



## **WATER RESOURCES RESEARCH GRANT PROPOSAL**

**Title:** Field Studies of Virus Transport Through Unsaturated Alluvium and Fractured Rock

**Focus Categories:** WW, TS, WU

**Keywords:** Viruses, Unsaturated Flow, Wastewater Irrigation, Groundwater Recharge

**Duration:** January 2000 to December 2000

**Federal funds requested:** \$11,858

**Non-Federal (matching) funds pledged:** \$24,978

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**Congressional district of university where the research is to be conducted:**  
Congressional District 5

**Statement of critical regional or state water problems**

The Arizona Groundwater Code was enacted in response to serious groundwater declines in central and southern Arizona. This regulation promulgated the formation of Active Management Areas (AMA) within the state, and stipulated that these areas must meet safe yield by the year 2025. The challenge of meeting safe yield has forced the state of Arizona to consider water conservation and augmentation methods. One such method is the recharging of groundwater through surface application of wastewater effluent. The systems used to recharge groundwater and dispose of effluent include infiltration basins, septic fields, and irrigation. Currently, effluent reuse accounts for approximately 3% of the projected water budget for the Tucson AMA (ADWR, 1999). While most large-scale wastewater treatment utilities sterilize effluent to destroy human enteric viruses, many smaller-scale operations not serviced by wastewater utilities may use disposal methods that threaten potable groundwater supplies. These include septic fields, leakage from

wastewater treatment plant lagoons, and agricultural practices, namely feedlots and irrigation of untreated effluent wastewater.

Wastewater containing animal enteric viruses may pose health and environmental risks, when discharged into the subsurface. Studies have been conducted to predict the manner in which these contaminants may be transported through saturated media (e.g., aquifer systems). However, these studies do not represent typical conditions associated with surface effluent application within the state of Arizona. We propose to conduct two field studies focused on examining the transport and fate of viruses during surface water recharge in two geologic settings common to wastewater application sites in the state of Arizona. These two geologic settings are an unconsolidated alluvium site typical of the hydrologic basins of the Basin and Range Physiographic Province, and a fractured bedrock site typical of hydrologic basins of the Transitional Physiographic Province. For these studies, we will use the virus PRD-1, which is a non-hazardous bacteriophage as an analog to human enteric viruses.

### **Statement of results or benefits**

The proposed research will examine virus fate and transport behavior in the two geologic settings, unconsolidated alluvium and fractured bedrock. This information will assist local, state, and federal officials in the design and compliance of regulatory and statutory guidelines for the protection of groundwater resources associated with surface application of wastewater.

More specifically, the proposed research will determine: 1) the maximum rate at which viruses can be transported through these geologic systems, 2) the concentrations at which viruses can be expected to be present at various distances from the release point, and 3) the duration that viruses will remain a viable threat to human health or the environment at different locations. These results will benefit the cost-effective and safe design of wastewater application systems within the state of Arizona.

### **Nature, scope, and objectives of the research**

Previous studies have been conducted to examine the transport of human enteric viruses through saturated media. Conversely, there has been little research on the infiltration and transport of viruses into unsaturated porous media (i.e. the vadose zone). The study of virus fate and transport through the vadose zone is crucial to understanding the risks posed to human health and the environment of various effluent disposal practices.

Preliminary laboratory studies have shown that the degree of saturation of the soil has a considerable effect on the rate of virus transport and on virus survival. One important aspect present during unsaturated conditions is the presence of the air-water interface. This interface can facilitate viral inactivation in several ways. First, viruses can partition to the air-water interface, thereby immobilizing those viruses from transport. Once viruses are lost from the flow field at the air-water interface they are more prone to microbial attack and inactivation due to stress.

In addition to the air-water interface, the generally limited flow of water present under unsaturated conditions decreases the rate of virus transport. It has been shown in laboratory studies that nearly ninety-nine percent of virus inactivation and removal occurs in unsaturated soil. However, even though virus concentration may be decreased by several orders of magnitude, the high concentrations present in wastewater may still lead to significant numbers of viruses entering vital groundwater resources. Therefore, it is of the utmost importance that unsaturated zone transport be studied under field conditions.

The proposed research will be conducted in two geologic settings: unconsolidated alluvium and fractured bedrock. Lab studies will be conducted prior to the proposed field studies to determine the extent of adsorption of PRD-1 to materials that are present in the unsaturated zones at the two field sites. Following the lab studies, field experiments will be conducted at the Apache Leap Research Site (ALRS) located near Superior, Arizona (fractured bedrock) and the University of Arizona Maricopa Agricultural Center (UofA MAC) (unconsolidated alluvium) located in Maricopa, Arizona. Field studies will include the infiltration of a conservative tracer (e.g. bromide), PRD-1, and latex microspheres at both sites. Concentrations and infiltration rates will be monitored during the course of the infiltration studies and will provide the basis for data analysis.

The transport and fate of viruses within fractured porous media has not been studied to a significant extent. The confines of the fractures may lead to partial filtration of virus particles. Conversely, exclusion of virus particles from the porous rock matrix may lead to more rapid transport. The welded-tuff at the ALRS site is slightly porous. Water molecules may diffuse into and out of the matrix of the welded tuff along the fracture walls. This process delays the transport of bulk water (and of dissolved solutes) compared to the rate of flow through the fractures alone. The virus particles, due to their larger size, are less likely to diffuse into the welded-tuff matrix and thereby will be more likely to remain within the fracture, and thus perhaps be transported more quickly than bulk water.

The unconsolidated alluvium site is representative of the geologic conditions associated with the large population centers and agricultural settings of the Phoenix and Tucson AMAs. The field location on the University of Arizona Maricopa Agricultural Center is a former agriculture field that has been developed to study surface water infiltration processes. The usage of former agricultural land is typical within the Phoenix and Tucson AMAs. Further, the dense irrigation system and shallow groundwater table (45 feet below ground surface) allow for study of the entire process of surface water application, vadose zone transport, and groundwater impact at this site.

## **Methods, procedures, and facilities**

### ***Laboratory Studies***

Preliminary laboratory studies will include experiments to determine the relationship between aqueous concentration and the relative adsorption of the bacteriophage PRD-1 to soil materials obtained from ALRS and the UofA MAC. This will be done using batch

techniques, varying initial virus aqueous concentrations. The enteric virus analog, PRD-1, is grown on a salmonella host and samples are quantified using plaque-forming-unit (pfu) analysis.

Approximately five grams of soil suspended in a 50 ml glass vessel will be subjected to constant stirring and inoculated with varying bacteriophage concentrations. Adsorption can be assumed to reach an equilibrium state after approximately twelve hours, at which time solution samples will be collected to determine the distribution of bacteriophage. Control studies to determine the influence of inactivation on aqueous concentration will be performed by utilizing a similar experimental setup without the soil. The solution and soil used in the study will be drawn from each site. These results will aid in a determination of the appropriate site-specific concentration to use in the larger field study and to demonstrate the potential relationship between aqueous concentration, inactivation, and adsorption present at the field sites.

An adsorption rate profile will also be measured using a specified bacteriophage concentration and the technique described above. Samples will be taken every ten to thirty minutes to determine the adsorption rate. This will indicate the potential relationship between aqueous concentration, adsorption, and inactivation that will occur under field conditions. Water chemistry tests will also be completed to accurately reproduce soil water conditions in the batch studies.

Flourescent latex microspheres will also be injected at both sites. The spheres, which are similar in size to the virus PRD-1, have negatively charged surfaces, similar to the virus. Unlike the virus, the microspheres are unlikely to degrade under field conditions. Thus, a comparison of virus recovery to microsphere recovery allows a direct analysis of virus inactivation rate for the field experiments. The adsorption of the microspheres will also be determined in a fashion similar to the virus.

## **Field Studies**

The field studies will be conducted at the ALRS and the UofA MAC. The ALRS site consists of a 9x9 meter plot, which is divided into nine 3x3 m subplots. The walls separating the subplots are constructed of reinforced concrete and have been coated with theroseal to make the walls waterproof. The site is highly instrumented. Each subplot has been instrumented with one neutron probe port (2-inch ID, 2.5 inches OD), five solution samplers (2-inch OD), and five tensiometers (1-inch OD, with access tube extended to the surface). The tensiometers and solution samplers are installed at depths of 0.5, 1.0, 2.0, 3.0, and 5.0 m. Separate water tanks and application systems will be maintained for each plot, so that the volume of water added to each plot can be measured. Field studies at the ALRS will be conducted to investigate the transport of viruses through fractured bedrock, in this case a welded-tuff.

The UofA MAC site consists of a 50x50 m plot designed for conducting controlled water flow and solute transport studies. This site contains numerous monitoring systems, including: 1) a 60 m trench excavated to 1.5 m depth, into which 13 instrumentation

clusters were installed at 5 m lateral spacing; 2) two vertical culverts (1.55 m diameter) drilled to 3 m depth with monitoring instruments installed radially around the islands into undisturbed soil; 3) vertical and horizontal access tubes into which permanently-installed (tensiometers and solution samplers) or portable sensors (neutron probe) were inserted; and 4) permanently-installed and portable geophysical sensors for monitoring bulk electrical conductivity and resistivity. The water application system at the UofA MAC site consists of a dense-network drip irrigation system. Field studies at the UofA MAC site will be conducted to investigate the transport of viruses through unconsolidated alluvium. Although water will be infiltrated over the entire array, a solution of the virus PRD-1 will be infiltrated over a 2x50 area of the array to simulate a line source.

A conservative tracer (bromide), bacteriophage (PRD-1), and latex microspheres will be infiltrated at each field site to investigate the transport of viruses in the unsaturated zones. PRD-1 will be used as an analog for the human enteric virus. The microspheres will serve a secondary comparison for virus fate and transport. All three tracers will be injected within the same pulse.

To aid in the design of the sampling and injection methods, a preliminary conservative tracer study will be conducted. This study has already been performed at the UofA MAC site. The conservative tracer will be mixed with water and infiltrated at an estimated rate of one centimeter per day at each site. Infiltration of both tracer pulses at ALRS will continue for 30 days and for 21 days at the UofA MAC site. At the end of the preliminary conservative tracer infiltration period, tracer-free water will be allowed to infiltrate for several more weeks. Samples will be collected from the solution samplers and measurements will be taken from the tensiometers at a specified interval. Results from the conservative tracer experiments will be used to design a sampling protocol for the virus infiltration experiments.

The PRD-1 and microsphere concentrations used during the infiltration experiments will be  $10^{12}$  plaque forming units per ml (PRD-1) or microspheres per ml. The PRD-1 and microspheres will be mixed with water and infiltrated at an estimated rate of one centimeter per day at each site. Infiltration of the PRD-1 and microspheres will continue for 30 days at the ALRS and for 21 days at the UofA MAC site. At the end of the tracer pulse water will be infiltrated for several more months. Samples will be collected from the solution samplers and measurements will be taken from the tensiometers at intervals determined from the conservative tracer test. The experiments will be numerically reproduced with a widely used unsaturated flow model.

## **Related Research**

Enteric virus contamination of groundwater supplies is of increasing concern. Potential sources of enteric viruses in groundwater include septic tanks, sewer leakage, sewer sludge beds, domestic solid disposal (landfills), and sewage oxidation ponds (Gerba et al. 1991). Over 120 distinct types of human pathogenic enteric viruses are known, included are enteroviruses, rotaviruses, hepatitis A, astroviruses, caliciviruses, norwalk, and other "small round" viruses (Gerba and Bitton 1984).

To develop guidelines for determining the siting of and the risks posed by waste-disposal sites, an understanding of the transport behavior of viruses in vadose zone systems is required. Few data sets on virus transport under well characterized field conditions are available (Gerba and Bitton 1984 and Bales et al, 1993). Because of the hazards and costs, the non-hazardous bacteriophage PRD-1 has been employed as an analogue to viruses of concern to human health (Bales et al. 1993-1 and Gerba et al., 1984).

Factors controlling virus fate in porous media are attachment to and detachment from the porous media surfaces, growth and inactivation, and advection and dispersion (Bales et al., 1991). Advection is a function of groundwater velocity. Dispersion depends on velocity, aquifer heterogeneity, and is scale dependent. Attachment and detachment of viruses is highly sensitive to groundwater chemistry including pH, ionic strength, and porous media composition (Gerba, 1984). Inactivation (degradation) of viruses depends strongly on temperature (Yates, et al., 1987).

Laboratory research on the transport of viruses through soil has been conducted by Kinoshito et al. (1993), and Bales et al. (1989) among others. However few controlled field studies have been conducted. Studies of viruses traversing through aquifers under waste-water infiltrations condition have been reported. Noonan and McNabb (1979) found bacteriophage T4 and 174 was transported over 900 meters from waste-water disposal site in a highly permeable alluvial aquifer. Another study of virus transport from infiltrating waste-water was accomplished by Gerba, et al. (1991).

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