57.66	0.00	57.78	0.00	57.90	0.00	58.01	0.00
58.13	0.00	58.25	0.00	58.37	0.00	58.48	0.00
58.60	0.00						

Table I-4. Output data for small-scale bromide tracer simulation using VIRALT.

Days	Br (mg/L)	Days	Br (mg/L)	Days	Br (mg/L)	Days	Br (mg/L)
0	0	1.10E-02	2.88E-20	2.20E-02	-5.30E-17	3.29E-02	1.61E-16
4.39E-02	6.52E-16	5.49E-02	2.71E-16	6.59E-02	-5.14E-15	7.69E-02	1.27E-14
8.78E-02	-3.03E-14	9.88E-02	6.30E-14	0.1098	-3.57E-15	0.1208	-6.10E-13
0.1318	1.29E-12	0.1427	5.40E-12	0.1537	-1.56E-11	0.1647	-6.50E-11
0.1757	8.27E-11	0.1867	6.77E-10	0.1976	5.42E-10	0.2086	-4.11E-09
0.2196	-1.64E-08	0.2306	-2.37E-08	0.2416	3.68E-08	0.2525	3.41E-07
0.2635	1.28E-06	0.2745	3.57E-06	0.2855	8.48E-06	0.2965	1.79E-05
0.3074	3.49E-05	0.3184	6.33E-05	0.3294	1.09E-04	0.3404	1.79E-04
0.3514	2.83E-04	0.3623	4.33E-04	0.3733	6.43E-04	0.3843	9.27E-04
0.3953	1.30E-03	0.4063	1.79E-03	0.4172	2.41E-03	0.4282	3.19E-03
0.4392	4.16E-03	0.4502	5.32E-03	0.4612	6.73E-03	0.4721	8.40E-03
0.4831	1.04E-02	0.4941	1.27E-02	0.5051	1.53E-02	0.5161	1.84E-02
0.527	2.18E-02	0.538	2.57E-02	0.549	3.01E-02	0.56	3.50E-02
0.571	4.04E-02	0.5819	4.64E-02	0.5929	5.29E-02	0.6039	6.00E-02
0.6149	6.78E-02	0.6259	7.61E-02	0.6368	8.51E-02	0.6478	9.48E-02
0.6588	0.1051	0.6698	0.1161	0.6808	0.1278	0.6917	0.1402
0.7027	0.1533	0.7137	0.167	0.7247	0.1815	0.7357	0.1967
0.7466	0.2125	0.7576	0.229	0.7686	0.2462	0.7796	0.2641
0.7906	0.2826	0.8015	0.3017	0.8125	0.3214	0.8235	0.3418
0.8345	0.3627	0.8455	0.3842	0.8564	0.4062	0.8674	0.4287
0.8784	0.4517	0.8894	0.4751	0.9004	0.4989	0.9113	0.523
0.9223	0.5475	0.9333	0.5722	0.9443	0.5971	0.9553	0.6222
0.9662	0.6475	0.9772	0.6728	0.9882	0.6981	0.9992	0.7234
1.01	0.7486	1.021	0.7736	1.032	0.7985	1.043	0.8232
1.054	0.8476	1.065	0.8716	1.076	0.8953	1.087	0.9186
1.098	0.9414	1.109	0.9638	1.12	0.9856	1.131	1.007
1.142	1.028	1.153	1.048	1.164	1.067	1.175	1.086
1.186	1.104	1.197	1.121	1.208	1.138	1.219	1.154
1.23	1.169	1.241	1.184	1.252	1.198	1.263	1.211
1.274	1.223	1.285	1.234	1.296	1.245	1.307	1.255
1.318	1.264	1.329	1.272	1.34	1.28	1.351	1.287
1.362	1.293	1.372	1.299	1.383	1.303	1.394	1.307
1.405	1.311	1.416	1.313	1.427	1.316	1.438	1.317
1.449	1.318	1.46	1.318	1.471	1.318	1.482	1.317
1.493	1.315	1.504	1.313	1.515	1.31	1.526	1.307
1.537	1.304	1.548	1.3	1.559	1.295	1.57	1.29
1.581	1.285	1.592	1.279	1.603	1.273	1.614	1.266
1.625	1.26	1.636	1.252	1.647	1.245	1.658	1.237
1.669	1.229	1.68	1.22	1.691	1.212	1.702	1.203
1.713	1.194	1.724	1.184	1.735	1.175	1.746	1.165
1.757	1.155	1.768	1.145	1.779	1.135	1.79	1.124
1.801	1.114	1.812	1.103	1.823	1.092	1.834	1.081
1.845	1.07	1.856	1.059	1.867	1.048	1.878	1.037
1.889	1.026	1.9	1.014	1.911	1.003	1.921	0.9917
1.932	0.9804	1.943	0.969	1.954	0.9576	1.965	0.9462
1.976	0.9348	1.987	0.9235	1.998	0.9121	2.009	0.9008
2.02	0.8895	2.031	0.8782	2.042	0.867	2.053	0.8558
2.064	0.8446	2.075	0.8335	2.086	0.8225	2.097	0.8115
2.108	0.8006	2.119	0.7898	2.13	0.779	2.141	0.7683

Table I-4. (continued)

Days	Br (mg/L)	Days	Br (mg/L)	Days	Br (mg/L)	Days	Br (mg/L)
2.152	0.7576	2.163	0.7471	2.174	0.7366	2.185	0.7262
2.196	0.7159	2.207	0.7056	2.218	0.6955	2.229	0.6854
2.24	0.6755	2.251	0.6656	2.262	0.6558	2.273	0.6462
2.284	0.6366	2.295	0.6271	2.306	0.6177	2.317	0.6084
2.328	0.5992	2.339	0.5901	2.35	0.5811	2.361	0.5722
2.372	0.5634	2.383	0.5547	2.394	0.5461	2.405	0.5376
2.416	0.5292	2.427	0.5209	2.438	0.5127	2.449	0.5047
2.46	0.4967	2.47	0.4888	2.481	0.481	2.492	0.4733
2.503	0.4657	2.514	0.4582	2.525	0.4509	2.536	0.4436
2.547	0.4364	2.558	0.4293	2.569	0.4223	2.58	0.4154
2.591	0.4086	2.602	0.4019	2.613	0.3953	2.624	0.3888
2.635	0.3823	2.646	0.376	2.657	0.3697	2.668	0.3636
2.679	0.3575	2.69	0.3516	2.701	0.3457	2.712	0.3399
2.723	0.3342	2.734	0.3285	2.745	0.323	2.756	0.3175
2.767	0.3121	2.778	0.3069	2.789	0.3016	2.8	0.2965
2.811	0.2914	2.822	0.2865	2.833	0.2816	2.844	0.2767
2.855	0.272	2.866	0.2673	2.877	0.2627	2.888	0.2582
2.899	0.2537	2.91	0.2494	2.921	0.245	2.932	0.2408
2.943	0.2366	2.954	0.2325	2.965	0.2285	2.976	0.2245
2.987	0.2206	2.998	0.2167	3.009	0.2129	3.02	0.2092
3.03	0.2056	3.041	0.2019	3.052	0.1984	3.063	0.1949
3.074	0.1915	3.085	0.1881	3.096	0.1848	3.107	0.1816
3.118	0.1783	3.129	0.1752	3.14	0.1721	3.151	0.1691
3.162	0.1661	3.173	0.1631	3.184	0.1602	3.195	0.1574
3.206	0.1546	3.217	0.1518	3.228	0.1491	3.239	0.1465
3.25	0.1439	3.261	0.1413	3.272	0.1388	3.283	0.1363
3.294	0.1339	3.305	0.1315	3.316	0.1291	3.327	0.1268
3.338	0.1245	3.349	0.1223	3.36	0.1201	3.371	0.1179
3.382	0.1158	3.393	0.1137	3.404	0.1117	3.415	0.1097
3.426	0.1077	3.437	0.1058	3.448	0.1039	3.459	0.102
3.47	0.1001	3.481	9.83E-02	3.492	9.66E-02	3.503	9.48E-02
3.514	9.31E-02	3.525	9.14E-02	3.536	8.98E-02	3.547	8.81E-02
3.558	8.65E-02	3.569	8.50E-02	3.579	8.34E-02	3.59	8.19E-02
3.601	8.04E-02	3.612	7.90E-02	3.623	7.75E-02	3.634	7.61E-02
3.645	7.48E-02	3.656	7.34E-02	3.667	7.21E-02	3.678	7.07E-02
3.689	6.95E-02	3.7	6.82E-02	3.711	6.70E-02	3.722	6.57E-02
3.733	6.45E-02	3.744	6.34E-02	3.755	6.22E-02	3.766	6.11E-02
3.777	6.00E-02	3.788	5.89E-02	3.799	5.78E-02	3.81	5.67E-02
3.821	5.57E-02	3.832	5.47E-02	3.843	5.37E-02	3.854	5.27E-02
3.865	5.17E-02	3.876	5.08E-02	3.887	4.99E-02	3.898	4.89E-02
3.909	4.80E-02	3.92	4.72E-02	3.931	4.63E-02	3.942	4.55E-02
3.953	4.46E-02	3.964	4.38E-02	3.975	4.30E-02	3.986	4.22E-02
3.997	4.14E-02	4.008	4.07E-02	4.019	3.99E-02	4.03	3.92E-02
4.041	3.85E-02	4.052	3.78E-02	4.063	3.71E-02	4.074	3.64E-02
4.085	3.57E-02	4.096	3.51E-02	4.107	3.44E-02	4.117	3.38E-02
4.128	3.32E-02	4.139	3.26E-02	4.15	3.20E-02	4.161	3.14E-02
4.172	3.08E-02	4.183	3.02E-02	4.194	2.97E-02	4.205	2.91E-02
4.216	2.86E-02	4.227	2.81E-02	4.238	2.76E-02	4.249	2.70E-02
4.26	2.65E-02	4.271	2.61E-02	4.282	2.56E-02	4.293	2.51E-02

Table I–4. (continued)

Days	Br (mg/L)	Days	Br (mg/L)	Days	Br (mg/L)	Days	Br (mg/L)
4.304	2.46E-02	4.315	2.42E-02	4.326	2.37E-02	4.337	2.33E-02
4.348	2.29E-02	4.359	2.25E-02	4.37	2.20E-02	4.381	2.16E-02
4.392	2.12E-02	4.403	2.08E-02	4.414	2.05E-02	4.425	2.01E-02
4.436	1.97E-02	4.447	1.93E-02	4.458	1.90E-02	4.469	1.86E-02
4.48	1.83E-02	4.491	1.80E-02	4.502	1.76E-02	4.513	1.73E-02
4.524	1.70E-02	4.535	1.67E-02	4.546	1.64E-02	4.557	1.61E-02
4.568	1.58E-02	4.579	1.55E-02	4.59	1.52E-02	4.601	1.49E-02
4.612	1.46E-02	4.623	1.44E-02	4.634	1.41E-02	4.645	1.38E-02
4.656	1.36E-02	4.667	1.33E-02	4.677	1.31E-02	4.688	1.28E-02
4.699	1.26E-02	4.71	1.24E-02	4.721	1.21E-02	4.732	1.19E-02
4.743	1.17E-02	4.754	1.15E-02	4.765	1.13E-02	4.776	1.11E-02
4.787	1.09E-02	4.798	1.07E-02	4.809	1.05E-02	4.82	1.03E-02
4.831	1.01E-02	4.842	9.88E-03	4.853	9.70E-03	4.864	9.52E-03
4.875	9.34E-03	4.886	9.17E-03	4.897	9.00E-03	4.908	8.83E-03
4.919	8.67E-03	4.93	8.51E-03	4.941	8.35E-03	4.952	8.20E-03
4.963	8.05E-03	4.974	7.90E-03	4.985	7.75E-03	4.996	7.61E-03
5.007	7.47E-03	5.018	7.33E-03	5.029	7.19E-03	5.04	7.06E-03
5.051	6.93E-03	5.062	6.80E-03	5.073	6.67E-03	5.084	6.55E-03
5.095	6.43E-03	5.106	6.31E-03	5.117	6.19E-03	5.128	6.08E-03
5.139	5.96E-03	5.15	5.85E-03	5.161	5.75E-03	5.172	5.64E-03
5.183	5.53E-03	5.194	5.43E-03	5.205	5.33E-03	5.215	5.23E-03
5.226	5.14E-03	5.237	5.04E-03	5.248	4.95E-03	5.259	4.86E-03
5.27	4.77E-03	5.281	4.68E-03	5.292	4.59E-03	5.303	4.51E-03
5.314	4.42E-03	5.325	4.34E-03	5.336	4.26E-03	5.347	4.18E-03
5.358	4.10E-03	5.369	4.03E-03	5.38	3.95E-03	5.391	3.88E-03
5.402	3.81E-03	5.413	3.74E-03	5.424	3.67E-03	5.435	3.60E-03
5.446	3.53E-03	5.457	3.47E-03	5.468	3.40E-03	5.479	3.34E-03
5.49	3.28E-03						

FINAL REPORT

FIELD EXPERIMENTS AND MODELING OF VIRAL TRANSPORT IN GROUNDWATER

Volume 2

LABORATORY STUDIES

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ABSTRACT

Groundwater, being a major source of drinking water, is also a source of waterborne illnesses, especially those viral in nature. Five bacteriophage (MS2, PRD1, PM2, Qβ, and ΦX174) were used to determine the fate of viruses in two Texas Aquifers. Batch adsorption and survival studies were conducted using laboratory microcosms containing sediment and groundwater. Phages exhibited decay rates between -0.01 and -0.5 logPFUs/ml/day. Phages exhibited maximum adsorption percentages negatively correlated with their isoelectric points(pI) ($R=-0.91$, $P=0.08$). Viral adsorption and transport studies were conducted using saturated PVC columns. In columns, adsorption was negatively correlated with their pI ($R=-0.9$, $P=0.08$). In transport columns phages with similar sizes (MS2, ΦX174, and Qβ) exhibited maximum C/C_os having direct correlation with their pIs ($R=1$, $P<0.005$). Field studies were conducted in the Brazos Alluvium. MS2 showed the ability to be transported 37 m under natural and forced gradients in concentrations of 10^1 and 10^3 PFUs/ml respectively. Thus, phages can survive for long periods of time and be transported over great distances in sandy aquifers like the Brazos Alluvium.

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CHAPTER 1

INTRODUCTION

1.1 PURPOSE OF STUDY

Approximately half of the population of the United States relies on groundwater for its drinking water (Corapcioglu and Haridas, 1984). In the future, the reliance on groundwater will continue to grow due to increasing population and industrialization. Thus, the potential for contamination of groundwater by domestic and industrial wastes will increase.

Little work to date has been done on the potential survival and transport of pathogenic viruses in Texas aquifers and soils (Dowd and Pillai, 1996, Dowd et al. 1996A). Since a significant portion of the Texas population relies on groundwater as it's source of drinking water (Dowd et al. 1995), the purpose of this study was to elucidate the fate and transport of pathogenic viruses in Texas aquifers.

1.2 OVERALL OBJECTIVE

The overall objective of this project was to use bacteriophages as viral indicators and conduct laboratory experiments and specific field experiments to elucidate the

potential fate and transport of pathogenic viruses in Texas aquifers. A majority of the laboratory studies focused on the Brazos Alluvium since the field site was located atop this aquifer.

1.3 SPECIFIC OBJECTIVES

The specific objectives of this study were:

1. To determine the survival characteristics of indicator viruses in the Rio Grande and Brazos Alluviums using laboratory microcosms.
2. To determine the adsorption characteristics of the indicator viruses in the Rio Grande and Brazos Alluvium using laboratory microcosms and continuous flow soil columns.
3. To determine the transport characteristics of the indicator viruses in the Rio Grande and Brazos Alluviums using laboratory saturated soil columns.
4. To determine the transport characteristics of the indicator viruses in the Brazos Alluvium using specific field experiments.

1.4 LITERATURE REVIEW

1.4.1 Survival Studies

Viral survival in soils has been shown to influence the extent and duration of contamination in both the soil and the associated aquifer (Sobsey et al., 1980). Hurst et al. (1980) compared and statistically evaluated the effects of biotic and abiotic soil factors to virus persistence. They used purified strains of enteric viruses (poliovirus type 1, echovirus type 1, coxsackievirus A9 and coxsackievirus B3, and simian rotavirus SA 11) and coliphages MS-2 and T2. The primary soil used was a loamy sand (FM soil), obtained near Phoenix, Arizona. Eight other soils from different regions of the United States, were also used in various parts of the experiments. Statistical analysis was performed using regression analysis to determine the decay rates and stepwise regression to determine the effects of the environmental variables. The factors they found significant were temperature, soil moisture, the presence of aerobic microorganisms, degree of adsorption, levels of resin-extractable phosphorus, exchangeable aluminum and soil pH. Overall, they determined that temperature ($p \leq 0.05$) and viral adsorption ($p \leq 0.05$) had the most significant effect

on the decay rates of the viruses and phages. They also developed an linear equation, decay rate = $0.1005 + 0.0025x_1 - 0.0008x_2 - 0.0007x_3 - 0.0510x_4$, to explain survival based on significant variables, where x_1 is the average percent adsorption, x_2 is resin-extractable phosphorus (ppm), x_3 is exchangeable aluminum (ppm) and x_4 is the saturation pH value for the soil. This was one of the more detailed survival experiments done and the statistical analyses were effectively utilized to interpret the collected data. It made use of a wide variety of enteric virus types and two phages, as well as various soil types and suspending media. Storage of the diluted samples at -20°C prior to sampling (which could tend to cause freeze-thaw disruption of viral tertiary structure and loss of viability of stressed organisms), could have potentially introduced error in these experiments. So also, the use of glass tubes which could have promoted significant viral adsorption to the glass surface. The experimental design did not include elution methods to account for viable soil-adsorbed virus which could have exhibited reversible adsorption under natural conditions.

Bitton et al. (1983) performed survival studies using three bacterial types, an enterovirus (poliovirus type 1) and a bacteriophage (f2). Unfortunately, only one source of

groundwater was used and only one environmental condition was employed. They suspended groundwater dilutions of virus and phage in 100 ml of groundwater and incubated in the dark at 22°C, sampling daily for 15 days. They found that poliovirus type 1 was relatively stable in the groundwater, whereas f2 declined very rapidly to below detection limits at 5 days. The f2 virus had an inactivation rate of - 0.059 LOG_{10} PFU/ml/hr and the poliovirus had an inactivation rate of only - 0.0019 LOG_{10} PFU/ml/day. They concluded that bacteriophages are unable to serve as indicators of viral pollution. Unfortunately, they based these conclusions on a limited data set (one bacteriophage in one groundwater sample).

Yates et al. (1984) used two enteric viruses, one bacteriophage, and eleven groundwater samples which were obtained from a wide variety of locations. They performed computerized statistical analysis using an intercorrelation matrix, and developed a regression equation. They suggested that it could be used to predict virus decay rates which could then be incorporated into mathematical modeling equations. They concluded that temperature was the main factor influencing viral survival with an R^2 of 0.775. The inclusion of one or two more bacteriophages and enteric viruses could have added greatly to the scope of the data.

Yahya et al. (1993) compared two bacteriophages (MS2 and PRD1) in order to determine their potential applicability as indicators for studying the fate and transport of pathogenic enteric viruses. They obtained groundwater from eight different wells in Arizona, three from Canada, and eight from Massachusetts. They used natural aquifer temperatures, as well as higher and lower temperatures, for each of the groundwater samples. They found that PRD1 survives better than MS2, that survival of the phages is greatly influenced by temperature, and that the two phages would serve well as models for studying the fate of enteric viruses in the environment.

The general rule for survival experiments is to make the data relevant. Many studies merely inoculate virus or phage in groundwater and measure decline in PFUs with time. To be applicable and relevant a study on viral survival should use several different groundwater samples, phages, viruses and conditions (e.g. temperatures). First a detailed study would determine the decay rates of the viruses under both natural and controlled temperatures and determine the chemical make-up of the groundwaters. It would then apply statistical analysis, such as stepwise multiple linear regression, to determine the significant factors influencing survival, and subsequently develop a

linear regression equation which takes these variables into account.

1.4.2 Adsorption Studies

Goyal and Gerba(1979) used 9 different soil types, 36 viruses and 5 bacteriophages. One gram of soil and 1 ml of phage suspended in distilled water were mixed and hand shaken. The samples were then placed upon a rotary shaker at 200 rpm for 30 min. The samples were centrifuged for 5 min. (2,500 x g) and the supernatant assayed for remaining viruses. The difference in titer between a control suspension (without soil) and the sample with the soil was determined and used in calculating percent viral adsorption. Product-moment correlation coefficients were then calculated for soil characteristics using the percent adsorption of the different viruses and phages. Their results indicated that adsorption was highly dependent on virus type and on soil type. The pH of the soil significantly affected the ability of viruses to adsorb, with lower pHs enhancing adsorption. They concluded that soil pH and adsorption were linearly correlated, until a certain pH, at which virus adsorption is significantly enhanced. In general, soils having a pH below 5.0 are good adsorbers and minimal adsorption occurs at soil pH values above five. Other factors they found that influenced adsorption, according to the statistical

analysis, were percent clay, percent sand, percent organic matter, total phosphorus, resin-extractable phosphorus, total iron, total aluminum, and exchangeable aluminum. These factors influenced the adsorption of at least 3 viruses, but no constant predictive pattern was found for all viruses. The one thing which could have improved the study would have been to shake the samples for 60 min., as opposed to 30 min., to ensure that steady state adsorption conditions had been reached. It might also have been preferable to use wastewater or groundwater instead of distilled water as the suspending media, as distilled water, having a low ionic strength, will tend to change the natural adsorptive capacity of the soils and viruses which is dependent on cationic interactions.

Gerba and Goyal(1982) used 14 virus types and 9 soil types in batch studies similar to their previous study. Viruses could be classified into two groups based on their adsorptive properties. Group I was greatly affected by soil variables such as pH, organic matter, and exchangeable iron content. Group II was found not to be significantly affected by any measured soil characteristic. They also found that certain bacteriophages were better indicators of certain enteroviruses. Considering the grouping of the viruses and the comparison of bacteriophages and enterovirus

adsorption, this paper gives information invaluable to future researchers. The grouping of the viruses is also helpful for selecting indicator viruses for field experiments.

Singh et al. (1986) attempted to determine whether a dye or a protein could be used to simulate viral transport. They used two dyes - amaranth, a positively charged dye, and methylene blue, which has a net negative charge. They also used three proteins with various isoelectric points, ferritin (pI4.5), myoglobin (pI7.3), and cytochrome-c (pI9.3). The dyes and proteins were individually suspended in distilled water and 2 ml of the suspension added to 0.5 grams of soil in centrifuge tubes. After different periods of shaking the tubes were centrifuged and the percentage of dyes and protein remaining in the supernatant were determined analytically. They found that no one substance tested could serve as an indicator for all viruses, but a two protein indicator system was possible to predict viral adsorption to soils. Though this was an interesting paper with a reasonable goal, they did not include the cost effectiveness of this approach, nor how the results of the resultant data would apply in the prediction of viral adsorption. Overall, the studies indicated that it was probably better to test the viruses themselves in the soil

of interest, the reason being that adsorption is highly variable, not only for virus type, but more importantly, for soil type.

1.4.3 Column Transport Studies

The transport of viruses in the subsurface was studied using laboratory soil columns. Lance et al. (1976) collected soil from a basin where secondary sewage effluent was dumped regularly and packed a 0.25 m long, 5 cm diameter column. They used ceramic samplers at various depths and added secondary sewage effluent containing about 3×10^4 polio virus type 1/ml and flooded the columns with this mixture for long periods of time at various infiltration rates (15-55 cm/day). They found that most of the viruses were removed from the infiltration medium as they passed through the first few centimeters of the column. The results also showed that virus movement is related to infiltration rate, with higher infiltration rates allowing greater mobility through the column. Studies were also done on the effects of drying-flooding cycles on viral transport. These studies showed that after drying, the virus failed to desorb when the infiltration rate was resumed. This suggested that drying the soil matrix had allowed the viruses to come in closer proximity to the soil particles and van-der-Waals forces had caused significant binding. It

was also determined that the addition of distilled water caused desorption of virus and subsequent movement through the columns suggesting a reversible adsorption phenomenon which was enhanced by lowering the ionic content of the system. Beef extract was also shown to cause a desorptive effect, though the information on this is cryptic at best. The results suggested a possible formation of biofilm layers in the top of the column which could have accounted for the removal of most of the viruses in this part of the column. They also used ceramic samplers which could tend to adsorb viruses themselves. The low infiltration rates along with the probable presence of a biofilm layer, could have been the major factors responsible for the low mobility of the virus through the column. They also failed to provide hydrodynamic data and chemical make-up on the column and the effluent, which is critical for researchers modeling or comparing results.

Lance et al. (1982) used two enteroviruses, Echo 1 and 29 in column adsorption studies, concentration studies, flow velocity studies, soil type studies, and virus-coliform comparison studies. For the adsorption studies, they used a 2.75 meter length of 10-cm (i.d.) PVC pipe containing 6 cm of pea gravel and 25 cm of loamy sand. Sewage effluent was applied using a Marriot siphon to maintain constant

hydraulic pressure on the top of the column. They flooded the column for 2 days with well water prior to adding injectate spiked with viruses (~10^5 PFU/ml). Sampling was performed using ceramic samplers at various depths and at the outlet line, and the flow rate was determined to be 50-60 cm/day. For the concentration studies, they flooded columns with different concentrations of viruses ranging from 10^2-10^5 PFU/ml and sampled during each of the flooding days. In the velocity studies, they used flow infiltration rates ranging from 0.6-2.5 meter/day and extracted water samples for each infiltration rate. In the different soil type experiments, they used 0.087 meter columns individually packed with one of three soil types. Finally, in the virus-coliform comparison they used poliovirus and a fecal coliform in soil transport columns. In the first experiment on soil adsorption, they found that leaching patterns of the Echo 29 virus was similar to previous experiments with polio virus with a significant amount (90%) of the virus adsorbed in the first 2 cm, while the Echo 1 virus showed only 77% adsorption in the first 2 cm. They also found that the addition of deionized water caused desorption of viruses near the top surface, but few moved past the 160 cm level in the columns. In the virus concentration studies, they found that increasing the concentrations did not affect the

percent adsorption or the leaching patterns. In the flow velocity studies, they found that increasing the flow rates increased the ability of the virus to travel through the columns, suggesting that adsorption is strongly related to flow rate. When comparing the different soil types they found that virus-removal efficiency varied among the different soils, but the leaching patterns for polio 1 and Echo 1 were similar for each soil type. Finally, in the virus-coliform comparison studies, they showed that penetration depths were the same, while leaching patterns were different. The coliform displayed a more linear adsorption pattern throughout the column, while the virus showed this only after the 40 cm depth though they did not mention the type of adsorption above 40 cm. It appears the effects of biofilm layers in the tops of these columns would explain many of these observations. The use of stainless steel samplers, as opposed to ceramic samplers would have been preferable, though they suggest that the ceramic showed little or no straining of the viruses used in the study. In general, this paper outlined several important factors which can affect the fate of virus in the subsurface, but future investigations would benefit from a more extensive description of soil and water chemistries, as well as the hydrodynamics of the column.

Lance and Gerba (1984) used the same basic design as their previous work, but they used a new technique to obtain saturation (lack of an air phase in the soil pore spaces) along with a desired flow rate. This was one of the first papers to determine the difference between saturated and unsaturated flow through soil. Their results suggest that virus penetration is greater in the saturated column. The actual penetration depth for the unsaturated column was 40 cm and for the saturated column it was 160 cm. As previously mentioned, the study fails to provide specific information. They only briefly describe how they prepared their saturated column and the description of the experimental design suggests that they probably did not achieve complete saturation of the column. Even with the large head of water that they maintain at the top of the column and the raising of the effluent tubing to provide back pressure, the column could have maintained air in the pore spaces, unless they saturated the column from the bottom forcing out the air. As in their previous paper, they failed to describe the soil chemistry and the hydrodynamic properties of the columns.

The paper by Powelson et al. (1990) addressed many of the limitations with the previous studies. They describe and diagram two new column designs. They used clear PVC to

enable them to see if preferential flow channels had been created along the walls of the column. They also used sandpaper to roughen the inside of the column perpendicular to the direction of water flow in order to prevent preferential flow of virus along the column walls. Several steps were also taken to ensure that the saturated column would be completely saturated. First, they filled the soil with CO_2 to displace the O_2, they then pumped water in from the bottom of the column to create positive pressure, forcing out any residual air, as the water moved upward through the column. The next improvement made on previous experiments was the use of stainless steel samplers, instead of ceramic ones, to ensure that no adsorption of the virus by sampling devices interfered with the results. Powelson and coauthors also described hydrodynamic properties of the columns, the soil chemistries, and the water chemistries. They attempted to sample the soil of the column afterwards, looking for adsorbed viable virus, using various elution techniques. They then used a mathematical model to predict the movement of the bacteriophage MS-2 through the column. Their conclusions were very conservative. They stated that MS2, under saturated conditions, showed little inactivation or adsorption. Under unsaturated conditions, MS-2 were strongly removed as they passed through the column. Even

after 18 pore volumes, the effluent concentration (C) did not approach the influent concentration (C_o). This paper also stated that they were unable to recover viruses from the soil using elution. The MS2 virus at 4°C survived for months in most groundwaters, especially under saturated conditions. MS2 showed a strong "bleeding" effect passing through soil profiles, which was due to either sieving (unlikely) or reversible adsorption. The modeling data was unusual, in that it indicated that more viruses may have been recovered than were put into the column. This suggests an inappropriate use of the error correction equation developed by Amoozegar-Fard (1983), which was originally intended for solute transport. It is possible this model could serve for modeling the results of virus transport, but their peak C/C_o was out of the range in which this model is effective (0.05-0.95). Another point is that displacing the O_2 in column with CO_2 would cause acidification of the column with the subsequent addition of H_2O ($CO_2 + H_2O \rightarrow H_2CO_3$). This paper contained all of the critical information on the column, soil and water. The experiment itself did have a number of problems, such as the elution attempt. A preferred approach would have been to run

several pore volumes of distilled water through the column to desorb unrecovered virus.

Bales and Li (1993) used silica beads as the porous media instead of soil. By doing so, they were able to delineate specific variables and determine how they affected transport. The variables studied were pH and hydrophobic organic content. The ionic strength of the feed solution was also varied to determine the ionic effects on movement. A comparison was made between MS2 phage and poliovirus to determine the effectiveness of the phage as an indicator. Finally they used 2.5% beef extract-glycine as an eluent to see if viable phage could be desorbed. The results suggested that as little as 0.001% hydrophobic organic content could enhance phage retention within the column. A bleeding or tailing effect was observed after initial breakthrough, over many pore volumes, with the relative size of the steady state decreasing as the hydrophobic content increased. They suggested that a simple way to model this effect was to use first order equations based on a rate coefficient for attachment. This attachment depended on the colloids sticking efficiency (i.e. net bonding energy to porous media). They also observed that more phage and virus retention occurred within the column at pH 5 than at pH 7, though poliovirus showed more retention at the higher pH

than MS2. Elution or desorption of viruses within the columns was dramatic, especially, when beef extract was introduced, suggesting that the retained viruses were both viable and their retention subject to reversal in response to chemical perturbations. They suggested that chemical perturbations associated with rainfall, for instance, may cause more release of virus than would occur over time under constant conditions. On the other hand, slow detachment (desorption) under "steady state conditions" could result in long-term release (bleeding) of viruses into groundwater. This paper greatly enhanced the understanding of several important factors influencing the fate of viruses in the subsurface. This type of study, though not comparable to natural conditions, does enlighten us as to the more important factors affecting transport of viruses through porous media. In the future, many more variables could be introduced (other viruses and phages, other environmental factors, other temperatures, other pH's, etc.), using the same type of highly controlled column experiments, to further elucidate how these factors affect transport.

Hinsby et al.(1994) looked at transport of viruses through fractured clay. In addition to two bacteriophages, they also used fluorescent microspheres to determine comparative flow rates and the applicability of microspheres

to simulate viral transport. A core sample of fractured clay was obtained and encased in a rubbery membrane and placed inside a triaxial pressure cell. This column was designed to simulate the in-situ pressure and temperature of the site from which it was taken. The column was saturated by flow from the bottom to the top and then inverted to create natural flow conditions. Flow was started through the column for two days before applying the tracers and then the head space was flushed and tracer flow started. Samples of effluent were taken every 5 min. and reservoir samples taken every hour. They also added tracer free effluent after 3 days to assess desorption. They detected tracer after 4 minutes and reached a steady state in the C/C_o within 1 hour, showing a 90% reduction in phages and a 99% reduction of microspheres. After adding tracer free influent, it took approximately 2 weeks to achieve virus free effluent. They state that microspheres are good surrogate tracers in that they can be obtained in high concentrations, they are easy to detect, are non-infectious, and their flow patterns are very similar to the phages. This experiment is very interesting since they have improved on column designs used by other studies. There was a lack of information on fracture size and layout. It appeared from the data and the type of aquifer material that there

was a very definite preferential flow through this column, meaning that the viruses were not actually going through the clay matrix but traveling through fractures. Though this does not affect the relevance of the data, it should have been considered in the results. Another point is that the cost of this type of column may be justifiable.

1.4.4 Field Transport Studies

Probably the most important aspect in studying the fate of virus in the subsurface is conducting actual field experiments. Laboratory experiments are useful for simulating and collecting data that is indicative of actual field experiments, but it is the actual introduction of viral tracers into the subsurface that demonstrates the actual fate and transport of viruses in saturated and unsaturated zones.

Romero (1970) briefly described the results of several pertinent groundwater pollution investigations and presented standards for governing "safe distances" between domestic well placement and sources of potential contamination. This paper was a review and provided interesting statistical data. He looked at two aspects of transport, downward movement and lateral movement in saturated zones. He does not elaborate on the experimental designs and the experiments also deal with bacterial transport. He states

that higher concentrations result in longer lengths of travel. Viruses travel further in nutrient laden waters and survival in the subsurface can be over 5 years under certain conditions. He also showed that viruses could travel up to 1,500 feet. The paper did provide evidence of the danger posed by viral and bacterial contamination of the subsurface and made preliminary recommendations intended to be used for domestic well placement.

Brown et al. (1979) looked at movement of coliphages and coliforms in septic tank leach fields under natural conditions using three types of soil with various clay content. They used naturally occurring coliforms and coliphages for most of the study. At the end of a two year study, they sampled the soil below the septic lines. This study was designed to simulate natural conditions. Unfortunately, the sampling methods were not efficient and the over all experimental design was suspect. The use of clay sampling cups and the low numbers of coliphages introduced (≅64 PFU/ml) were probably not sufficient to assure detectability. When you consider that an infected individual will shed 10^6 Rotavirus/ml, looking at such low numbers of coliphages did not provide a good estimate of possible scenarios. The authors did spike the leachate with

10^9 f-2 phage, but at the time the samplers were dry and the clay lysometers had cracked. Phage are notorious for preferential flow and from the experimental design it appears that the phage could have avoided the samplers altogether. Another probable reason that they failed to detect any coliphage may have been attributed to the formation of biofilms. In essence, this study had a good premise for natural condition studies, but the authors failed to use extensive or even appropriate sampling techniques to ensure that they detected movement of the coliphage.

Bales et al. (1989) established a field site using steel drive point wells closely spaced with holes in the samplers at various levels. They also had lateral wells to either side of the final well to enable them to look for dispersion or pluming effects. They introduced f2 phage and sodium bromide (a conservative chemical tracer) into the source well and sampled (over time) using tygon tubing and a hand-operated pump to extract samples from the wells. They found that the f2 phage travels almost twice as fast as the anionic chemical tracer. The phage also showed evidence of dispersion, but not retardation, due to adsorption. This experiment was well designed and based on laboratory data using column, batch adsorption, and batch survival studies.

The f2 phages are notorious for rapid inactivation under certain subsurface conditions and are not always good tracers. It would also have been advisable to have determined whether f2 phages show increased inactivation in the presence of sodium bromide. The same tygon tubing was used for each sampling round. They cleaned the tubing with chlorine and sodium thiosulfate before each sampling. Tygon tubing could potentially retain trace amounts of the cleansing solutions and the effects of this on the viability of later sampling rounds was not determined.

Powelson et al. (1993) compared the transport of the two phages (MS2 and PRD1) with sodium bromide (a conservative chemical tracer). They looked at the effects of infiltration rates on virus removal, compared removal in soil to inactivation in effluent and groundwater, and studied the effects of secondary vs. tertiary treated sewage effluent on removal of viruses during transport through soil. They built two experimental basins (one for tertiary, the other for secondary effluent), each of which had been spiked with MS2, PRD1, and sodium bromide. The basins were steel lined and contained stainless steel suction samplers at a variety of depths. The basins were kept in a flooded condition for seven days while effluent was being collected. The results indicated that infiltration rates declined with

each succeeding test due to formation of an algal mat or subsurface clogging. This was critical when rates were partially restored after a period of drying. They found that the concentrations of the effluents sampled reached a steady-state followed by a decline late in the experiment, which was probably due to longer travel time as the infiltration rate declined. They showed that secondary treated effluent caused slower infiltration rates due to greater hydrophobic content in the effluent. They revealed that PRD1 was removed faster than MS2, possibly due to some kind of size exclusion. This study which was one of the more efficiently designed field experiments, was able to provide very comprehensive data. The design of the field basins was one of the best examples of how to do this type of infiltration study under natural conditions. The researchers set out specific objectives and were able to give accurate answers to these questions, as well as other observations.

Paul et al. (1995) looked at how sewage disposal, such as septic tanks, injection wells and illegal cesspits, potentially affect the quality of the porous limestone subsurface environment. They introduced bacteriophage PRD1 and ΦHSIC, which is a marine bacteriophage, into a septic

tank and a simulated injection well and looked for the phages in adjacent surface canals. They also injected fluorescent microspheres to compare to the movement of the phages. The basic design of the septic tank experiment was to flush the tracers down the toilet and then look for movement through the subsurface soil into surface waters. The injection well study was conducted by introducing the phage into a 13.5 meter screened PVC well over 5 hours and looking for the viruses appearance in monitoring wells and in surface waters. In the septic tank study, large numbers of the tracers were detected at the monitoring well 20 meters from the seed site within 7 hours. They were also detected after 11 hours at one of the surface water sampling sites 167 meters from the seed site. This was surprising, considering that after 23 hours they were detected at the closest surface water sampling site. The tracers were also detected up stream of the seed site and at an offshore monitoring site. Similar results were obtained with the injection well study. The tracers showed considerable lateral transport, as well as some vertical movement. The results suggested a connection between septic tank drain fields, well discharge areas, and surface marine water contamination. This study is indicative of the dangers involved in on-site disposal practices, the potential

dangers of contamination from septic tank leach fields, and the dangers of subsurface aquifer recharge.

Bales et al. (1995) studied the transport of bacteriophage PRD1, fluorescent labeled bacteria, and fluorescent latex microspheres under natural gradient conditions in Cape Cod, Massachusetts. The tracers were added over two hours by gravity feed and monitored over a distance of 12 meters as they moved with the natural flow of groundwater. The samples were taken from multilevel monitoring wells using a peristaltic pump over a 23 day period. The field site was laid out grid-like so that plume spreading could be determined. The results suggested that phages exhibited reversible adsorption based on the bleeding effects. The results indicated that in sandy media, phage attenuation and narrow plume spread could confine contamination to a few meters if initial concentrations are not too high. This paper had concise and comprehensive data to show movement, plume spread and breakthrough curves over time, depth, and distance.

1.4.5 Mathematical Modeling

Amoozegar-Fard et al. (1983) developed a very simple model to describe solute movement through soil. In general, it was an error function correction model with two unknowns. It dealt with one dimensional flow which results in a

sigmoidal curve that stabilizes with a $C/C_o = 1$. Though this model was made for the flow of solutes such as chromium and calcium, it still was able to predict, to a certain extent, the flow of viruses through soils. Many researchers have used this model to describe the flow of bacteriophages through soil columns (Powelson et al., 1990). This model may not be appropriate as exhibited in the Powelson paper and had many limitations when dealing with viruses. It did not take into account pH, soil type, temperature and many other factors which effect virus flow to a greater extent than solute transport. It could work well for many applications, such as long columns with slow flow rates, as long as the initial pre-error function C/C_o is between 0.05 and 0.95. This model is recommended for its simplicity and relative accuracy when dealing with solutes.

Corapcioglu and Haridas (1986) introduced an equation which was postulated to predict microbial movement in soils. The model predicts spatial and temporal distribution in one or two dimensions. The bacterial transport was modeled using rate processes and the viruses assumed to have equilibrium partitioning. This model deals with many factors that influence microbial movement, including clogging and declogging of the soils, survival, chemical perturbations, bacterial and viral type, pH, soil moisture,

soil composition, as well as the possibility of growth, by taking into account the presence of oxygen and nutrients. This paper is highly comprehensive concerning the factors that influence the fate of microorganisms, especially viruses. The modeling equation appeared efficient in predicting movement and was applicable to most situations. It was somewhat conservative in its estimates of movement, which is desirable when using the model to predict the placement of drinking wells in relation to a contamination source such as septic tank leach fields. A field and column experiment was used to test the data where the model appears to work well. It seems this model has few flaws other than its slightly complex nature, which when used to model field data, would make it necessary to estimate certain input variables. This was an impressive publication which took into account an large variety of variables which could effect the movement of microorganisms under any soil condition.

Yates and Ouyang (1992) looked at the flow of viruses through unsaturated zones. This mathematical model was also highly comprehensive, and, unlike many models, it coupled the flow of water, viruses, and heat through the soil. It was predictive on the basis of changing temperature in the various soil depths and their effect on the survival of

viruses. This model was not as conservative in its estimates of movement, as was the previous model by Corapcioglu and Haridas (1984), and tended to under-predict the movement of viruses, especially when considering cases where preferential flow may occur. This model was highly comprehensive concerning viral inactivation as a function of temperature.

It is important to do laboratory and field experiments to help increase our understanding of the fate of microorganisms, especially those viral in nature, and use this information to create predictive models to define safe placement zones for domestic wells in relation to possible sources of contamination. Unfortunately, many of the laboratory studies are of limited usefulness and applicability for one or more of the following reasons: (Sobsey et al. 1980) (i) investigation of only a few soil materials, usually a sand or clay; (ii) use of distilled water instead of wastewater; (iii) use of bacteriophages or only a single type on enterovirus; and (iv) use of only sterilized water and soil material. The field experiments provide only limited information because of their marked differences in characteristics and operating conditions, as well as their apparent differences in virus removal efficiencies. In general, this means that laboratory

experiments are generally too site specific and field experiments have not been designed using quality control measures and operating principles.

Mathematical models should be designed to take into account the changing conditions which occur as viruses travel through the vertical soil profile and move with horizontal water flow. They should respond to soil type, as well as viral type, and the wide variety of factors which influence their adsorption and survival. When doing site specific work, which is ultimately necessary, researchers should not attempt to extend their observations and conclusions beyond the scope of their research. When doing specific studies such as adsorption, survival or column experiments, not to mention field studies, researchers should provide a wide variety of information on soil and water chemistries, as well as, as much information as possible on hydrodynamic conditions.

Future work will soon be concentrating more and more on very site specific studies centering around field experimentation. Because no one subsurface environment can be used to predict the fate of virus in any other subsurface environment. Field sites should be set up in such a way that the data obtained can be compared to other field studies. In order to do this, field sites should be

constructed using universal operating principles and quality control. Thus, in order to provide comprehensive data, researchers will have to combine forces. Analytical scientists, soil scientists, civil engineers, environmental microbiologists, and computer scientists will all have to work closely together. Soil scientists and analytical scientists are needed to provide accurate data on chemical composition of soil, sediment, and groundwater; civil engineers are needed to properly meter and construct field sites; microbiologists are needed to run laboratory experiments and viral analysis, and mathematicians and computer scientists are needed to interpret data and develop mathematical models.

CHAPTER 2

MATERIALS AND METHODS

2.1 BACTERIOPHAGES AND HOST BACTERIA

MS2 (Fig. 1)is a 24 nm (Average diameter) bacteriophage which was enumerated on *Escherichia coli* ATCC 15597. PRD1 (Fig. 2) is a 63 nm (Avg. diam.) bacteriophage (Lenczewski, 1993) which was enumerated using *Salmonella typhimurium* LT-2. Qβ is a 24 nm (Avg. diam.) bacteriophage which was enumerated using *E. coli* ATCC 17853 (Fig. 3). ΦX174 (Fig. 4) is a 27 nm (Avg. diam.) bacteriophage which was enumerated using *E. coli* ATCC 13706. PM2 (Fig. 5) is a 60 nm (Avg diam.) bacteriophage which was enumerated on *Alteromonas espejia* ATCC 27025. Selected physiochemical characteristics of the phages are given in Table 1. Electron microscopic procedures are given in Appendix B.

2.2 PHAGE ENUMERATION

2.2.1 Double Agar Overlay

This method was described by Adams et al. (1959). For MS2 and PRD1 respective host cells were grown to mid to late log growth phase in Tryptic Soy Broth (TSB), in a shaking incubator at 37°C (This temperature is required in

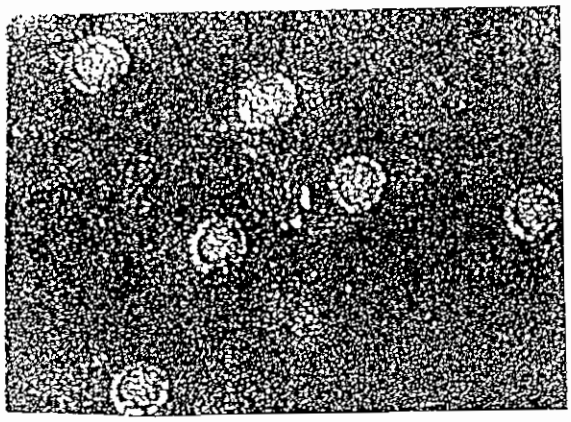

Figure 1

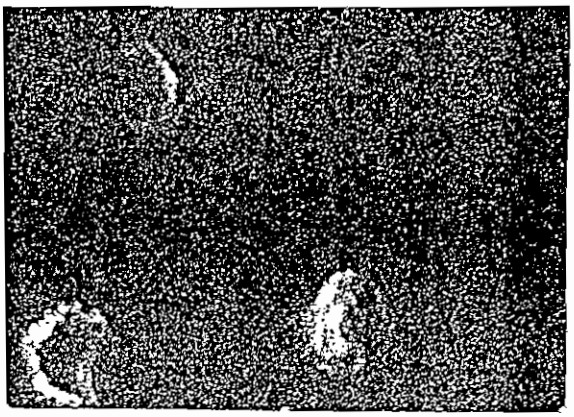

Figure 2

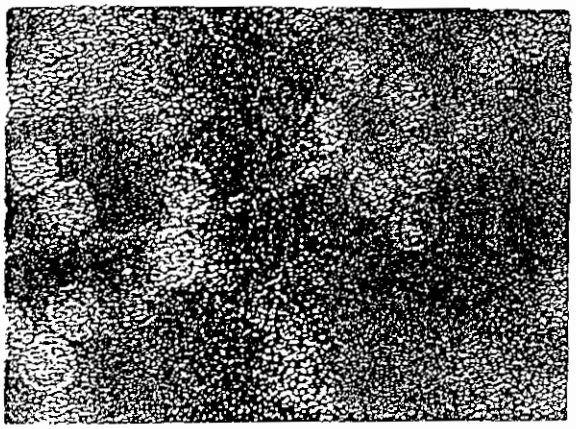

Figure 3

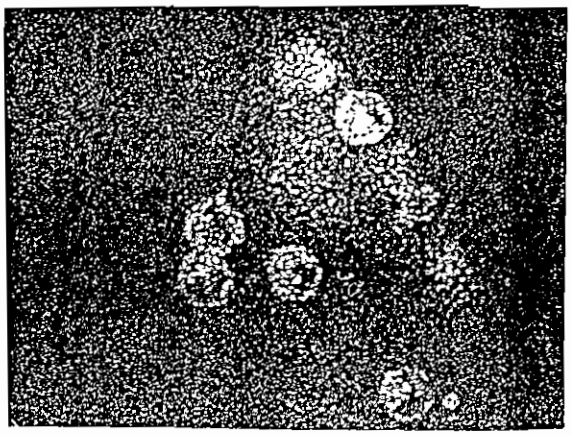

Figure 4

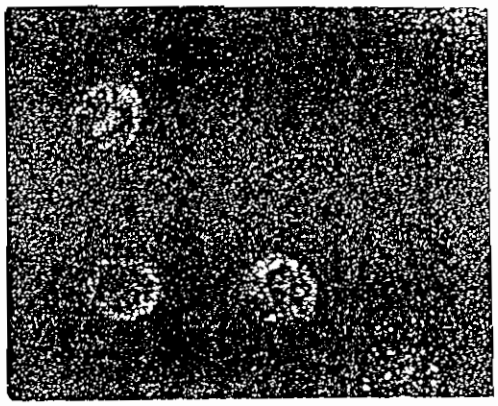

Figure 5

Table 1. SELECTED PHYSICAL AND CHEMICAL CHARACTERISTICS OF BACTERIOPHAGES

Subgroup	Group	Diameter (nm)	Iso electric point (pI)	Buoyant density (g/ml, CsCl)
MS2	Leviviridae I	24	3.9	1.42
PRD1	Tectiviridae	63	4.2	1.28
ΦX174	Microviridae	27	6.6	1.41
Qβ	Leviviridae III	24	5.3	1.45
PM2	Corticoviridae	60	7.3	1.28

Subgroup	S	Lipids (%)	nucleic acid (nature)	G+C (%)	Weight (Mdaltons)
MS2	79	0	R1L	51	3.8
PRD1	357	16	D2L	51	70
ΦX174	114	0	D1C	44	6.7
Qβ	84	0	R1L	51	4.2
PM2	230	13	D2C	43	48.8

note: D = DNA, R = RNA, 1 = single stranded, 2 = double stranded, L = linear, C = circular.
ref. Ackermann and Michael (1987).

order for the organisms to produce the F-pilus). For enumeration 0.2 ml of the host culture was placed in molten soft agar test tubes (0.75% agar at 55°C), 1.0 ml of groundwater (or 0.1 ml of an appropriate dilution) was added, and this molten mixture was then briefly vortexed and poured out onto Tryptic Soy Agar (TSA) plates and swirled to spread the molten agar over the plate. The molten agar was then allowed to solidify and incubated at 37°C for 12-18 hours. Three to 300 plaques were counted at each dilution with over 300 deemed Too Numerous To Count (TNTC). At least three replicate plates and three duplicate platings were performed whenever possible in order to provide a mean and standard error both for plating and experimental errors.

2.2.2 Membrane Elution

This method of phage assay was employed to increase the detection sensitivity in field studies. This method, which was described by Sobsey et al. (1990) increased the detection sensitivity from 1 PFU/ml to 1 PFU/100 ml. This method involved filtering 100 ml of groundwater amended to a final concentration of 0.05M $MgCl_2$, through a negatively charged type HA, 47mm diameter, 0.45µm pore size, cellulose-mixed acetate membrane filter (Millipore Corporation, Bedford, MA). The groundwater sample was vacuum filtered

through 500 ml capacity filter holders at a flow rate of no more than 200 ml per minute. This effectively caused the formation of Mg^{2+} (cation) bridges between the negatively charged phage and the negatively charged membrane surface, trapping the virus which would normally pass through a membrane of this polarity with such a large pore size.

The virus particles were then eluted from the membrane using 10 ml of an elution solution (1.5% beef extract, 1.5% glycine, pH 8.3). Not only did this method concentrate the virus but it removed background bacteria from groundwater samples. This eluent was then assayed for virus using the double agar overlay method.

2.2.3 Dilution Blanks

All dilutions were made in 9 ml distilled deionized (DDI) water containing 0.5% NaCl and 0.1% gelatin. Preliminary studies indicated that this solution would not cause significant phage inactivation for periods up to several months.

2.3 HIGH TITER LYSATE PRODUCTION

High titer lysates were produced using a stock 10^7 PFU/ml of the bacteriophage. The appropriate host was grown up to mid log growth phase in 15 ml TSB test tubes at

shaking at 37°C. After this 1.5 ml of the phage stock was inoculated into the turbid host culture. This mixture was then vortexed gently and 0.5 ml of the host phage mixture then mixed with 52°C molten TSA (0.75% soft agar) tubes, and poured over TSA plates. The soft agar was allowed to solidify for several minutes and then incubated overnight at 37°C. After the incubation, 10 ml of a 1X tris buffered saline [stock solution diluted 1:10; 31.6 g Tris base, 81.8 g NaCl, 3.73 g KCl, 0.57 g anhydrous Na_2HPO_4, dissolved in 807 ml of distilled H_20 and adjusted to pH 7.4] was poured over the plates. Using a sterile glass spreading rod, the layer of soft agar was scraped off the lower TSA layer and suspended in the elution solution. This slurry was then poured into 250 ml centrifuge bottles and vigorously shaken for one hour at 21°C to allow the virus to diffuse from the agar.

In order to insure that the viral lysates, especially those intended for field studies, were free of bacterial contamination, several rounds of centrifugations were conducted at speeds high enough to pellet any bacterial debris but allow phage particles to remain suspended (6,000 x g, 10 min, 4°C). After several rounds of centrifugation the lysate was then filter sterilized through 0.45 µm pore

size membrane filters to remove any remaining bacterial contamination. After these purification steps, 10 X 200µL aliquots of the lysate were plated out on TSA and incubated at 27°C to determine if any bacterial growth occurred. If there were no colonies, the lysate stocks were deemed ready for use.

2.4 GROUNDWATER AND AQUIFER MATERIAL

Aquifer sediment and groundwater were obtained from the Brazos Alluvium (Burleson County, Texas). Aquifer sediment and groundwater was also obtained from the Rio Grande alluvium (El Paso County, Texas) for some of the laboratory studies. The groundwater chemistries of the two aquifers are shown in Table 2.

2.5 LABORATORY MICROCOSM STUDIES

2.5.1 Survival Studies

Survival studies were performed using 50 ml centrifuge tubes (Falcon 2098) containing 5 grams of aquifer material and 9 mls of groundwater to which 1 ml of a known concentration of groundwater suspended viruses were added. They were kept under both shaking and non-shaking conditions (in the dark at the ambient temperature of the aquifer) and

Table 2. GROUNDWATER CHEMICAL CHARACTERISTICS FOR THE BRAZOS AND RIO GRANDE ALLUVIUMS

Parameters	Rio Grande Alluvium	Brazos Alluvium
Sodium (mg/L)	440	94
Potassium (mg/L)	11.4	4.3
Calcium (mg/L)	210	149
Magnesium (mg/L)	44.2	35
Fluoride (mg/L)	2.66	0.1
Cloride (mg/L)	349	48
Nitrate (mg/L)	59.6	0.2
Phosphate (mg/L)	9.14	0.03
Sulfate (mg/L)	751	59
Alkalinity (mg/L) as $CaCO_3$	336	260
hardness (mg/L) as $CaCO_3$	709	516
Lead (µg/L)	3.0	5.0
Nickel (µg/L)	15.7	10.0
Chromium (µg/L)	3.3	10.0
Total Nitrogen	0.0123(%)	0.9 (µg/L)
pH (avg.)	7.36	7.10
Conductivity (mmhos/cm)	2.25	1.17
Temperature (avg.)	23°C	21°C

sampled periodically for phage titers. This was done by centrifuging (1,000 xg, 2 min.) to pellet the sediment and sampling the supernatant. The changes in titer over time were compared to initial counts (C_o) to determine the inactivation rate. Shaking survival studies were also performed using 25 ml of groundwater only (no sediment) in 50 ml conical centrifuge tubes in order to determine the difference in the inactivation rate due to the presence of the sediment. The ability of MS2 to survive in the presence of high concentrations of sodium bromide was also determined. Sodium bromide is a conservative chemical tracer used in field studies to determine aquifer characteristics. Sodium bromide is relatively non-reactive in the soil matrix and exhibits no inactivation as would a viral tracer. Thus, in order to compare the transport properties of the MS2 virus with that of the conservative tracer the virus had to be able to survive in the presence of high weight/volume concentrations. Studies were done at two high concentrations (needed for detection in large scale field studies) 200g/L and 600g/L.

2.5.2 Adsorption Studies

Batch adsorption studies were performed using 50 ml centrifuge tubes (Falcon 2098) containing 5 grams of aquifer material and 9 mls of groundwater to which 1 ml of a known

concentration of viruses (10^7-10^{10}) were added. The effects of soil quantity, virus titer, and volume of groundwater were determined in preliminary studies for use in flask adsorption studies. A 2:1 (weight:volume) ratio of sediment to groundwater was determined to provide reproducible results with MS2 viruses. This ratio was used in all flask studies. Due to the varying burst size of different phage and subsequent variations in lysate titer it was necessary to use titers between 10^6 and 10^9 PFU/ml. Two hour periods were determined to be necessary for the adsorption of the virus to come to a steady state condition where little or no additional adsorption took place. This time period was also adequate to ensure that the effects of inactivation were minimal.

Viral lysate was diluted in groundwater to reduce the amount of the suspending media (TSB) and used as a stock for all inoculations. This was to provide the same inoculation concentrations for each tube. One ml of the groundwater diluted stock was then added to the time-zero tube which was vortexed, serial diluted, and the virus enumerated to provide an initial, pre-adsorptive phage concentration (C_o). The remaining tubes containing groundwater and sediment were inoculated and immediately placed in a vigorously shaking incubator at 21°C. One tube at a time was removed at

various time increments (10 min, 20 min, 40 min, and 90 min) and centrifuged at 1000 x g for 2 minutes in order to sediment the soil. The supernatant was sampled and assayed for remaining virus titers, which were compared to the time-zero concentration. These values were used to determine total percent adsorption as well as the rates of adsorption.

2.6 LABORATORY COLUMN STUDIES

2.6.1 Column Preparation

Clear polyvinyl chloride (PVC) cylinders, 5.2 cm in diameter, were used to prepare the soil columns. This type of design has been used in the past by Powelson et al. (1990). The studies were performed using columns of various heights (0.24, 0.38, 0.76, and 0.99 meters) and were prepared to observe the differences in flow characteristics such as breakthrough curves and C/C_o, at each length.

Two holes were drilled in the PVC 5 cm from each end using a power drill and an appropriate size drill bit. Using the drill, a 1 meter flexible extension, and a circular wire rasp, the insides of the columns were roughened perpendicular to the angle of flow. This was to prevent preferential flow along the column walls. The column was then washed free of any debris using DI water

followed by groundwater. Tapered hose connections were inserted into the two holes by carefully tapping them into place. These connections were then sealed on the outside of the column with clear silicon. One end of each column was closed up using PVC caps and sealed with PVC sealant, so that the chemicals were not exposed to surfaces associated with virus. Finney Aquarium Filter Floss (Finney World Class Pet Products, Inc., Cincinnati, Ohio), was prewetted in groundwater and inserted into the bottom of the column so that when soil was added it only covered the bottom 5.2 cm, effectively covering the injection port. Preliminary studies showed that filter floss did not adsorb or inactivate viruses and the pore spacing of the floss was much higher than the soil matrix. It was thus determined that it would not affect the admittance of the virus into the soil column and should ensure initial optimal dispersion throughout the soil matrix.

Moistened soil was poured into the columns in 50 mm increments stirred and the walls of the columns tapped with a rubber mallet to ensure that the soil settled uniformly. Plastic funnels with the tips removed were filled lightly with filter floss and fitted upside down on the top of the soil column to maintain the packed volume of the column while allowing free flow of groundwater and virus. These

plugs were then sealed with clear silicone so that the silicone was not exposed to the soil column interior and the columns and the groundwater intended to be used in the column were allowed to sit for several days at the natural temperature of the aquifer (21°C) in order to reach a steady temperature condition throughout the soil matrix. A schematic representation of a transport column is presented in Figure 6.

2.6.2 Column Operation

Saturation was accomplished by forcing water in from the bottom of the columns creating positive pressure throughout the column. A Spencer Veristaltic Power Dispenser and Pump (Manostat Corp., Barrington, IL) set at the lowest flow rate setting(1.5), which maintained a steady flow rate in the columns was used to pump the groundwater from a carboy into the bottom injection port of the columns. Approximately 2-4 pore volumes of virus free groundwater were pumped up through the column to ensure sufficient saturation and steady state conditions. For the 2 pore volume injection studies, after initial saturation, the tubing was attached to 2.1 pore volumes of the virus inoculated groundwater (in a separate carboy) which was injected into the columns before virus free influent was added. Up to 10 pore volumes of virus free groundwater was

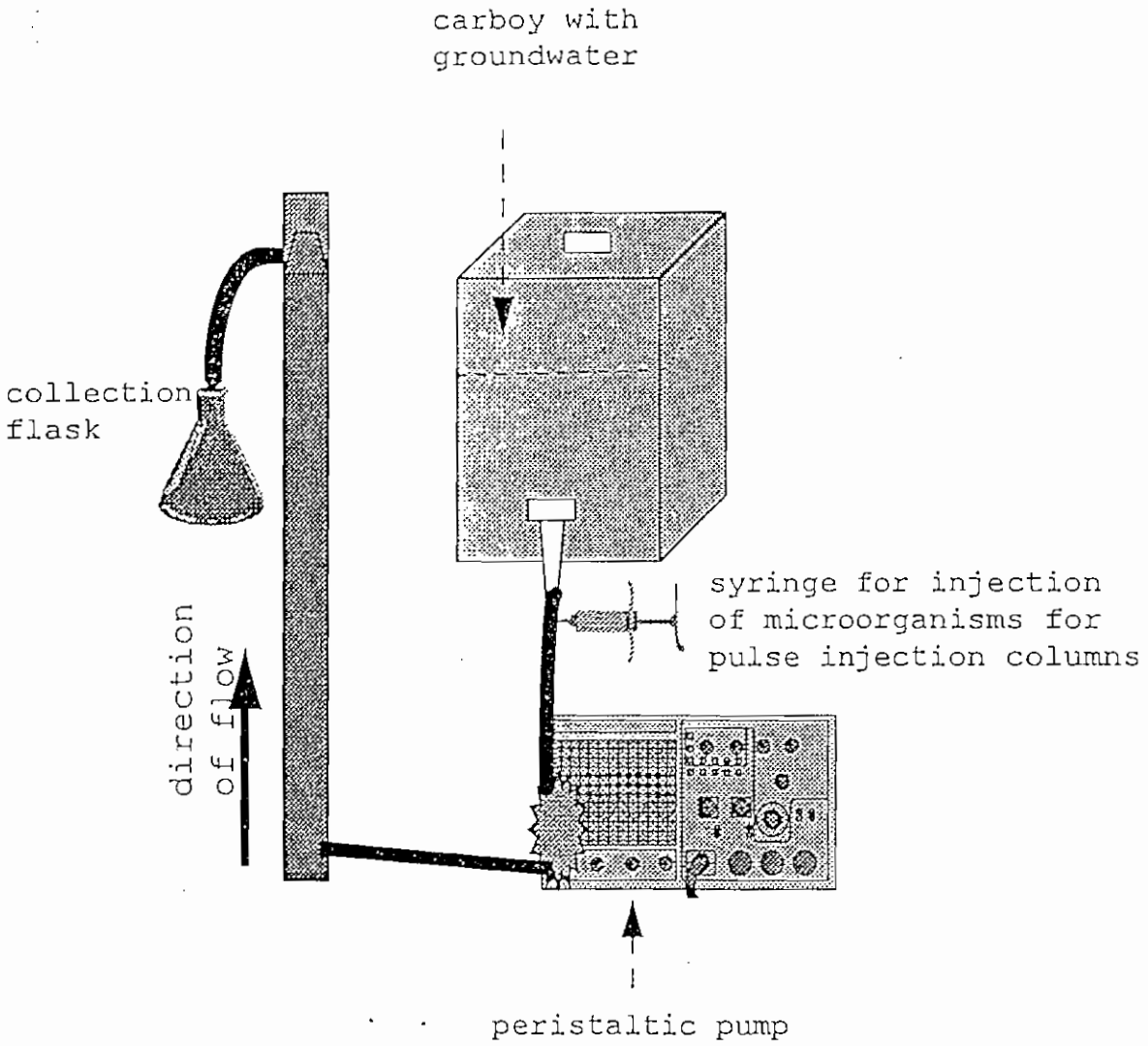

FIGURE 6. SCHEMATIC REPRESENTATION OF A VIRUS TRANSPORT COLUMN

added after viral injection. Flow conditions and pressure were maintained in a steady state throughout the experiments, except when the water supplies were being changed. For the pulse injection studies, the flow was maintained throughout the experiment and the virus pulse injected directly into the peristaltic line. As soon as the virus containing groundwaters were applied to the columns, sampling of the effluent was begun. Samples were also taken from the virus containing groundwater to determine the injected virus concentration and this value was termed the C_o. Samples were also taken from this virus containing groundwater toward the middle, and at the end of the injection to determine any change in concentration over the time of the injection.

2.6.3 Column Sampling

Effluent samples calibrated as pore volumes, or fractions of pore volumes of effluent were collected from the columns for enumeration. Samples were collected in sterilized Erlenmeyer flasks with volumes twice that of the effluent volume being collected. The collected samples were then swirled vigorously and two, 1 ml aliquot samples taken for every 50 ml of groundwater collected. The lower detection limit of the columns was 1 plaque forming unit per

ml (PFU/ml) through the first two pore volumes and 0.1 PFU/ml for subsequent pore volumes.

2.6.4 Continuous Column Adsorption Studies

Continuous column adsorption studies were performed using columns constructed as described above with a few design alterations (Fig. 7). Clinical I.V. tubing connections (Travenol Continu-flo®, Travenol Laboratories, Inc., Deerfield, IL) were used to create sampling ports at the top and the bottom of the column by inserting the needle ports in the peristaltic pump line, creating an injection port at the bottom of the column and a sampling port at the top.

The column was fully saturated by passing three pore volumes through the system, after which the circuit was closed and the column allowed to stabilize until it maintained a constant pressure at the injection port (7.5 psi). Groundwater diluted virus lysate was then injected using a syringe and a 20 gauge needle. Samples were obtained by injecting 1 ml of groundwater into the sampling port (to maintain the column volume), waiting several seconds and withdrawing 1 ml. These samples were immediately inoculated into preservative dilution blanks and used for enumeration. Samples were obtained over periods of

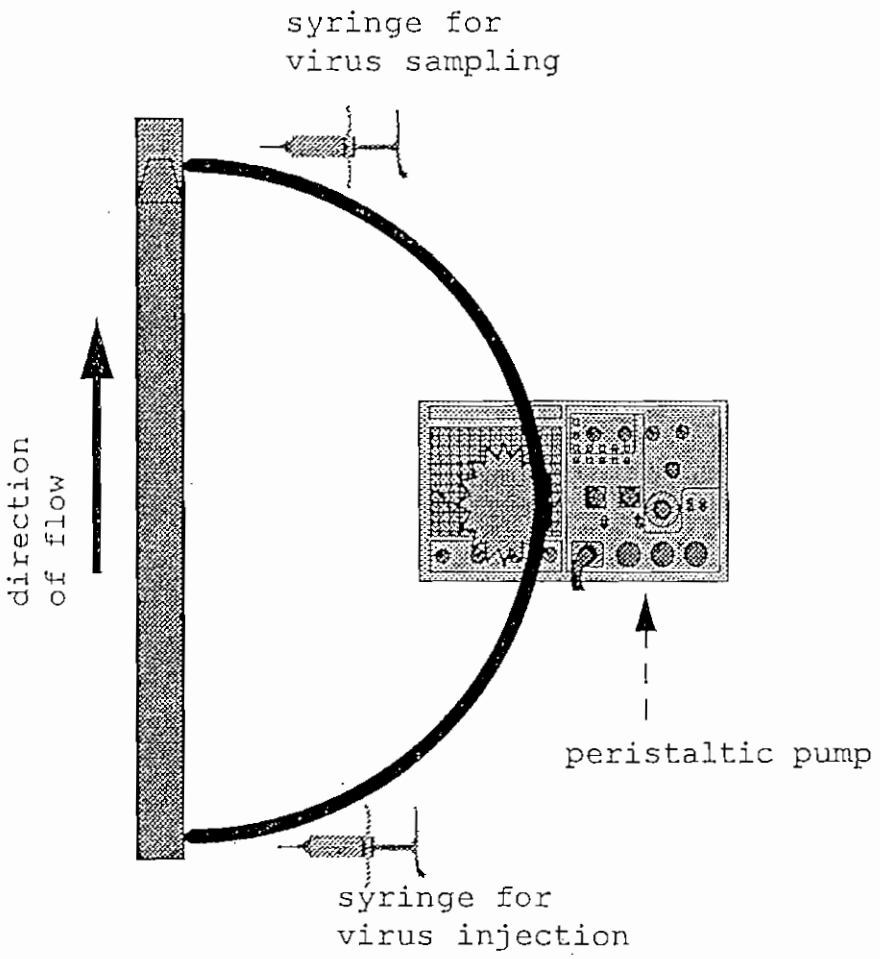

FIGURE 7. SCHEMATIC REPRESENTATION OF A CONTINUOUS FLOW COLUMN

time to coincide with the flask adsorption studies (2.5 min, 5 min, 10 min, 20 min, 40 min, 60 min, 120 min, and 240 min).

2.6.5 Ten Meter Horizontal Column Studies

A 9.30 meter column was constructed by creating 8 column segments as described above (Fig. 8). The injection and three sampling ports were located at the beginning, at 1.77 meters, at 4.5 meters, and the end segments of the column respectively. Segments were put together using PVC connections and sealed using a minimal amount of PVC sealant. Each joining was sealed using clear silicone on the outside only. Groundwater flow was begun using a peristaltic pump. The column was packed in ice to maintain temperatures close to the ambient temperature of the aquifer. For the pulse injection study a high titer viral lysate was injected in a 30 ml slug. Samples were collected every 6 hours at each of the three sampling sites using 2000 ml beakers packed in blue ice. Subsamples (150 ml) were collected from these beakers in sterile polypropylene bottles and stored at 4°C until analysis. After detection at the 9.3 meter sampling port the flow was increased by placing an in-line flow valve which increased the input pressure from 10 psi to 20 psi to determine the effects of increased flow rate. Twenty four hours later, deionized

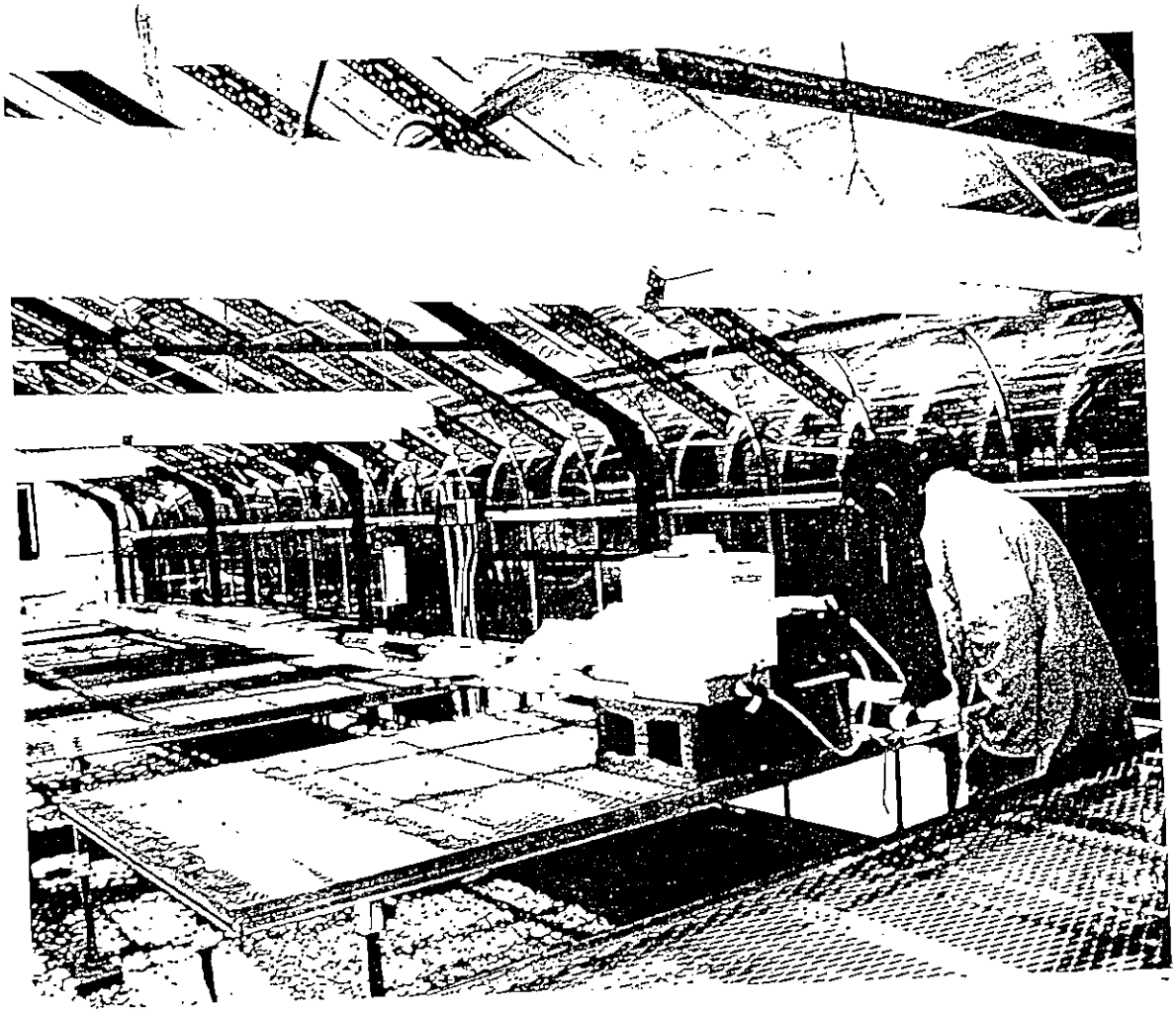

Figure 8. PHOTOGRAPH OF THE 9.3 METER HORIZONTAL COLUMN

water was injected in place of the groundwater to determine its effect on flow and recovery.

A 9.3 meter two pore volume injection horizontal column study was also conducted. Only one sampling port, located at 9.3 meters, was used for this experiment. The purpose of the study was to compare the transport of the bacteriophage to a conservative chemical tracer (sodium bromide). Two pore volumes (12 liters) of groundwater was inoculated with 30 ml of MS2 lysate, 30 ml of PRD1 lysate and 200 mg/L of sodium bromide. Two pore volumes of virus free groundwater was initially run through the column in order to saturate the system and then flow of the tracers was begun. Sample collection was begun during the virus free groundwater saturation in order to determine a time zero concentration or background level of bromide. After the tracer injection was begun the samples were collected over a 2-4 hour period through 5 pore volumes. After the initial two pore volumes of groundwater containing the tracers was injected 3 pore volumes of tracer free groundwater flow was flushed through the system.

2.7 FIELD STUDIES

2.7.1 Site Description

A five-hectare research site of the Brazos River flood plain, operated by Dr. Clyde Munster, at the Texas A&M University research farm near College Station, Texas, has been TNRCC approved for the research. The aquifer has been instrumented for groundwater research. It is an unconfined aquifer, the surface layer at the site is a Ships clay unit approximately 6.1 meters deep. Beneath the clay surface is a sand and gravel layer that extends to a depth of approximately 19.8 meters. The aquifer grades from fine sand at the top to a coarse sand mixed with gravel at the bottom (Fig. 10). The water table is typically located in the sand aquifer at a depth of approximately 9.1 meters. The aquifer is underlain by an impermeable shale formation (Vogel et al, 1996). The physiochemical parameters of the aquifer are listed in Table 3.

2.7.2 Monitoring Well Description

A total of ten well nests, an injection well, and a pumping well (Fig. 9) are installed at the site. Each well nest has four monitoring wells. An injection well, two well nests, and the pumping well were used under forced and natural gradient conditions. The distance from the injection well to the pumping well is 113 meters. Two

TABLE 3. SELECTED SOIL CHARACTERISTICS FOR THE BRAZOS AND RIO GRANDE ALLUVIUMS

Parameter	Brazos Alluvium	Rio Grande Alluvium
Texture	sandy	sandy
Sand %	95	91
Silt %	2	7
Clay %	3	2
Total porosity	0.36	.27
Particle density	2.5	2.63
Bulk density	1.61	1.92
Velocity (natural gradient)	0.5m/day*	
Velocity (forced gradient	1.0m/day*	

*Reference from Dr. Clyde Munster (personal communication)

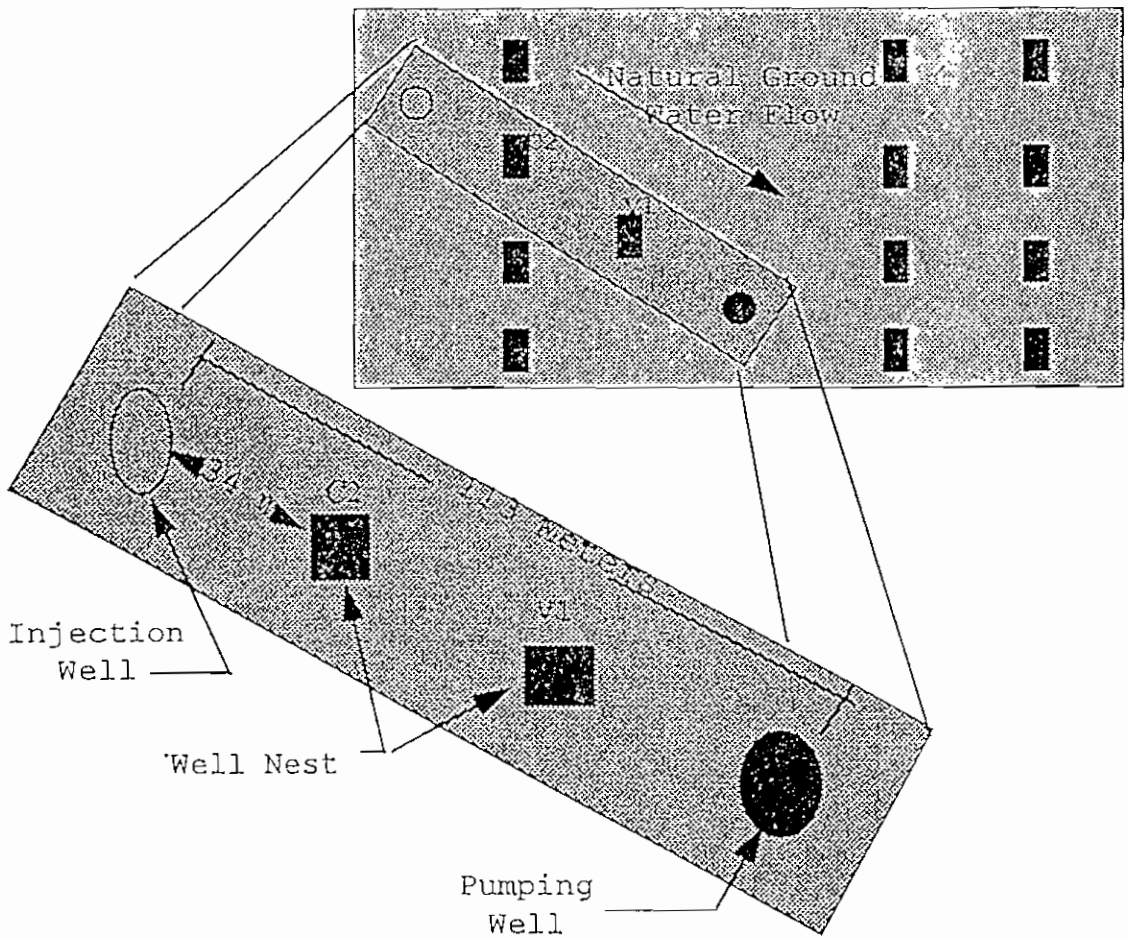

FIGURE 9. SCHEMATIC REPRESENTATION OF THE BRAZOS ALLUVIUM FIELD SITE LAYOUT

monitoring well nests are spaced evenly between these. The wells are all in line with the predicted natural flow of the aquifer. All monitoring wells were 51 mm in diameter with flush threaded, polyvinyl chloride(PVC) well casings with 152.4 mm long well screens.

The short well screens are located at different depths to provide discrete sampling points within the aquifer (Fig. 10). In addition to the well nests, three monitoring wells are labeled "water table" (A-wt, Bwt, and C-wt) wells. These wells are screened the entire depth of the aquifer. A 203.2 mm diameter pumping well with a 150.8 mm diameter submersible pump rated at 760 liters per minute, was also installed at the pumping well.

2.7.3 Sample Injection

Fifty gallons of site groundwater and 1-5 liters of virus lysate were mixed in a 500 liter tank with a controlled flow valve. The injection solution was allowed to flow by gravity into the well. The flow rate was periodically checked and adjusted. The inoculated groundwater contained virus concentrations of around $10^{?}$ - 10^{12} PFU/ml. This groundwater was then injected by gravity flow directly into the injection well and subsequently into the saturated zone of the aquifer. The effects of sunlight and temperature on the survival of the bacteriophages during

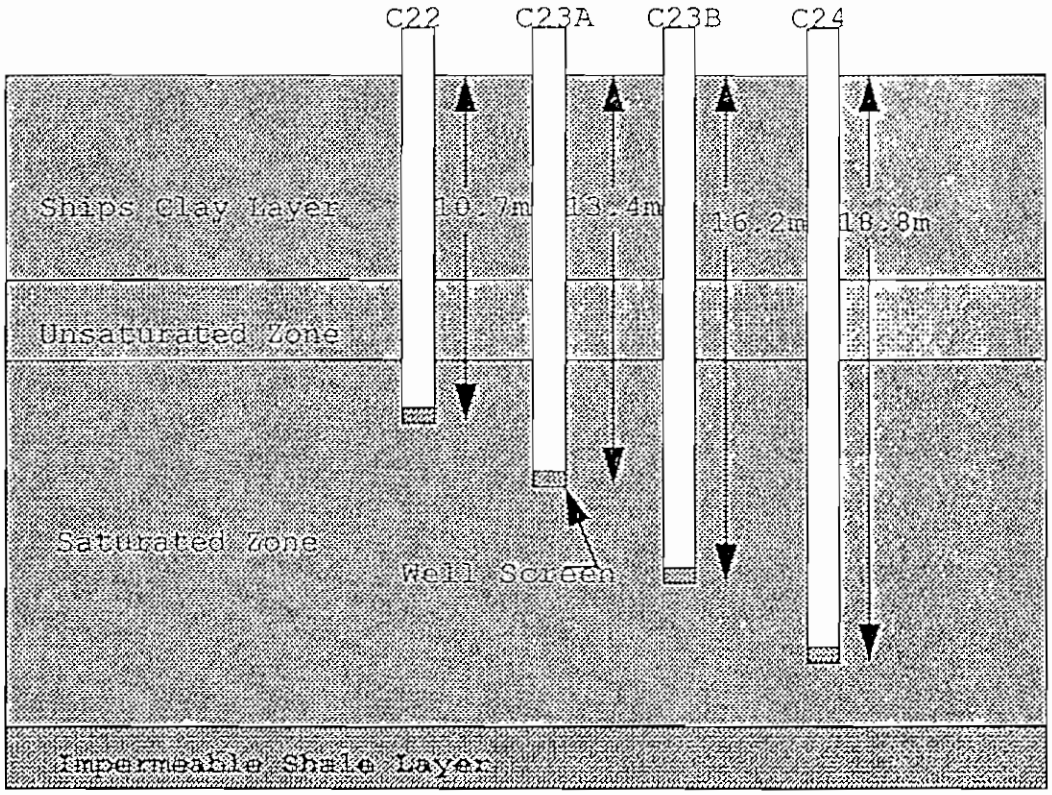

FIGURE 10. SCHEMATIC REPRESENTATION OF THE WELL NEST DESIGN WITH SOIL PROFILE

the injection period was minimized by spraying the outside of the tank with reflective paint and packing the tank with ice.

Since the injection well was fully screened throughout the depth of the aquifer, a double packer system (Fig. 11) was used to inject the virus into a discrete section of the well screen. The packer system consisted of inflatable bladders which partition off a 400 mm section of the well and the tracer was then injected between the bladders.

2.7.4 Sample Collection

After injection of virus contaminated groundwater commenced, samples were taken from the C2 and V1 wellnests and the pumping well. The water samples were measured for pH, temperature, and the water level in each of the wells. Nearby wellnests and the water table wells at the research site were also monitored, though less frequently, to detect bacteriophage movement not directly in-line with the pumping well.

Samples from the wells were obtained using sterile PVC bailers, 5 gallon buckets, and fresh lengths of rope for each well sampled. Three well volumes of water were bailed from each well before the sample was taken and the purged water was dumped off site. Two 120 ml groundwater samples were collected in sterile polypropylene bottles from each

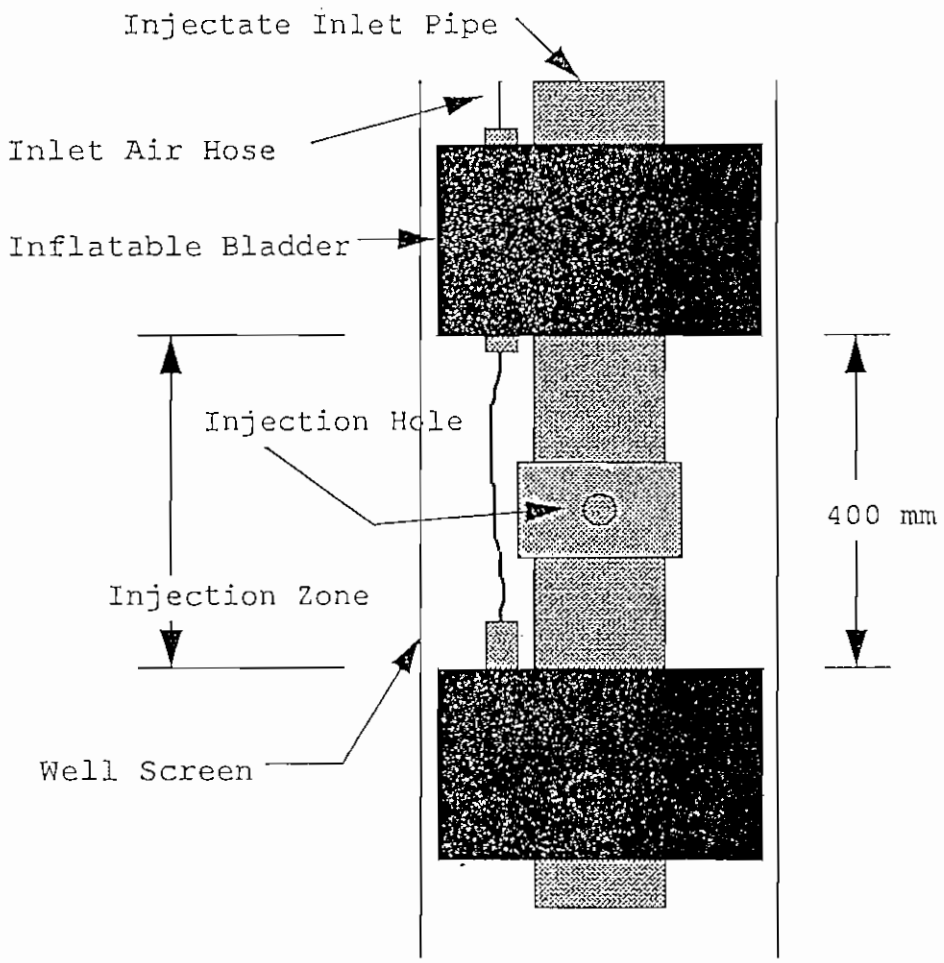

FIGURE 11. SCHEMATIC REPRESENTATION OF THE FIELD INJECTION DOUBLE PACKER SYSTEM

well. One set of the samples was then shipped overnight packed in "blue ice" to the Texas A&M Research Center at El Paso, Texas. All samples were collected in the field by Mr. Jason Vogel, a Graduate Research Assistant in the Agricultural Engineering Department of Texas A&M University at College Station, Texas.

2.7.5 Sample Analysis

The groundwater samples were transported to El Paso on blue ice within 24 hours of sampling and were assayed for bacteriophages using the double agar overlay method. Three 1 ml samples were plated out on each of the host strains for the two viruses (PRD1 and MS2) and two 1 ml samples were inoculated into preservative dilution blanks for archival purposes. Antibiotics were used in the assay to control background bacteria from the groundwater and keep it from interfering with the enumeration. Preliminary studies showed the use of erythromycin at 8µg/ml did not decrease the size or numbers of plaques during assays. The hosts were grown in TSB containing resistance levels of the antibiotic and the agar plates used in the overlay method were also amended with erythromycin. The two hosts had some natural resistance to the antibiotics, so finding mutants with resistance was not necessary. The organisms had to be

cultured with the antibiotic in order for them to grow well when performing the double agar overlay. After the initial assay, 100 ml of the groundwater was subjected to filter concentration and elution and then assayed using the double agar overlay method.

2.7.6 Natural Gradient Study

To characterize the large-scale transport of viruses under natural gradient in the sandy aquifer at the research site, a virus transport field study was started on November 16, 1995. For this study 190 liters of groundwater was mixed with 1 liter of MS2 bacteriophage with a resultant concentration of 2.4 X 10^9 PFU/ml. This solution was injected at a depth of approximately 18.3 meters below the surface over a 32 hour period. Sampling at the well nests was conducted every other day for a period of 32 days, with a total of 75 groundwater samples obtained from the C2, V1, B2 wellnests, and C-wt well during the natural gradient virus tracer study. Samples for the natural gradient test were assayed using the double agar overlay method (Vogel et al. 1996).

2.7.7 Forced Gradient Study

To characterize the large scale transport of viruses under a forced gradient, gradients were created by the site pumping well. For all forced gradient studies, the pumping

well was run for up to a week to create a steady state flow condition before virus injection. The pumping well was started on January 13, 1996, pumping approximately 700 liters per minute to create the forced gradient. On January 20, 1996, the gradient had stabilized. At this time 190 liters of groundwater was mixed in the field with 3 liters of MS2 and PRD1 bacteriophage solutions at a concentration of 3.2×10^{11} ad 6.4×10^{9} PFU/ml, respectively. This solution was injected at a depth of approximately 18.3 meters below the surface over a 32 hour period. The MS2 and PRD1 injection concentrations from tank samples collected throughout the injection was 5×10^{9} and 1×10^{8} PFU/ml, respectively. Sampling was conducted everyday for a period of 30 days, with a total of 182 samples being obtained from the C2 well nest, V1 well nest, and the pumping well during the first forced gradient virus tracer study. The 100 ml membrane concentration, elution method was used for this analysis making the detection limit a theoretical 1 PFU/100 ml. The recovery efficiency of the elution method was tested prior to the study using the same conditions expected in the field study. The efficiency of recovery for MS2 ranged between 20% and 100% with a mean of 47.5 %. The recovery of PRD1 was less satisfactory if not as variable

with percentages of recovery ranging from 2.8% to 8.4% with a mean of 5.2% (Vogel et al. 1996).

2.8 Statistical Analysis and Data Handling

All statistical analyses was performed with Sigmastat® and Sigmaplot® software (Jandel Corporation, San Rafael, CA). The statistical methods to be used were predetermined and then double checked for applicability using the Sigmastat Statistic Wizard function of the Jandel Scientific software.

For survival studies the decay rates were determined using a best fit linear regression equation applied to the logrithmically plotted data points and the decay rate defined as the slope of this regression line. Maximum adsorption percentages were calculated by dividing the initial concentration of virus in the supernatant in the system at time zero (prior to adsorption) by the resultant concentration in the supernatant after two hours. Spearman Rank Order Correlation analysis was used to determine the viral characteristics which correlated to the maximum adsorption characteristics. Spearman Rank Order Correlation analysis is used to measure the strength of association between pairs of variables without specifying which variable is dependent or independent. Other correlations were

determined using Pearson Product Moment Correlation. The Pearson product moment correlation is used to measure the strength of association between pairs of variable without regard to which is dependent or independent, the relationship, if any, between the variables is a straight line, and the residuals are normally distributed with constant variance. C/C_os were calculated by dividing the effluent or resultant experimental concentrations (C) by the initial or pre-experimental concentrations (C_o).

CHAPTER 3

RESULTS

3.1 SURVIVAL STUDIES

3.1.1 Survival of Phages in the Brazos Alluvium

The decay rates, based on regression analysis, for MS2 in a shaking microcosm with Brazos Alluvium groundwater and sediment, after taking into account the adsorption effects (This was done by allowing the adsorption reach a steady state where no more adsorption was taking place befor time zero concentration were recorded), was - 0.2 $\log_{10}$PFU/ml/day (Fig. 12), while in a non-shaking microcosm, the decay rate was - 0.3 $\log_{10}$PFU/ml/day. Survival of MS2 in a microcosm with groundwater alone was - 0.16 $\log_{10}$PFU/ml/day. PRD1 showed lower decay rate than MS2. In a shaking microcosm containing sediment, after adsorption, PRD1 exhibited a decay rate, based on regression analysis, of -0.081 $\log_{10}$PFU/ml/day (Fig. 13), while in a non-shaking microcosm with sediment the decay rate was - 0.089 $\log_{10}$PFU/ml/day. In groundwater alone the decay rate of PRD1 was - 0.055 $\log_{10}$PFU/ml/day. In a microcosm containing sediment and groundwater Qβ exhibited a decay rate of - 0.5 $\log_{10}$PFU/ml/day (Fig. 14) and ϕX174 had a decay rate of -0.32 $\log_{10}$PFU/ml/day (Fig. 15). MS2 also showed the

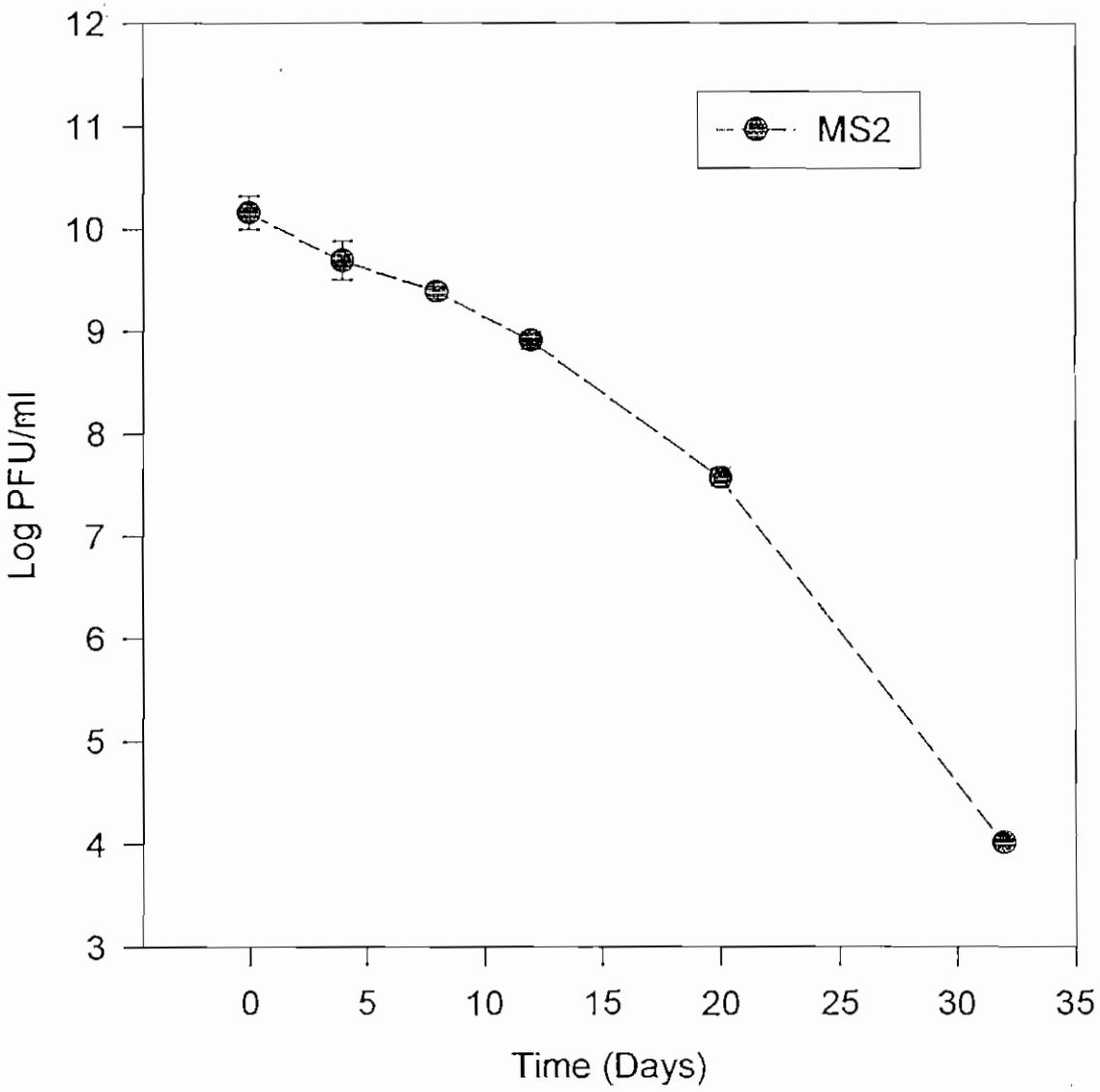

FIGURE 12. BATCH SURVIVAL OF MS2 IN THE BRAZOS ALLUVIUM

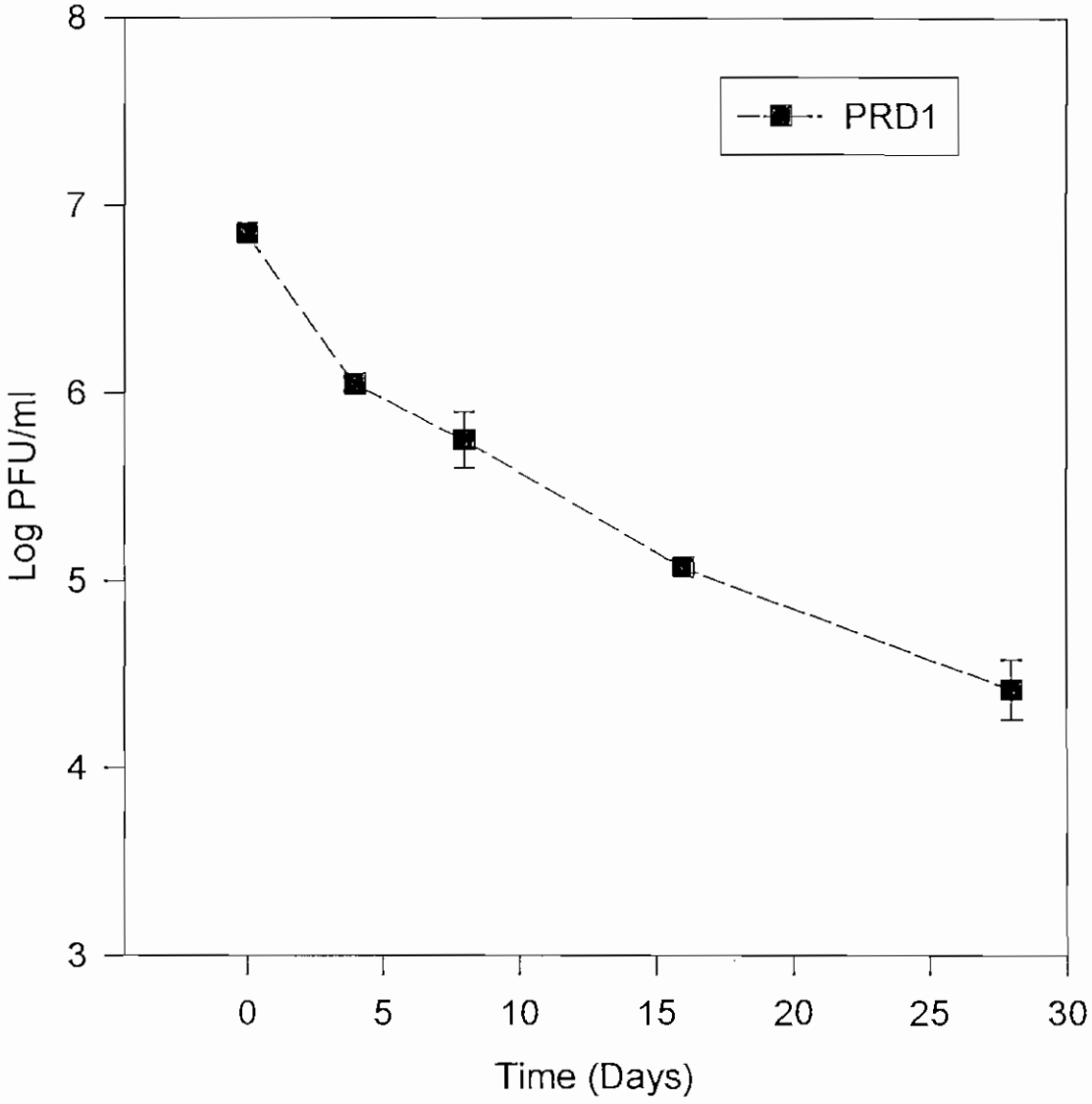

FIGURE 13. BATCH SURVIVAL OF PRD1 IN THE BRAZOS ALLUVIUM

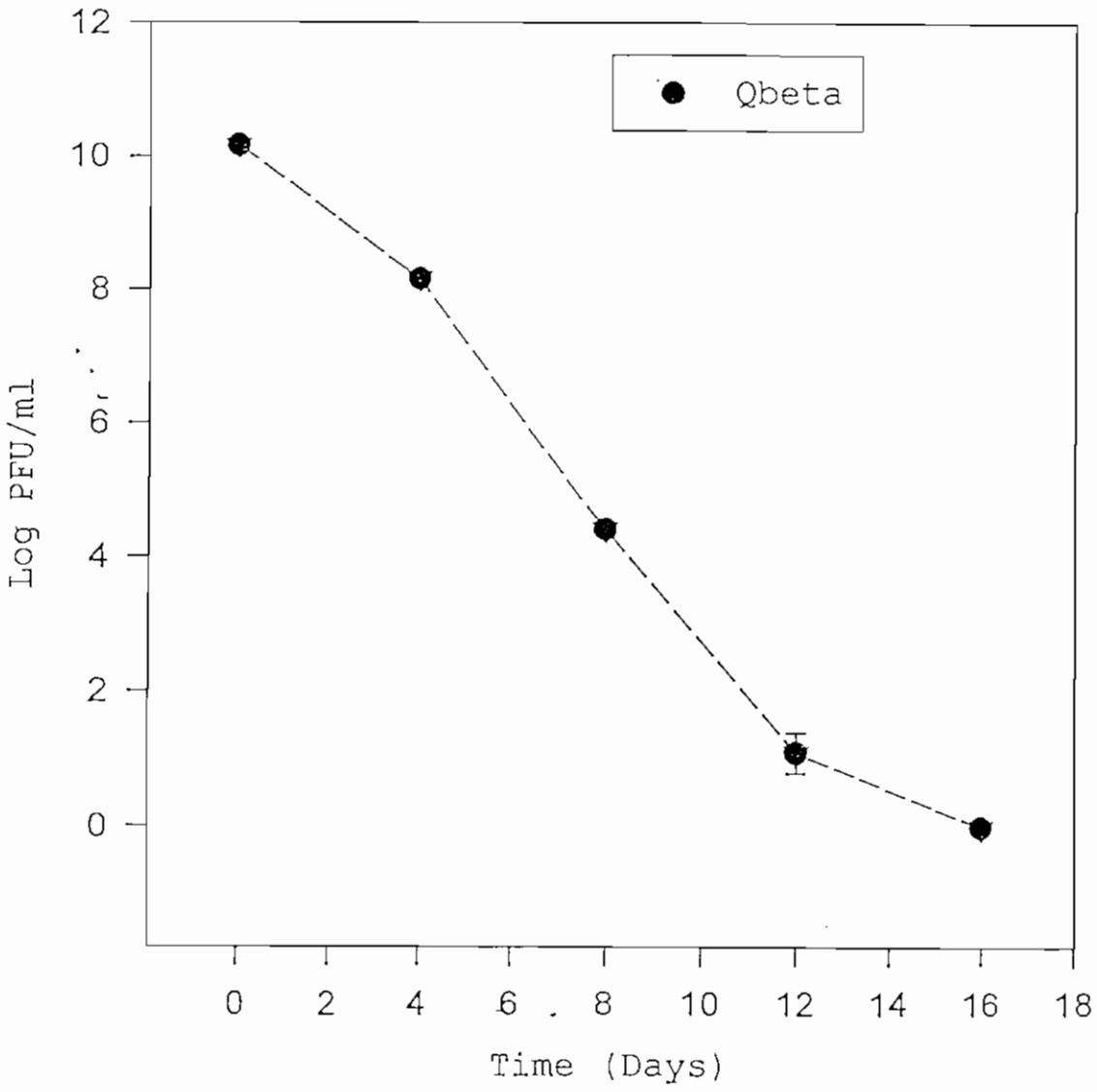

FIGURE 14. BATCH SURVIVAL OF Qbeta
IN THE BRAZOS ALLUVIUM

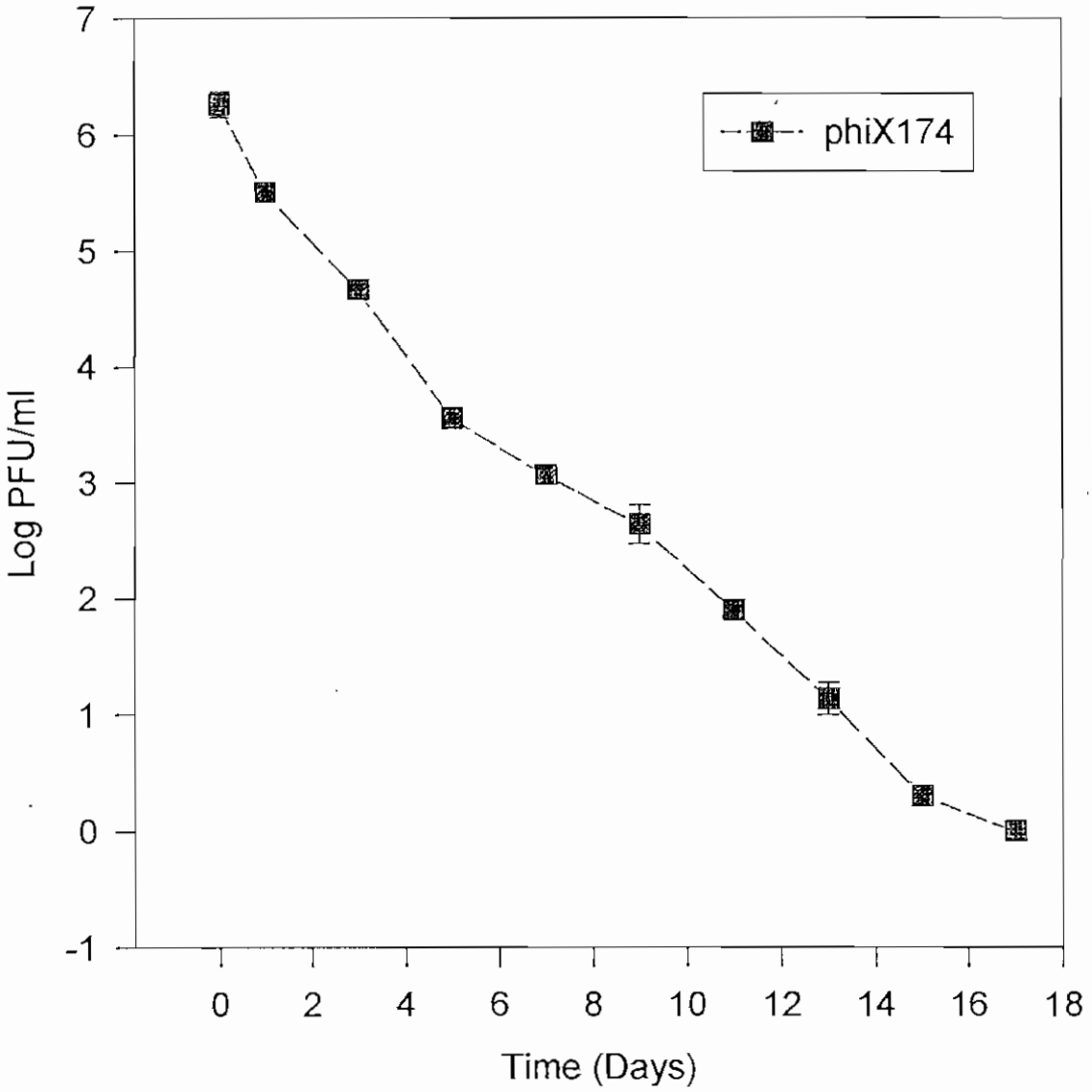

FIGURE 15. BATCH SURVIVAL OF phiX174 IN THE BRAZOS ALLUVIUM

ability to survive for long periods of time in the presence of 200g/L of sodium bromide (-0.17 log PFU/ml/day decline), while at a higher concentration (600g/L) the virus was inactivated within 4 days (Fig. 16).

3.1.2 Survival of Phages in the Rio Grande Alluvium

The decay rates for MS2, based on regression analysis, in a shaking microcosm with Rio Grande Alluvium aquifer sediment (Fig. 17) and groundwater, was - 0.85 log_{10}PFU/ml/day. PRD1 once again showed a slightly lower decay rate than MS2, with an average decay rate of - 0.71 log_{10}PFU/ml/day decline (Fig. 18). This suggested a difference in the effects of these two groundwaters on the survival of these bacteriophage, possibly due to the groundwater chemistries (Table 2).

3.2 ADSORPTION STUDIES

3.2.1 Flask Adsorption Studies

Using Brazos Alluvium aquifer sediment and Brazos Alluvium groundwater the rates of adsorption of each of the bacteriophages was determined. Percent adsorption was also calculated by determining the point of greatest adsorption and dividing it by the initial concentration. The percent adsorption was used in statistical analysis in determining phage associated factors, such as phage *pIs* (isoelectric

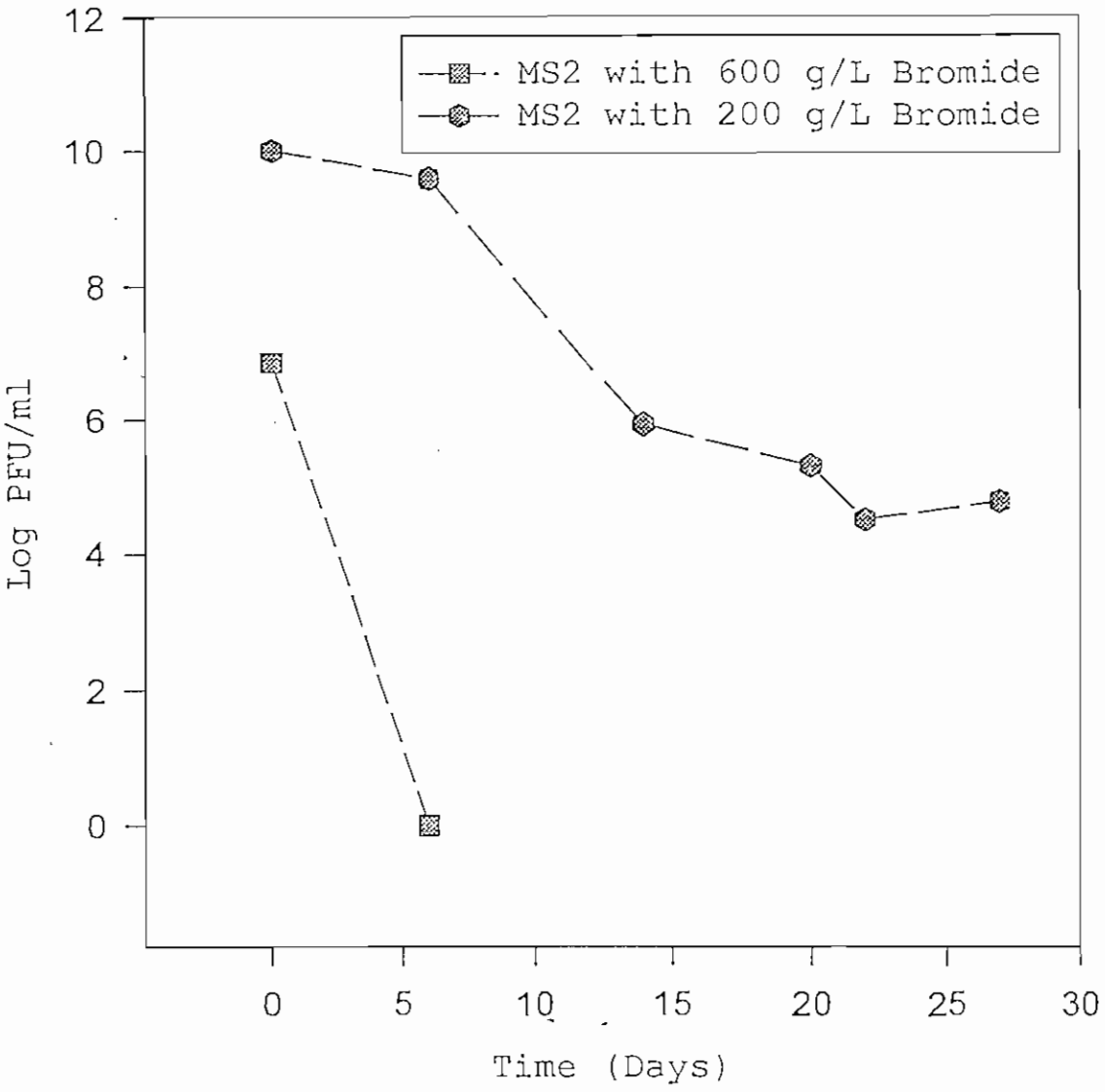

FIGURE 16. BATCH SURVIVAL OF MS2 WITH BROMIDE IN THE BRAZOS ALLUVIUM

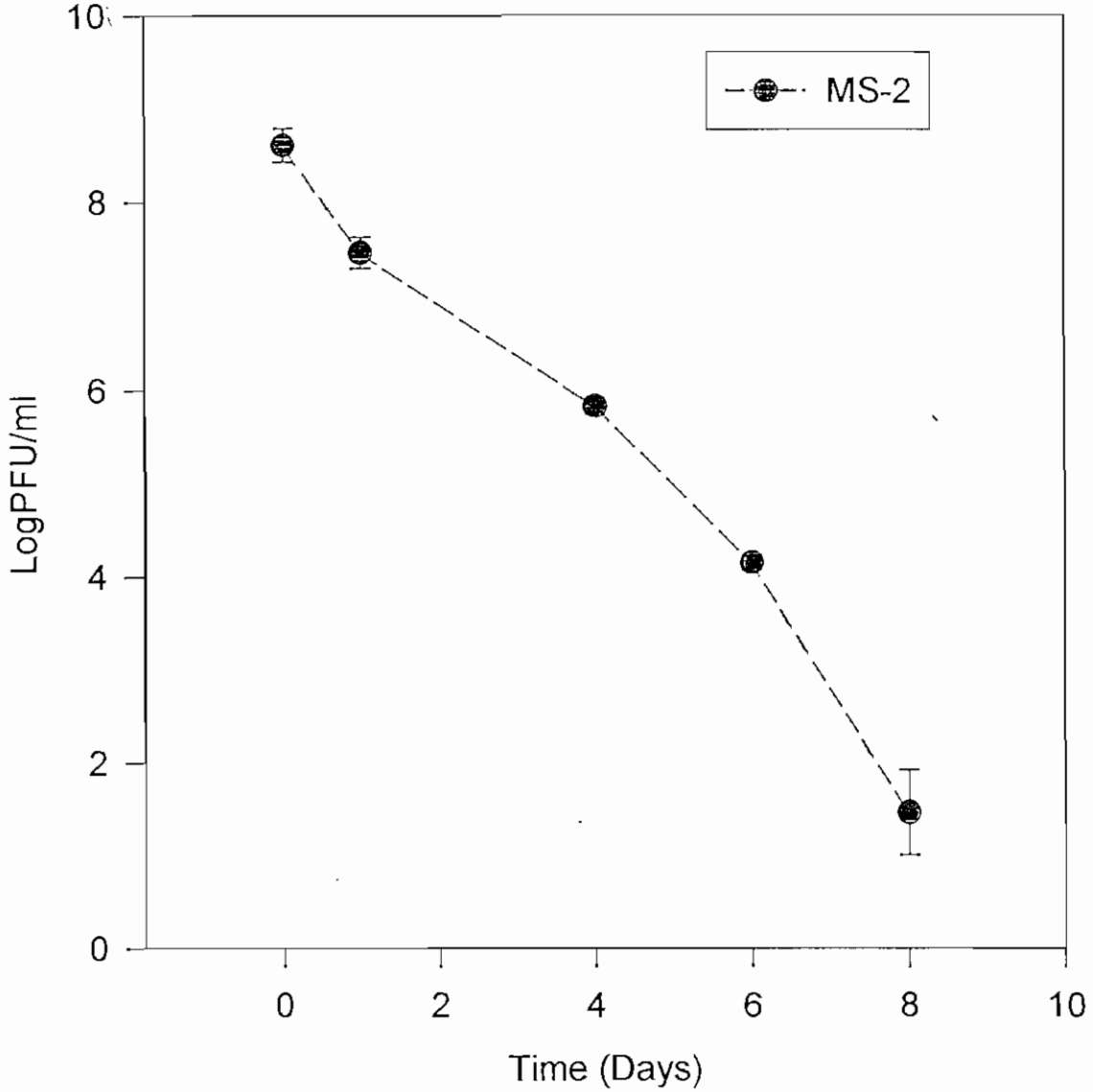

FIGURE 17. BATCH SURVIVAL OF MS2 IN THE RIO GRANDE ALLUVIUM

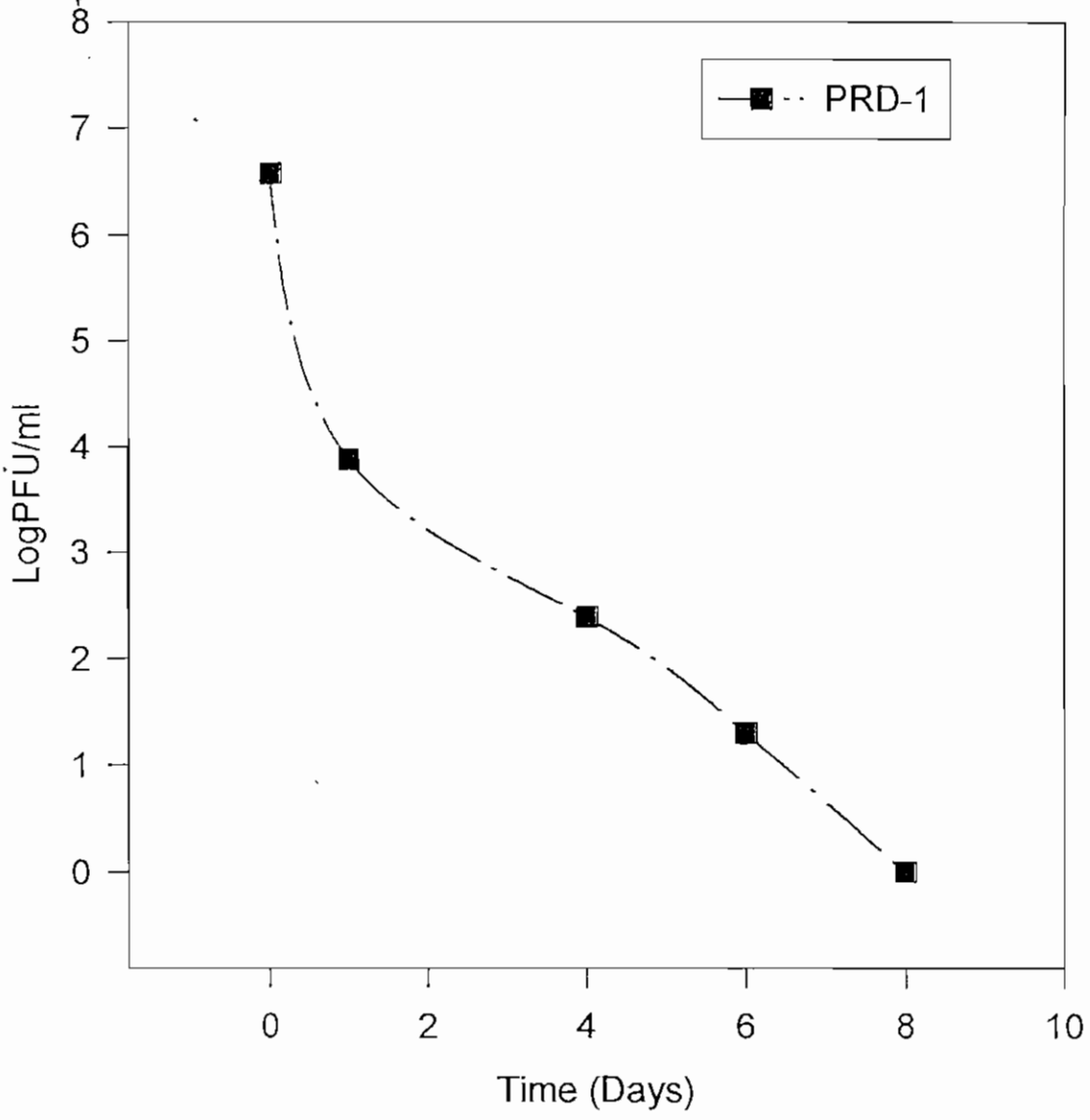

FIGURE 18. BATCH SURVIVAL OF PRD1 IN THE RIO GRANDE ALLUVIUM

points), which may potentially affect the adsorption and subsequent transport of virus in the subsurface. Adsorption plotted over time for the five different phages is presented in Figures 19-23. MS2 showed the greatest amount of adsorption with 99.96% of phage removed from the groundwater phase. PRD1 showed 99% adsorption, Qβ showed 98% adsorption , ϕX174 showed 92% adsorption, and PM2 showed only 88% adsorption. Since the only variable within these experiments are the virus types, the Spearman Rank Order Correlation analysis function of Sigmastat software (Jandel Scientific Software) was used to determine which viral characteristic correlated with the adsorption percentage. The viral characteristics used were isoelectric point, molecular weight, buoyant density, and diameter. The only relationship that was evident was between the adsorption and the isoelectric point of the phage. The correlation analysis showed that as the isoelectric point increased the adsorption decreased. The correlation coefficient was equal to - 0.9 with 92% confidence. This suggested that the interaction of the aquifer pH and the isoelectric point of the virus (phage) will create a wide variety of adsorptive characteristics.

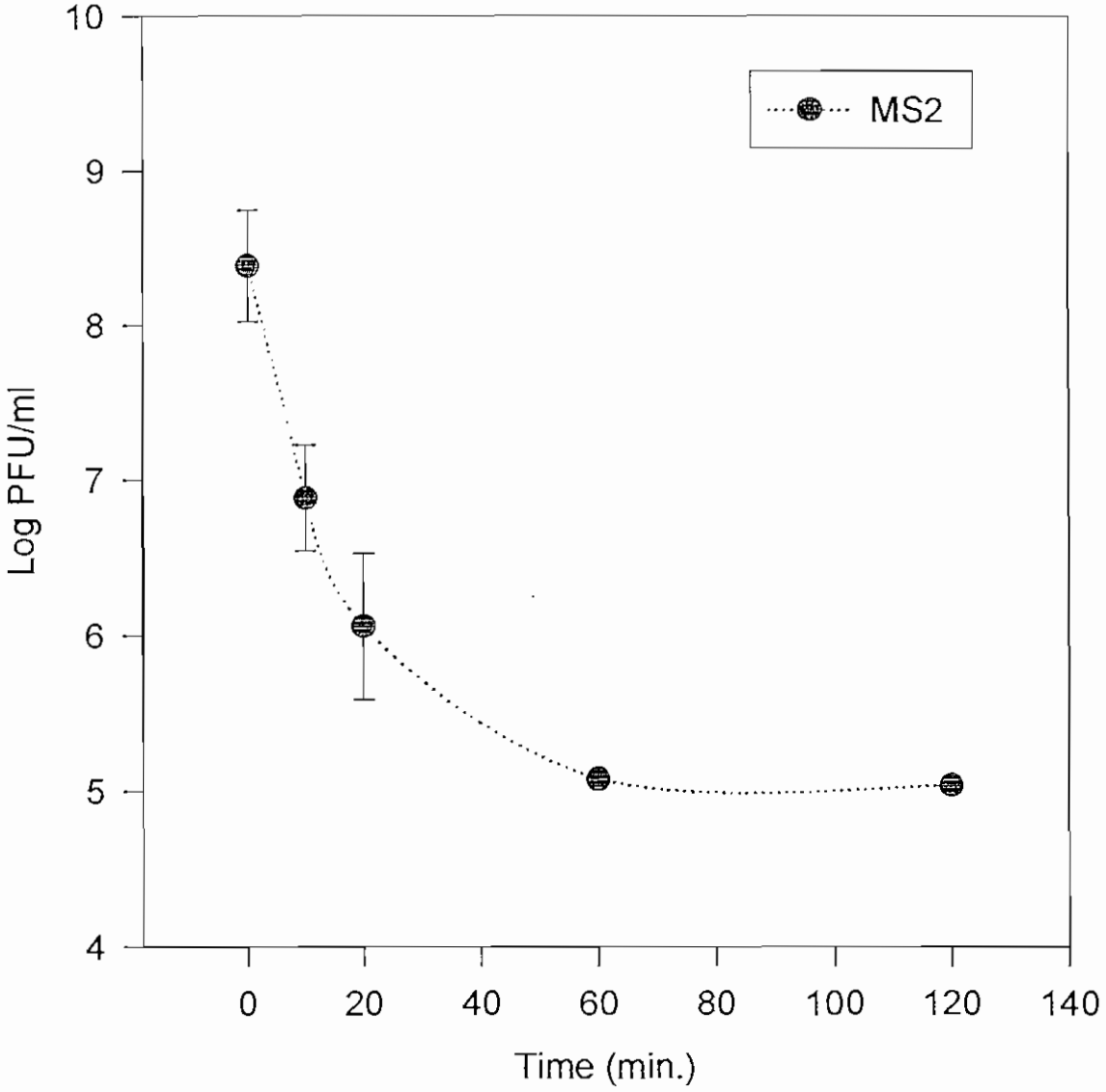

FIGURE 19. BATCH ADSORPTION OF MS2 IN THE BRAZOS ALLUVIUM

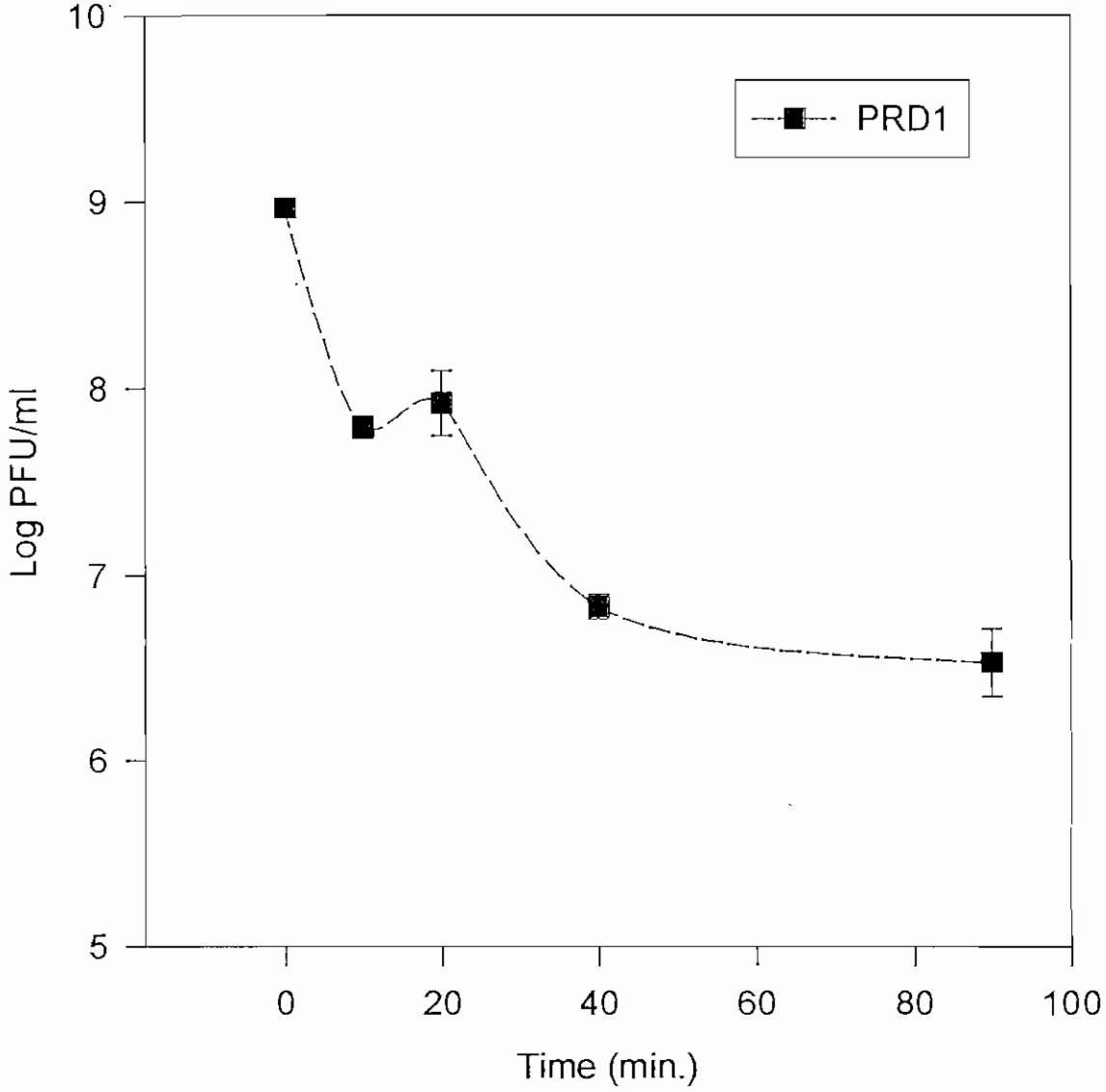

FIGURE 20. BATCH ADSORPTION OF PRD1 IN THE BRAZOS ALLUVIUM

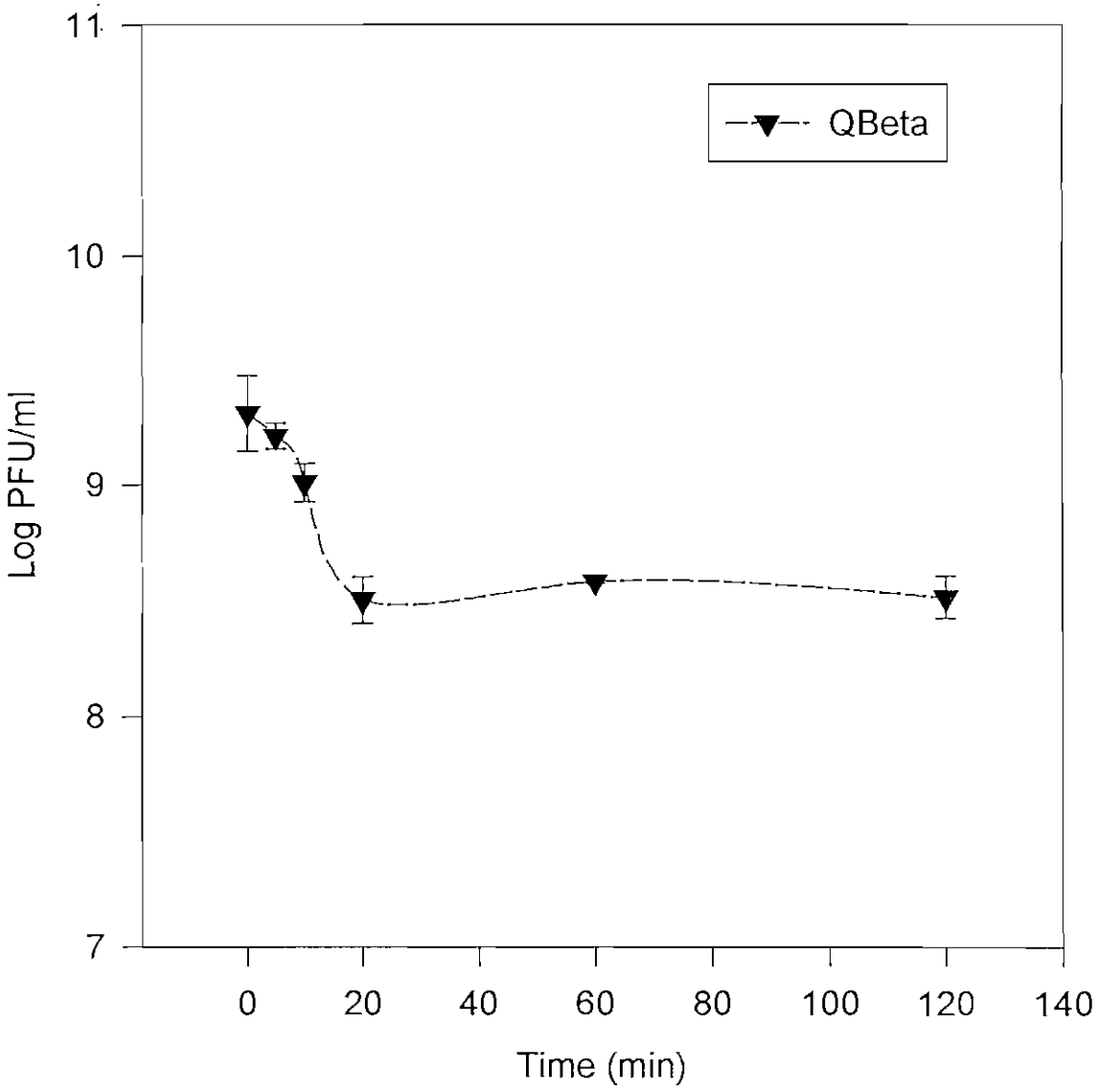

FIGURE 21. BATCH ADSORPTION OF Qbeta IN THE BRAZOS ALLUVIUM

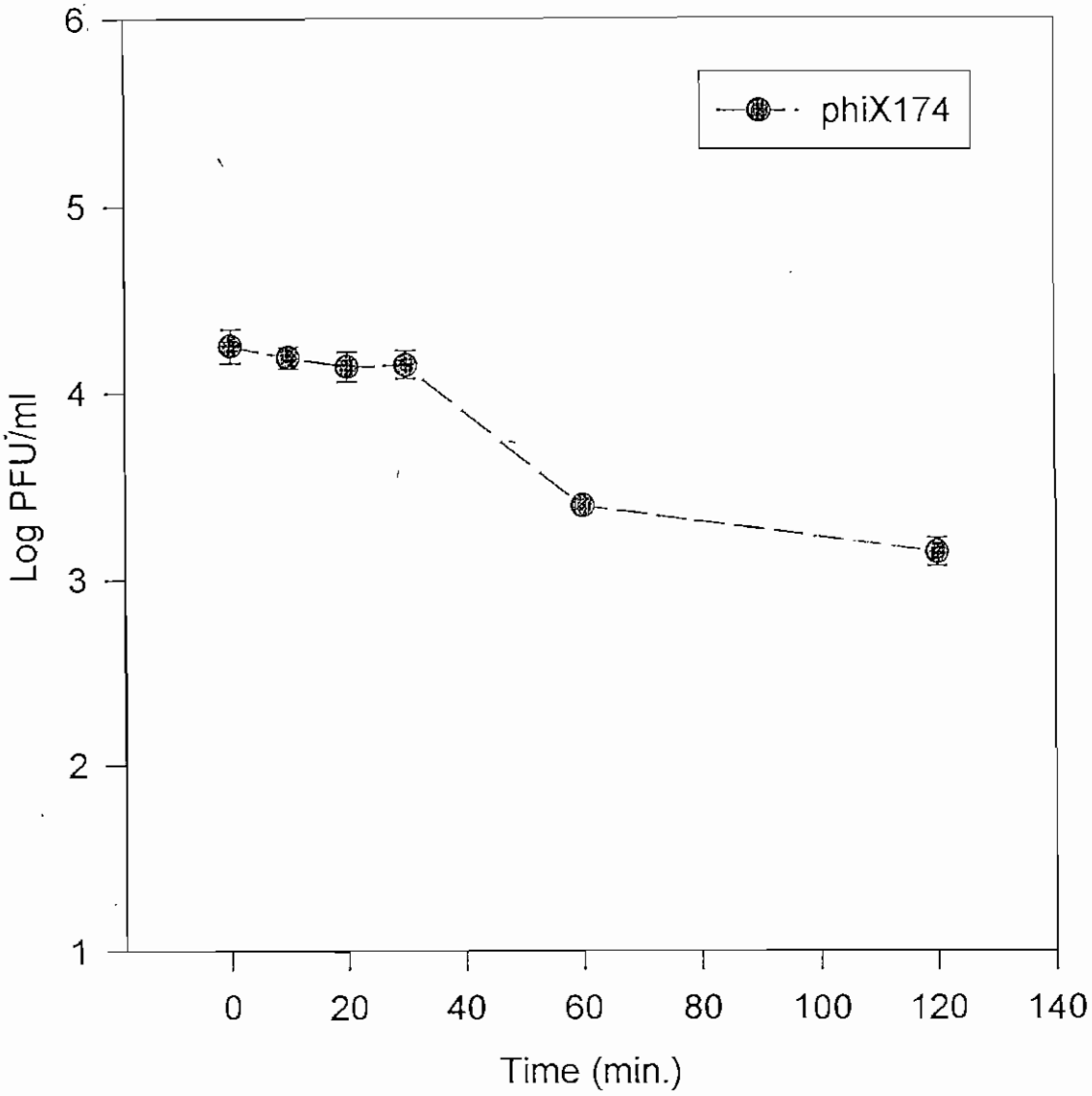

FIGURE 22. BATCH ADSORPTION OF phiX174 IN THE BRAZOS ALLUVIUM

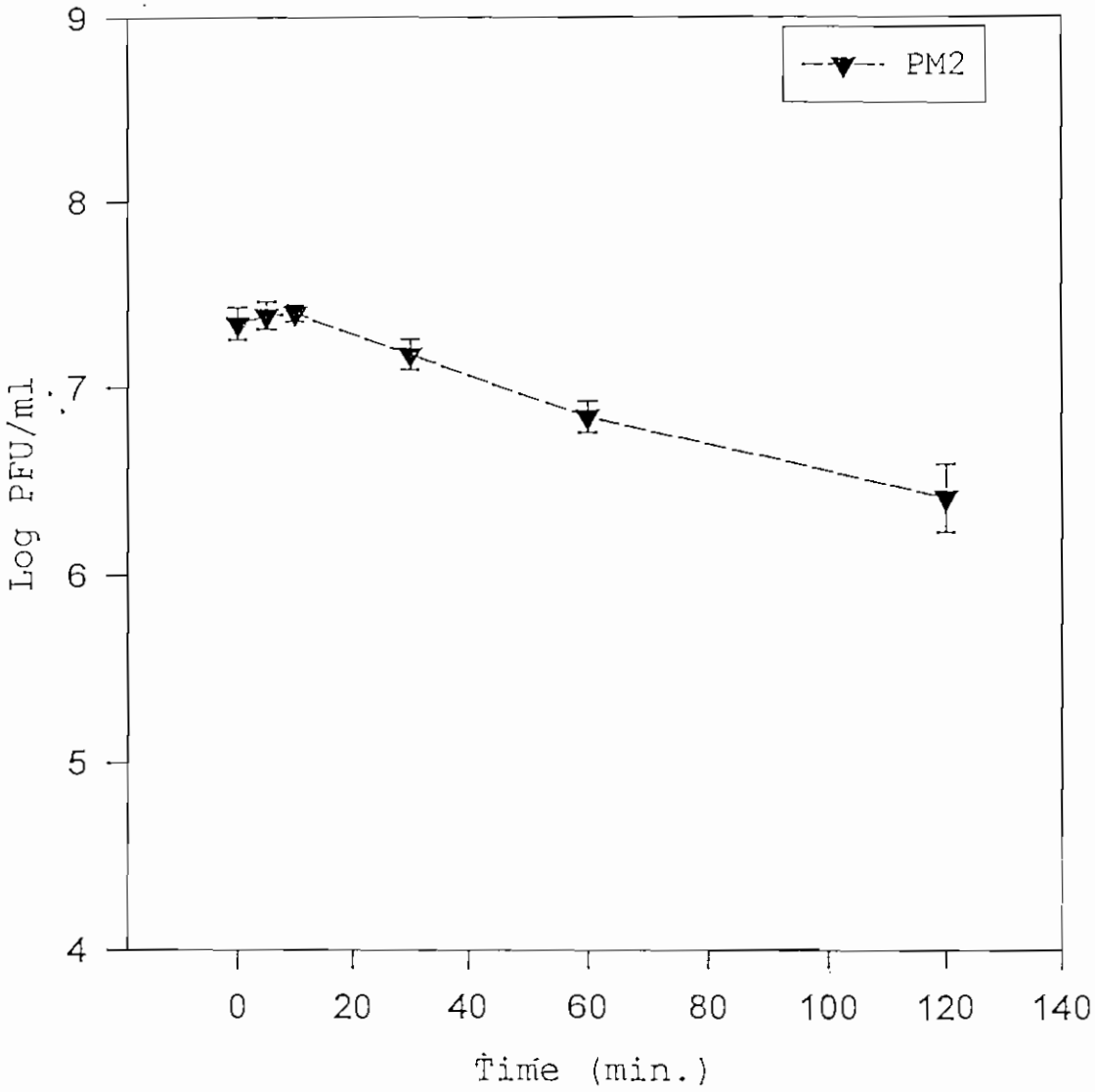

FIGURE 23. BATCH ADSORPTION OF PM2
IN THE BRAZOS ALLUVIUM

The adsorptive properties of MS2 were determined in a total of three soil types which were analyzed for their effects on adsorption, Rio Grande Alluvium sediment, Brazos Alluvium sediment, and Rio Grande Field soil (Field 13 soil). In the Field 13 soil, MS2 exhibited little or no adsorption (29%) after 90 minutes, while in the Rio Grande Alluvium sediment MS2 showed 95% adsorption over 90 minutes (Fig. 24). In the Brazos Alluvium, MS2 showed the same behavior as with the first study, exhibiting 99.9% adsorption over 90 minutes. This displays the variability of virus adsorptive behavior between different soil types.

3.2.2 Continuous Column Adsorption Studies

Adsorptive behavior was also studied with the use of a novel continuous column experiment, which served as a closed reactor simulating transport and adsorption of phage through a soil matrix over time. This seemed to be a more realistic condition than shaking flask studies. MS2 showed 99.4% phage removal after 4 hours, PRD1 showed 99% removal after 4 hours, Qβ showed 97% removal, ΦX174 showed 85% adsorption, and PM2 showed 80% adsorption. The plots for the continuous column are presented in Figures 25-29. These values coincided extremely well with the viral batch adsorption studies. This suggested that the continuous column provided

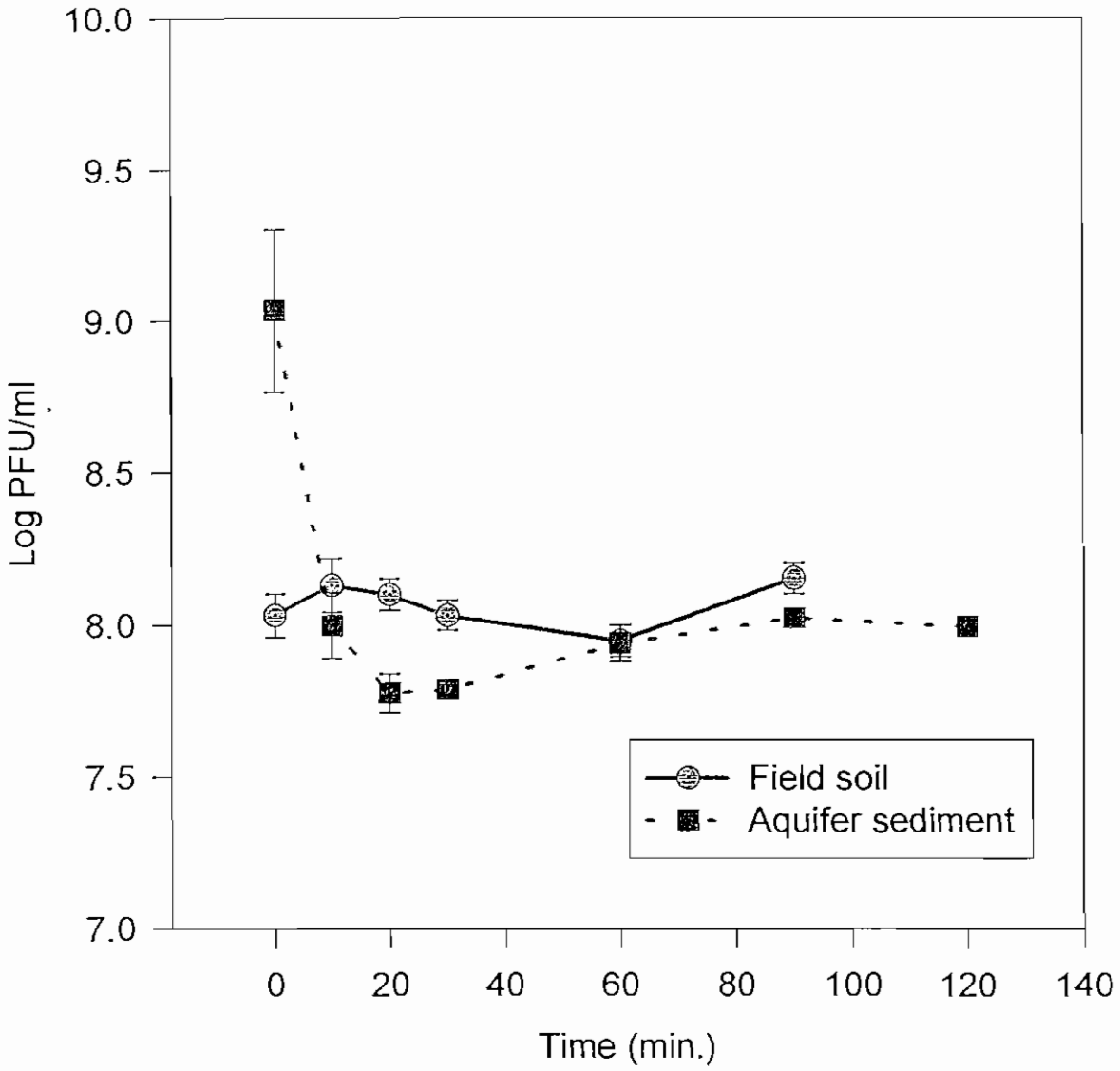

FIGURE 24. BATCH ADSORPTION OF MS2 IN THE RIO GRANDE ALLUVIUM

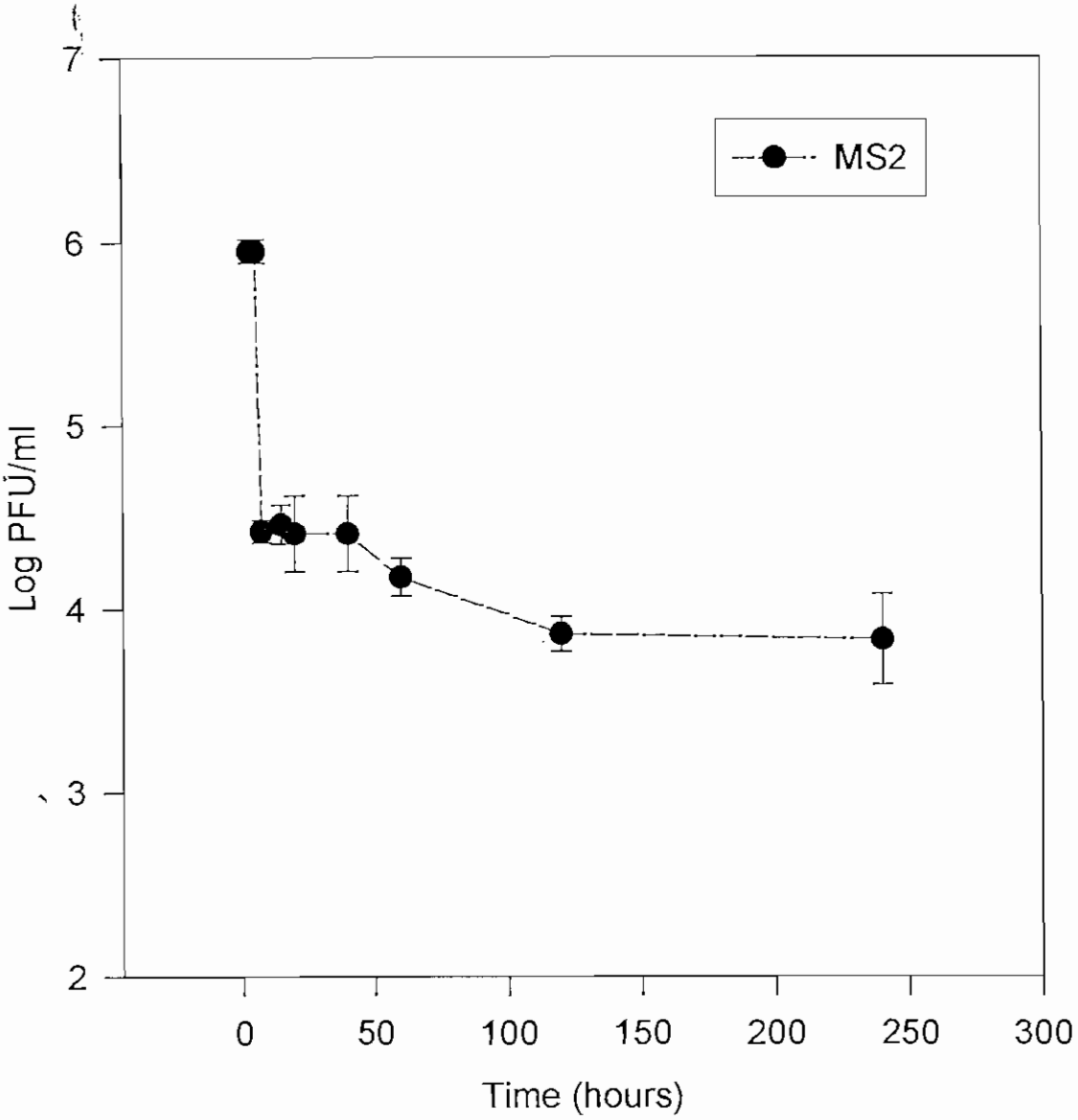

FIGURE 25. CONTINUOUS COLUMN ADSORPTION OF MS2 IN THE BRAZOS ALLUVIUM

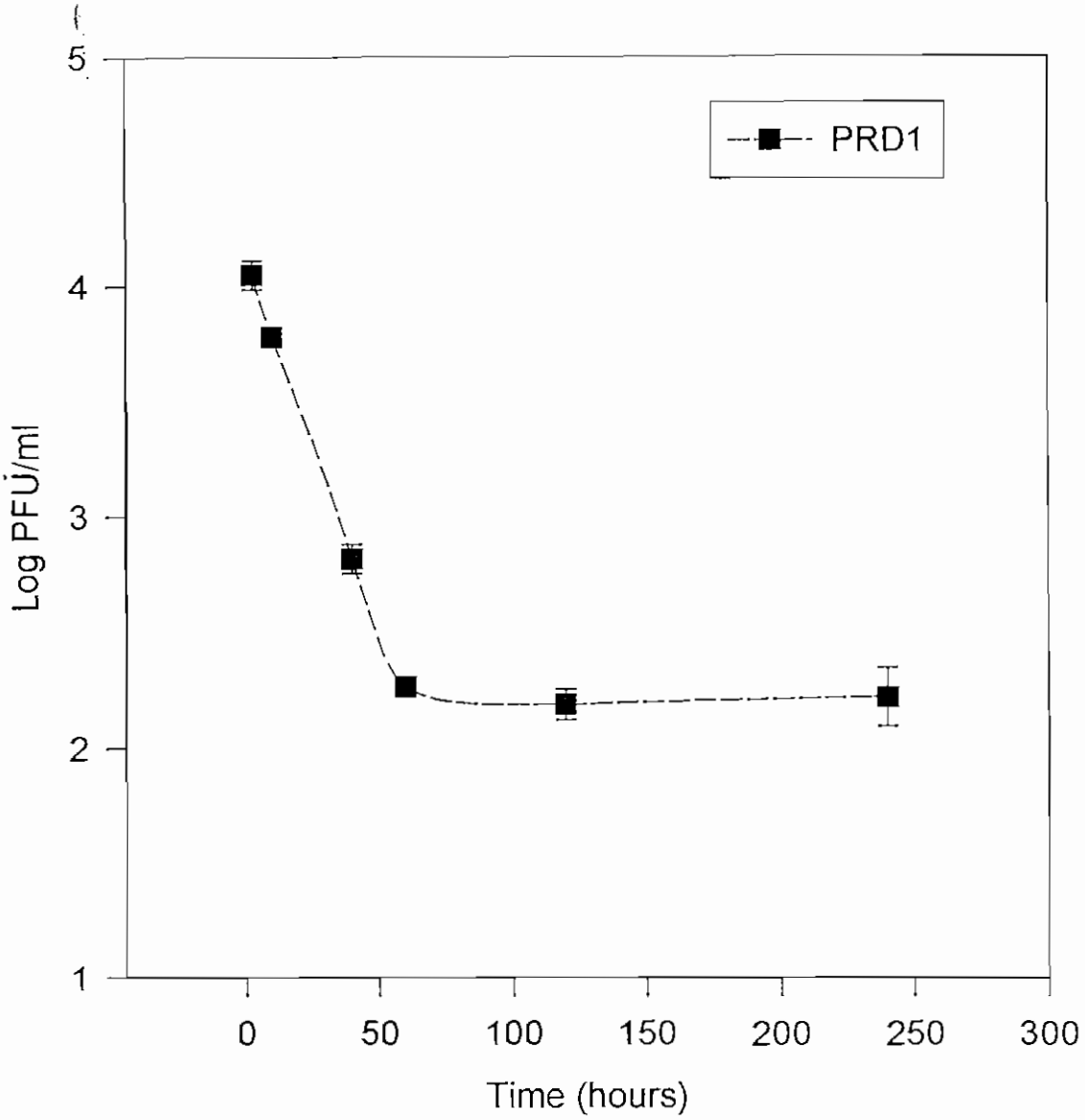

FIGURE 26. CONTINUOUS COLUMN ADSORPTION OF PRD1 IN THE BRAZOS ALLUVIUM

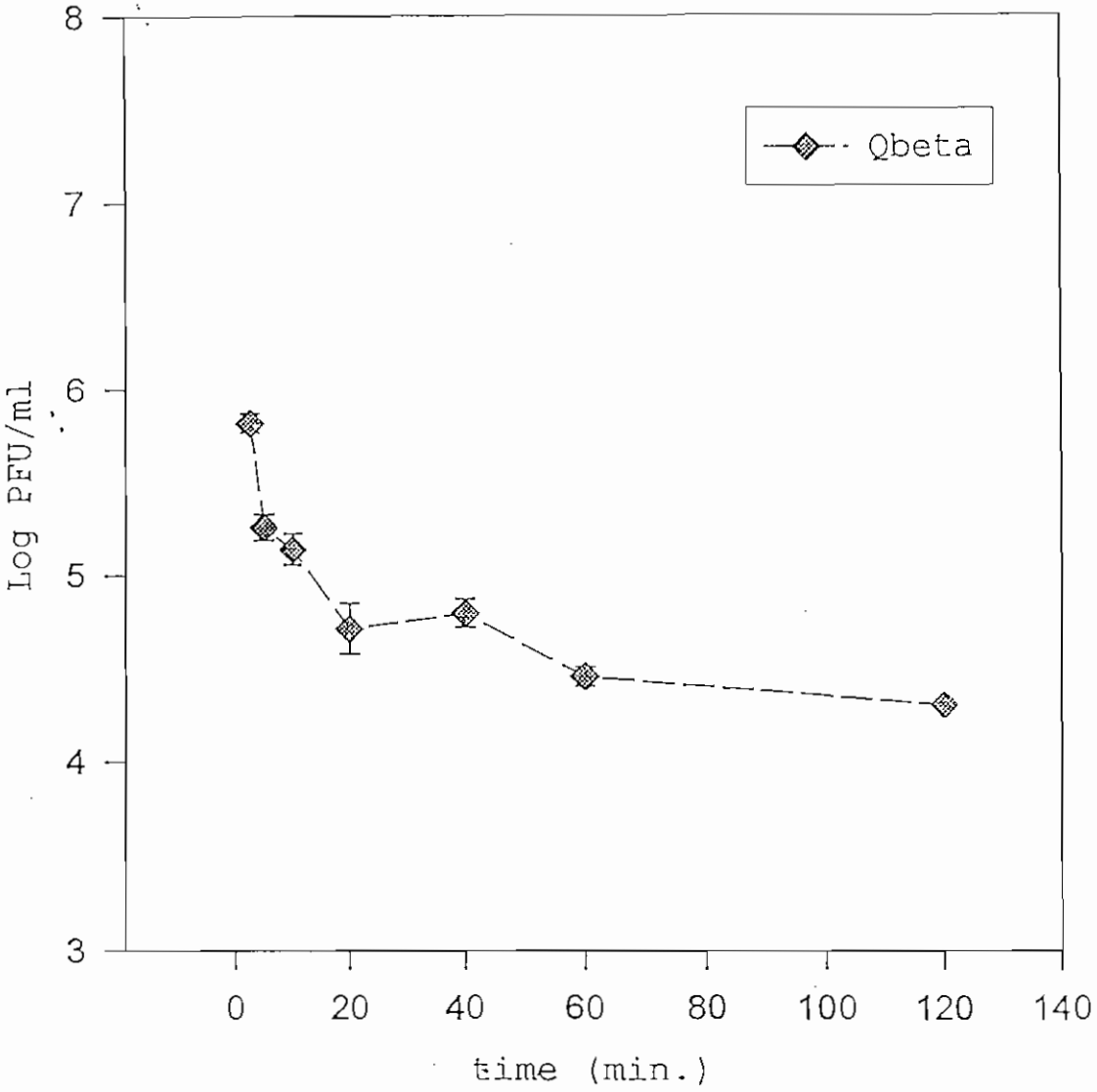

FIGURE 27. CONTINUOUS COLUMN ADSORPTION OF Qbeta IN THE BRAZOS ALLUVIUM

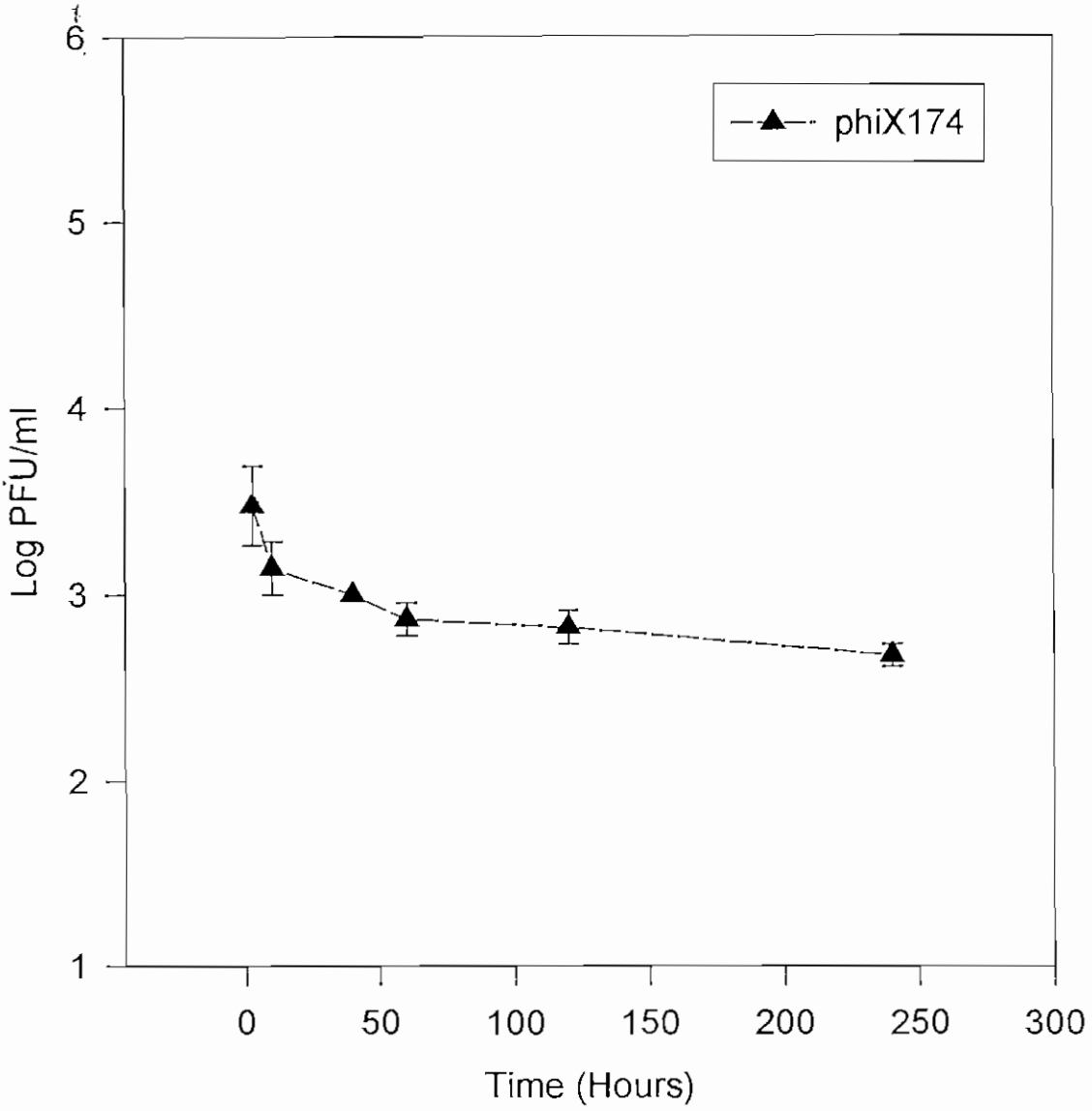

FIGURE 28. CONTINUOUS COLUMN ADSORPTION OF phiX174 IN THE BRAZOS ALLUVIUM

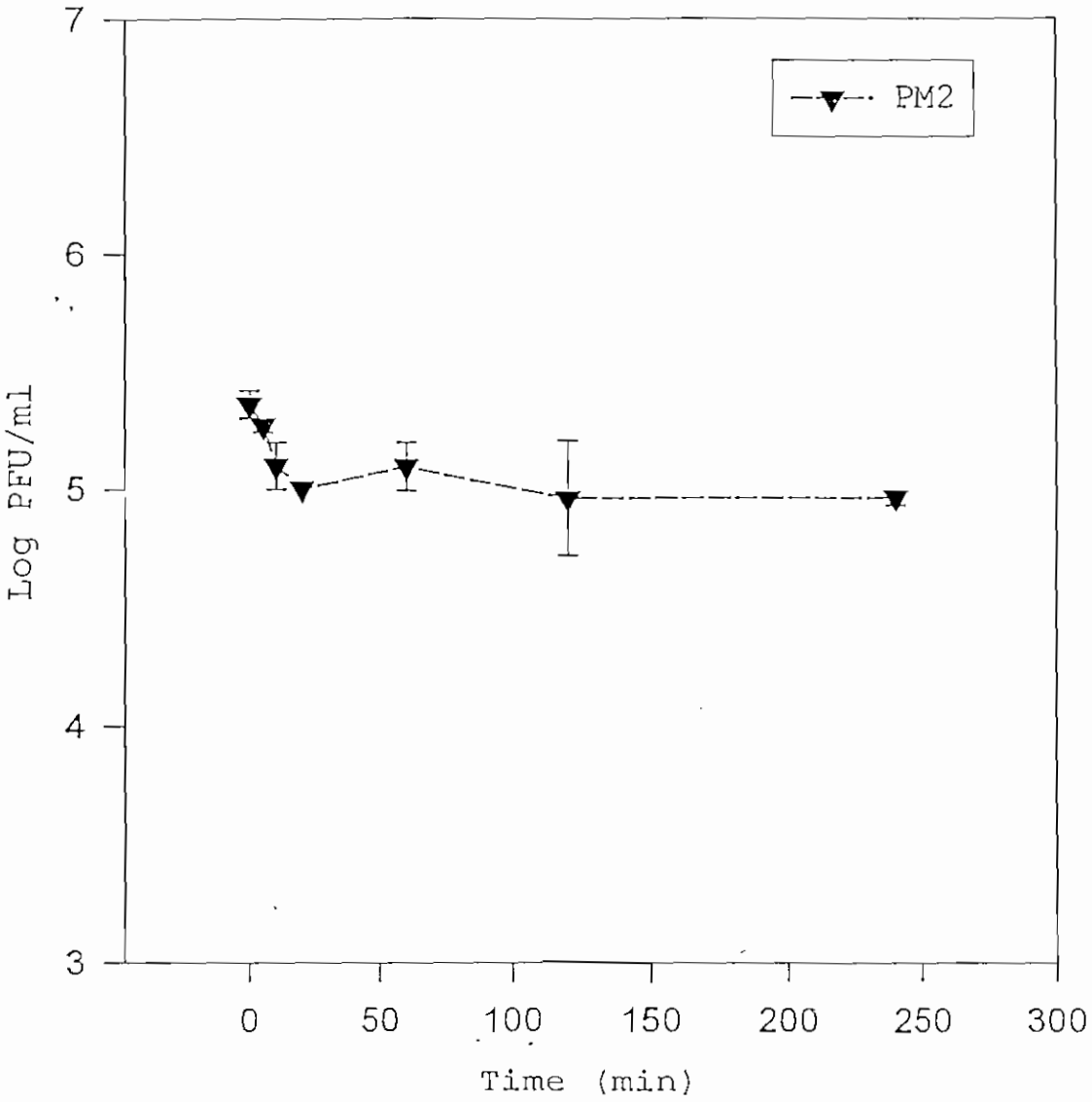

FIGURE 29. CONTINUOUS COLUMN ADSORPTION OF PM2 IN THE BRAZOS ALLUVIUM

accurate adsorption curves. Spearman Rank Order Correlation (SROC) analysis was also run on this adsorption data to determine the extent adsorption correlated with the isoelectric points of the virus. The resultant correlation coefficient was equal to -0.90 with a confidence of 83.3%. SROC analysis was also run to determine the exact correlation of the continuous column to the batch adsorption data. The correlation coefficient between the two types of adsorption studies was 0.7 with 80% confidence.

3.3 COLUMN TRANSPORT STUDIES

3.3.1 Pulse Injection Transport Studies

3.3.1.1 0.24 Meter Column

MS2 transport-Most of the MS2 passed through the 0.24 meter column after 12 pore volumes. Mass balance shows that a total of 1.56×10^{11} total plaque forming units or 12.6 % of the total virus injected remained in the 0.24 meter soil column even after 12 pore volumes of virus free feed solution had flushed through the column (Fig. 30). The flow rate of the system was 200 ml/min. (the slowest steady state obtainable with the peristaltic pump) at 0.24 meters. The volume of the column was equal to 400 ml, thus 1 pore volume equaled to 144 ml. The experiment lasted only 8.64 minutes;

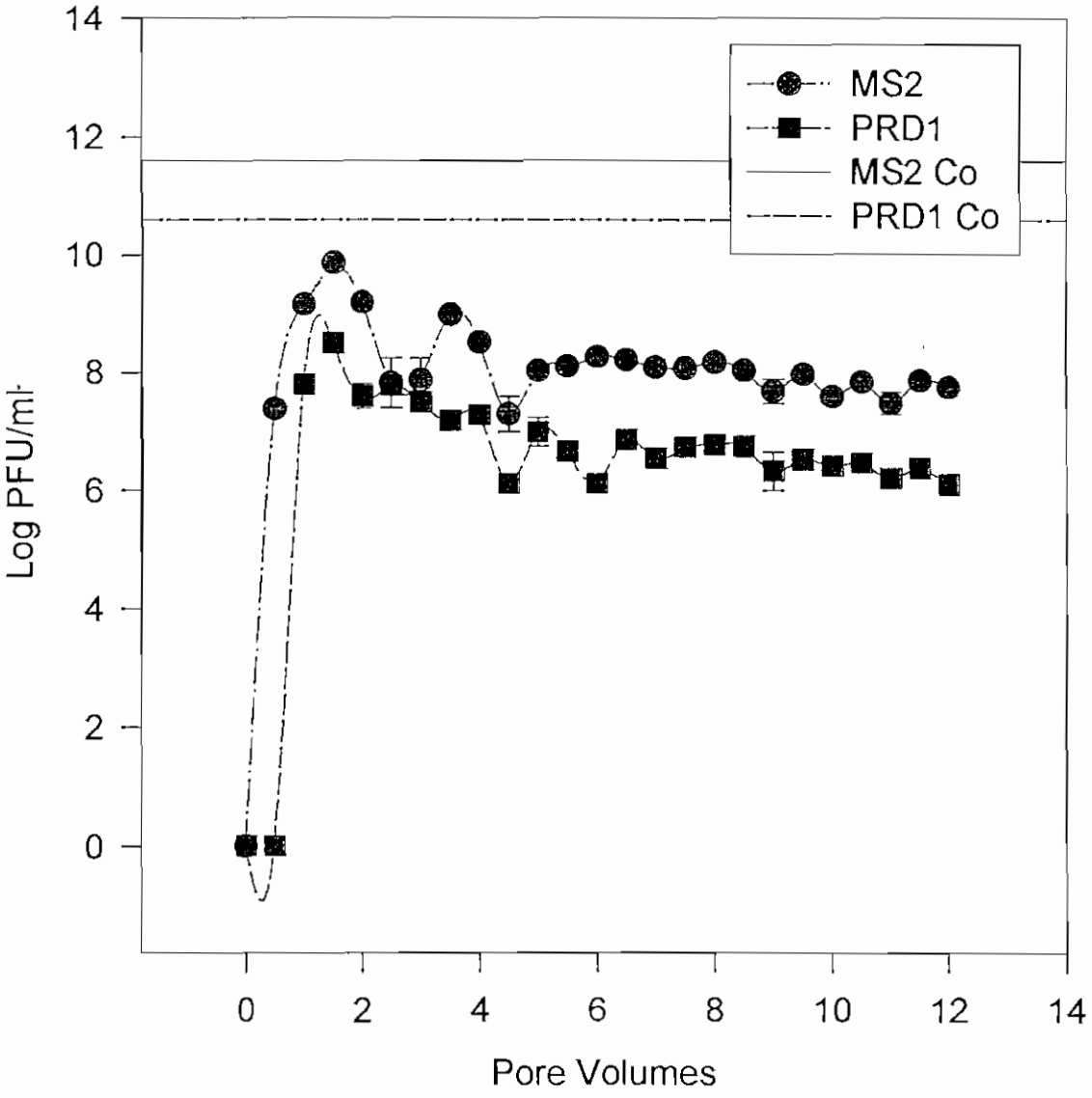

FIGURE 30. TRANSPORT OF MS2 AND PRD1 IN THE BRAZOS ALLUVIUM USING A 0.24 METER PULSE INJECTION COLUMN

therefore, based on the flask adsorption studies maximal adsorption probably had not occurred. The maximum C/C_o was 0.018 (1.8×10^{-2}) for MS2 at 1.5 pore volumes, thus it can be seen that there was a retardation effect occurring in the column. This effect could be seen as reversible adsorption, or, more probably, the effects of relatively high tortuosity within the soil matrix.

PRD1 transport-PRD1 was retained considerably within the soil column (Fig. 30) with only 4.47×10^{10} total virus particles passing through, leaving 7.83×10^{10} total PFUs within the system. A very high percentage (63%) of PRD1, when compared to MS2 remained in the column after 12 pore volumes of virus free groundwater had been flushed through the system. This seems to indicate that PRD1 was subject to considerably more retardation than MS2. This retardation effect is especially evident when you consider flask adsorption studies where PRD1 did not adsorb to soil as efficiently as MS2. PRD1 seems unable to navigate the tortuosity of the matrix as efficiently as MS2. MS2 was seen after 0.5 pore volumes had passed through the system, whereas PRD1 was not seen until after 1.0 pore volume had been flushed through the column. The maximum C/C_o was 0.008 (8.0×10^{-3}) for PRD1 at 1.5 pore volumes (lower than the MS2 C/C_o), which is further indication that there was

more retardation for PRD1, even though it exhibited less adsorption in batch and continuous column studies. This increased retardation of PRD1 is possibly due to its larger surface area (62 nm for PRD1 and 24 nm for MS2). Another possible explanation was that the larger PRD1 was more influenced by preferential flow patterns and must seek out larger pore spaces and channels when navigating the column's soil matrix, thus, at least in this column, making its path longer and subsequently slower. It can also be postulated that this retardation may be caused by increased London (van der Waals) force interaction of the larger surface area of the PRD1 virus.

3.3.1.2 0.36 Meter Column

MS2 transport-In the 0.36 meter column MS2 left only 32% (3.96×10^{11}) of total virus (1.23×10^{12}) within the column after 12 pore volumes (Fig. 31). There was a 153 % increase in virus removal when comparing it to the 0.24 meter column with only a 50% increase in column size. Thus, there seemed to have been adsorption occurring. The flow rate decreased 21.5%, maintaining a steady flow of 157 ml/min. throughout the experiment. The maximum C/C_o was 0.005 (5.0×10^{-3}) PFU/ml for MS2 at 2 pore volumes, which was a 72% decrease in C/C_o. The length of the experiment was 16.5 minutes, thus the mean residence time of the virus in the column was

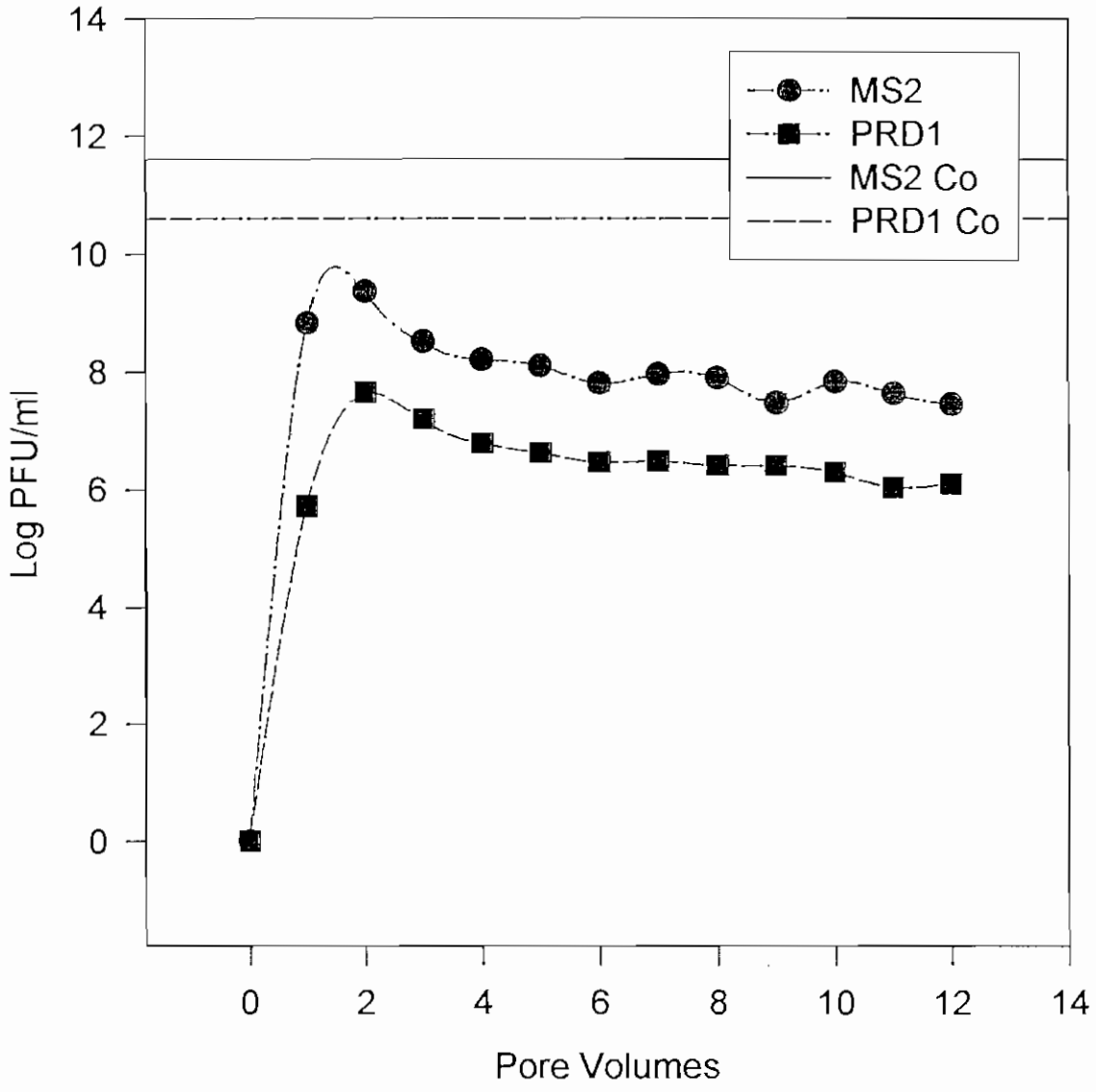

FIGURE 31. TRANSPORT OF MS2 AND PRD1 IN THE BRAZOS ALLUVIUM USING A 0.36 METER PULSE INJECTION COLUMN

probably enough in this experiment for adsorption to occur. It could also be seen from the high numbers of virus still being shed from the column even after 12 pore volumes that it was a reversible adsorption phenomena.

PRD1 transport-PRD1 had only 15 % (1.83 X 10^{10} PFUs) of the total virus (1.2 X 10^{11} PFUs) pass through the column after the 12 pore volumes of virus free groundwater was flushed through (Fig. 31). There was a total of 1.02 X 10^{11} PFUs (85%) remaining in the column after the 12 pore volumes which was a 32% increase in virus removal. This was not as significant as the 153% increase in removal seen with the MS2 phage. The maximum C/C_o was 0.001 (1.1 X 10^{-3}) for PRD1 at 2 pore volumes. This was also indicative of some type of particle size exclusion occurring within the column. PRD1 did not exhibit the large increase in adsorption percentage (when compared to the 0.24 meter column) like that exhibited by MS2. This corresponded to the adsorption data, suggesting that viral adsorption is not a linear phenomenon.

3.3.1.3 0.76 Meter Column

MS2 transport-The 0.76 meter column is a 111% increase in size over the 0.36 meter column and a 216% increase in size over the 0.24 meter column. A total of 1.16 X 10^{12} virus particles (94%) of the 1.23 X 10^{12} total virus particle

injected remained in the column after 12 pore volumes virus free effluent had passed through (Fig. 32). This was a 192% increase in virus removal over the 0.36 meter column and a 643% increased removal over the 0.24 meter column. The steady state flow rate decreased by 48% compared to the 0.36 meter column and 59% when compared to the 0.24 meter column. The length of the experiment was 68 minutes, which gives more than adequate time for most adsorption to occur when considering the mean residence time. The maximum C/C_o was 1.0×10^{-4} for MS2 at 2 pore volumes in the 0.76 meter column.

PRD1 transport-The total numbers of virus passing through the system after 12 pore volumes was 4.69×10^{9} PFUs (3.9%) of the total 1.2×10^{11} PFUs introduced into the system (Fig. 32). The virus fraction (97.1%) remaining in the column represented an increased particle removal of 10% and 46% over the 0.36 and 0.24 meter column, respectively. The maximum C/C_o was 7.0×10^{-5} for PRD1 at 1 pore volume. The C/C_o for PRD1 was again lower than MS2 within this column, lending further strength to the observation that PRD1 exhibits more retardation within the soil matrix. The MS2 exhibited a much larger percentage increase in removal compared to PRD1. This would tend to confirm the adsorption data, especially when considering the decrease in relative

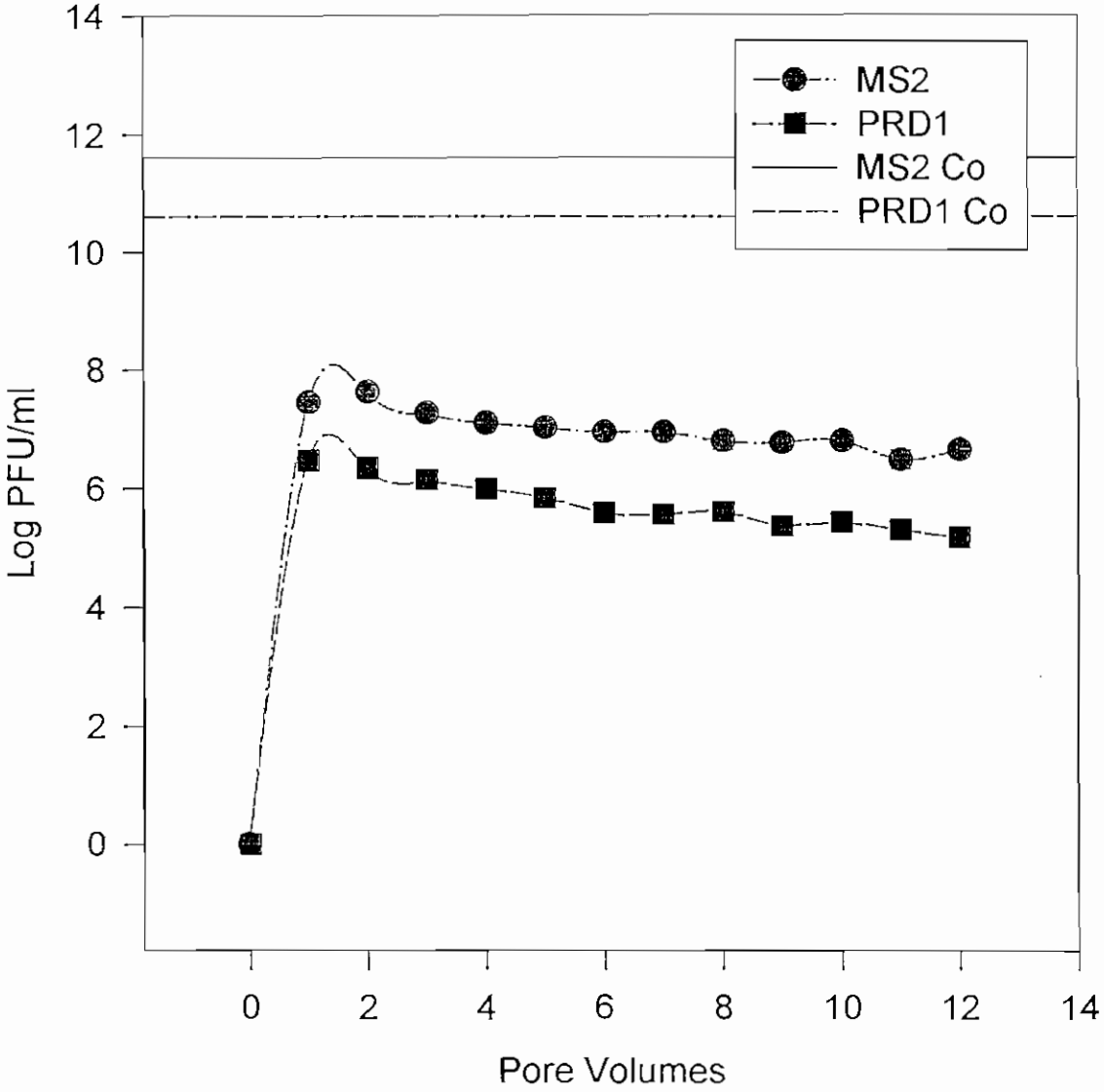

FIGURE 32. TRANSPORT OF MS2 AND PRD1 IN THE BRAZOS ALLUVIUM USING A 0.76 METER PULSE INJECTION COLUMN

flow rate and the increased residence time within the system.

3.3.1.4 0.99 Meter Column

MS2 transport-The 0.99 meter column was a 30% increase in size over the 0.76 meter column, a 175% increase in size over the 0.36 meter column, and a 312% increase in size over the 0.24 meter column. A total of 1.16×10^{12} virus particles (96%) of the 1.23×10^{12} total virus particle injected remained in the column after 12 pore volumes (Fig. 33). This was only a 1.7% increased removal compared to the 0.76 meter column, a 197% increase in virus removal over the 0.36 meter column, and a 656% increased virus particle removal over the 0.24 meter column. The steady state flow rate decreased by 56% compared to the 0.36 meter column and 65% when compared to the 0.24 meter column. The length of the experiment was 110 minutes, which gives more than adequate time for complete adsorption to occur. The maximum C/C_o was 3.4×10^{-5} for MS2 at 2 pore volumes.

PRD1 transport-The total numbers of PRD1 virus passing through the system after 12 pore volumes was 1.34×10^{9} PFUs (2%) of the total 1.2×10^{11} PFUs introduced into the system (Fig. 33). The 98% of the virus retained in the column represent an increased particle removal of 3.5%, 16%, and

retained within the column. A total of 1.77 X 10^{11} PFUs of PRD1 were introduced into the column in 30 mls. Only 2.1 X 10^{9} PFUs of PRD1 were recovered from the column leaving 99.9% within the column. These numbers represent totals throughout the whole experiment including when the flow rate was increased and the deionized water was injected.

3.3.1.5.1 1.77 Meter Sampling Port

MS2 transport-The 1.77 meter sampling port exhibited some signs of preferential flow as can be seen by the relatively high C/C_o of 2.0 X 10^{-5}. When compared to the C/C_o of the 0.99 meter column, the 1.77 meter column shows only a slight decrease (Fig. 34). After the peak C/Co, which reached the sampling port within four hours the concentrations declined steadily over 60 hours. At 68 hours the flow rate was increased, which essentially doubled the flow rate out of the 1.77 meter port. There was a brief increase in the C/C_o, which reached 2.5 X 10-7 and declined. At 80 hours the deionized water was added and within 2 hours the C/C_o again increased to 1.6 X 10^{-5}, almost reaching the original C/C_o. Thus, it can be seen that two major factors influencing the transport of MS2 virus within this aquifer column are the flow rate and the ionic content.

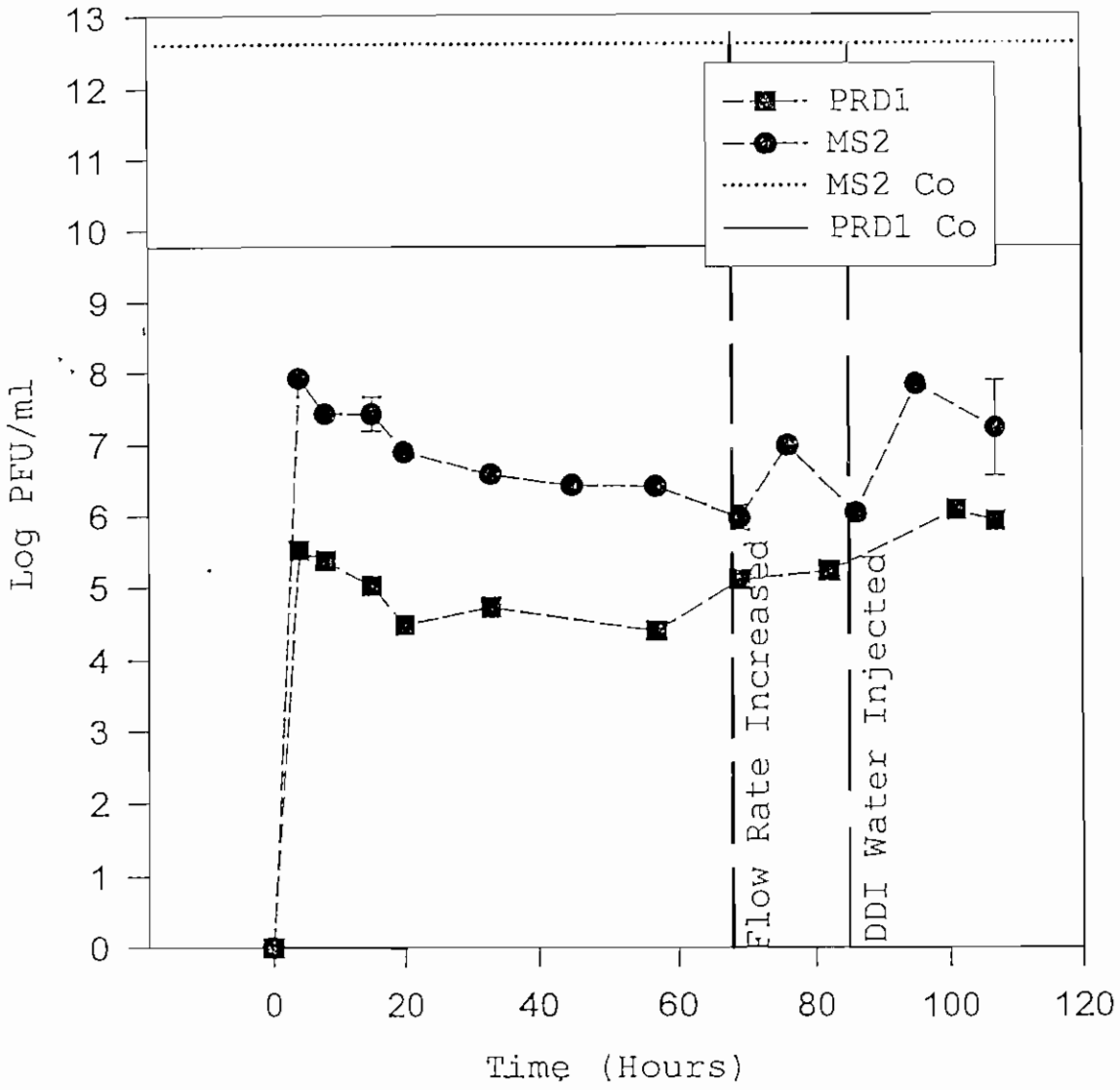

FIGURE 34. TRANSPORT OF MS2 AND PRD1 IN THE BRAZOS ALLUVIUM USING A 9.3 METER PULSE INJECTION COLUMN AT THE 1.77 METER SAMPLING PORT

PRD1 transport-PRD1 reached a maximum C/C_o of 0.00056 (5.6×10^{-5}) at the 1.77 meter sampling port; thus, it exhibited considerable signs of preferential flow compared to the 0.76 meter column (Fig. 34). The flow rate increase did not have a large effect on the recovery of PRD1 at the 1.77 meter point, though the deionized water did seem to mobilize adsorbed virus at this sampling port.

3.3.1.5.2 4.5 Meter Sampling Port

MS2 transport-MS2 reached the 4.5 meter point within 4 hours. MS2 reached a maximum C/C_o of 6.19×10^{-11} after 20 hours, with an average of 6 PFU/ml, and then declined over the next 40 hours. This C/C_o is probably accurate, considering the preferential flow up to 1.77 meters (Fig. 35). After the flow rate was increased, there was a desorbing effect exhibited by MS2 which quickly increased the C/C_o to 1.15×10^{-10} at 82 hours. After flow of deionized water, there was an even larger desorbing effect where the C/C_o increased to 1.7 X 10-6, which was close to the C/C_o at the 1.77 meter sampling port. This indicates that there was a large amount of MS2 particles adsorbed between the 1.77 and the 4.5 meter sampling ports, which were desorbed both by an increased flow rate and the addition of a low ionic solution.

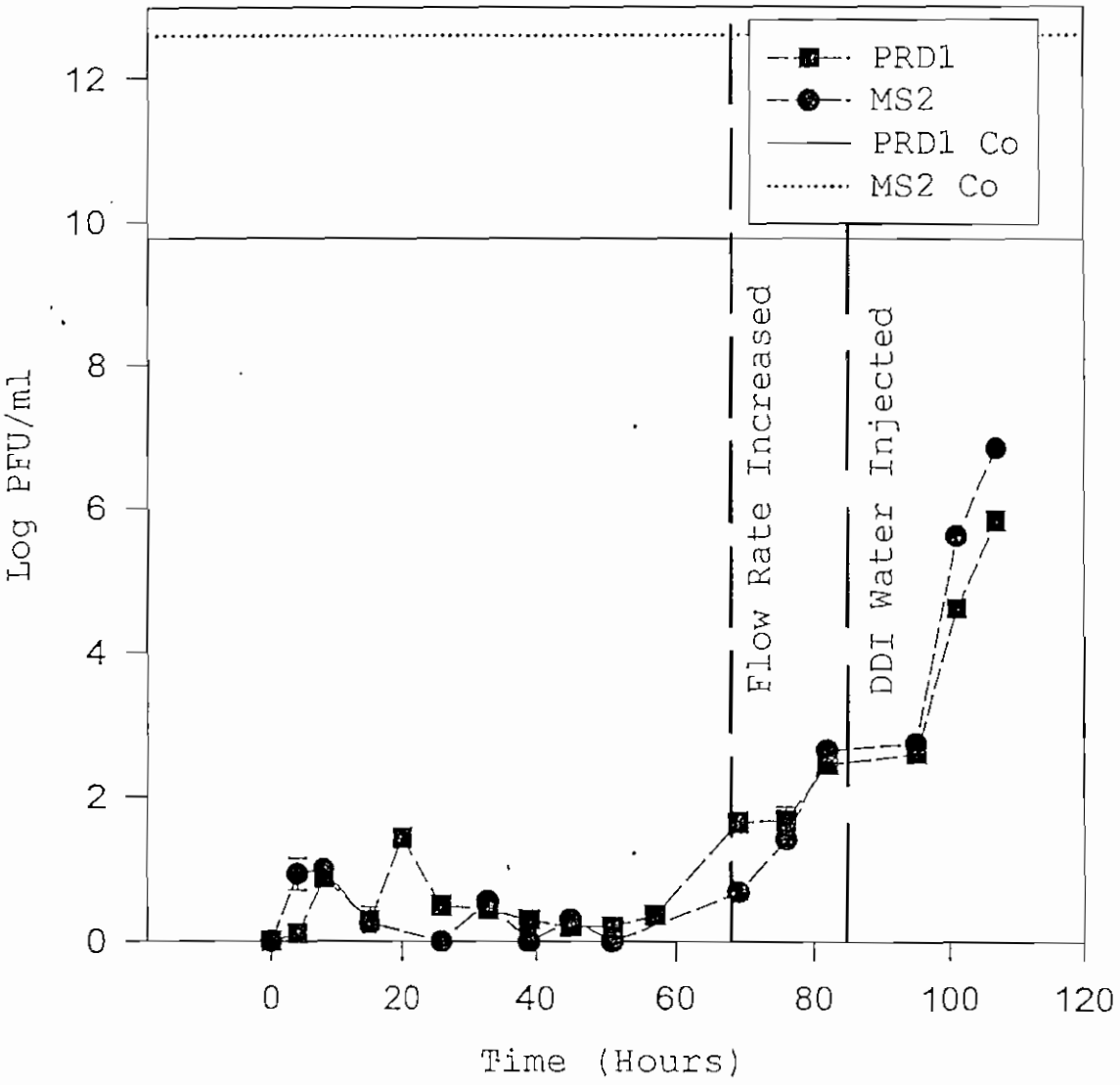

FIGURE 35. TRANSPORT OF MS2 AND PRD1 IN THE BRAZOS ALLUVIUM USING A 9.3 METER PULSE INJECTION COLUMN AT THE 4.5 METER SAMPLING PORT

PRD1 transport-The maximum C/C_o was 4.5×10^{-9} for PRD1 at the 4.5 meter port with numbers reaching 34 PFUs/ml within 20 hours. Thus the overall numbers were higher than those for MS2, as was the C/C_o (Fig. 35). These results tend to confirm the adsorption data. As with MS2, the increase in flow rate increased the C/C_o, as did the flushing of the system with deionized water, which increased the C/C_o to 1.7×10^{-4} at 107 hours (12 hours after the deionized water was started). This was considerably higher than the PRD1 C/C_o at the 1.77 meter sampling port. This tremendous desorption tends to indicate that the major factor controlling PRD1 transport is the ionic content of the groundwater system. It also indicates that the PRD1 was able to transport well over 1.77 meters, before most of the adsorption took place.

3.3.1.5.3 9.3 Meter Sampling Port

MS2 transport-A maximum C/C_o of 1.48×10^{-12} for MS2 indicates that within 9.3 meters all of the virus were removed from the groundwater flow. There was no detection of MS2 until after 15 hours, after which it dropped below detection again until 51 hours (Fig. 36). The numbers dropped below detection again until the flow rate was increased, and after the deionized water was added there were detects again. There were no numbers above 1 PFU/ml

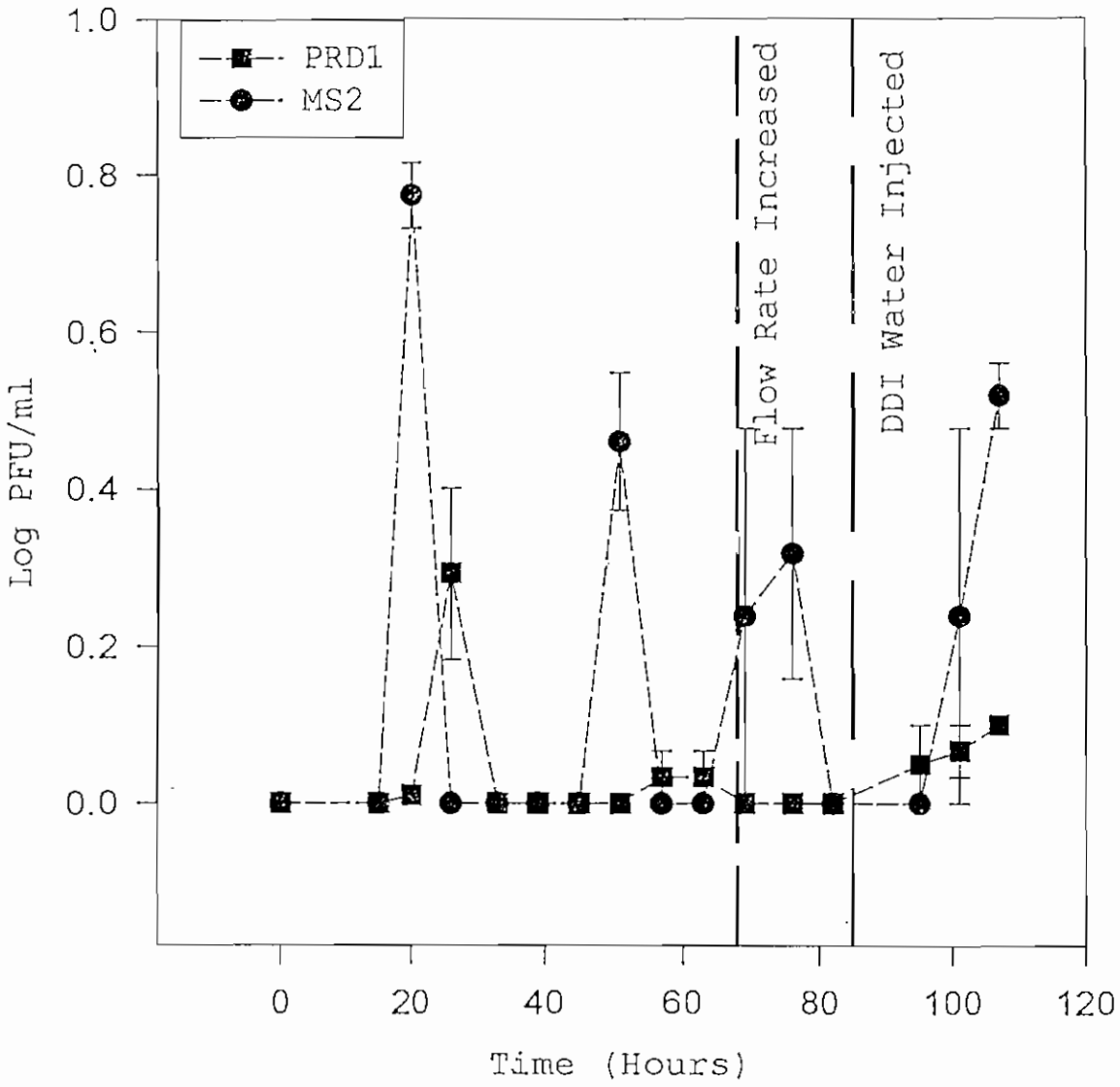

FIGURE 36. TRANSPORT OF MS2 AND PRD1 IN THE BRAZOS ALLUVIUM USING A 9.3 METER PULSE INJECTION COLUMN AT THE 9.3 METER SAMPLING PORT

after the initial peak of 6 PFU/ml. It was determined that there was no chance for the effects of the deionized water to reach the 9.3 meter point before the termination of the experiment.

PRD1 transport-PRD1 reached a maximum C/C_o of 3.3×10^{-10}, exhibiting the same sporadic pattern of detection as MS2 (Fig. 36). These results seem to indicate that there is a reversible adsorption that is continually occurring, which could have great public health significance if the same pattern of desorption were to occur over long distances.

3.3.1.6 Rio Grande Alluvium Pulse Injection Column Studies

The Rio Grande column studies were done using 0.24 meter columns using the same protocol as for the Brazos Alluvium pulse injection column studies. The virus was introduced as a 3ml pulse. MS2 was first detected after the first half pore volume. It reached a maximum C/C_o of 9.97×10^{-3} during pore volume 2.5 and slowly declined through the sixth pore volume (Fig. 37). A total of 6.84×10^{9} PFUs of MS2 were introduced in a two ml pulse. The total number of virus particles recovered over 6 pore volumes is equal to 5.41×10^{9}, which is 79% of the total virus particles introduced. The 21% of the virus particles left in the 0.24 meter soil matrix after being flushed with 6 pore volumes of virus free groundwater represents over 1.4×10^{9} MS2 virus

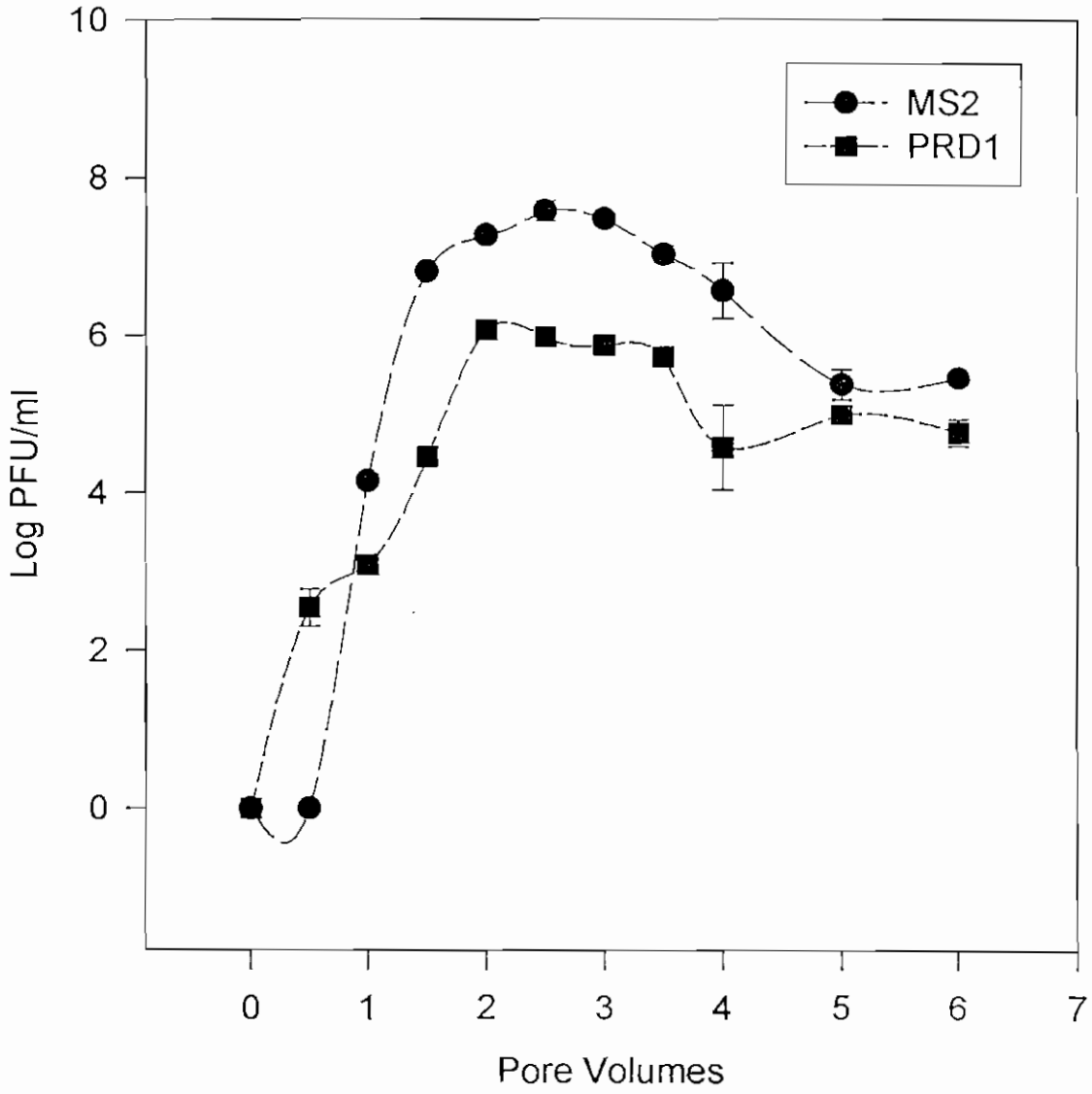

FIGURE 37. TRANSPORT OF MS2 AND PRD1 IN THE RIO GRANDE ALLUVIUM USINGA 0.24 METER PULSE INJECTION COLUMN

particles. PRD1 was first detected half a pore volume sooner than MS2. It reached a peak C/C_o of 0.014 (1.4×10^{-2}) during pore volume 2.5, along with MS2, and slowly declined through the sixth pore volume. A total of 1.51×10^8 PFUs of PRD1 were introduced in a two ml pulse. The total number of PRD1 particles recovered after 6 pore volumes equals 1.7×10^8 PFUs. This means that virtually none of the PRD1 virus particles introduced remained within the soil matrix after being flushed with 6 pore volumes of virus free groundwater. This is interesting in that the first PRD1 were detected in the effluent during the first half pore volume and at the sixth pore volume 6.3×10^4 PFUs were still detected. The mass balance suggests that 112% of the virus introduced into the system were recovered. Thus there was obviously some problem with the initial enumeration of the C_o value. This can be explained when considering the 10% experimental error, inherent in any dilution based enumeration. This also suggests that PRD1 was able to move easily through the Rio Grande Alluvium sediment as compared to MS2. It also seems that it had less retention in the Rio Grande Alluvium than in the Brazos Alluvium. MS2, on the other hand, had more retention in the Rio Grande Alluvium than in the Brazos Alluvium. This was

suggestive of how variable and site specific virus transport can be.

3.3.2 Two Pore Volume Injection Column Studies

3.3.2.1 0.78 Meter Two Pore Volume Injection Columns

MS2 transport-Two pore volumes (1032 ml) of groundwater containing 2.135 X 10^9 PFU/ml was introduced into the column after the initial saturation of the column. The MS2 broke through after 0.5 pore volumes had been introduced. A total of 1.23 X 10^{12} PFUs were recovered over 12 pore volumes (Fig. 38), which is 56% of the total 2.2 X 10^{12} MS2 virus introduced. After the initial feed solution was switched to virus free groundwater, there was a significant increase in virus release for one pore volume, after which the concentration declined. The maximum C/C_o was 0.50 at 3 pore volumes, after which the virus concentration slowly decreased through the 12th pore volume.

PRD1 transport-A total of 3.09 X 10^{10} PFUs 2.99 X 10^7 of PRD1 were introduced into the column in 2 pore volumes. Over 69% of the virus remained in the column after 12 pore volumes (Fig. 39). The first breakthrough of PRD1 occurred after 0.5 and steadily climbed until after virus free groundwater flow was begun. As with MS2, the PRD1 reached a peak after the beginning of virus free flow and then steadily declined

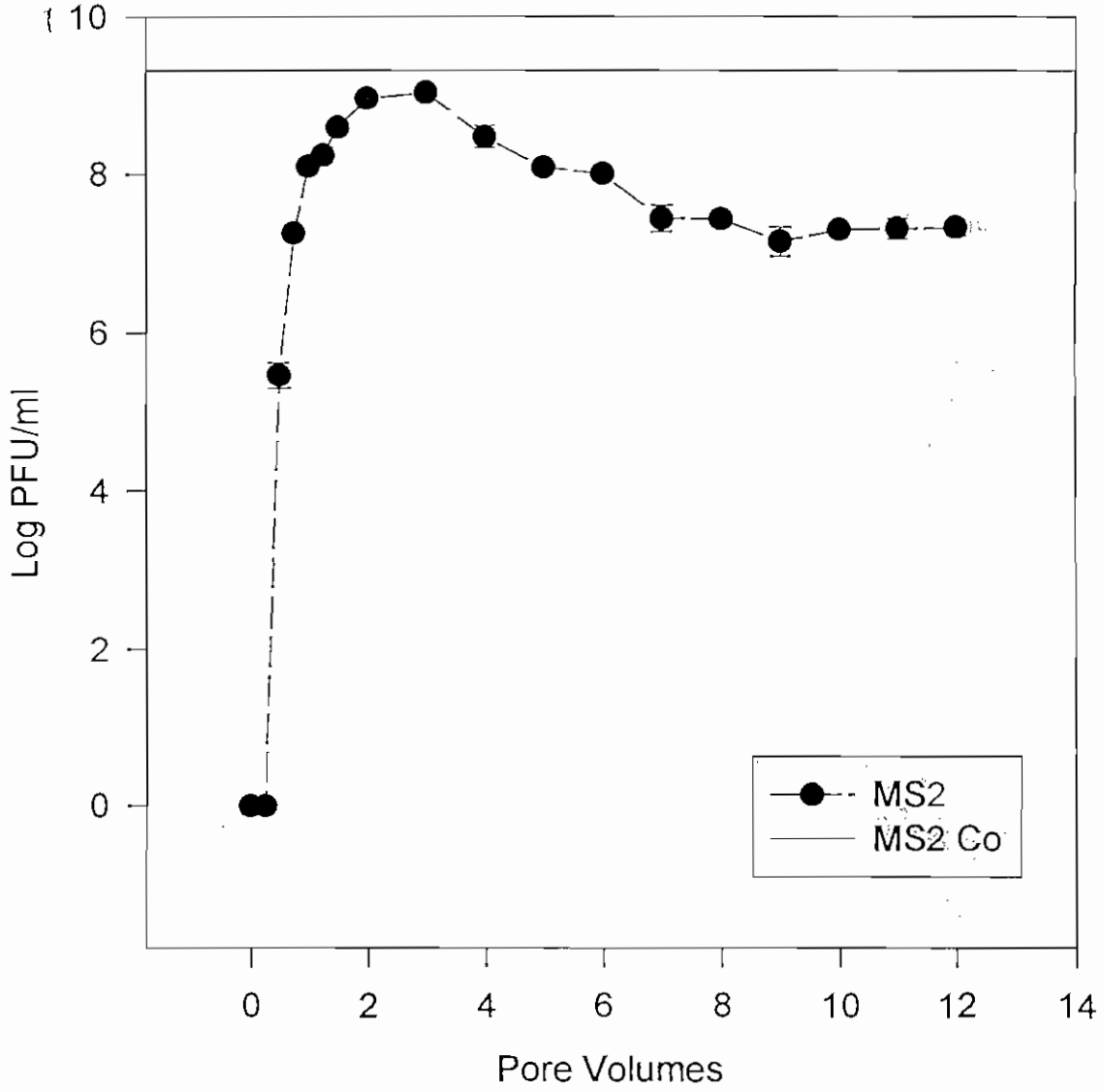

FIGURE 38. TRANSPORT OF MS2 IN THE BRAZOS ALLUVIUM USING A 0.76 METER TWO PORE VOLUME INJECTION COLUMN

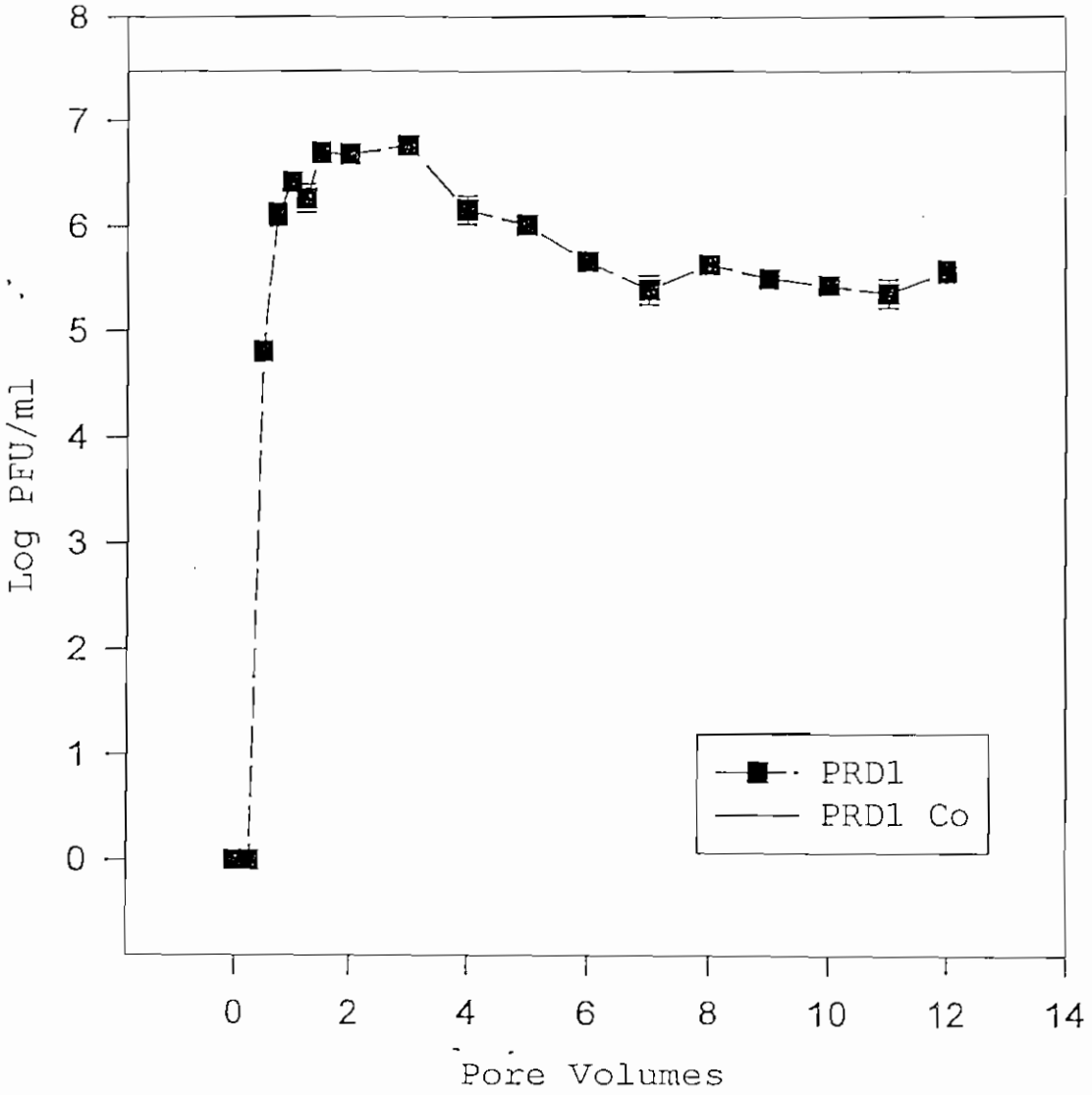

FIGURE 39. TRANSPORT OF PRD1 IN THE BRAZOS ALLUVIUM USING A 0.76 METER TWO PORE VOLUME INJECTION COLUMN

through the 12th pore volume. The maximum C/C_o at 3 pore volumes was 0.19. This confirms that PRD1 is strained within the soil matrix, compared to the smaller MS2 virus, which exhibited greater adsorption in batch studies than PRD1, and thus should have passed through the soil matrix with greater ease. With an average porosity of 0.35, the pore space was many thousand times the size of either virus. Thus, in theory, straining of virus should have been negligible. There seems little else, however, to explain the phenomenon displayed within these soil columns.

ΦX174 transport-A total of 2.02×10^9 PFUs of ΦX174 was introduced in 1052 ml (2 pore volumes) of groundwater with a resultant injection concentration of 1.92×10^6 PFUs/ml. A total of 1.98×10^9 PFUs were recovered over 10 pore volumes (Fig. 40), which left 5.0×10^7 PFUs of ΦX174 within the soil column which is only 2.5% of the virus introduced. The first breakthrough of ΦX174 occurred within the first 0.25 pore volumes, after which the concentration climbed quickly, reaching a peak at 3 pore volumes, which corresponds to a C/C_o of 0.834. After this peak, the viral concentration of the virus particles in the effluent declined rapidly over the next 7 pore volumes. This overall curve and breakthrough characteristic corresponds to the curves

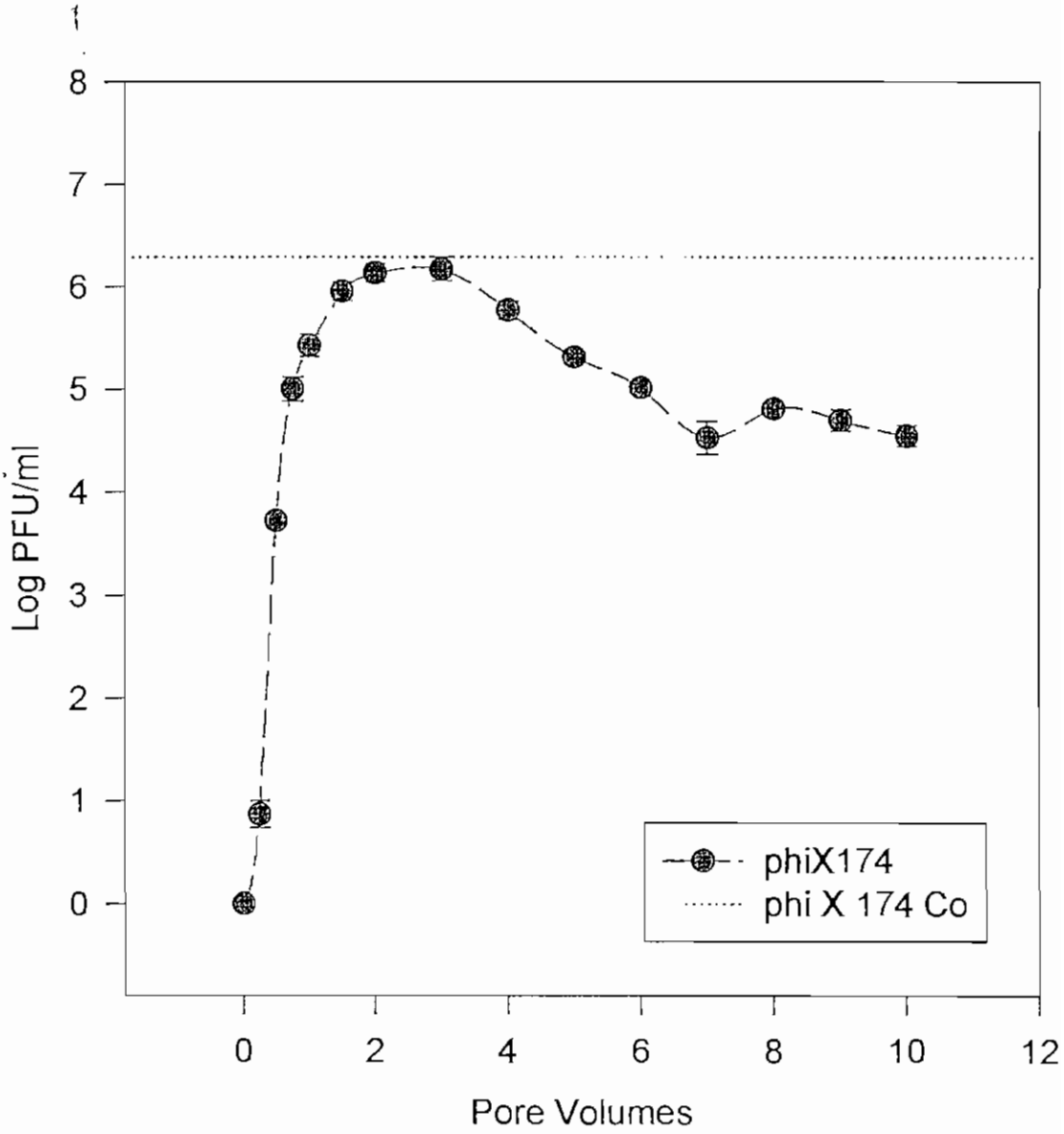

FIGURE 40. TRANSPORT OF phiX174 IN THE BRAZOS ALLUVIUM USING A 0.76 METER TWO PORE VOLUME INJECTION COLUMN

exhibited by MS2 and PRD1 in the 2 pore volume injection studies. The maximum C/C_o was extremely high considering the C/C_os of MS2 and PRD1 within this column. This corresponds to the adsorption characteristics displayed in the batch and continuous column studies. ΦX174 is the same size as MS2 but its isoelectric point is 6.2, thus it does not adsorb as readily to the soil matrix as the MS2, which has a isoelectric point of 3.9. Though PRD1 has an isoelectric point similar to MS2, it is a much larger particle with a much higher molecular weight.

Qβ transport-A total of 6.69×10^7 PFUs of Qβ was introduced in 1052 ml (2 pore volumes) of groundwater with a resultant injection concentration of 6.36×10^4 PFUs/ml. A total of 3.11×10^7 PFUs (Fig. 41) were recovered over 8 pore volumes, which left 3.6×10^7 PFUs of Qβ within the soil column which is 53% of the virus introduced. The first breakthrough of Qβ occurred within the first 0.5 pore volumes, after which the concentration climbed quickly, reaching a peak at 2.5 pore volumes, which corresponds to a C/C_o of 0.68. After this peak, the concentration of the virus particles (in the effluent) declined rapidly over the next 6 pore volumes. This overall curve and breakthrough characteristic was similar to the curves exhibited by MS2,

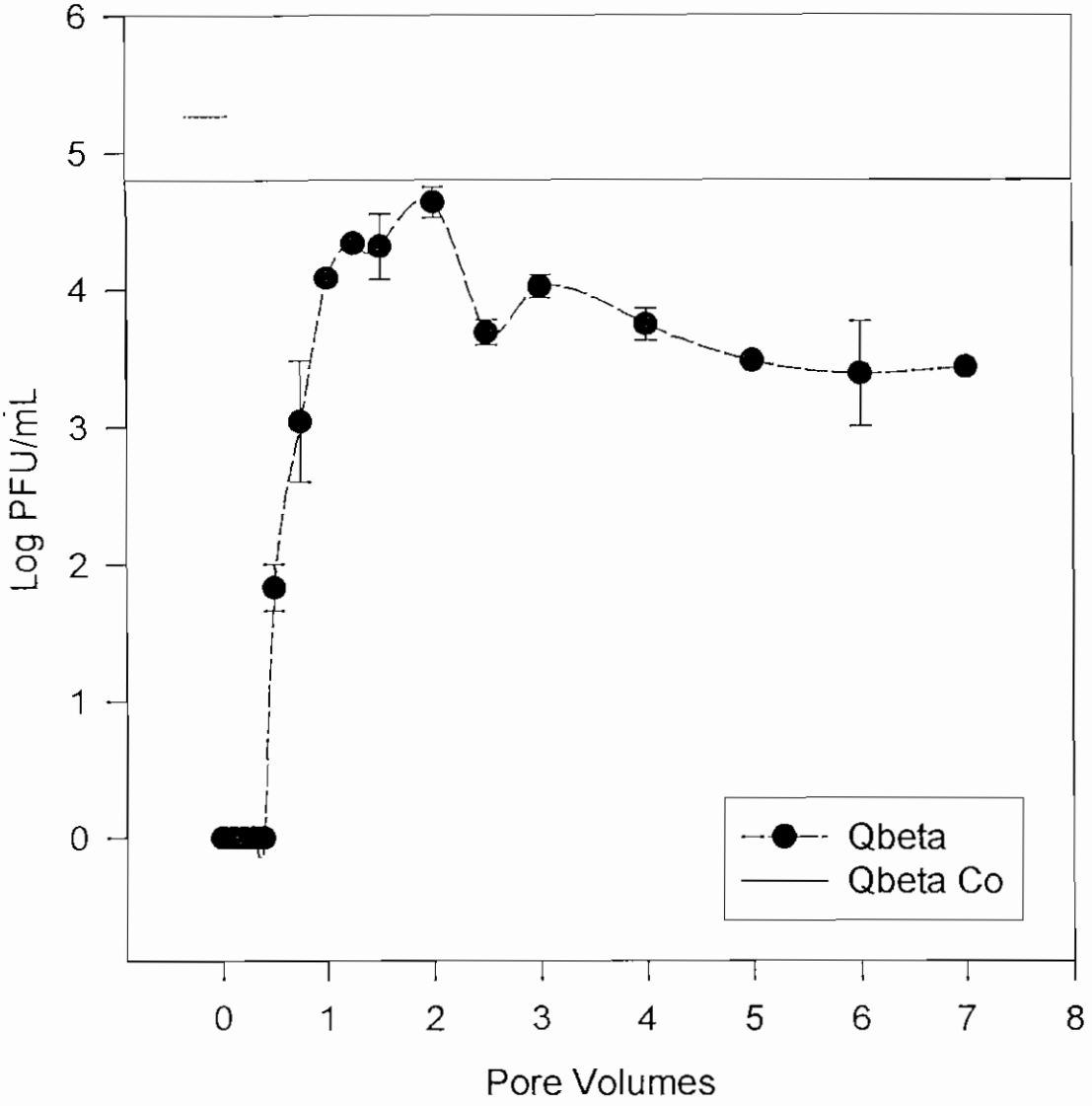

FIGURE 41. TRANSPORT OF Qbeta IN THE BRAZOS ALLUVIUM USING AN 0.76 METER TWO PORE VOLUME INJECTION COLUMN

ΦX174, and PRD1 in the 2 pore volume injection studies. The maximum C/C_o was high considering the C/C_os of MS2 and PRD1 within this column, though lower than the ΦX174. This corresponded to the adsorption characteristics displayed in the batch and continuous column studies. Qβ is the same size as MS2, but its isoelectric point is 5.3, thus it does not adsorb as readily to the soil matrix compared to MS2, which has an isoelectric point of 3.9 and thus a stronger negative charge at the pH of the aquifer.

PM2 transport-A total of 1.72×10^{10} PFUs of PM2 was introduced in 1050 ml (2 pore volumes) of groundwater, with a resultant injection concentration of 1.635×10^{7} PFUs/ml. A total of 5.26×10^{9} PFUs (Fig. 42) were recovered over 12 pore volumes, which left 1.19×10^{10} PFUs of PM2 within the soil column, which is 30.6% of the virus introduced. The first breakthrough of PM2 occurred within the first 0.25 pore volumes, after which the concentration climbed quickly, reaching a peak at 2 pore volumes, which corresponds to a C/C_o of 0.44. After this peak, the concentration of the virus·particles (in the effluent) dropped drastically (two logs) within the next half pore volume. After this drop, which corresponded to the addition of virus free influent, the concentration in the effluent declined slowly over the

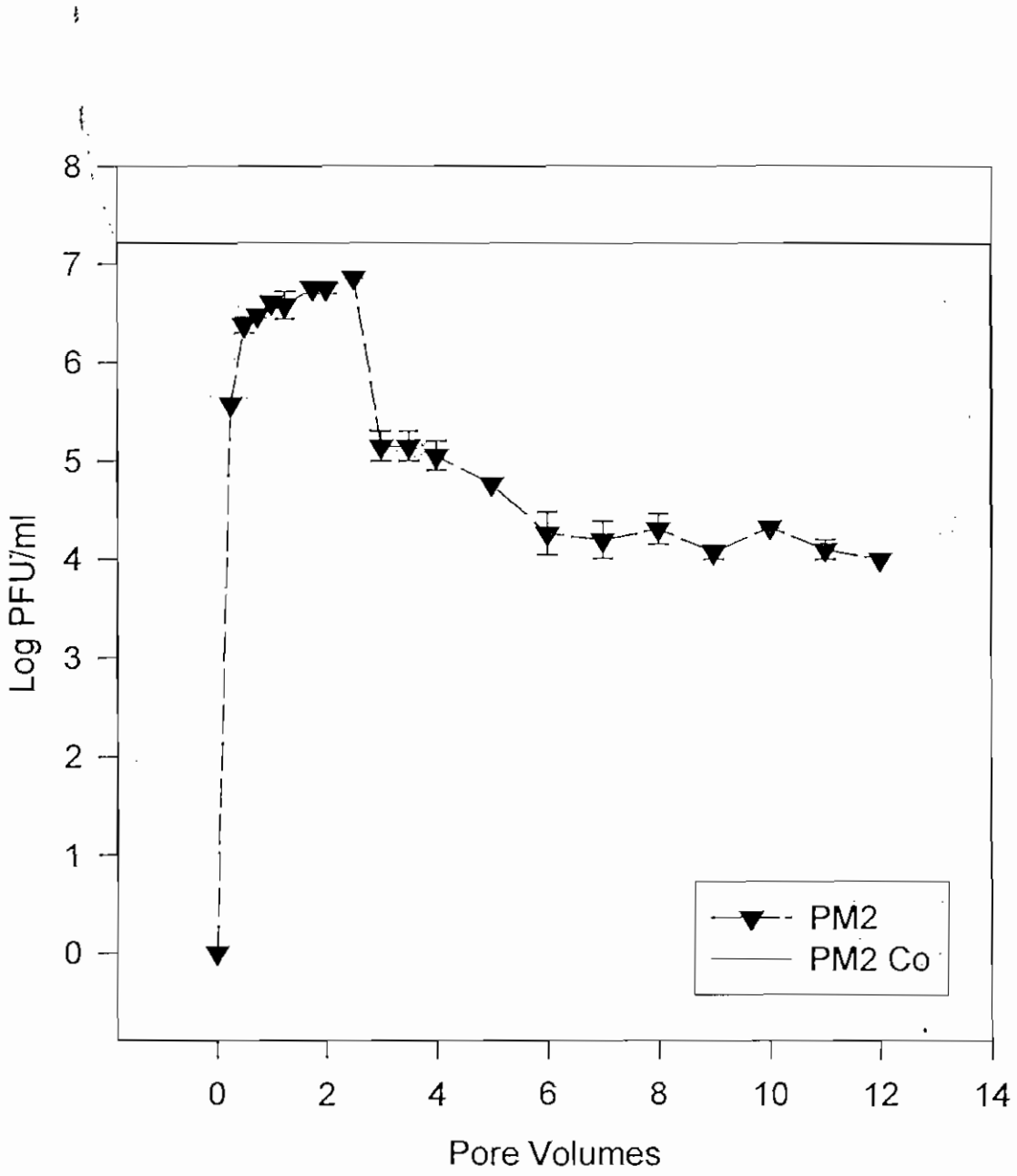

FIGURE 42. TRANSPORT OF PM2 IN THE BRAZOS ALLUVIUM USING AN 0.76 METER TWO PORE VOLUME INJECTION COLUMN

next ten pore volumes. PM2, which was the same size as PRD1, had a *pI* of 7.3, which made it nearly neutral in the pH of the aquifer (7.1). Thus it should have showed little or no ionic interaction with the soil matrix. The effect of the isoelectric point (pI) was evident when compared to PRD1, which was the same size as PM2, but a lower pI equal to 4.2. PRD1 showed a C/C_o of 0.19, while PM2 showed a much higher C/C_o. Thus it can be seen that the major factors influencing adsorption in the Brazos Alluvium was the isoelectric point of the virus, while the size of the virus affected the transport within the sandy soil matrix. This size effect was evident when compared to ΦX174 (diameter 27nm, pI 6.6), which had a lower pI yet displayed a higher C/C_o when compared to PM2 (60nm).

3.3.2.2 Ten Meter Two Pore Volume Injection Column

MS2 transport-A total of 2.99×10^{13} was inoculated into 12 liters of groundwater (2 pore volumes) with a resultant concentration of 2.49×10^{9} PFU/ml. A total of 3.96×10^{7} PFUs of MS2 (Fig. 43) were recovered from the column in just over 5 pore volumes. This means that 2.99×10^{13} MS2 virus particles remained within the column and only a small percentage of the total was recovered (1.33×10^{-5}). The first detection of virus occurred within 4 hours, in low

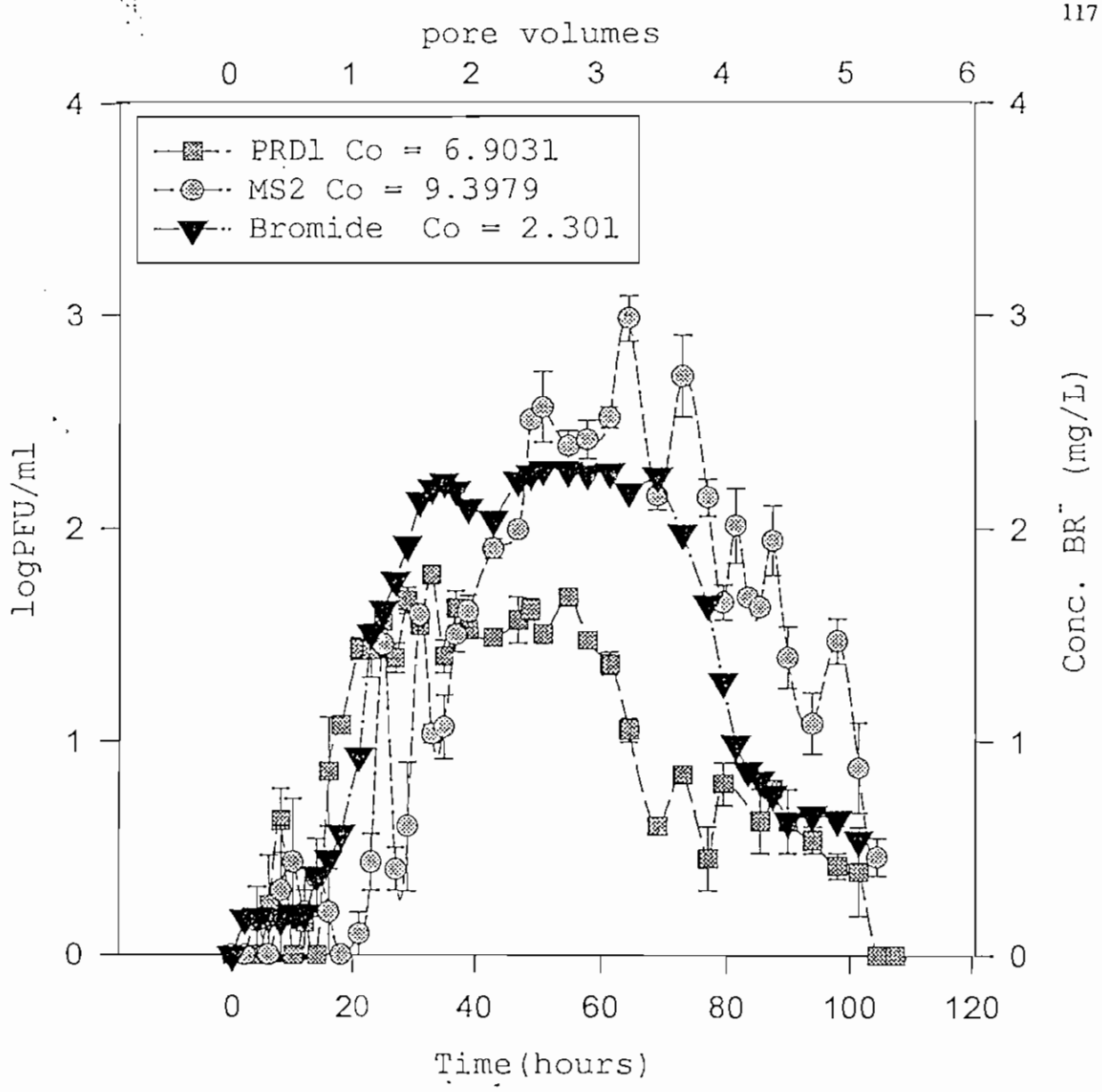

FIGURE 43. TRANSPORT OF MS2, PRD1, AND BROMIDE IN THE BRAZOS ALLUVIUM USING A 9.3 METER HORIZONTAL TWO PORE VOLUME INJECTION COLUMN

concentrations. This baseline of low detection continued through the first 20 hours of the study, when the effluent virus concentrations began to climb. This time frame (20 hours) corresponds to around 1 pore volume. These observations are similar to those seen in the 0.78 meter 2 pore volume columns. As with the smaller column, the peak was reached just after 3 pore volumes with a C/C_o value of 3.894×10^{-7}. After this peak the concentration of the virus in the effluent declined over the next 20 hours reaching the lower detection limit at 5.43 pore volumes (107.5 hours).

PRD1 transport-A total of 1.21×10^{12} PRD1 virus particles was introduced into the column in two pore volumes, with a resultant injectate concentration (C_o) of 8.003×10^{6} PFU/ml. A total of 4.33×10^{5} virus particles was recovered from the column over 5.4 pore volumes, for a recovery percentage of 3.5×10^{-5} (Fig. 43). The first breakthrough of the PRD1 occurred after 4 hours, as with the MS2 virus, and as with MS2, low level detection continued through the 20th hour, at which time the concentrations began to climb, reaching a maximum concentration in the effluent at 55 hours (corresponding to 1.9 pore volumes), with a maximum C/C_o of 5.98×10^{-6}. After the peak was reached around the second pore volume, the virus continued

to be shed from the column at this relative concentration through the third pore volume, when there was a sharp decline in the C/C_o through the next two pore volumes. The virus levels dropped below detection at 104.5 hours which corresponds to 5.2 pore volumes.

Bromide transport-A total of 2.4 grams of sodium bromide was introduced into the column in two pore volumes (Fig. 43), with a resultant injectate concentration (C_o) of 200 mg/L. The first significant breakthrough of the sodium bromide occurred after 12 hours. Low level detection continued through the 20th hour, at which time the concentrations began to climb, reaching a maximum concentration in the effluent at 55 hours (corresponding to 1.9 pore volumes), with a maximum C/C_o of 0.98. After the peak was reached, around the beginning third pore volume, the sodium bromide continued to be shed from the column at this relative concentration through the third pore volume, when there was a sharp decline in the C/C_o through the next two pore volumes, until the concentration dropped below detection at 104.5 hours, which corresponds to 5.2 pore volumes.

3.4 FIELD STUDIES

3.4.1 Natural Gradient Field Study

The C22 well was located 34 meters from the injection well at 9.8 meters below the surface. The first detection of virus occurred 10 days after injection. Two peaks occurred at the C22 well nest, one at 12 and one at 20 days (Fig. 44), exhibiting a bimodal transport phenomenon. This could be explained if the initial plume was split into two different fractions which followed different flow paths of varying tortuosities. One of the plume fractions could thus have arrived earlier than the second. The maximum C/C_o at the C22 well of 1.45×10^{-9} occurred on the 22nd day of the study. The C23A well was located at 12.8 meters below the surface. The first detection at this well was also on the 10th day of the study and the peak occurred at 20 days (Fig. 45), with a C/C_o of 1.67×10^{-9}. The C23B well, located at 15.5 meters below the surface, exhibited two peaks which coincided with the peaks at the C22 well (Fig. 46). The maximum C/C_o was 1.87×10^{-9} and occurred at day 20, as did the peak at the C22 well. Finally, at the C24 well, this same phenomenon occurred with two peaks coinciding with the C22 and the C23B. The highest C/C_o of 1.25×10^{-9} occurred on the 12 day however (Fig. 47). The sampling frequency may have been such that the major peaks and maximum C/C_o levels

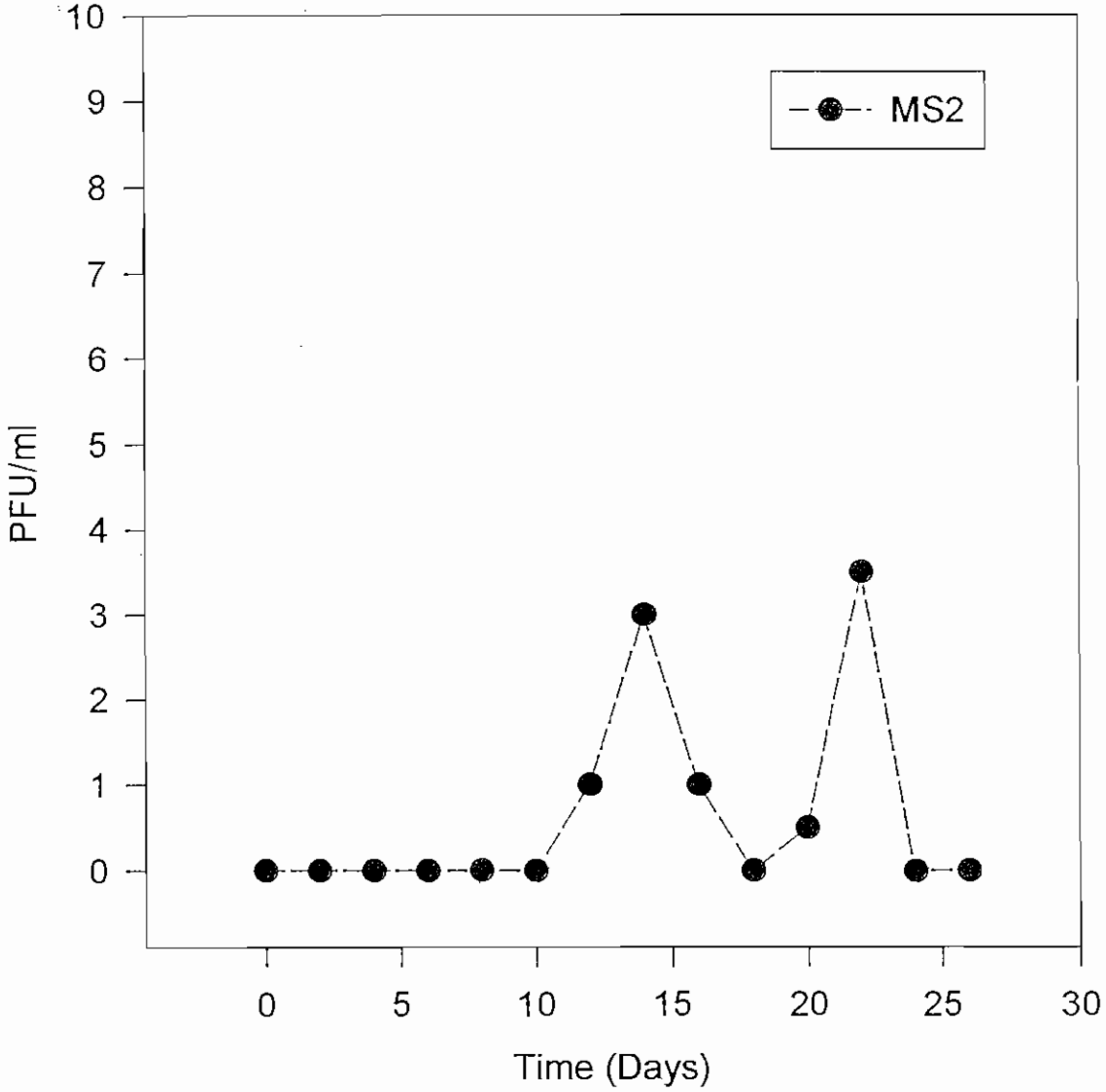

FIGURE 44. BREAKTHROUGH OF MS2 AT THE C22 WELL UNDER NATURAL GRADIENT FIELD CONDITIONS IN THE BRAZOS ALLUVIUM

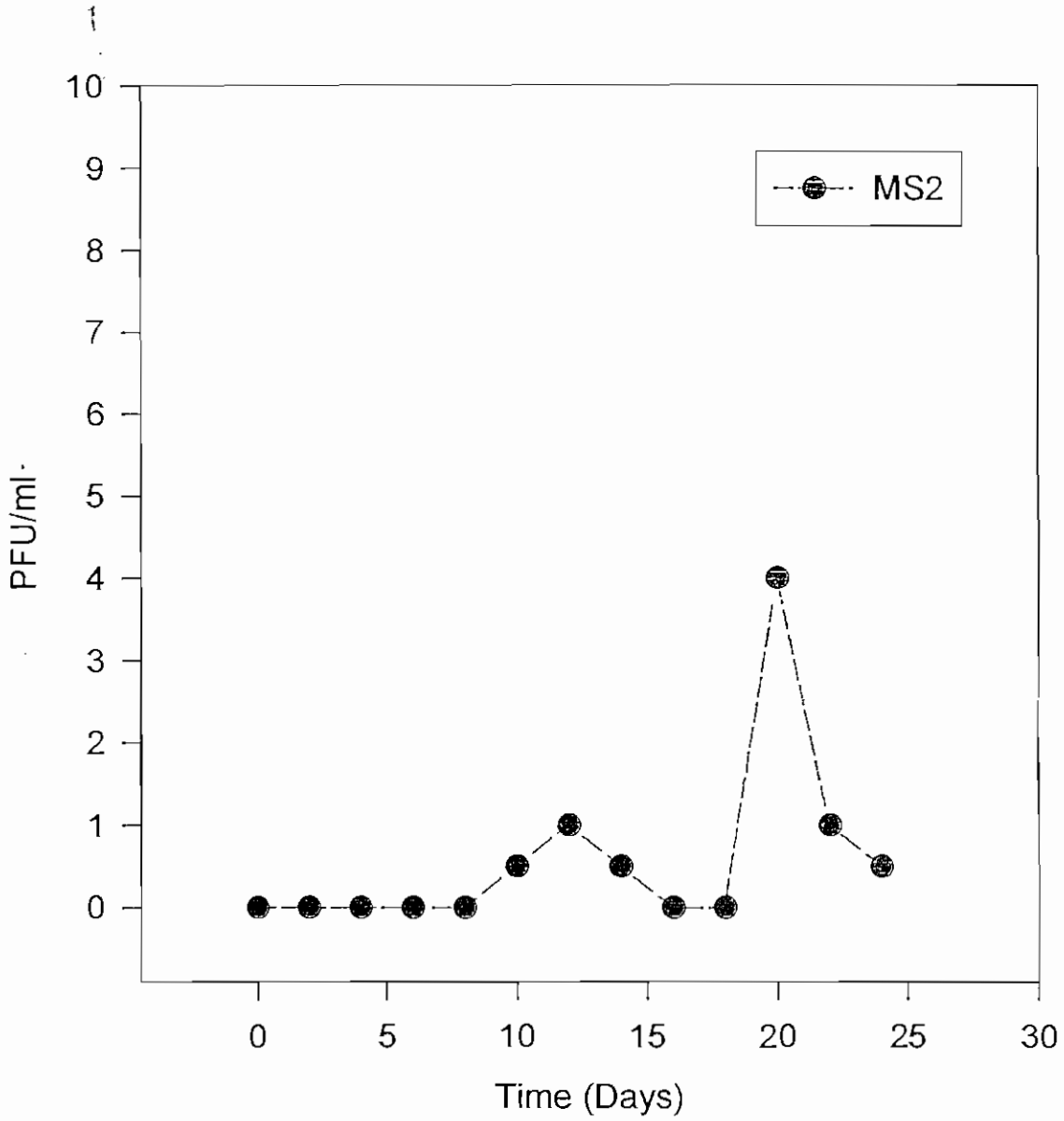

FIGURE 45. BREAKTHROUGH OF MS2 AT THE C23A WELL UNDER NATURAL GRADIENT FIELD CONDITIONS IN THE BRAZOS ALLUVIUM

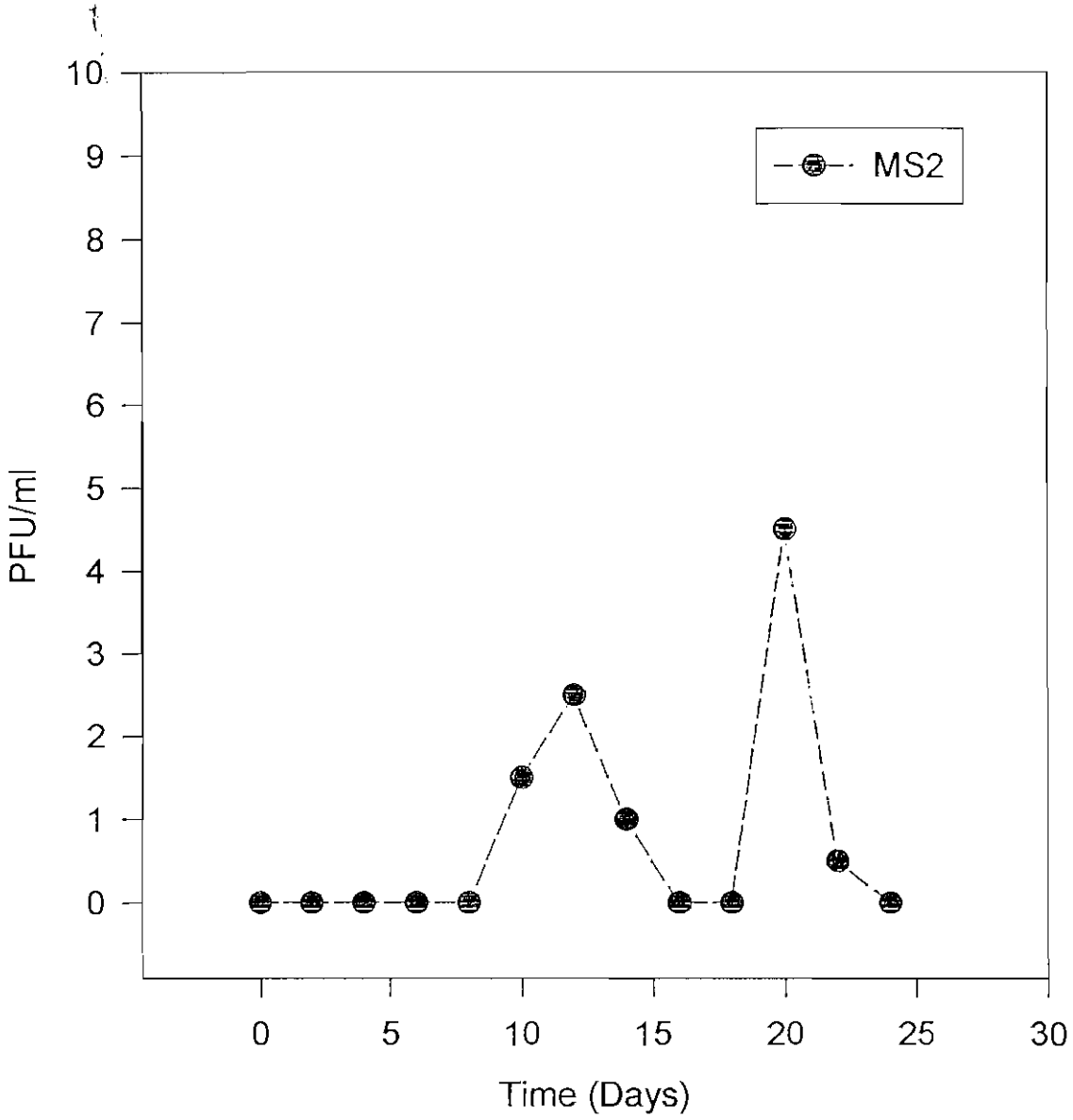

FIGURE 46. BREAKTHROUGH OF MS2 AT THE C23B WELL UNDER NATURAL GRADIENT FIELD CONDITIONS IN THE BRAZOS ALLUVIUM

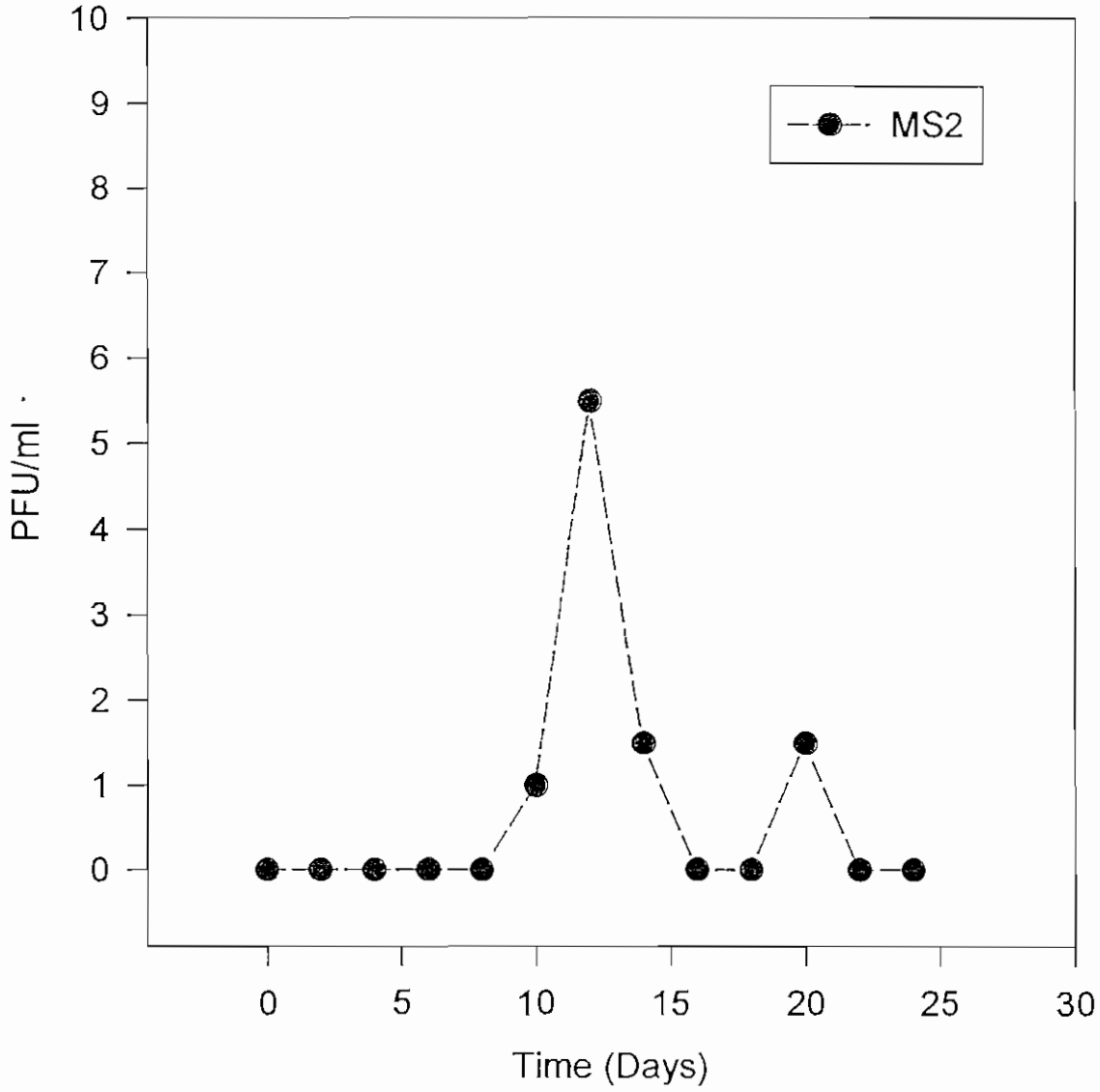

FIGURE 47. BREAKTHROUGH OF MS2 AT THE C24 WELL UNDER NATURAL GRADIENT FIELD CONDITIONS IN THE BRAZOS ALLUVIUM

at this well nest arrived and passed the vicinity of this well nest between samplings. The mere fact that there were any detections at all under the natural gradient at 37 meters is indicative of the potential for viral transport.

3.4.2 Forced Gradient Field Study

The concentrations of MS2 and PRD1 in the injectate was 5.9×10^9 and 1.0×10^8 PFUs/ml, respectively. In order to determine the presence of any background virus left in the aquifer from the previous study, preinjection samples were collected and analyzed. There were no background levels detected in any of the samples taken before or after the pump was turned on and the aquifer reached steady state conditions.

The first detection occurred on the second day at relatively low levels suggesting that there were background virus levels not detected previously. This in itself is highly significant in that the virus if they were background from the natural gradient study, had been in the aquifer for well over 60 days, which is over the maximum survival time predicted in the laboratory microcosms. What may be more significant is the possibility of the secondary explanation, which is that the virus had traveled over 30 meters in 2 days in an aquifer with an average flow velocity around 1 meter/day.

The C23A well exhibited background levels up until day 6, when a small peak averaging 4.18 PFU/ml of MS2 occurred. After this time the levels declined to background levels until day 15, when the numbers of MS2 reached 5.4 PFU/ml. The concentrations climbed for 2 days, reaching a peak of 150.3 PFU/ml, which was a C/C_o of 2.54×10^{-8} (Fig. 48). This C/C_o was considerably higher than the peak under natural gradient conditions. It was interesting to note, however, that there was some evidence of the bimodal phenomenon exhibited during the first study. At the C23B well, the levels were over a log lower, with a peak of 11.5 PFU/ml MS2 occurring at day 15. The C/C_o for this well reached a maximum of 1.59×10^{-9}, which was comparable to the C/C_os during the natural gradient study (Fig. 49). The deepest well (C24), which was at the same depth as the virus was injected, showed the highest numbers. The levels first rose above the "background levels" on the 12th day of the study and climbed steadily over the next 5 days, reaching a peak of 661.7 PFUs/ml of MS2 on day 17 (Fig. 50). This was an effective C/C_o of 1.12×10^{-7}, which was comparable to the 4.5 meter sampling port during the 9.3 meter column study after the addition of DI water. These are incredible levels considering the distance traveled and the results of the column studies. The flow velocity of the forced gradient

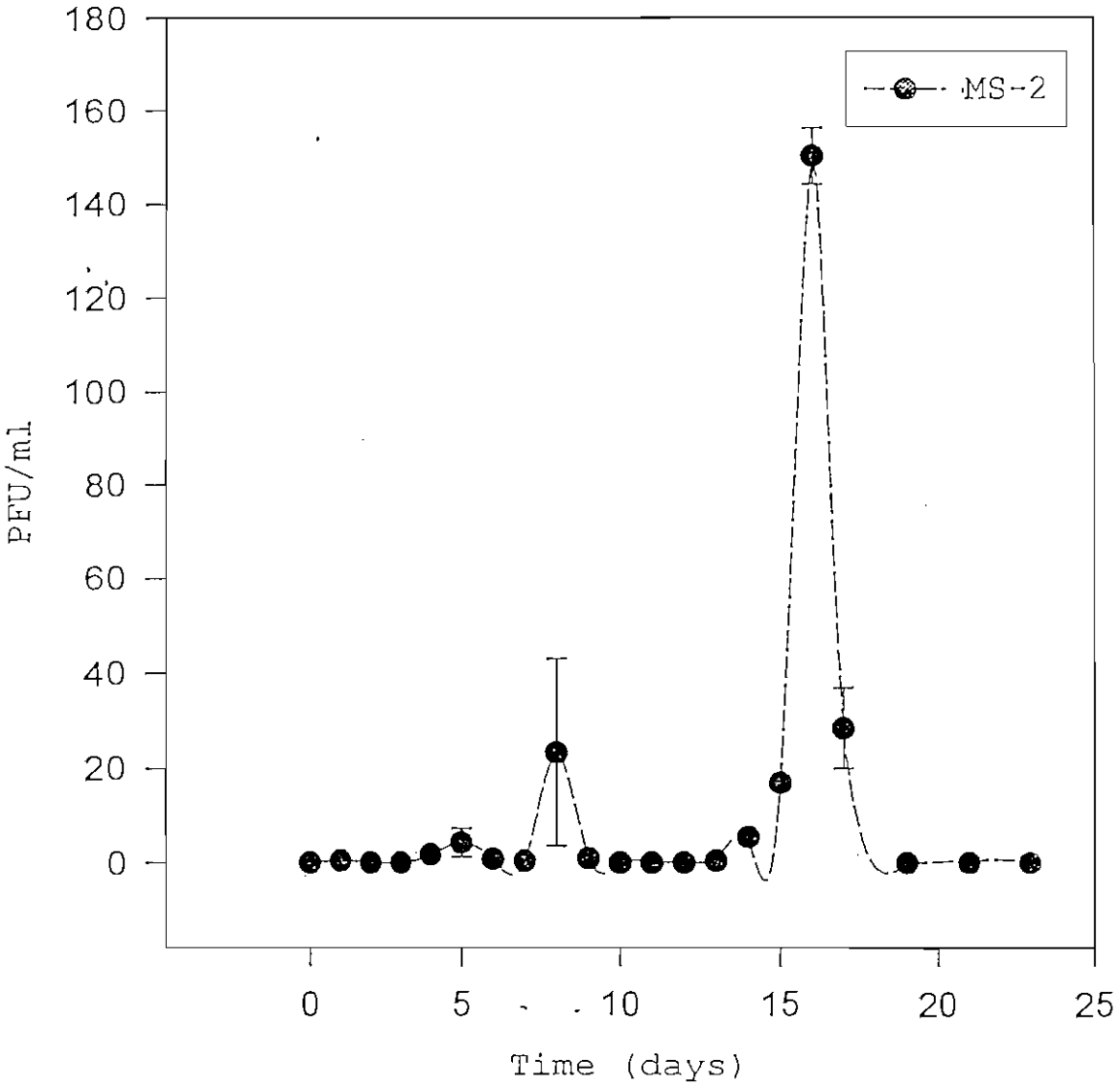

FIGURE 48. BREAKTHROUGH OF MS2 AT THE C23A WELL UNDER FORCED GRADIENT FIELD CONDITIONS IN THE BRAZOS ALLUVIUM

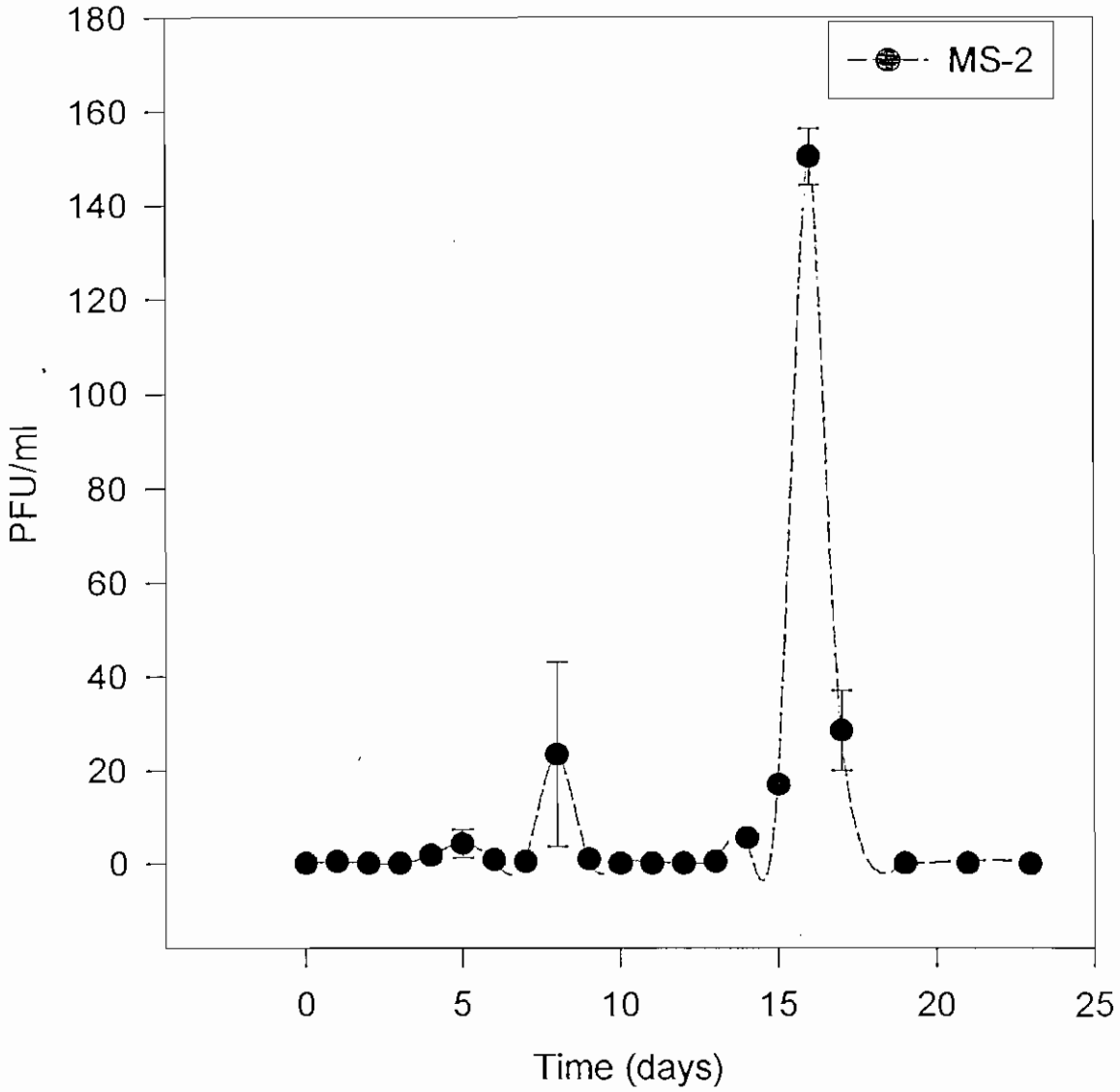

FIGURE 49. BREAKTHROUGH OF MS2 AT THE C23A WELL UNDER FORCED GRADIENT FIELD CONDITIONS IN THE BRAZOS ALLUVIUM

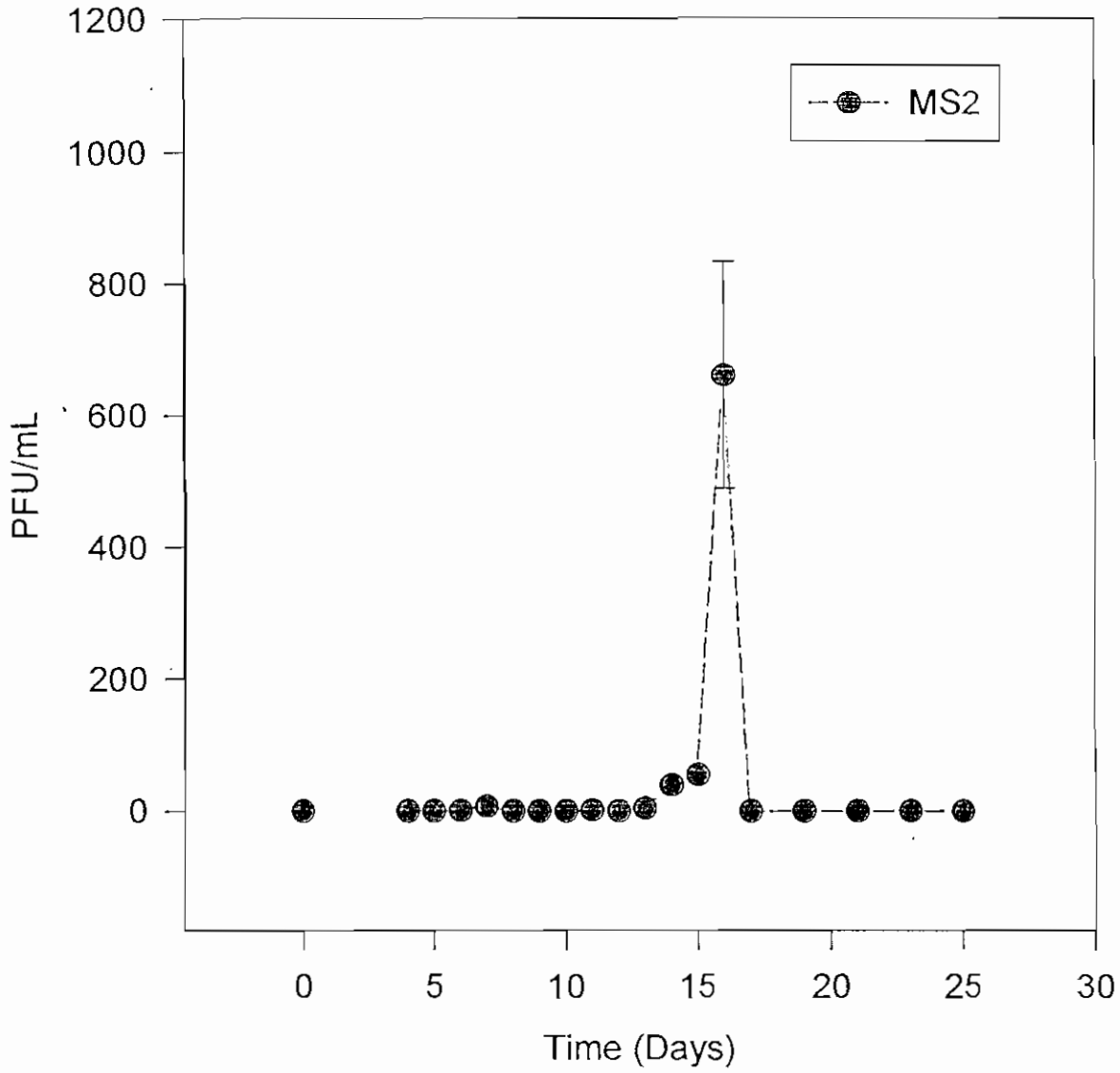

FIGURE 50. BREAKTHROUGH OF MS2 AT THE C24 WELL UNDER FORCED GRADIENT FIELD CONDITIONS IN THE BRAZOS ALLUVIUM

study was close to 1 meter/day which indicates that the viruses were traveling considerably faster than the average flow velocity in the aquifer. PRD1 was not detected during this field study, though during the 11 day, the 14 day, and the 16 day levels corresponding to 0.5 PFUs/ml were detected.

CHAPTER 4

DISCUSSION

4.1 SURVIVAL

One of the key factors influencing the transport of virus within an aquifer is viral inactivation. The phages (MS2, PRD1, Qβ, and ΦX174) displayed the ability to survive for very long periods of time within the Brazos Alluvium aquifer. Since these viruses can be considered as indicators of pathogenic viruses, it is evident that if aquifers do become contaminated with pathogenic viruses the viruses could potentially survive for long periods of time. Hurst et al. (1980), indicate that the main environmental factor influencing survival is temperature. Other factors which they indicate have an influence on survival are adsorption (which is reversible and not true decay), phosphorus, aluminum, and pH. This information corresponds to the results of the survival studies. In the Rio Grande Alluvium, where the phosphorus content, cation content, and the pH are higher, MS2 and PRD1 do not survive as long as they do in the Brazos alluvium. All the phage studied displayed the ability to survive for long periods of time in the Brazos Alluvium especially PRD1. This corresponds to the publication by Yahya et al. (1984) which showed that

PRD1 survived longer than MS2 under all conditions. Bitton et al. (1983) stated that the low survival rates of bacteriophage made them in appropriate as indicators. All the phage studied (MS2, PRD1, ΦX174, PM2, and Qβ) would serve as good indicators, in the Brazos Alluvium, based on their survival rates. Finally it was found that MS2 displayed the ability to survive in concentrations of sodium bromide, equal to 200g/L, for up to 30 days. However, in concentrations of 600g/L the MS2 virus titer was inactivated within 4 days. Thus, sodium bromide at levels of 200g/L was determined to be an adequate tracer concentration for use in conjunction with lare scale viral transport studies in the Brazos Alluvium.

4.2 ADSORPTION

The adsorptive properties of phages are extremely variable (Gerba and Goyal, 1979). In the Brazos Alluvium, where the only variable introduced was the phage type, the adsorptive percentages ranged from 81.4% to 99.96% adsorption. The use of correlation analysis shows that the isoelectric point of the virus particle may be the physiochemical property which is responsible for the variability. Thus the pH of the aquifer material interacts with the isoelectric point of the phage. The pH of the

Brazos Alluvium ranges from 6.9 to 7.1, thus, the relative strength of the negative charge on the surface of the virus particle increases as the isoelectric point decreases. Thus it can be assumed that as the strength of the negative charge increases, the adsorptive properties are further enhanced and increase. The high correlation coefficient (- 0.90) indicate this relationship (Fig. 51), suggesting that adsorption increases in inverse proportion to the isoelectric point of the virus particle. The second major factor, besides the virus type, is the soil type, as can be displayed by the variability of the adsorptive characteristics of the MS2 phage in the Brazos Alluvium sediment, the Rio Grande Alluvium sediment, and the El Paso Field 13 agricultural top soil. The adsorptive percentages ranged from 29% to 99.99% adsorption, indicating that the type of soil introduces another extreme variable. Thus it can be seen that adsorptive behavior is extremely site specific and virus specific. These conclusions agree with the conclusions of Goyal and Gerba (1979) and Goyal and Gerba (1982). The conclusions on the isoelectric point stated previously, go beyond these publications into factors, not directly related to the soil, which influence adsorption. The conclusions do however relate to the paper

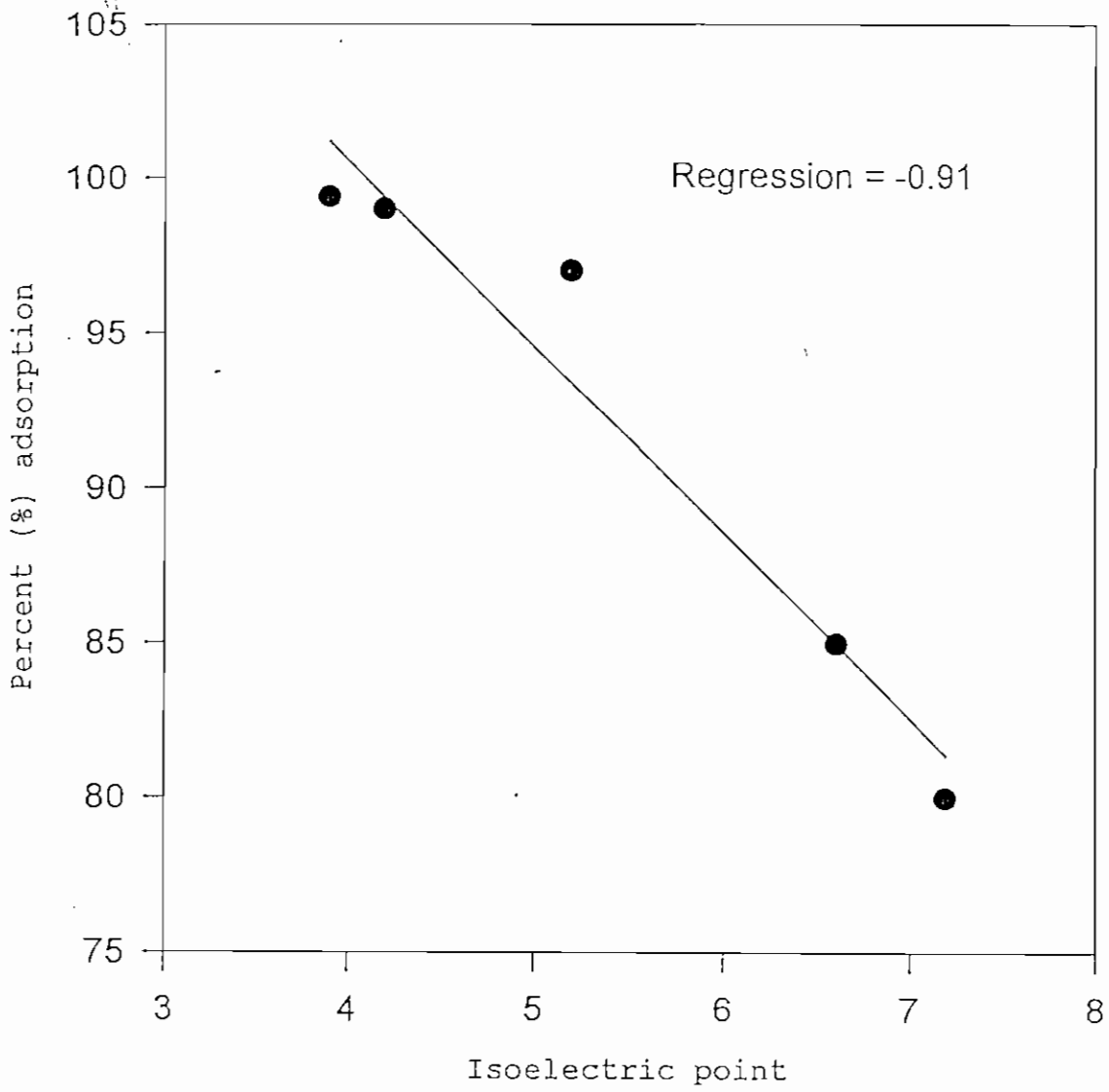

Figure 51. CORRELATION OF ISOELECTRIC POINT TO MAXIMUM ADSORPTION PERCENTAGE

by Singh et al. (1986) which concluded that two proteins, one with a high isoelectric point and one with a low isoelectric point, could predict viral adsorption to soils.

4.3 COLUMN STUDIES

A comparison was made between MS2, ΦX174, Qβ, PM2, and PRD1 to determine the differences in their transport characteristics. In the pulse injection columns in all cases the larger PRD1 particle (62 nm diam), exhibited more retardation in the soil matrix than the smaller MS2 (24 nm diam). The adsorption during transport is non-linear as can be seen when comparing the different sized column. After the 0.38 meter column the adsorption does become more linear in nature as determined by C/C_o analysis. This is the same phenomenon seen by Lance et al. (1982). The results also agree with the results of Powelson et al. (1990) Which stated that relatively little viral adsorption occurs under saturated conditions. The 0.78 m pulse injection columns also displays a lower C/C_os for MS2 and PRD1, than the two pore volume columns. This may be explained by the higher concentration of organic matter being introduced in the pulse column which causes increased adsorption. Bales and Li (1993) saw this same effect in their controlled column

experiments when 0.01% hydrophobic organic content caused increased adsorption. They also showed that increasing flow rate caused a decrease in adsorption which agrees with the results of the ten meter pulse injection column where increasing the flow rate caused increased C/C_o at the all the sampling ports. The addition of DDI water caused a much greater effect on the adsorption of the virus resulting in a much higher C/C_o than just increasing the flow rate. This, agrees with the results of Bales and Li (1993) who displayed similar results with the addition of DI water.

In the two pore volume injection columns the higher isoelectric point of the ΦX174 and the Qβ seem to cause an effect in their transport. These viruses, which have structural and size properties similar to MS2, exhibit much higher C/C_o values (Fig. 52) therefore less adsorption. When considering a soil matrix which is essentially negatively charged this should indicate that some type of charge repulsion should take place with the more negatively charged viral particles. MS2 should thus, when considering this factor alone, exhibit less adsorption than the viruses with higher pIs and be repulsed from the soil surface and confined to the middle pore spaces. There are two layers of cations associated with the negatively charged soil particles termed the Stern double layer (Bohn et al., 1979).

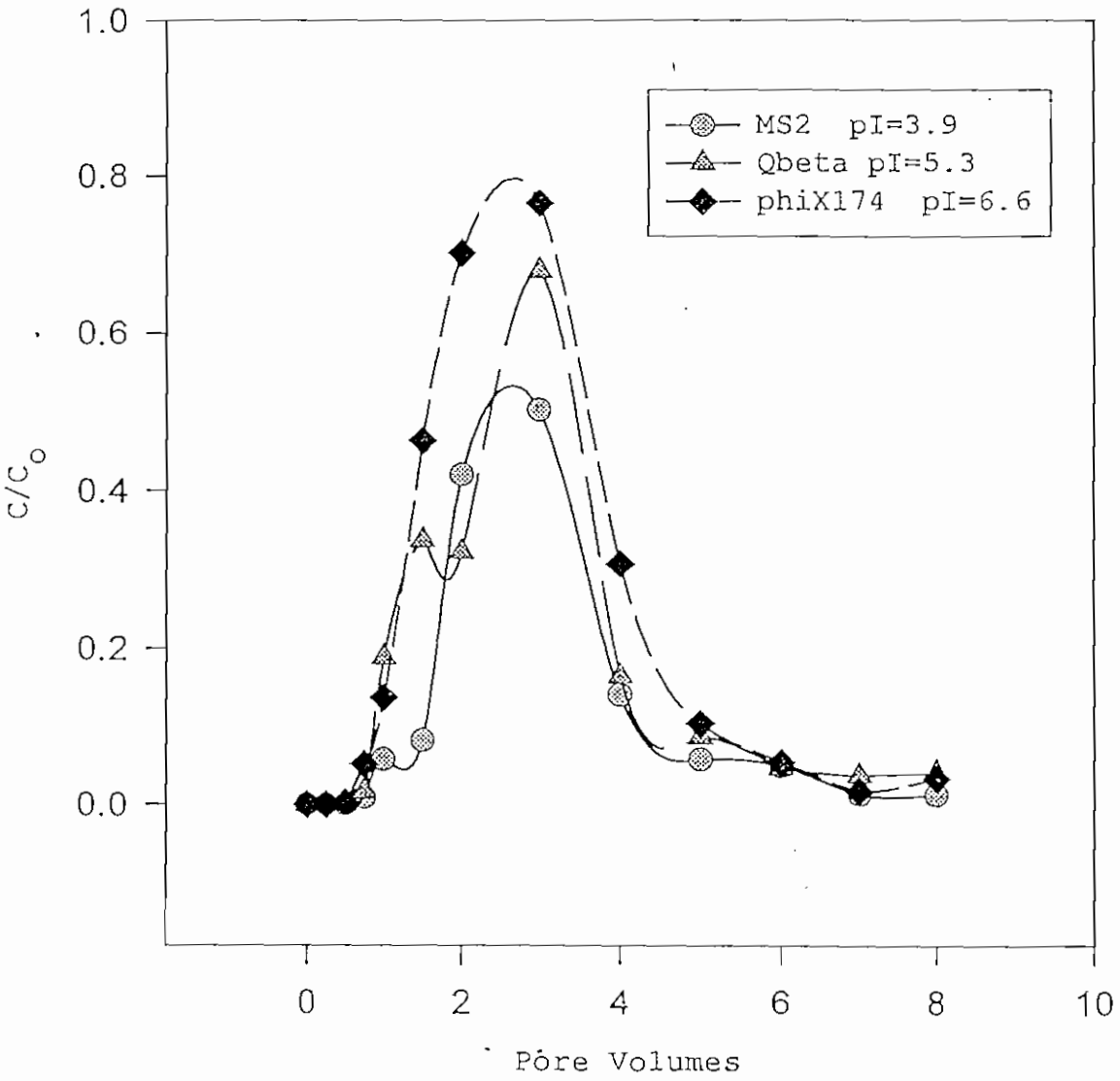

Figure 52. TWO PORE VOLUME INJECTION COLUMN C/Co VALUES FOR THE 24-27 nm PHAGES MS2, Qbeta, AND phiX174

The layer closest to the soil layer is a rigid layer and the layer next to this is a diffuse region of cations. These cation layers neutralize the negative charge of the soil mineral. These layer in turn create a cation excess which attracts anions such as virus particles closer to the soil layer where cation exchange with subsequent adsorption can occur (Jury et al., 1991). This was not the case with the PRD1 phage, which though it has a higher isoelectric point than MS2, has lower C/C_os in all cases. Pearson Product Moment Correlation analysis was run to determine the correlation between the maximum C/C_os of MS2, Qβ, and ΦX174 to their isoelectric points (Fig 53). There was exact correlation with a coefficient of 1.0 and confidence at the 99.7% level. This indicates that as the isoelectric point of the phage increases, the maximum C/C_o increases.

When considering the size of the phages it might be tempting to consider some sort of sieving or size exclusion phenomenon is occurring with the PRD1 phage and the PM2 phage. Especially, when considering the isoelectric point effects of the smaller phage. PM2 which has the highest isoelectric point (7.2), should be relatively unreactive with the soil chemistry (pH 7.1), yet it shows a lower C/C_o than the smaller ϕX174 which has a lower IP (6.2). But, if you consider that the pore spacing in the soil matrix is

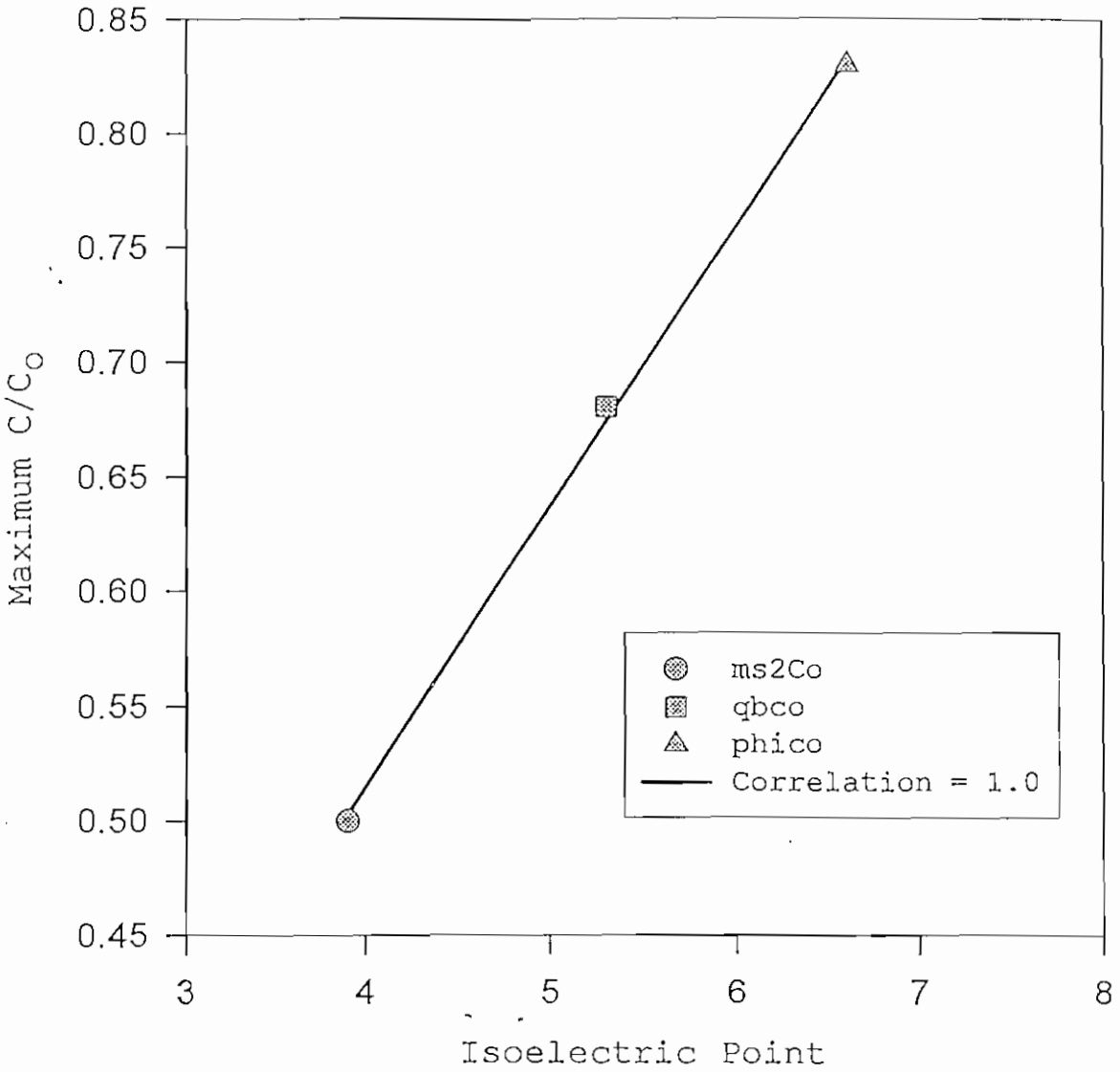

Figure 53. CORRELATION OF THE MAXIMUM C/Co VALUES OF MS2, Qbeta, AND phiX174 TO THEIR ISOELECTRIC POINT

many thousand times larger than the virus particles, sieving should be negligible. The primary type of adsorption which occurs in the soil matrix can be described as London (van der Waals) forces (Corapcioglu and Haridas, 1984). This explanation for the increased size exclusion is also an attractive explanation for the differences in the transport characteristics of the phages. These dipole interaction operate only over very short distances, so the molecules (virus and soil) must be in close proximity in order for the forces to have any effect. The key factor that makes this interaction attractive, when related to this study, was the fact that the larger the atom, the farther its electrons are from the nuclei and consequently the greater its polarizability. Therefore, the chance of occurrence and the magnitude of these London dispersion forces should increase with an increase in the surface area of the phage. A problem with this hypothesis for the retardation of the PRD1 is that London forces operate only over very short distances. The energy of this interaction varies as $1/r^6$, so that doubling the distance between particles decreases the energy by a factor of 2^6 (Brown and LeMay, 1977). A final hypothesis to explain the greater relative retardation of PRD1 and PM2 is that the larger phages are unable to find a path directly through the soil matrix, and thus must find

a route of increasing tortuosity, as compared to the smaller phages. The PRD1 and the PM2 retardation could also be a result of a combination of these factors making the interaction highly virus specific.

As with the survival and adsorption experiments, the pulse columns using Rio Grande Alluvium aquifer material display the site specific nature of virus transport, as well as the particle size exclusion phenomenon of the larger PRD1 virus. PRD1 is retained less within the matrix of the Rio Grande Alluvium than the Brazos Alluvium, whereas the opposite is true for the MS2 viruses, which are retained more within the Brazos Alluvium than in the Rio Grande Alluvium. This is possibly an effect of the higher pH and cation content of the Rio Grande Alluvium.

4.5 FIELD STUDIES

MS2 displayed the ability to migrate over 30 meters, in the Brazos Alluvium, under natural gradient conditions. Other controlled field studies done in sandy aquifers have also shown the ability of phages to migrate over great distances (Paul et al. 1995; Bales et al. 1995). Thus it can be assumed that phages can be used to model enteric virus movement (under natural and forced gradients

conditions) due to their stability in groundwater and similarities in size and physiochemical properties with viruses of public health significance.

Under forced gradients, MS2 showed the ability to move over 30 meters, in relatively high concentrations (8×10^2 PFU/ml), moving faster than the average flow rate of the aquifer. The average flow rate in the aquifer is around 1 meter per day, while the virus moved over 30 meters in less than 15 days. This, can be explained by the fact that channels are present in the center of soil pore spaces that move faster than the average velocity over the whole of the aquifer. This can be easily explained by example when considering size exclusion chromatography where larger particles travel faster than smaller particles. Thus we can see that the virus show size exclusion while the groundwater does not. Mathematical models used for granting variances, should take this into account when determining the flow rate of the aquifer.

CHAPTER 5

CONCLUSIONS

Bacteriophage can survive for long periods of time in some aquifers, such as the Brazos Alluvium, while in others, such as the Rio Grande Alluvium, their ability to survive is reduced. This suggests that survival is highly site specific. Different phages also display a wide variety of adsorptive characteristics which can be explained statistically as an effect of their relative isoelectric point(pI). In column and batch adsorption studies in the Brazos alluvium, the adsorption of phages decrease with an increase in the pI. This effect is also evident in transport columns when considering the maximum C/C_o of phages with the same relative size. A size exclusion phenomenon is evident when considering the two larger phages. PRD1 and PM2 show increased retardation in their breakthrough curves when comparing pI, size, and maximum C/C_os with those of the smaller phages. Thus the pI and the size of the phages appears to influence transport. Finally, MS2 shows the ability to move over considerable distances (37 m), under natural and forced gradients, in relatively high concentrations (10^3PFU/ml), in the Brazos Alluvium.

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