# Effects of Environmental Variables and Soil Characteristics on Virus Survival in Soil

CHRISTON J. HURST,[1] CHARLES P. GERBA,[1]* AND IRINA CECH[2]

*Department of Virology and Epidemiology, Baylor College of Medicine,[1] and University of Texas School of Public Health,[2] Houston, Texas 77030*

Because of the increasing emphasis placed upon land application as a means of wastewater disposal, it is important to evaluate the influences of different factors upon virus survival in soil. The objective of this study was to measure the effects of various environmental variables on virus persistence. Test samples of soil were placed in vials, and the soil was wetted with suspensions of virus in either distilled water, unchlorinated secondary sewage effluent, or mixtures of effluent and water. The viruses used were coxsackieviruses A9 and B3, echovirus 1, poliovirus 1, rotavirus SA11, and bacteriophages T2 and MS2. The rate of virus inactivation was evaluated statistically with regard to conditions under which the vials were incubated and to the soil characteristics. The factors that were found to influence virus survival were temperature, soil moisture content, presence of aerobic microorganisms, degree of virus adsorption to the soil, soil levels of resin-extractable phosphorus, exchangeable aluminum, and soil pH. Overall, temperature and virus adsorption to soil appeared to be the most important factors affecting virus survival.

Viruses contained in wastewater that is applied to soil can persist in the environment for prolonged periods of time. Indigenous enteroviruses have been isolated from soils of rapid infiltration basins receiving primary and secondary sewage effluents (7). Indigenous enteroviruses have also been shown to survive for 28 days in soil of a cypress dome after application of secondary sewage effluent onto the dome soil (17). Seeded polioviruses were shown by Tierney et al. (16) to survive in soil after irrigation with effluent for 11 days during the summer and 96 days during the winter. Because viruses can persist in soil, the land disposal of wastewater poses a potential risk of resultant human illness. To evaluate the extent of this risk, survival of viruses in soil must be carefully studied and the factors affecting virus persistence in soil must be elucidated.

A few studies have been conducted concerning the effects of environmental conditions on survival of viruses in soil. Murphy et al. (11) found that survival of mouse encephalomyelitis virus was greater in soil at neutral pH than in soil that had been adjusted to either pH 3.7 or pH 8.5, and Bagdasaryan (3) determined that the survival time of enteroviruses was shorter in loamy soil than in sandy soil. Lefler and Kott (9) observed that poliovirus and bacteriophage f2 survived equally well in sand saturated with distilled water, with distilled water containing cations, with tap water, and with oxidation pond effluent.

In general, poliovirus appears to persist for longer periods of time at 4 to 8°C than at 20 to 37°C (9, 13, 18). Virus survival also appears to be generally greater under sterile conditions than under nonsterile conditions, although these tests have so far been run only under aerobic conditions (3, 11). No definite reason has been given for the apparent difference in virus survival in soil aerobically under sterile versus nonsterile conditions; however, Bagdasaryan (3) concluded that the difference was so small as to hardly be attributable to the antagonistic effect of soil microflora. Published reports concerning the effects of soil moisture upon virus survival are conflicting and indicate no clear trend (3, 9, 13, 18).

The purpose of this study was to compare and statistically evaluate the effects of these factors upon virus survival in one system. Experiments were performed to compare the possible antagonistic effects of soil and sewage effluent microflora upon virus survival under anaerobic as well as aerobic conditions, to examine the effects of temperature and of soil moisture upon virus survival, and to examine the possible role(s) which soil properties play in persistence of viruses.

## MATERIALS AND METHODS

**Cells and cell cultures.** Buffalo green monkey (BGM) kidney cells, a continuous green monkey kidney cell line, were used for growth of virus stocks and assay of viruses. These cells were cultured in Eagle

minimum essential medium (14) supplemented with 10% fetal bovine serum, 5% tryptose-phosphate broth, 0.03% glutamine, 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 0.15% sodium bicarbonate. Maintenance medium was the same as supplemented minimum essential medium but with a reduction in fetal bovine serum to 2% and an increase in sodium bicarbonate to 0.25%.

MA-104 cells, a continuous fetal rhesus monkey kidney cell line, were used in the propagation and assay of simian rotavirus SA11. These cells were grown in Eagle minimum essential medium supplemented with 10% fetal bovine serum, 5% tryptose-phosphate broth, 2% essential vitamins, 0.25% glucose, 0.03% glutamine, 0.075% sodium bicarbonate, 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 25 μg of gentamicin per ml.

**Virus and virus assays.** The enteroviruses used included plaque-purified strains of poliovirus 1 (strain LSc-2ab), echovirus 1 (strain Farouk), coxsackievirus A9 (strain Bozek), and coxsackievirus B3 (strain Nancy). Enterovirus stocks were grown in BGM cells. Thirty-two-ounce (ca. 960-ml) bottles of BGM cells were inoculated at a multiplicity of infection of 0.2 to 1.0 and incubated for 1 h at 37°C, and maintenance medium without serum was added back to the bottles. Upon the appearance of 4+ cytopathic effect, bottles containing virus were rapidly frozen and thawed three times, and cell debris was centrifuged out. Virus stock was stored at −20°C until use. Enterovirus assays were done by the plaque-forming unit method as described by Melnick and Wenner (10). When necessary, enterovirus samples were diluted in Tris buffer [containing (per liter) 3 g of tris(hydroxymethyl)aminomethane, 8 g of NaCl, 0.38 g of KCl, 0.1 g of $Na_2HPO_4$, 1 g of dextrose, 0.1 g of $MgCl_2$, 0.1 g of $CaCl_2$, and 0.002 g of phenol red and (per ml) 100 U of penicillin and 100 μg of streptomycin].

Simian rotavirus SA11 stocks were MA-104 infected cell lysates prepared by freeze-thawing, sonication, and low-speed centrifugation. SA11 virus was assayed by the plaque-forming unit method of Smith et al. (15). Samples containing rotavirus were diluted in a 5% tryptose-phosphate broth solution (BBL Microbiology Systems, Cockeysville, Md.). When necessary for preventing growth of contaminating microorganisms, nystatin (E. R. Squibb & Sons, Princeton, N.J.) was added to the agar overlay medium used for both enteroviruses and rotavirus at a final concentration of 100 U/ml. Neomycin sulfate (E. R. Squibb & Sons) was also added to the agar overlay medium when necessary at a final concentration of 10 μg/ml.

Two different coliphages were used: MS2 and its host *Escherichia coli* ATCC 15597, obtained from the American Type Culture Collection, Rockville, Md., and T2 along with its host *E. coli* B, obtained from G. Schaiberger (University of Miami, Miami, Fla.). Both host bacteria were maintained in culture on Trypticase soy broth (BBL).

Bacteriophage stocks were prepared by the following procedure. Agar plates confluent with plaques were overlaid with 5 ml of Trypticase soy broth. After 3 h at room temperature, the plates were gently swirled several times and the broth was decanted. The sus-

pension of free bacterial cells was then centrifuged at 1,600 × *g* for 15 min to sediment the bacterial cells. The supernatant fluid was passed through an 0.45-μm-pore-size HA membrane filter (Millipore Corp., Bedford, Mass.) which had previously been washed with sterile Trypticase soy broth. Samples (0.4 ml) of the filtrate were stored in ampoules at −70°C until used in the experiments.

The bacteriophages were assayed essentially by the procedure described by Adams (1). The bottom agar for the phage assays consisted of Trypticase soy broth containing 15 g of agar per liter. The top agar for the phage assays consisted of Trypticase soy broth containing (per liter) 7 g of agar and 1 ml of 1 M $CaCl_2$. The plating procedure consisted of mixing together 3 ml of top agar, 0.1 ml of virus sample, and 1 ml of log-phase host cell suspension. This mixture was then spread over a layer of bottom agar. Bacteriophage plaques were counted after 24 h of incubation at 37°C.

**Soils.** The main soil used in the virus survival studies was a loamy sand obtained from the Salt River bed near Phoenix, Ariz. (FM soil). This soil is identical to that used in the rapid infiltration beds at the Flushing Meadows experimental wastewater land treatment site and has been used previously for studying virus removal during filtration of wastewater by soil (8). Several other soils were also used for a portion of the experiments. These soils were Vernon, Clarita, Windhorst, Chigley, unclassified soil sample, Pomello fine sand, Anthony, and Rubicond. The latter eight soil samples were obtained through the courtesy of J. F. McNabb and C. G. Enfield (U.S. Environmental Protection Agency, Ada, Okla.) and have been characterized extensively (4). The characteristics of the nine soils are presented in Table 1.

**Experimental procedure for determining virus survival in soil.** The procedure used for the laboratory studies on virus survival was as follows. Two-gram amounts of soil were placed into 4-dram (16-ml), screw-capped glass vials, and the soil was then wetted with virus suspended in either unchlorinated secondary sewage effluent, distilled water, or a mixture of effluent and water. The vials were loosely capped and either incubated in GasPak anaerobic jars containing generated $H_2$-$CO_2$ atmosphere (GasPak generators and anaerobic jars purchased from BBL), with wet paper towels to provide humidity and prevent desiccation, or incubated aerobically in humid chambers constructed of plastic tubs partially filled with wet, crushed rock and covered tightly with aluminum foil. Before placement in the aerobic humid chambers, the crushed rock was washed with tap water to remove extraneous organic material and autoclaved for 1 h to prevent growth of mold during the experiment.

Three survival experiments were performed. The design for the first study involved soil wetted with either distilled water, unchlorinated sewage effluent, or a mixture of 25 or 50% sewage effluent in distilled water. For the first study, half of the water-effluent combinations were sterilized by autoclaving before addition of the virus, and the vials containing soil were also sterilized by autoclaving before addition of the virus suspensions. Incubation of one-third of the vials containing virus was done at each of the temperatures

TABLE 1. *Characteristics of soils used in this study*

| Soil type | Virus adsorption (%) | | | Surface area (m²/g) | Clay (%) | Silt (%) | Sand (%) | Organic matter (%) | Saturation pH | Cation exchange capacity (meq/100 g) | Conductivity (μmohs/cm) | Phosphorus | | Iron | | Aluminum | | Calcium | | Magnesium | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Poliovirus 1 | T2 | MS2 | | | | | | | | | Total (ppm) | Resin-extractable (ppm) | Total (%) | Ex-changeable (ppm) | Total (%) | Ex-changeable (ppm) | Total (%) | Ex-changeable (ppm) | Total (%) | Ex-changeable (ppm) |
| Vernon | 100 | 100 | 100 | 84 | 39 | 13 | 48 | 0.3 | 4.5 | 32.0 | 420 | 15 | 0.7 | 1.8 | 3.4 | 4.8 | 340 | 0.24 | 1,430 | 0.37 | 300 |
| Anthony | 82 | 70 | 30 | 38 | 13 | 10 | 77 | 0.3 | 8.2 | 4.2 | 305 | 265 | 72 | 2.0 | 2.8 | 5.5 | <50 | 0.80 | 1,260 | 0.40 | 150 |
| Rubicond | 56 | 64 | 34 | 18 | 4 | 4 | 92 | 0.4 | 5.5 | 5.6 | 90 | 147 | 2.5 | 0.7 | 5.0 | 1.5 | <50 | 0.18 | 260 | 0.09 | 6 |
| Windhorst | 94 | 100 | 47 | 155 | 42 | 43 | 15 | 1.4 | 4.9 | 53.0 | 320 | 110 | 2.8 | 3.5 | 5.2 | 5.8 | 70 | 0.46 | 3,080 | 0.56 | 860 |
| Chigley | 99 | 86 | 39 | 52 | 28 | 13 | 59 | 1.4 | 8.0 | 23.0 | 410 | 382 | 7.8 | 2.0 | 4.0 | 5.1 | <50 | 5.80 | 2,340 | 1.52 | 340 |
| FM | 100 | 36 | 17 | | 3 | 8 | 89 | 0.9 | 7.8 | | | | | | | | | | | | |
| Pomello fine sand | 42 | 8 | 69 | | 3 | 8 | 89 | 3.6 | 7.1 | 6.5 | | | | | | | | | | | |
| Clarita | 95 | 90 | 17 | 203 | 54 | 20 | 26 | 4.2 | 7.1 | 71.0 | 450 | 255 | 6.5 | 3.1 | 0.8 | 6.0 | <50 | 0.92 | 1,400 | 0.73 | 940 |
| Unclassified sample | 100 | 95 | 56 | 105 | 36 | 24 | 40 | 0.8 | 8.0 | 30.0 | 1,300 | 764 | 18 | 3.2 | 3.6 | 8.7 | <50 | 2.15 | 7,020 | 1.04 | 600 |

(1, 23, and 37°C). Half of the vials were incubated under aerobic conditions, and the other half were incubated under anaerobic conditions. In all, 48 different sets of incubation conditions were used. The initial virus titers for vials of FM soil wetted with either distilled water, sewage effluent, or diluted effluent were all $1.4 \times 10^4$ plaque-forming units per g for those samples containing poliovirus and $2.1 \times 10^5$ plaque-forming units per g for those samples containing echovirus. The soil moisture in this first study as well as the third study was 15% of the soil by wet weight. This moisture value was chosen because it was the average that we have obtained for soil samples in field studies on virus survival. FM soil was chosen for this study because it is currently being used for the rapid infiltration of sewage effluent.

For the second survival study, virus was incubated aerobically at 23°C under nonsterile conditions in soil wetted with a 50% solution of sewage effluent. Five different sets of vials were used in this study, with the difference in incubation conditions being the moisture content within the vials. The amounts of moisture, expressed as percentages of wet weight of the vial contents, were 5, 10, 15, 25, and 40%. At soil moisture contents up to 15%, no freestanding liquid was present in the vials. Some freestanding liquid was present at 25% moisture, and a much greater amount was present at a moisture content of 40%. Incubation of all vials was done under nonsterile aerobic conditions at 23°C.

The third survival study entailed comparisons between the survivals of poliovirus 1, echovirus 1, coxsackieviruses A9 and B3, rotavirus SA11, and bacteriophages T2 and MS2 in the nine different soils. For this study, all soil samples were adjusted to 15% moisture by addition of a 50% solution of sewage effluent, and incubation was done under nonsterile conditions at 23°C.

For all of these laboratory studies a group of 10 to 15 replicate vials with identical conditions were incubated together so that on each sampling date one of each type of vial was removed intact, and the contents were diluted in Tris buffer and frozen at −20°C until assayed. Dilution of the vial contents before freezing was accomplished by the addition of 4 ml of Tris buffer containing 2% fetal bovine serum for soil containing enteroviruses or serum-free buffer for samples containing rotavirus or bacteriophages. Assay of the samples was by direct inoculation.

Two steps were used in analysis of the survival data (Fig. 1). The first step was to determine slope values of the virus survival curves as a function of time. Virus survival was expressed as the logarithm of the ratio $N_T/N_0$ (called the survival ratio), where $N_0$ equals the virus titer at the beginning of the experiment and $N_T$ equals the virus titer at elapsed time $T$. A $\log_{10} N_T/N_0$ value of −1 equals 10% of the initial titer, −2 equals 1% of the initial titer, and −3 equals 0.1% of the initial titer. The slopes of the survival curves were determined by regression analysis, using the Statistical Package for the Social Sciences (SPSS) computer program (12). The survival data appeared, generally, to be well represented by the linear equation $y = at + b$, in which $t$ is time in days and coefficients $a$ and $b$ are, respectively, the slope and the intercept as
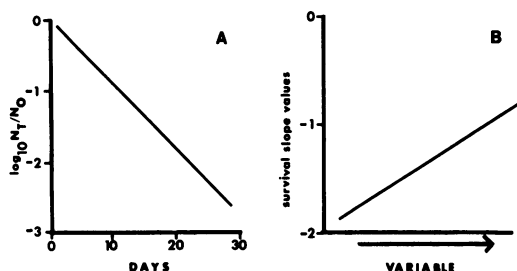
FIG. 1. *Format used for regression analysis. (A) Survival slope determination—survival slopes expressed as the rate of $log_{10}$ change in virus titer per day. (B) Survival slope values versus independent variable—the independent variables used in this stage of the analysis represented experimental conditions or soil characteristics. The example shown in (B) indicates a positive correlation between the variable and virus survival.*

determined by least-squares analysis. The second step was to evaluate by stepwise regression the effects on survival slope value of the experimental variables temperature, level of sewage effluent, aerobic or anaerobic atmosphere, sterile or nonsterile environment, soil moisture content, virus adsorption to soil, and soil characteristics. This step involved the plotting of bivariate scatter diagrams and computation of a stepwise multiple regression. In this analysis the survival slope values were taken as the "response" or "dependent" variables ($y$), which were expected to be functionally dependent on incubation conditions and soil characteristics (denoted by $x_1, x_2, \ldots x_n$). The multiple regression model was: $y = c + a_1x_1 + a_2x_2 + \ldots a_nx_n$. A $P$ value was calculated in each step of the regression, reflecting the statistical significance of the regression coefficients of each entry. For references on computational procedures used, the reader is referred to chapters 18 and 20 of the SPSS manual (12).

## RESULTS

**Effects of environmental conditions on poliovirus survival in FM soil.** The survival results for various experimental conditions are shown in Fig. 2 through 5 on a time scale for various concentrations of sewage effluent used to wet the soil. It appeared from these plots that temperature had a large effect on survival of the virus, with inactivation rates increasing as temperature increased. It also appeared that virus survival under aerobic nonsterile conditions was less than that occurring under the other three sets of conditions—aerobic sterile and both anaerobic sterile and anaerobic nonsterile. To further examine the possible difference between virus survival under aerobic nonsterile conditions versus the other sets of conditions, the data were plotted with respect to temperature. The graph of virus survival at 23°C (Fig. 6) indicated that virus survivals under both anaerobic sterile and anaerobic nonsterile conditions as well as

under aerobic sterile conditions were similar. Virus survival under aerobic nonsterile conditions was distinctly lower than those under the other three sets of conditions. Thus, the presence of aerobic microorganisms appears to have deleteriously affected virus survival. Anaerobic microorganisms, at least those which existed in the soil and sewage used in this study and under an $H_2$-$CO_2$ atmosphere, did not appear to affect virus survival. The influence of aerobic microorganisms was also evident, but to a lesser degree, with samples incubated at 37°C. This apparently reduced degree of influence of aerobic microorganisms at 37°C may have been due to the more pronounced influence of thermal effects upon virus survival at that temperature. There was no discernible effect of aerobic microorganisms upon virus survival for samples incubated at 1°C, possibly as a result of the much slower growth and metabolism rates of the microorganisms at this lower temperature.

In the first step of regression analysis of the data from this survival study, the rate of change in virus titer, expressed as $log_{10} N_T/N_0$, appeared to be linear with time. Exceptions occurred in some instances when virus inactivation was minimal over a long period of time, i.e., with incubation at 1°C. In these cases random errors in virus sampling and assaying, in addition to possible differences in evolution of the microbial environment inside the vials, made it difficult to obtain clearly significant slope values. In such instances the calculated slope values were taken to be statistically nondifferent from zero.

Bivariate scatter diagrams produced in the second step of the analysis evaluating effects of individual environmental factors on virus survival indicated the following: (i) the survival slopes and incubation temperatures (Fig. 7) showed a statistically significant reciprocal correlation ($P \leq 0.01$), indicating that virus survival decreased with increasing temperature, and (ii) the concentration of sewage effluent used to wet the soil did not correlate with virus survival ($P = 0.38$). These two findings held under all conditions of aerobic or anaerobic atmosphere and sterile or nonsterile incubation. The observed difference between survival slope values obtained for virus survival under aerobic nonsterile conditions versus those obtained under aerobic sterile conditions was not statistically significant ($P = 0.156$).

**Effects of soil moisture on poliovirus survival in FM soil.** Figure 8 is the scatter diagram of average survival slopes derived from the second virus survival study against soil moisture content. Linear correlation of survival slope values with soil moisture appears to be inadequate or inappropriate ($P = 0.03$) to describe the effect

of soil moisture on virus survival. It seems, how-ever, that there was a nonlinear trend inasmuch as virus survival decreased as the soil moisture content increased up to 15% and then increased with the presence of additional amounts of liq-uid. The saturation level of this soil appeared to be between 15 and 25%; thus, survival may have been at its minimum point near the soil moisture saturation point.

**Effects of soil characteristics on virus survival.** The results obtained from the study of the effects of soil characteristics on virus survival are plotted with respect to soil type in Fig. 9. In Fig. 9H, the survivals of four additional viruses, coxsackieviruses A9 and B3, echovirus 1, and rotavirus SA11 were compared with those of poliovirus 1 and the two bacteriophages in FM soil. The survivals of these four additional viruses were not studied in any of the other soils,

and, therefore, the survival slope values obtained for these viruses were not included in the step-wise multiple regression.

The analysis of bivariate scatter diagrams of the virus survival slopes versus various soil char-acteristics indicated that virus survival corre-lated significantly ($P \le 0.05$) with virus adsorp-tion to the soil and with soil saturation pH. The significance of resin-extractable phosphorus was marginal ($P = 0.056$). None of the other soil characteristics demonstrated a statistically sig-nificant correlation with virus survival. Loga-rithmic transformation of the independent vari-ables (the variable of soil pH was not trans-formed, since it is normally represented as a logarithm) did not substantially improve the fit between the soil characteristics and virus sur-vival.

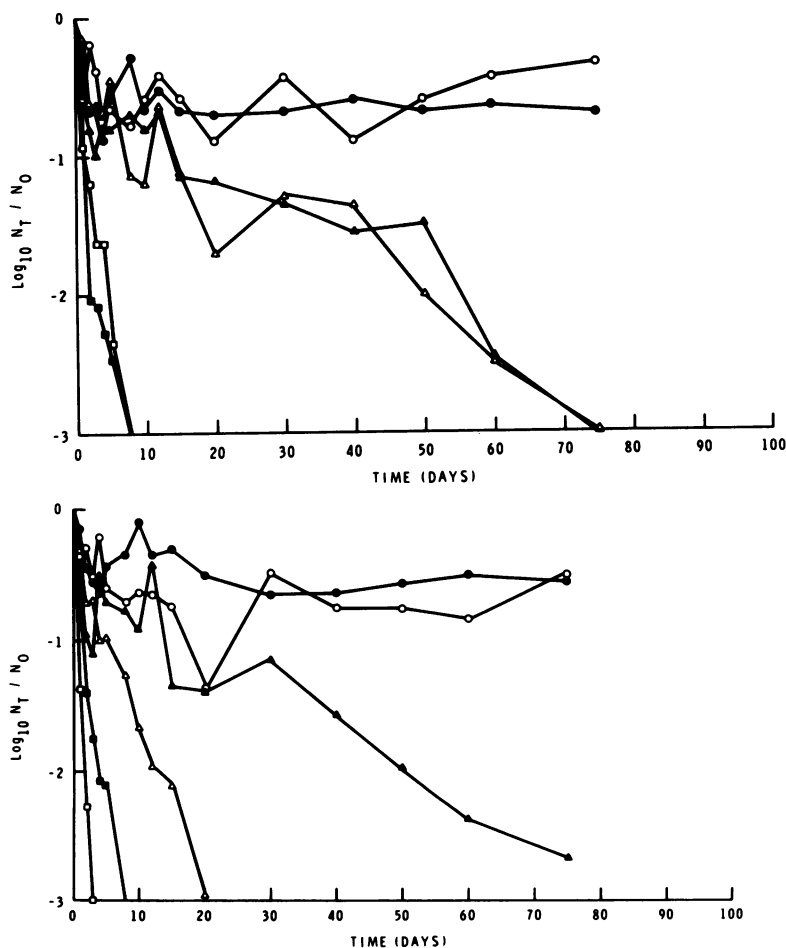Stepwise multiple regression analysis of the



FIG. 2. *Poliovirus 1 survival in FM soil wetted with distilled water under sterile (top) and nonsterile (bottom) conditions. Symbols:* O, *1°C, aerobic;* ●, *1°C, anaerobic:* △, *23°C, aerobic:* ▲, *23°C, anaerobic:* □, *37°C, aerobic;* ■, *37°C, anaerobic.*
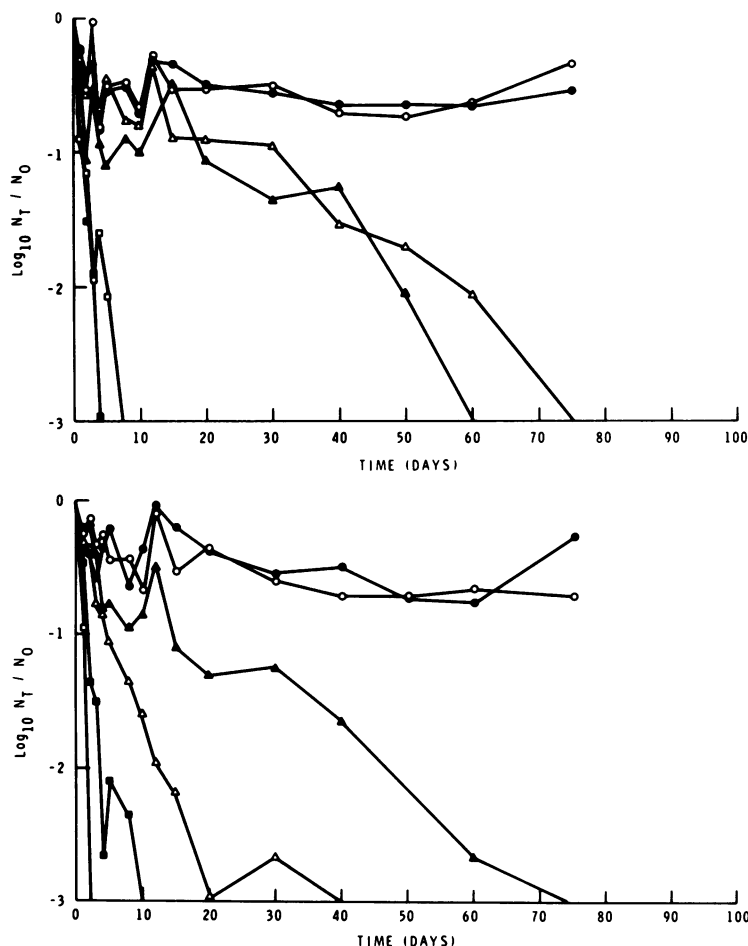
FIG. 3. *Poliovirus 1 survival in FM soil wetted with 25% sewage effluent under sterile (top) and nonsterile (bottom) conditions. Symbols: see legend to Fig. 2.*

virus survival slope values as dependent variables and soil characteristics listed in Table 1 as independent variables was then performed. Of the 19 soil characteristics listed in Table 1, the 10 that were ranked highest in the stepwise multiple regression analysis are presented in Table 2. Virus adsorption to soil accounted for 23% of the variance, extractable phosphorus accounted for an additional 11%, exchangeable aluminum accounted for about 4%, and pH accounted for about 13% (Table 2). In combination, these four variables accounted for a total of approximately 51% of all variance in the experiment.

None of the six variables listed below pH in Table 2 were statistically significant, and they represented a total of only 13% change in $R$ square. The final equation derived from these results thus contains only the first four variables, i.e., adsorption to soil, extractable phosphorus,

exchangeable aluminum, and soil pH. The bivariate scatter diagrams of the survival slope values obtained for incubation of the different viruses in the nine soil types versus these four soil characteristics are shown in Fig. 10.

## DISCUSSION

The results from evaluating effects of environmental variables on virus survival indicated that temperature was a significant predictor of virus survival under all sets of conditions. This is in agreement with the work of many research groups that have evaluated the survival of viruses in the environment. Virus survival was not significantly affected by the concentration of sewage effluent, a finding which held true for both sterile and nonsterile conditions, as well as aerobic and anaerobic incubation conditions. Our finding that the presence of sewage did not influence virus survival is of interest, since vi-
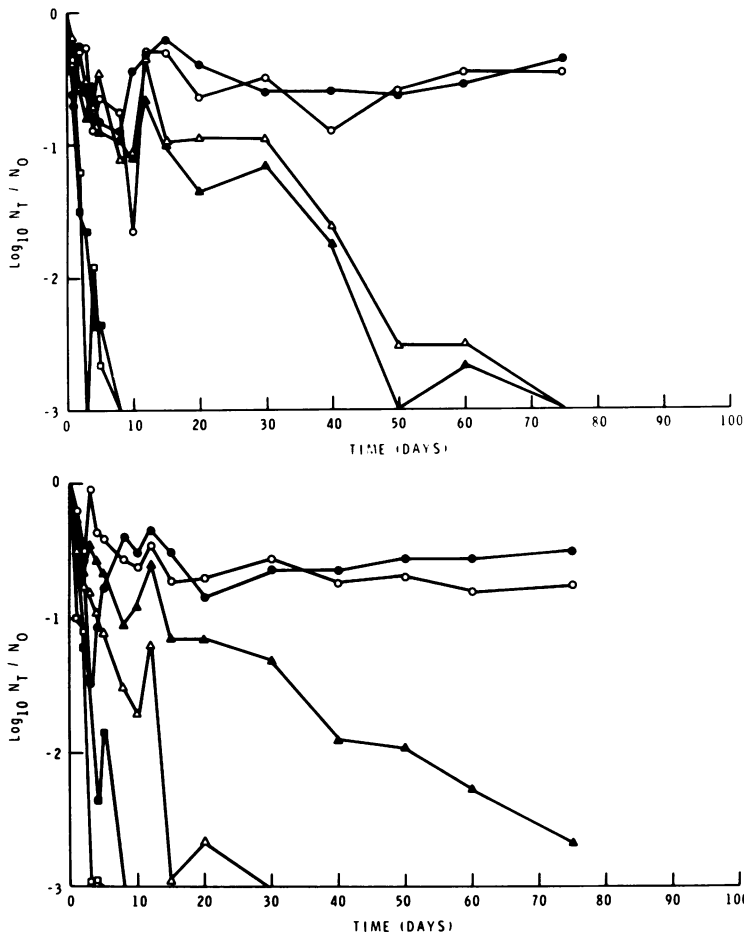
FIG. 4. *Poliovirus 1 survival in FM soil wetted with 50% sewage effluent under sterile (top) and nonsterile (bottom) conditions. Symbols: see legend to Fig. 2.*

ruses have been shown to survive longer in sewage-polluted water than in nonpolluted water (2), possibly due to the presence of organic matter contributed by sewage to the polluted water. Inasmuch as our results showed that sewage effluent did not substantially influence virus survival, we would like to suggest that the practice of diluting sewage effluent with other water before land application will, in itself, probably not affect virus survival in the soil.

The presence of aerobic microorganisms appeared to result in decreased virus survival (Fig. 2 through 6), although the results of a *t*-test performed on data evaluating poliovirus survival under aerobic nonsterile conditions, contrasted with aerobic sterile, anaerobic sterile, and anaerobic nonsterile conditions, indicated that these two groups of conditions were not significantly different statistically ($P = 0.156$). The results of a *t*-test to compare poliovirus survivals

under aerobic nonsterile versus only aerobic sterile conditions likewise did not indicate statistical significance.

Virus survival did not correlate linearly with soil moisture content. The scatter diagram of virus survival versus soil moisture content did, however, appear to show a general trend; i.e., virus survival appeared to decrease as the soil moisture content increased up to the soil saturation point. Virus survival then increased as more liquid was added to the system beyond the saturation point. Possible reasons that could account for this apparently enhanced survival of virus at both low and high soil moistures would include soil moisture-level-dependent differences in the extent of virus adsorption to the soil and mechanisms of adsorption. Moisture-level-dependent differences in microbial growth rates might also affect virus survival.
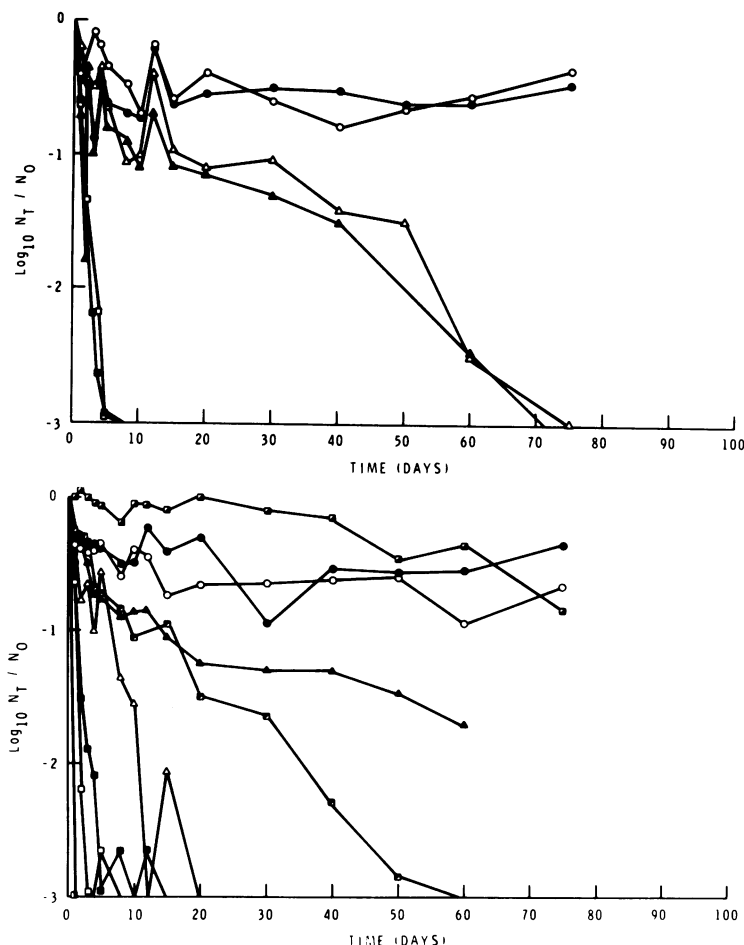
The multiple regression equation derived from

FIG. 5. *Poliovirus 1 and echovirus 1 survival in FM soil wetted with 100% sewage effluent under sterile (top) and nonsterile (bottom) conditions. Symbols: for poliovirus 1, see legend to Fig. 2; for echovirus 1,* ▲, *1°C, aerobic;* ◪, *23°C, aerobic;* ⊡, *37°C, aerobic.*

comparison of virus survival in the nine different soils includes virus adsorption to soil, soil resin-extractable phosphorus, soil exchangeable aluminum, and soil pH. These four variables accounted jointly for 51% of all variance in the survival data. Sources of variance in the experiment not related to soil characteristics included human error in sampling, variation in evolution of the microbial environment between the different vials, variation which naturally exists in the plaque assay method, and perhaps other factors presently unknown to us.

The fact that adsorption of viruses to soil significantly affected virus survival is of great importance. This finding indicates a dilemma insofar as virus inactivation during land treatment is concerned. On one hand, concern for public health would, of necessity, require that

land treatment sites be developed on soils with high virus adsorptive capacity. This is required to minimize the possibility of viruses applied to soil reaching groundwater. On the other hand, virus survival is likely to be greatest in those soils that would be most effective in preventing groundwater contamination. Because results from this study comparing virus survivals aerobically and anaerobically under nonsterile conditions showed that virus survival is apparently prolonged under anaerobic conditions, virus reaching the groundwater in an anaerobic environment might have a subsequently prolonged lifetime of infectivity.

The effect that soil pH was found to have upon virus survival might be mediated through virus adsorption to the soil. It was found in a recent study (6) that soil pH was the major

variable affecting adsorption of viruses to soil, with adsorption increasing as pH decreased.

Both soil exchangeable aluminum levels and resin-extractable phosphorus levels are important affectors of virus survival, as was evidenced by their position in the stepwise regression equa-

tion. Exchangeable aluminum only (that which would be soluble as cations in soil water), and not total aluminum, was indicated as being important in the stepwise regression equation. Previous work on concentration of virus from water has indicated that the presence of aluminum
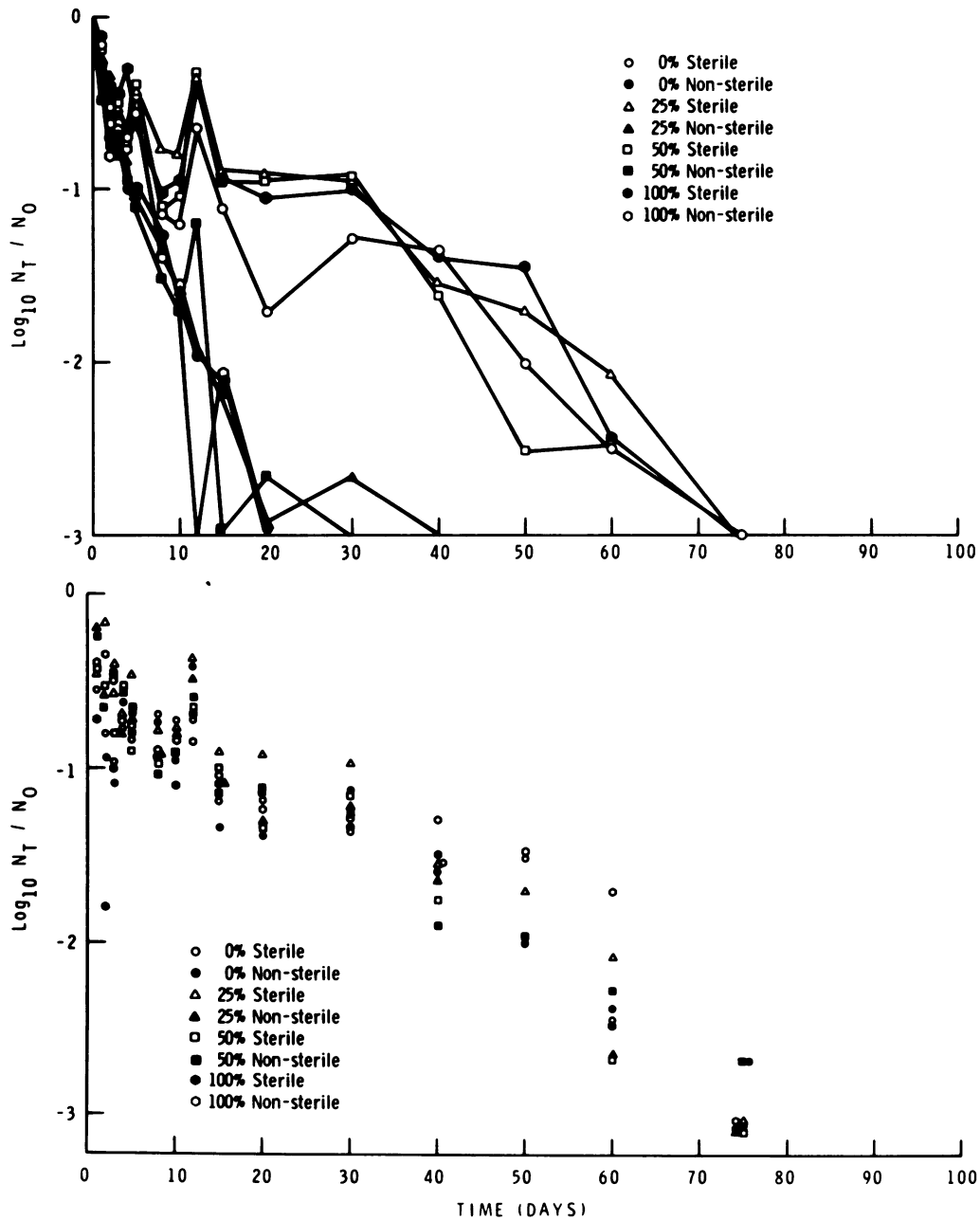


FIG. 6. *Poliovirus 1 survival in FM soil under aerobic (top) and anaerobic (bottom) conditions at 23°C.*

cations increases the adsorption of viruses to filters (5). In this role of aiding virus adsorption to filters, aluminum is much more effective than either magnesium or calcium salts, and neither exchangeable magnesium nor exchangeable calcium significantly affected the stepwise regression equation. Thus, the correlation between virus survival and soil exchangeable aluminum was possibly due to an increase in virus adsorption to the soil particles at higher aluminum levels.

The level of resin-extractable phosphorus in soil is in effect a measure of the amount of available phosphate anions in the soil. The addition of phosphate anions to a soil suspension can result in elution of adsorbed virus particles from soil (D. H. Taylor, A. R. Bellamy, and A. T. Wilson, Water Res., in press). The finding that virus survival increases as the level of resin-extractable phosphorus decreases may also have a relationship to previous work on concentration of virus from water. Virus has been shown to precipitate from water during alum and lime flocculation in relation to the precipitation of phosphorus. In the latter case, phosphate anions, when free in solution, may have acted to inhibit virus adsorption to the calcium and aluminum precipitates.

The soil variables of exchangeable aluminum, for which correlation with virus survival had a positive direction, and resin-extractable phosphorus, for which correlation with virus survival was reciprocal, were retained in the final model equation even though their individual correlation with the survival slopes was not statistically significant ($P < 0.05$). In combination with the two other soil characteristics of virus adsorption and soil pH, the above characteristics provide a



FIG. 8. Scatter diagram of the survival slope values versus soil moisture content from the second survival study; $R = -0.31$, significant at the 0.31 level.

TABLE 2. Stepwise regression equation for virus survival slopes versus soil characteristics

| Variable | Significance of regression coefficients | $R$ square | Overall significance of total model |
|---|---|---|---|
| Adsorption to soil | 0.010 | 0.235 | 0.010 |
| Extractable phosphorus | 0.056 | 0.345 | 0.006 |
| Exchangeable aluminum | 0.257 | 0.381 | 0.010 |
| Soil saturation pH | 0.026 | 0.508 | 0.003 |
| % Silt | 0.349 | 0.529 | 0.005 |
| % Organic matter | 0.265 | 0.558 | 0.007 |
| Conductivity | 0.177 | 0.599 | 0.007 |
| Surface area | 0.619 | 0.605 | 0.014 |
| Exchangeable calcium | 0.416 | 0.620 | 0.022 |
| Total cation-exchange capacity | 0.302 | 0.646 | 0.028 |

model capable of explaining 51% of the variance between the survival slopes and which is statistically significant ($P \leq 0.05$). Elimination from the four-variable equation of either exchangeable aluminum separately or of both variables (exchangeable aluminum and resin-extractable phosphorus) together resulted in loss of the significance of soil saturation pH in describing virus survival in the test soils. Although the results of the survival studies cannot prove this hypothesis, it would appear that the effects of soil pH upon virus survival are mediated through chemical equilibria, among which are the charge states and relative concentrations of aluminum and phosphorus.

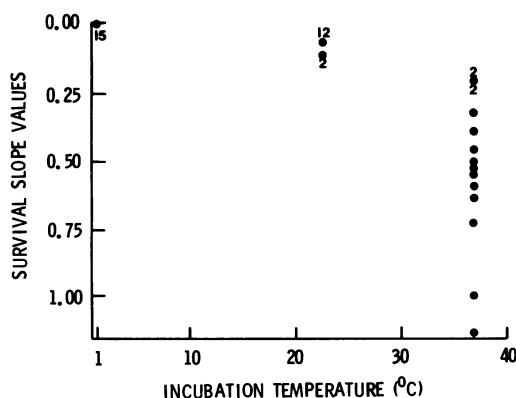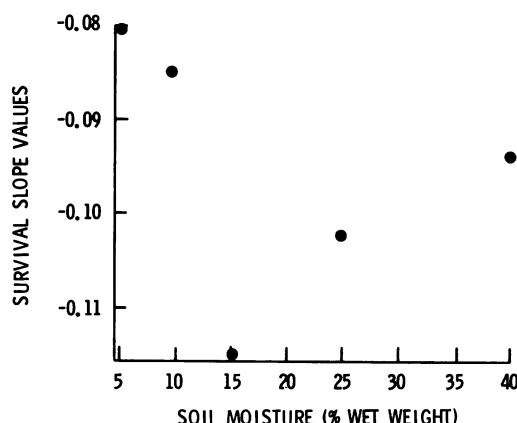The final stepwise multiple regression equation derived from this study is: $y = 0.1005 +$
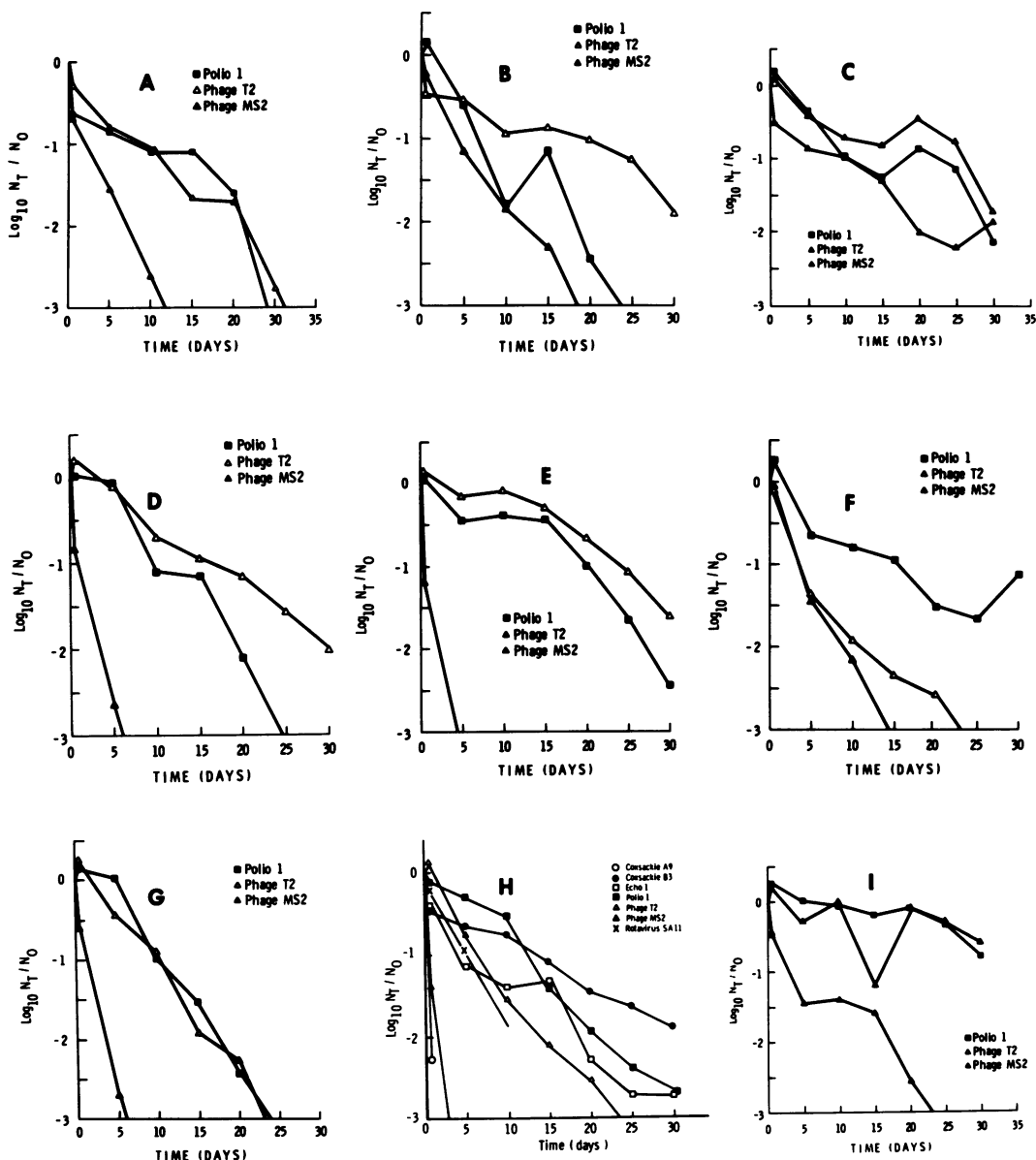


FIG. 7. Scatter diagram of the survival slope values versus incubation temperature from the first survival study; $R = -0.68$, significant at the 0.00001 level.

FIG. 9. *Comparative survivals of viruses in different soils. The soils tested were: (A) Vernon, (B) Clarita, (C) Windhorst, (D) Chigley, (E) unclassified soil sample, (F) Pomello, (G) Anthony, (H) FM, and (I) Rubicond.*

$0.0025x_1 - 0.0008x_2 - 0.0007x_3 - 0.0510x_4$, where, for a given soil, $y$ is the average of the survival slope values for the three viruses under the conditions of the experiment, $x_1$ is the average percent adsorption of all three viruses to the soil, $x_2$ is the resin-extractable phosphorus value (parts per million) for the given soil, $x_3$ is the exchangeable aluminum value (parts per million) for the given soil, and $x_4$ is the saturation pH value for the given soil.

It would be strenuous to use regression equations of the type developed in this study for actually predicting virus survival under natural field conditions of constantly changing temperature and soil moisture. Instead, the value of such derived equations lies in estimating virus survival in different soil types based upon their known physical properties.
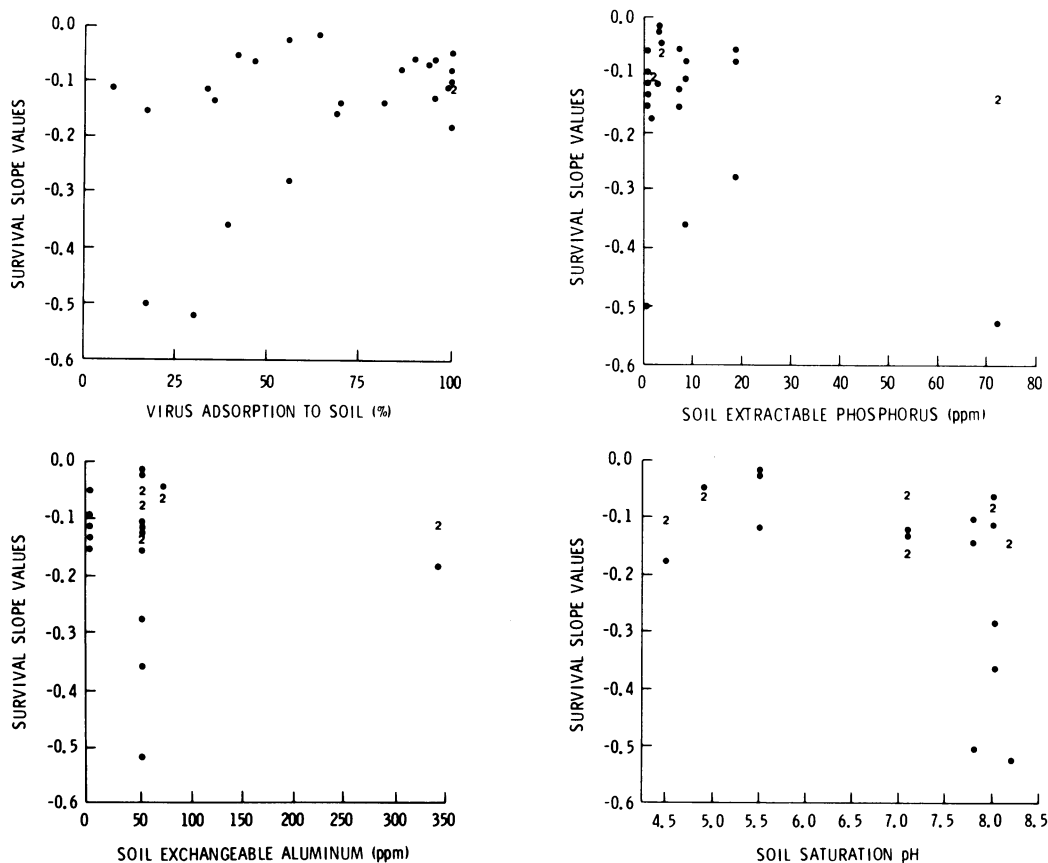
Fig. 10. *Scatter diagrams of the survival slope values versus soil characteristics from the third survival study. Virus adsorption to soil: R = 0.48, significant at the 0.0052 level. Soil extractable phosphorus: R = −0.34, significant at the 0.040 level. Soil exchangeable aluminum: R = 0.08, significant at the 0.34 level. Soil saturation pH: R = −0.39, significant at the 0.022 level.*

## ACKNOWLEDGMENTS

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