VIRUS TRANSPORT AND SURVIVAL

IN SATURATED AND UNSATURATED FLOW THROUGH SOIL COLUMNS

by

David Keith Powelson

A Dissertation Submitted to the Faculty of the
DEPARTMENT OF SOIL AND WATER SCIENCE
In Partial Fulfillment of the Requirements
For the Degree of
DOCTOR OF PHILOSOPHY
In the Graduate College
THE UNIVERSITY OF ARIZONA

1 9 9 0

THE UNIVERSITY OF ARIZONA
GRADUATE COLLEGE

As members of the Final Examination Committee, we certify that we have read the dissertation prepared by David Keith Powelson

entitled VIRUS TRANSPORT AND SURVIVAL IN SATURATED AND UNSATURATED FLOW THROUGH SOIL COLUMNS

and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

James R. Simpson — Date 10/30/90

Arthur W. Warrick — Date 10/30/90

Ian L. Pepper — Date 10/30/90

Charles P. Gerba — Date 10/30/90

Date

Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copy of the dissertation to the Graduate College.

I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

Dissertation Director James R. Simpson — Date 10/30/90

STATEMENT BY AUTHOR

This dissertation has been submitted in partial fulfillment of requirements for an advanced degree at The University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the Library.

Brief quotations from this dissertation are allowable without special permission, provided that accurate acknowledgment of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the Graduate College when in his or her judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

SIGNED: David Keith Powelson

ACKNOWLEDGEMENTS

This research was supported in part by U.S. Environmental Protection Agency through contract CR-81147, and in part by the University of Arizona through graduate research assistantship funds.

I would like to thank Dr. C. P. Gerba and Dr. J. R. Simpson for supervising this project and for always being available for advice. Dr. S. Kutz-Bradford performed the MS-2 virus adsorption tests on the porous stainless steel and ceramic materials (supporting experiment 2). Dr. A. W. Warrick made valuable suggestions on mathematically fitting the data, and Dr. R. C. Bales offered important editorial comments.

TABLE OF CONTENTS

page

LIST OF ILLUSTRATIONS 8

LIST OF TABLES 10

ABSTRACT 11

CHAPTER 1. INTRODUCTION 13

Definitions 13

Extent and Impact of Virus in Water 13

Factors in Virus Removal 15

Electrostatic effects 15

Hydrophobic effects 17

Other adsorption considerations 19

Unsaturated flow effects 19

Variability in removal 22

Other Reasons for Studying Virus Transport 24

Sampling Devices 25

Purpose of this Research 27

CHAPTER 2. MATERIALS AND METHODS 28

Virus 28

Soil Columns 29

Water 32

Samplers 35

Eluants 35

Mathematical Fitting 36

CHAPTER 3. RESULTS AND DISCUSSION 42

Overview 42

Saturated Versus Unsaturated Flow 42

Effect of Organic Matter on Unsaturated Transport 53

Bromide Transport 58

CHAPTER 4. POSSIBLE REMOVAL MECHANISMS AND IMPLICATIONS 60

Effect of Unsaturation on Adsorption 60

Inactivation Mechanisms 63

Implications for Water Treatment 66

Future Research 68

CHAPTER 5. CONCLUSIONS 70

APPENDIX A. DATA FROM SUPPORT EXPERIMENTS (SE) 71

SE1: Soil Water Volume Versus Water Potential Relationships 71

SE2: Virus Removal by Samplers 73

SE3: Virus Survival with KBr 76

SE4: Batch Virus Removal by Soil 78

SE5: Virus Removal by Container Material 80

APPENDIX B. DATA FROM TRANSPORT EXPERIMENTS (TE) 82

Legend for Transport Experiments 82

TE1: Short Term Test 82

TE2: Medium Term Test 84

TE3: Long Term Test with Ponding 86

TE4: Testing the Effect of Different Flow Rates 89

TE5: All Column Effluent Extracted through a Sampler 91

TE6: Saturated Versus Unsaturated Flow 93

TE7: Unsaturated to Ponded 95

TE8: Longer Term Unsaturated to Ponded 97

TE9: Unsaturated to Ponded like TE3 99

TE10: Fresh Soil Versus Leached Soil 102

TE11: Saturated Versus Unsaturated and Eluant Test 105

TE12: Sludge Pretreatment Versus Untreated 108

APPENDIX C. A COMPARISON OF MATHEMATICAL MODELS 110

REFERENCES 116

LIST OF ILLUSTRATIONS

Figure page

1. Experimental apparatus showing solution application, sample extraction, and pressure measurement methods. Only one of the six pairs of sampler and manometer tubing is shown 30

2a. The relative virus concentrations versus pore volumes and fitted breakthrough curves at 0.20 m. $(C/C_o)_s$ is the apparent steady-state value. The markers are labeled by transport experiment number and column letter. The curves were constructed from Eq. [4] 45

2b. The relative virus concentrations versus pore volumes and fitted breakthrough curves at 0.40 m. 46

2c. The relative virus concentrations versus pore volumes and fitted breakthrough curves at 0.80 m. 47

2d. The relative virus concentrations versus pore volumes and fitted breakthrough curves at 1.05 m. 48

3. The relative virus concentration profiles under saturated flow (TE6E) at four sampling times. The average linear velocity was 0.24 m d^{-1} 50

4. The relative virus concentration profile under unsaturated flow (TE3 and TE9) from day 3 (one pore volume at 1.05 m) to day 9, and fitted curve using Eq. [4] and [5], where $u = 4.62\ d^{-1}$, z is depth (m), $v = 0.37\ m\ d^{-1}$, $G = 3\ m^{-1}$, and $L = 0.05$ m . . . 51

5. The relative virus concentrations versus pore volumes at the 1.05 m depth and fitted breakthrough curves in transport experiment 10. $(C/C_o)_s$ is the apparent steady-state value. The curves were constructed from Eq. [4]. 55

6. The relative virus concentrations versus pore volumes at the 1.05 m depth and fitted breakthrough curves in transport experiment 12. $(C/C_o)_s$ is the apparent steady-state value. The curves were constructed from Eq. [4]. 57

7. The relative virus concentration profile with depth from TE10 and TE12 on day 7 (1.8-1.9 pore volumes). The fitted curves were constructed from Eq. [4] and [5], where z is depth (m), $v = 0.27\ m\ d^{-1}$, $G = 3\ m^{-1}$, $L = 0.05$ m, and the organic $u_o = 0.81\ d^{-1}$ and the leached $u_l = 3.1\ d^{-1}$ 59

8. A conceptual model of virus adsorption and inactivation at the air-water interface. "H" represents hydrophobic groups, and "-H" represents dissolved organic matter that is negatively charged at pH 8.1. The net negative charge on the virion keeps it from sorbing to the soil solids. Hydrophobic groups on the virion tend to be excluded from water, and permit the virion to be adsorbed to the air-water interface. Surface tension distorts adsorbed virus leading to inactivation. If the air-water interface is coated with an organic microlayer the virus does not adsorb and does not become inactivated. 67

9. Curves generated by MISCIB and BTHRU at depths of (a) 0.4 m and (b) 1.05 m; $(C/C_o)_s$ is the apparent steady-state relative concentration. The MISCIB curves are the same as in Fig. 2b and 2d. For the Curve labeled BTHRU1, $k_p = 22.5\ cm^3 / g$ and $k_f = 4.87 \times 10^{-6} / s$; for BTHRU2, $k_p = 113.4\ cm^3 / g$ and $k_f = 4.87 \times 10^{-6} /s$; and for BTHRU3, $k_p = 113.4\ cm^3 /g$ and $k_f = 4.38 \times 10^{-6}/s$. 112

10. The depth profile showing steady-state relative virus concentrations from days 3 to 8 of TE3 and TE9 (each point is the average of four replicate columns), the curve generated by the equations of Jury et al. (1987), and the curve generated by BTHRU1 for day 3. 115

LIST OF TABLES

Table page

1. Soil characteristics ... 31
2. Water characteristics ... 33
3. Summary of transport experiments and major variables ... 43
4. Saturated versus unsaturated study fitting parameters for relative concentration versus pore volume curves (Fig. 2) ... 44
5. Eluant effect on the partition coefficient, k_p, TE11 ... 52
6. Partition coefficients k_p and number balances for five columns using TSB as the eluant ... 54

ABSTRACT

Water with entrained disease-causing virus entering soil normally passes through water saturated and unsaturated regions before reaching the groundwater. Twelve experiments were conducted to evaluate the effect of saturated versus unsaturated flow, and the effect of organic matter in unsaturated flow on the survival and transport of a virus, MS-2 bacteriophage, in soil columns. Additional experiments were conducted to characterize the soil, to assure that the experimental equipment did not remove virus, and to determine the extent of reversible adsorption of virus to soil.

The virus were added to well water and applied to soil columns 0.052 m in diameter and 1.05 m long. KBr was used as a chemical tracer. In two experiments organic matter in the soil water was increased by using soil humic material or extract from sewage sludge. The soil material was Vint loamy fine sand (a sandy, mixed, hyperthermic Typic Torrifluvent) mixed with recent alluvium. Water samples were extracted from 0.10, 0.20, 0.40, and 0.80 m depths through porous stainless steel samplers, and from the 1.05 m depth through the percolate tube. Four different eluants were tested to remove virus from soil, and one, tryptic soy broth, was used to elute virus after three transport experiments.

For saturated flow the virus concentrations reached the influent concentration in less than 2 pore volumes (T), with a retardation coefficient R at 1.05 m = 0.80. For unsaturated flow with low organic matter the relative concentrations reached steady-state values $(C/C_o)_s$ ranging from a mean of 27% of inflow at 0.20 m (5 to 18 T) to a mean of 5% at 1.05 m (1 to 3.3 T). Under unsaturated conditions with increased organic matter, virus $(C/C_o)_s$ at 1.05 m was from 41% to 49% of influent, 8 to 10 times greater than with low organic matter. Elution permitted calculation of a partition coefficient k_p essentially equal to 0 (saturated average k_p = -0.07 mL/g, SD = 0.15 mL/g; unsaturated average k_p = 0.28 mL/g, SD = 0.40 mL/g), indicating little or no adsorption of virus to soil solids.

Under unsaturated flow conditions enhanced removal of this virus occurs, and the removed virus are apparently inactivated. Organic matter reduced the removal of virus during transport by unsaturated flow. Virus concentrations reached and maintained a steady-state, exponentially-declining profile with depth.

CHAPTER 1.

INTRODUCTION

Definitions

Some terms that will be used in this report are defined in detail in an overview of virus structure by Harrison (1990): the capsid is a protein shell directly surrounding viral nucleic acid, a virion is the entire virus particle, a virus is a collection of virions from a single type, and viruses are several types of virus. A plaque forming unit, pfu, is the number of virions required to form one infection center or plaque on a layer of the host cells. For the MS-2 virus used in this research, a pfu may be considered to represent a single infectious virion. MS-2 is a nonenveloped bacteriophage with icosahedral symmetry, and its host is Escherichia coli. "Coliphages" are bacteriophages such as MS-2 that attack E. coli. When virus are no longer infective they are usually called "inactivated," although terms such as "decay," " half-life," and "killed" have been used by various investigators. A "$\log_{10}$ reduction" is a reduction in virus concentration by a factor of ten; for example, 10^5 pfu/mL to $10^{2.5}$ pfu/mL is a 2.5 $\log_{10}$ reduction. The "isoelectric point" is the pH at which a virion is electrokinetically uncharged.

Extent and Impact of Viruses in Water

The disposal of sewage by infiltration through soil is believed to be an effective means of removing most pathogenic virus as long as the waste is exposed to several meters of unsaturated flow (Reneau et al., 1989). However, the soil factors that influence removal of virus are poorly understood, and a single virus particle may cause infection (Plotkin and Katz, 1966; Ward and Akin, 1984). The risks of infection, disease, and mortality from water contaminated with virus has been calculated. For example, one hepatitis A virus in 10^4 L of drinking water over a lifetime results in a 7% risk contracting the disease (Gerba and Haas, 1988). More than 10^6 infectious virus particles may be excreted per gram of feces by infected persons, and concentrations as high as 10^5 infectious virus particles per liter have been detected in raw sewage

(WHO, 1979). The use of contaminated groundwater was responsible for 51% of all waterborne disease outbreaks in the United States during 1971-1982, and of these 18% were caused by virus and 69% were unidentified cases of gastroenteritis (Craun 1985). It is likely that most of the unidentified cases were caused by virus.

The presence of pathogenic viruses in wells and drinking water in most parts of the world has been well documented (Gerba, 1987; Payment and Armon, 1989; Gerba and Rose, 1990). In a survey of 99 wells in Israel, Marzouk et al. (1979) found enteroviruses in 20% of the ground-water samples. Virus were found in the ground water 27.5 m beneath a field of hay on clay loam irrigated with sewage effluent (Goyal, et al., 1984). In Minnesota Goyal et al. (1989) detected human pathogenic virus in 10 of 26 ground water sources; and on two occasions coliphage were isolated from samples in which coliform bacteria were absent. Since the presence of coliform bacteria is the standard criterion indicating water contamination, this finding underlines the importance of monitoring virus transport to ground water.

Virus in wastewater has a strong tendency to sediment with sludge during sewage treatment (Cliver, 1976), so sludge should be disposed of in such a way that the virus is inactivated before it reaches ground water. The treated wastewater with the sludge removed also contains virus. Gerba et al. (1984) obtained samples of ground water near 6 sewage treatment infiltration basins; viruses were isolated from as deep as 30 m and from as far as 100 m from the basins. Septic tanks, however, are the most frequently reported cause of ground water contamination in the US (Yates, 1985). Vaughn et al. (1983) detected virus originating from septic tanks that had passed through 3.6 m of unsaturated soil and 67 m of saturated soil.

Once virus contaminate an aquifer, water treatment cannot be depended on to prevent these virus from infecting consumers of the water. Keswick et al. (1985) found rotavirus in 3 out of 26 samples of finished drinking water from a full-scale drinking water treatment plant that met acceptable limits for turbidity, total coliform bacteria, and residual chlorine. In Mexico rotavirus

was detected in 11 of 11 tapwater samples (Rao, 1982). Most common pathogenic viruses and coliphages are more resistant to chlorination than are Escherichia coli. The presence of coliphage in 82 out of 147 potable water samples strongly suggested to El-Abagy, et al. (1988) that pathogenic viruses can survive the normal treatment and disinfection processes.

Factors in Virus Removal

Electrostatic effects

Several mechanisms have been proposed to explain the loss of virus from soil water including electrostatic and hydrophobic interactions with the soil matrix (Preston and Farrah, 1988; Sobsey and Shields, 1987). Electrostatic attractions of virus to soil would appear to be favorable only under conditions where the pH of percolating fluid is between the isoelectric point of the virus and that of the soil (Vaughn and Landry, 1983, p. 180). Zerda et al. (1985) studied the adsorption of poliovirus 1, reovirus 1 and 3, and bacteriophages MS-2 and T2 to charge modified silica. They found that all viruses adsorbed exclusively to negatively charged silica at pH values below their isoelectric points, and all virus adsorbed exclusively to positively charged silica at pH values above their isoelectric points. In neutral solution and most natural waters, viruses are negatively charged (as was the case in the current experiments), and thus there occur strong electrostatic repulsion forces between viruses and similarly charged surfaces (USEPA, 1977, p. D14; Gerba, 1983).

Although silica and most clays have net negative charge in the neutral pH range, there are positive sites at clay edges, metal oxides, and $CaCO_3$ deposits. Some soil material isoelectric points are: kaolinite (4.7), quartz (2.0), and calcite (10.0) (Sposito, 1984, p. 84). Iron oxides, particularly magnetite, display a high affinity for viruses (Gerba and Bitton, 1984). Schiffenbauer and Stotzky (1982) found that pretreatment of kaolinite and montmorillonite with sodium metaphosphate, which binds to positively charged sites on clays, reduced the adsorption of bacteriophage T1, indicating that T1 was adsorbed primarily to anion exchange sites.

Charge on the virion is also not spread uniformly, but is concentrated where there are charged groups, resulting in variations in charge over the virion surface (Mix, 1974). Multivalent cations such as Ca^{2+} may complex with hydroxo sites of the protein surface of virus and form positive sites for adsorption to negative media (cation bridging) (Mix, 1974; Jenkins et al., 1980). Lipson and Stotzky (1983) found that the adsorption of reovirus 3 to montmorillonite depended on the type of cation in solution, with strength of adsorption varying according to: Al > Ca > Mg > Na > K. They also point out (Lipson and Stotzky, 1987) that the surface pH of clay minerals is presumably 3 to 4 units lower than the bulk pH of the suspension. This would permit proton transfer from the surface of the clay to the virion making it positively charged and permitting electrostatic adsorption. It is not clear, however, how an electrostatically repelled virus can approach and remain in this low pH region long enough for proton transfer and adsorption to occur.

At ionic strength greater than about 10^{-2} M the electrostatic diffuse layer shrinks and electrostatic repulsion is overpowered by van der Waals attraction (Stumm and Morgan, 1981, p. 659). Lipson and Stotzky (1983) found that 10^{-2} M solution was more effective than 10^{-3} M in adsorbing reovirus 3 to clay minerals, apparently by reducing electrostatic repulsion.

At an intermediate ionic strength often found in ground water (10^{-3} M) van der Waals attraction creates a "secondary minimum" at the outer fringe of the electrostatic effect where adsorption may occur (Stumm and Morgan, 1981, p. 659). However, this weak attraction may be counteracted by stirring or thermal diffusion (Hiemenz, 1977, p. 426).

Low ionic strength water such as rain tends to desorb virus from soil. Apparently this low salt water expands the diffuse layer which prevents close approach of the virus to a similarly charged surface. By alternating effluent (electrical conductivity = 0.073 S/m) and distilled water as infiltrating liquids through loamy sand, Dubiose et al. (1976) were able to demonstrate that distilled water effectively elutes poliovirus 1. Wellings et al. (1975) isolated virus from sandy soil

infiltrated with sewage effluent at 1.5 m but not at 3.0 m until after heavy summer rains. (The rain also created saturated conditions which may have contributed to the virus transport.) Roper and Marshall (1974) adsorbed a tailed coliphage to saline sediment, and by diluting the sea water in steps found a rapid increase in desorption which coincided with the dispersal of sediment colloidal material.

Hydrophobic effects

Under the pH conditions of the current research (pH = 8.1) it is likely that hydrophobic effects would be the most important force removing virus from the water flow (Murray, 1980; Farrah et al., 1981). Although this effect is sometimes called hydrophobic adsorption, it might be better called hydrophobic exclusion, since the main force is due to water molecules "squeezing" hydrophobic material out of bulk water. This effect is greater than one would calculate from van der Waals' attraction between hydrophobic material alone. Hydrophobic attraction involves decreasing interfacial contact area between nonpolar side chains and water, resulting in the formation of hydrogen bonds between water molecules (Mix, 1974). Entropy is increased when organic molecules displace water molecules at a hydrophobic surface resulting in greater stability of the system (Jury, 1986).

Hydrophobic attraction appears to be almost entirely related to the structure of water and can be increased by changing the structure of water in the direction of greater order (Hatefi and Hanstein, 1969). Antichaotropic anions (SO_4^{2-} or Cl^- as opposed to SCN^- or NO_3^-) are less disruptive of water structure, and promote hydrophobic attraction and "salting out" of nonelectrolytes (Hatefi and Hanstein, 1969). Farrah (1982) found that antichaotropic ions such as F^-, Mg^{2+}, and citrate^{4-} promoted the adsorption of MS-2 to membrane filters at pH 9.5. Presumably these ions increased the structure of water which made the water less able to accommodate the hydrophobic groups on the virus.

Partly hydrophobic organic matter, however, interferes with virus adsorption to containers

and microporous filters with larger pore size than the diameter of the virus. Ward and Winston (1985) showed that the degree of adherence of virus to glass and polypropylene containers was inversely related to the turbidities of the water; they proposed that this was probably due to the scarcity of competitive binding substances in the clear waters. With electronegative filters, humic acid concentrations above 10 mg/L prevented poliovirus 1 adsorption (Guttman-Bass and Catalano-Sherman, 1985). Humic acid decreased adsorption of poliovirus type 1 to membrane filters (Guttman-Bass and Catalano-Sherman, 1986); complexation of the virus with humic materials was not observed. Sobsey and Hickey (1985) found that soluble organic matter reduced the effectiveness of poliovirus 1 collection by membrane filters. They performed a single particle analysis and found a high percentage of single virus particles in suspension with humic and fulvic acids. This indicated that soluble organic matter interfered with filter effectiveness by competing with the virus rather than by complexing with it.

Similarly, organic matter appears to reduce virus removal by soil. A strong negative correlation was found between poliovirus 2 adsorption to 34 minerals and soils, and the content of organic matter in the substrate (Moore et al., 1981). Gerba et al. (1981) correlated eight viruses including MS-2 with 14 soil characteristics; they found that amount of organic matter was negatively correlated with virus adsorption ($r = -0.492$), and it was the second most strongly correlated factor after pH. Viruses adsorb poorly to muck soil (Moore et al., 1982; Gerba and Bitton, 1984, p. 74). Overgrowth of soil with bacterial exopolymer significantly decreased adsorption of virus resulting in a negative correlation between virus removal from soil water and percent organic carbon (Fuhs et al., 1981, p. 2244). Schaub and Sagik (1975) observed that the addition of 0.6 mg/mL of serum protein reduced adsorption of encephalomyocarditis virus to clay from 95.8 to 16%. Lipson and Stotzky (1984) concluded that decreased adsorption of reovirus to montmorillonite complexed with lysozyme, chemotrypsin, and ovalbumin probably resulted from the blockage of negatively charged sites on the clay by each protein. Dizer et al. (1984) found

that running soil columns with secondary treated wastewater instead of distilled water allows more enterovirus to pass through sand columns. They attributed this to the high organic and surfactant load in the wastewater which disrupted the hydrophobic bonding of the virus to sand. Stagg et al. (1977) found that the presence of 3 mg of organic carbon per liter of 0.05 M $MgCl_2$ solution reduced adsorption of MS-2 bacteriophage to bentonite from 97 to 35%. Bixby and O'Brien (1979) found that fulvic acid complexed with MS-2 bacteriophage, and prevented adsorption of virus to soil. Lance and Gerba (1984a) found that organic compounds in sewage counteracted the effect of higher salt content and lessened the adsorption of poliovirus to soil. The only exception seems to be the research of Landry et al. (1979) who found that exposure to sewage effluent enhanced a very coarse soil's ability to adsorb virus. This may have been due to plugging of the soil surface which resulted in unsaturated flow and greater removal of virus.

Other adsorption considerations

Filtration is unlikely to be a major factor in the removal of virus by sandy soil since the size of suspended particles needs to be greater than 0.07 times the particle size of the medium to allow bridging or blocking (Bouwer, 1984); by this rule MS-2 virus are about 280 times too small to filter by 0.1 mm sand. It should be remembered, however, that there are porous fabrics of silt and clay in even sandy soil, and organic materials as well. The mycelia of fungi are often extended throughout the soil, and particles are entrapped and tied together (Stevenson, 1982, p. 396). Sphaerothilus, a genus of sheathed bacteria, form filaments 1-2 μm wide of protein-polysaccharide-lipid complex (Brock, et al., 1984, p. 658). Foster (1981) observed polysaccharide fibers 8 to 10 nm in diameter stretching across fine pores in soil crumbs to form an open mesh in which small stacks of clay platelets became entangled. As soil dehydrates surface tension effects would tend to concentrated polysaccharides in crevices between adjacent clay platelets (Foster, 1981), and this might contribute to the increased removal of virus in unsaturated conditions.

Unsaturated flow effects

Unsaturation of soil results in a number of physical effects that influence virus transport. The strength of adsorptive forces might be enhanced in unsaturated conditions since the largest pores are filled with air, and the viruses are forced closer to the particle surfaces (Lance and Gerba, 1984b). A particle suspended in laminar flow in a small pore may experience a torque due to differences in flow velocity across its diameter, and this sets up a rotational motion causing movement in the direction of lower fluid velocity (Vinten and Nye, 1985). This tendency of colloids to migrate to regions of lower shear is maintained even against an appreciable concentration gradient (Hunter and Alexander, 1963). This may in part be due to the lower self diffusion coefficient for colloids, which is of the order of 10^{-11} m^2 s^{-1}, compared to small molecules which is of the order of 10^{-9} m^2 s^{-1} (Hiemenz, 1986, p. 81). The degree of dispersion of a chloride breakthrough curve under unsaturated conditions may be three to four times greater than that when the soil is saturated due to an extremely wide range in microscopic pore velocities associated with the nearly emptied pores in unsaturated conditions (Nielsen and Biggar, 1962). The accelerated arrival of contaminants at a discharge point and increased "tailing" are characteristic features of dispersion resulting from some parts of the contaminant plume moving faster than the average water velocity (Anderson, 1984, p. 37). Nielsen et al. (1986) described the effects of unsaturated flow on solute transport models; some important considerations are: the diffuse layer becomes more dominant in its effect on hydraulic conductivity and ion exchange, hysteresis must be taken into account, and flow may concentrate in preferred paths. Colloids such as virus may show more dispersion than solutes because they are concentrated in the larger pores with greater hydraulic conductivity (K). [$K = r^2/(8\eta)$, for a cylinder of radius r, where η is viscosity according to Poiseuille's law (Hillel, 1982, p. 91-94).]

Unsaturated conditions in the field have usually been found to reduce virus transport. A survey of the ground water beneath three sewage treatment plants showed that greater human

virus removal was achieved when the depth to ground water was greater (Vaughn et al., 1978). Cogger et al. (1988) found that one septic tank leach field with 31-45 cm depth to ground water gave a 2.5 $\log_{10}$ reduction in bovine enterovirus 1, whereas another field with 61-90 cm depth to ground water gave a 3.6 $\log_{10}$ reduction. Sewage disposal in rivers may be altered when seepage is restricted during low flows because of plugging by fine sediments, creating unsaturated conditions for infiltration (Schultz et al., 1976).

Laboratory studies support the observation that greater virus removal occurs in unsaturated conditions. Yeager and O'Brien (1979) observed a decline in poliovirus 1 infectivity with soil water content (no flow); and the rate of inactivation increased markedly at less than 0.012 (kg kg^{-1}) moisture. Lance and Gerba (1984b) found that saturated flow through "flushing meadows" loamy sand resulted in 7% recovery of poliovirus at a depth of 0.10 m, but unsaturated flow resulted in only 0.5% recovery. Vaughn et al. (1981) found that infiltrating a poliovirus 1 suspension at 0.06 m/h through coarse sand resulted in a concentration 0.5% of influent at 2.25 m, whereas infiltrating at 0.005 to 0.02 m/h which produced drier conditions resulted in a concentration 0.05% of influent at 2.25 m. Duboise et al. (1974) applied T7 coliphage on sandy soil columns under continuous (saturated) or intermittent (mostly unsaturated) flow conditions. By the end of the experiments 2 to 9% of the virus applied to the saturated columns were collected in the effluent, whereas only 1.2% were recovered from the unsaturated columns. Initially, however, the unsaturated columns showed higher virus concentrations in the effluent, which probably reflected higher dispersion in the unsaturated columns. The saturated versus unsaturated part of the current research (Powelson et al., 1990) shows that under unsaturated flow conditions at 1.05 m MS-2 virus concentration was about 5% of concentration under saturated conditions.

The current study extended that of Bradford (1987) which found that MS-2 removal by saturated flow through 0.85 m of "flushing meadows" soil at 4°C averaged 11.3% (SD = 19.8, n

= 10). The large standard deviation may have been in part due to the normal variability of the bacteriophage assay. The flow rates Bradford used were relatively high for this loamy fine sand (average linear velocity v = 4.20 m d^{-1}), and this may have resulted in excessive preferential flow and variation in effluent virus concentration. Nevertheless, Bradford's results indicate very little virus removal under saturated flow conditions.

Variability in removal

Virus adsorption and inactivation varies greatly, depending on the type of virus, type of soil, water content, pH, temperature, and salt content of the water (Gerba, 1984). The virus isoelectric point and the pH determine the charge on the virus and tendency to adsorb to charged surfaces including soil. Goyal and Gerba (1979) conducted batch studies with 28 viruses and 9 soils and found adsorption ranging from 0.01 to 99.9%. During recharge of wastewater through sand Jansons et al. (1989a) recovered echovirus 2 at a depth of 9 m in ground water from a well 14 m from the recharge basin, whereas seeded poliovirus were not isolated beyond a depth of 1.5 m below the basin. Drewry and Eliassen (1968) reported 50 to 99.99% removal of bacteriophage f2 by mixing with batches of various soils for 24 h. Yet Schaub and Sorber (1977) recovered f2 at 47% of the applied concentration after 72 hours of flow through 18 m of unsaturated silty sands and gravel.

Viral inactivation rates vary widely from one virus to another. The review by Reddy et al. (1981) stated that the half life of contamination-indicator viruses in soil varied from 4.7 h for coxsackievirus A-9 to 415.8 h for poliovirus. Yates et al. (1986) found that MS-2 decay rates ranged from 0.068 to 0.71 log PFU d^{-1} in Tucson ground water, and used this information to recommend septic tank setback distances. They cautioned that hepatitis A virus may persist for much longer periods than other enteroviruses so that the use of MS-2 may lead to an underestimation of the survival times of hepatitis A virus in ground water.

Under some conditions virus removal by soil is very efficient. Bitton, et al. (1984) applied

poliovirus 1 and echovirus 1 to fine sand columns 0.33 m long, allowed these viruses to leach for 2.5 months by natural rainfall, and found no virus observable in the leachate. By simulating the natural rainfall in Denmark for seven months on unsaturated sandy soil, Jorgensen (1985) found that soil samples below 0.035 m did not contain detectable amounts of the applied Coxsackievirus B3 and adenovirus 1. At the Flushing Meadows Wastewater Treatment Facility near Phoenix, Arizona, Gilbert et al. (1976) reported no detectable virus after secondary treated sewage infiltrated through 3 m of unsaturated fine loamy sand and 6 m of saturated coarse sand and gravel. (The "flushing meadows" fine loamy sand was the same soil material used in this research.) Pepper et al. (1981) used 0.60 m long columns planted to bermudagrass and infiltrated secondary treated sewage seeded with poliovirus 1, echovirus 5, and Coxsackie B3; and found 99% removal of the viruses.

Batch studies of virus adsorption and inactivation may give very different results from column transport studies. For example, Koya and Chaudhuri (1977) infiltrated MS-2 with well water (pH = 7.8) through 15 cm of silt by periodic ponding and found the maximum output concentration was 0.3 % of the influent. They attributed this loss to adsorption by the soil because their batch studies indicated adsorption of MS-2 to silt was 7×10^7 pfu/g. However, the current research (support experiment 4) and that of Goyal and Gerba (1979) indicate that at pH 7.8 there should be very little adsorption of MS-2. Koya and Chaudhuri used the normal method of determining batch adsorption by measuring the concentration of MS-2 remaining in the supernatant and assuming the removed virus were adsorbed to the soil. This method does not take into account adsorption to the container and inactivation of the virus. A true estimate of reversible adsorption to soil can only be obtained by eluting virus from the soil. Consequently, the current research did determine virus adsorption by elution (transport experiments 9, 10, and 11). Koya and Chaudhuri's column study results may have been due to unsaturated flow conditions.

Theoretically, more virus should be adsorbed in a soil column than in a batch experiment. Miller et al. (1989) show that in the thermodynamically open flow system, the sorbent is exposed to a much greater total mass of sorbate than in the closed batch system, so that the potential exists for much greater sorption in the flow system. In addition, competition from desorbed antecedent species will be less important in flow systems because these species are removed in the effluent solution, which should also contribute to greater adsorption in a flow system.

Other Reasons for Studying Virus Transport

Tracing the movement of pathogenic microorganisms in ground water is essential to understanding and correcting ground water contamination. Bacteriophages appear to be the microorganisms most suited as a microbial tracer due to their size, ease of assay, and lack of pathogenicity (Keswick et al., 1982). In 47% of 25 samples of drinking water from Lima, Peru, coliphages were the only contamination indicator organisms present (Ratto, et al., 1989). Although bacteria are normally 10 to 100 times larger than virus, dwarf bacteria 80 nm in diameter, in the virus size range, have been observed with a transmission electron microscope (Bae et al., 1972).

The study of virus transport may help improve our understanding of the transport of other colloids. Any particle which has some linear dimension between 1 nm and 1 μm is considered a colloid (Hiemenz, 1977, p. 1). McDowell-Boyer et al. (1986) reviewed the incidence, mechanisms, and mathematical modelling of particle transport through soil. They emphasized that transport of pollutants adsorbed to mobile particles is dominated by particle removal mechanisms not related to the partitioning of pollutant between a mobile and aqueous phase and an immobile, adsorbed phase. Colloids may act as a third phase to increase the amount of contaminant transported by ground water. Plutonium and americium disposed of at a liquid seepage site adsorbed to colloids and migrated up to 30 m, in contrast to predictions that were based on laboratory measurements of radionuclide binding to immobile subsurface material which

forecast that migration would be limited to a few millimeters (McCarthy and Zachara, 1989). The herbicides atrazine (K_{oc} = 1850) and linuron (K_{oc} = 6750) are likely to be strongly bound to natural estuarine colloids (K_{oc} is the organic carbon/water partition coefficient)(Means and Wijayaratne, 1982). DDT adsorbed on sewage effluent solids and was observed at greater depths than expected due to colloid transport (Vinten et al., 1983). Colloidal particles may be formed of ferrous phosphate during infiltration of sewage, and metals or hydrophobic compounds may sorb to these mobile colloids resulting in enhanced transport (Gschwend and Reynolds, 1987).

Sampling Devices

The study of unsaturated transport of virus requires the use of sampling devices that are able to extract soil water that is at less than atmospheric pressure. Litaor (1988) reviewed the types of soil solution samplers and factors involved in their use. Of the many considerations in the selection of appropriate soil water samplers, two are critical in this research. First, the sampler pore size must be suitable to permit extraction of a sufficient flux of water from soil held at the experimental water potential. Second, the sampler must not adsorb the material of interest. In the case of viruses, adsorption is dependent on the isoelectric point of the virus, pH, and the electrostatic and hydrophobic nature of the sampler port material.

When extracting soil solution in unsaturated conditions, soil air is kept out of the samplers by the water menisci across the pores of the sampler surface. Water is extracted when a potential lower than that of the soil water is created in the sampler. Soil air has a stronger extraction gradient since it is at a higher pressure (atmospheric) than the unsaturated soil water; consequently, air will break through into the sampler if the water's tensile strength across the sampler pores is exceeded. The relationship between pore size and air entry may be approximated by the capillary rise equation for free height of rise of water in a vertical capillary tube with one end of the tube submerged in water. A soil system or porous material may be thought of as having an effective pore radius equivalent to the capillary radius. The capillary rise

equation may be expressed as:

$$r = (2 \gamma \cos \kappa)(\phi \rho_w)^{-1} \qquad [1]$$

where r (m) is the radius of the pore, γ is the surface tension of water (0.075 J m^{-2} at 4° C), κ is the contact angle, ϕ (J kg^{-1}) is the potential energy difference across the air-water meniscus, and ρ_w is the density of water (1000 kg m^{-3} at 4° C) (Danielson and Sutherland, 1986).

From Eq. [1] it is clear that a greater range of unsaturated conditions may be sampled with a smaller pore size. However, the pore size of the samplers must be large enough so that they do not clog with fine soil particles, and to permit high enough flow velocity to obtain the necessary volume in a reasonable amount of time. By Poiseuille's law the mean velocity (v_c) for fluid flow in a cylinder is:

$$v_c = \Delta p \, r^2 / (8 \eta L)$$

where Δp is the pressure gradient, η is fluid viscosity, and L is cylinder length (Hillel, 1982, p. 92). In general, ideal samplers have a large enough pore size to prevent clogging, yet a small enough pore size to block the entry of air into the samplers.

The contact angle κ in Eq. [1] is the angle the surface of a liquid makes with a surface of another material. It is a measure of the attraction of the liquid to the material; a zero contact angle indicates the strongest attraction. Eq. [1] shows that at a given potential the largest pore size is obtained when the contact angle is as small as possible. Since ceramics, glass, and metals generally have smaller contact angles than hydrocarbons, such as plastics (Landolt-Bornstein, 1956, p.473-479), it is likely that a porous sampler made of ceramic, glass, or metal would permit larger pore size than plastic filters.

Ceramics, glass, and oxidized metals, however, tend to readily adsorb virus (Gerba, 1984). Ceramic sampling ports have been found to vary in their suitability for testing viruses and bacteria. Dazzo et al. (1974) found porcelain cup soil water samplers (pore size 3 to 8 μm) to reduce the fecal coliform concentrations 10^2 to 10^7 fold. Schaub et al. (1982) found that passing a sample

through porous ceramic caused about a 99% loss of f2 bacteriophage. Coxsackievirus B3 titers were decreased approximately 2 log units due to the use of ceramic probes in lysimeter studies (Damgaard-Larsen et al., 1977). Wang et al. (1980) found a ceramic that caused no more than 11% loss. However, its use is limited to very wet soils due to the large pore size (20 μm) and consequent air-entry potential difference of about 3.2 J/kg. Brown et al. (1979) used a ceramic sampler that passed coliphages, although they did not report the efficiency.

Stainless steel samplers seem to be the most promising for virus sampling, and may be durable and chemically resistant. Technology of sintering metal powders into porous objects has been well developed for flow regulation with pore sizes ranging from 0.5 to 100 μm. It is apparent from the number of different soil water samplers available and their potential for virus retention, that samplers must be individually tested for their retention of virus or bacteria prior to use in soil column studies.

Purpose of this Research

The purpose of this research was to evaluate the effects of saturated versus unsaturated flow on the transport of MS-2 bacteriophage. Breakthrough curves and equilibrium concentrations were fit to existing mathematical models (Amoozegar-Fard et al, 1983; Jury et al., 1987). To determine whether virus not detected in the soil water were adsorbed or inactivated, samples of soil were eluted to recover adsorbed virus and the distribution coefficients calculated. A number balance to account for all the input virus was determined to assess viral inactivation for saturated and unsaturated flow. The effects of natural humic material and sewage sludge organic matter on virus transport through unsaturated soil were evaluated. A test of whether the effects were due to complexation or competition for adsorption sites was conducted. Possible mechanisms of virus adsorption and inactivation in unsaturated flow are discussed.

CHAPTER 2.

MATERIALS AND METHODS

Virus

MS-2 was chosen as the model virus since it has structural properties similar to those of many human enteric viruses but safer to use with a more reliable assay (Yates et al., 1985). It has a diameter of 25 nm and an isoelectric point of pH_{iep} 3.9 (Overby, et al., 1966). Because of its small size and low isoelectric point MS-2 can be considered a "worst case" virus; it should be transported farther through most soils than human virus. Human water-transported virus generally have pH_{iep} 5.0 to 8.2 (Gerba, 1984). The amino acid sequences of the capsid protein of MS-2 and other RNA phages have been mapped (Weber and Konigsberg, 1975); these amino acids include hydrophobic and hydrophilic groups. The MS-2 capsid differs from that of another bacteriophage used in transport studies, f2, in the replacement of just 1 amino acid out of 131. Some important characteristics of MS-2 and other F-specific RNA bacteriophages are:

> [They] infect their host cells via primary adsorption to the sides of F- or sex-pili produced by E. coli or related bacteria possessing the F-plasmid. They belong to the Levivivridae-family, have a diameter of 20-27 nm and consist of a single-stranded RNA molecule encapsulated by a protein coat. Hence they are related in structure and size to the enteroviruses. Strains such as MS-2 and f2 have been shown to be relatively resistant to inactivation. The major exception seems to be the highly oxidizing disinfectants such as free hyporchlorous acid (Havelaar and Pot-Hogeboom, 1988).

The MS-2 virus used in this research was obtained from American Type Culture Collection (ATCC 15597B1) and grown on bacterial lawns of E. coli (ATCC 15597) as described by Adams (1959). The virus was assayed by the plaque forming unit method (Adams, 1959) on trypticase soy agar (Difco, Detroit, MI). A soil water sample was serially diluted in tris [Trizma Base (trishydroxymethyl aminomethane), Sigma Chemical Co., St. Louis, MO] buffered saline before mixing with the host cells growing in 3% tryptic soy broth (Difco, Detroit, MI). The mixture was poured onto agar plates, incubated at 37°C for 12-24 hours, and enumerated by counting the holes (plaques) in the host lawn. Only the dilutions that resulted in 15-150 plaques per plate were

counted. Each reported virus concentration is the average of two duplicate plates. To minimize virus inactivation the experiments were conducted in a walk-in refrigerator at 4°C.

Soil Columns

The soil material was obtained from the Flushing Meadows Wastewater Renovation Project on the floodplain of the Salt River near Phoenix, Arizona. It consisted of Vint loamy fine sand (a sandy, mixed, hyperthermic Typic Torrifluvent) mixed with recent alluvium (Table 1). This soil material was used in the research of Gilbert et al. (1976) and Lance and Gerba (1984). The actual soil material used in this research was collected previously for one of Dr. C. P. Gerba's projects. The soil classification was determined by locating the source of the soil on the U.S. Soil Conservation Service soil survey of E. Maricopa and N. Pinal Co., AZ, sheet 9, near the intersection of the Salt River and Center St.

Clear polyvinyl chloride (PVC) cylinders, 0.052 m inside diameter, were used to hold 1.05 m-long soil columns. This soil column design has been previously used in virus transport studies (Wang et al., 1981). Twelve mm diameter holes were drilled in the cylinders at 0.05, 0.10, 0.20, 0.40, and 0.80 m depths for the water samplers and soil access ports, and a four mm hole at 1.05 m for the effluent tube (Fig. 1). (Every depth was not sampled in most experiments; see Appendix B.) To extend the unsaturated section of a soil column the water at the bottom must be kept at negative pressure head (using, for example, a suction or siphon arrangement) (Bouwer, 1984, p. 27). The effluent tube of these columns extended 0.20 m below the column thus providing a negative head. To assure that the non-soil materials did not adsorb or inactivate the virus, a cylinder with samplers installed was filled with virus stock, and a water sample was taken daily and assayed for virus concentration (Appendix A).

To fill the cylinders, air dried soil was crushed, passed through a 2 mm screen, poured down a tube into the cylinders in 50 mm increments, and stirred to prevent layering. The columns were weighed and tapped to settle the soil to the desired volume creating a bulk densities of 1.50

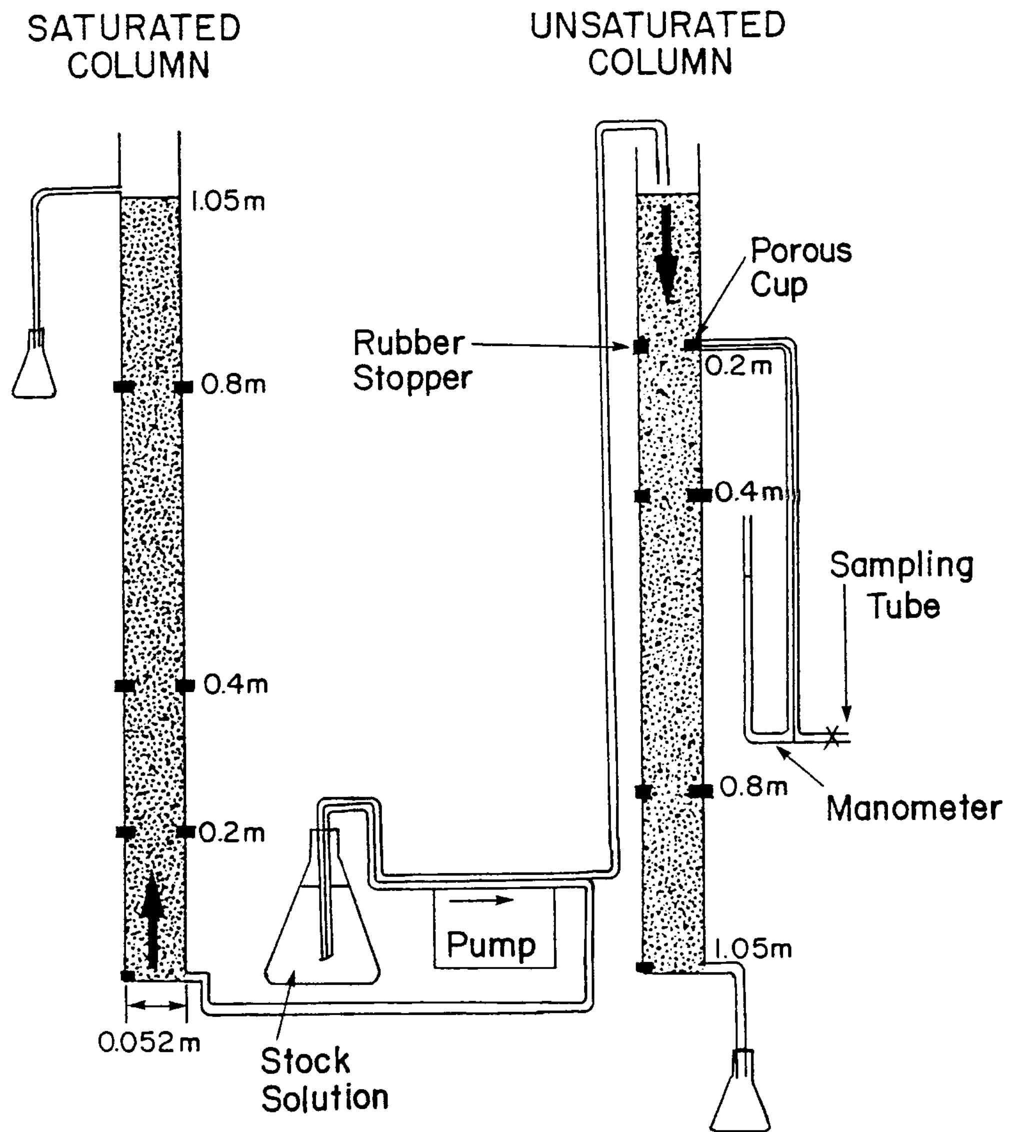


Fig. 1. Experimental apparatus showing solution application, sample extraction, and pressure measurement methods. Only one of the six pairs of sampler and manometer tubing is shown.

Table 1. Soil characteristics.

Source: Flushing Meadows Water Treatment Facility, Phoenix, AZ

Parameter	Value
Texture†:	loamy fine sand
clay	9. %
silt	10. %
sand	81. %
0.05-0.10 mm	24. %
0.10-0.25 mm	34. %
0.25-0.50 mm	11. %
0.50-1.00 mm	9. %
1.00-2.00 mm	3. %
pH‡	8.0
Ca§	2.44 cmol kg^{-1}
Na§	0.04 cmol kg^{-1}
K§	0.65 cmol kg^{-1}
Mg§	1.44 cmol kg^{-1}
Al + H§	0.
Cation exchange capacity§	8.44 $cmol_c$ kg^{-1}
CO_3¶	0.
HCO_3¶	0.04 $cmol_c$ kg^{-1}
Cl#	0.16 $cmol_c$ kg^{-1}
SO_3††	0.08 $cmol_c$ kg^{-1}
TDS in soil extract‡‡	0.0070 %
EC in soil extract§§	0.02 S m^{-1}
$CaCO_3$¶¶	2.0 %
Organic carbon##	0.29 %
Surface area†††	20.5 m^2 g^{-1}
Bulk densities	1.51 to 1.55 Mg m^{-3}

† Clay and silt by pipette method (Gee and Bauder, 1986), sand by sieving.
‡ In 0.01 M CaCl2, mixed 2:1 with soil (McLean, 1982).
§ Extractable cations, ammonium acetate method (Thomas, 1982).
¶ From saturated paste and titration.
# From saturated paste and $AgNO_3$ precipitation.
†† From saturated paste and $BaCl_2$ turbidity.
‡‡ Total dissolved solids by evaporation from saturated paste.
§§ Electrical conductivity of saturated paste.
¶¶ Pressure calcimeter method (Nelson, 1982).
Walkley-Black procedure (Nelson and Sommers, 1982).
††† Ethylene glycol monoethyl ether sorption (Carter et al., 1986).

to 1.56 Mg m^{-3}. After the soil was moistened, cores were removed through the holes and the samplers installed.

The columns designated for saturated flow were given additional treatments to reduce two problems. 1) To minimize flow between the soil and the container wall, bypassing the soil, the inside of the cylinder walls was roughened. This was accomplished by rotating the cylinders around a sandpaper-covered rod and thereby making scratches perpendicular to the direction of water flow. 2) Air may become trapped in wetting soil and lead to incomplete saturation. This problem was minimized by filling the soil with CO_2 before moistening and again before saturation (Drewry and Eliassen, 1968; Constantz et al., 1988), and by pumping the experimental virus suspension in from the bottom to create positive pressure throughout the column. Attempts to saturate the soil by ponding the surface (TE7 and TE8, Appendix B) were not successful. Water potential and water content measures indicated no trapped air remained in the soil.

Water

Water from a 70-m deep well located in Tucson, AZ was used (Table 2). The influent stock was applied with a peristaltic pump. Water flow conditions were established before applying the virus, and the daily conditions maintained as listed in Appendix B with each transport experiment. The infiltration rate was low enough to prevent ponding of the unsaturated columns. The influent stock was pumped at the same rate to the paired columns in the saturated vs unsaturated studies; however, due to backpressure, the saturated columns had a slightly lower infiltration rates than the unsaturated columns. Water contents were determined by weighing or by estimation from the water potential-volume relationship (SE1), as noted in Appendix B. Water potentials were measured from the water level in the manometer tubes. All soil columns except those labeled as "fresh soil" were leached with more than 2 pore volumes of well water to assure that the concentration of organic matter in the soil water had declined to less than 61 mg/L.

Table 2. Water characteristics.

Parameter	Value
Source: Martin St. well, Univ. Arizona, Tucson	
static level	69.9 m
bottom of bore	183. m
Added tracer	0.119 g KBr L^{-1}
pH	8.1
Electrical conductivity†	0.057 S m^{-1}
O.M., influent‡	10. mg L^{-1}

Average Hydrodynamic Conditions

	Sat. vs Unsat. Study		Organic Matter Study
	Unsaturated	Saturated	Unsaturated
Θ_v§ (m^3 m^{-3})	0.35	0.41	0.35
v¶ (m d^{-1})	0.37	0.24	0.27
ϕ# (J kg^{-1})	-3.2	7.7	-3.4

† At 25°C, including added KBr. Without KBr, EC = 0.037 S m^{-1}.

‡ Organic matter in groundwater, including the virus stock; 105°C to 550°C weight-loss (Rabenhorst, 1988).

§ Volumetric water content.

¶ Average linear velocity. $v = I / \Theta_v$, where I is infiltration (m d^{-1}).

Water potential at 0.4 m depth.

Organic matter concentrations of water samples were estimated by a method modified from Rabenhorst (1988). A 120 mL water sample was dried at 105°C; the residue was then heated to 550°C for 20 minutes in air; the weight lost was taken to be the organic matter in the sample. This method provided a relative measure of the organic matter concentrations, but the concentrations should not be considered absolute determinations. Davies (1974) used a similar method to determine organic matter contents of soil and found it correlated well ($r = 0.994$) with the method of Walkley and Black (1934); but at lower organic matter contents there was more divergence. This non-standard method was used because an instrument to measure total organic carbon was not readily available.

Potassium bromide was used as chemical tracer in many of the experiments. Smith and Davis (1974) successfully used Br as a tracer in soil columns, finding that it moved 1.05 to 1.64 times as fast as if it had been uniformly associated with all soil water. Bromide concentrations were determined using a Corning bromide electrode, a Corning double junction reference electrode, and a Beckman ϕ70 pH meter. Because of the small volume of sample (1 to 2 mL), a subsample of 0.5 mL was diluted in 4.5 mL of distilled water to make the volume required by the electrodes. This dilution requirement was the reason for the relatively high KBr influent concentration (10^{-3} M). The diluted sample was mixed with an equal volume of 0.2 M KNO_3 to buffer the ionic strength, making a total volume of 10 mL for measurement. Electrode potential is linearly related to log [Br]. The measured millivolts (mv) were converted to Br concentrations (mmol/L) using a standard curve.

For use in transport experiment (TE) 12 sewage sludge organic matter (SSOM) was obtained by taking the supernatant of autoclaved primary sewage sludge from the Ina Road Sewage Treatment Plant, Tucson, AZ. The supernatant was diluted with well water to produce an influent solution with an electrical conductivity of 0.057 S m^{-1} and organic matter concentration of 203 mg L^{-1}; these values were chosen to be nearly the same as those of the fresh soil effluent.

One leached column was pre-treated with one pore volume of this solution; the other leached column was untreated. Virus mixed with this solution was then applied to both columns.

Samplers

The water samplers selected for the transport experiments were made of cup-shaped sintered stainless steel with 2 μm pore size, 25.4 mm length, 10 mm outer diameter, and air-entry potential of -12.7 J/kg (Mott Metallurgical Corp., Farmington Industrial Park, Farmington, CT 06032). Two Tygon tubes were sealed into the cup opening with epoxy glue to make a manometer tube and a sampling tube (Fig. 1).

The procedure for sampling water from unsaturated columns was: 1) lower the manometer tube about 0.2 m and allow 2-3 mL of water to be extracted (this required 30 to 60 minutes), 2) return the manometer to the original position and open the sampling tube, thereby siphoning water from the sampler, 3) discard the first mL which was in the sampling tube and collect the next mL. The saturated soil water was at greater than atmospheric pressure, so samples could be taken by simply releasing the clamp on the Tygon tube attached to the sampler. The 1.05 m samples were taken from the percolate collected over the preceding sampling period.

There were some modifications of these column designs and extraction procedures in individual transport experiments as noted in Appendix B.

Eluants

A material used to remove virus adsorbed to soil or a filter is called an eluant. The best eluant depends on the type of virus and adsorbent. Of nine eluants tested by Bitton et al. (1979) to recover poliovirus 1 from sand, the most efficient were: casein, non-fat dry milk, beef extract, and glycine-EDTA. Sobsey et al. (1980) found beef extract superior to glycine in eluting reovirus 3 and adenovirus from filters. Elution of enteric viruses from estuarine sediments was most efficiently accomplished with lecithin rather than casein or beef extract (Johnson et al., 1984).

In this research four different eluants were tested at the end of transport experiment 11: glycine (0.05 M); beef extract ("Beef Extract V", 1.5%, BBL, Cockeysville, MD); humate ("humic acid, sodium salt", 1.5%, Aldrich Chemical, Milwaukee, WI), adjusted to pH 8.7; and TSB (tryptic soy broth, Difco, Detroit, MI, 3%, pH 7.3). Glycine and beef extract are substances commonly used to elute viruses adsorbed to various surfaces (A.P.H.A., 1985, p. 950); the solutions were adjusted to pH 8.7 for this virus. Humate and TSB were also thought capable of replacing virus on adsorption sites.

At the end of transport experiment 11 about 4 g of soil was scooped from each of the sampling depths and divided among the four eluants. After 1.5 h at 4°C each sample was mixed, diluted into more of the same eluant, and assayed for virus in the same manner as the water samples. The resulting virus concentrations were due to adsorbed virus plus virus in the soil water. The volume of water in each sample was calculated from its wet weight, and the wet and dry weights of the soil column. The virus concentration in the soil water at each depth was known from the extracted water samples. By subtracting the number of virus in the sample soil water from that found in the eluant assay, the number of virus associated with the solids was determined. Since TSB was already being used as the growth medium for the host E. coli, TSB was used as the standard eluant for three transport experiments (TE9, TE10, and TE11).

Mathematical Fitting

A transport equation for microbes should include a decay term as well as the usual convective, dispersive, and retardation terms used to describe chemical transport. The governing transport equation of VanGenuchten (1981) may be general enough to be used for this purpose. Assuming volumetric water content (Θ_v) and the water velocity (v) remain constant, the governing transport equation may be simplified to his Eq. [6]:

$$D(\partial^2 C/\partial z^2) - v(\partial C/\partial z) - R(\partial C/\partial t) = uC - \gamma \qquad [2]$$

where D is the dispersion coefficient, C is concentration, z is distance or depth, v is average linear

water velocity ($v = q / \Theta_v$, where q is the Darcian flux), t is time, u is the decay constant, γ is the liquid phase source term, and R is the retardation factor ($R = 1 + \rho_b k_p / \Theta_v$, where k_p is the partion coefficient).

The partition coefficient k_p indicates the degree of adsorption:

$$k_p = S/C \qquad [3]$$

where S is the adsorbed virus per gram of solid, and C is the virus per mL of soil water. A k_p of 0 indicates no adsorption, a large positive k_p indicates a large amount of adsorption, and a negative k_p is theoretically impossible. Yates et al. (1987) show that Eq. [3] is derived from the Langmuir isotherm:

$$S = (k_L\ C\ S_{max})/(1 + k_L\ C)$$

where k_L is the Langmuir constant related to the bonding energy and S_{max} is the maximum adsorbed concentration when all the active surface sites are occupied. The Langmuir isotherm reduces to the linear form of eq. [3] when the adsorbed state is weakly favored and the liquid phase concentration is small so that $k_L\ C << 1$; and in that case $k_L\ S_{max} = k_p$.

Some investigators (Burge and Enkiri, 1978; Gerba, 1984) have used the Freundlich isotherm to describe the adsorption process:

$$S = k_F\ C^n$$

where k_F and n are constants. This isotherm does not assume homogeneity among active sites for adsorption as does the Langmuir, but requires two constants for a solution.

A number of investigators have used different forms of Eq. [2] to model virus transport. Vilker and Burge (1980) assumed that virus adsorption is characterized by a large number of sites, but that equilibrium strongly favors the liquid-phase over the adsorbed phase resulting in highly dispersed breakthrough curves. The curves they generated may be adequate for a specific virus, water, and soil condition, but are too simplified for general use. If water removal occurs from the system, as by plant root uptake, Eq. [2] may be modified with a water sink function (e.g.,

$W(z) = A \exp(-Bz))$ (Lomen et al., 1984). Pekdeger and Matthess (1983) used Eq. [2] in vectorial form to predict bacteria and virus transport in ground water. Corapcioglu et al. (1987) refined the adsorption and decay terms by defining a decay rate in the adsorbed state R_{ds}:

$$\partial(\rho\ k_p\ C)/\partial t = R_a + R_{ds}$$

where R_a is the rate of deposition of particles on the solid. Yates and Yates (1988) noted that modelling virus transport should include a consideration of organic matter since this can act not only as a competitor for virus adsorption sites, but also as an eluting agent.

It is difficult to use Eq. [2] to predict virus transport using results as diverse as have been previously reported. This diversity may be due in part to variability in the decay constant u, which in the current research appears to be a function of depth and organic matter. This will be discussed below. As Yates et al. (1987) concluded, "[The] mathematical capabilities far exceed our basic understanding of the behavior of viruses in soils and groundwater systems." Nevertheless, it has been possible to empirically fit the data from the current research to equations with some theoretical basis which may help in the formulation of a comprehensive mathematical model in the future.

Amoozegar-Fard et al. (1983) solved Eq. [2] for $u = 0$, $\gamma = 0$, $C\ (z,0) = 0$, $C\ (0,t) = C_o$, and $\partial C/\partial z\ (\infty,t) = 0$, giving:

$$C^* = 0.5\ \mathrm{erfc}\ [(R\ z - v\ t)\ (4\ D\ R\ t)^{-0.5}]$$

where C^* is relative concentration ($C^* = C/C_o$ for a conservative tracer). By setting $t = T\ L\ /\ v$, where T is pore volumes:

$$(R\ L\ v\ /\ 4\ D\ T)^{0.5} - (L\ v\ T\ /\ 4\ D\ R)^{0.5} = \mathrm{erfc}^{-1}\ (2\ C^*)$$

(Amoozegar-Fard et al., 1983). By letting $R = a/b$ and $D = Lv/(4ab)$, where a and b are parameters found by least squares fitting of tracer breakthrough data:

$$C^* = 0.5\ \mathrm{erfc}\ [(a - b\ T)\ T^{-0.5}] \qquad [4]$$

If $u \neq 0$, (a/b) is a composite of the effects of retardation and decay.

It was necessary to scale Eq. [4] to fit the current data. C* increases from zero to one, but the experimental relative concentrations, C/C_o, did not reach one. The experimental C/C_o values were determined by dividing C, the concentration of virus detected at a given depth and time, by C_o, the influent concentration of virus at that time calculated from the linear regression of the concentrations of the inflow samples over the course of the experiment. Consequently, it was necessary to use $(C/C_o)_s$, the apparent steady-state relative concentration, as a scaling factor. $(C/C_o)_s$ was found by averaging the C/C_o values after they stopped increasing. The measured C/C_o values were divided by $(C/C_o)_s$, Eq. [4] fit to these scaled-up values, and the resulting C* values multiplied by $(C/C_o)_s$ to generate the data-fitting C/C_o vs T curves.

After the virus concentrations reached steady-state, the concentration profiles with depth declined exponentially. Iwasaki (1937) found that organic and inorganic microscopic particles in a sand filter declined exponentially with depth, and the data could be fit by:

$$C/C_o = \exp(-\lambda_o z)$$

where λ_o is initial impediment modulus and z is depth of the sand layer. Burge and Enkiri (1978) used a similar equation, with time as the independent variable, to fit the results of a batch study. They mixed bacteriophage ϕX174 with loamy sand by inverting the solution at 60 cycles per minute. The fraction of the initial concentration of varied with time (t) as:

$$C/C_o = \exp(-k_1 t)$$

where k_1 is the first order rate constant.

Hoeks (1981) solved Eq. [2] for two different soil layers after a steady-state had been reached for D = 0 and $\partial C/\partial t = 0$:

$$C/C_o = \exp -[u_2 (t-t_1) / (1+R_2) + u_1 t_1 / (1 + R_1)]$$

where u_1 and u_2 are decay constants in the upper and lower regions respectively, R_1 and R_2 are retardation coefficients for the upper and lower regions respectively, and t_1 is the time to reach the boundary between the regions. This equation fits the current data well. However, it requires

measurement or estimation of five parameters.

Another model for transport and decay through different layers that requires only 3 parameters and produces a smoother curve than that of Hoeks (1981) is model of Jury et al. (1987). Although it was developed to describe the biodegradation of pesticides, data from the current research were consistent with it. To use this model the soil profile was divided into two regions: a surface zone ($0 < z \leq L$) and a transition zone ($L \leq z < 1.05$ m). In the surface zone the steady-state concentration was:

$$(C/C_o)_{ss} = \exp(-uz/v) \qquad [5]$$

where z is depth (m) and v (m d^{-1}) is the average linear velocity. Their Eq. [9] and [11] may be combined:

$$(C/C_o)_{st} = \exp\{(-1/v)\int_0^{z_{st}} u \exp[-G(z-L)]\,dz\}$$

and solved to give the steady-state concentration in the transition zone:

$$(C/C_o)_{st} = \exp\{(-u/Gv)[1 + GL - \exp(L - z)]\} \qquad [6]$$

where G (m^{-1}) is the depth constant and L (m) is the depth of the boundary between the surface and transition zone. (Their Eq. [14]: $(C/C_o)_{st} = \exp([-u/Gv]\{1 - \exp[-G(z - L)]\})$ appears to be in error.) The value for G (3 m^{-1}) was taken from Jury et al. (1987), and L was estimated to be about 0.05 m to fit the rapid decline in concentration near the surface. The specific removal rate u was then adjusted to fit Eq. [6] to the steady-state virus concentrations at the 1.05 m depth.

This model has three "adjustable" parameters (L, u, and G) that permit it to fit almost any set of data that declines with depth, whether or not its use is appropriate. However, each of these parameters may be approximated independently. L must be at a change in the soil conditions or at an inflection point in the experimental curve. Parameter u may be measured in batch

experiments or be estimated from the steepness of the experimental curve above L. G may be found by measuring the decline with depth in numbers of bacteria or whatever produces the decay rate.

CHAPTER 3

RESULTS AND DISCUSSION

Overview

The transport experiments (TE) with column letters and major variables are listed in Table 3 (see Appendix B for details of the experiments). Some experiments were of short duration, had technical problems such as cracking of the soil column or plugging of the samplers, or were not controlled for organic matter. These experiments (TE1, TE2, TE4, TE5, and TE7) will not be discussed in this chapter, but short summaries are provided with the data in Appendix B.

Saturated Versus Unsaturated Flow

The a and b parameters found by fitting Eq. [4] to combined saturated studies (TE6E and TE11L) and combined unsaturated studies (TE3C, TE3D, TE9H, and TE9I) are listed in Table 4. These experiments were the best indicators of the effects of saturated and unsaturated flow because of their long durations and comparable conditions. The retardation (R) values at 1.05 m were 0.80 for saturated flow (Table 4), which might be expected due to size or charge exclusion from part of the water volume. R is not calculated for unsaturated flow because the decay coefficient $u \neq 0$ (Eq. [4]). Calculations of u in unsaturated conditions are made below.

Figures 2a to 2d show the relative concentration (C/C_o) of virus versus pore volumes past the indicated depths. The curves were constructed using Eq. [4]. There was considerable variability in the assay as indicated by an average $r^2 = 0.37$ for the influent concentration regressions for TE3, TE6, TE9, and TE11. Scatter in the (C/C_o) data may be due to variability in the MS-2 assay and to variability between columns. Consequently, the averaged results reported below should be viewed with caution when looking for small differences. Under saturated conditions at the 0.20 m depth the steady-state relative concentration $(C/C_o)_s = 1.26$, a surprisingly large value indicating an accumulation of virus above the influent concentration. This may be due to anion sieving (Bolt, 1982), size sieving, or variability of the bacteriophage assay.

Table 3. Summary of transport experiments and major variables.

Exp.	Column†	Saturation‡	Organics§	Duration
				--days--
TE1	A	U	L	1
	B	U	L	1
TE2	A	U	L	5
	B	U	L	5
TE3	C	U	L	10
	D	U	L	10
TE4	C	U	L	6
	D	U	L	6
TE5	Ks¶	U	O	5
	Ls	U	O	5
TE6	D	U	L	9
	E	S	L	9
TE7	F	U	O	9
TE8	G	U	L	11
TE9	H	U	L	8
	I	U	L	8
TE10	J	U	O	7
	K	U	L	7
TE11	K	U	L	6
	L	S	L	6
TE12	J	U	O	8
	K	U	O	8

† Each new or repacked soil column was given a different letter.
‡ S = saturated; U = unsaturated.
§ L = leached; O = high organic matter in soil water.
¶ s = short column (0.1 m).

Table 4. Saturated versus unsaturated fitting parameters for relative concentration versus pore volume curves (Fig. 2).

Depth m	$(C/C_o)_s$†	a‡	b‡	R§	C.D.¶	k_p,est# mL/g
			Unsaturated			
0.20	0.27	2.10	0.83	††	0.52	††
0.40	0.17	0.95	1.08	††	0.43	††
0.80	0.07	2.11	2.04	††	0.53	††
1.05	0.05	3.01	4.01	††	0.66	††
			Saturated			
0.20	1.26	1.11	1.00	1.11	0.93	0.03
0.40	1.10	0.80	1.29	0.62	0.75	-0.10
0.80	1.00	4.41	3.89	1.13	1.00	0.04
1.05	0.98	2.97	3.71	0.80	0.87	-0.05

† Relative concentration at the apparent equilibrium value.

‡ Breakthrough curve parameters.

§ Retardation coefficient, R = a / b. R<1 indicates that the virus were being transported faster than the average linear velocity of the soil water.

¶ Coefficient of determination for direct C/C_o.

Partition coefficient estimated from:

$k_p,est = (R - 1)\,\Theta_v / \rho_b$

where R = retardation coefficient, Θ_v = volumetric water content, and ρ_b = bulk density.

†† R and k_p,est cannot be calculated from a and b in unsaturated conditions due to enhanced inactivation of the virus ($u \neq 0$ for Eq. [4]).

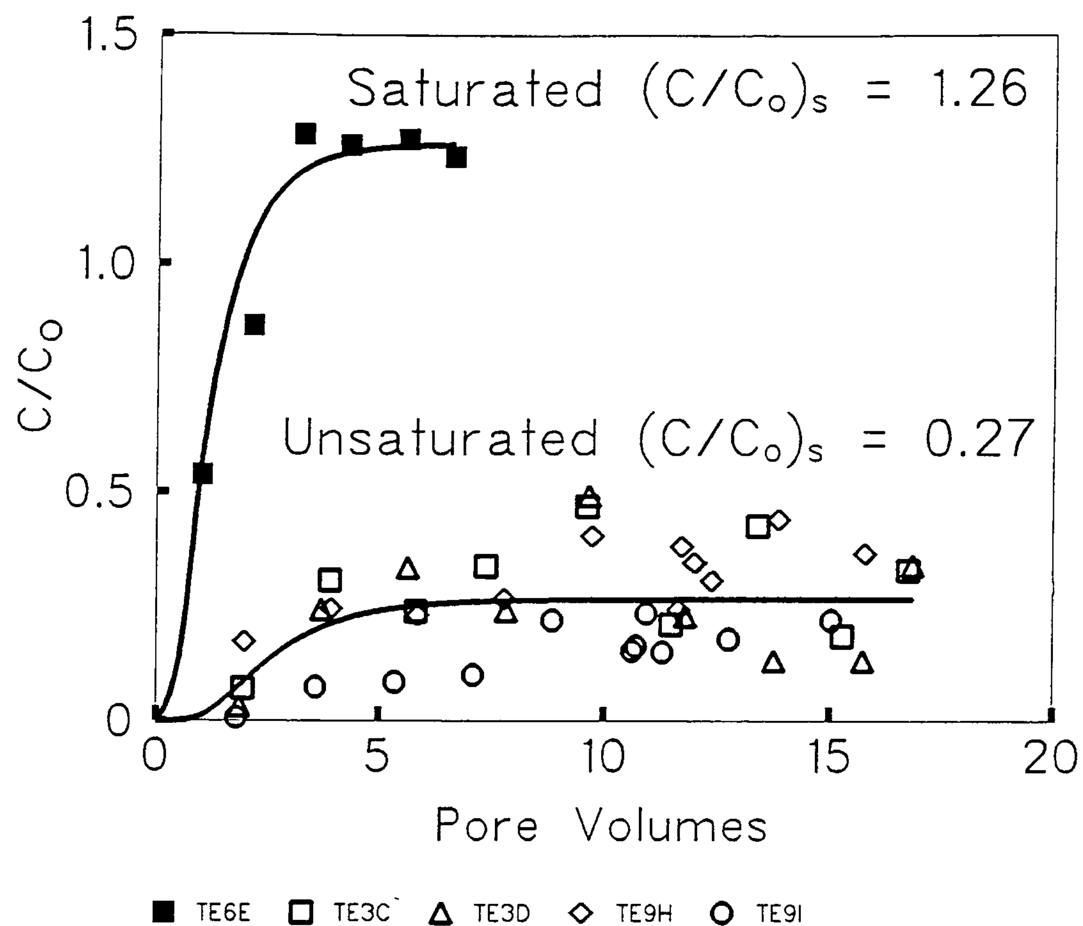


Fig. 2a. The relative virus concentrations versus pore volumes and fitted breakthrough curves at 0.20 m. $(C/C_o)_s$ is the apparent steady-state value. The markers are labeled by transport experiment number and column letter. The curves were constructed from Eq. [4].

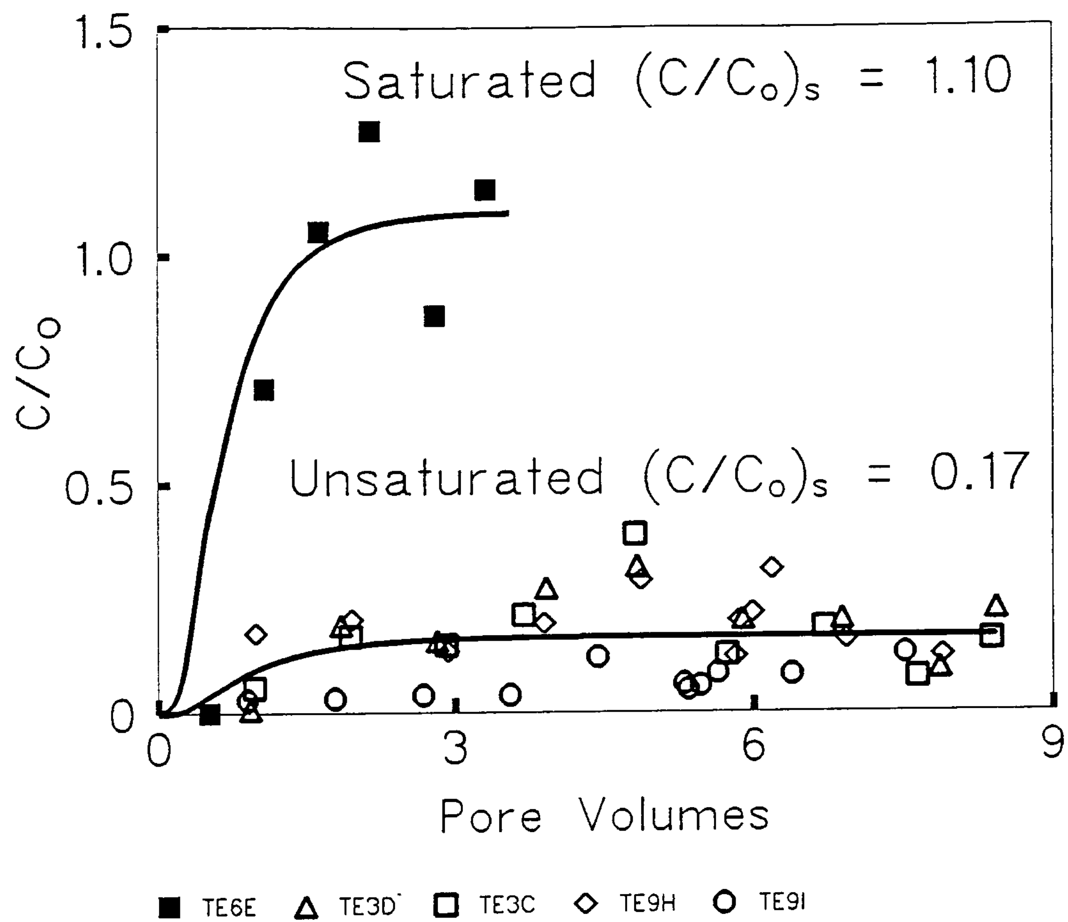


Fig. 2b. The relative virus concentrations versus pore volumes and fitted breakthrough curves at 0.40 m.

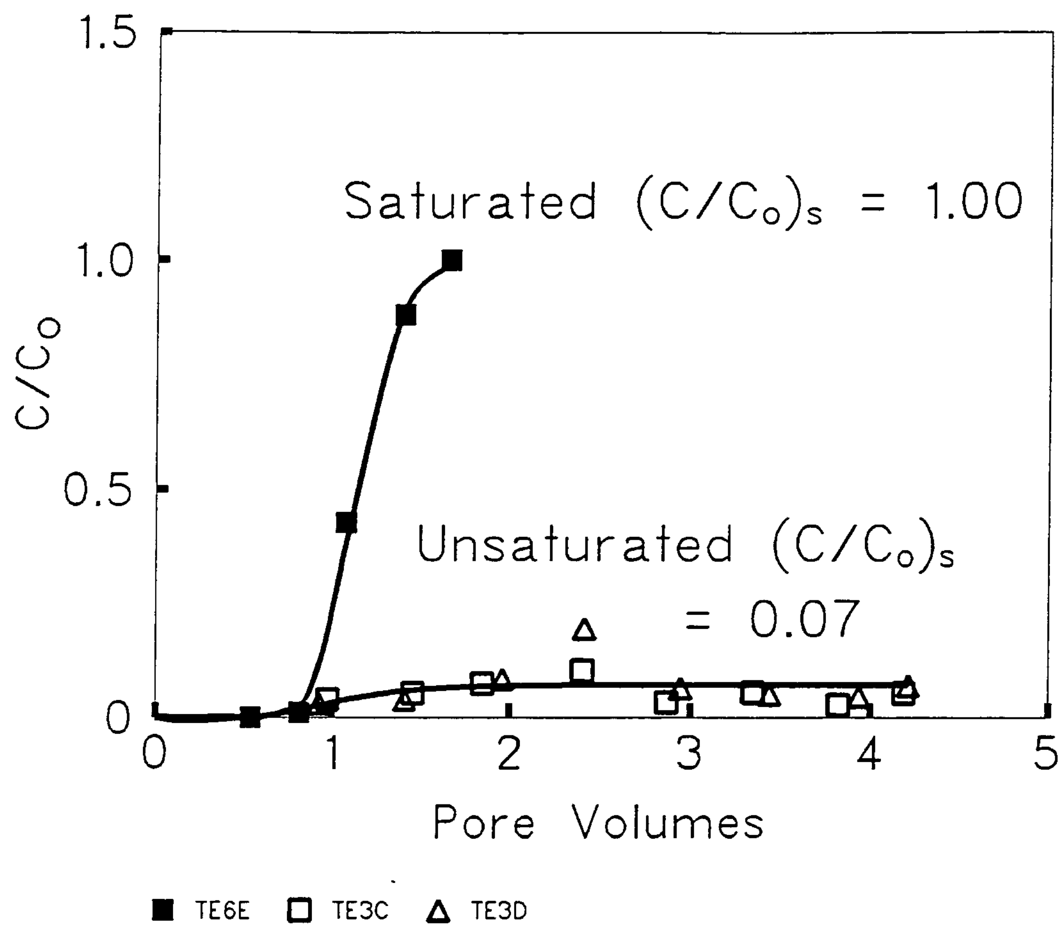


Fig. 2c. The relative virus concentrations versus pore volumes and fitted breakthrough curves at 0.80 m.

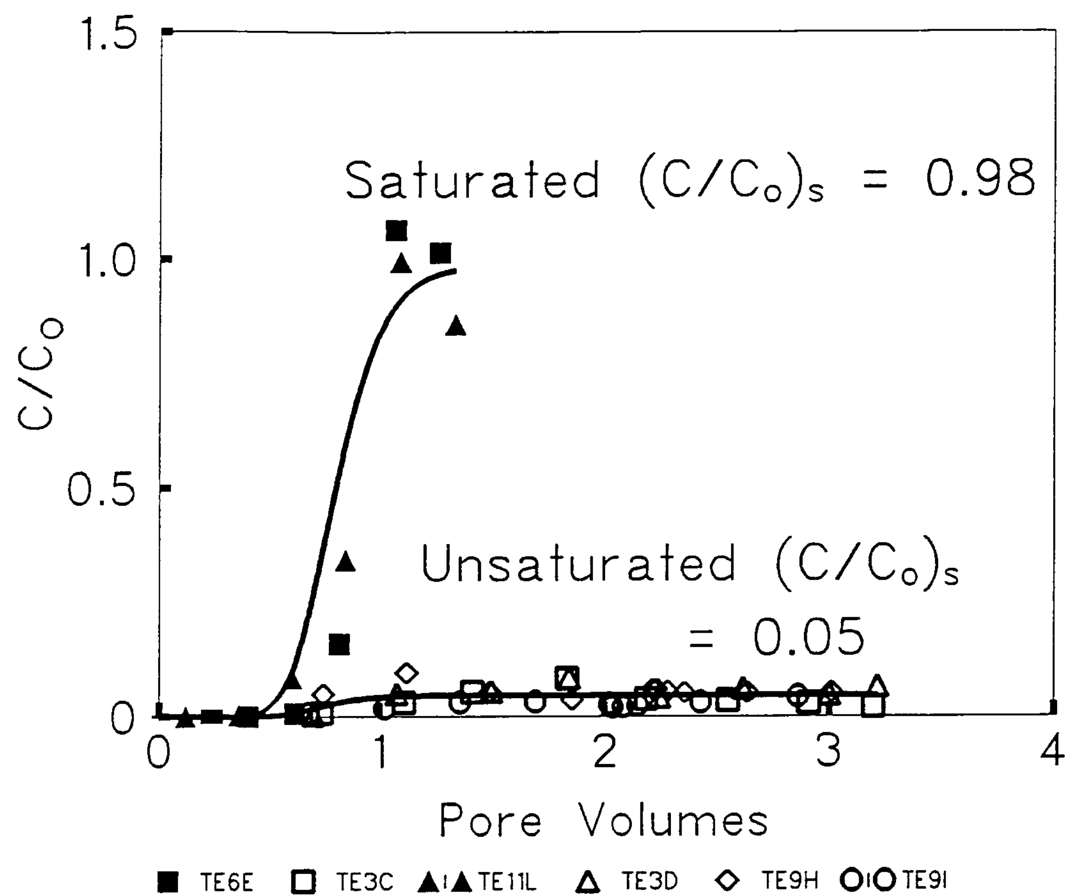


Fig. 2d. The relative virus concentrations versus pore volumes and fitted breakthrough curves at 1.05 m.

The higher concentration at shallow depth can also be clearly seen in Fig. 3. By day 5 the relative concentrations essentially equal one throughout the saturated profile. Under unsaturated conditions the $(C/C_o)_s$ values declined sharply with depth (Fig. 4). Even after 18 pore volumes at the 0.20 m depth (Fig. 2a), the relative concentration continued at about 0.27. At 1.05 m $(C/C_o)_s$ was only 0.05 after more than 3 pore volumes. Virus concentration profiles that do not increase with the passage of many pore volumes have been observed in saturated flow (Lance et al., 1982). Their results, however, are complicated by uncertainty about the degree of saturation of the column and the decay rate of the virus in non-refrigerated conditions.

Figure 4 compares the virus concentrations after steady-state was reached with the curve derived from Eq. [5] and [6]. The small value of L for this data (0.05 m) indicates that the active surface zone is very shallow for these columns. The specific decay rate u for this data is so large (4.62 d^{-1}) that all the virus would have been removed from the soil water before reaching a depth of 0.92 m if u was not modified by G beginning at 0.05 m. Before this model could be used predictively, the effect of environmental variables such as volumetric water content, pH, and virus type on each of the parameters L, u, and G would have to be quantified.

The model of Jury et al. (1987) assumes the decline in concentration is due to decay. It is possible, however, that this decline could be due to adsorption to a large number of sites at a slow rate. To examine this possibility I attempted to recover any adsorbed virus by testing four different soil eluants (Table 5). The most effective eluant was beef broth (average k_p = 0.03 mL/g, SD = 0.27 mL/g). The magnitude of the SD indicates that k_p was not different from zero, indicating that the virus were not reversibly adsorbed to the soil. TSB, the eluant used in TE9 and TE10, gave similar results (average k_p = -0.02 mL/g, SD = 0.13 mL/g). The negative k_p values resulted when fewer virus were detected in the combined soil solids and soil water than in the same soil water volume extracted from the soil with the porous samplers. To check the accuracy of the k_p values, estimated k_p values were calculated from the retardation coefficients (Table 4);

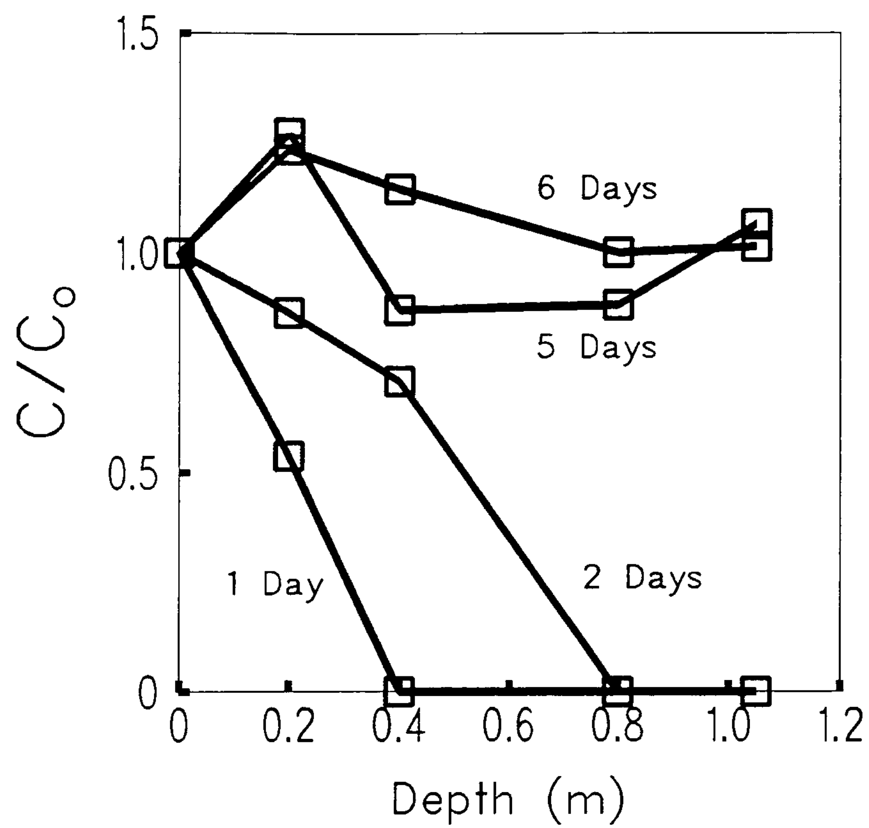


Fig. 3. The relative virus concentration profiles under saturated flow (TE6E) at four sampling times. The average linear velocity was 0.24 m d^{-1}.

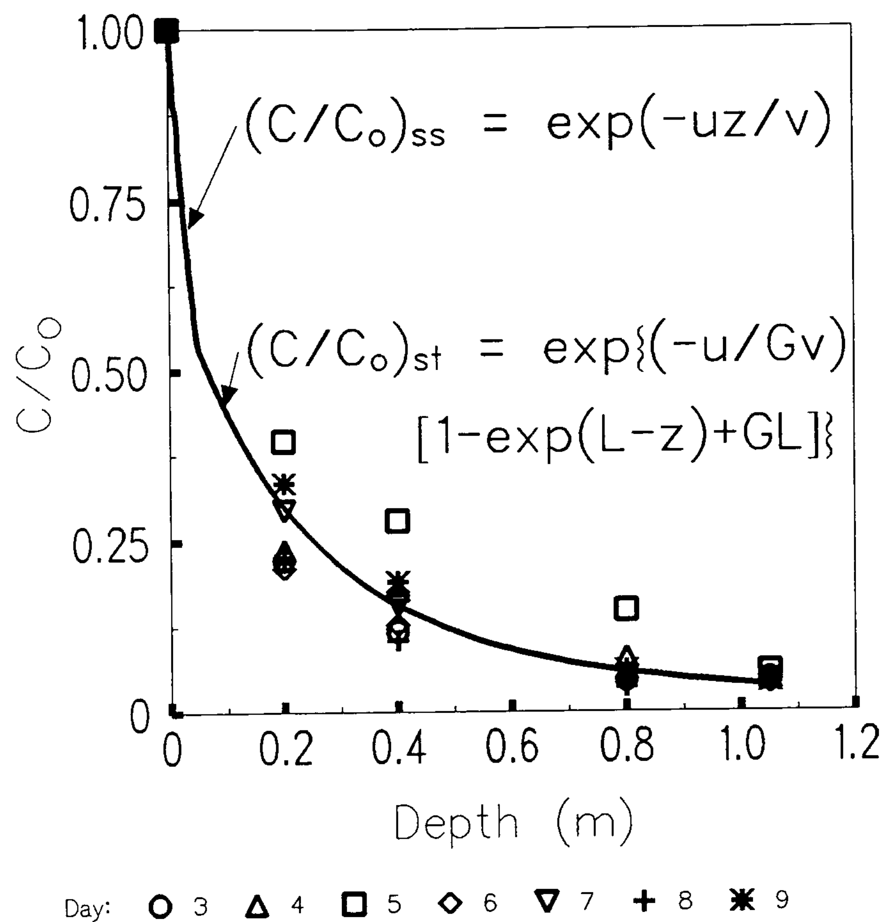


Fig. 4. The relative virus concentration profile under unsaturated flow (TE3 and TE9) from day 3 (one pore volume at 1.05 m) to day 9, and fitted curve using Eq. [5] and [6], where $u = 4.62\ d^{-1}$, z is depth (m), $v = 0.37\ m\ d^{-1}$, $G = 3\ m^{-1}$, and $L = 0.05$ m.

Table 5. Eluant effect on the partition coefficient, k_p, TE11.

Depth	Eluant: TSB	Glycine	Beef	Humate	Avg.	SD†
m	mL/g					
			Unsaturated k_p			
0.00	0.15	0.30	0.54	0.31	0.32	0.16
0.40	-0.03	-0.07	0.02	0.01	-0.01	0.04
1.05	-0.05	-0.09	0.00	-0.08	-0.05	0.04
Avg.	0.03	0.05	0.19	0.08	0.09	
SD	0.11	0.22	0.30	0.21	0.20	
			Saturated k_p			
0.00	0.10	-0.10	0.05	0.20	0.06	0.13
0.40	-0.17	-0.20	-0.20	-0.20	-0.19	0.01
1.05	-0.14	-0.20	-0.22	-0.15	-0.18	0.04
Avg.	-0.07	-0.17	-0.12	-0.05	-0.10	
SD	0.15	0.06	0.15	0.22	0.14	
			Overall			
Avg.	-0.22	-0.06	0.03	0.02	-0.01	
SD	0.13	0.19	0.27	0.20	0.19	

† Standard deviation of sample

both calculations resulted in values for k_p near zero.

The partition coefficients using TSB as the eluant for four unsaturated columns and one saturated column are presented in Table 6. Although the average k_p for the unsaturated columns was greater than zero (0.28 mL/g, SD = 0.40), the SD again indicates that k_p cannot be considered different from zero.

At the end of these experiments number balances of virus were tallied (Table 6). The balance included: 1) virus in the cumulative effluent, 2) virus remaining in the column water, and 3) virus inactivated due to the transit time through the column, estimated from the inactivation rate in the influent stock. A $k_p = 0$ was assumed, so no virus were considered adsorbed to soil solids. Under saturated conditions 80% of the virus were accounted for, whereas under unsaturated conditions an average of only 27% were accounted for. It appears that more virus are being inactivated during unsaturated flow.

Effect of Organic Matter on Unsaturated Transport

In TE10 (fresh soil versus leached soil) the organic matter concentration in the effluent water of the fresh soil decreased from 188 to 97 mg L^{-1} in two pore volumes (T), and the leached soil effluent had a mean organic matter concentration of 68.0 mg L^{-1} (SD = 10.9, n = 4). These values are high for soil water; this may have been due to: 1) the recent alluvial origin of the soil material, 2) the breakup of the soil during drying and sieving, or 3) the method used to measure the organic matter.

The relative virus concentration (C/C_o) at the 1.05 m depth in the fresh soil increased to 0.47 of inflow (SD = 0.016, n = 3) while C/C_o in leached soil increased to only 0.05 (SD = 0.017, n = 4) (Fig. 5). The virus concentrations maintained these steady-state values [$(C/C_o)_s$] from one T until the end of the experiment at two T. Since $(C/C_o)_s/2$ was reached before one pore volume it appears that the virus were transported faster than the average water flow.

Table 6. Partition coefficients k_p, and number balances for five columns using TSB as the eluant.

Depth m	U(TE9H)	U(TE9I)	U(TE10K)	U(TE11K)†	U(avg.)	U(SD‡)	S(TE11L)†
	Column Type (U=unsaturated, S=saturated)						
	Partition coefficients, k_p (ml/g)						
0.00	1.25	0.95	0.37	0.15	0.68	0.51	0.10
0.20	0.26	0.63	0.06	nd§	0.32	0.29	nd
0.40	0.20	0.18	-0.08	-0.03	0.07	0.14	-0.17
0.80	nd	nd	-0.02	nd	-0.02	nd	
1.05	nd	nd	-0.02	-0.05	-0.04	0.02	-0.14
Avg.	0.57	0.58	0.07	0.02			-0.07
SD	0.59	0.39	0.18	0.11			0.15
Overall					0.28	0.40	
	Number Balance (No. accounted for / No. input)¶						
	0.33	0.31	0.15	0.28	0.27	0.08	0.80

† Data also shown in Table 5.

‡ Standard deviation of sample.

§ No data

¶ $k_p = 0$

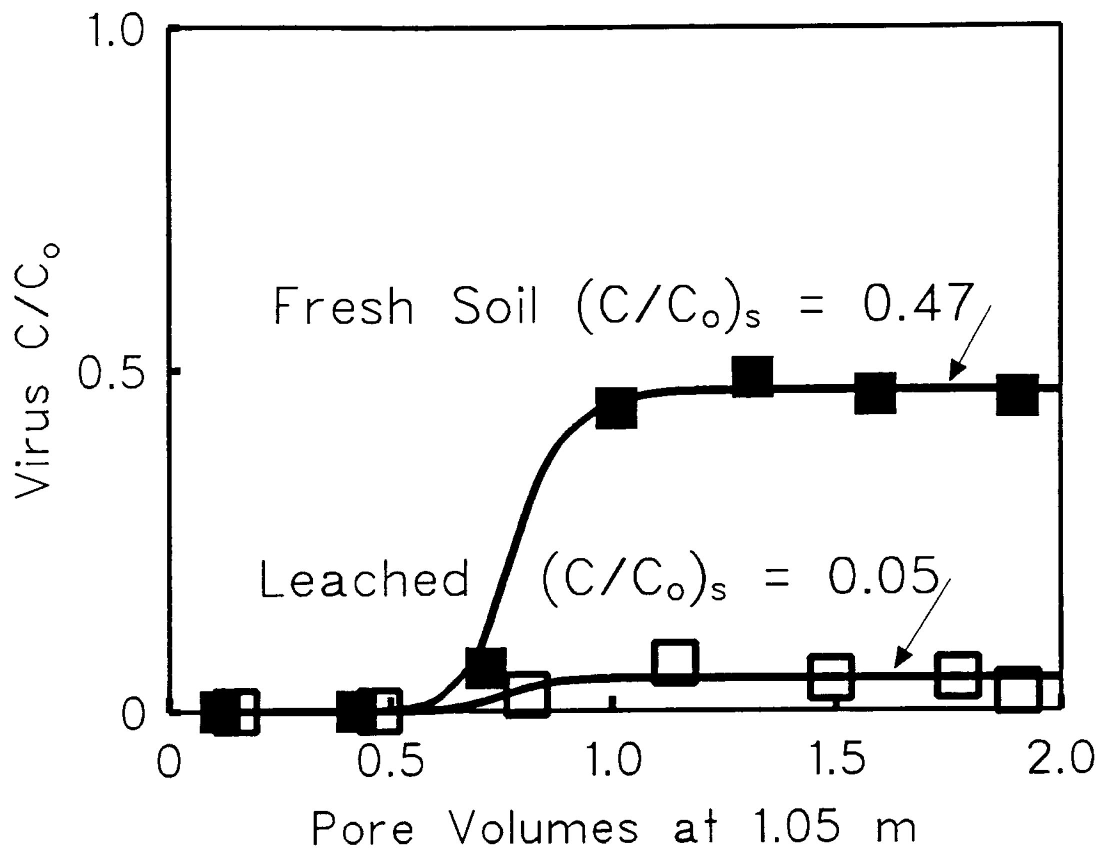


Fig. 5. The relative virus concentrations versus pore volumes at the 1.05 m depth and fitted breakthrough curves in transport experiment 10. $(C/C_o)_s$ is the apparent steady-state value. The curves were constructed from Eq. [4].

In TE12 (sludge pretreatment versus untreated) the soil water organic matter derived from sewage sludge organic matter (SSOM) decreased in transit through the columns. The influent concentration from added SSOM was 203 mg L^{-1}, and at two T the effluent mean was 37.9 mg L^{-1} (SD = 6.5, n = 2). The organic matter concentrations in the water leaving the columns in TE12 were lower than in the leached column in TE10 (68.0 mg l^{-1}). This may have been due to more leaching of the columns used in TE12, resulting in lower concentrations of resistant humic material in the effluent.

It was anticipated that pretreatment of a leached column with SSOM would permit more virus to be transported than in an untreated leached column when the influent contained SSOM. This would indicate that organic matter competition rather than complexation protects virus from removal. Instead, the steady-state value for the pretreated column [$(C/C_o)_s$ = 0.41, SD = 0.038, n = 4] was slightly less than that of the untreated column [$(C/C_o)_s$ = 0.49, SD = 0.063, n = 2] (Fig. 6). This tends to support complexation as the dominant mechanism protecting virus during unsaturated flow. However, virus breakthrough was retarded slightly more in the untreated column compared to the pretreated column. This may indicate that the initial front of virus was removed by the untreated column, and only after the influent organic matter began to compete with the virus did the steady-state virus concentrations appear in the effluent. The results from this experiment do not clearly support either complexation or competition as the dominant effect of organic matter on virus transport.

It is interesting that the virus concentrations in the effluent from fresh soil in TE10 and from both columns in TE12 had very similar steady-state values, $0.41 \leq (C/C_o)_s \leq 0.49$. The nature of the organic matter in the two experiments was different: natural humic material in TE10 and primary sludge extract in TE12. Further research is needed to determine if the similar steady-state values were a coincidence, or if the values are characteristic of these soil and water content

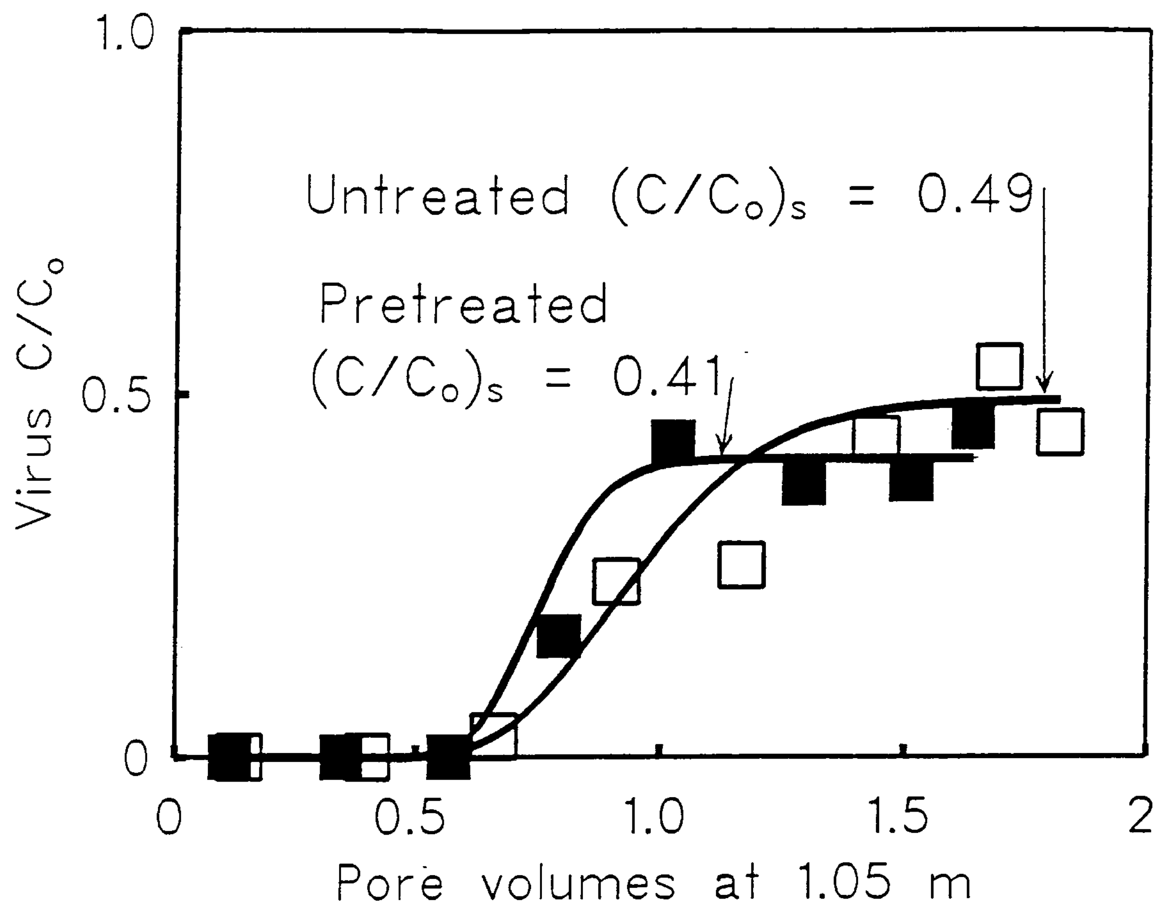


Fig. 6. The relative virus concentrations versus pore volumes at the 1.05 m depth and fitted breakthrough curves in transport experiment 12. $(C/C_o)_s$ is the apparent steady-state value. The curves were constructed from Eq. [4].

conditions. The data from these three columns were combined to generate the "organic" profile in Fig. 7.

The specific removal rates (u) found from fitting the data to the model of Jury et al. (1987) indicated that the rate under the higher organic matter conditions was 0.26 (SD = 0.031, n = 3) of the rate under leached conditions (Fig. 7). The specific removal rate under organic conditions was $u_o = 0.81\ d^{-1}$ (SD = 0.096, n = 3), and under leached conditions it was $u_l = 3.1\ d^{-1}$.

In comparing the leached column parameters with the unsaturated columns of the saturated versus unsaturated study, the steady-state values at 1.05 m are the same [$(C/C_o)_s$ = 0.05], but u_l in TE10 is less than the previous average unsaturated u_l (mean $u_l = 4.6\ d^{-1}$, SD = 0.45, n = 4). This was due to the slower linear velocity in TE10 ($v = 0.27\ m\ d^{-1}$ versus $v = 0.37\ m\ d^{-1}$).

Bromide Transport

The Br data are compiled and commented on with the transport experimental data in Appendix B. Sampling was not frequent enough at early times in these experiment to provide useful breakthough curves. Also, the need for dilution created considerable scatter in the data. Consequently, the Br results will not be discussed further in this chapter.

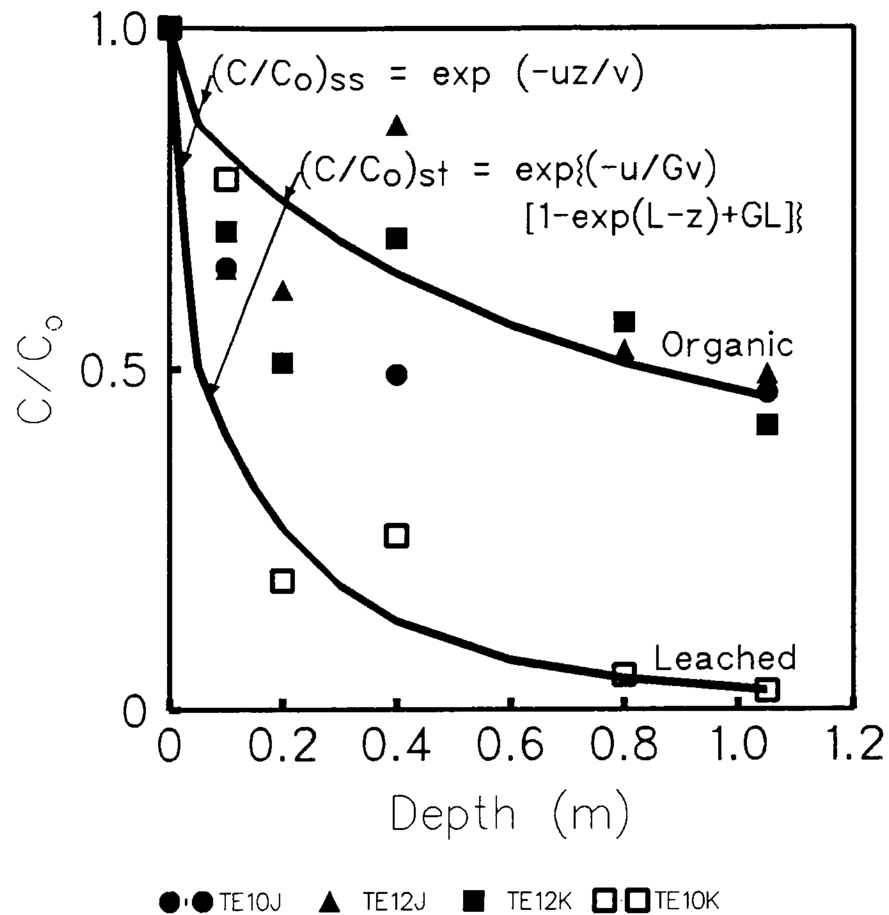


Fig. 7. The relative virus concentration profile with depth from TE10 and TE12 on day 7 (1.8-1.9 pore volumes). The fitted curves were constructed from Eq. [5] and [6], where z is depth (m), $v = 0.27\ m\ d^{-1}$, $G = 3\ m^{-1}$, $L = 0.05$ m, and the organic $u_o = 0.81\ d^{-1}$ and the leached $u_l = 3.1\ d^{-1}$.

CHAPTER 4.

POSSIBLE REMOVAL MECHANISMS AND IMPLICATIONS

Effect of Unsaturation on Adsorption

In the unsaturated conditions of this research the conducting pores probably were very large compared to the size of the virus (25 nm) and the effective range (about 10 nm) of the electrostatic and hydrophobic removal mechanisms associated with soil particles. The "capillary rise equation" (Eq. [1]) may be used to estimate the size of the largest water filled pores in the soil. If the contact angle κ is estimated to be 47° (Letey et al., 1962), and the potential difference ϕ across the air-water meniscus has the typical experimental value of 3.2 J kg^{-1}, the calculated largest water-filled pores have a radius of about 32 μm. This is 1280 times the size of a virion, giving it plenty of room to avoid contact with removal mechanisms associated with soil particles.

Unsaturated flow velocities are often slower than saturated, and it has been shown that lower flow velocities result in more complete adsorption and lower levels of transported virus (Wang et al., 1981). In this experiment, however, the unsaturated flow velocities were faster than the saturated flow by a factor of 1.5, so the effect of velocity does not explain the results.

It seems likely that a qualitatively different process is occurring in unsaturated flow. The introduction of air-water interfaces may provide additional surfaces for adsorption of the partially hydrophobic virus. It is possible that bulk water tends to exclude the virus and force them to air-water interfaces in unsaturated conditions. There are no electrostatic forces keeping virus away from the air-water interface as there are from the soil solids. Dead-end pores created by air-water interfaces away from the preferred flow paths are likely to have lower fluid velocity and collect suspended particles such as virus. Near the flow paths, a virion may be rolled across an air-water interface by higher fluid velocity in the center of the pore, and thereby increasing the possibility of establishing a hydrophobic "bond."

Large bodies of water have been found to have a surface microlayer consisting of partly

hydrophobic organic molecules to which organisms and particles adhere (Norkrans, 1980). In all nonpolluted locations natural air-water boundaries are dominated by glycoprotein and proteoglycan type films (Baier, 1975). Bulk organic matter was concentrated in an estuarine microlayer, with humic acids tending to partition into the surface in preference to fulvic materials (Guerin, 1989). Particulate amino acids were found to adsorb to the sea surface microlayer (Hendricks and Williams, 1985). In pure water, without an organic microlayer, layers thicker than 1 μm can be considered to have properties of bulk liquid (Clifford, 1975). The presence of surface-active organic films, however, may cause water molecules next to the interface to orient into 'icelike' clathrate structures, resulting in additional stabilization of the boundary layer to a depth of 50 μm (Hardy et al., 1987).

The formation of microlayers may be promoted by physical, chemical, and biological forces. Bubbles selectively adsorb inorganic and organic matter and microorganisms during their upward path through water resulting in selective concentration of these substances in thin films at the air-water interface (Blanchard and Parker, 1977). Baier (1972) observed that after 24 h of cultivation with sewage bacteria (on light lubricating oil over distilled water) a coherent surface matrix was being set up by the propagating biological material.

The association of microbes with the air-water interface has been observed in aqueous systems. Bandoni and Koske (1974) observed bacteria, protozoans, fungi, and other small organisms inhabiting the surface film after moist forest litter was submerged in water. Bacteriophages T2 and T4 were concentrated by a factor of 50 in droplets from the surface film of bursting bubbles (Baylor et al., 1977). Bubbles in surf produced 200 times more viruses per mL of aerosol than were present in the surf (Waldichuck, 1982). Sieburth (1976) found that bacteria were concentrated by a factor of 4×10^4 in the sea surface microlayer. In soil the air-water interface may also be considered a site for the formation of a microlayer and hydrophobic adsorption.

The air-water surface area in soil σ_i (m^2) may be estimated by equating two measures of the work w (J) required to remove water from saturated soil:

$$dw = \gamma \, d\sigma = \phi(M) \, dM \qquad [7]$$

where γ is the surface tension (0.075 N m^{-1} at 4°C), and $\phi(M)$ is the water potential (J kg^{-1}) as a function of M (kg), the mass of water in the column.

The function $\phi(M)$ was calculated from the water release curve of a sample of the soil material (SE1) packed to the typical experimental bulk density (1.55 Mg m^{-3}). In the region of the experimental water content the data fit a spline function (r^2 = 0.994):

$$\phi(M) = -5.94 + 6.42\,M, \qquad 0.925 \geq M \geq 0.809$$

$$\phi(M) = -59.7 + 72.8\,M, \qquad 0.809 \geq M \geq 0.716$$

The soil column saturated water mass (M_{sat}) was 0.925 kg, and the experimental water mass (M_i) was 0.781 kg.

Integrating the right side of Eq. [7] gives:

$$\int \phi(M) \, dM =$$

$$\int_{M=0.925}^{0.809} -5.94 + 6.24M \, dM + \int_{M=0.809}^{0.781} -59.7 + 72.8M \, dM$$

$$= 0.0939 J$$

Equating this to the change in surface area measure of work:

$$\int_{\sigma=0}^{\sigma_i} \gamma \, d\sigma$$

results in σ_i = 1.25 m^2. This surface includes films on particles and menisci.

MS-2 virus particles are 25 nm in diameter, therefore about 2.0 X 10^{15} virus would be required to form a single layer on σ_i. Since the maximum number of virus removed by passage through a column was about 1.3 X 10^9, only a small fraction (6.5 X 10^{-7}) of the air-water surface area could have been covered with virus. The surface area of the air-water interface in the

columns was certainly large enough to adsorb all of the virus removed from the soil water without need for decay, desorption, or multi-layer adsorption.

Inactivation Mechanisms

Very few of the virus removed by unsaturated flow in experiments TE9, TE10, and TE11 could be recovered by elution of the soil. Apparently, virus removal is due to inactivation rather than reversible adsorption. Virus may be inactivated by physical disruption of the capsid tertiary structure, oxidation, or microbial attack (Hurst et al., 1980; Gerba, 1984). A combination of these effects may be important. As Duboise et al. (1979) noted, "Alteration of enterovirus surface protein configurations by the adsorption process and any consequent effects upon viral susceptibility to proteolytic degradation apparently have not been examined."

The inactivation mechanism appears to operate at different rates at different depths. The data show a rapid decline in virus concentration in the first 0.05 m followed by a more gradual decline. Vaughn et al. (1981) found that during unsaturated flow through coarse sand poliovirus concentration declined rapidly to 0.75 m, but the same concentration occurred at a depth of 0.75 m and 2.25 m.

Strain differences in a virus population that result in some viruses being adsorbed and inactivated rapidly and others slowly could contribute to the observed distribution of virus concentrations with depth. Lipson and Stotzky (1983) found that a fixed proportion of reovirus adsorbed to clay regardless of the concentration of virus added; this may have been a result of a heterogeneity in the net charge of the virus particles within a given population. Approximately 10% of a Herpes simplex virus type 1 (HSV1) population was not adsorbed to clay, which indicated that their surface was different from that of 90% of the HSV1 population (Stotzky, 1986, p. 399). Taylor et al. (1981) found two forms of poliovirus 2 with isoelectric points of 4.5 and 7.5. Lance and Gerba (1980) found that the depth to which poliovirus moved did not increase with increasing concentration of applied virus. They proposed that the differences in strength of the

negative charge among members of a given viral population could account for the adsorption of some viruses near the soil surface while others moved farther through the profile.

In the current research it is possible that the high surface degradation rate was due to peristaltic application of the influent. The changing shape and location of air-water interfaces near the drip point could increase adsorption of virus compared to the steady water flow deeper in the column. Some of the microscopic turbulence created by changes in water content are described by Childs (1969, p. 122):

> The greatest suction which can be maintained by the [air-water] interface corresponds to the sharpest curvature which can be accommodated in the channel through which the interface is being withdrawn, and the sharpest curvature occurs at the narrowest part...At the imposed suction required to get the interface beyond [this point], no position of rest can be found until the interface has withdrawn completely from the "cell" and has taken up a position in the next channel...In fact some water is left behind: the saddle-shaped masses are reduced by water withdrawal as suction is increased, but at the moment of instability their connection with the withdrawing main body is ruptured, and they spring back as isolated water rings round the points of contact between the particles.

In the model of Jury et al. (1987) the rapid decline in pesticide near the surface is attributed to a constant bacterial degradation rate, and the decrease in this rate in the transition zone to the a decrease in the population of bacteria (accounted for by G in eq. [6]). The soil in the current research was not sterile, so it is possible that bacterial enzymes contributed to the decline in virus concentration. Protease digested the protein capsid of the Coxsackie virus, but was ineffective against poliovirus (Bitton, 1978, p. 278). Cliver and Herrman (1972) tested six bacterial species and found only Bacillus subtilis and Pseudomonas aeruginosa gave firm evidence of antiviral activity against Coxsackie virus A9. Walter et al. (1985) report that cyanobacteria and algae are capable of inactivating enteric viruses. Cheo (1980) found that tobacco mosaic viruses were relatively stable in sterile soils, but were rapidly inactivated when placed in a nonsterile soil environment. Bacterial activity was the suspected cause of inactivation since the presence of high concentrations of bacteriocidal agents reduced the rate of inactivation. Poliovirus 1 and reovirus 3 survived two to three times longer in sterile than in non-sterile samples,

suggesting that microbial activity plays some role in virus inactivation in soils (Sobsey et al., 1980). Feachem et al. (1983, p. 146) reported that virus inactivation occurred much more rapidly under nonsterile aerobic soil conditions than under sterile aerobic conditions, or under sterile or nonsterile anaerobic conditions. In seawater the major virus inactivating agents appear to be of bacteriological nature on the basis of the physical size and the susceptibility of the antiviral factor to thermal and antibiotic treatments (Girones et al., 1989). Ward et al. (1986) found 27 out of 27 bacterial isolates from creek water produced enzymes that inactivated echovirus. Herrmann et al. (1974) labeled coxsackievirus A9 and poliovirus 1 with ^{14}C leucine; the more rapid inactivation in lake than in sterile lake water and the retention of the label on the 220 nm filters suggests microbial utilization of virus coat protein. On the other hand, LaBelle and Gerba (1982) found that the presence of bacterial nutrients enhanced virus survival, possibly by virus adsorption to the resulting bacterial population.

Jansons et al. (1989) found that dissolved oxygen concentration was the most significant factor in the loss of virus infectivity in groundwater (Jansons et al., 1989). This could be due biological degradation, unsaturated flow, or possibly chemical oxidation.

Physical adsorption to mineral and soil surfaces resulted in a loss of infectivity of reovirus (Moore et al., 1982). Adsorption of virus to the air-water interface may also physically disrupt and inactivate the virus due to surface tension effects. Proteins spread on the air-water interface are structurally altered in such a way that they will not redissolve (Adamson, 1982, p.155). Poliovirus were irreversibly inactivated in sludge through dewatering, not because of an increase in the ratios of surface area to volume, but because of some event that takes place during the evaporation process (Ward and Ashley, 1977). When tobacco mosaic virus suspension was aerated by bubbling, the virus underwent rapid degradation which was attributed to air-liquid surface force (Cheo, 1980). By spraying aerosols containing MS-2 virus through air of varying humidity Trouwborst and DeJong (1973) reported the maximum inactivation occurred at 70 to 75%

relative humidity when the aerosol particle is fluid, and the inactivation was less at lower relative humidity where aerosol particles were expected to be the solid state. They suggested that inactivation of MS-2 in aerosols could be explained by surface inactivation at the air-water interface. Trouwborst et al. (1974) found that phages T_1, MS-2, and Semliki Forest virus were rapidly inactivated by bubbling air or nitrogen gas through the suspension. The inactivation was attributed to the net radial stress on the partially hydrophobic virus at the air-water interface, which is proportional to the air-water surface tension.

The increased unsaturated transport of virus with organic matter may be due to a lower surface tension, since almost all organic substances found in natural waters reduce the interfacial tension (Stumm and Morgan, 1981, p. 606). Hunter and Liss (1981) report that the adsorption of surface active species on lake water may reduce the surface tension by 40%. Trouwborst and DeJong (1973) found that when the surface active substance OED (oxyethylene docosylether and oxyethylene octadecylether) formed a monolayer around aerosol droplets containing MS-2 virus, inactivation was reduced by a factor of more than 100. A conceptual model of some of the factors involved in virus adsorption and inactivation in unsaturated conditions is depicted in Fig. 8.

Implications for Water Treatment

The increased removal of virus by unsaturated flow suggests that waste water recharge facilities should provide for unsaturated flow, possibly by maintaining enough depth to ground water or by reducing surface hydraulic conductivity. This latter effect normally occurs with time as the surface pores become clogged with particulate matter and algae growth. Care must be taken to assure that saturated pulses of water (fingers) that tend to form below restricting layers (Baker and Hillel, 1990) do not reach the ground water.

This research supports the policy of reducing the amount of organic matter in water to improve virus removal. A site that had been irrigated with sewage for 40 years had a buildup of

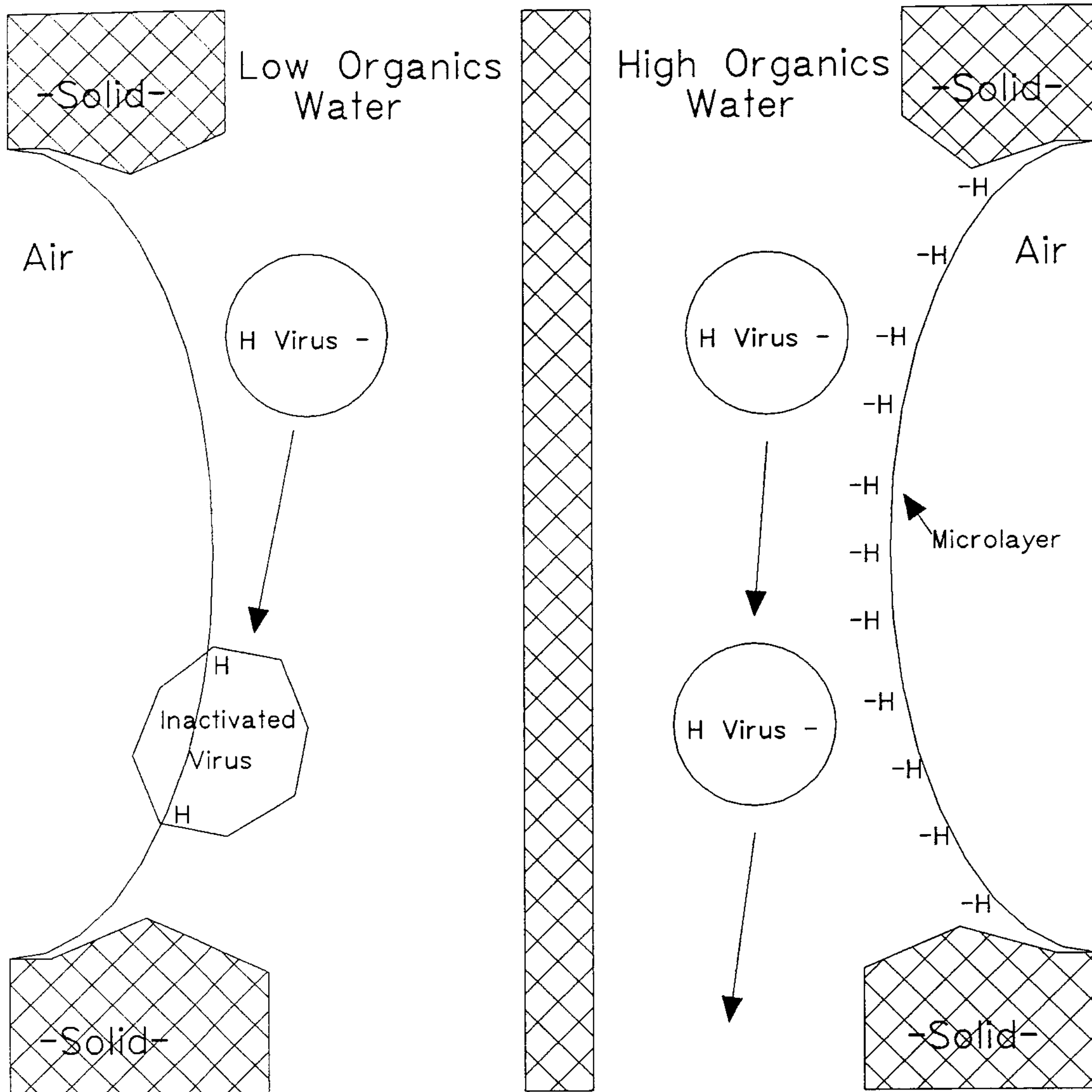


Fig. 8. A conceptual model of virus adsorption and inactivation at the air-water interface. "H" represents hydrophobic groups, and "-H" represents dissolved organic matter that is negatively charged at the experimental pH. The net negative charge on the virion keeps it from sorbing to the soil solids. Hydrophobic groups on the virion tend to be excluded from water, and permit the virion to be adsorbed to the air-water interface. Surface tension distorts adsorbed virus leading to inactivation. If the air-water interface is coated with an organic microlayer the virus does not adsorb and does not become inactivated.

organic coatings and decreased virus adsorption (Fuhs et al., 1985). Bixby (1980) advised that organic soils should be viewed cautiously as land disposal sites. Gerba et al. (1975) found that poliovirus removal from wastewater effluent was greatly improved by reducing the amount of organics by lime coagulation.

Future Research

It would be important to explore the effects of unsaturated flow and organic matter in a field situation to make the results more useful for water treatment. The same virus, sampling device, water, soil, and other factors should be used if possible. The field research could then be extended to more realistic conditions using sewage effluent, native soil, and groundwater sampling.

Research into the mechanisms of virus removal may provide basic data that would be useful in general colloid transport studies and other areas of soil science. Does adsorption to the air-water interface actually the cause removal of virus? If so, the transport of other materials may be influenced by adsorption to the air-water interface, and transport models should take this form of adsorption into account. Electron microscopy of the air-water interface might show virus adsorption. This would be challenging given the difficulty in preserving small soft objects like virus in the dense soil matrix. Transmission electron microscopy might be possible by obtaining thin sections of the air-water interface from a "soil" made of soft material. Scanning electron microscopy might be possible by rapidly freezing the soil and observing broken surfaces. Would it be possible to completely cover the air-water interface with virus to increase the steady-state concentration at a given depth? Could a surfactant be used to alter the hydrophobicity and surface tension at the air-water interface with resulting effects on virus removal?

Both the field and microscopy areas of research might be facilitated by developing virus analogs. The technology of making latex beads and colloids of other materials has been advancing rapidly. It is possible to attach carboxyl, amino, and other groups to the beads which

could give them surface properties similar to various viruses. Some beads are fluorescent or magnetic, which would make it easier to measure concentrations.

It may be possible to use genetic engineering or other biochemical techniques such as antibody-antigen reactions to permit identification of small quantities of a specific virus in groundwater. Any technology that can supplement growing virus on specific host cells as the means of enumeration would facilitate virus transport research.

CHAPTER 5.

CONCLUSIONS

1) Under saturated conditions MS-2 bacteriophage showed little adsorption or inactivation due to passage through loamy sand. The saturated average partition coefficient k_p was -0.07 (SD = 0.15). Relative concentrations (C/C_o) reached one in less than two pore volumes with the average retardation coefficient equal 0.92.

2) Under unsaturated conditions MS-2 was strongly removed from the soil water. After the passage of as many as 18 pore volumes, the virus concentrations did not approach the influent concentration, but maintained steady-state relative concentrations $(C/C_o)_s$ that declined exponentially with depth, from 0.20 m where $(C/C_o)_s = 0.27$, to 1.05 m where $(C/C_o)_s = 0.05$. The resulting depth profiles were consistent with a mathematical model developed by Jury et al. (1987).

3) Effectively none of the virus removed in the unsaturated conditions could be recovered from the soil by elution. The average partition coefficient k_p was 0.28 (SD = 0.40), so k_p should not be considered different from zero. The removed and non-recoverable virus were assumed to be inactivated.

4) The steady-state values were higher if substantial dissolved organic matter occurred with the virus; 8 to 10 times as much virus reached the 1.05 m depth when the soil water contained 1.4 to 3.0 times as much organic matter from natural humus or primary sewage sludge.

APPENDIX A.

DATA FROM SUPPORT EXPERIMENTS (SE)

SE1: Soil Water Volume Versus Water Potential Relationships

Volumetric water content vs potential relationships were established using a tension plate assembly with hanging water column (Hillel, 1982, p. 85). A buchner funnel with a 1 bar porous ceramic plate was connected by a 1/16" ID flexible tube to a buret graduated in mL, and the apparatus filled with tap water. The soil material was poured into a brass cylinder and given 2 to 3 cycles of wetting and drying until the soil volume was constant. Starting at near saturation, the buret was lowered about 0.10 m and when the meniscus level in the burette stabilized the volume extracted and the distance between the meniscus and the surface of the soil was recorded. This process was repeated until a potential of less than -0.80 m was reached, and then the buret was raised in increments to near saturation. The wet and oven-dry (105°C) weight of the soil was determined, and the volumetric water content (Θ_v) determined for each potential of the desorption and sorption curve.

The Θ_v versus soil water potential for seven different bulk densities of the experimental soil material were measured. Some of the variation is due to the difference in bulk densities, and some may be due to random variation in soil packing.

In those transport experiments where Θ_v was estimated from the measured potential, the volume-potential results for bulk density of 1.55 Mg m^{-3} was used since most of the transport experiments had bulk densities around this value. For example, at a typical potential of -0.29 m the volumetric water content was considered to be 0.346.

SE1: Soil water volume versus potential (poten.) relationships

bulk den. (Mg m^-3)	Desorp. or Sorp.	Vol. Water (m^3/m^3)	Poten. (m)	bulk den. (Mg m^-3)	Desorp. or Sorp.	Vol. Water (m^3/m^3)	Poten. (m)
1.45	Desorp.	0.31	-0.03	1.52	Desorp.	0.416	-0.05
		0.299	-0.175			0.413	-0.06
		0.277	-0.391			0.406	-0.23
		0.26	-0.61			0.398	-0.45
		0.223	-0.787			0.338	-0.65
		0.187	-0.939			0.321	-0.97
	Sorp.	0.202	-0.755			0.301	-1.32
		0.225	-0.553			0.169	-2.02
		0.253	-0.364		Sorp.	0.173	-1.31
		0.269	-0.19			0.192	-0.83
		0.3	-0.03			0.219	-0.6
						0.272	-0.41
1.52	Desorp.	0.345	-0.03			0.307	-0.05
		0.333	-0.19				
		0.324	-0.443	1.51	Desorp.	0.43	-0.05
		0.31	-0.645			0.422	-0.22
		0.231	-1.12			0.417	-0.34
						0.411	-0.53
1.55	Desorp.	0.357222	-0.04			0.298	-0.8
		0.353056	-0.17				
		0.346111	-0.29	1.52	Desorp.	0.361	0
		0.335	-0.437			0.355	-0.24
		0.315556	-0.733			0.313	-0.66
	Sorp.	0.319722	-0.555			0.239	-0.97
		0.324583	-0.421			0.151	-1.93
		0.332222	-0.267		Sorp.	0.196	-0.88
		0.341944	-0.131			0.233	-0.54
		0.321111	-0.733			0.252	-0.09
						0.289	0
1.49	Sorp.	0.229	-0.63				
		0.416	-0.01				
	Desorp.	0.411	-0.14				
		0.377	-0.56				
		0.2	-1.17				
		0.163	-1.65				

SE2: Virus Removal by Samplers

The removal of MS-2 coliphage by stainless steel and ceramic soil water samplers was tested by Susan Kutz-Bradford. The samplers selected had appropriate pore sizes for use in future experiments on the transport of virus through unsaturated soil columns. Each stainless steel or ceramic soil water sampler was glued into a clear polypropylene tube, using epoxy to make a water tight seal. The filter devices (sampler and tube) were disinfected by chlorination. Free chlorine was neutralized using a 10% sodium thiosulfate solution. A sterile rubber septum was fitted over the open end of the plastic tube. A stock suspension of MS-2 bacteriophage was diluted in dechlorinated tab water to achieve the influent concentrations listed.

A 5 ml portion of the virus suspension was placed into individual sterile syringes fitted with 27 gauge needles. The suspensions were then individually passed through the two sampling devices. Samples of the influent and effluent suspensions were collected into sterile 25 mL polypropylene containers. Adsorption of the virus to the syringes, rubber septa, and tubing was determined by passing the suspension through filtration devices that did not have the porous ceramic or stainless steel. Influent and effluent MS-2 samples were appropriately diluted in Tris buffer. MS-2 samples were kept at 4°C prior to being assayed.

Recoveries of MS-2 coliphage from two different types of soil samplers are shown. No decrease in phage numbers was detected in the devices that did not have the samplers and therefore any decrease in titer was assumed to be due to the soil water sampler. Passing the virus through the porous stainless steel resulted in no loss of virus. The decrease due to the ceramic ranged from 46 to 89.5% of the original titer, and the average loss was 74%. Consequently, the stainless steel samplers were used for the transport experiments.

The material from which the soil sampler is made and also possibly its configuration appears to have an impact on the retention of virus. Although pore size per se is not considered to be a factor in the retention of virus particles (retention is mainly due to adsoption and not a

physical entrapment of the particles), a smaller pore size may provide a greater surface area in which the virus may encounter a greater number of appropriate electrostatic forces resulting in possible adsorption sites. Scanning electron micrographs of the ceramic samplers show finer and more angular particles than those of the stainless steel samplers. The ceramic filters are probably kaolinite in nature. The adsorption of viruses to clay minerals is well documented and the retention of MS-2 by the ceramic samplers may be due to their clay composition.

Soil water samplers (in sampling ports at various soil depths) in soil columns provide an effective way to monitor the transport of virus. It would appear from this study that one type of soil water sampler may not be suitable for use in tracing the movement of different types of virus and bacteria through soil. A sampler must be selected based on the type of material or organism to be tested, the level of unsaturation in the soil, and the type of soil used in the column study. A thorough investigation on the retention of the virus or bacteria by the soil water sampler should be done prior to soil column experiments.

SE2: Virus removal by samplers.

Cntl = control, the plastic tube without sampler
SS = porous stainless steel disk, 1 um pore size
Soil Measurement, Tucson, AZ
CR = porous ceramic disk, 0.6 um pore size
Soil Moisture, Santa Barbara, CA

VIRUS CONCENTRATIONS#

Material	Rep.	influent	effluent
		-----pfu/mL-----	
SS Cntl	1	7.9E+07	1.0E+08
SS	1	7.1E+07	8.5E+07
SS	2	7.5E+07	7.9E+07
SS	3	9.4E+07	6.1E+07
CR Cntl	1	2.2E+05	2.9E+05
CR	1	1.9E+05	2.0E+04
CR	2	3.2E+05	4.0E+04
CR	3	3.9E+05	2.1E+05

Assay performed by Susan Kutz-Bradford

SE3: Virus Survival with KBr

The survival of MS-2 with KBr was tested in well water (influent solution) and in soil water (sample solution). Three glass bottles were filled with virus solution. The first contained virus in 100 mL of well water. The second had virus, 100 mL well water, and 0.013 g KBr (10^{-3} M). The third had virus, 100 mL soil water extracted through the stainless steel samplers at the 0.80 m depth of a column of the experimental soil material, and 0.012 g KBr (10^{-3} M). Virus and Br concentrations were measured at 0, 3, 6, and 13 days.

Virus titers and Br concentrations in well water and soil water were measured over a 13 day period, which was longer than any of the transport experiments. Some of the variation in the data may be due to variability between the four separate assays. However, the overall results showed that neither the virus titer nor the bromide concentrations were reduced over this time period. Consequently, KBr was used as a chemical tracer in many of the transport experiments. Since virus titer did not decrease in soil water, samples could be stored at 4° C in glass test tubes for as long as 13 days before assaying. (Normally, samples were assayed in less than 5 days.)

SE3: Virus survival with KBr.

January 1988
Glass bottles
Bottle 1: 100 mL well water + virus
Bottle 2: 100 mL well water + virus + 0.013 g KBr
Bottle 3: 100 mL soil water + virus + 0.012 g KBr

VIRUS CONCENTRATIONS

Bottle	Time (d) 0	3	6	13
	pfu/mL			
1	147000	174000	164500	190000
2	173000	174000	194500	188500
3	269000	137000	206000	162500

BROMIDE CONCENTRATIONS #

Bottle	Time (d) 0	3	6	13
	mmol/L			
2	1.046	1.08	0.779	0.964
3	1.046	0.954	0.846	0.918

Bromide concentrations from Corning select ion electrode

SE4: Batch Virus Removal by Soil

MS-2 removal by the experimental soil was tested in a batch study using three glass flasks. In flask one was virus plus 300 mL well water. In flask two was virus, 300 mL well water, and 1 g soil. In flask three was virus, 300 mL well water, and 10 g soil. Samples were extracted through the porous steel samplers and Tygon tubing at 0, 0.04, 0.25, and 1 day.

Although this experiment was conducted for only one day, it is likely that most of the possible removal had occurred because of the mixing of the solution after each sampling period. The results indicate that there is no loss of MS-2 by adsorption to soil in a batch study under these conditions of pH, soil type, and temperature.

SE4: Batch virus removal by soil.

May 1988
Glass flasks
Flask 1: 300 mL well water + virus
Flask 2: 300 mL well water + virus + 1 g soil
Flask 3: 300 mL well water + virus + 10 g soil

Samples extracted through porous stainless steel and Tygon

VIRUS CONCENTRATIONS

	Time of sample (d)			
	0.00	0.04	0.25	1.00
Flask	pfu/mL			
1	644000	678000	804000	772000
2		684000	682000	892000
3		694000	638000	1056000

SE5: Virus Removal by Container Materials

Because virus adsorption or inactivation by container material could influence the results, MS-2 removal by the containers used in this study was tested. The materials were polypropylene (used in the influent stock bottle), polyvinyl chloride (PVC was used in the soil column cylinder), and Pyrex glass (used in the sample tubes). Each container contained 500 mL well water, virus, and 10^{-3} M KBr. Samples were taken through a porous stainless steel cup and Tygon tubing at 0, 1, 3, 5, 8, and 11 days for virus assay. In addition, Br concentrations were measured at 0, 5, and 11 days.

The inactivation rate of the virus in the PVC cylinders was found to be essentially the same as in glass or polypropylene containers: the concentration decreased by about 4% of initial value per day. The Br concentrations were not affected by storage in any of these materials.

SE5: Virus removal by container material

June 1988
Container P: Polypropylene
Container V: Polyvinyl chloride
Container G: Glass

Each contained 500 mL well water + 10^-3 M KBr + virus.
Samples extracted through porous stainless steel and Tygon.

VIRUS CONCENTRATIONS

	Time of sample (d)					
	0	1	3	5	8	11
Container	pfu/mL					
P	85000	92000	83000	110000	24500	63500
V	143000	159500	100500	92500	82500	90000
G	127500	172500	148000	102000	127000	66000

BROMIDE CONCENTRATIONS

	Time of sample (d)					
	0	1	3	5	8	11
Container	mmol/L					
P	0.96			0.96		1.06
V	1.06			1.06		1.06
G	1.01			0.96		0.96

APPENDIX B.

DATA FROM TRANSPORT EXPERIMENTS (TE)

Legend for Transport Experiments

TE = Transport experiment

col = column letter

b = bulk density

w = volumetric water content determined by weighing

w,est = volumetric water content estimated from volume-potential curve (SE1) at b = 1.55 Mg m^{-3}.

q = infiltration rate

p = water potential

pfu = plaque forming unit

1.05 m samples taken from outflow collected over preceding sampling period.

TE1: Short Term Test

The primary purpose of this experiment was to test the application and extraction procedures, and to see how many samples were needed early in an experiment. The virus did not reach 0.8 m by the end of the experiment, and the concentrations at shallower depths increased gradually. Consequently, more frequent sampling was not necessary.

TE1: Short term test.

November 1987
b = 1.50 Mg/m^3   w,est = 0.36
q = 0.187 m/d   p = -0.20 m

Col	Depth (m)	Time (d) 0	0.08	0.25	1
		pfu/ml			
A,B	0	7370			6000
A	0.05	0	2010	2830	7055
A	0.2	0	0	0	600
A	0.8	0	0	0	0
B	0.05	0	315	2030	5665
B	0.2	0	0	0	2125
B	0.8	0	0	0	0

TE2: Medium Term Test

In this experiment two replicate columns were run under the same unsaturated conditions to test the variance between columns. In column A, a horizontal crack in the soil formed at the 0.40 m level on day 2. This restricted the unsaturated flow of water and produced nearly saturated conditions above this level. The crack was formed when the lower portion of soil settled while the upper portion was held up by rigid samplers. This problem was remedied in later experiments by gluing the porous cups to flexible Tygon tubing that extended into the soil. The higher concentrations of virus in column A reflect the higher water content.

Several anomalous values from column B, 0.05 m sampler, were recorded. The sampling in this experiment was done with a needle through a septum stopper, and this sampler had a leaking stopper. This problem was remedied by replacing the septum-and-needle extraction method with the two-tube-lifting-manometer method in later experiments as described in "Materials and Methods."

The general trends in column B indicate the initial fronts of Br and MS2 are transported at nearly the same rate. The Br concentrations from all ports tend toward $C/C_o = 1$. The MS-2 concentrations appear to reach an approximately steady value that decreases with depth and does not increase to the influent concentration. For example, on day 3 at 0.20 m $C/C_o = 0.83$ and at 0.80 m $C/C_o = 0.38$, and on day 5 at 0.20 m $(C/C_o) = 0.56$ and at 0.80 m $(C/C_o) = 0.21$.

TE2: Medium term test.
February 1988

Column A
b = 1.52 Mg/m^3   w,est = 0.36
q = 0.187 m/d   p = 0.02 m

Column B
b = 1.53 Mg/m^3   w,est = 0.35
q = 0.187 m/d   p = -0.24 m

VIRUS CONCENTRATIONS

Col	Depth (m)	0	1	2	3	4	5
		Time of sample (d) ---pfu/mL---					
A,B	0	7750		10200			7150
A	0.05		3100	4250	10000	11700	12900
A	0.2		5100	1800	8000	6950	4700
A	0.4		3850	4350	6700	3900	5850
A	0.8		0	785	4100	6600	1800
B	0.05		6600	1150	5600	2800	6700
B	0.2		4550	3450	5750	3350	4100
B	0.4		600	3350	5300	1700	1550
B	0.8		0	895	2200	1150	600

BROMIDE CONCENTRATIONS

Col	Depth (m)	0	1	2	3	4	5
		Time of sample (d) ---mmol/L---					
A,B	0	0.897		0.777			0.987
A	0.05		0.855	0.530	0.741	0.897	0.941
A	0.2		0.674	0.345	0.642	0.741	0.741
A	0.4		0.584	0.612	0.674	0.855	0.815
A	0.8	0.000	0.000	0.204	0.642	0.897	0.707
B	0.05		0.741	0.126	0.345	0.777	0.815
B	0.2		0.674	0.345	0.460	0.460	0.674
B	0.4		0.146	0.329	0.506	0.482	0.506
B	0.8	0.000	0.000	0.380	0.674	0.897	0.987

TE3: Long Term Test with Ponding

To see if virus would eventually occupy all adsorption sites and the virus concentration in the soil reach the influent level this 10-day experiment was conducted. On day 9 the flow rate was doubled by adding additional tubes of well water without virus or bromide. After 5 h the surface had ponded, and the extra flow was stopped.

On day 2 some virus had reached the 1.05 m level, whereas the bromide was not detectable. This indicates that the virus was moving slightly ahead of the bromide as might be expected from size exclusion of the virus from part of the soil water volume. The decrease of virus concentration with depth was approximately stable from day 2 to 9. On day 10, 19 h after the original flow rate was restored, the virus concentrations had increased. Since the concentrations at the 0.05 and 0.20 m depths were greater than the influent, it appears that some adsorbed virus were mobilized. (The virus concentrations at 0.40 m depth should also be considered greater than influent since water at this depth had infiltrated with half the usual concentration of virus and bromide due to dilution on day 9.)

Bromide concentrations on day 3, 1.05 m depth, had reached $C/C_o = 1$. C/C_o at the 0.40 and 0.80 m levels were still less than 1. It is not clear why this occurred. Some of the variability in bromide concentrations may have been due to the dilution procedure which could have caused errors in the volume measurement, as well as variations in electrode sensitivity. The influent bromide was diluted on day 9, and the pulse of dilution showed up at the 0.40 m level on day 10.

TE3: Long term test with ponding.
March 1988

Column C, day 0-9
b = 1.56 Mg/m^3   w,est = 0.35
q = 0.138 m/d   p = -0.25 m

Column C, day 9-10
b = 1.56 Mg/m^3   w,est = 0.35
q = 0.169 m/d   p = -0.21 m

Column D, day 0-9
b = 1.54 Mg/m^3   w,est = 0.34
q = 0.137 m/d   p = -0.30

Column D, day 9-10
b = 1.56 Mg/m^3   w,est = 0.35
q = 0.164 m/d   p = -0.19 m

VIRUS CONCENTRATIONS (days 6 to 10 on next page)

Col	Depth	Time of sample (d)					
		0	1	2	3	4	5
	(m)	pfu/mL					
C,D	0.00	89500	34500	105500	79500	104500	67000
C	0.05		17000	100000	61500	58000	67500
C	0.20		5950	24000	17500	23000	30000
C	0.40		4500	13000	10700	14500	25000
C	0.80		0	3000	3850	5000	6550
C	1.05		0	650	2200	3600	5300
D	0.05		32500	60000	44000	54000	81000
D	0.20		2650	19000	24500	16400	31500
D	0.40		650	15000	11500	18500	20500
D	0.80		0	2400	2800	5750	12500
D	1.05		0	350	3650	3700	5350

BROMIDE CONCENTRATIONS (days 6 to 10 on next page)

Col	Depth	Time of sample (d)					
		0	1	2	3	4	5
	(m)	mmol/L					
C,D	0.00	1.028	0.935	1.078	1.012	1.012	1.012
C	0.05	0.000	0.809	1.027	0.965	0.877	0.920
C	0.20	0.000	0.668	0.848	0.877	1.012	1.012
C	0.40	0.000	0.607	0.890	0.760	0.920	1.061
C	0.80	0.000	0.000	0.848	1.012	1.061	1.012
C	1.05	0.000	0.000	0.000	1.061	1.012	1.061
D	0.05	0.000	0.809	1.077	1.012	1.012	1.012
D	0.20	0.000	0.203	0.890	0.965	0.691	0.877
D	0.40	0.000	0.000	0.933	0.965	0.965	1.012
D	0.80	0.000	0.000	0.579	0.628	0.920	0.965
D	1.05	0.000	0.000	0.000	1.061	0.965	1.012

TE3 (Continued).

VIRUS CONCENTRATIONS

Col	Depth	Time of sample (d) 6	7	8	9	10 #
	(m)	pfu/mL				
C,D	0.00	57000	45000	31000	41500	44000
C	0.05	29500	30000	27000	25500	40500
C	0.20	12500	23000	9150	14500	43500
C	0.40	7400	10000	3700	6900	20000
C	0.80	1950	2900	1350	2350	4800
C	1.05	2200	1850	1400	900	3350
D	0.05	25500	20400	18000	27500	58000
D	0.20	13600	7150	6550	15000	23000
D	0.40	12000	11000	4650	9900	12000
D	0.80	3800	2750	2250	3050	5150
D	1.05	2450	3350	2350	2900	5100

BROMIDE CONCENTRATIONS

Col	Depth	6	7	8	9	10 #
	(m)	mmol/L				
C,D	0.00	1.012	0.965	1.061	0.988	0.928
C	0.05	0.965	0.836	1.012	0.942	0.856
C	0.20	0.965	0.965	0.965	0.942	0.942
C	0.40	1.012	1.061	1.012	0.988	0.613
C	0.80	1.012	1.061	0.965	1.036	0.988
C	1.05	1.061	1.012	0.965	1.087	0.988
D	0.05	1.012	0.965	1.012	0.988	0.942
D	0.20	0.965	0.836	0.965	0.898	0.816
D	0.40	1.061	1.061	0.920	0.988	0.643
D	0.80	1.012	0.725	0.797	0.942	0.856
D	1.05	0.965	0.965	0.920	1.036	0.988

After day 9, q was doubled for 5 h creating ponding, then returned to the previous q.

TE4: Testing the Effect of Different Flow Rates

This experiment compared a typical infiltration rate (0.16 m/d, column D, the "wetter soil") with a low rate (0.055 m/d, column C, the "drier soil"). The wetter soil had much higher virus concentrations. For example, on day 4 the wetter soil (Θ_v = 0.364) virus concentrations were 6 to 96 times greater than the drier soil (Θ_v = 0.346). Since the virus concentrations were still increasing in the drier soil when the flow rates were increased on day 4, it is not clear whether the lower concentrations were due to lower water content or to insufficient time to establish steady-state conditions.

When the flow rates were increased on day 4, a slight pulse of higher virus concentrations occurred on day 5 in the drier soil at 0.40 m and in the wetter soil at 0.80 m. However, there were no increases to $C/C_o > 1$ as in the previous experiment. Mobilization of adsorbed virus, if it occurred at all, was much less than in the previous experiment.

Bromide concentrations showed a high degree of hydrodynamic dispersion. For example, in the wetter soil at 0.80 m the passage of 0.55 pore volumes (T) resulted in Br $C/C_o = 0.06$; at 2.2 T, Br $C/C_o = 0.79$. This suggests the presence of preferred flow paths in the soil column.

TE4: Testing effect of different flow rates.
June 1988

Column C (dry), day 0-4:
b = 1.56 Mg/m^3 w,est = 0.346
q = 0.055 m/d p = -0.46 m

Column C, day 4-6:
b = 1.56 Mg/m^3 w,est = 0.368
q = 0.24 m/d p = -0.08 m

Column D (wet), day 0-4
b = 1.54 Mg/m^3 w,est = 0.364
q = 0.16 m/d p = -0.23 m

Column D, day 4-6
b = 1.54 Mg/m^3 w,est = 0.368
q = 0.24 m/d p = -0.11 m

VIRUS CONCENTRATIONS

Col	Depth (m)	Time of sample (d) 0	1	2	3	4	4.77	5	6
		pfu/mL							
C,D	0.00	95000	94500	70000	91500	82500	84000	84000	85000
C	0.20	0	3150	5400	6350	6000	36000	32000	65500
C	0.40	0	0	150	1900	1250	13000	37000	28000
C	0.80	0	0	0	0	150	7000	14000	13500
D	0.20	0	28000	35000	43500	37500	44500	64000	47500
D	0.40	0	23000	30500	37000	29500	41000	29000	46000
D	0.80	0	0	6350	15500	14450	21500	33000	34000

BROMIDE CONCENTRATIONS

Col	Depth (m)	Time of sample (d) 0	1	2	3	4	4.77	5	6
		mmol/L							
C,D	0.00	0.997	0.997	0.997	0.997	0.997	0.997	0.997	0.997
C	0.20	0.000	0.227	0.536	0.619	0.714	0.951	0.951	0.906
C	0.40	0.000	0.052	0.238	0.403	0.619	0.997	0.906	0.864
C	0.80	0.000	0.043	0.037	0.060	0.384	0.951	0.951	0.951
D	0.20	0.000	0.619	0.785	0.864	0.906	0.906	0.906	1.046
D	0.40	0.000	0.590	0.785	0.824	0.906	0.997	0.951	1.046
D	0.80	0.039	0.057	0.302	0.619	0.785	0.906	0.906	0.997

TE5: All Column Effluent Extracted through a Sampler

The purpose of this experiment was to use short columns (0.10 m long) to facilitate calculation of Θ_v by keeping the columns on a scale for weighing. To maintain unsaturated flow in a short column, negative pressure must be applied to the water at the bottom of the column. This was accomplished by extracting all the flow through a porous stainless steel cup and through a tube with its outlet 2.10 m (Ks) or 0.88 m (Ls) below the soil surface.

The virus concentrations on days 1 and 2 illustrate the effect of Θ_v on the average linear velocity, with the drier column effluent having higher virus concentrations. It is unclear why the drier column had higher virus concentrations after day 2.

This experiment had to be terminated after 5 days because outflow declined and water content increased in both columns. The wetter column ponded on day 2 and the drier column ponded on day 5. Apparently a biofilm formed on the porous cups which restricted flow. This was confirmed after the experiment by flushing the cups with dilute bleach which oxidized the biofilm and restored the flow. Porous stainless steel may not be suited for extracting large amounts (>300 mL) of water from non-sterile media.

TE5: All column effluent extracted through samplers.
July 1988

Column Ks
b = 1.52 Mg/m^3   w = 0.216 - 0.368
q = 0.049 m/d   p = -2.10 m

Column Ls
b = 1.51 Mg/m^3   w = 0.346 - 0.403
q = 0.049 m/d   p = -0.88 m

VIRUS CONCENTRATIONS

Col	Depth (m)	Time of sample (d)# 0	1	2	3	4	5
		pfu/mL					
Ks,Ls	0.00		4000000			4200000	
Ks	0.10		1300000	3400000	3650000	3850000	
Ls	0.10		195000	2250000	2300000	2550000	

VOLUMETRIC WATER CONTENTS (w)

Col	Time of sample (d) 0	1	2	3	4	5	
	m^3/m^3						
Ks	0.216	0.232	0.239	0.267	0.328	0.368	=pond##
Ls	0.346	0.389	0.403	=pond	pond	pond	

Samples taken from outflow collected over previous sampling period.
pond = standing water on surface.

TE6: Saturated Versus Unsaturated Flow

This experiment had three objectives: 1) compare saturated versus unsaturated flow on virus transport, 2) use higher virus concentrations (about 50 times higher) and a long application period to see if adsorption sites would fill and permit C/C_o to increase to one, and 3) test the effect of ponding on the mobilization of virus from the unsaturated column.

The virus concentrations in the unsaturated column (D) reached steady-state on day 3. C/C_o values were about the same as in TE4. In spite of the higher virus concentrations $(C/C_o)_s$ did not increase. The ponding of column D on days 8 and 9 resulted in higher virus concentrations as in TE4, but not as high as the saturated column. The saturated column (E) had little loss of virus, and C/C_o increased to one after flow of 1 to 2 pore volumes.

The attempt to saturate column D on day 7 was not entirely successful. The outlet of the column was clamped, and when the surface ponded a Mariotte siphon was started and the outlet unclamped. This kept the surface ponded; however, during the next two days the manometers showed that negative pressures (about -1.5 J kg^{-1}) persisted. Apparently, the hydraulic conductivity near the surface was less than the rest of the column. This lack of saturation probably explains why the virus concentrations in column D did not reach $C/C_o = 1$, as in column E.

TE6: Saturated versus unsaturated flow.
August 1988

Column D (unsaturated), day 0-7:
b = 1.54 Mg/m^3   w,est = 0.34
q = 0.095 m/d   p = -0.33 m

Column D, day 8-9:
b = 1.54 Mg/m^3   w,est = 0.36
q = 0.222 m/d   p = -0.18 m

Column E (saturated), day 0-9
b = 1.51 Mg/m^3   w,est = 0.43
q = 0.088 m/d   p = no data

VIRUS CONCENTRATIONS (Days 6 to 9 below)

Col	Depth	Time of sample (d) 0	1	2	3	4	5
	(m)	pfu/mL					
D,E	0.00	4000000	5000000	4900000	5225000	5550000	4800000
D	0.20		330000	1725000	3400000	4200000	3100000
D	0.40		49000	1075000	2785000	2475000	2250000
D	0.80		0	450	655000	530000	605000
D	1.05		0	0	1200	130500	140000
E	0.20		2710000	4150000	5850000	5450000	5200000
E	0.40		0	3400000	4800000	5500000	3550000
E	0.80		0	50	49000	1850000	3600000
E	1.05		0	50	29500	685000	4350000

VIRUS CONCENTRATIONS (Continued)

Col	Depth	Time of sample (d) 6	7	8	9
	(m)	pfu/mL			
D,E	0.00	4250000	2600000	2250000	3450000
D	0.20	3250000	785000	2500000	2800000
D	0.40	1700000	1045000	2600000	1800000
D	0.80	620000	132500	1040000	950000
D	1.05	160000	37500	390000	1200000
E	0.20	4750000	1450000	2350000	1700000
E	0.40	4400000	1950000	2350000	1950000
E	0.80	3850000	2250000	2400000	3350000
E	1.05	3900000	2500000	2550000	3750000

TE7: Unsaturated to Ponded

The objective of this experiment was to test the effect of changing unsaturated flow to saturated. The method of saturation was to raise the outlet tube to maintain a ponded condition. After ponding the manometers indicated positive pressure, but the water content did not reach saturation probably due to entrapped air.

The virus concentrations were high all through the experiment. Ponding had no effect on the concentrations. This column had been packed with fresh soil. In hindsight, it is likely that the high level of soil water organic matter resulted in the high virus concentrations. It is interesting that most of the loss of virus occurred in the first 0.20 m. This may be due to greater leaching and less organic matter in the soil water in the shallow zone.

TE7: Unsaturated to ponded
September 1988

Column F, day 0-4:
b = 1.53 Mg/m^3 w = 0.357
q = 0.105 m/d p = -0.58 m
fresh soil

Column F #, day 5-9:
b = 1.53 Mg/m^3 w = 0.394
q = 0.093 m/d p = 0.218 m

VIRUS CONCENTRATIONS

Col	Depth	Time of sample (d)					
		0	1	2	3	4	5
	(m)	pfu/mL					
F	0.00	900000	840000	620000	515000	937500	885000
F	0.20				350000	647500	960000
F	0.40				745000	817500	762500
F	0.80				320000	865000	562500
F	1.05		0	0	105000	780000	757500

VIRUS CONCENTRATIONS (Continued)

Col	Depth	Time of sample (d)			
		6	7	8	9
	(m)	pfu/mL			
F	0.00	1340000	1150000	1150000	1500000
F	0.20	825000	765000	765000	675000
F	0.40	810000	585000	585000	900000
F	0.80	585000	715000	715000	650000
F	1.05	870000	640000	640000	660000

Nearly saturated. Less than 0.01 m-deep pond produced by raising the outlet tube.

TE8: Longer Term Unsaturated to Ponded

The purpose of this experiment was to determine if increasing soil water content to ponding would mobilize adsorbed virus. The same ponding technique as TE7 was used. The virus concentrations declined with each depth increment as seen in all the unsaturated transport experiments except TE7. After ponding on day 6, there was no increase in titer at the 0.10, 0.40, and 1.05 m, so the mobilization hypothesis was not supported. The ponded flow did not produce $C/C_o = 1$. It is possible that enough air remained in the soil during ponded flow to remove virus (volumetric air content only decreased from the unsaturated level of 0.066 to the ponded level of 0.039).

On day 2 at 1.05 m virus $C/C_o = 0.016$ and [Br] = 0. This supports the idea that the initial front of a colloid tends to be transported faster than solutes.

TE8: Longer term unsaturated to ponded.
October 1988

Column G, day 0-6:
b = 1.50 Mg/m^3 w = 0.367
q = 0.155 m/d p = -0.41 m

Column G, day 7-11:
b = 1.50 Mg/m^3 w = 0.394
q = 0.145 m/d p = nd

VIRUS CONCENTRATIONS (Day 6 to 11 below)

		Time of sample (d)					
Col	Depth	0	1	2	3	4	5
	(m)	pfu/mL					
G	0.00	98000	49000	52000	52500	40000	53000
G	0.10						
G	0.40						
G	0.80						
G	1.05	0	0	850	13100	12350	14850

BROMIDE CONCENTRATIONS (Day 6 to 11 below)

		Time of sample (d)					
Col	Depth	0	1	2	3	4	5
	(m)	mmol/L					
G	0.00	0.087					
G	1.05	0.000	0.000	0.000	0.077	0.087	0.087

VIRUS CONCENTRATIONS (Continued)

		Time of sample (d)					
		pfu/mL					
Col	Depth	6	7	8	9	10	11
	(m)						
G	0.00	50000	35500	50500	39000	39500	30500
G	0.10	33000	35500				
G	0.40	23000	19000				
G	0.80	16000	21000				
G	1.05	15000	12000	11500	15000	18000	13000

BROMIDE CONCENTRATIONS (Continued)

		Time of sample (d)					
		mmol/L					
Col	Depth	6	7	8	9	10	11
	(m)						
G	0.00						
G	1.05	0.091	0.087	0.095	0.095	0.091	0.091

TE9: Unsaturated to Ponded like TE3

This experiment replicated TE3 primarily to see if the pulse of virus "released" after ponding could be observed again. In addition to duplicating the same bulk density and flow rate as TE3, virus adsorbed to the soil were eluted with TSB, and the organic matter in the soil water leaving the columns was measured. Also, column H was put through an unsaturated to ponded to unsaturated cycle.

The virus concentrations reached and maintained levels that declined with depth as in TE3. The increase in water content on day 6 did not mobilize virus.

The elution of adsorbed virus at the end of the experiment permitted calculation of partition coefficient k_p (Eq. [3]). S (pfu/g) was calculated by subtracting the virus in the water of the soil sample from the total pfu assayed from the eluted sample. The virus in the soil water was determined from the mass of the soil sample, Θ_v, and the virus concentration C (pfu/mL) measured in the soil water sample at the given depth.

TE9: Unsaturated to ponded like TE3.
December 1988

Column H, day 0-6:
b = 1.55 Mg/m^3 w = 0.344
q = 0.133 m/d p = -0.37 m

Column H, day 6.04-6.33:(ponded)
b = 1.55 Mg/m^3 w = 0.346
q = 0.152 m/d p = -0.29 m

Column I, day 0-6
b = 1.55 Mg/m^3 w = 0.345
q = 0.122 m/d p = -0.40 m

Column I, day 6.04-8:(ponded)
b = 1.55 Mg/m^3 w = 0.346
q = 0.147 m/d p = -0.31 m

VIRUS CONCENTRATIONS (Day 6 to 8 on next page)

Col	Depth (m)	Time of sample (d) 0	1	2	3	4	5
		pfu/mL					
H,I	0.00	21000	20000	10950	17500	11500	15500
H	0.20	0	3150	4100	3550	3700	5050
H	0.40	0	3150	3400	2050	2700	3600
H	1.05	0	0	825	1450	743	445
I	0.20	0	130	1250	1300	1400	2750
I	0.40	0	510	505	580	515	1500
I	1.05	0	0	103	275	418	408

BROMIDE CONCENTRATIONS (Day 6 to 8 on next page)

Col	Depth (m)	Time of sample (d) 0	1	2	3	4	5
		mmol/L					
H,I	0.00	1.006	1.090	1.006	1.006	1.090	1.006
H	0.20	0.000	0.673	0.823	0.892	0.928	0.928
H	0.40	0.000	0.729	0.928	1.006	1.006	1.006
H	1.05	0.000	0.000	0.264	0.767	0.967	0.967
I	0.20	0.000	0.597	0.857	0.928	0.966	0.966
I	0.40	0.000	0.508	0.928	0.966	0.966	1.047
I	1.05	0.000	0.000	0.089	0.592	0.986	1.007

VIRUS ELUTED FROM SOIL WITH TRYPTIC SOY BROTH (Day 6 to 8 on next page)

Col	Depth (m)	0	1	2	3	4	5
		pfu/g					
H	0.00						
H	0.20						
H	0.40						
I	0.00						
I	0.20						
I	0.40						

ORGANIC MATTER IN WATER (Day 6 to 8 on next page)

Col	Depth (m)	Time of sample (d) 0	1	2	3	4	5
		mg/L					
H,I	1.05	48				50	

TE9 (Continued).

Column H, day 7-8:
b = 1.55 Mg/m^3 w = 0.346
q = 0.134 m/d p = -0.38 m

VIRUS CONCENTRATIONS (Continued)

Col	Depth	6	6.04	6.17	6.33	7	8
		Time of sample (d)					
	(m)	pfu/mL					
H,I	0.00	11100	5499	5410	5291	10150	7450
H	0.20	2700	4200	3750	3250	4050	2900
H	0.40	1350	2200	2350	3300	1450	975
H	1.05	655	645	583	540	475	418
I	0.20	1700	1800	2550	1600	1650	1750
I	0.40	675	530	620	905	750	1015
I	1.05	290	235	220	255	275	348

BROMIDE CONCENTRATIONS (Continued)

Col	Depth	6	6.04	6.17	6.33	7	8
		Time of sample (d)					
	(m)	mmol/L					
H,I	0.00	0.966	0.510	0.509	0.509	1.006	1.047
H	0.20	0.892	0.892	0.966	0.729	0.892	0.928
H	0.40	0.966	0.966	1.006	0.966	0.729	0.928
H	1.05	0.986	0.986	0.986	0.986	1.027	1.047
I	0.20	0.966	0.928	0.966	0.892	0.857	0.790
I	0.40	1.006	0.966	1.006	0.928	0.597	0.790
I	1.05	1.047	1.047	0.988	0.988	1.047	0.988

VIRUS ELUTED FROM SOIL WITH TRYPTIC SOY BROTH (Continued)

Col	Depth	6	6.04	6.17	6.33	7	8
		Time of sample (d)					
	(m)	pfu/g					
H	0.00						9944
H	0.20						741
H	0.40						194
I	0.00						7495
I	0.20						1096
I	0.40						179

ORGANIC MATTER IN WATER (Continued)

Col	Depth	6	6.04	6.17	6.33	7	8
		Time of sample (d)					
	(m)	mg/L					
H,I	1.05						53

TE10: Unsaturated Flow; Fresh Soil Versus Leached Soil

Some of the variability in the preceding transport experiments seemed to correlate with the degree of leaching of the soil. For example, TE7 had freshly packed soil columns, and virus were transported at high levels. This experiment sought to quantify the concentration of organic matter in the soil water and test the effect of fresh versus leached soil on virus transport.

Organic matter in the effluent of the fresh soil column (J) declined from 187.7 to 96.7 mg/L in six days. The leached column (K) organic matter was between 82.5 to 56.2 mg/L. The virus concentration at 1.05 m in the fresh soil maintained a steady-state $(C/C_o)_s = 0.45$, whereas in the leached soil the steady-state $(C/C_o)_s = 0.05$. $(C/C_o)_s$ values were calculated by averaging the C/C_o values after they stopped increasing. Elution of the soil samples at the end of the experiment resulted in low k_p average values of 0.015 for the fresh soil column and 0.067 for the leached soil column. Negative values of eluted pfu/g resulted when the number of virus attributed to the water in the soil sample exceeded the total number eluted. The surface had relatively higher k_p values ($k_{p,J} = 0.311$ and $k_{p,K} = 0.366$), indicating a higher level of reversibly adsorbed virus near the surface. The mass balance resulted in 51% of virus added to the fresh soil accounted for, and only 15% of the virus added to the leached soil accounted for.

TE10: Fresh soil versus leached soil.
January 1989

Column J, fresh soil:
b = 1.52 Mg/m^3 w = 0.312
q = 0.103 m/d p = -0.27 m

Column K, leached soil:
b = 1.55 Mg/m^3 w = 0.343
q = 0.114 m/d p = -0.49 m

VIRUS CONCENTRATIONS

Col	Depth	Time of sample (d) 0	1	2	3	4	5	6	7
	(m)	pfu/mL							
J,K	0.00	1485000	1065000	1100000	1005000	1210000	1270000	1090000	1075000
J	0.10								700000
J	0.20								140000
J	0.40								530000
J	0.80								145000
J	1.05		0	0	74000	515000	555000	515000	500000
K	0.10								840000
K	0.20								205000
K	0.40								275000
K	0.80								54000
K	1.05		0	0	27000	80000	53000	54000	29500

BROMIDE CONCENTRATIONS

Col	Depth	Time of sample (d) 0	1	2	3	4	5	6	7
	(m)	mmol/L							
J,K	0.00	0.967			1.006				
J	1.05		0.000	0.000	0.067	0.892	0.967	0.967	0.967
K	1.05		0.000	0.000	0.208	0.967	0.967	0.967	0.967

(Continued on next page)

TE10 (Continued).

VIRUS ELUTED FROM SOIL WITH TRYPTIC SOY BROTH

Col	Depth (m)	Time of sample (d) 0	1	2	3	4	5	6	7
		pfu/g							
J	0.00								334107
J	0.10								-21692
J	0.20								-30239
J	0.40								15903
J	0.80								-22529
J	1.05								-65005
K	0.00								393929
K	0.10								85395
K	0.20								36860
K	0.40								-23059
K	0.80								-893
K	1.05								-653

ORGANIC MATTER IN WATER

Col	Depth (m)	Time of sample (d) 0	1	2	3	4	5	6	7
		mg/L							
J,K	0.00	10.4		10.4		10.4		10.4	
J	1.05	187.7		125		94.2		96.7	
K	1.05	56.2		67.5		65.8		82.5	

TE11: Saturated Versus Unsaturated and Eluant Test

Column L was pretreated with CO_2 to increase the degree of saturation. This worked well resulting in Θ_v (0.421) nearly equal to the calculated porosity (0.415).

The saturated versus unsaturated results confirm the conclusions of TE6: in unsaturated flow virus concentrations decline with depth, and in saturated flow virus C/C_o increases to 1.

None of the eluants tested was clearly superior in desorbing the virus. Although beef extract was the most effective eluant (k_p,avg = 0.03) we decided to keep using TSB (k_p,avg = -0.02) (Table 4) as the standard eluant to avoid adding another compound that might influence the virus assay. (TSB was already being used as the nutrient media for the E. coli host cells.)

Since none of the eluants released large numbers of virus, inactivation is the most likely explanation for the removal of virus in unsaturated flow.

TE11: Saturated versus unsaturated and eluant test.
March 1989

Column K, unsaturated:
b = 1.55 Mg/m^3   w = 0.353
q = 0.101 m/d   p = -0.37 m

Column L, saturated:
b = 1.55 Mg/m^3   w = 0.421
q = 0.107 m/d   p = nd

VIRUS CONCENTRATIONS

Col	Depth (m)	0	1	2	3	4	5	6
		Time of sample (d)						
		pfu/mL						
K,L	0.00	47500	41500	54000	45500	61000	55000	28000
K	0.40							13700
K	1.05		5	5	1200	4950	9050	7605
L	0.40							54925
L	1.05		5	150	4050	16100	46000	38870

(Continued on next page)

TE11 (Continued)

VIRUS ELUTED FROM SOIL
ELUANTS: T=TSB, G=GLYCINE, B=BEEF, H=HUMATE

Col,Eluan	Depth	Time of sample (d)						
		0	1	2	3	4	5	6
	(m)	pfu/g						
K,T	0.00							6937
K,G	0.00							13543
K,B	0.00							24047
K,H	0.00							14083
K,T	0.40							-444
K,G	0.40							-1102
K,B	0.40							369
K,H	0.40							222
K,T	1.05							-455
K,G	1.05							-871
K,B	1.05							-14
K,H	1.05							-791
L,T	0.00							4446
L,G	0.00							-4685
L,B	0.00							2129
L,H	0.00							9108
L,T	0.40							-12305
L,G	0.40							-14105
L,B	0.40							-14184
L,H	0.40							-13781
L,T	1.05							-6732
L,G	1.05							-10098
L,B	1.05							-10706
L,H	1.05							-7319

ORGANIC MATTER IN WATER

Col	Depth	Time of sample (d)						
		0	1	2	3	4	5	6
	(m)	mg/L						
K	1.05	42		37				100
L	1.05	238		178				125

TE12: Unsaturated Flow; Sludge Pretreatment Versus Untreated

This experiment used another form of organic matter in unsaturated flow to compare with TE10. The influent to both columns contained organic matter from primary sewage sludge.

The organic matter was almost entirely removed in flow through the columns, declining from 203 mg/L to an average of 30 mg/L.

Pretreatment with sludge (column K) had little effect on $(C/C_o)_s$ values. Both pretreated and untreated had $(C/C_o)_s$ at 1.05 m close to the fresh soil value in TE10 [$0.41 \leq (C/C_o)_s \leq 0.49$], which was 8 to 10 times the value for leached soil. This supports the idea that organic matter protects virus from removal in unsaturated flow.

TE12: Sludge pretreatment versus untreated.
June 1989

Column J, untreated:
b = 1.52 Mg/m^3   w,est = 0.340
q = 0.0922 m/d   p = -0.28 m

Column K, sludge pretreated:
b = 1.55 Mg/m^3   w,est = 0.353
q = 0.0844 m/d   p = -0.32 m

VIRUS CONCENTRATIONS

Col	Depth (m)	0	1	2	3	4	5	6	7	8
		Time of sample (d)								
		pfu/mL								
J,K	0.00	2785000	2700000	3750000	2400000	2550000	2330000	2650000	2150000	2700000
J	0.10								1590000	
J	0.20								1515000	
J	0.40								2110000	
J	0.80								1295000	
J	1.05		0	0	76500	655000	695000	1120000	1335000	1080000
K	0.10								1725000	
K	0.20								1250000	
K	0.40								1700000	
K	0.80								1395000	
K	1.05		0	10	15	450000	1140000	965000	950000	1095000

ORGANIC MATTER IN WATER

Col	Depth (m)	0	1	2	3	4	5	6	7	8
		Time of sample (d)								
		mg/L								
J,K	0.00	203								
J	1.05	0			28				43	
K	1.05	13			16				33	

APPENDIX C.

A COMPARISON OF MATHEMATICAL MODELS

Virus relative concentrations (C/C_o) versus time (breakthrough curves) were fit by scaling the equations of Amoozegar-Fard et al. (1983), Eq. [4]. A computer program based on these equations, MISCIB (Dept. Soil and Water Science, Univ. AZ), was used to find the least squares fit. This is a statistical approach that generates two fitting parameters, a and b, which may be related to the standard parameters of retardation (R) and dispersion coefficient (D):

$$R = a / b$$

$$D = z\, v / (4\, a\, b)$$

where z is depth (cm) and v is average linear velocity (cm/s). In the current research, the virus in unsaturated conditions were not conserved, but were apparently inactivated. Consequently, R and D could not be determined using MISCIB. After C/C_o reached a steady-state, $(C/C_o)_s$ versus depth points (profile curves) were fit using equations of Jury et al. (1987), Eq. [5] and [6].

It would be advantageous to use a single mathematical model that would fit the data using standard parameters of R, D, k_f. Parameter k_f, the forward rate coefficient, is used when there is a kinetic component to adsorption equilibrium. The decay constant u, representing virus inactivation, may be equated with k_f. Cameron and Klute (1977) were able to combine equilibrium and kinetic adsorption to model pesticide, nutrient, and metal transport in soils with both breakthrough and profile curves.

An attempt to apply this approach was made using the computer program BTHRU (Dept. Hydrology, Univ. AZ) to generate breakthrough curves. BTHRU requires input of the Peclet number (P), R, and omega (Ω):

$$P = v\, z / D$$

$$R = (k_p\, \rho_b / \Theta) + 1$$

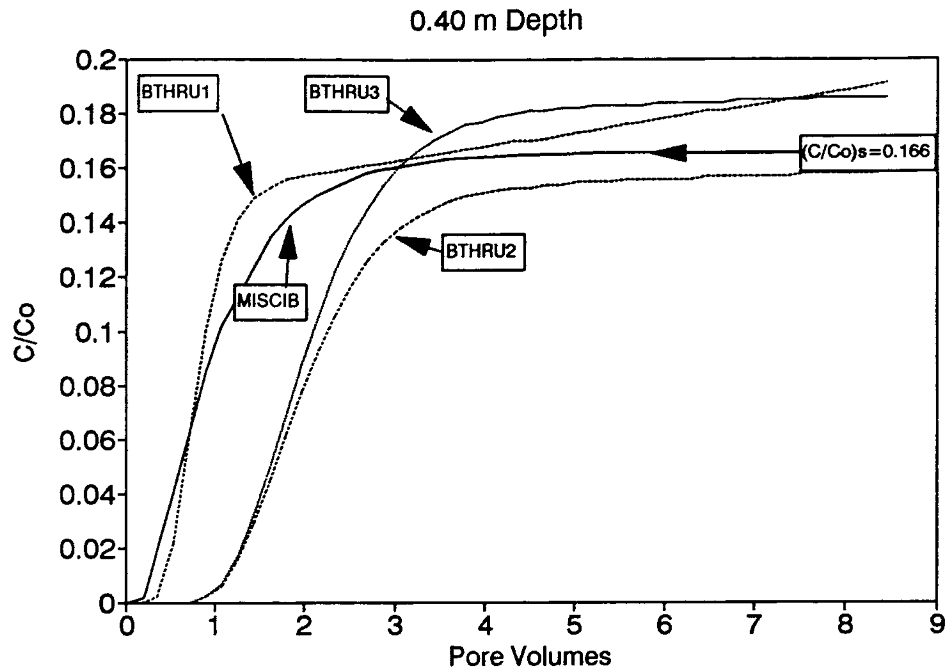
$$\Omega = (k_f\, \rho_b\, z) / (\Theta\, v)$$

where v is the average linear velocity (0.000428 cm/s), z is depth (cm), D (cm^2/s) is the dispersion coefficient of the soil column, k_p (cm^3/g) is the partition coefficient, ρ_b is the bulk density (1.54 g cm^{-3}), Θ (0.35) is the volumetric water content, and k_f is the forward adsorption rate coefficient. D was estimated by entering the bromide breakthrough data of TE3 in MISCIB, resulting in an average D of 0.00143 cm^2/s. The curves generated by BTHRU were fit by eye to the data.

Using BTHRU is was possible to generate curves that approximately fit the data (Fig. 2b and 9a). It was necessary to pick a large value for k_p to cause the curves to decrease in slope near the steady-state value. The forward rate coefficient k_f was then adjusted to generate a curve that matched the steady-state value at 3 pore volumes [for 0.4 m depth, $(C/C_o)_s = 0.17$]. Different coefficients were tried, but those that generated the BTHRU1 curve gave the best fit at the 0.4 m depth:

Curve	k_p(cm^3/g)	k_f(/s)
BTHRU1	22.5	4.87×10^{-6}
BTHRU2	113.4	4.87×10^{-6}
BTHRU3	113.4	4.38×10^{-6}

The curves in Fig 9a indicate that when k_p is increased from 22.5 to 113.4 the curve becomes more level at the steady state value, but that the curve is shifted too far to the right or retarded (BTHRU2). Also, the C/C_o value at 3 pore volumes becomes slightly less. By reducing k_f the C/C_o value is increased (BTHRU3), but the retardation remains. The BTHRU curve for the 1.05 m depth (Fig. 9b) was constructed using BTHRU1 values for k_p and k_f. The MISCIB curve is from Fig. 2d. It is apparent that the BTHRU coefficients would have to be adjusted for each depth to fit the data.



a.

Fig. 9. Curves generated by MISCIB and BTHRU at depths of (a) 0.4 m and (b) 1.05 m; $(C/C_o)_s$ is the apparent steady-state relative concentration. The MISCIB curves are the same as in Fig. 2b and 2d. For the curve labeled BTHRU1, k_p = 22.5 cm^3 / g and k_f = 4.87 X 10^{-6} /s; for BTHRU2, k_p = 113.4 cm^3 / g and k_f = 4.87 X 10^{-6} /s; and for BTHRU3, k_p = 113.4 cm^3 / g and k_f = 4.38 X 10^{-6} /s.

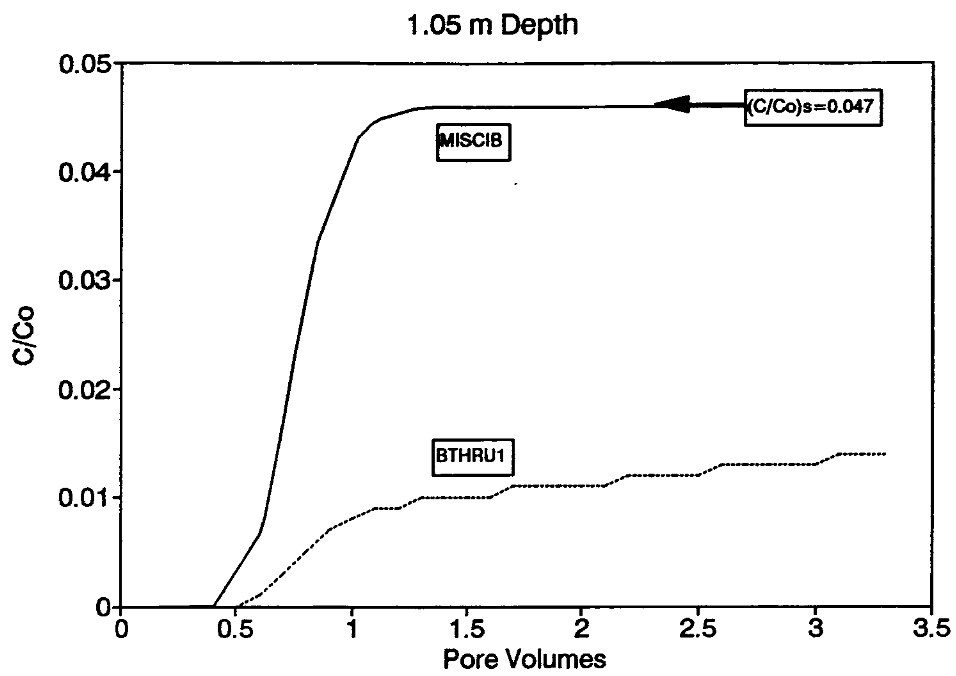


Fig. 9b.

The BTHRU1 C/C_o values for day 3 are plotted in Fig. 10, along with the data from days 3 to 8 of TE3 and TE9 (each point is an average of 4 replicate columns), and the curve derived from Jury et al. (1987). The BTHRU values are closely represented by:

$$C/C_o = \exp(-4.5\ z)$$

but the values increase slowly with time. The data after three days do not show a tendency to change with time, and the data declines more rapidly at shallow depth than the BTHRU1 results. The Jury equations fit the data well.

It appears that BTHRU does not fit the data as well as MISCIB and the Jury equations. Furthermore, the goals of determining the standard parameters k_p (or R) and k_f were not achieved. The actual k_p, as determined by elution of the soil, was near zero; k_p used in adjusting the output of BTHRU to the data was simply a fitting parameter. The rate coefficient, k_f was also essentially a fitting parameter since it had to be adjusted when k_p or depth was changed. Both MISCIB and BTHRU require three fitting parameters: MISCIB requires a, b, and the steady-state value $(C/C_o)_s$ for scaling; BTHRU requires k_p, k_f, and depth. The output of BTHRU can be used to generate a profile curve that approximates the data without resorting to another model such as that of Jury et al. (1987). However, the Jury equations fit the data much better, and the curve does not change with time.

The essential problem for mathematically describing these data is that virus inactivation, the decay constant u in the basic transport equation [2], appears to be a function of depth, which neither BTHRU nor MISCIB allow for. Further mathematical solutions are required to find a single model for these data.

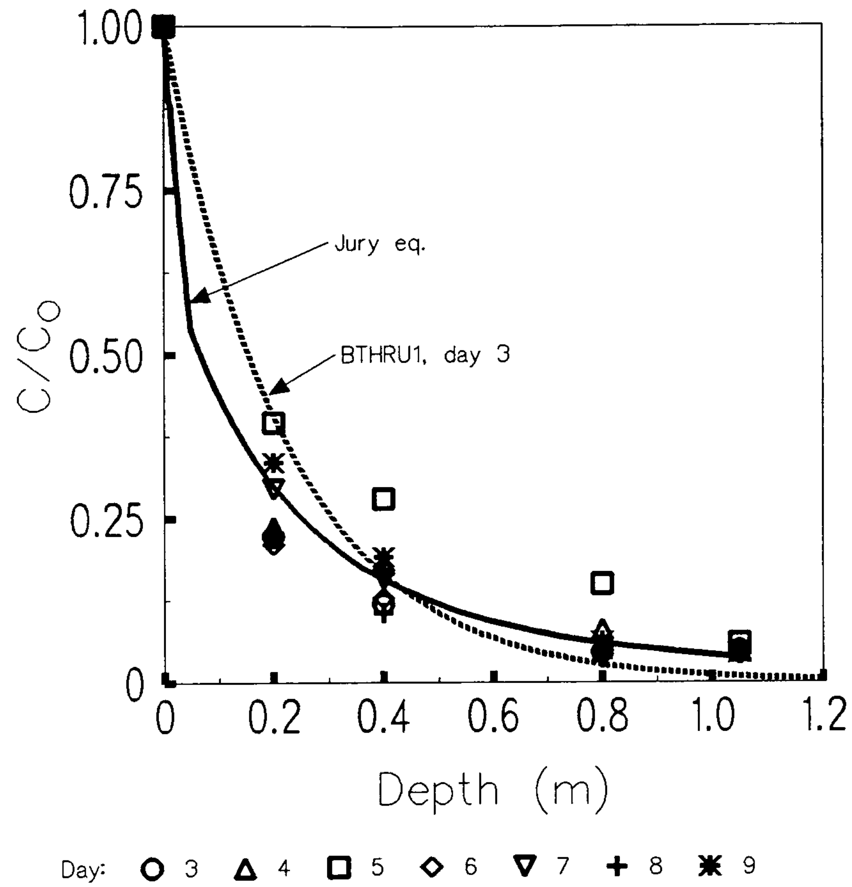


Fig. 10. The depth profile showing steady-state relative virus concentrations from days 3 to 8 of TE3 and TE9 (each point is the average of four replicate columns), the curve generated by the equations of Jury et al. (1987), and the curve generated by BTHRU1 for day 3.

REFERENCES

Adams, M.H. 1959. Bacteriophages. Interscience Publishers, Inc. New York.

Adamson, A.W. 1982. Physical chemistry of surfaces. Wiley, NY.

American Public Health Association. 1985. Standard methods for the examination of water and wastewater. 16th ed. Am. Public Health Assoc., Washington, D.C., p 950.

Amoozegar-Fard, A., A.W. Warrick, and W.H. Fuller. 1983. A simplified model for solute movement through soils. Soil Sci. Soc. Am. J. 47:1047-1049.

Anderson, M.D. 1984. Movement of contaminants in groundwater: Groundwater transport--advection and dispersion. p. 37-38. In Geophysics Study Committee of the National Research Council, Studies in Geophysics Groundwater Contamination. National Academy Press, Washington, DC.

Bae, H.C., E.H. Costa-Robles, and L.E. Casida. 1972. Microflora of soil as viewed by transmission electron microscopy. Appl. Microbiol. 23:637-648.

Baier, R.E. 1972. Organic films on natural waters; their retrieval, identification and modes of elimination. J. Geophys. Res. 77:5062.

Baier, R.E. 1975. Applied chemistry at protein interfaces. Adv. Chem. Ser. 145:1-25.

Baker, R.S., and D. Hillel. 1990. Laboratory tests of a theory of fingering during infiltration into layered soils. Soil Sci. Soc. Am. J. 54:20-30.

Bandoni, R.J., and R.E. Koske. 1974. Monolayers and microbial dispersal. Science 183:1079-1081.

Baylor, E.R., V. Peters, and M.B. Baylor. 1977. Water-to-air transfer of virus. Science 197:763-764.

Bitton, G. 1978. Survival of enteric viruses. p. 277-289. In R. Mitchell (ed.) Water pollution microbiology. Wiley, NY.

Bitton, G., M.J. Charles, and S.R. Farrah. 1979. Virus detection in soils: a comparison of four recovery methods. Can. J. Microbiol. 25:874-880.

Bitton, G., O.C. Pancorbo, and S.R. Farrah. 1984. Virus transport and survival after land application of sewage sludge. Appl. Environ. Microbiol. 47: 905-909.

Bixby, R.L. 1980. Transport of viruses through organic soils and sediments. J. Environ. Eng. Div. 106:674-675.

Bixby, R.L., and D.J. O'Brien. 1979. Influence of fulvic acid on bacteriophage adsorption and complexation in soil. Appl. Environ. Microbiol. 38:840-845.

Blanchard, D.C. 1975. Bubble scavenging and the water-to-air transfer of organic material in the sea. Adv. Chem. Ser. 145:360-387.

Blanchard, D.C., and B.C. Parker. 1977. The freshwater to air transfer of microorganisms and organic matter. p. 627-658. In J.J. Cairns (ed.) Aquatic microbial communities. Garland, NY.

Bolt, G.H. 1982. Electrochemical phenomena in soil and clay systems. p. 387-432. In G.H. Bolt (ed.) Soil chemistry, B. physico-chemical models. Elsevier Scientific Publishing Co., Amsterdam, the Netherlands.

Bouwer, H. 1984. Elements of soil science and groundwater hydrology. p. 11-31. In G. Bitton and C.P. Gerba (ed.) Groundwater pollution microbiology. Wiley, NY.

Bradford, A.W. 1987. Transport of MS-2 virus through saturated soil columns. M.S. thesis, Univ. Arizona, Tucson

Brock, T.D., D.W. Smith, and M.T. Madigan. 1984. Biology of microorganisms. Prentice-Hall, Englewood Cliffs, NJ.

Brown, K.W., H.W. Wolf, K.C. Donnelly, and J.F. Slowey. 1979. The movement of fecal coliforms and coliphages below septic lines. J. Environ. Qual. 8: 121-125.

Burge, W.D., and N.K. Enkiri. 1978. Virus adsorption by five soils. J. Environ. Qual. 7:73-76.

Cameron, D.R., and A. Klute. 1977. Convective-dispersive solute transport with a combined equilibrium and kinetic adsorption model. Water Resour. Res. 13:183-188.

Carter, D.L., M.M. Mortland, and W.D. Kemper. 1986. Specific surface. p. 413-423 In A. Klute (ed.) Methods of soil analysis. Part 1. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.

Cheo, P.C. 1980. Antiviral factors in soil. Soil Sci. Soc. Am. J. 44:62-67.

Childs, E.C. 1969. An introduction to the physical basis of soil water phenomena. Wiley, London.

Clifford, J. 1975. Properties of water in capillaries and thin films. In F. Franks (ed.) Water: a comprehensive treatise. Plenum Press, NY.

Cliver, D.O. 1976. Surface application of municipal sludges. p.77-79. In L.B. Baldwin (ed.) Virus aspects of applying municipal waste to land. Univ. Florida, Gainesville.

Cliver, D.O., and J.E. Herrman. 1972. Proteolytic and microbial inactivation of enteroviruses. Water Research 6:797-805.

Cogger, C.G., L.M. Hajjar, C.L. Moe, and M.D. Sobsey. 1988. Septic system performance on a coastal barrier island. J. Environ. Qual. 17:401-408.

Constantz, J., W.N. Herkelrath, and F. Murphy. 1988. Air encapsulation during infiltration. Soil Sci. Soc. Am. J. 52: 10-16.

Corapcioglu, M.Y., N.M. Abboud, and A. Haridas. 1987. Governing equations for particle transport in porous media. p.296-302. In J. Bear and M.Y. Corapcioglu (ed.) Advances in transport phenomena in porous media. Martinus Nijhoff Pub., Dordrecht, The Netherlands.

Craun, G.F. 1985. A summary of waterborne illness transmitted through contaminated groundwater. J. Environ. Hlth. 48: 122-127.

Damgaard-Larsen, S., K.O. Jensen, E. Lund, and B. Nissen. 1977. Survival and Movement of Enterovirus in Connection with Land Disposal of Sludges. Water Research 11:503-508.

Danielson, R.E., and P.L. Sutherland. 1986. Porosity. p.443-461 In A. Klute (ed.) Methods of soil analysis. Part 1. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.

Davies, B.E. 1974. Loss-on-ignition as an estimate of soil organic matter. Soil Sci. Soc. Am. Proc. 38:150-151.

Davis, J. E., and R. L. Sinsheimer. 1963. The replication of bacteriophage MS-2. J. Mol. Biol. 6:203-207.

Dazzo, F. B., and D. F. Rothwell. 1974. Evaluation of porcelain cup soil water samplers for bacteriological sampling. Appl. Environ. Microbiol. 27:1172-1174.

Dizer, H., A. Nasser, and J.M. Lopez. 1984. Penetration of different human pathogenic viruses into sand columns percolated with distilled water, groundwater, or wastewater. Appl. Environ. Microbiol. 47:409-415.

Drewry, W.A., and R. Eliassen. 1968. Virus movement in groundwater. J. Wat. Pollut. Control Fed. 40:R257-R271.

Dubiose, S.M., B.E. Moore, and B.P. Sagik. 1976. Poliovirus survival and movement in a sandy forest soil. Appl. Environ. Microbiol. 31:536-543.

Duboise, S.M., B.E. Moore, C.A. Sorber, and B.P. Sagik. 1979. Viruses in soil systems. CRC critical reviews in microbiology, Nov.:245-285.

Duboise, S.M., B.P. Sagik, B.E.D. Moore, and J.F. Malina. 1974. Virus migration through soils. In J.F. Malina and B.P. Sagik (ed.) Virus survival in water and wastewater systems. Univ. Texas, Austin.

El-Abagy, M.M., B.J. Dutka, and M. Damel. 1988. Incidence of coliphage in potable water supplies. Appl. Environ. Microbiol. 54:1632-1633.

Farrah, S.R. 1982. Chemical factors influencing adsorption of bacteriophage MS2 to membrane filters. Appl. Environ. Microbiol. 43:659-663.

Farrah, S.R., D.O. Shah, and L.O. Ingram. 1981. Effects of chaotropic and antichaotropic agents on elution of poliovirus adsorbed on membrane filters. Proc. Natl. Acad. Sci. USA 28(2):1229-1232.

Feachem, R.G., D.J. Bradley, H. Garelick, D.D. Mara. 1983. Sanitation and disease. Wiley, Chichester, UK.

Foster, R.C. 1981. Polysaccharides in soil fabrics. Science 214:665-667.

Fuhs, G.W., M. Chen, L.S. Sturman, and R.S. Moore. 1985. Virus adsorption to mineral surfaces is reduced by microbial overgrowth and organic coatings. Microb. Ecol. 11:25-39.

Fuhs, G.W., L.S. Sturman, D.H. Taylor, and R.S. Moore. 1981. Evaluation of soils for suitability for virus adsorption. Proc. Water Reuse Symp. II 3:2241-2250.

Funderberg, S. W., B. E. Moore, C. A. Sorber, and B. P. Sagik. 1979. Method of soil column preparation for the evaluation of viral transport. Appl. Environ. Microbiol. 38:102-107.

Gee, G.W., and J.W. Bauder. 1986. Particle-size analysis. p. 383-409. In A. Klute (ed.) Methods of soil analysis. Part 1. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.

Gerba, C.P. 1983. Methods for recovering viruses from the water environment. p. 20-22. In G. Berg (ed.) CRC Press, Boca Raton, FL.

Gerba, C.P. 1984. Applied and Theoretical Aspects of Virus Adsorption to Surfaces. Advances In Applied Microbiology 30: 133-168.

Gerba, C.P. 1987. transport and fate of viruses in soils: field studies. In V.C. Rao and J.L. Melnick (ed.) Human viruses in sediments, sludges, and soils. CRC Press, Boca Raton, FL.

Gerba, C.P., and G. Bitton. 1984. Microbial pollutants: their survival and transport pattern to groundwater. p. 74-83. In C.P. Gerba and G. Bitton (ed.) Groundwater pollution microbiology. Wiley, NY.

Gerba, C.P., S.M. Goyal, I. Cech, and G.F. Bogdan. 1981. Quantitative assessment of the adsorptive behavior of viruses to soils. Environ. Sci. Technol. 15:940-944.

Gerba, C.P., and C.N. Haas. 1988. Assessment of risks associated with enteric viruses in contaminated drinking water. p. 489-494. In J.J. Lichtenberg et al. (ed.) Chemical and biological characterization of sludges, sediments, dredge spoils, and drilling muds. Am. Soc. for Testing and Materials, Philadelphia, PA.

Gerba, C. P. and J. C. Lance. 1978. Poliovirus removal from primary and secondary sewage effluent by soil filtration. Appl. Environ. Microbiol. 36:247-251.

Gerba, C.P., Marzouk, Y., Manor, Y., Idelovitch, E., and Vaughn, J.M. 1984. Virus removal during land application of wastewater: a comparison of three projects. p. 1518-1529 In AWWA Research Foundation, Future of Water Reuse, vol. 3, Denver, CO.

Gerba, C.P., and J.B. Rose. 1990. Viruses in source and drinking water. p. 380-396. In G.A. McFeters (ed.) Drinking water microbiology. Springer-Verlag, NY.

Gerba, C.P., M.D. Sobsey, C. Wallis, and J.L. Melnick. 1975. Adsorption of poliovirus onto activated carbon in wastewater. Environ. Sci. Tech. 9:727-731.

Gilbert, R.S., C.P. Gerba, R.C. Rice, H. Bouwer, C. Wallis, and J.L. Melnick. 1976. Virus and bacteria removal from wastewater by land treatment. Appl. Environ. Microbiol. 32:333-338.

Girones, R., J. Jofre, and A. Bosch. 1989. Natural inactivation of enteric viruses in seawater. J. Environ. Qual. 18:34-39.

Goyal, S.M., D. Amundson, R.A. Robinson, and C.P. Gerba. 1989. Viruses and drug resistant bacteria in groundwater of southeastern Minnesota. J. Minn. Acad. Sci. 55:58-62.

Goyal, S.M., and C.P. Gerba. 1979. Comparative adsorption of human enteroviruses, simian rotavirus, and selected bacteriophages to soils. Appl. and Environ. Microbiol. 38: 241-247.

Goyal, S.M., B.H. Keswick, and C.P. Gerba. 1984. Viruses in groundwater beneath sewage irrigated cropland. Water Research 18: 299.

Gschwend, P.M., and M.D. Reynolds. 1987. Monodisperse ferrous phosphate colloids in an anoxic groundwater plume. J. Contaminant Hydrology 1:309-327.

Guerin, W.R. 1989. Phenanthrene degradation by estuarine surface microlayer and bulk water microbial populations. Microb. Ecol. 17:89-104.

Guttman-Bass, N., and J. Catalano-Sherman. 1985. Effects of humic materials on virus recovery from water. Appl. Environ. Microbiol. 49:1260-1264.

Guttman-Bass, N., and J. Catalano-Sherman. 1986. Humic acid interference with virus recovery by electropositive microporous filters. Appl. Environ. Microbiol. 52:556-561.

Hardy, J.T., E.A. Crecelius, L.D. Antrim, V.L. Broadhurst, C.W. Apts, J.M. Gurtisen, and T.J. Fortman. 1987. the sea-surface microlayer of Puget Sound: part II, concentrations of contaminants and relation to toxicity. Mar. Env. Res. 23:251-271.

Harrison, S.C. 1990. Principles of virus structure. p. 37-61. In B.N. Fields et al. (ed.) Virology. Raven Press, NY.

Hatefi, Y, and W.G. Hanstein. 1969. Solubilization of particulate proteins and nonelectrolytes by chaotropic agents. Proc. Natl. Acad. Sci. USA 62:1129-1136.

Havelaar, A.H., and W.M. Pot-Hogeboom. 1988. F-specific RNA-bacteriophages as model viruses in water hygiene: ecological aspects. Wat. Sci. Tech. 20:399-407.

Hendricks, S.M., and P.M. Williams. 1985. Dissolved and particulate amino acids and carbohydrates in the sea surface microlayer. Mar. Chem. 17:141-163.

Herrmann, J.E., K.D. Kostenbader, Jr., and D.O. Cliver. 1974. Persistence of enteroviruses in lake water. Appl. Microbiol. 28:895-896.

Hiemenz, P.C. 1977. Principles of colloid and surface chemistry. Marcel Dekker, NY.

Hoeks, J. 1981. Analytical solutions for transport of conservative and nonconservative contaminants in groundwater systems. Water, Air, and Soil Pollution 16:339-350.

Hunter, R.J., and A.E. Alexander. 1963. Surface properties and flow behavior of kaolinite, III, flow of kaolinite solutions through a silica column. J. Colloid Sci. 18:846-862.

Hunter, K.A., and P.S. Liss. 1981. Organic sea surface films. p. 259-298. In E.K. Duursma and R. Dawson (ed.) Marine organic chemistry. Elsevier, Amsterdam.

Hurst, C.J., C.P. Gerba, and I. Cech. 1980. Effects of environmental variables and soil characteristics on virus survival in soil. Appl. Environ. Microbiol. 40: 1067-1079.

Iwasaki, T. 1937. Some notes on sand filtration. J. Am. water Works Assoc. 29: 1591-1597.

Jansons, J., L.W. Edmonds, B. Speight, and M.R. Bucens. 1989a. Movement of viruses after artificial recharge. Wat. Res. 23: 293-299.

Jansons, J., L.W. Edmonds, B. Speight, and M.R. Bucens. 1989b. Survival of viruses in groundwater. Wat. Res. 23:301-306.

Jenkins, S.R., C.A. Copperthite, J.S. Barton, C.G. Jordan. 1980. Removal of bacteriophage Escherichia coli T, by sand columns using Ca^{2+} as a filter aid. Water Research 14:437-440.

Johnson, R.A., R.D. Ellender, and S. Tsai. 1984. Elution of enteric viruses from Mississippi estuarine sediments with lecithin-supplemented eluents. Appl. Environ. Microbiol. 48:581-585.

Jorgensen, P.H. 1985. Examination of the penetration of enteric viruses in soils under simulated conditions in the laboratory. Water Science and Technology 17:197-199.

Jury, W.A. 1986. Adsorption of organic chemicals onto soil. In S.C. Hern and S.M. Melancon (ed.) Vadose zone modeling of organic pollutants. Lewis Pub., Chelsea, MI.

Jury, W.A., D.D. Focht, and W.J. Farmer. 1987. Evaluation of pesticide groundwater pollution potential from standard indices of soil-chemical adsorption and biodegradation. J. Environ. Qual. 16:422-428.

Karr, G. R. and J. B. Hendricks. 1986. Superfluid flow in porous stainless steel. Cryogenics. 26:114-116.

Keswick, B.H., D.-S. Wang, and C.P. Gerba. 1982. The use of microorganisms as ground-water tracers: a review. Ground Water 20:142-149.

Keswick, B.H., C.P. Gerba, J.B. Rose, and G.A. Toranzos. 1985. Detection of rotavirus in treated drinking water. Water Sci. Tech. 17:1-6.

Koya, K.V.A., and M. Chaudhuri. 1977. Virus retention by soil. Prog. Water Technol. 9: 43-52.

LaBelle, R., and C.P. Gerba. 1982. Investigations into the protective effect of estuarine sediments on virus survival. Water Research 16:469-478.

Lance, J.C., and C.P. Gerba. 1980. Poliovirus movement during high rate of land filtration of sewage water. J. Environ. Qual. 9:31-34.

Lance, J.C., and C.P. Gerba. 1984a. Effect of ionic composition of suspending solution on virus adsorption by a soil column. Appl. Environ. Microbiol. 47:484-488.

Lance, J.C., and C.P. Gerba. 1984b. Virus movement in soil during saturated and unsaturated flow. Appl. Environ. Microbiol. 47: 335-337.

Lance, J. C., C. P. Gerba, and J. L. Melnick. 1976. Virus movement in soil columns flooded with secondary sewage effluent. Appl. Environ. Microbiol. 32:520-526.

Lance, J.C., C.P. Gerba, and D.-S. Wang. 1982. Comparative movement of different enteroviruses in soil columns. J. Environ. Qual. 11: 347-351.

Lance, J. C., and F. D. Whisler. 1972. Nitrogen balance in soil columns intermittently flooded with secondary sewage effluent. J. Environ. Qual. 1:180-186.

Landolt-Bornstein. 1956. Zahlenwerte und funktionen, Springer-Verlag, Berlin. 535p.

Landry, E.F., J.M. Vaughn, M.Z. Thomas, and C.A. Beckwith. 1979. Adsorption of enteroviruses to soil cores and their subsequent elution by artificial rainwater. Appl. Environ. Microbiol. 38:680-687.

Larkin, E. P., J. T. Tierney, J. Lovett, D. van Donsel, and D. W. Francis. 1978. Land application of sewage wastes: potential for contamination of foodstuffs and agricultural soils by viruses, bacterial pathogens and parasites. vol.2, p.215-223. In State of knowledge in land treatment of wastewater (Proceedings of the International Symposium, Hanover, NH). U. S. Government Printing Office, Washington, D.C.

Letey, J., J. Osborn, and R.E. Pelishek. 1962. Measurement of liquid-solid contact angles in soil and sand. Soil Sci. 93: 149-153.

Lipson, S.M., and G. Stotzky. 1983. Adsorption of reovirus to clay minerals: effects of cation-exchange capacity, cation saturation, and surface area. Appl. Environ. Microbiol. 46:673-682.

Lipson, S.M., and G. Stotzky. 1984. Effect of proteins on reovirus adsorption to clay minerals. Appl. Environ. Microbiol. 48:525-530.

Lipson, S.M., and G. Stotzky. 1987. Interactions between viruses and clay minerals. In V.C. Rao and J.L. Melnick (ed.) Human viruses in sediments, sludges, and soils. CRC Press, Boca Raton, FL.

Litaor, M.I. 1988. Review of soil solution samplers. Water Resources Research 24:727-733.

Lomen, D.O., P.J. Tonellato, and A.W. Warrick. 1984. Salt and water transport in unsaturated soil for non-conservative systems. Agricultural Water Management 8:397-409.

Marzouk, Y., S.M. Goyal, and C.P. Gerba. 1979. Prevalence of enteroviruses in ground water of Israel. Ground Water 17:487-491.

McCarthy, J.F., and J.M. Zachara. 1989. Subsurface transport of contaminants. Environ. Sci. Technol. 23:496-502.

McDowell-Boyer, L.M., J.R. Hunt, and N. Sitar. 1986. Particle transport through porous media. Water Resources Res. 22:1901-1921.

McLean, E.O. 1982. Soil pH and lime requirement. p. 199-223. In A.L. Page et al. (ed.) Methods of soil analysis, Part 2. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.

Means, J.C., and R. Wijayaratne. 1982. Role of natural colloids in the transport of hydrophobic pollutants. Science 215:968-970.

Melnick, J. L., H. A. Wenner, and C. A. Phillips. 1979. Enteroviruses. p. 471-534 In E. H. Lennette and N. J. Schmidt (eds.) Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections. 5th edition. American Public Health Assoc., Washington, D.C.

Miller, D.M., M.E. Sumner, and W.P. Miller. 1989. A comparison of batch- and flow-generated anion adsorption isotherms. Soil Sci. Soc. Am. J. 53:373-380.

Mix, T.J. 1974. The physical chemistry of membrane-virus interaction. Dev. Ind. Microbiol. 15:136-142.

Moore, R.S., D.H. Taylor, M.M. Reddy, and L.S. Sturma. 1982. Adsorption of reovirus by minerals and soils. Appl. Environ. Microbiol. 44:852-859.

Moore, R.S., D.H. Taylor, L.S. Sturman, M.M. Reddy, and G.W. Fuhs. 1981. Poliovirus adsorption by 34 minerals and soils. Appl. Environ. Microbiol. 42:963-975.

Murray, J.P. 1980. Physical chemistry of virus adsorption and degradation on inorganic surfaces: Its relation to wastewater treatment. USEPA Rep. 600/2-80-134. USEPA, Cincinnati, OH.

Nelson, D.W., and L.E. Sommers. 1982. Total carbon, organic carbon, and organic matter. p. 539-579. In A.L. Page et al. (ed.) Methods of soil analysis, Part 2. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.

Nelson, R.E. 1982. Carbonate and gypsum. p. 181-196 In A.L. Page et al. (ed.) Methods of soil analysis, Part 2. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.

Nielsen, D.R., and J.W. Biggar. 1962. Miscible displacement III: theoretical considerations. Soil Sci. Soc. Am. Proc. 26:216-221.

Nielsen, D.R., M.T. VanGenuchten, and J.W. Biggar. 1986. Water flow and solute transport processes in the unsaturated zone. Water Resour. Res. 22:89S-108S.

Norkrans, B. 1980. Surface microlayers in aquatic environments. Adv. Microb. Ecol. 4: 51-81.

Overby, L.R., G.H. Barlow, R.H. Doi, M. Jacob, and S. Spiegelman. 1966. Comparison of two serologically distinct ribonucleic acid bacteriophages. J. Bacteriology 91:422-448.

Payment, P., and R. Armon. 1989. Virus removal by drinking water treatment processes. CRC Crit. Rev. Environ. Control 19:15-31.

Pekdeger, A., and G. Matthess. 1983. Factors of bacteria and virus transport in groundwater. Environmental Geology 5:49-52.

Penrose, W.R., W.L. Polzer, E.H. Essington, D.M. Nelson, and K.A. Orlandini. 1990. Mobility of plutonium and americium through a shallow aquifer in a semiarid region. Environ. Sci. Technol. 24:228-234.

Pepper, I.L., W.R. Kneebone, and P.R. Ludovici. 1981. Water reclamation by the use of soil-turfgrass systems in the southwest USA. National Technical Information Service PB82-168626.

Plotkin, S.A., and M. Katz. 1966. The minimal infective doses of viruses for man by the oral route. p. 151-166. In G. Berg (ed.) Transmission of viruses by the water route. Wiley, NY.

Powelson, D.K., J.R. Simpson, and C.P. Gerba. 1990. Virus transport and survival in saturated and unsaturated flow through soil columns. J. Environ. Qual. 19:(In press).

Preston, D.R., and S.R. Farrah. 1988. Activation thermodynamics of virus adsorption to solids. Appl. Environ. Microbiol. 54: 2650-2654.

Rabenhorst, M.C. 1988. Determination of organic and carbonate carbon in calcareous soils using dry combustion. Soil Sci. Soc. Am. J. 52:965-969.

Rao, V.C. 1982. Introduction to environmental virology. p. 1-13. In C.P. Gerba and S.M. Goyal (ed.) Methods in environmental virology. Marcel Dekker, NY.

Ratto, A., B.J. Dutka, C. Vega, C. Lopez, and A. El-Shaarawi. 1989. Potable water safety assessed by coliphage and bacterial tests. Wat. Res. 23:253-255.

Reddy, K.R., R. Khaleel, and M.R. Overcash. 1981. Behavior and transport of microbial pathogens and indicator organisms in soils treated with organic wastes. J. Environ. Qual. 10:255-266.

Reneau, R.B.,Jr., C. Hagedorn, and M.J. Degen. 1989. Fate and transport of biological and inorganic contaminants from on-site disposal of domestic wastewater. J. Environ. Qual. 18: 135-144.

Roper, M.R. and K.C. Marshall. 1974. Modification of the interaction between Escherichia coli and bacteriophage in saline sediment. Microb. Ecol. 1:1-13.

Schaub, S. A., H. T. Bausum, and G. W. Taylor. 1982. Fate of Virus in Wastewater Applied to Slow-Infiltration Land Treatment Systems. Appl. Environ. Microbiol. 44: 383-394.

Schaub, S.A., and B.P. Sagik. 1975. Association of enteroviruses with natural and artificially introduced colloidal solids in water and infectivity of solids-associated virions. Appl. Microbiol. 30:212-222.

Schaub, S.A. and C.A. Sorber. 1977. Virus and bacteria removal from wastewater by rapid infiltration through soil. Appl. Environ. Microbiol. 33: 609-619.

Schiffenbauer, M., and G. Stotzky. 1982. Adsorption of coliphages T1 and T7 to clay minerals. Appl. Environ. Microbiol. 43:590-596.

Schultz, T.R., J.H. Randall, L.G. Wilson, and S.N. Davis. 1976. Tracing sewage effluent recharge--Tucson, Arizona. Groundwater, Nov./Dec.

Sieburth, J.M. 1976. Dissolved organic matter and heterotrophic microneustron in the surface microlayers of the North Atlantic. Science 194:1415-1418.

Slade, J. S. and B. J. Ford. 1983. Discharge to the environment of viruses in wastewater, sludges, and aerosols. In G. Berg (ed.) Viral Pollution of the Environment. CRC Press, Inc. Boca Raton, Florida.

Smith, S.J., and R.J. Davis. 1974. Relative movement of bromide and nitrate through soils. J. Environ. Qual. 3:152-155.

Sobsey, M.D., C.H. Dean, M.E. Knuckles, R.A. Wagner. 1980. Interactions and survival of enteric viruses in soil materials. Appl. Environ. Microbiol. 40:92-101.

Sobsey, M.D., J.S. Glass, R.R. Jacobs, W.A. Rutala. 1980. Modification of the tentative standard method for improved virus recovery efficiency. J. Am. Water Works Assoc. 72:350-355.

Sobsey, M.D., and A.R. Hickey. 1985. Effects of humic and fulvic acids on poliovirus concentration from water by microporous filtration. Appl. Environ. Microbiol. 49:259-264.

Sobsey, M.D., and P.A. Shields. 1987. Survival and transport of viruses in soils: model studies. p.155-177. In V.C. Rao and J.L. Melnick (ed.) Human viruses in sediments, sludges, and soils. CRC Press, Boca Raton, FL.

Sposito, G. 1984. The surface chemistry of soils. Oxford Univ. Press, NY.

Stagg, C.H., C. Wallis, and C.H. Ward. 1977. Inactivation of clay-associated bacteriophage MS-2 by chlorine. Appl. Environ. Microbiol. 33:385-391.

Stevenson, F.J. 1982. Humus chemistry: genesis, composition, reactions. Wiley, NY.

Stotzky, G. 1986. Influence of soil mineral colloids on metabolic processes, growth, adhesion, and ecology of microbes and viruses. p. 305-412. In P.M. Huang and M. Schnitzer (ed.) Interactions of soil minerals with natural organics and microbes. Soil Sci. Soc. Am. Inc., Madison, WI.

Stumm, W., and J.J. Morgan. 1981. Aquatic chemistry. 2nd ed. John Wiley & Sons, New York.

Thomas, G.W. 1982. Exchangeable cations. p.159-165. In A.L. Page et al. (ed.) Methods of soil analysis, Part 2. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.

Taylor, D.H., R.S. Moore, and L.S. Sturman. 1981. Influence of pH and electrolyte composition on adsorption of poliovirus by soils and minerals. Appl. Environ. Microbiol. 42:976-984.

Trouwborst, T., and J.C. DeJong. 1973. Interaction of some factors in the mechanism of inactivation of bacteriophage MS2 in aerosols. Appl. Microbiol. 26:252-257.

Trouwborst, T., S. Kuyper, J.C. DeJong, and A.D. Plantinga. 1974. Inactivation of some bacterial and animal viruses by exposure to liquid-air interfaces. J. gen. Virol. 24:155-165.

U.S. Environmental Protection Agency. 1977. Process design manual for land treatment of municipal wastewater. EPA 625/1-77-008 (COE EM1110-1-501).

VanGenuchten, M.T. 1980. Analytical solutions for chemical transport with simultaneous adsorption, zero-order production, and first-order decay. J. Hydrology 49:213-233.

VanGenuchten, M.T., and W.J. Alves. 1982. Analytical solutions of the one-dimensional convective-dispersive solute transport equation. USDA, Tech. Bul. 1661. U.S. Gov. Printing Office, Washington, DC.

Vaughn, J.M., E.F. Landry, C.A. Beckwith, M.Z. Thomas. 1981. Virus removal during groundwater recharge: effects of infiltration rate on adsorption of poliovirus to soil. Appl. Environ. Microbiol. 41:139-147.

Vaughn, J.M., and E.F. Landry. 1983. Viruses in soils and groundwater. p. 165-201. In G. Berg (ed.) Viral pollution of the environment. CRC Press, Boca Raton, FL.

Vaughn, J.M., E.F. Landry, L.J. Baranosky, C.A. Beckwith, M.C. Dahl, and N.C. Delihas. 1978. Survey of human virus occurrence in wastewater-recharged groundwater on Long Island. Appl. Environ. Microbiol. 36:47-51.

Vaughn, J.M., E.F. Landry, M.Z. Thomas. 1983. Entrainment of viruses from septic tank leach fields through a shallow, sandy soil aquifer. Appl. Environ. Microbiol. 45: 1474-1480.

Vilker, V.L., and W.D. Burge. 1980. Adsorption mass transfer model for virus transport in soils. Water Research 14:783-790.

Vinten, A.J.A., and P.H. Nye. 1985. Transport and deposition of dilute colloidal suspensions in soils. J. Soil Science 36:531-541.

Vinten, A.J.A., B. Yaron, and P.H. Nye. 1983. Vertical transport of pesticides into soil when adsorbed on suspended particles. J. Agricultural and Food Chemistry 32:662-664.

Waldichuck, M. 1982. Air-sea exchange of pollutants. p. 186-189. In G. Kullenberg (ed.) Pollutant transfer and transport in the sea. CRC Press, Boca Raton, FL.

Walter, R., J. Durkop, B. Friedman, and H.J. Dobberkau. 1985. Interactions between biotic and abiotic factors and viruses in a water system. Water Sci. Technol. 17:139-151.

Walter, R., W. Macht, J. Durkop, R. Hecht, U. Hornig, and P. Schulze. 1989. Virus levels in river waters. Wat. Res. 23:133-138.

Wang, D.-S., C.P. Gerba, and J.C. Lance. 1981. Effect of Soil Permeability on Virus Removal Through Soil Columns. Appl. Environ. Microbiol. 42: 83-88.

Wang, D.-S., J. C. Lance, and C. P. Gerba. 1980. Evaluation of various soil water samplers for virological sampling. Appl. Environ. Microbiol. 39:662-664.

Ward, R.L and E.W. Akin. 1984. Minimum infective dose of animal viruses. CRC Critical Reviews in Environmental Control 14: 297-310.

Ward, R.L., and C.S. Ashley. 1977. Inactivation of enteric viruses in wastewater sludge through dewatering by evaporation. Appl. Environ. Microbiol. 34:564-570.

Ward, R.L., D.R. Knowlton, P.E. Winston. 1986. Mechanism of inactivation of enteric viruses in fresh-water. Appl. Environ. Microbiol. 52:450-459.

Ward, R.L., and P.E. Winston. 1985. Development of methods to measure virus inactivation in fresh waters. Appl. Environ. Microbiol. 50:1144-1148.

Weber, K., and W. Konigsberg. 1975. Proteins of the RNA phages. p. 51-84. In N.D. Zinder (ed.) RNA phages. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Wellings, F. M., A. L. Lewis, C. W. Mountain, and L. V. Pierce. 1975. Demonstration of virus in groundwater after effluent discharge into soil. Appl. Microbiol. 29:751-757.

Wellings, F.M., A.L. Lewis, C.W. Mountain, and L.M. Stark. 1975. Virus consideration in land disposal of sewage effluents and sludge. Florida Scientist 38:202-207.

Wiley, D.C., and J.J. Skehel. 1990. Viral membranes. p. 63-85. In B.N. Fields et al. (ed.) Virology. Raven Press, NY.

World Health Organization. 1979. Human viruses in water, wastewater, and soil. Technical Report 639, WHO, Geneva.

Yates, M.V. 1985. Septic tank density and ground water contamination. Ground Water 23:586-591.

Yates, M.V., C.P. Gerba, and L.M. Kelly. 1985. Virus persistence in groundwater. Appl. Environ. Microbiol. 49:778-781.

Yates, M.V., and S.R. Yates. 1988. Modeling microbial fate in the subsurface environment. CRC Critical Reviews in Environmental Control 17:307-344.

Yates, M. V., S. R. Yates, J. Wagner, and C. P. Gerba. 1987. Modeling Virus Survival and Transport in the Subsurface. J. Contaminant Hydrol. 1: 329-345.

Yates, M.V., S.R. Yates, A.W. Warrick, and C.P. Gerba. 1986. Use of geostatistics to predict virus decay rates for determination of septic tank setback distances. Appl. Environ. Microbiol. 52:479-483.

Yeager, J.G., and R.T. O'Brien. 1979. Enterovirus inactivation in soil. Appl. Environ. Microbiol. 38:694-701.

Zerda, K.S., C.P. Gerba, K.C. Hou, and S.M. Goyal. 1985. Adsorption of viruses to charge-modified silica. Appl. Environ. Microbiol. 49:91-95.