UNC-WRRI-81-168

SURVIVAL AND FATE OF ENTERIC VIRUSES IN SOIL TREATMENT SYSTEMS FOR WASTEWATER

by

Mark D. Sobsey
Associate Professor of Environmental Microbiology

Cheryl H. Dean
Graduate Research Assistant

Robert R. Jacobs
Graduate Research Assistant

Maurice E. Knuckles
Graduate Research Assistant

Ray A. Wagner
Graduate Research Assistant

Department of Environmental Sciences and Engineering
University of North Carolina at Chapel Hill
Chapel Hill, North Carolina 27514

The work upon which this publication is based was supported in part by funds provided by the Office of Water Research and Technology, U. S. Department of the Interior, Washington, D.C., through the Water Resources Research Institute of The University of North Carolina as authorized by the Water Research and Development Act of 1978.

Project No. A-090-NC
Agreement No. 14-34-0001-7070

ACKNOWLEDGMENTS

This study was carried out with the generous assistance of many persons. We especially thank B.L. Carlile, S. Weed, L. Aull, D.J. Osborne and L. Stewart, Soil Science Department, North Carolina State University, Raleigh, North Carolina, for assistance in selecting, obtaining and analyzing soil materials and wastewaters, and for their cooperative efforts with pilot scale soil columns. During the course of parts of this study M.D. Sobsey was supported by a Public Health Service Research Career Development Award from the National Institute of Environmental Health Sciences (5-K04-ES-00026).

DISCLAIMER STATEMENT

ABSTRACT

There were marked differences in the abilities of different soil materials to remove and retain viruses from settled sewage, but for each soil material the behavior of two different viruses, poliovirus type 1 and reovirus type 3, was often similar. Virus adsorption to soil materials was rapid, the majority occurring within 15 min. Clayey materials efficiently adsorbed both viruses from wastewater over a range of pH and total dissolved solids levels. Sands and organic soil materials were comparatively poor adsorbents, but in some cases their ability to adsorb viruses increased at low pH and with the addition of total dissolved solids or divalent cations. Viruses in suspensions of soil material in settled sewage survived for considerable time periods, despite microbial activity. In some cases virus survival was prolonged in suspensions of soil materials compared to soil-free controls. Although sandy and organic soil materials were poor virus adsorbents when suspended in wastewater, they gave >95% virus removal from intermittently applied wastewater as unsaturated, 10-cm-deep columns. However, considerable quantities of the retained viruses were washed from the columns by simulated rainfall. Under the same conditions, clayey soil material removed >99.9995% of the viruses from applied wastewater, and none were washed from the columns by simulated rainfall.

In pilot scale studies with 5-foot long and 6-inch diameter columns having 3-foot, unsaturated upper zones and 2-foot, saturated bottom zones, extensive (≥ 99.999998 percent) poliovirus and reovirus reductions occurred when septic tank effluent containing 3-6 x 10^4 PFU of viruses per ml was applied at a rate of 1.3 inches (3 cm) per day over 8-week periods. Furthermore, no viruses were detected in extracts made from the column soil materials after column operation, thus indicating that retained viruses were extensively inactivated. This extensive virus retention and inactivation with 5-foot long columns was obtained with soil materials that had relatively poor virus adsorbent capabilities. These results suggest that extensive virus reductions in soils can be achieved with soils that have poor virus adsorbing abilities if there is a sufficient depth of unsaturated flow. However, the effects of periodic rainfall on virus retention and inactivation in soils must be further investigated.

TABLE OF CONTENTS

Page

OBJECTIVES

MATERIALS AND METHODS

RESULTS AND DISCUSSION

Page

LIST OF TABLES

LIST OF FIGURES

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

There were marked differences in the abilities of eleven different soil materials to remove and retain viruses from settled sewage. However, for each type of soil material the behavior of two markedly different viruses, poliovirus type 1 and reovirus type 3, was often similar. Considering the differences in size and surface properties, including isoelectric points (pI of poliovirus type 1, strain LSc = 7.15; and of reovirus type 3, Dearing = 3.9)(Sobsey, M.D., R.R. Jacobs and W.A. Rutala, unpublished results), the similarities in adsorption to soil materials were unexpected. These findings are in contrast to those of Goyal and Gerba (1979) and Landry et al. (1979), who found that virus adsorption and retention by soil materials varied greatly with virus type and strain. Further studies with additional types and strains of enteroviruses and reoviruses would be necessary to determine if the soil types used in this present study show the variability in virus adsorption and retention reported by these other workers.

Experiments on the kinetics of virus adsorption to soil material suspended in wastewater showed that most of the adsorption occurred within 15 min. and did not vary appreciably during a 90-min. contact period. Similar results have been reported for the adsorption of bacteriophage T4 to kaolinite (Carlson et al., 1968) encephalomyocarditis viruses to montmorillonite (Schaub and Sagik, 1975) and poliovirus to sandy loam (Goyal and Gerba, 1979), with the majority of adsorption occurring within 20 min. or less.

The effects of pH and TDS on virus adsorption to suspensions of soil materials in wastewater depended primarily on the type of soil material. Kaolinite and B-horizon materials of Cecil, Davidson, and Norfolk extensively adsorbed both poliovirus and reovirus over a wide range of pH and TDS levels. Bentonite extensively adsorbed reovirus at all pH and TDS levels tested, whereas poliovirus adsorption was less extensive at all pH levels but was markedly improved at higher TDS levels. Ponzer organic muck adsorbed reovirus more efficiently than poliovirus, although the adsorption of the latter virus was greatly improved at low pH and high TDS levels. The three sands, Lakeland, Fripp and commercial sand as well as loam and organic topsoils from the Tidewater region of North Carolina were generally poor adsorbents for both viruses. Virus adsorption of Fripp and Lakeland was improved at lower pH and higher TDS levels. The addition of $MgCl_2$ to Fripp suspensions enhanced virus adsorption to both soil materials and wastewater solids more effectively than NaCl. This enhanced virus adsorption by added salts, especially at lower pH levels, is consistent with the results of previous studies in which virus adsorption to various suspended solids and soil materials was enhanced at lower pH levels and more effectively by divalent than by monovalent cations (Bitton et al., 1976; Carlson et al., 1968; Lefler and Kott, 1974; and Moore et al., 1975).

None of the eluents tested was capable of extensively eluting adsorbed viruses from a majority of the soil materials, but an organic material, nutrient broth, was more effective than either distilled water or 3.5% NaCl. The most extensive elution was from Lakeland sand and Ponzer organic muck. These findings are in contrast to those of some previous studies in which organic materials and distilled water readily desorbed virus from clay and silicate particles (Carlson et al., 1968; and Lo and Sproul, 1977).

Viruses in suspensions of soil materials in settled sewage survived for considerable periods of time, even when the suspensions were microbially active. Similar protection against inactivation by adsorption to soil and mineral particulates has been reported for viruses in seawater (Gerba and Schaiberger, 1975) and exposed to chlorine (Boardman and Sproul, 1977; and Stagg et al., 1977). The results of the experiments on virus survival in soil suspensions suggest that viruses in saturated soils may remain infectious for considerable periods of time, even at a moderate temperature of 20°C where the soil would be microbially active.

The extent of virus removal and retention in 10-cm-long, unsaturated soil columns receiving intermittent applications of wastewater or simulated rainwater depended primarily on the type of soil material. Virus removal from columns of a soil material with a high clay content, Norfolk sandy clay loam, removed >99.9995% of the viruses from wastewater applied at both high and low hydraulic loadings over a 58-day period. Furthermore, none of the applied viruses were washed out of the columns by simulated rainfall. Sandy and organic soil materials, which were poor virus adsorbents when suspended in wastewater, were capable of ≥95% virus removal from wastewater as columns. However, considerable quantities of the removed viruses were washed out of the columns under simulated rainfall conditions. These findings with sandy and organic soil columns are consistent with those of other workers who have observed the release and migration of viruses in soil columns by applications of distilled and other low-TDS waters simulating rainfall (Duboise et al., 1976; Lance et al., 1976; Landry et al., 1979; Lefler and Kott, 1974; and Moore et al., 1978). However, the results of experiments with Norfolk columns indicate that some types of soil materials have such a strong affinity for viruses that little, if any, release and migration will occur under rainfall conditions.

Fecal coliform reductions in soil columns exceeded virus reductions by 1.3 to 3.3 orders of magnitude under high and low hydraulic loadings of wastewater. The specific reasons why fecal coliform bacteria were removed by soils more efficiently than poliovirus were not investigated in this study. One possible reason is that bacteria, being larger than viruses, can be removed in soils by three mechanisms: entrapment between soil particles, adsorption to soil particles, and sedimentation on the "downstream" side of soil particles. However, viruses are so small that they are probably removable in soils primarily by adsorption, unless they are associated with larger particles. Another possibility is that the difference in bacteria and virus removal efficiency by soils reflects differences in surface properties that influence their adsorption behavior. The validity of these hypotheses must await the finding of future studies designed to address these issues.

The observation that soils removed fecal coliforms more extensively than poliovirus suggests that fecal coliforms may not be suitable indicators for enteric virus reductions in land application systems for wastewater. This suggestion supports the findings of recent field studies on virus and indicator bacteria contamination of groundwaters in which enteroviruses were isolated from groundwater samples containing no detectable fecal coliforms in 100-ml volumes (Marzouk et al., 1979).

The results of pilot-scale studies indicated that 5-foot long soil columns containing an upper 3-foot unsaturated zone and receiving an hydraulic load of 1.3 inches per day were capable of achieving extensive virus and fecal coliform bacteria reductions from applied wastewater. Furthermore, extensive inactivation of the retained viruses occurred, as indicated by the lack of infectious viruses remaining in the soil column materials.

It is important to note that this extensive virus retention and inactivation was achieved in soil materials that were relatively poor virus adsorbents, based on their ability to adsorb viruses under saturated conditions as soil suspensions in wastewater. However, it should also be noted that the pilot scale columns did not receive simulated rainfall. Therefore, it cannot be known if such extensive virus retention and inactivation would occur under actual field conditions where periodic rainfall might cause greater movement and penetration of viruses through the soil material. The results of studies with miniature soil columns indicated that simulated rainfall could indeed result in extensive virus movement through at least some soil materials, especially sandy and organic soils. Further pilot scale soil column studies should be conducted using simulated rainfall in order to determine if this will cause increased virus movement and breakthrough in column effluents.

INTRODUCTION

The survival and fate of enteric viruses in the soil is an important consideration in land treatment of municipal, domestic, and animal wastes that has not been adequately evaluated. Potential human and animal health hazards from enteric viruses are associated with a variety of land application systems for human and animal wastes such as groundwater recharge, crop irrigation, household septic waste disposal, and municipal solids waste landfill.

Virus survival in soils and the nature and degree of virus retention by soil materials will influence the extent of virus contamination of both the soil and the associated groundwater. A number of laboratory and field studies have established that virus retention by and survival in soils is influences by such factors as soil type; chemical characteristics of the applied water, including pH, and concentrations of total dissolved solids (TDS), polyvalent cations and organics; and hydraulic characteristics, including areal loading and application frequency (see reviews in Baldwin et al., 1976; Bitton, 1975; and Gerba et al., 1975). The results of many of the laboratory studies are of limited usefulness and applicability for one or more of the following reasons: (i) investigation of only a few soil materials, usually a sand or clay; (ii) use of distilled or tap water instead of wastewater; (iii) use of bacteriophages or only a single type of enterovirus; and (iv) use of only sterilized water and soil material. The few reported field studies on viruses in land treatment systems have also provided only limited information because their marked differences in characteristics and operating conditions and their apparent differences in virus removal efficiencies have not led to the development of rational design and operation principles for virus control (Gilbert et al, 1976; Moore et al., 1978; Schaub and Sorber, 1977; and Wellings et al., 1975).

This report described the results of laboratory and pilot scale studies on the interactions and survival of representatives of two different enteric virus groups in eleven different soil materials in settled sewage. Additional variables studies include: (i) hydraulic conditions, (ii) soil microbial activity, and (iii) such wastewater characteristics as pH and the concentrations of TDS and divalent cations.

OBJECTIVES

General Objective

To determine the interactions, survival and fate of enteric viruses in soil treatment systems for wastewater.

Specific Objectives

To characterize the short term interactions of enteric viruses with soil-wastewater mixtures under controlled laboratory conditions.

To assess the survival and fate of enteric viruses in wastewaters applied to laboratory scale columns containing North Carolina soils or soil components.

To evaluate enteric virus removal from a treated wastewater effluent by pilot scale soil treatment systems.

MATERIALS AND METHODS

Soil Materials for Small-Scale Laboratory Studies

Eight widely different soil materials were used in laboratory studies, and their characteristics are summarized in Table 1. In addition, organic matter content, as determined by ignition tests, was 80.1% for Ponzer and <1% for the other seven soil materials.

Viruses

The viruses used in this study were poliovirus type 1, strain LSc, and reovirus type 3, strain Dearing. Virus growth, preparation, and assay procedures are described elsewhere (Sobsey et al., 1978). Samples for virus assay were diluted in phosphate-buffered saline containing 2% heat-inactivated fetal calf serum and antibiotics (400 U of penicillin, 400 μg of streptomycin, and 200 μg of kanamycin per ml).

Wastewaters

All small-scale experiments were done with settled sewage that was prepared by settling raw, domestic sewage for 3 hours in the laboratory and collecting the upper half of the liquid from the settling containers. The settled sewage was then dispensed into 100- to 250-ml volumes and stored at -20°C. The average composition of the settled sewage was as follows: TDS = 290 mg of NaCl per liter, pH = 7.6, total volatile solids = 110 mg/l, total organic carbon = 46 mg/l, Biochemical Oxygen Demand (5-day measurement) 60 mg/l, Kjeldahl-N = 24 mg/l, NH_2-N = 22 mg/l, NO_3 + NO_2-N<0.3 mg/l, total phosphate = 5 mg/l, Ca^{+2} = 12 mg/l, Mg^{+2} = 4 mg/l. These analyses were performed according to the procedures in Standard Methods (American Public Health Association, 1976).

Fecal Coliform Analysis

Fecal coliforms were analyzed by the modified A-1 multiple tube fermentation procedure (Hunt and Springer, 1975) or by the membrane filter procedure (American Public Health Association, 1976).

Short-Term Virus Adsorption in Soil-Wastewater Suspensions

Virus adsorption was studied in short-term, batch-type experiments with virus-containing soil suspensions in wastewater. Such variables as contact time, type of soil material, pH, and concentrations of TDS and divalent cations were investigated. As a general experimental procedure, weighed quantities of air-dried soil material were added to enough settled sewage in polypropylene beakers containing Teflon-coated stir bars to give 1 or 5% (wt/vol) suspensions having final volumes of 50 or 100 ml. In some experiments the pH, TDS, or $MgCl_2$ levels of the suspensions were adjusted before making them up to the final volumes. After such adjustments, these suspensions were magnetically premixed at several hundred revolutions per minute for 30 minutes to allow them to reach room temperature (24 ± 1°C) and to otherwise stabilize, especially with regard to disegregation of air-dried soil lumps. While mixing continued, no more than 1.0 ml of stock virus was then added to give an initial concentration of about 5 x 10^4 plaque-forming units per ml. Control samples consisted of only settled sewage and virus. All samples were magnetically mixed at several hundred revolutions per minute throughout the entire experimental period. At each sampling time, 1.0-ml volumes were withdrawn from the beakers, initially diluted 10-fold in 9 ml of diluent, and then diluted another 10-fold for subsequent virus assay.

To determine the extent of virus adsorption to soil material at each sampling time, a 10-ml volume was also withdrawn from each beaker and immediately centrifuged at 5,000 to 10,000 x g for 10 to 15 min. to sediment the soil material. The resulting supernatant was diluted 10- and 100-fold and assayed for viruses. The amount of virus adsorbed to the sedimented soil material was considered to be the difference between the amount of virus in the uncentrifuged sample and the supernatant resulting from centrifugation. For experiments on the kinetics of virus adsorption to soil materials, the pH and TDS levels were measured at regular intervals during the experiment but were not adjusted or controlled. For experiments on the effects of pH on virus adsorption, the samples were adjusted to the desired pH levels with small volumes of 0.1 N HCl or NaOH, and, if necessary, small volumes of 16% NaCl were added to give a TDS concentration of 350 mg of NaCl per liter. Sample pH levels were checked at regular intervals during the course of the experiment and, if necessary, readjusted to the desired level. For experiments on the effects of TDS concentration on virus adsorption, the samples were adjusted to different TDS concentrations by adding small amounts of 16% NaCl. Sample TDS levels were checked at regular intervals during the course of the experiment to verify that appreciable changes did not occur. To study the effects of added divalent cations on virus adsorption to Fripp sand, small volumes of concentrated $MgCl_2$ solution were added to the samples, and they were then adjusted to the desired pH and conductivity levels as already described.

Elution of viruses adsorbed to soil materials after short contact times was also studied. Virus-containing soil suspensions in settled sewage were prepared as previously described, and, except where noted otherwise, the samples were adjuted to pH 4.5 and a TDS of 3,500 mg of NaCl per liter to obtain maximum virus adsorption. After an adsorption period of 45 to 60 min., 1-ml samples were centrifuged as previously described. The resulting supernatants were sampled for virus assay and then decanted. The sedimented soil materials were resuspended to 10-ml volumes in different eluent fluids, and after adjusting to pH 7.5 with 0.1 N HCl or NaOH (pH 5.0 for Ponzer soil), 1-ml samples were taken for virus assay. The following eluent fluids were used: distilled water, 3.5% NaCl buffered with 0.007 M sodium phosphate, and 4% nutrient broth (pH 7.5). After 15 min., these suspensions were centrifuged as before, and the resulting supernatants were assayed for viruses. The proportion of viruses eluted was determined from the virus titers of the soil material suspensions in eluent fluid and the supernatants resulting from centrifugation. The virus titers of the soil material suspensions in the eluent fluid were also compared with the virus titers of the initial suspensions in wastewater to confirm that no extensive loss of virus infectivity had occurred.

Virus Survival in Soil-wastewater Suspensions

Experiments were done to determine virus survival in sterile and nonsterile soil-wastewater suspensions. One and 5% (wt/vol) suspensions of soil material in settled sewage were prepared in 500-ml-capacity polypropylene, widemouth, screwcap bottles to give final volumes of 300 ml after adjusting to pH 6.5 (pH 5.5 for Ponzer) with 0.1 N HCl or NaOH. Four replicate suspensions were prepared for each soil material. Two of the four suspensions were sterilized by autoclaving for 15 min. After the autoclaved suspensions cooled, 1.5 ml of penicillin-streptomycin-kanamycin mixture was added to give final concentrations of 200 Units/ml, 200 µg/ml and 100 µg/ml, respectively; and, if necessary, the pH was readjusted. After bringing to 20°C, sterile and nonsterile suspensions were inoculated with no more than 1 ml of stock reovirus or poliovirus to give an initial concentration of about 5 x 10^5 plaque-forming units per ml. Control samples consisted of only settled sewage and virus (and antibiotics in sterile samples). The suspensions were mixed for several minutes, and 1-ml volumes were taken for virus assay. For sterile suspensions, weekly 1-ml samples were also plated on nutrient agar to confirm that sterility was being maintained.

Survival and Fate of Poliovirus in Miniature Soil Columns

To study the survival and fate of viruses in a system that was physically more representative of an intact, unsaturated soil than were suspensions of soil material in wastewater, experiments were done with small, unsaturated columns of soil materials to which virus-laden wastewater was intermittently applied. Miniature soil columns were made from the barrels of disposable 60-ml polypropylene syringes (length = 13.3 cm; diameter = 2.6 cm) by placing a small wad of polypropylene fibers in the bottom of the barrel and then packing with soil material to a height of 10 cm. Columns of Fripp, Lakeland, Ponzer, and B-horizon material of Norfolk were used as representatives of a wide range of different soil types, including porous sands, organic soils and clay soils. The columns were conditioned by first saturating with distilled water and draining overnight. Throughout the experiment the columns were kept at 20 ± 1°C in the dark.

In the first phase of the soil column experiment, 13.5 ml, corresponding to 2.54 cm, of settled sewage containing 5 x 10^4 plaque-forming units of poliovirus per ml was applied all at once to each soil column two times per week, thus giving a weekly application rate of 5.08 cm. The column effluents were allowed to drain freely overnight into sterile, 17-ml-capacity, polystyrene test tubes that were tightly connected to the column outlets. However, it was observed that overnight column drainage was not essential because all of the column effluent was produced within 1 hr. after each wastewater application. A sample of the virus-containing wastewater influent was kept with the soil columns overnight to serve as a control for virus survival in wastewater. After the column effluents and controls were collected, they were diluted and assayed for both fecal coliform bacteria and poliovirus. This first phase of the soil column experiment lasted 37 days from the initial application of virus-containing settled sewage.

Both the sandy loam topsoil and the organic topsoil were collected near the community of Plymouth in the Tidewater region of the North Carolina coastal plain. The sand the gravel were obtained commercially. These materials were hand-packed into the columns at approximately 6% moisture by weight.

Tidewater region soils were selected for this study because they are from a problem region of chronic septic tank failure. It was of interest to determine virus removal and survival in these soils when they were used for wastewater treatment under idealized pilot scale conditions.

Operation and Dosing of Pilot Scale Columns

The columns were first conditioned with tapwater and then with septic tank effluents (STE) for a total period of 6 months prior to the addition of test viruses in STE. For the first 3 months the columns were dosed daily with 3 cm of tapwater. After 3 months of tapwater dosing, the columns began to be dosed with STE. They were dosed with 300 ml of STE twice daily, corresponding to an hydraulic loading rate of 3.3 cm (1.3 inches) per day. At each dosing, the STE was added over a 30-minute period at a rate of 10 ml/min. Hydraulic residence times in the columns, which were determined experimentally by supplementing STE with 1000 mg/l chloride and then monitoring chloride concentrations in the column effluents, averaged about 2 weeks. The columns were conditioned with STE for 3 months before adding viruses to the system. Column temperatures were maintained at 25°C. During the study period, column effluent samples of 100 ml were collected 2 or 3 times weekly for virus assay. Samples were also periodically collected for the determination of fecal coliform bacteria (FC), chemical oxygen demand (COD), total ammonium and nitrate nitrogen (TN, NH_4-N, and NO_3-N, respectively) and acid hydrolyzable ortho- and polyphosphate (PO_4-P). All chemical and fecal coliform analyses were done as previously described (Stewart, 1976).

The virus dosing schedule during the study period was as follows. After the 3 month conditioning period with STE, the columns were dosed with STE containing 6×10^4 PFU/ml of added poliovirus type 1, strain LSc, for a period of 8 weeks. This was followed by an 8-week period of dosing with STE containing no added viruses. After this 8-week "rest" period, the columns were then dosed for 8 weeks with STE containing added reovirus type 3, at a concentration of 3.7×10^4 PFU/ml. This was also followed by an 8-week rest period during which the columns were dosed with STE containing no added viruses. After this final 8-week period, the contents of each column were removed and fractionated as a function of depth, and each fraction was analyzed for its virus content.

Virus Concentration from Effluents of Pilot Scale Columns

The 100-ml column effluent samples were too large in volume to be economically assayed in their entirely for viruses without first concentrating the viruses to a smaller amount of fluid. Therefore, viruses in these samples were first concentrated 5- to 10-fold by adsorption to and elution from 47 mm diameter fiberglass-asbestos-epoxy filters, 2.0 and 0.45 µm pore size, in series (Types AA200 and AA45, respectively, Cox Instrument Division, Lynch Corp, Detroit, Michigan). To promote efficient virus

Beginning on day 38, the application schedule for the columns was modified to simulate the occurrence of rain shortly after wastewater application. Virus-contaminated, settled sewage was applied to the columns in 13.5-ml amounts twice weekly as before. However, the column effluents were collected for only 3 hr. after wastewater application instead of overnight, and they were set aside.

A 13.5-ml volume of sterile, distilled water was then applied to each column all at once to simulate a 2.5-cm rainfall. The column effluents from the distilled water applications were then separately collected overnight. The collected column effluents from the wastewater application as well as the control wastewater samples were stored with the columns overnight. All three types of samples were then assayed for fecal coliform bacteria and poliovirus. A total of four applications of wastewater and distilled water were made over a 2-week period (days 38, 41, 45, and 48). Beginning on day 52, the application schedule of these same columns was again changed, this time to simulate increased wastewater loads and application frequencies. The distilled water applications simulating rainfall were eliminated. The wastewater application frequency was increased to 13.5 ml (2.54 cm) per day, corresponding to a weekly wastewater loading of 17.8 cm. A total of seven daily applications were made from days 52 through 58. As before, column effluents were collected overnight and then assayed for fecal coliforms and poliovirus as were the stored control samples of applied wastewater.

Pilot Scale Columns and Their Soil Materials

Pilot scale soil absorption systems consisted of soil columns constructed of acrylic plastic. Each column was 5 feet long and 6 inches in diameter and had an unsaturated upper zone of 3 feet and a saturated bottom zone of 2 feet. The soil composition of the upper zone was either loamy sand topsoil or a 1:1 mixture of loamy sand topsoil and commercial sand. The saturated 2 foot bottom zone was either gravel or a 1:2 mixture of organic topsoil (27% organic matter) and medium sand. The properties of the individual soil material components are summarized in Table 2. There were four different columns; one for each possible combination of upper and lower zone material.

Column 1: Loamy sand topsoil upper zone
2:1 organic topsoil-sand bottom zone

Column 2: Loamy sand topsoil upper zone
gravel bottom zone

Column 3: 1:1 mixture of loamy-sand topsoil + sand upper zone
2:1 organic topsoil-sand bottom zone

Column 4: 1:1 mixture of loamy sand topsoil + sand upper zone
gravel bottom zone.

Liquid was added to the columns through a 1.9 cm-thick porous block of the type which is sometimes used for septic tank soil absorption lines as an alternative to perforated pipe. The block material was used in the form of a hollow cube with acrylic side walls and porous block for the cube top and bottom (Figure 1).

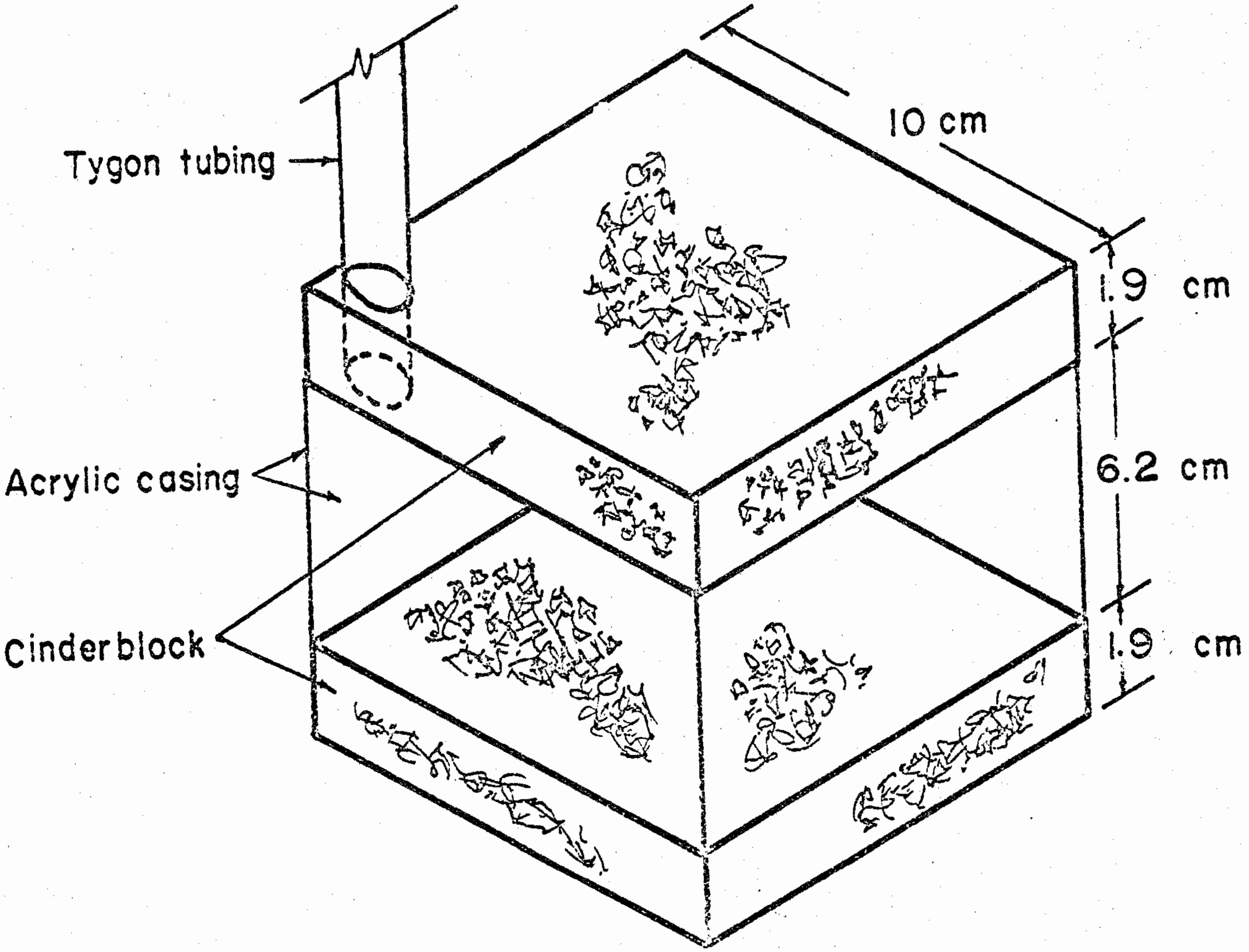

Figure 1. Construction details of cinderblock cube.

adsorption to the filters, the samples were adjusted to either pH 3.5 (for poliovirus) or pH 5.5 (for reovirus) and either 0.0005 M $AlCl_3$ (for poliovirus) or 0.05 M $MgCl_2$ (for reovirus) was added. The conditioned samples were then filtered through the above filter series and the filtrates were discarded. The filters were washed with 10 ml of 0.14 N NaCl to remove excess $AlCl_3$ or $MgCl_2$, and these filtrates were discarded. Adsorbed viruses were then eluted from the filter with 7 to 9 ml of either glycine (0.05 M)-NaOH, pH 11.5 (for poliovirus) or 4% nutrient broth (Difco, Detroit, Michigan), pH 9.0 (for reovirus). The eluates were adjusted to pH 7.5 with pH 1.5 glycine (0.05 M)-HCl and supplemented with fetal calf serum (2%), penicillin (400 Units/ml) and streptomycin (400 μg/ml). The entire concentrates were then assayed for viruses by the plaque technique as previously described.

Virus Recovery from Fractionated Soil Materials of Pilot Scale Columns

At the termination of column operation with STE dosing, it was of interest to determine the levels of viruses that had not migrated through the columns but remained associated with the soil materials in an infectious state. Attempts were made to recover these viruses from the soil materials as a function of column depth by using three different fluids to elute soils-associated viruses: alkaline glycine, distilled water and nutrient broth.

After the 8-week "rest" period that followed the reovirus dosing period, the soil materials of each column were removed in fractions according to column depth. The upper, unsaturated zone material of each column was collected and composited as 5 fractions: 0-3, 3-6, 6-12, 12-18 and 18-36 inches. Composite soil samples were also taken from the saturated bottom zones of columes 1 and 3. To elute and recover adsorbed viruses, 10 to 50 gram quantities of each fraction were resuspended in one of the three different media at a dilution ratio of 1:7 (w/v) in an effort to desorb the infectious viruses present.

In the recovery procedure using alkaline glycine fifty-gram samples of composited soil material were resuspended in 350 ml of 0.05 M glycine. While mixing with a magnetic stirrer, the suspensions were adjusted to pH 10.5 with 1.0 and 0.1 N NaOH to promote virus elution from the soil materials. The suspensions were then centrifuged at 2000 x g for 30 minutes to remove soil materials. Viruses in the resulting supernatants were concentrated to a smaller volume by adsorption to and elution from microporous filters as follows. The supernatants were adjusted to pH 5.5 with 1.0 and 0.1 N HCl and $MgCl_2$ was added to a final 0.05 M concentration to promote virus adsorption to adsorbent filters. Each sample was then filtered through a 47 mm diameter filter series consisting of a fiberglass per filter (type AP25, Millipore Corp.) and a fiberglass-asbestos-epoxy microporous filter (0.45 μm pore size, type AA45, Cox Instrument Div., Lynch Corp.) to adsorb viruses. The filtrate was discarded and the adsorbed viruses were then eluted from the filters with a 15-ml volume of nutrient broth in 0.14 N NaCl, pH 9.0. The eluate was treated with antibiotics and assayed for viruses.

Viruses were recovered from soil materials with distilled water by suspending another 50-gram sample of each composite fraction of soil material in 350 ml of sterile distilled water and adjusting to pH 8.0. The suspension was centrifuged and viruses in the resulting supernatant were further concentrated and assayed as described above for samples treated wtih alkaline glycine.

To recover viruses from column fractions using nutrient broth, 10 grams of soil materials were resuspended in 70 ml of 4% nutrient broth, pH 9.0 and briefly shaken by hand to elute adsorbed viruses. The samples were then centrifuged at 2,000 x g to sediment the soil material. The resulting supernatant was decanted, supplemented with antibiotics and divided into two equal volumes for subsequent poliovirus and reovirus assay, respectively.

RESULTS AND DISCUSSION

Short-term Virus Adsorption to Soil Materials Suspended in Wastewater

Kinetics of virus adsorption to soil materials. The results of the kinetic studies on virus adsorption to soil materials suspended in settled sewage (Table 3) indicate that adsorption, if any, is rapid, with most of it occurring within 15 min. The two sands, Lakeland and Fripp, were relatively poor adsorbents for both viruses. Ponzer was a poor adsorbent for poliovirus, but considerably better for reovirus with about 80% adsorption. Bentonite effectively adsorbed reovirus, but its adsorptive ability for poliovirus was much lower and even decreased over the 90-min. contact period. Kaolinite adsorbed poliovirus and reovirus with average efficiencies of 96 and 89%, respectively. B-horizon materials of Cecil, Davidson and Norfolk were excellent adsorbents for both viruses, with adsorption efficiencies consistently >99%. It should be noted that because only one-fifth as much soil material was used in the bentonite and kaolinite suspensions, the results are not directly comparable to those for the other six soil materials. Although some virus adsorption occurred in the control samples containing no soil material, the majority of both viruses remained in the supernatant after centrifugation. Virus adsorption in the controls was probably to the suspended sewage solids that sedimented upon centrifugation. Because most of the virus adsorption to soil materials occurred within 15 min., contact times of 45 to 60 min. were used in subsequent short-term virus adsorption experiments to insure that relatively stable conditions were achieved with respect to adsorption equilibria.

Effect of pH on virus adsorption. The results of experiments on virus adsorption to soil materials as a function of pH are summarized in Table 4. In control samples there was little poliovirus adsorption between pH 4.5 and 7.5 and little reovirus adsorption between pH 5.5 and 7.5. However, at pH 3.5, 47% of the poliovirus infectivity in the control was removed by centrifugation, and for reovirus the infectivity reduction by centrifugation was 58 and 99% at pH 4.5 and 3.5, respectively. This virus infectivity reduction in acidic controls was probably due to virus adsorption to sedimentable sewage solids. At all pH levels tested, B-horizon materials of Cecil, Davidson, and Norfolk adsorbed >99% of both viruses. Kaolinite adsorbed both viruses with at least 90% efficiency at all pH levels, and adsorption efficiency increased to >99% with decreasing pH. Bentonite adsorbed reovirus with >99% efficiency at all pH levels, but it was considerably less effective for poliovirus, with adsorption efficiencies ranging from 70 to 91%. The adsorption efficiency of Ponzer varied not only with the virus type but also with pH. Ponzer adsorption was >90% for reovirus between pH 3.5 and 5.5, but for poliovirus it was >90% only at pH 3.5. Virus adsorption efficiency of Ponzer was considerably lower at the higher pH levels tested. Fripp was a generally poor virus adsorbent at all pH levels tested, except for reovirus at pH 4.5 for which the adsorption efficiency was 74%. For Lakeland the adsorption efficiency for both viruses was markedly increased as pH decreased, with 97 and 99% adsorption efficiency for poliovirus and reovirus, respectively, at pH 4.5. The improved virus adsorption efficiency obtained at low pH levels for some samples may be partly attributable to sewage solids rather than soil materials. However. the extent of virus adsorption at low pH was greater in the suspensions of soil materials than in the controls, thus indicating that the soil materials were responsible for some adsorption.

Effect of total dissolved solids concentration on virus adsorption. As shown by the results in Table 5, increasing the TDS concentrations of the settled sewage suspensions at pH 6.5 (pH 5.5 for Ponzer) produced increased virus adsorption with some of the soil materials tested. For both control and Fripp samples, virus adsorption efficiency was low at all TDS levels, although poliovirus adsorption by Fripp increased to 40% at a TDS concentration of 3,500 mg of NaCl per liter. Reovirus adsorption by Lakeland did not exceed 30%, whereas poliovirus adsorption was greater, reaching 94% at a TDS concentration of 3,500 mg of NaCl per liter. At pH 5.5, Ponzer adsorbed reovirus more efficiently than poliovirus, with 90% adsorption of the former and only 63% adsorption of the latter at the highest TDS level, and for both viruses, adsorption efficiency improved somewhat with increasing TDS concentration. With poliovirus, bentonite also gave increased adsorption efficiency with increasing TDS concentration, whereas reovirus adsorption was $\geq$99% at all TDS levels. B-horizon materials of Cecil, Davidson, and Norfolk adsorbed both viruses with >99% efficiency, and kaolinite adsorbed poliovirus with $\geq$99% efficiency at all TDS levels.

Effect of added $MgCl_2$ on virus adsorption to Fripp. Because the results of the previous experiments showed that Fripp was a generally poor virus adsorbent at all pH TDS levels tested, the effect of increased divalent cation concentration, specifically Mg^{+2}, on virus adsorption by Fripp was investigated. $MgCl_2$ at added concentrations of 0.01 and 0.001 M enhanced poliovirus adsorption at pH 7.5 and 4.5, but reovirus adsorption was enhanced only at pH 4.5 (Table 6). Added $MgCl_2$ and lower pH also caused greater virus adsorption in the soil-free controls, particularly for reovirus, but the extent of virus adsorption in the control was generally less than that in the corresponding Fripp sample. It should be noted that even at 0.001 M, the added $MgCl_2$ appreciably increased the divalent cation concentration of the settled sewage, which was only about 0.00016 and 0.0003 M for Mg^{+2} and Ca^{+2}, respectively.

Elution of adsorbed viruses. None of the three media was capable of eluting either virus from all seven of the soil materials tested (Table 7). The degree of virus elution from the soil materials varied with the types of soil materials, eluents, and viruses. The least effective eluent was buffered 3.5% NaCl, which eluted a substantial amount of only reovirus from Lakeland sand. The effectiveness of the NaCl solution in eluting adsorbed viruses is consistent with the previous observation that increasing concentrations of NaCl (TDS) tended to enhance virus adsorption by some soil materials. Distilled water at pH 7 was a somewhat more effective elution medium than buffered 3.5% NaCl, eluting considerable quantities of both viruses from Lakeland as well as poliovirus from Ponzer and B-horizon Cecil. The most effective eluent was nutrient broth, but it was more effective for poliovirus than reovirus. Nutrient broth eluted $\geq$10% of adsorbed poliovirus from Lakeland, Ponzer, bentonite, and B-horizon material of Cecil, but it eluted $\geq$10% of adsorbed reoviruses only from B-horizon material of Cecil and Norfolk.

Virus Survival in Soil-wastewater Suspensions

The results of experiments on virus survival in sterile and nonsterile suspensions of soil materials in settled sewage are summarized in Table 8 in terms of the time in days for 99% of the total, initial viruses to become inactivated (T-99). As shown by the mean T-99 values, both viruses survived longer in sterile than in nonsterile samples, thus suggesting that microbial activity plays at least some role in virus inactivation in soils. The survival of the two viruses in corresponding nonsterile samples was generally similar, but in some of the sterile samples, such as bentonite and Ponzer, the survival of the two viruses was markedly different. Poliovirus survival was lowest in both sterile and nonsterile Ponzer at pH 5.5, but this was not directly attributable to pH effects, because poliovirus survival in a pH 5.5, soil-free control (not shown in Table 8) was higher than in the Ponzer samples. Reovirus survival in nonsterile samples was lowest in the soil-free control, followed by Ponzer, whereas in sterile samples it was the lowest in bentonite, followed by the soil-free control. In some cases, viruses survived considerably longer in soil-containing suspensions than in soil-free controls, suggesting that viruses in soils may be protected against inactivation.

Survival and Fate of Poliovirus in Miniature Soil Columns

Summarized in Table 9 are the results of the initial phase of the soil column experiment in which two 2.54-cm applications of settled sewage were made per week. Column performance for each wastewater application is expressed at $\log_{10}$ reduction, which was computed by converting the organism concentrations of the applied wastewater and column effluents to $\log_{10}$ value, and then subtracting the latter from the former. Mean column performance over the entire period is also shown as percent organism reduction. In general, the 10-cm-deep columns of all four soil materials produced considerable poliovirus and fecal coliform reductions, with fecal coliform reductions being consistently greater. Mean fecal coliform reductions exceeded mean poliovirus reductions by 1.3 to 3.3 orders or magnitude, depending upon column material. The extent of poliovirus and fecal coliform reduction remained relatively stable throughout the entire test period, except for poliovirus reductions by the Lakeland columns, which decreased somewhat after 23 days. B-horizon material of Norfolk, which was an excellent virus adsorbent in the previous experiments with soil suspensions, also produced extensive poliovirus and fecal coliform reductions in the column system, averaging >4.3 and >5.6 orders of magnitude, respectively. Fripp, Lakeland, and Ponzer columns were capable of producing mean $\log_{10}$ poliovirus reductions of 1.9, 3.5 and 2.0, respectively, even though they were often ineffective virus adsorbents in soil suspensions.

Effect of simulated rainfall on poliovirus retention. Table 10 shows the results of the experiments on simulated rainfall effects on poliovirus retention, in terms of the $\log_{10}$ virus reduction in the column wastewater effluent (i.e., the difference in the virus concentrations of the applied wastewater and the corresponding column effluent) and the $\log_{10}$ virus concentration in the column effluent resulting from the simulated rainfall (distilled water application). The column effluents resulting from the wastewater applications showed about the same degree of virus reduction as that in the first phase of the column experiments when only wastewater was applied, especially if the comparison is limited to the later times of the first experimental phase. In the columns of B-horizon Norfolk, none of the viruses retained from the applied wastewater appeared in the column effluents from the subsequent rainwater application. However, the amounts of virus washed out of the Fripp, Lakeland, and Ponzer columns by the rainwater were so great that they exceeded the amounts of virus retained from the wastewater application of the same day. Therefore, some of the viruses washed out of these columns by the simulated rainfall were from previous wastewater applications, suggesting that retained viruses survived long enough in the soils to accumulate to higher concentrations than those in the applied wastewater. The results of this experiment indicate that with some soil materials considerable migration of viruses retained from wastewater is possible as a result of rainfall, even under conditions of unsaturated flow. However, the results with the columns of B-horizon Norfolk indicate that some soil materials having a strong affinity for viruses may not release their adsorbed viruses under rainfall conditions.

Effect of increased hydraulic loading on virus retention. Increasing the wastewater loading 3.5 times by going from two to seven 2.54-cm applications per week resulted in little change in the virus retention efficiency of the soil columns. Virus reduction by all four soil materials was similar to those in the previous two phases of the column experiment (Table 11). Thus, the increased hydraulic loading either had no effect on soil column performance or the columns were not operated long enough for the effects to be detected. Fecal coliform reductions at the increased hydraulic loading were lower in some soil columns than those at the lower application rate, but the columns were still producing $\log_{10}$ fecal coliform reductions of about 3.5 to >5.7 (99.97 to >99.9998%).

Survival and Fate of Poliovirus and Reovirus in Pilot Scale Soil Columns

Reductions of chemical constituents, fecal coliform bacteria and viruses in column effluents. The performance of the columns for non-viral parameters during the entire 32-week virus study period is summarized in Table 12.

COD reductions in the columns averaged 73%. There was a substantial decrease in ammonia nitrogen and the conversion of much of the nitrogen in the STE to the nitrate form. Phosphorous removals in the columns averaged about 95%. Of particular interest are the fecal coliform reductions. Although the FC concentration in the STE averaged 5.1 x 10^5 colonies/100 ml, no FC were detected in any of the column effluents during the entire 32-week study period. The coliform reductions were in excess of 99.9998% or greater than 5.7 orders of magnitude.

Although septic tank effluent containing poliovirus type 1 at an average concentration of 6.05 x 10^6 PFU/100 ml was fed into the columns for a total period of 8 weeks, the 39 column effluent samples (100 ml each) collected throughout this 8-week period of poliovirus feed and for a subsequent 8-week period contained polioviruses only rarely (Table 13). The differences in the extent of virus presence in the effluents of the 4 columns were minor, and all columns produced extensive virus reductions. Based upon the poliovirus concentration in the STE and the average poliovirus concentration in the effluents of the 4 columns, the average virus reduction was in excess of 99.999998 percent or about 7.7 orders of magnitude.

Following the 8-week rest period after poliovirus dosing when STE with no added viruses was fed into the columns, the columns were dosed with STE containing reoviruses. Septic tank effluent containing reovirus Type 3 at an average concentration of 3.7×10^6 PFU/100 ml was fed into the columns for 8 weeks. Column effluent samples for virus assay were collected during this 8-week period as well as during an additional 8-week period of dosing with STE containing no added reoviruses. No reoviruses were detected in any of the 38, 100 ml samples collected from each column during this 16-week period (Table 14). Based on the average reovirus concentration in the STE, the virus reduction in the columns was in excess of 99.9999993 percent or more than 8.1 orders of magnitude.

Occurrence of viruses in fractionated column soil material. Because little or no virus appeared in soil column effluents, an effort was made to recover viruses from fractionated soil materials taken from the columns. No viruses were detected in any of the soil fractions using any of the 3 elution procedures (alkaline glycine, distilled water or nutrient broth). If all of the added viruses had been uniformly retained in the columns with no virus inactivation, the average concentrations of poliovirus and reovirus would have been 4.2×10^4 and 2.6×10^4 PFU per gram of soil, respectively. Because no viruses were detected in any of the 5 to 50 gram soil fraction extracts, extensive virus inactivation must have occurred in the columns.

Short-term Virus Adsorption to Soil Materials Used in Pilot Scale Columns When Suspended in Wastewater.

The results of the studies with pilot scale soil columns showed that 5-foot long columns containing 3 feet of unsaturated soil materials were capable of extensive virus reductions from applied wastewater. It was of interest to determine if the extensive virus reductions were attributable to exceptional virus adsorption abilities of the soil materials used in the columns or to other factors related to the columns and their operating characteristics. Previous experiments with a variety of other soil materials showed that there were considerable differences among them when they were tested for short-term virus adsorption efficiency as suspensions in wastewater (Tables 3 through 6). Therefore, a series of experiments was done to determine the short-term poliovirus and reovirus adsorption efficiencies of the three main types of soil materials used in the pilot scale columns.

The general procedures used in these experiments were similar to those described for the other soil materials previously tested for virus adsorption as suspensions in settled sewage, with the following exceptions: suspension volumes were 150 ml, contact time was 180 minutes, and neither pH nor conductivity was controlled. The results of these experiments (Table 15) indicate that all three soil materials tested were relatively poor virus adsorbents, with little or no increase in virus adsorption beyond that observed in soil-free control samples of settled sewage. These findings suggest that soil materials with inherently poor virus adsorption abilities have the potential to achieve extensive virus reductions from applied wastewater when there is a sufficient depth for unsaturated flow.

TABLE 1. Characteristics of Soil Materials Used in Laboratory Studies.

Soil Series	Taxonomic Series	Location	Horizon	pH	Texture				CEC[a]
					% Sand	% Silt	% Clay	Class	me./100 gm.
Fripp	Mixed, thermic Typic Udpisamment	Atlantic Beach, N.C.	C	4.7	98.8	0	1.2	Sand	9.9
Lakeland	Thermic, coated Typic Quartzpisamment	Goldsboro, N.C.	C	4.3	92.9	3.3	3.7	Sand	14.2
Ponzer	Loamy, mixed, dysic, thermic Terric Medisaprist	Hyde Co., N.C.	-[b]	3.6	NT[e]	NT	NT	Much	37.5
Bentonite	-[c]	Wyoming	-[c]	NT	<1	<1	>99	Clay[d]	204
Kaolinite	-[c]	Georgia	-[c]	NT	<1	<1	>99	Clay[d]	24.7
Cecil	Clayey, kaolinitic, thermic Typic Hapludult	Chatham Co., N.C.	Bt	4.6	46.0	18.9	35.1	Sandy Clay	18.7
Davidson	Clayey, kaolinitic, thermic Rhodic Paleudult	Pittsboro, N.C.	Bt	4.9	11.9	36.2	51.9	Clay	25.1
Norfolk	Fine loamy, siliceous, thermic; Typic Paleudult	Clayton, N.C.	Bt	4.6	62.6	7.4	30.0	Sandy clay loam	22.3

[a] Cation exchange capacity. [b] Collected from a depth of 20-35 cm below surface.

[c] Gifts of D. S. Weed, Soil Science Dept., N.C. State Univ., Raleigh, N.C. [d] Clay-size particles obtained by sedimentation method (Jackson, 1969). [e] NT = not tested.

TABLE 2. Characteristics of Soil Materials Used in Pilot Scale Columns

Designation	Taxonomic Series	Horizon Depth (cm)	Texture (%):			Class	Organic Matter
			Sand	Silt	Clay		
Loam Topsoil	Pactolus series, Aquic Quartipsamment, Siliceous, thermic, coated	Ap, 0-20	83	13	4	Sandy loam	1.9%
Organic topsoil	Cape Fear series Typic Umbraquult Clayey, mixed, thermic (kaolinitic)	Ap 0-20	52	20	28	Sandy clay loam	27%
Sand*	-	-	Commercial, washed				
Gravel	-	-	Smooth stone, 1.3-1.9 cm diameter				

*Grading: very course = 20.5%, coarse = 63.5%, medium = 14%, fine = 2%

TABLE 3. Kinetics of Virus Adsorption to Soil Materials in Settled Sewage[a]

	Percentage of total initial poliovirus adsorbed								
Time (min.)	Control	Lakeland	Fripp	Ponzer	Bentonite	Kaolinite	Cecil (B)	Davidson (B)	Norfolk (B)
15	NT[b]	21	0	28	86	98	>99	>99	>99
30	NT	27	6	28	80	97	>99	>99	>99
90	6	32	5	34	60	94	>99	>99	>99
	Percentage of total initial reovirus adsorbed								
15	NT	25	36	80	>99	88	>99	>99	>99
30	NT	<1	28	82	>99	88	>99	>99	>99
90	22	<1	38	82	>99	91	>99	>99	>99

[a] Suspensions were 5% (w/v) except Bentonite and Kaolinite which were 1%. Sample pH levels: Controls = 7.6, Lakeland = 7.4, Fripp = 7.2, Ponzer = 4.6, Bentonite = 6.3, Kaolinite = 7.5, Cecil (B) = 6.8, Davidson (B) = 7.4, and Norfolk (B) = 6.6. Sample TDS levels ranged from 200-410 mg/l NaCl.

[b] NT = not tested.

TABLE 4. Effect of pH on Virus Adsorption to Soil Materials in Settled Sewage[a]

	Percentage of total initial poliovirus adsorbed to:								
pH	Control	Lakeland	Fripp	Ponzer	Bentonite	Kaolinite	Cecil (B)	Davidson (B)	Norfolk (B)
7.5	10	59	8	NT[b]	91	98	>99	>99	>99
6.5	6	76	<1	38	70	99	>99	>99	>99
5.5	7	90	9	34	75	99	>99	>99	>99
4.5	16	97	31	19	91	>99	>99	>99	>99
3.5	47	NT	NT	97	NT	NT	NT	NT	NT
	Percentage of total initial reovirus adsorbed								
7.5	22	4	20	NT	99	92	>99	>99	>99
6.5	7	54	<1	55	>99	NT	>99	>99	>99
5.5	9	85	51	92	>99	NT	>99	>99	>99
4.5	58	99	74	93	>99	>99	>99	>99	>99
3.5	99	NT	NT	98	NT	NT	NT	NT	NT

[a] Suspensions were 5% (w/v) except Bentonite and Kaolinite which were 1%; Contact time was 60 minutes; TDS concentration was 350 mg/l NaCl.

[b] NT = not tested.

TABLE 5. Effect of Total Dissolved Solids Concentration on Virus Adsorption to Soil Materials in Settled Sewage[a]

TDS (mg/l)[b]	Control	Fripp	Lakeland	Ponzer	Bentonite	Kaolinite	Cecil (B)	Davidson (B)	Norfolk (B)
	Percentage of total initial poliovirus adsorbed to:								
350	5	1	65	35	48	98	>99	>99	>99
1,000	10	16	84	58	85	>99	>99	>99	>99
3,500	5	40	94	63	99	>99	>99	>99	>99
	Percentage of total initial reovirus adsorbed								
350	18	13	24	80	99	NT[c]	>99	>99	>99
1,000	6	31	30	89	>99	NT	>99	>99	>99
3,500	17	13	19	90	>99	NT	>99	>99	>99

[a] Suspensions were 5% (w/v) except for Bentonite and Kaolinite which were 1%. Samples were pH 6.5, except Ponzer which was pH 5.5; contact time was 60 min.

[b] as NaCl.

[c] NT = not tested.

TABLE 6. Effect of Added $MgCl_2$ on Virus Adsorption to Fripp Sand[a]

Added $MgCl_2$ (M)	pH	Percentage of total initial virus adsorbed			
		Poliovirus		Reovirus	
		Control	Fripp	Control	Fripp
0.01	7.5	47	98	11	17
	4.5	62	99	94	>99
0.001	7.5	47	96	12	18
	4.5	42	96	92	98
None	7.5	24	33	23	25
	4.5	32	54	43	56

[a] Fripp samples were 5% (w/v) sand; controls contained only settled sewage; all samples were brought to a TDS concentration of 2,000 mg/l as NaCl by adding NaCl; temperature = 20 ±1°C; contact time was 60 min.

TABLE 7. Elution of Viruses Adsorbed to Soil Materials

Elution Medium	Lake-land	Pon-zer	Ben-tonite	Kao-linite	Cecil (B)	David-son (B)	Norfolk (B)
	Percentage of total adsorbed poliovirus eluted						
Distilled water	18	49	8	3	15	6	<1
Buffered, 3.5% NaCl	<1	<1	7	<1	<1	<1	<1
Nutrient broth	33	40	25	<1	10	<1	<1
	Percentage of total adsorbed reovirus eluted						
Distilled water	25	3	<1	5	<1	<1	<1
Buffered, 3.5% NaCl	37	<1	<1	2	<1	<1	4
Nutrient broth	8	3	2	4	21	6	17

TABLE 8. Virus Survival in Non-Sterile and Sterile Suspensions of Soil Material in Settled Sewage[a]

Sample	Time for 99% virus inactivation (days)			
	Poliovirus		Reovirus	
	Non-Sterile	Sterile	Non-Sterile	Sterile
Control	34	86	9	38
Fripp	25	69	20	113
Lakeland	47	154	23	61
Ponzer	9	18	17	146
Bentonite	37	83	23	8
Kaolinite	49	95	30	257
Cecil (B)	80	167	110	233
Davidson (B)	46	73	35	111
Norfolk (B)	47	109	51	139
Mean	42	95	35	123

[a] Samples contained 5% (w/v) soil material, except Bentonite and Kaolinite, which contained 1%. Controls contained only settled sewage with virus. Temperature = 20 ±1°C; pH = 6.5; except Ponzer = 5.5.

TABLE 9. Poliovirus and Fecal Coliform Reduction in Miniature Soil Columns[a]

Time (days)	$\log_{10}$ poliovirus reduction				$\log_{10}$ fecal coliform reduction			
	Fripp	Lakeland	Ponzer	Norfolk (B)	Fripp	Lakeland	Ponzer	Norfolk (B)
0	4.3	4.3	1.8	1.9	>5.6	>5.6	>5.6	>5.6
3	1.4	4.2	2.3	4.2	5.0	4.8	5.4	>5.4
6	1.4	3.2	2.1	4.6	4.9	5.0	5.4	>5.4
9	1.2	>4.5	1.7	>4.5	4.2	4.1	4.7	>5.2
13	1.1	3.1	2.0	>4.5	4.8	4.3	5.0	>5.6
16	1.3	3.0	1.9	>4.4	5.5	4.8	5.6	>5.8
20	3.0	>4.4	4.4	>4.4	4.3	4.3	5.0	>5.0
23	1.7	>4.1	1.5	>4.1	5.3	5.5	4.6	>5.8
26	1.7	2.5	1.6	>4.9	4.8	5.1	5.4	>5.6
30	1.8	2.3	1.3	>4.8	>4.8	>4.8	6.0	>6.3
34	1.0	2.6	1.6	>5.0	NT[b]	NT	NT	NT
Mean	1.9	3.5	2.0	>4.3	4.9	4.8	5.3	>5.6
Mean Percent Reduction	98.7	99.97	99	>99.995	99.9987	99.9984	99.9995	>99.9997

[a] Two 2.54-cm (1-in.) applications of settled sewage per week. Mean $\log_{10}$ poliovirus and fecal coliform concentrations in the applied sewage were 4.5 and 5.2, respectively.

[b] NT = not tested.

TABLE 10. Effect of Simulated Rainfall on Poliovirus Retention by Miniature Soil Columns[a]

Time (days)	Log_{10} poliovirus conc. in applied wastewater	Log_{10} poliovirus reduction from applied wastewater				Log_{10} poliovirus concentration in rainwater column effluent			
		Fripp	Lakeland	Ponzer	Norfolk (B)	Fripp	Lakeland	Ponzer	Norfolk (B)
38	4.3	1.6	2.4	1.0	>4.3	3.6	3.3	3.2	<0
41	4.8	1.3	1.9	1.6	>4.8	3.7	3.7	3.4	<0
45	4.5	1.6	1.9	1.5	>4.5	3.2	3.5	3.4	<0
48	4.5	1.9	1.8	1.7	>4.5	3.1	3.3	3.1	<0
Mean Log_{10}	4.5	1.6	2.0	1.4	>4.5	3.4	3.4	3.3	<0

[a] Twice-weekly applications of 2.54 cm (1 in.) of settled sewage followed after 3 hr by an equal volume of sterile distilled water. Mean $\log_{10}$ poliovirus concentration in the applied sewage was 4.5.

TABLE 11. Effect of Increased Hydraulic Loading on Poliovirus and Fecal Coliform Retention in Miniature Soil Columns[a]

Time (days)	Log$_{10}$ poliovirus reduction				Log$_{10}$ coliform reduction			
	Fripp	Lakeland	Ponzer	Norfolk (B)	Fripp	Lakeland	Ponzer	Norfolk (B)
52	2.2	2.0	1.6	>4.6	5.9	5.5	5.5	>6.3
53	1.5	1.4	1.4	>4.7	4.0	<3.7	<3.7	>5.2
54	1.4	1.9	1.9	>4.7	<3.4	3.5	4.1	>5.8
55	1.2	1.5	1.7	>4.7	3.6	3.7	4.7	>6.4
56	1.2	1.3	1.8	>4.6	2.6	2.1	3.8	>5.3
57	1.3	1.3	2.1	>4.7	2.6	2.4	3.9	>4.9
58	1.4	1.4	1.9	>4.7	3.7	3.8	4.7	>6.0
Mean	1.5	1.5	1.8	>4.7	∿3.7	∿3.5	∿4.3	>5.7

[a] Daily 2.54-cm (1-in.) applications of settled sewage; mean log$_{10}$ poliovirus and fecal coliform concentrations in the applied sewage were 4.7 and 5.3, respectively.

TABLE 12. Performance of Pilot Scale Columns for Non-viral Parameters[a]

Parameter	Applied STE	Effluent concentrations for column:				Mean effluent concentrations, all columns
Chemical oxygen demand (mg/l)	248	66	68	84	48	66
Ammonia nitrogen (mg/l as N)	44	0.5	0.5	0.2	0.1	0.3
Nitrate nitrogen (mg/l as N)	N.D.*	23	21	20	25	22.2
Acid hydrolyzable phosphate (mg/l as P)	16.6	0.1	1.5	0.2	1.9	0.9
Fecal coliforms (colonies/100 ml)	5.1×10^5	N.D.[b]	N.D.	N.D.	N.D.	N.D.

[a] Results are mean values based upon 6-7 samples for chemical parameters and weekly samples for fecal coliforms collected over the 32-week period of virus studies.

[b] N.D. = not detected

TABLE 13. Reduction of Poliovirus in Septic Tank Effluent in Pilot Scale Soil Columns[a]

Column Number	Number Positive / Number of Samples	Column Effluent Virus Concentration (MPN/100 ml)[b]	Virus Reduction Percent	$\log_{10}$
1	4/39	0.11	99.999998	7.7
2	4/39	0.11	99.999998	7.7
3	2/39	0.05	99.9999991	8.0
4	7/39	0.20	99.999997	7.5

[a] Column effluent sample volumes were 100 ml.
Poliovirus concentration in the applied STE averaged 6.05 x 10^6 PFU/100 ml during the 8-week poliovirus dosing period.

[b] Most probable number of viruses per 100 ml of column effluent.

TABLE 14. Reduction of Reovirus in Septic Tank Effluent in Pilot Scale Soil Columns[a]

Column Number	Number Positive / Number of Samples	Column Effluent Virus Concentration (MPN/100 ml)[b]	Virus Reduction Percent	$\log_{10}$
1	0/38	<0.03	>99.9999993	>8.1
2	0/38	<0.03	>99.9999993	>8.1
3	0/38	<0.03	>99.9999993	>8.1
4	0/38	<0.03	>99.9999993	>8.1

[a] Column effluent sample volumes were 100 ml
Reovirus concentration in the applied STE averaged 3.7 x 10^6 PFU/100 ml during the 8-week reovirus dosing period.

[b] Most probable number of viruses per 100 ml of column effluent.

TABLE 15. Virus Adsorption to Soil Materials used in Pilot Scale Soil Columns when Suspended in Settled Sewage*

Soil Material	Percent Adsorption of:	
	Poliovirus	Reovirus
Control (no soil)	16	16
Loamy sand	15	0
Organic	11	10
Sand	21	7

*Suspensions were 5% (wt/vol); Sample pH levels: control = 7.85, loamy sand = 7.2, organic = 6.65, sand = 7.9; sample TDS levels ranged from 118-275 mg of NaCl per liter; temp. = 26°C; contact time = 180 min. Results of triplicate trials of each condition.

LITERATURE CITED

American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1976. Detection of enteric viruses in water and wastewater, section 913. In Standard Methods for the examination of water and wastewater, 14th ed. American Public Health Association, Washington, D.C.

Baldwin, L.B., J.M. Davidson, and J.F. Gerber (ed.). 1976. Virus aspects of applying municipal waste to land. University of Florida and U.S. Environmental Protection Agency, Gainesville, Fla.

Bitton, G. 1975. Adsorption of viruses onto surfaces in soil and water. Water Res. 9:473-484.

Bitton, G., O. Pancorbo, and G.E. Gifford, 1976. Factors affecting the adsorption of poliovirus to magnetite in water and wastewater. Water Res. 10:978-980.

Boardman, G.C., and O.J. Sproul. 1977. Adsorption as a protective mechanism for poliovirus. Paper 14A-a. In Proceedings of the Annual Conference of the American Water Works Association, Anaheim, California. American Water Works Association, Denver, Colo.

Carlson, G.F., Jr., F.E. Woodward, D.F. Wentworth, and O.J. Sproul. 1968. Virus inactivation on clay particles in natural waters. J. Water Pollut. Contr. Fed. 47:93-103.

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

Gerba, C.P., C. Wallis, and J.L. Melnick. 1975. Fate of wastewater bacteria and viruses in soil. J. Irrig. Drain. Div., Proc. Am. Soc. Div. Eng. 101:157-174.

Gerba, C.P., and G.E. Schaiberger. 1975. The effects of particulates on virus survival in seawater. J. Water Pollut. Contr. Fed. 47:93-103.

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

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

Hunt, D.A., and J.A. Springer. 1975. Comparison of two rapid test procedures with the standard EC test for the recovery of fecal coliform bacteria from shellfish growing water, p. 41-48. In D.S. Wilt (ed.), Proceedings of the Tenth National Shellfish Sanitation Workshop. Food and Drug Administration, Washington, D.C.

Jackson, M.L. 1969. Soil chemical analysis-advanced course, 2nd ed. Published by the author, Dept. of Soil Science, Univ. of Wisconsin, Madison.

Lance, K.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.

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

Lefler, E., and Y. Kott. 1974. Virus retention and survival in sand, p. 84-91. In J.F. Maline, Jr. and B.P. Sagik (ed.), Virus survival in water and wastewater systems, Center for Research in Water Resources, University of Texas, Austin.

Lo, S.H. and O.J. Sproul. 1977. Poliovirus adsorption from water onto silicate minerals. Water Res. 11:653-658.

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

Moore, B.E., B.P. Sagik, and C.A. Sorber. 1978. Land application of sludges: minimizing the impact of viruses on water resources, p. 154-167. In Proceedings of the Conference on Risk Assessment and Health Effects of Land Application of Municipal Wastewater and Sludges, 12-14 December 1977. Center for Applied Research and Technology, University of Texas, San Antonio.

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.

Sobsey, M.D., R.J. Carrick, and H.R. Jensen. 1978. Improved methods for virus detection in oysters. Appl. Environ. Microbiol. 36:121-128.

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

Stewart, Larry W. 1976. Evaluation of simulated alternative treatments of septic tank effluent. Master's Technical Report, N.C. State University. 42 pages.

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