Virus Movement In Groundwater Systems

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WATER RESOURCES RESEARCH CENTER

Publication No. 4

In Cooperation With

ENGINEERING EXPERIMENT STATION

Research Report No. 18

UNIVERSITY OF ARKANSAS

Fayetteville

1969

PROJECT COMPLETION REPORT

PROJECT NO: A-005-ARK AGREEMENT NO.: 14-01-0001-1072

Starting Date: September 1965 Completion Date: June 1968

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ABSTRACT

VIRUS MOVEMENT IN GROUNDWATER SYSTEMS

The purpose of this study is to investigate the extent to which soil acts as an agent in the transmission of waterborne viruses. Since many waterborne outbreaks of viral diseases have involved small well-water supplies contaminated by effluents from subsurface wastewater disposal systems, there is a great need for such information.

Results of this study show that virus adsorption by soils is greatly affected by the pH, ionic strength, and soil-water ratio of the soil-water system and various soil properties. Also, it is shown that one cannot predict the relative virus adsorbing ability of a particular soil based on the various tests normally used to characterize a soil. It is shown that virus movement through a continuous stratum of common soil under gravity flow conditions and with intermittent dosing should present no health hazard if usual public health practices relating to locating water supply wells are followed. Test results also indicate no greater or lesser movement of virus through soils with a highly polluted water than with a non-polluted water.

Drewry, William A.
VIRUS MOVEMENT IN GROUNDWATER SYSTEMS
A-005-ARK
Research Project Technical Completion Report, February, 1969
KEYWORDS--viruses*/bacteriophage/water pollution*/septic tanks/
soil disposal fields*/soil contamination

ACKNOWLEDGMENT

The work upon which this report is based was supported with funds provided by the Office of Water Resources Research, U.S. Department of the Interior, as authorized under the Water Resources Research Act of 1964, P.L. 88-379 as amended by P.L. 89-404.

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CHAPTER I

INTRODUCTION

Enteric viruses have been found in wastewater and wastewater treatment plant effluents by numerous investigators and several outbreaks of viral diseases have been attributed to waterborne virus. Extensive reviews of these investigations have been presented by Mosley (1) and Drewry (2). Infectious hepatitis virus and polio virus appear to be the causative agents in most waterborne outbreaks of viral diseases to date. Since more than 70 new enteric viruses have been discovered over the past 20 years, it may well be possible that even more occurences of disease will be traced to waterborne viruses, especially as new epidemiological and diagnostic techniques are developed in the field of medicine. Since most of the waterborne outbreaks of viral diseases have involved small driven or drilled well water supplies contaminated by cesspool or septic tank wastewater disposal systems, there is a great need to know to what extent wastewater-contaminated soil acts as an agent in the transmission of viruses.

Relatively few studies on virus removal by water and wastewater treatment processes have been undertaken and even fewer have included sand or soil as a virus-retention medium. Many uncertainties exist regarding the distance of travel of virus particles as discharged into the soil with waterborne human wastes, and research has hardly begun on

this subject. The rules among public health agencies on the relative locations of wells and cesspools or septic tanks are based on studies, often rather ill defined, on the removal of Coliform bacteria in soils. The only recent well defined studies on virus movement through soils are those of Drewry (2), Drewry and Eliassen (3) and Tanimoto, et al (4).

The studies by Drewry (2) and Drewry and Eliassen (3) involved the use of four selected California soils and saturated flow conditions. Viruses used in these studies included T1 and T2 bacteriophage. Among other things, conclusions reached from these studies included: (1) that the ability of a soil to adsorb virus particles cannot be judged on the basis of the various tests which are normally used to characterize a soil (clay content, silt content, ion-exchange capacity, etc.) and (2) virus movement through continuous strata of common soils under saturated flow conditions should present no great hazard to water supply wells. The study by Tanimoto, et al (4) involved the use of three Hawaiian Island soils and the T4 bacteriophage. Column experiments performed under unsaturated flow conditions showed that two the the soils were very effective for virus removal. The third soil, which was a gravel-sized cindery material, proved to be ineffective for virus retention.

The original specific aims of this study were:

a. To tag animal viruses with radioisotopes and to establish a quantitative relationship between the specific activity of a culture and the number of virus particles.

b. To perform static and dynamic soil-virus studies to

determine the effects of soil and water properties on virus retention or movement.

CHAPTER II

EXPERIMENTAL PROCEDURES AND MATERIALS

Soil

Soils used in this study were obtained from various sites at the University of Arkansas Agricultural Experiment Station, Fayetteville, Arkansas. The four soils selected are of types being used for subsurface disposal of effluents from cesspools and septic tank systems. Selection of the various soils was such that there significant differences in soil properties, i.e., clay content, cation exchange capacity, grain size distribution, etc. Laboratory analyses of the soils were performed in the Sanitary Engineering Laboratory at the University of Arkansas. Complete analyses of the various soils has been reported elsewhere by Reece (5) and are summarized in Table 1A.

Water

Water used in this study included both water prepared in the laboratory and septic tank effluents collected from existing, operating septic tank installations. Four standard waters were used and are referred to as Water Nos. 1, 2, 3, and 4. Water No. 1 was prepared in the laboratory using distilled water to which Na_2HPO_4 was added to make a 0.005 molar solution. Water No. 2 was prepared using distilled water to which NaCl and $NaHCO_3$ were added to concentrations of 200 mg/l and 340 mg/l respectively. Waters No. 3 and No. 4 were septic tank effluents collected from two different septic tank installations. The properties of these waters are presented in

TABLE 1A

Soil Properties

Soil No.	Grain Size Analysis, Percent					Sp.Gr.	pH	Organic Carbon, Percent	Surface Area, M^2/g	Cation Exchange Capacity, me/100g
	Larger than 2 mm	2 mm to 0.074 mm	Silt, .074 to .005 mm	Clay, .005 to .001 mm	Colloids, less than .001 mm					
1	2.9	17.7	60.0	11.0	8.4	2.68	5.5	0.29	27.8	4.5
2	0.1	1.8	78.7	14.5	4.9	2.61	6.2	1.07	17.9	5.3
3	3.3	8.4	58.3	13.0	17.0	2.68	5.7	0.40	32.8	6.9
4	2.5	19.9	60.6	10.0	7.0	2.82	4.7	0.44	55.3	8.2

Tables 1 and 2. Any other water used was distilled water to which varying concentrations of various chemicals were added. The chemical concentration is given with the other test results whenever such a water was used.

Virus

Influenza virus, strain PR8, was chosen for initial work on radioisotope tagging. A search of the literature revealed that little work had been performed in tagging PR8 with I-131 but an examination of the available data on the properties of PR8 indicated that it should be possible. Also, selection of PR8 for initial work was based, to a great extent, on the relative ease with which PR8 can be cultivated in the laboratory and on the relatively large amount of data available on the properties of the virus.

Initial experiments showed that fairly large amounts of the virus could be grown at low cost and in a minimum of time. Also, it was found that tagging PR8 with I-131 could be carried out satisfactorily. However, PR8 proved not to be as good for this type of work as was originally thought. As the research requires an assay system, it was believed that enumeration of the virus particles by means of plaque formation on chick embryo monolayer would serve the purpose. However, it became evident that Influenza PR8 cannot be well adapted to this type of assay. The virus is a poor plaque former on chick embryo monolayer and as any other type of biological assay is relatively insensitive (such as hemagglutination), the use of PR8 was discontinued. An electron microscope may serve the enumeration purpose for some future study but one was not

TABLE 1

CHARACTERISTICS OF WATER NO.3

Parameter	Units	Value
pH		6.8
Alkanlinity	mg/l $CaCO_3$	620
Conductivity	micromhos/cm	750
Solids, Total	mg/l	5370
Solids, Total, Volatile	mg/l	3580
Suspended Solids, Total	mg/l	4840
Suspended Solids, Volatile	mg/l	3510
B.O.D.	mg/l	3200
C.O.D.	mg/l	8000

TABLE 2

CHARACTERISTICS OF WATER NO.4

Parameter	Units	Value
pH		7.4
Alkalinity	mg/l as $CaCO_3$	380
Conductivity	micromhos/cm	230
Solids, Total	mg/l	2580
Solids, Total, Volatile	mg/l	980
Suspended Solids, Total	mg/l	1355
Suspended Solids, Volatile	mg/l	220
B.O.D.	mg/l	60
C.O.D.	mg/l	295

available for this project. Some work was also done with New Castle Disease Virus (NDV) but the results were less than satisfactory. Thus, time and budget considerations dictated that a bacteriophage be used as a model for animal viruses.

Use of bacteriophage of the T-coliphage series was ruled out even though most sanitary engineering virus investigations to date have involved their use. Bacteriophage of the T-coliphage series all have a tail structure which animal viruses apparently do not. Also, reaction between the tail structure and inorganic substances appear to cause a splitting of the protein and DNA fractions of these viruses (3,6) which seriously affect test result interpertation. These considerations led to the selection of f2 bacteriophage as the test virus.

The f2 bacteriophage used in this study was the Zinder strain, specific to Escherichia coli (K12 Hfr D). Bacteriophage f2 is a small virus with an equivalent spherical diameter of about 22 millimicrons and has no apparent tail structure as do the T-coliphage. Also, the f2 virus contains RNA as do most of the animal viruses rather than DNA as do most of the bacteriophage. Growth procedures for stock cultures of f2 and enumeration techniques used in this study were essentially those described by Loeb and Zinder (7) and have been presented in detail by Reece (5). Virus from these stock cultures were used in all static or batch experiments of this study. Dynamic or column experiments required the use of radioisotope-tagged virus to provide a means of measuring the virus distribution on the column itself at any given time. P-32 was used as the tracer to give a beta energy, 1.71 Mev, large enough so that virus concentrations at various depths within a soil column could be measured directly

by means of a radiation detector located externally adjacent to the column.

The medium for growth of the tagged bacteriophage contained the following constituents (in grams per liter): Neopeptone, 10; dextrose, 1; NaCl, 8.5; $CaCl_2$, 0.22; and yeast extract, 0.10. The procedure used for growth and tagging was as follows:

1. Time = 0.00 hours. Add 2-10 mc P-32 to 1 liter of growth medium. Inoculate with 10 ml of an 18 hour culture of E. coli, K12. Shake in water bath at 37°C.
2. Time = 3.50 hours. Add f2 bacteriophage using a 5 to 1 ratio of bacteriophage to bacteria. The growth medium should contain about 2.8×10^8 bacteria per ml at this time. Continue to shake in water bath.
3. Time = 4.50 hours. Add EDTA to make growth solution 0.2 M. Continue to shake.
4. Time = 4.75 hours. Add 25 mg/l lysozyme to growth solution. Continue to shake.
5. Time = 5.00 hours. Remove flask from shaker and place in refrigerator at approximately 4°C.
6. Time = approximately 11 hours. The bacterial cells should be completely lysed by this time. Remove bacterial debris by centrifugation at 6000 rpm for 10 minutes (Swinging bucket clinical type centrifuge). Make supernatant 2.0M with ammonium sulfate and refrigerate for 6 to 12 hours.
7. Remove precipitate by centrifugation at 15,000 rpm for 10 minutes (International Model HT). Resuspend the precipitate in 0.02M phosphate buffer, pH 7.5, using 40 ml buffer per liter of growth medium.

8. Layer up to 5 ml of the suspension on a 15 cm deep by 2.5 cm diameter column of Biogel P-200, prepared in 0.02M phosphate buffer, pH 7.5. Elute with 0.02M phosphate buffer, pH 7.3, and collect fractions. The virus passes through the column with the void volume and so is collected very quickly.

Determination of the radioactivity content of the final tagged virus stocks was performed by evaporating suitable sized aliquots of the virus solution on aluminum planchets. The counting system consisted of an end-window flow counter, Baird-Atomic Model 821C, in a low background shield, Baird-Atomic Model 800D, and a Baird-Atomic Model 530 Spectrometer. Virus and radioactivity concentrations of the tagged virus solutions used for the column experiments are presented in Chapter III along with the other column data.

Static Experiments

Static tests consisted of mixing a small sample of soil with water containing the viruses, shaking for a given length of time, then centrifuging to separate the water from the soil so that a virus count could be made. A detailed description of this technique has been presented by Reece (5). Unless otherwise stated in the test results section, the soil sample weight was 7 grams, the water volume was 7 ml, and the mixing time was 24 hours. All such tests were performed in duplicate using sterile media and aseptic techniques insofar as possible.

Dynamic Experiments

Column studies, using intermittant flow conditions (i.e., dosing at intervals) were used to simulate virus migration in groundwater aquifers. Column influent and effluent radioactivity was monitored using the technique and gas-flow proportional counting system described earlier.

Soil columns used in this study were prepared by carefully packing dry soil to the desired depth in a 28 mm diameter chromatographic tube (Sargent No. S-18825-35, Size H, 600 mm length) using the fritted glass disc supplied with the column for the soil support. The columns were dosed with water of the type to be used in the experiment for several days prior to adding virus in order to simulate field conditions as near as possible. Detailed information on each column is provided in Table 16, Chapter III. All columns used were of the downflow type with gravity flow. This condition was selected to prevent displacement of the soil under flow conditions.

A radioactivity detection system was designed and constructed to measure the P-32 radioactivity (contained within the virus particles) retained on the soil columns. This column scanning device is shown in Figure 1. A Baird-Atomic Model 815CL scintillation probe is contained in a lead and stainless steel shield with a 1.00 cm light-tight collimated slit. This shielded detector is mounted on a motor driven platform such that the detector can be positioned against the column and moved up or down the column to any desired position. The signal generated in the detector is transmitted to the Model 530 Spectrometer as described earlier. Radioactivity measurements

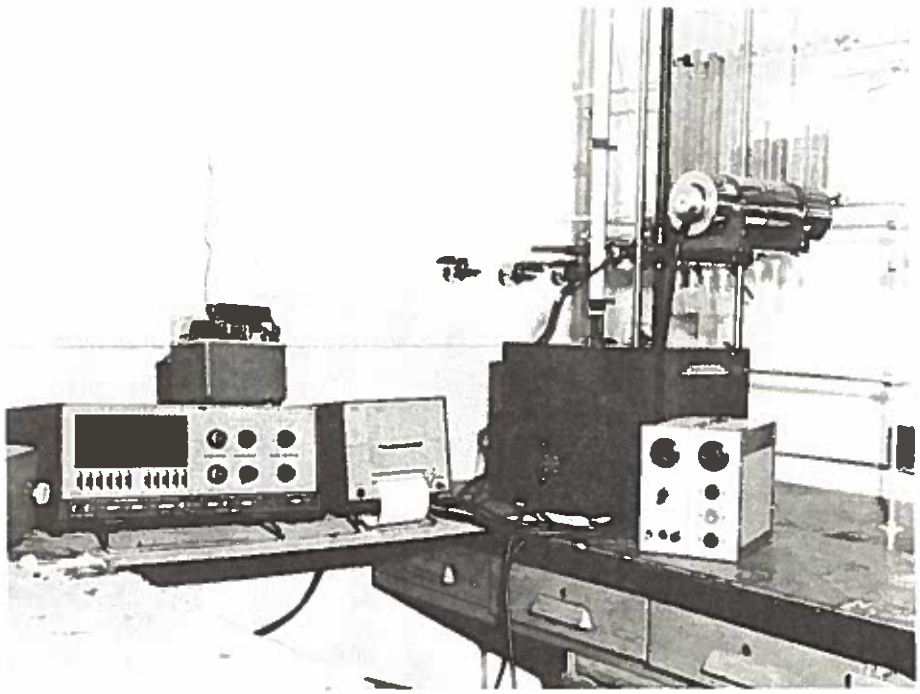

FIGURE 1. Column Scanning Device.

are recorded on a digital printout device, Baird-Atomic Model 620-2 Printer, connected to the spectrometer. All column radioactivity measurements were made at 1 cm intervals from top to bottom of the soil columns. All radioactivity measurements made relating to a given tagged virus culture and column experiment were corrected for radioactive decay to a given, arbitrarily selected, time and date. The date selected in all cases corresponded to the day the tagged culture was grown. This technique is similar to the one used by Drewry (2) in another study. As in the static experiments, all tests were performed using sterile media and aseptic techniques insofar as possible.

CHAPTER III

EXPERIMENTAL RESULTS

Static Experiments

Rate of virus sorption by soils and virus concentration effect studies for all four soils have been presented elsewhere by Reece (5) and will not be shown here. However, the significance of these studies will be covered in the discussion of results, Chapter IV.

Table 3 shows the results of an experiment to determine the effect of variable virus concentration on sorption by all four soils using a septic tank effluent for the liquid phase. Except for Soil No. 2 the removal was well over 99 percent at all virus concentrations.

Tables 4 through 7 show the results of static experiments designed to show the effects of varying ionic strength of the liquid phase on virus sorption by all four soils. With the exception of Soil No. 2 the removal in all cases was well over 99 percent. The results using Soil Nos. 1 and 3 indicate a slight decrease in percent removal with increasing ionic strength. Soil No. 4 results indicate a slight increase and then a slight decrease in percent removal as the ionic strength increases. Soil No. 2 results indicate just the opposite, i.e., a decrease and then an increase in percent removal as ionic strength increases.

Tables 8 through 15 show the effects of varying the soil-water ratio on virus sorption by all four soils using two different waters. The results using Water No. 1 show the percent removal increasing with

TABLE 3

VIRUS ADSORPTION, SOILS 1 THROUGH 4, WATER NO.4

Soil No.	Virus Concentration, PFU/ml Initial	Final	pH*	Percent Virus Adsorbed
1	6.50×10^{8}	1.20×10^{3}	6.7	99.999+
1	6.50×10^{6}	1.25×10^{1}	6.7	99.999+
1	6.50×10^{4}	1.00×10^{0}	6.7	99.998
2	6.50×10^{8}	3.65×10^{7}	6.7	94.385
2	6.50×10^{6}	6.18×10^{5}	6.7	90.493
2	6.50×10^{4}	9.98×10^{3}	6.7	84.647
3	6.50×10^{8}	4.80×10^{3}	6.1	99.999+
3	6.50×10^{6}	4.05×10^{1}	6.1	99.999+
3	6.50×10^{4}	1.10×10^{0}	6.1	99.998
4	6.50×10^{8}	2.13×10^{2}	5.6	99.999+
4	6.50×10^{6}	4.00×10^{1}	5.6	99.999+
4	6.50×10^{4}	0.50×10^{0}	5.6	99.999+

* Of the soil-water-virus mixture.

TABLE 4

VIRUS ADSORPTION AS AFFECTED BY IONIC STRENGTH
SOIL NO.1

Water			pH*	Virus Concentration, PFU/ml		Percent Removal
$NaHCO_3$, mg/l	NaCl, mg/l	Ionic Strength		Initial	Final	
0	0	0	6.00	6.36×10^7	3.60×10^1	99.999+
336	100	0.0057	6.40		8.00×10^1	99.999+
672	200	0.0114	6.50		4.64×10^2	99.999+
1008	300	0.0171	6.80		3.00×10^3	99.995
1344	400	0.0228	6.80		6.15×10^3	99.990
1680	500	0.0285	6.90	6.36×10^7	5.40×10^3	99.992

* Of the soil-water-virus mixture.

TABLE 5

VIRUS ADSORPTION AS AFFECTED BY IONIC STRENGTH
SOIL NO. 2

Water			pH*	Virus Concentration, PFU/ml		Percent Removal
$NaHCO_3$, mg/l	NaCl, mg/l	Ionic Strength		Initial	Final	
0	0	0	7.20	4.50×10^9	3.32×10^8	92.623
336	100	0.0057	7.10		1.37×10^9	69.560
672	200	0.0114	7.15		1.78×10^9	60.450
1008	300	0.0171	7.20		5.97×10^8	86.734
1344	400	0.0228	7.30		1.81×10^8	95.978
1680	500	0.0285	7.38	4.50×10^9	1.33×10^8	97.045

* Of the soil-water-virus mixture.

TABLE 6

VIRUS ADSORPTION AS AFFECTED BY IONIC STRENGTH
SOIL NO.3

Water			pH*	Virus Concentration, PFU/ml		Percent Removal
$NaHCO_3$, mg/l	NaCl, mg/l	Ionic Strength		Initial	Final	
0	0	0	6.25	4.20×10^7	8.40×10^1	99.999+
336	100	0.0057	6.65		2.78×10^2	99.999+
672	200	0.0114	6.90		4.59×10^2	99.999
1008	300	0.0171	7.10		7.00×10^2	99.998
1344	400	0.0228	7.30		1.55×10^3	99.996
1680	500	0.0285	7.30	4.20×10^7	1.50×10^3	99.996

* Of the soil-water-virus mixture.

TABLE 7

VIRUS ADSORPTION AS AFFECTED BY IONIC STRENGTH
SOIL NO.4

Water			pH*	Virus Concentration, PFU/ml		Percent Removal
$NaHCO_3$, mg/l	NaCl, mg/l	Ionic Strength		Initial	Final	
0	0	0	4.55	1.60×10^7	1.20×10^1	99.999+
336	100	0.0057	4.70		1.50×10^0	99.999+
672	200	0.0114	4.90		5.00×10^0	99.999+
1008	300	0.0171	5.30		5.50×10^0	99.999+
1344	400	0.0228	5.50		1.00×10^1	99.999+
1680	500	0.0285	5.65	1.60×10^7	1.05×10^1	99.999+

* Of the soil-water-virus mixture.

TABLE 8

VIRUS ADSORPTION AS AFFECTED BY SOIL-WATER RATIO
SOIL 1, WATER 1

Water, ml	Soil, gm		Virus Concentration, PFU/ml Initial	Final	Percent Removal
7	7.0	6.40	4.50×10^8	3.75×10^8	16.67
↑	3.0	6.90	↑	2.67×10^8	40.67
↑	1.0	7.23	↑	2.40×10^8	46.67
↑	0.50	7.40	↑	2.16×10^8	52.00
↑	0.10	7.63	↑	2.00×10^8	55.56
↑	0.050	7.70	↑	1.42×10^8	68.44
↑	0.010	7.83	↑	1.07×10^8	76.22
↑	0.0050	7.85	↑	8.30×10^7	81.56
↑	0.0010	7.88	↑	6.75×10^7	85.00
7	0.00010	7.95	4.50×10^8	1.07×10^8	76.22
7	0.000010	7.95	4.50×10^8	1.18×10^8	73.78

TABLE 9

VIRUS ADSORPTION AS AFFECTED BY SOIL-WATER RATIO
SOIL 2, WATER 1

Water, ml	Soil, gm	pH	Virus Concentration, PFU/ml		Percent Removal
			Initial	Final	
7	7.0	7.35	3.00×10^8	2.24×10^8	25.33
↑	3.0	7.40	↑	1.86×10^8	38.00
↑	1.0	7.45	↑	1.67×10^8	44.33
↑	0.50	7.38	↑	1.38×10^8	54.00
↑	0.10	7.50	↑	1.23×10^8	59.00
↑	0.050	7.53	↑	1.08×10^8	64.00
↑	0.010	7.50	↑	8.80×10^7	70.67
↑	0.0050	7.53	↑	5.30×10^7	82.33
↑	0.0010	7.50	↑	2.90×10^7	90.33
↑	0.00010	7.50	↑	7.51×10^6	97.50
7	0.000010	7.50	3.00×10^8	1.63×10^6	99.46

TABLE 10

VIRUS ADSORPTION AS AFFECTED BY SOIL-WATER RATIO
SOIL 3, WATER 1

Water, ml	Soil, gm	pH	Virus Concentration, PFU/ml		Percent Removal
			Initial	Final	
7	7.0	6.90	3.00×10^8	3.75×10^5	99.87
	3.0	7.25		2.00×10^5	99.93
	1.0	7.43		1.00×10^5	99.97
	0.50	7.68		7.40×10^5	99.75
	0.10	7.90		1.31×10^6	99.56
	0.050	7.95		2.61×10^6	99.13
	0.010	8.10		4.52×10^6	98.49
	0.0050	8.10		5.15×10^6	98.28
	0.0010	8.10		8.26×10^6	97.25
	0.00010	8.10		1.25×10^7	95.83
7	0.000010	8.10	3.00×10^8	1.95×10^7	93.50

TABLE 11

VIRUS ADSORPTION AS AFFECTED BY SOIL-WATER RATIO
SOIL 4, WATER 1

Water, ml	Soil, gm	pH	Virus Concentration, PFU/ml		Percent Removal
			Initial	Final	
7	7.0	6.10	3.00×10^8	1.53×10^8	47.33
	3.0	5.50		1.13×10^8	62.33
	1.0	6.35		7.75×10^7	74.17
	0.50	6.83		7.95×10^7	73.50
	0.10	7.38		6.30×10^7	79.00
	0.050	7.55		4.80×10^7	84.00
	0.010	7.69		3.38×10^7	88.73
	0.0050	7.78		2.45×10^7	91.83
	0.0010	7.81		1.35×10^7	95.50
	0.00010	7.83		9.75×10^5	99.67
7	0.000010	7.88	3.00×10^8	3.40×10^5	99.89

TABLE 12

VIRUS ADSORPTION AS AFFECTED BY SOIL-WATER RATIO
SOIL 1, WATER 2

Water, ml	Soil, gm	pH	Virus Concentration, PFU/ml		Percent Removal
			Initial	Final	
7	7.0	5.88	4.50×10^8	7.15×10^3	99.99+
↑	3.0	6.35	↑	5.70×10^3	99.99+
↑	1.0	6.78	↑	2.40×10^7	94.67
↑	0.50	7.08	↑	5.50×10^7	87.78
↑	0.10	7.48	↑	6.65×10^7	85.22
↑	0.050	7.65	↑	7.80×10^7	82.67
↑	0.010	7.78	↑	1.45×10^7	96.78
↑	0.0050	7.80	↑	4.10×10^6	99.09
↑	0.0010	7.88	↑	1.51×10^6	99.66
↑	0.00010	8.30	↑	9.30×10^5	99.79
7	0.000010	8.30	4.50×10^8	3.00×10^5	99.93

TABLE 13

VIRUS ADSORPTION AS AFFECTED BY SOIL-WATER RATIO
SOIL 2, WATER 2

Water, ml	Soil, gm	pH	Virus Concentration, PFU/ml		Percent Removal
			Initial	Final	
7	7.0	7.10	3.00×10^8	2.67×10^8	10.67
↑	3.0	7.05	↑	2.35×10^8	21.67
↑	1.0	7.20	↑	1.73×10^8	42.33
↑	0.50	7.25	↑	1.28×10^8	57.33
↑	0.10	7.25	↑	1.01×10^8	66.33
↑	0.050	7.30	↑	8.45×10^7	71.83
↑	0.010	7.30	↑	5.18×10^7	82.73
↑	0.0050	7.30	↑	2.30×10^7	92.33
↑	0.0010	7.35	↑	2.97×10^6	99.01
↑	0.00010	7.63	↑	1.19×10^6	99.60
7	0.000010	7.80	3.00×10^8	6.80×10^5	99.77

TABLE 14

VIRUS ADSORPTION AS AFFECTED BY SOIL-WATER RATIO
SOIL 3, WATER 2

Water, ml	Soil, gm	pH	Virus Concentration, PFU/ml		Percent Removal
			Initial	Final	
7	7.0	7.03	3.00×10^8	2.75×10^5	99.91
↑	3.0	6.85	↑	4.50×10^4	99.98
↑	1.0	7.13	↑	6.30×10^6	97.90
↑	0.50	7.25	↑	8.50×10^7	71.67
↑	0.10	7.62	↑	3.00×10^8	0.00
↑	0.050	7.78	↑	2.93×10^8	2.33
↑	0.010	7.98	↑	3.28×10^7	89.07
↑	0.0050	8.15	↑	1.00×10^7	96.67
↑	0.0010	8.33	↑	1.33×10^7	95.57
↑	0.00010	8.50	↑	1.65×10^7	94.50
↓			↓		
7	0.000010	8.50	3.00×10^8	7.23×10^6	97.59

TABLE 15

VIRUS ADSORPTION AS AFFECTED BY SOIL-WATER RATIO
SOIL 4, WATER 2

Water, ml	Soil, gm	pH	Virus Concentration, PFU/ml		Percent Removal
			Initial	Final	
7	7.0	4.63	3.00×10^8	3.00×10^8	0.00
↑	3.0	5.08	↑	2.10×10^8	30.00
↑	1.0	5.65	↑	7.75×10^7	74.17
↑	0.50	6.20	↑	4.90×10^7	83.67
↑	0.10	6.83	↑	3.15×10^7	89.50
↑	0.050	7.03	↑	1.40×10^7	95.33
↑	0.010	7.19	↑	8.15×10^6	97.28
↑	0.0050	7.30	↑	6.45×10^6	97.85
↑	0.0010	7.41	↑	5.47×10^6	98.18
↑	0.00010	7.50	↑	4.10×10^6	98.63
↓ 7	0.000010	7.61	3.00×10^8	2.57×10^6	99.14

decreasing soil concentrations for Soils No. 1, 2, and 4. A reverse trend is indicated for Soil No. 3. Results using Water No. 2 and Soils No. 2 and 4 also show increasing percent virus removal with decreasing soil concentrations. However, results with both Soils No. 1 and 3 show a decrease and then an increase in percent removal as the soil concentration is decreased with extreme results indicated for Soil No. 3.

Dynamic Experiments

Dynamic experiments included column runs using all four soils and three different waters for a total of twelve column setups. All pertinant column information including the feeding or dosing schedules for all columns is presented in Table 16. The feed schedule as shown in Table 16 is interepted as follows: For Column No. 2. At time zero, 15 ml of water containing the indicated virus concentration was added to the top of the column. When this volume of water had just entered the soil(at the end of one day in this case) another dose was applied (25 ml). This process was repeated until all the tagged virus solution prepared for a given column was used or until 20 days of operation were recorded (two or three of the columns were kept in operation for slightly longer periods).

Tables 17 through 28 show the percent of total virus on a column at a given time that was retained at various depth intervals. Using Soil Column No. 1 as an example, after 15 days of operation 16.1 percent of the virus on the column at that time were retained in the interval below the 1 cm level and above the 2 cm level, both levels being measured from the top of the column, i.e., depth interval 1-2 cm.

TABLE 16

SOIL COLUMN CHARACTERISTICS AND FEED SCHEDULE

Column No.	1	2	3	4	5	6
Soil No.	1	2	3	4	1	2
Water No.	2	2	2	2	3	3
Column Dia., cm	2.8	2.8	2.8	2.8	2.8	2.8
Wt. Dry Soil, gm	184.3	164.9	168.0	169.1	181.1	156.9
Soil Depth, cm	20.2	20.0	19.8	20.0	20.0	19.9
Bulk Density, gm/cm^3	1.48	1.34	1.38	1.37	1.47	1.28
Virus Added:						
Total Count, PFU	5.25×10^9	6.30×10^9	6.30×10^9	1.00×10^{12}	8.25×10^9	7.05×10^1
Radioactivity, CPM	1.86×10^6	7.60×10^5	7.60×10^5	3.02×10^6	2.56×10^6	2.70×10^6
Water Added:						
Time, Days-ml	0-17.5 2-25.0 5-25.0 14-25.0	0-15.0 1-25.0 2-25.0 3-25.0 6-25.0 8-25.0 10-25.0 14-25.0 17-25.0	0-15.0 1-25.0 9-25.0 12-25.0	0-15.0 4-25.0 15-25.0	0-15.0 2-25.0 5-25.0 8-25.0 12-25.0 16-25.0 20-25.0	0-15.0 1-25.0 4-25.0 7-25.0 10-25.0 14-25.0 18-25.0 20-25.0

Column No.	7	8	9	10	11	12
Soil No.	3	4	1	2	3	4
Water No.	3	3	1	1	1	1
Column Dia., cm	2.8	2.8	2.8	2.8	2.8	2.8
Wt. Dry Soil, gm	173.8	168.7	178.1	156.4	170.0	172.1
Soil Depth, cm	21.4	19.9	19.3	19.5	21.2	19.3
Bulk Density, gm/cm^3	1.32	1.38	1.50	1.30	1.36	1.4
Virus Added:						
Total Count, PFU	7.05×10^{10}	4.65×10^{11}	4.65×10^{11}	4.65×10^{11}	4.65×10^{11}	4.65×1
Radioactivity, CPM	2.70×10^6	3.86×10^6	3.86×10^6	3.86×10^6	3.86×10^6	3.86×1
Water Added:						
Time, Days-ml	0-15.0 1-25.0 4-25.0 7-25.0 10-25.0 14-25.0 19-25.0	0-15.0 3-25.0 11-25.0	0-15.0 3-25.0 5-25.0 11-25.0 14-25.0 19-25.0	0-15.0 3-25.0 5-25.0 7-25.0 11-25.0 13-25.0 14-25.0 17-25.0 19-25.0	0-15.0 3-25.0 6-25.0 14-25.0 18-25.0	0-15.0 5-25.0 11-25.0 17-25.0

TABLE 17

VIRUS DISTRIBUTION ON SOIL COLUMN NO.1

Time, Days	Percent of Total Virus Column Depth Interval, cm							
	0-1	1-2	2-3	3-4	4-5	5-10	10-15	15-20
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	93.1	2.2	0.4	0.7	0.5	0.8	2.3	0.0
2	86.9	3.4	0.6	1.1	1.0	4.1	1.5	1.4
3	56.3	17.5	8.1	4.5	1.8	5.1	1.4	5.3
4	43.3	17.2	9.7	10.1	9.1	7.1	1.6	1.9
6	40.8	14.5	8.7	7.8	6.7	17.7	3.7	0.1
8	36.7	23.3	8.4	9.3	6.9	11.4	0.8	3.2
9	42.0	15.5	8.9	8.5	6.2	13.3	5.6	0.0
10	42.6	19.2	9.3	7.8	8.8	6.1	4.3	1.9
11	41.3	18.7	8.5	6.8	6.8	15.0	0.1	2.8
12	41.6	24.5	8.6	6.8	5.0	9.3	3.4	0.8
14	46.6	20.4	7.5	6.9	7.3	7.3	2.4	1.6
15	41.7	16.1	8.5	9.7	7.5	12.6	3.3	0.6
17	38.5	16.1	7.4	8.3	7.9	11.1	7.5	3.2
18	45.0	20.7	7.4	6.7	6.2	9.6	4.3	0.1
20	42.8	18.2	6.8	7.7	4.8	11.9	3.8	4.0
21	41.5	23.5	8.3	4.4	6.5	12.5	1.2	2.1
23	39.0	22.4	8.9	4.9	5.3	8.6	6.4	4.5
24	39.1	19.5	10.0	7.5	4.2	12.6	2.2	4.9
26	37.8	16.3	5.1	5.0	4.7	19.6	10.6	0.9
27	36.3	14.2	4.2	3.5	4.5	20.9	6.1	10.3
28	34.0	23.5	5.4	10.3	5.0	12.3	5.6	3.9
30	40.8	22.5	6.3	3.9	5.8	13.1	0.0	7.6
31	46.2	13.0	9.5	4.6	0.0	12.2	8.6	5.9
33	34.9	13.5	6.1	4.5	6.8	22.5	9.4	2.3
37	33.5	16.8	10.5	5.4	7.8	18.2	4.0	3.8
38	23.7	21.5	6.8	5.8	7.0	18.5	16.7	0.0

TABLE 18

VIRUS DISTRIBUTION ON SOIL COLUMN NO.2

Time, Days	Percent of Total Virus Column Depth Interval, cm							
	0-1	1-2	2-3	3-4	4-5	5-10	10-15	15-20
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	33.9	11.8	10.4	13.4	7.9	18.1	1.9	2.6
3	27.1	10.4	7.7	5.0	5.9	23.6	15.8	4.5
4	29.2	12.0	6.5	8.2	4.9	18.5	12.7	8.0
5	24.5	9.8	9.6	5.2	2.6	24.2	13.8	10.3
6	25.6	11.9	5.1	5.5	1.9	29.3	17.5	3.4
8	30.5	12.7	3.4	2.7	6.9	14.8	11.7	17.3
10	21.2	10.5	7.5	4.0	6.0	19.5	22.8	8.7
11	21.0	10.0	9.6	5.1	3.6	14.7	22.5	13.5
13	25.6	13.0	6.3	3.9	5.2	15.4	13.7	16.9
15	26.8	12.6	9.3	3.8	5.3	17.6	15.1	9.5
17	31.2	4.5	5.0	8.6	10.8	14.9	2.8	22.2
18	23.2	10.4	6.6	11.3	7.7	20.5	11.5	8.8
20	30.7	11.6	4.0	6.5	0.9	22.1	11.9	12.3
21	35.2	12.3	5.9	0.3	2.5	26.9	8.9	8.0

TABLE 19

VIRUS DISTRIBUTION ON SOIL COLUMN NO.3

Time, Days	Percent of Total Virus Column Depth Interval, cm							
	0-1	1-2	2-3	3-4	4-5	5-10	10-15	15-20
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	41.4	18.3	13.0	5.4	6.3	3.5	7.8	4.3
2	40.6	18.6	11.6	10.8	3.5	7.7	0.6	6.6
3	52.8	22.7	11.2	6.4	0.0	0.4	3.6	2.9
4	48.5	13.6	13.9	13.4	0.9	4.5	1.5	3.7
5	39.7	16.4	12.2	13.2	5.1	6.7	5.7	1.0
7	33.9	8.5	9.8	9.6	9.5	16.3	10.0	2.4
9	45.2	11.6	11.8	7.1	4.1	12.6	2.1	5.5
10	30.7	6.6	10.3	18.1	12.5	7.3	3.4	11.1
12	32.1	17.4	15.4	11.6	2.8	10.3	3.9	6.5
14	29.0	12.2	7.2	8.8	4.2	18.3	12.1	8.2
17	38.1	13.1	11.3	6.5	4.2	16.7	9.8	0.3
19	45.7	6.5	13.0	8.5	0.0	5.7	8.9	11.7
21	54.5	13.8	7.6	9.8	2.2	9.8	1.8	0.5
22	45.3	14.2	4.5	18.3	0.0	14.2	3.5	0.0
23	35.8	10.5	13.9	9.1	11.8	18.9	0.0	0.0

TABLE 20

VIRUS DISTRIBUTION ON SOIL COLUMN NO.4

Time, Days	Percent of Total Virus Column Depth Interval, cm							
	0-1	1-2	2-3	3-4	4-5	5-10	10-15	15-20
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	66.4	24.1	3.6	0.0	1.5	2.2	1.2	1.0
5	60.9	25.5	6.9	1.5	0.3	2.8	1.8	0.3
6	55.4	26.6	7.3	2.4	1.5	3.6	0.5	2.7
7	54.5	25.2	11.2	2.2	0.9	2.5	1.7	1.5
8	51.6	25.4	7.9	3.1	1.5	3.8	4.0	2.7
11	51.2	22.4	9.5	4.3	1.6	2.9	2.7	5.4
13	59.7	25.9	5.8	2.0	1.2	4.5	0.0	0.9
14	57.2	23.5	8.3	2.7	1.2	1.7	2.4	3.0
15	52.7	22.6	6.1	2.4	1.7	7.0	4.1	3.4
17	55.1	27.2	7.7	2.7	1.8	3.5	0.8	1.2
19	50.2	23.3	8.3	2.0	1.7	5.5	6.7	2.3
20	54.4	23.2	9.3	2.9	1.0	7.0	1.3	0.9
21	49.8	18.9	13.4	4.9	0.5	10.5	1.6	0.4
22	57.0	19.2	11.4	4.5	2.3	3.9	1.1	0.6
26	55.2	18.1	7.4	6.5	0.8	9.7	1.1	1.2

TABLE 21

VIRUS DISTRIBUTION ON SOIL COLUMN NO.5

Time, Days	Percent of Total Virus Column Depth Interval, cm							
	0-1	1-2	2-3	3-4	4-5	5-10	10-15	15-20
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	50.3	27.0	11.4	2.5	0.9	2.2	4.1	1.6
2	41.8	20.7	11.5	10.8	6.3	1.7	2.7	4.5
5	45.2	17.7	10.1	8.5	7.6	8.0	2.2	0.7
7	47.1	17.8	10.4	9.0	8.9	5.5	0.2	1.1
8	42.1	19.2	8.1	8.3	9.1	7.9	4.9	0.4
9	42.2	18.0	9.1	8.6	8.2	9.1	2.3	2.5
11	45.2	15.9	9.4	9.8	10.7	9.0	—	—
13	41.7	13.6	10.8	8.3	6.9	14.7	1.4	2.6
14	42.4	14.6	10.0	8.6	7.4	13.8	2.4	0.8
15	40.5	14.5	7.9	10.1	9.1	14.8	2.0	1.1
16	41.8	12.7	7.3	10.4	7.9	14.6	3.7	1.6
18	45.5	12.3	8.3	8.6	8.1	14.1	1.9	1.2
20	45.4	17.4	8.1	10.0	7.1	12.0	—	—

TABLE 22

VIRUS DISTRIBUTION ON SOIL COLUMN NO.6

Time, Days	Percent of Total Virus Column Depth Interval, cm							
	0-1	1-2	2-3	3-4	4-5	5-10	10-15	15-20
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	53.5	26.8	9.6	3.4	0.4	3.3	0.8	2.2
3	48.9	16.0	10.9	7.8	7.4	7.9	0.7	0.4
4	37.8	14.1	12.3	12.7	12.0	9.4	1.6	0.1
5	35.4	11.5	13.8	12.9	12.8	11.3	2.0	0.3
7	38.9	12.0	10.6	11.5	13.8	10.9	0.6	1.7
8	38.3	12.8	11.7	10.0	13.1	11.9	1.9	0.3
9	39.4	12.2	11.4	11.9	9.3	14.6	0.3	0.9
10	35.7	14.1	10.7	14.4	10.0	12.7	2.0	0.4
12	34.8	14.3	12.5	12.3	10.2	15.7	0.2	0.0
13	36.0	14.6	14.6	11.8	7.2	12.1	2.0	1.7
14	38.6	14.2	12.1	11.1	6.6	15.2	1.3	0.9
15	38.0	12.9	11.9	11.1	7.2	16.1	0.4	2.4
16	36.9	14.3	9.8	11.0	9.0	15.5	0.9	2.6
17	36.7	14.4	10.6	10.4	9.7	13.7	3.0	1.5
19	37.6	14.3	10.5	10.1	9.5	14.1	3.3	0.6
20	40.6	14.6	10.0	9.4	8.4	15.7	0.7	0.6

TABLE 23

VIRUS DISTRIBUTION ON SOIL COLUMN NO.7

Time, Days	Percent of Total Virus Column Depth Interval, cm							
	0-1	1-2	2-3	3-4	4-5	5-10	10-15	15-20
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	60.6	25.2	7.4	2.2	0.7	3.2	0.7	0.0
4	41.4	19.8	16.4	11.0	2.7	5.1	1.8	1.8
5	38.1	22.9	15.8	12.3	6.2	3.4	1.0	0.3
7	32.9	16.1	9.9	12.0	11.6	13.8	3.3	0.4
8	34.0	16.3	10.6	12.1	9.2	14.2	1.8	1.8
9	35.1	17.8	12.1	11.2	8.5	11.5	2.0	1.8
10	35.8	15.1	12.0	11.0	8.5	12.9	2.7	2.0
13	39.0	18.6	12.4	10.7	8.1	9.2	1.9	0.1
14	39.2	16.5	12.2	11.0	6.4	9.0	3.7	2.0
15	39.9	15.8	10.8	11.2	6.5	12.8	1.7	1.3
16	41.6	15.2	10.3	7.7	7.3	9.5	5.9	2.5
17	38.9	14.4	9.3	6.2	7.2	10.6	4.6	8.8
19	37.7	14.0	10.4	7.3	5.7	11.7	9.7	3.5
20	40.0	15.7	9.5	8.8	7.8	12.2	3.0	3.0

TABLE 24

VIRUS DISTRIBUTION ON SOIL COLUMN NO.8

Time, Days	Percent of Total Virus Column Depth Interval, cm							
	0-1	1-2	2-3	3-4	4-5	5-10	10-15	15-20
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	51.9	34.3	7.2	2.9	1.7	1.3	0.2	0.5
4	45.4	31.3	11.0	4.4	2.3	4.1	0.7	0.8
5	42.3	33.6	16.4	3.0	1.4	1.3	1.4	0.6
6	45.2	30.7	16.1	2.8	2.0	2.0	0.7	0.5
7	49.1	29.2	13.4	2.6	1.8	1.7	1.5	0.7
10	36.7	26.8	18.7	6.0	3.2	7.1	0.9	0.6
11	38.6	26.8	19.0	6.9	3.1	3.7	1.1	0.9
12	39.6	28.3	18.2	6.7	2.8	2.4	0.8	1.2
13	38.3	27.0	19.4	6.8	2.5	4.4	1.6	0.0
14	38.9	26.8	16.8	7.5	2.0	4.7	3.1	0.2
17	35.4	25.6	18.6	8.9	2.3	6.5	2.2	0.5
19	33.6	20.4	23.7	9.4	2.0	6.4	2.1	2.4
20	27.0	18.1	20.7	10.5	2.8	6.8	5.6	8.5

TABLE 25

VIRUS DISTRIBUTION ON SOIL COLUMN NO.9

Time, Days	Percent of Total Virus Column Depth Interval, cm							
	0-1	1-2	2-3	3-4	4-5	5-10	10-15	15-20
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	55.0	25.3	10.7	3.9	0.9	1.6	2.0	0.6
4	45.9	19.8	12.1	9.9	7.4	3.7	0.8	0.4
5	42.4	15.4	14.2	13.5	8.6	4.0	0.9	1.0
7	37.7	10.2	8.7	11.9	10.7	19.1	1.6	0.1
10	29.5	12.8	8.5	9.5	7.9	24.6	4.6	2.6
11	29.5	15.9	7.6	7.1	8.5	29.7	1.6	0.1
12	28.8	17.1	8.2	8.1	7.1	27.5	2.1	1.1
13	27.4	16.7	7.2	7.5	6.0	25.8	5.4	4.0
14	27.5	19.7	7.1	5.9	7.0	29.3	3.3	0.2
17	28.7	20.9	7.0	6.4	7.4	27.7	1.6	0.3
19	30.1	26.3	7.6	3.6	5.5	23.3	2.8	0.8
20	30.6	27.6	6.6	3.7	5.4	25.3	0.0	0.8

TABLE 26

VIRUS DISTRIBUTION ON SOIL COLUMN NO.10

Time, Days	Percent of Total Virus Column Depth Interval, cm							
	0-1	1-2	2-3	3-4	4-5	5-10	10-15	15-20
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	40.9	15.7	10.3	7.4	5.6	17.3	2.3	0.5
4	33.4	17.7	5.9	3.0	5.1	20.3	13.7	0.9
5	27.3	6.8	4.7	4.2	5.0	31.5	20.2	0.3
6	29.5	9.7	4.6	3.9	3.2	23.3	25.6	0.2
7	26.8	7.1	4.6	2.6	2.5	20.1	24.8	11.5
10	25.4	7.1	4.0	2.5	5.3	18.1	25.2	12.4
11	29.0	7.2	3.8	2.3	4.5	16.1	23.4	13.7
13	30.3	7.3	3.5	3.1	2.8	13.9	22.7	16.4
14	30.9	7.8	3.4	3.0	2.5	13.8	22.1	16.5
17	32.6	10.3	4.3	3.5	2.1	16.4	17.1	13.7
19	37.8	15.5	5.7	3.7	3.1	10.8	8.2	15.2
20	37.5	18.9	5.0	3.2	3.8	9.4	12.2	10.0

TABLE 27

VIRUS DISTRIBUTION ON SOIL COLUMN NO.11

Time, Days	Percent of Total Virus Column Depth Interval, cm							
	0-1	1-2	2-3	3-4	4-5	5-10	10-15	15-20
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	32.1	29.8	20.0	9.1	3.3	3.2	2.1	0.4
4	28.3	28.4	18.9	11.0	6.1	5.7	0.9	0.7
5	24.7	25.1	18.4	14.1	9.9	6.8	0.6	0.4
6	21.7	23.6	17.9	13.9	10.9	9.7	1.2	1.1
7	21.0	23.8	16.0	15.3	10.6	11.4	0.5	1.4
10	19.5	20.1	17.5	14.2	10.3	16.6	1.4	0.4
11	16.6	18.8	18.0	14.2	11.9	18.2	1.5	0.8
13	20.2	22.1	15.0	11.6	11.0	17.5	0.9	1.7
14	20.8	22.0	14.5	11.6	11.7	18.3	0.7	0.4
18	17.3	16.4	19.2	16.6	8.7	20.2	1.6	0.0
19	18.2	16.9	19.1	13.3	9.5	19.9	1.0	2.1
20	19.4	16.4	13.6	14.3	10.2	22.0	1.9	2.2

TABLE 28

VIRUS DISTRIBUTION ON SOIL COLUMN NO.12

Time, Days	Percent of Total Virus Column Depth Interval, cm							
	0-1	1-2	2-3	3-4	4-5	5-10	10-15	15-20
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	73.3	15.9	4.6	1.3	1.3	1.2	2.3	0.1
4	54.6	17.8	14.8	6.1	2.5	2.2	1.3	0.7
5	39.0	28.0	17.4	7.4	2.9	4.0	0.6	0.7
6	41.2	25.9	17.7	8.5	2.8	2.3	0.6	1.0
7	41.8	25.5	17.8	8.4	3.4	2.0	0.9	0.2
10	34.6	24.0	18.3	11.1	6.1	3.0	1.5	1.4
11	34.3	25.0	18.6	12.3	5.2	2.9	1.2	0.5
13	39.5	22.3	18.4	10.1	5.5	1.6	2.3	0.3
14	42.5	21.0	20.3	10.1	4.8	0.6	0.6	0.1
17	39.2	19.9	18.8	11.5	4.1	3.7	1.2	1.6
19	34.4	21.0	19.4	14.0	4.3	5.3	1.0	0.6
20	36.8	20.9	20.5	14.3	4.0	2.1	1.2	0.2

Tables 29 through 40 are effluent histories for the twelve columns. The entire effluent for each column was analyzed for virus and/or radioactivity at various intervals during the column runs. Table 41 shows the results of standard tests on the effluents of those columns using septic tank effluent as the liquid phase, Columns No. 5, 6, 7, and 8.

TABLE 29

EFFLUENT HISTORY, COLUMN NO.1

Time, Days	Virus, PFU/ml	Radioactivity, CPM/ml
1 ↓ 38	NO VIRUS DETECTED	NO RADIOACTIVITY DETECTED

TABLE 30

EFFLUENT HISTORY, COLUMN NO.2

Time, Days	Virus, PFU/ml	Radioactivity* CPM/ml
2	0	7.0
3	0	21.0
5	0	35.0
6	0	67.0
7	0	19.0
8	0	82.0
11	0	81.0
14	0	117.0
15	6.0	137.0
17	0	159.0
18	0	98.0
19	0	—
21	0	59.0

* Total Radioactivity Recovered, Approx. 2%

TABLE 31

EFFLUENT HISTORY, COLUMN NO.3

Time, Days	Virus, PFU/ml	Radioactivity, CPM/ml
1	NO VIRUS DETECTED	0
2		0
4		0
5		4.0
7		0
9		0
10		1.0
12		0
16		0
17		0
19		0
21		0

TABLE 32

EFFLUENT HISTORY, COLUMN NO.4

Time, Days	Virus, PFU/ml	Radioactivity, CPM/ml
1	0	0
4	0	0
5	—	0
6	—	0
7	0	0
8	0	0
11	—	0
13	0	9.5
14	0	0
15	0	4.0
17	0	0
18	0	2.0
19	0	7.0

TABLE 33

EFFLUENT HISTORY, COLUMN NO.5

Time, Days	Virus, PFU/ml	Radioactivity, CPM/ml
1	0	0
2	—	0
7	0	0
8	0	6
9	0	11
11	0	2
13	0	0
15	0	7
18	0	0
20	0	0

TABLE 35

EFFLUENT HISTORY, COLUMN NO.7

Time, Days	Virus, PFU/ml	Radioactivity, CPM/ml
3	—	0
4	0	3
7	0	0
8	—	0
9	0	0
10	0	0
12	—	0
13	0	6
14	—	0
15	—	0
16	0	0
17	0	0
19	0	0
20	0	11

TABLE 34

EFFLUENT HISTORY, COLUMN NO.6

Time, Days	Virus, PFU/ml	Radioactivity, CPM/ml
5	0	0
7	—	0
8	—	0
9	0	0
10	—	9
12	0	0
13	—	0
14	0	4
15	—	1
16	0	0
17	0	16
19	—	27
20	0	19

TABLE 36

EFFLUENT HISTORY, COLUMN NO.8

Time, Days	Virus, PFU/ml	Radioactivity, CPM/ml
0 ↕ 20	NO VIRUS DETECTED	NO RADIOACTIVITY DETECTED

TABLE 37

EFFLUENT HISTORY, COLUMN NO.9

Time, Days	Virus, PFU/ml	Radioactivity, CPM/ml
3	0	—
4	0	4
5	0	—
7	0	16
11	—	16
14	0	—
17	0	41
19	0	26
20	0	28

TABLE 38

EFFLUENT HISTORY, COLUMN NO.10

Time, Days	Virus, PFU/ml	Radioactivity, CPM/ml
3	0	6
4	0	18
5	0	70
7	0	176
11	0	1,212
13	0	592
14	0	1,054
19	0	2,128
20	0	2,158

TABLE 39

EFFLUENT HISTORY, COLUMN NO.11

Time, Days	Virus, PFU/ml	Radioactivity, CPM/ml
3	0	0
5	0	0
6	0	0
7	0	0
11	0	8
14	0	0
18	0	0
20	0	0

TABLE 40

EFFLUENT HISTORY, COLUMN NO.12

Time, Days	Virus, PFU/ml	Radioactivity, CPM/ml
3	0	0
5	28	8
6	—	16
7	16	—
11	—	12
13	0	21
17	0	14
20	0	6

TABLE 41

EFFLUENT CHARACTERISTICS OF SOIL COLUMNS 5, 6, 7, and 8.

Parameter	Column 5	Column 6	Column 7	Column 8
Conductivity, micromhos/cm	695	829	587	508
Alkalinity, mg/l as $CaCO_3$	118	328	94	—
BOD, mg/l	86	37	31	145
COD, mg/l	260	313	146	17

CHAPTER IV

DISCUSSION OF RESULTS

Static Experiments

Initial experiments on adsorption of f2 bacteriophage have been reported by Reece (5). These initial studies showed that most of the adsorption takes place during the first few minutes of soil-water-virus contact, under the static test conditions used in this study, and is essentially complete after 24 hours. The adsorption studies carried out by Reece (5) also showed the adsorption process to be characterized by the Freundlich isotherm with the constants being such that for all practical purposes the process could be represented by linear isotherms. Density of adsorption sites on the soils appeared to be relatively low and with a considerable range from soil to soil. Nevertheless, adsorption of well over 99 percent of the virus particles was obtained under static test conditions.

Virus adsorption of well over 99 percent was obtained on Soils No. 1, 3, and 4 using septic tank effluent (Water No. 4) as shown in Table 3. Soil No. 2 exhibited decreasing adsorption with decreasing virus concentrations. Reece (5) also obtained the poorest removal with Soil No. 2 using Water No. 2. This might be explained by the fact that Soil No. 2 has less surface area per

unit weight than the other soils (see Table 1A) and thus simply has fewer adsorption sites available. Also, Soil No. 2 contained considerably more organic carbon per unit weight than the other soils (see Table 1A) and the organic matter present could have occupied some of the adsorption sites. The fact that a wastewater containing 900 mg/l organic matter (volatile suspended solids, Table 2) was used as the liquid phase did not appear to affect overall virus adsorption, i.e., the organic matter present in the wastewater did not present much, if any, competition for adsorption sites.

Results of ionic strength effects for Soils No. 1 and 3 are as expected (Table 4 and 6). Adsorption is high and decreases slightly as ionic strength increases. The decrease in adsorption is expected because of the increase in pH of the soil-water system as the ionic strength increases. This agrees well with the explanation presented by Drewry (2) and Drewry and Eliassen (3) concerning the amphoteric nature of the protein coated virus particles. Adsorption with Soil No. 4 (Table 7) was so complete that it would be moot to comment one way or the other on the results. Results with Soil No. 2 present no easy explanation (Table 5). It is noticed that as adsorption decreased with increasing ionic strength the pH also decreased. Then as ionic strength further increased both adsorption and pH increased. This would seem to go against logic but more likely is simply the result of complex physicochemical reactions within the soil-water-virus system. In any case no further explanation will be attempted here.

Tables 8 through 15 show that, in general, virus adsorption by soils increases per unit weight of soil as the soil concentration decreases. Exceptions are noted for Soil No. 3 with Water No. 1 and Soil No. 1 with Water No. 2 where a decrease in adsorption was obtained with decreasing soil concentrations. However, in both cases the adsorption was high at all soil concentrations. No explanation is offered for the results with Soil No. 3 and Water No. 2. These figures are the average of results from duplicate tests and the results were nearly the same both times. These results serve to show that adsorption by natural waterborne suspended matter can serve to help purify virus contaminated waters but also serve to show that such cannot be depended upon in all cases. Thus, as Drewry (2) has pointed out; that while it is logical to believe that there should be some property of soil which would indicate the relative virus adsorbing power this factor has not yet been discovered. Examination of the results of the static tests of this study seem to support this view. While soils appear to be good adsorbers of virus particles in general, it would seem that actual laboratory or field measurements are needed to determine this for any particular soil, i.e., soil analysis information alone, as usually presented, will not suffice.

Dynamic Experiments

Portions of the data on soil column virus distribution (Tables 17 through 28) are plotted as percent of total virus on the columns for various depth intervals as a function of time and are shown

in Figures 2 through 13. The results are not consistant from column to column by any means. Nearly all columns show a decrease in virus retained in the top 1 to 2 cm for periods ranging from about 10 to 20 days. This could be due in part to possible disturbances in those soil layers during dosing operations. Below the top one to two cm depths the results are quite varied from column to column. In some, Column 12 for example, the virus concentration in successive 1 cm depth intervals increased over the length of the column run. In others, Column 9 for example, the virus concentration increased for awhile and then began to decrease. In still others, Columns 1 and 11 for example, the virus concentration appeared to remain constant for most of the latter portion of the run. In all cases, by the end of the column runs, 20 days minimum, over 75 percent of the virus applied were retained in the upper 10 cm of the soil columns.

Only with Columns 2, 9, 10, and 12 did significant amounts of radioactivity wash through the columns (Tables 29 through 40). Only Columns 2 and 12 passed any detectable viable virus particles. No other columns passed any detectable viable virus particles and Columns 1, 8, and 11 passed no detectable radioactivity. Thus, it is concluded that after passage through a few centimeters of soils such as those used in this study a water should be essentially, if not completely, free of virus particles. This assumes, of course, a continuous strata of soil. This agrees quite well with the work of Drewry (2) where several California soils were tested under saturated flow conditions.

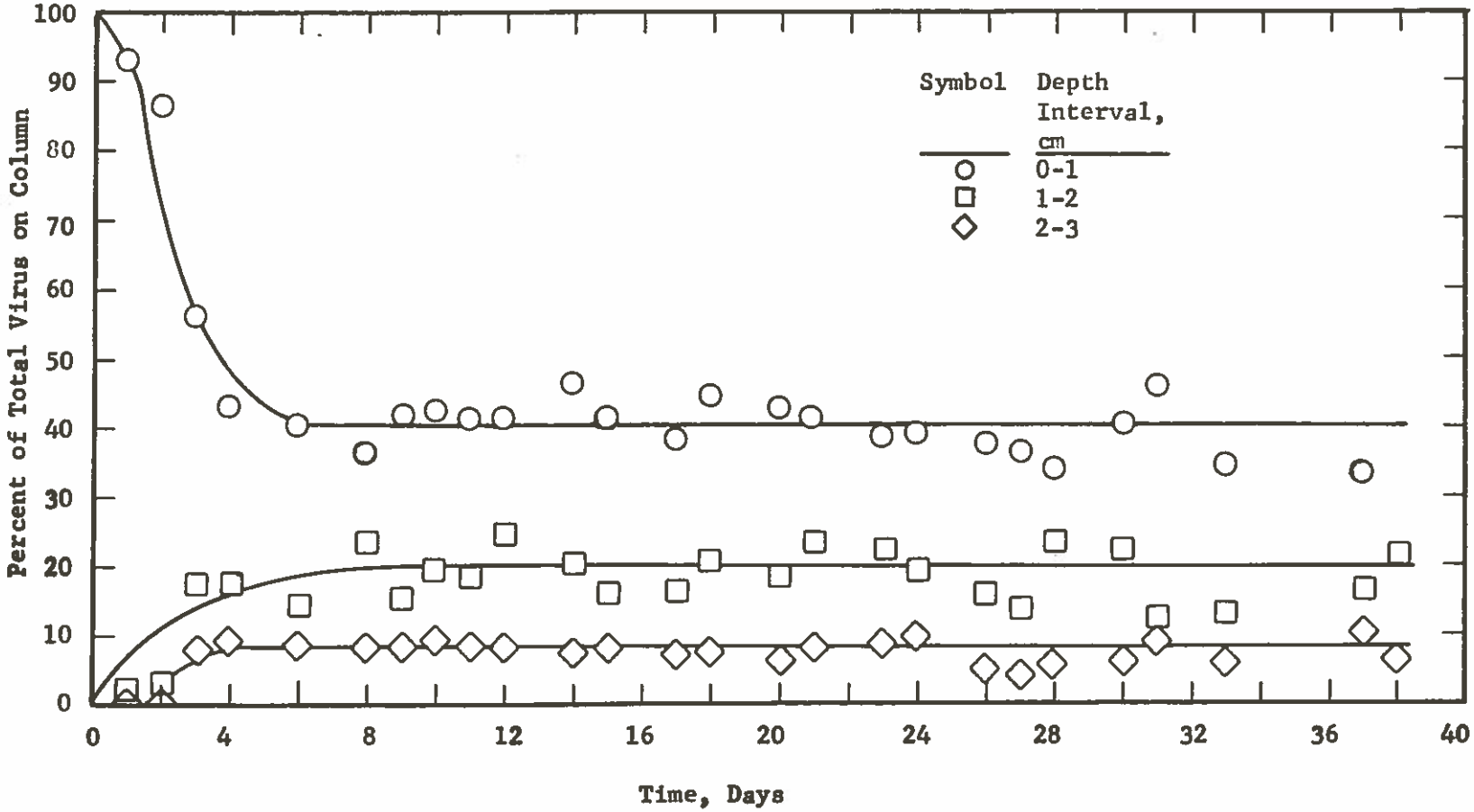

FIGURE 2. Virus Distribution on Soil Column No. 1.

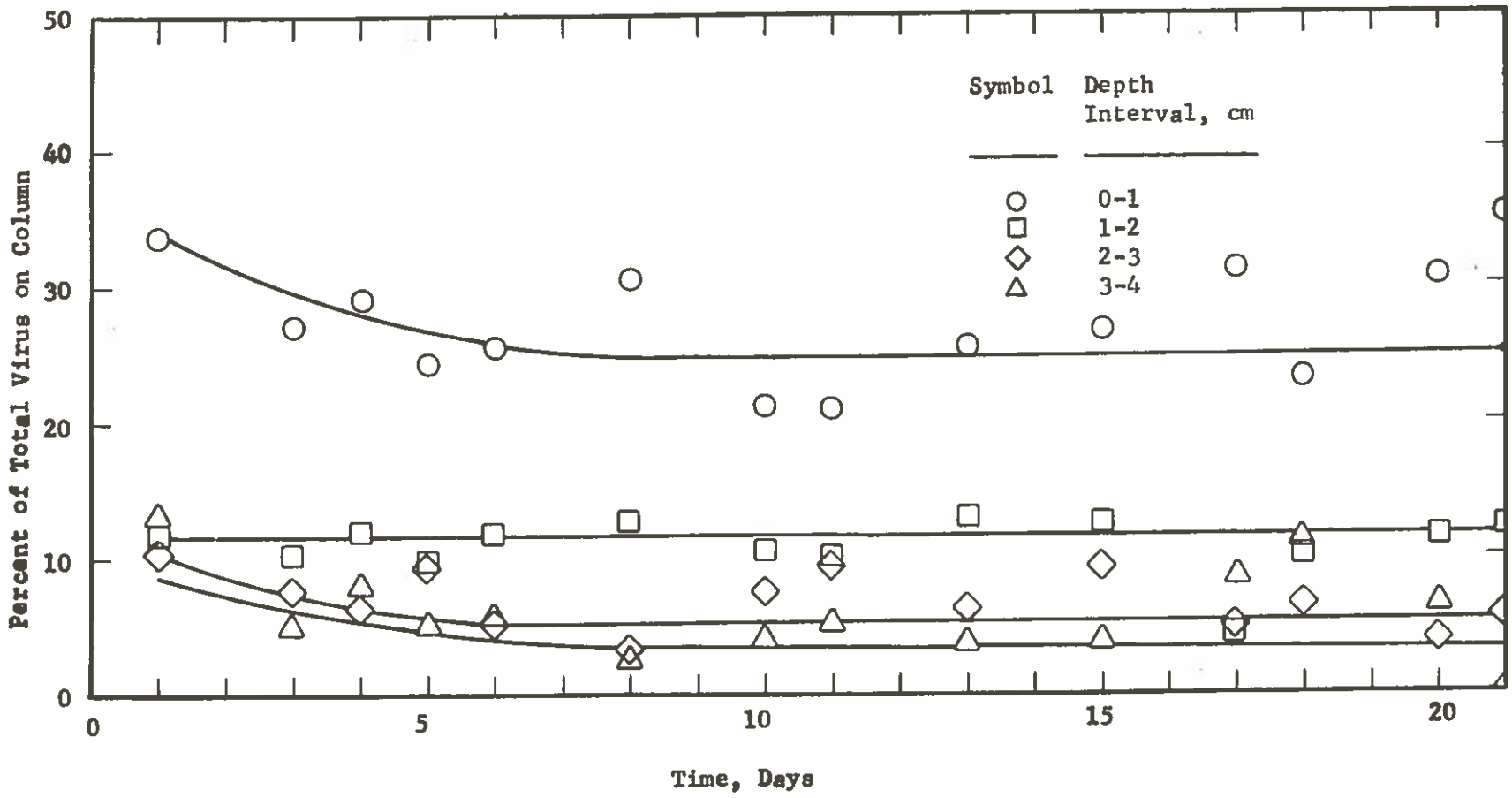

FIGURE 3. Virus Distribution on Soil Column No. 2.

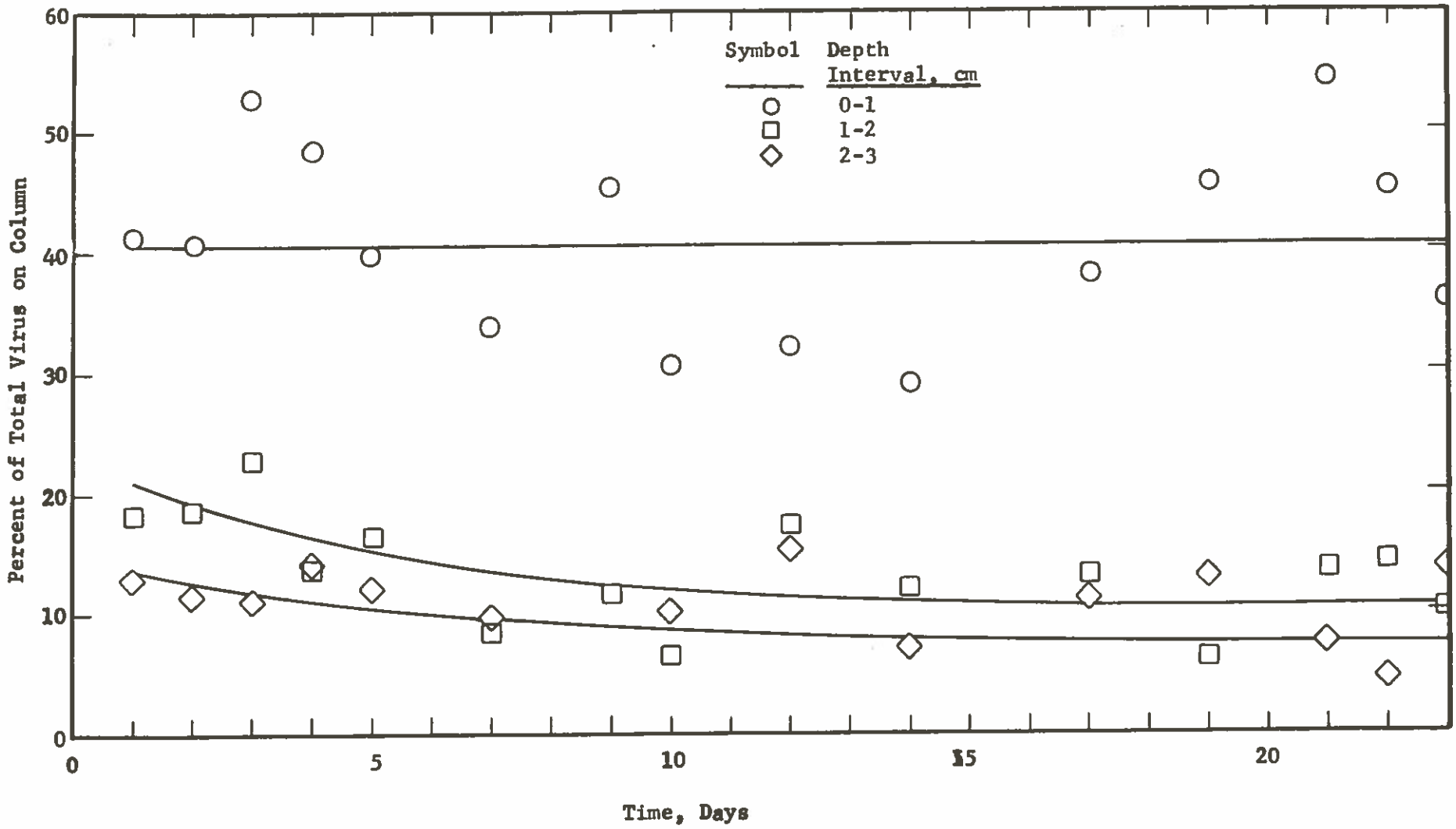

FIGURE 4. Virus Distribution on Soil Column No. 3.

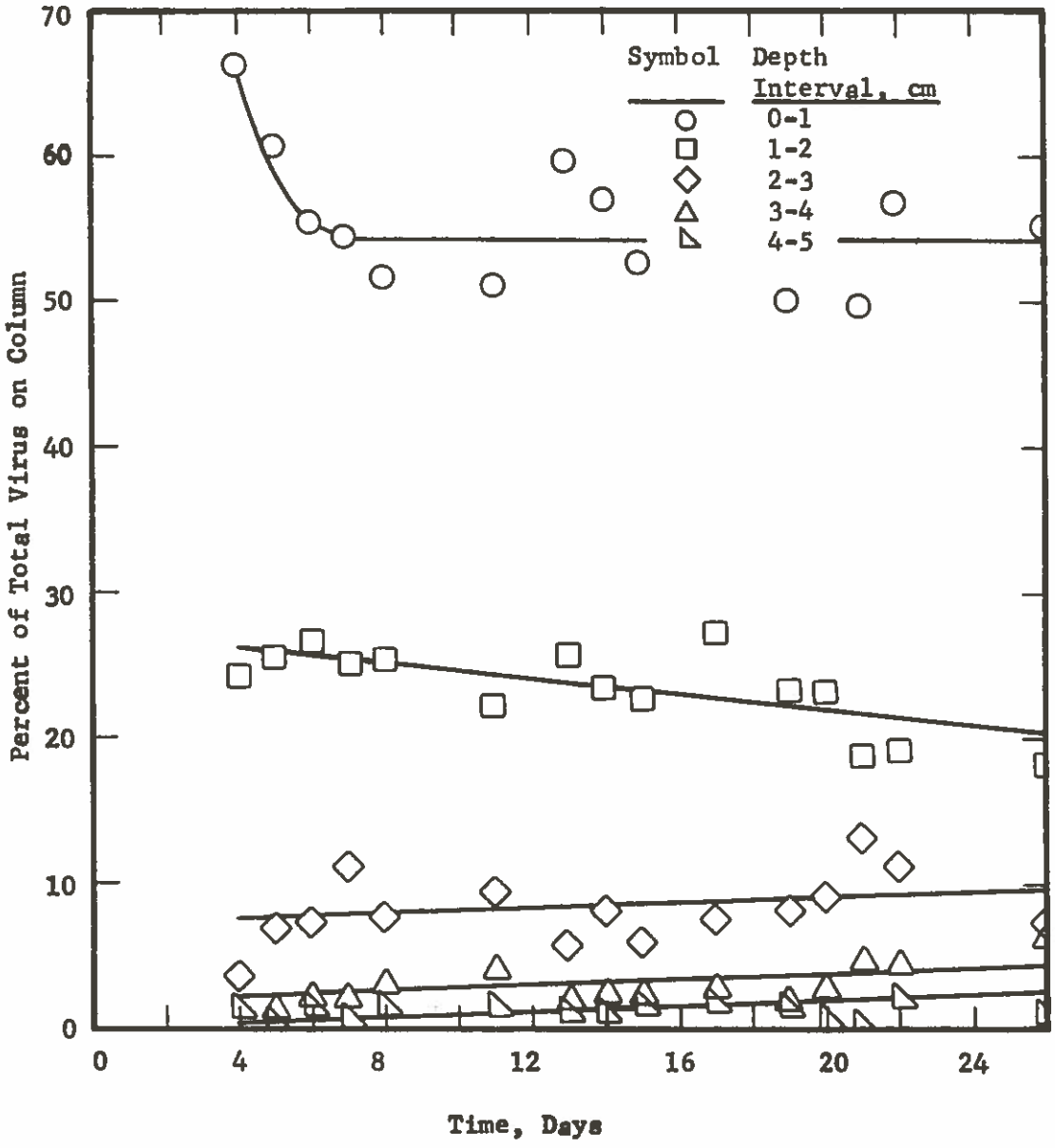

FIGURE 5. Virus Distribution on Soil Column No. 4.

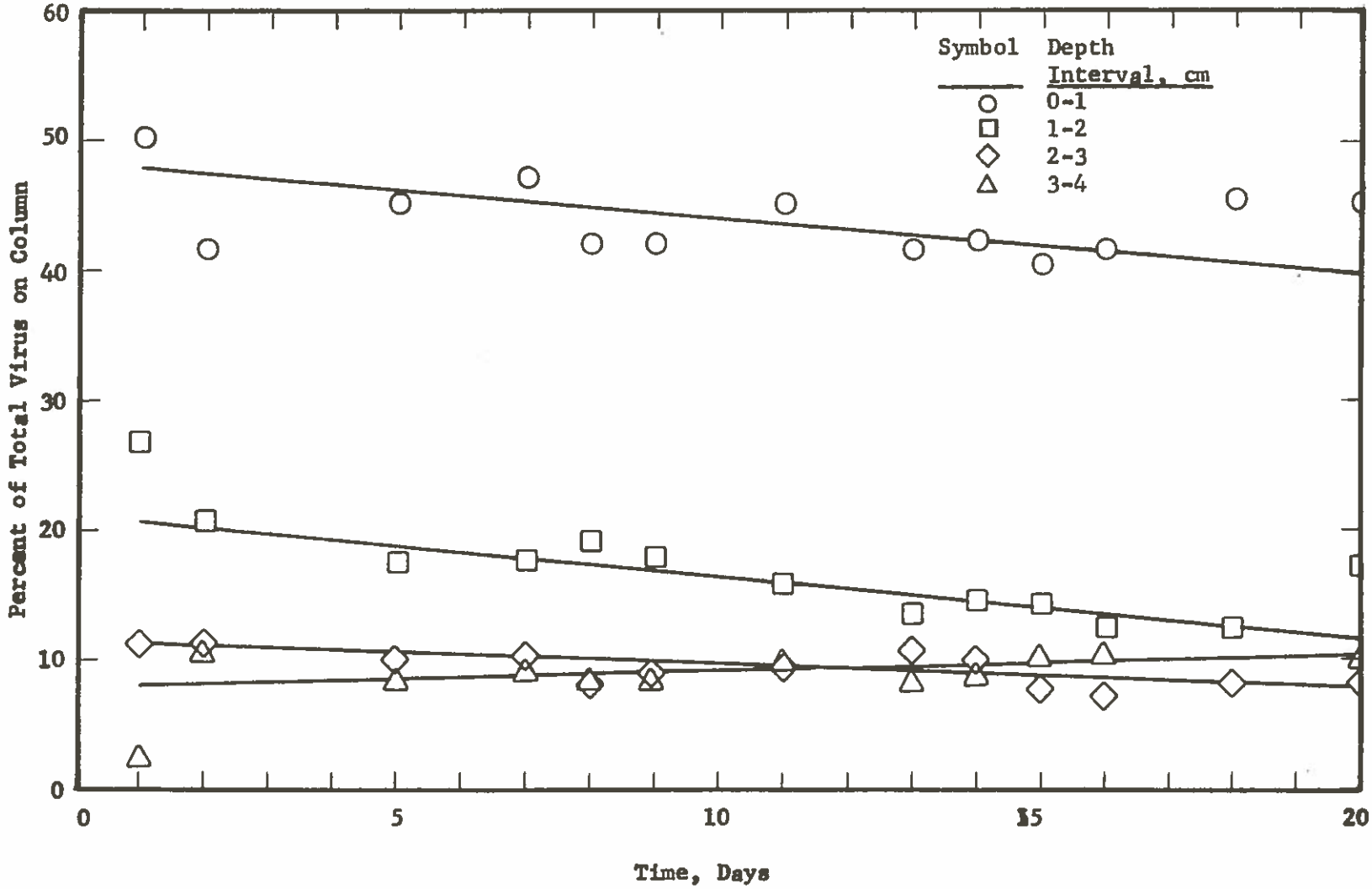

FIGURE 6. Virus Distribution on Soil Column No. 5.

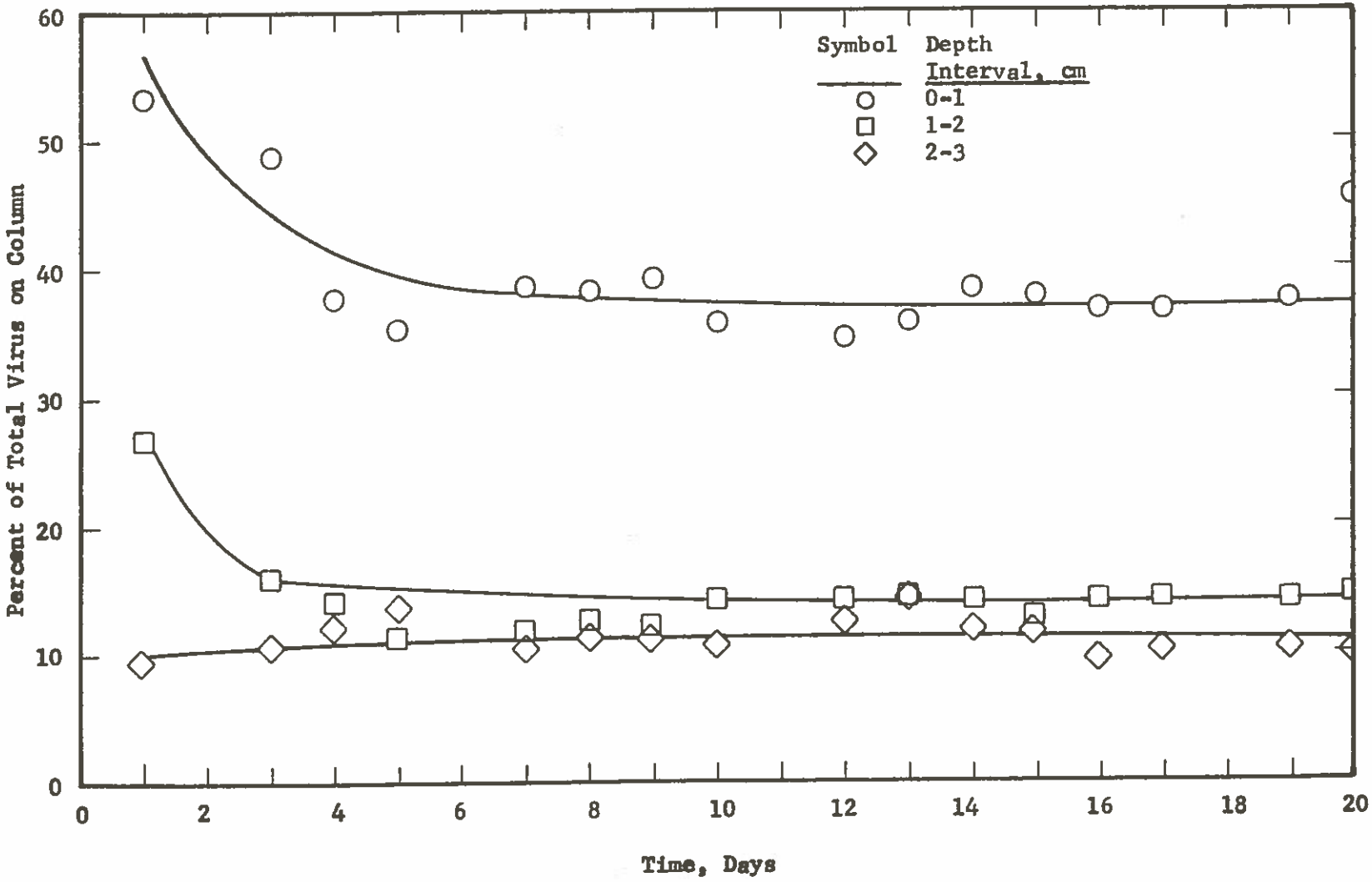

FIGURE 7. Virus Distribution on Soil Column No. 6.

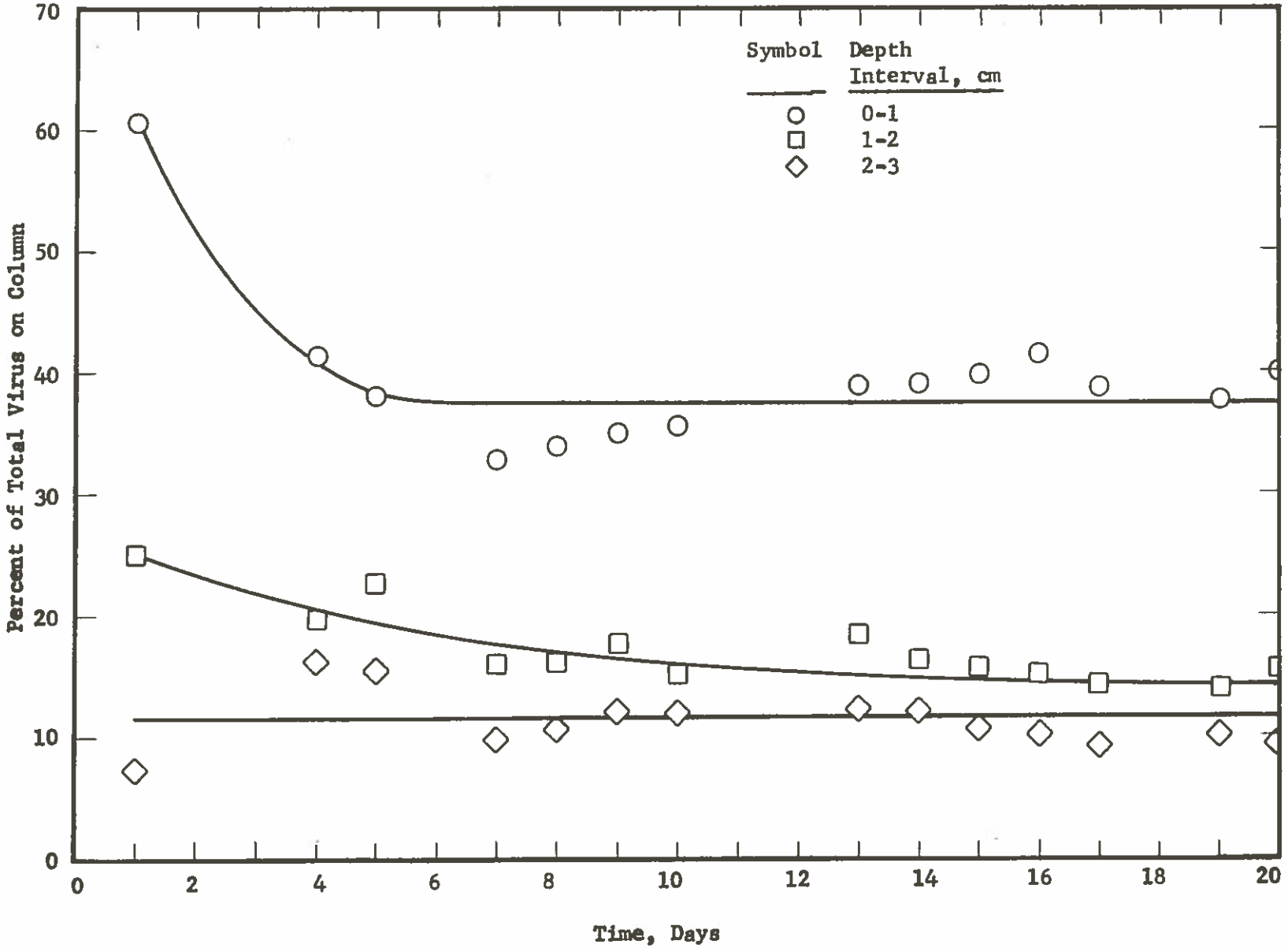

FIGURE 8. Virus Distribution on Soil Column No. 7.

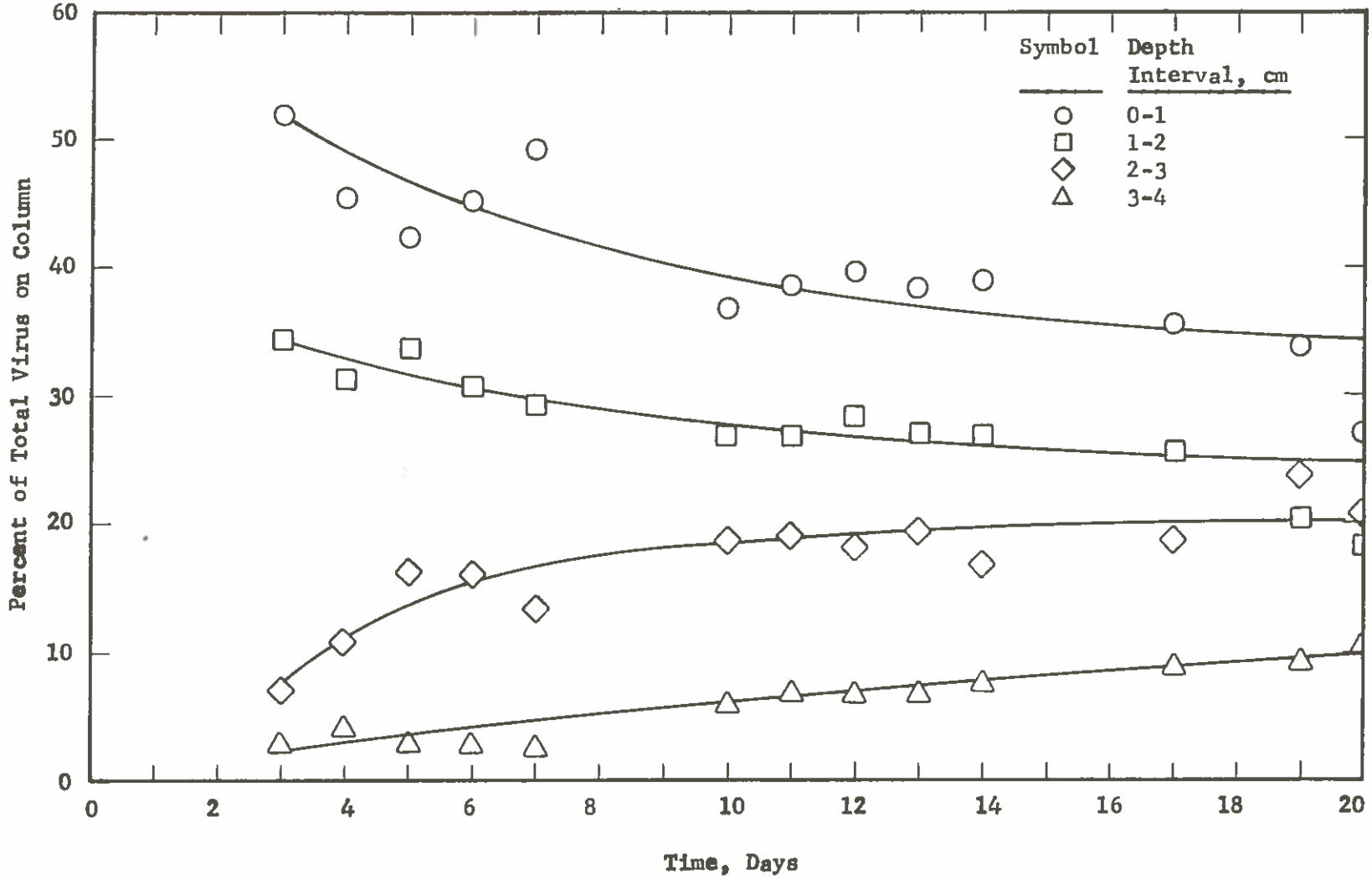

FIGURE 9. Virus Distribution on Soil Column No. 8.

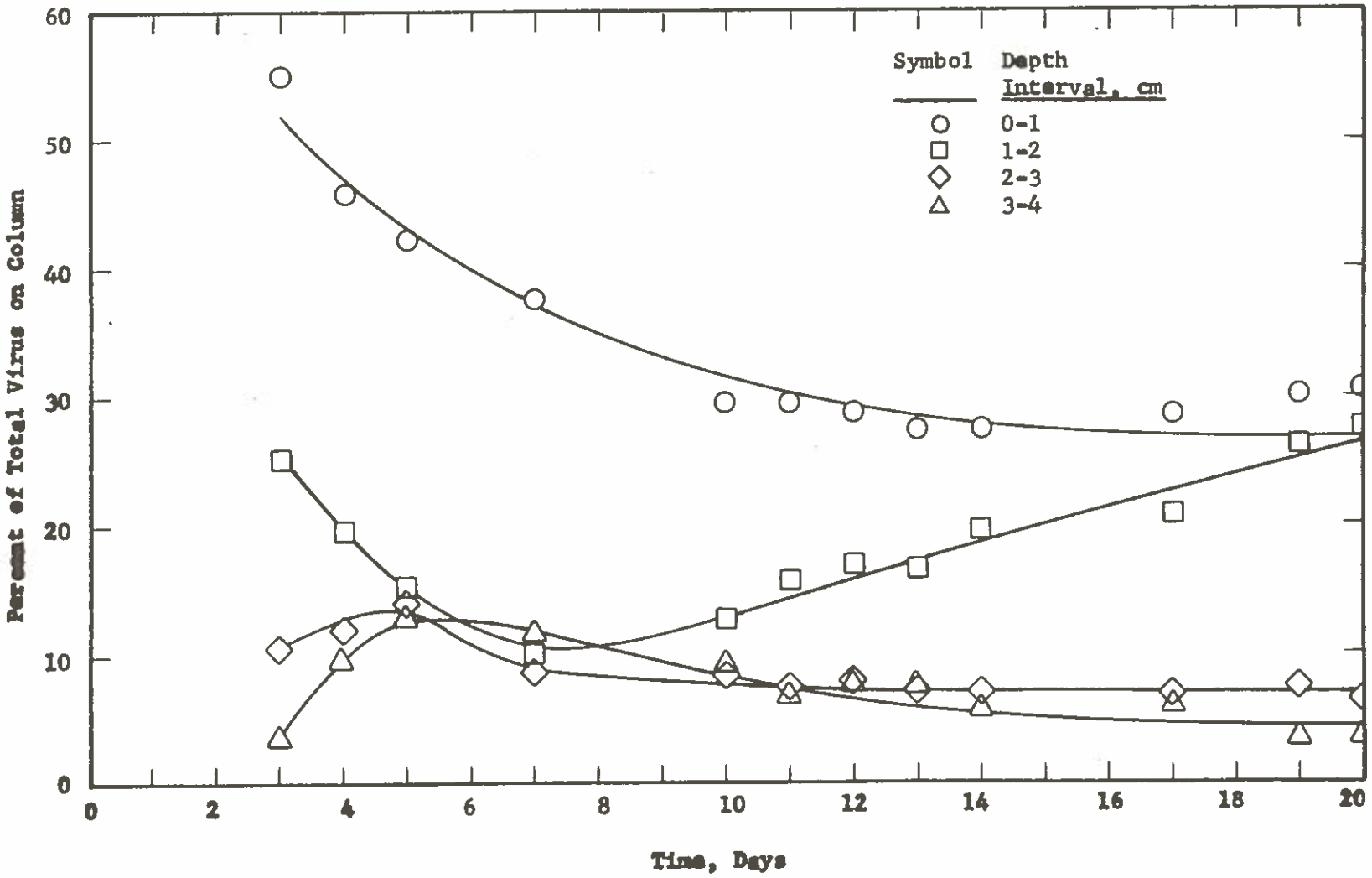

FIGURE 10. Virus Distribution on Soil Column No. 9.

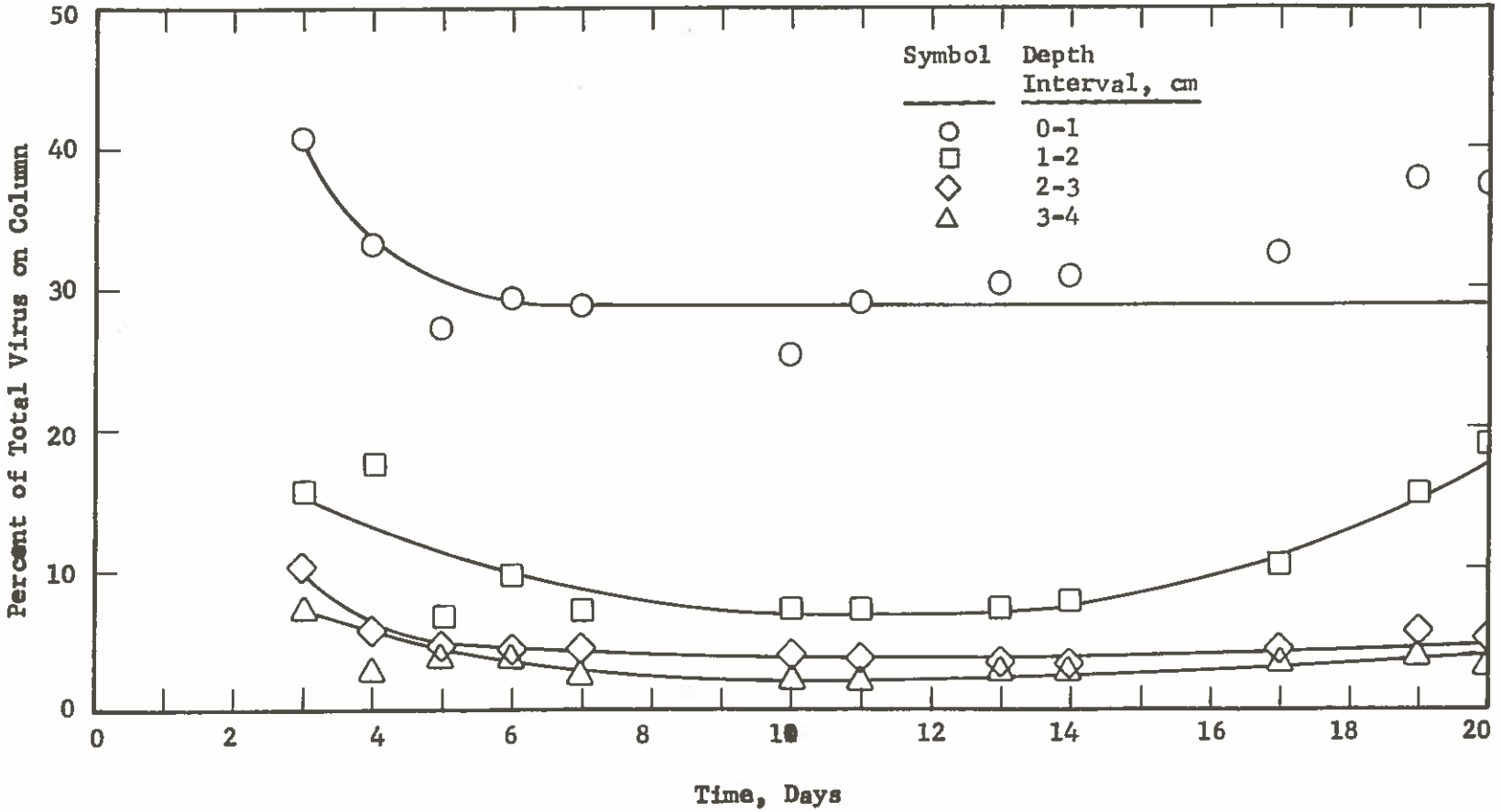

FIGURE 11. Virus Distribution on Soil Column No. 10.

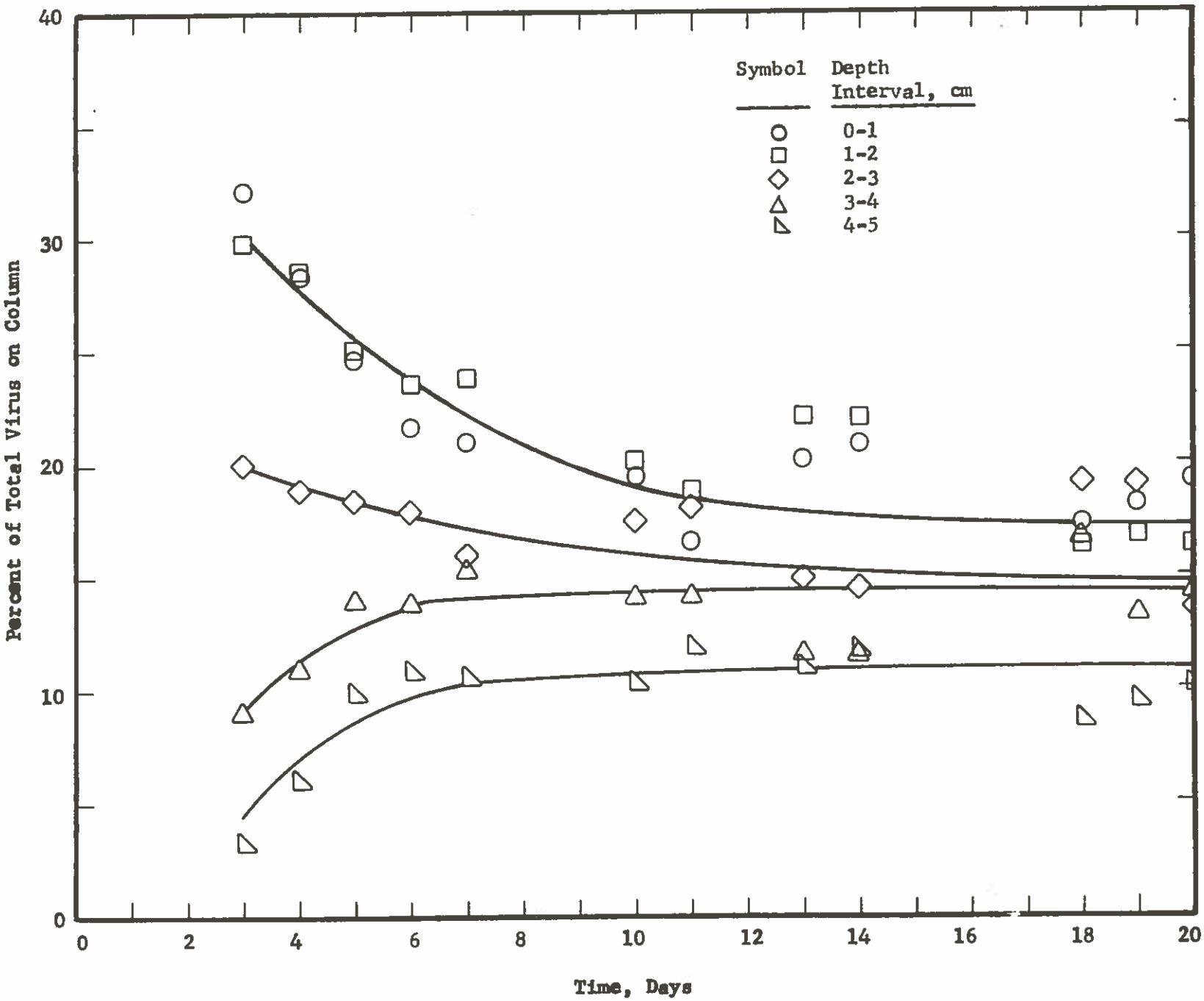

FIGURE 12. Virus Distribution on Soil Column No. 11.

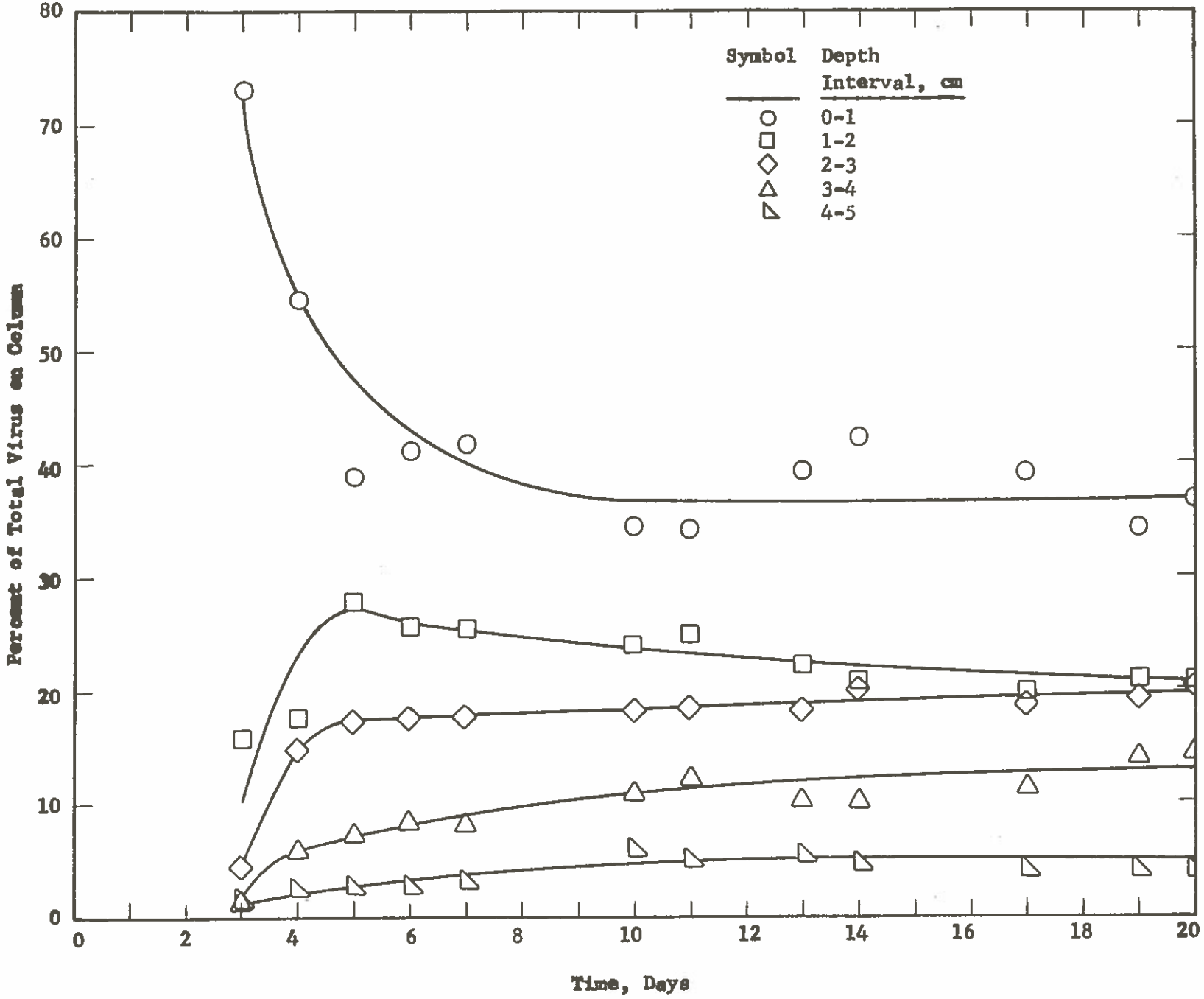

FIGURE 13. Virus Distribution on Soil Column No. 12.

When the effluent characteristics of Columns 5, 6, 7, and 8 (Table 41) are compared with the influent characteristics (Table 1) it is seen that passage through a few centimeters of soil is also an efficient process for removing other wastewater contaminants. Also, as shown in the static test results, use of a highly polluted wastewater did not significantly affect the ability of a soil to adsorb virus particles.

Flow rates through the various columns was quite varied as shown by the varying intervals between column dosings (see Table 16). The average flow rate between dosings was quite varied for each column. For example, the average rate for Column No. 10 varied from 0.008 cu m/day/sq m to 0.041 cu m/day/sq m. The lowest average rate for all columns was 0.0037 cu m/day/sq m for Column No. 4 while the highest average rate for all columns was 0.0406 cu m/day/sq m for Columns 2 and 10. This high rate is below the desired minimum percolation rate of about 1 inch per hour (0.62 cu m/day/sq m) according to many studies (8,9,10). However, Robeck, et al (11) indicate that suitable rates may be as low as 0.12 cu m/day/sq m. It is not expected that higher flow rates would significantly affect the virus retention capacity of the soils used in this study. Drewry and Eliassen (3) obtained similar results using California soils under saturated flow conditions with flow rates as high as 0.41 cu m/day/sq m. Also, it should be noted that higher flow rates than those attained in this study would probably be attained under field conditions with the same soil types. Lower laboratory

flow rates are caused by the increased bulk density in the soil columns brought about by grinding and repacking the soil.

On the basis of the results of this and other studies (2,3) it would appear that where a continuous stratum of common soil exists between the drain field of septic tanks and the water supply well that usual public health practices are more than adequate for protection from viral pollution of water. Normal practice calls for placing water supply wells 100 to 150 feet upstream from septic tank and cesspool drain fields.

CHAPTER V

CONCLUSIONS

1. Bacteriophage f2 can serve as a useful model for animal viruses in general and can serve as a useful tool in developing an understanding of virus movement through porous media.

2. Virus retention by soils is an adsorption process and is affected by many properties of the soil-water system.

3. Virus adsorption by soils is greatly affected by the pH, ionic strength, and soil-water ratio of the soil-water system and soil properties themselves. However, the effect of increasing or decreasing any one of these soil-water system parameters is not predictable with any degree of certainty for soils in general. Also, one cannot predict the relative virus adsorbing ability of a particular soil based on the various tests which are normally used to characterize a soil.

4. There appears to be no greater or lesser movement of virus through soils with a highly polluted water than with a non-polluted water.

5. Virus movement through a continuous stratum of common soil under gravity flow conditions and with intermittent dosing should present no health hazard if usual health practices relating to locating water supply wells are enforced.

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