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Persistence of Viruses in Desert Soils Amended with Anaerobically Digested Sewage Sludge

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Pima County, Ariz., is currently investigating the potential benefits of land application of sewage sludge. To assess risks associated with the presence of pathogenic enteric viruses present in the sludge, laboratory studies were conducted to measure the inactivation rate (k = $\log_{10}$ reduction per day) of poliovirus type 1 and bacteriophages MS2 and PRD-1 in two sludge-amended desert agricultural soils (Brazito Sandy Loam and Pima Clay Loam). Under constant moisture (approximately -0.05×10^5 Pa for both soils) and temperatures of 15, 27, and 40°C, the main factors controlling the inactivation of these viruses were soil temperature and texture. As the temperature increased from 15 to 40°C, the inactivation rate increased significantly for poliovirus and MS2, whereas, for PRD-1, a significant increase in the inactivation rate was observed only at 40°C. Clay loam soils afforded more protection to all three viruses than sandy soils. At 15°C, the inactivation rate for MS2 ranged from 0.366 to 0.394 $\log_{10}$ reduction per day in clay loam and sandy loam soils, respectively. At 27°C, this rate increased to 0.629 $\log_{10}$ reduction per day in clay loam soil and to 0.652 in sandy loam soil. A similar trend was observed for poliovirus at 15°C (k = 0.064 $\log_{10}$ reduction per day, clay loam; k = 0.095 $\log_{10}$ reduction per day, sandy loam) and 27°C (k = 0.133 $\log_{10}$ reduction per day, clay loam; k = 0.154 $\log_{10}$ reduction per day, sandy loam). Neither MS2 nor poliovirus was recovered after 24 h at 40°C. No reduction of PRD-1 was observed after 28 days at 15°C and after 16 days at 27°C. At 40°C, the inactivation rates were 0.208 $\log_{10}$ reduction per day in amended clay loam soil and 0.282 $\log_{10}$ reduction per day in sandy loam soil. Evaporation to less than 5% soil moisture completely inactivated all three viruses within 7 days at 15°C, within 3 days at 27°C, and within 2 days at 40°C regardless of soil type. This suggests that a combination of high soil temperature and rapid loss of soil moisture will significantly reduce risks caused by viruses in sludge.

More than 120 different viruses are excreted in human feces and urine and may find their way into sewage (9). These viruses can cause a variety of symptoms and diseases including diarrhea, nausea, vomiting, poliomyelitis, hepatitis, and meningitis. An infected individual may excrete as many as 10^{10} viruses per g of feces and may continue shedding these viruses for up to 50 days (11). In raw sewage, the estimated average density of enteric viruses is approximately 7,000/liter (11). Because enteric viruses are known to concentrate in sludge, the fate of these viruses during sludge treatment and after land application is of importance. It has been estimated that half of the waterborne illnesses in the United States are due to microbial contamination of groundwater supplies (5).

Despite these statistics, there is little evidence of contamination of groundwater sources by viruses from sludge-amended soils (18). There are few studies which report the recovery and survival of indigenous enteroviruses from sewage sludge-amended soils. Pepper and Gerba (13) reported recovering viruses from a freshly amended field in Avra Valley, Ariz., but were unable to recover any infectious units 24 h after soil amendment. Van Sluis et al. (20) were unable to recover viruses from an experimental farm in Riverside, Calif., even though viruses were isolated in the sludge used to amend that soil. Sorber and Moore (17) were able to recover viruses from a sludge burial site in Montana 6 months after the last sludge disposal. Unfortunately, no initial sample was taken so that an initial inactivation rate could not be determined. In Florida during the dry season, Bitton et al. (4) showed that viruses were inactivated 8 days after sludge injection, suggesting that a combination of evaporation and warm (27°C) soil temperatures would significantly reduce risks of viral contamination of groundwater sources. In laboratory studies using conditioned chemical- and lime-stabilized sludges, Pancorbo et al. (12) showed that poliovirus adsorbed to the sludge solids and could not be mobilized from the sludge layer even with rainwater. This led to the conclusion that viruses immobilized in the sludge layer would be eventually inactivated and thereby not be expected to pose a threat of viral contamination of groundwater sources beneath sludge disposal sites.

Pima County, Ariz., located in the unique ecosystem of the desert Southwest, is currently investigating the benefits of land application of sewage sludge. Applied as liquid containing approximately 1 to 2% solids, sludge benefits the soil by both its fertilizer and irrigation values. The combination of arid conditions and soil temperatures in excess of 20°C at least 9 months (March through November) of the year may lead to the rapid inactivation of enteric pathogens. Currently, the U.S. Environmental Protection Agency (19) has proposed standards for the reduction of indicator pathogens during treatment of sewage sludge. To meet the requirements for viruses, a 97% or greater reduction and/or removal is needed. Despite this, the number of viruses in sludge after anaerobic digestion can be as high as 1,000/liter of liquid sludge (16). Considering the low infectious dose for virus and that approximately 72,000 liters of sludge are applied per ha, the number of viruses could pose a risk to public health if these viruses (as many as 10^7/ha) remained infectious and contaminated ground- and surface-water sources.

The objectives of this project were to determine the

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inactivation rates of three representative viruses seeded in sludge-amended soil by (i) soil type (sandy loam soil versus clay loam soil), (ii) soil moisture (constant versus desiccation), and (iii) soil temperatures typical of the arid southwestern United States.

MATERIALS AND METHODS

Growth and purification of poliovirus. Monolayers of Buffalo green monkey kidney (BGM) cells were grown in 32-oz. (0.946-liter) bottles at 37°C for 5 days, rinsed twice with 15-ml volumes of prewarmed (37°C) Tris-buffered saline (stock solution diluted 1:10; stock solution consisted of 63.2 g of Trizma base [Sigma Chemical Co., St. Louis, Mo.], 163.6 g of NaCl, 7.46 g of KCl, and 1.13 g of anhydrous Na_2HPO_4 dissolved in 1,600 ml of distilled water [final pH, 7.2]), and infected with stock poliovirus type 1 (LSc) to achieve an approximate multiplicity of infection of 0.01. Prewarmed tissue culture medium (minimal essential medium [MEM]; Irvine Scientific Co., Santa Ana, Calif.), supplemented with 2% fetal calf serum, glutamine (200 mM), kanamycin (100 μg/ml), penicillin or streptomycin (100 IU or 100 μg, respectively per ml), and mycostatin (10^4 IU/ml) was added (total volume, 30 ml) to each bottle after the virus was allowed to adsorb to the cells for 45 min at 37°C. The infected monolayers were incubated at 37°C, and, after approximately 72 h, the cells were frozen (−10 to −60°C) and thawed (37°C) three times to liberate virus from the cells. This suspension was extracted with Freon (1,1,2-trichlorotrifluoroethane; high-performance liquid chromatography grade; Aldrich, Milwaukee, Wis.) to reduce organic matter and disperse viral aggregates. Cellular debris was removed by centrifugation for 10 min at 15,300 × *g* (JA-14 rotor; Spinco Division, Beckman Instruments, Palo Alto, Calif.). The aqueous layer containing the virus was removed by pipette, the titer of the virus was measured, and the material was frozen at −20°C until needed.

Preparation of bacteriophage MS2 and PRD-1. An 18- to 24-h culture of *Escherichia coli* (ATCC 15597) grown in tryptic soy broth (TSB; Difco, Detroit, Mich.) was transferred (0.1 ml) to fresh TSB and grown for 3 to 6 h at 37°C with continuous shaking. Stock MS2 (ATCC 15597B) was serially diluted in Tris buffer to an approximate concentration of 10^5 PFU/ml. A 1-ml suspension of the host and 0.1 ml of the phage dilution were mixed in molten overlay agar (top agar; TSB with 1% Bacto Agar; Difco) and poured onto presolidified tryptic soy agar (TSA, 1.5% agar; Difco) petri dishes. After 18 to 24 h of incubation at 37°C, 6 to 7 ml of sterile Tris buffer was added to petri dishes with confluent plaques, and the dishes were allowed to sit for a maximum of 1 h to allow the phage to diffuse from the agar surface. The liquid fraction was recovered from the plates with the aid of a pipette and centrifuged (15,300 × *g*), the resulting supernatant was dispensed into a sterile bottle, the titer of the virus was measured, and the supernatant was stored at 4°C until used.

Bacteriophage PRD-1 (obtained from Junetsu Ito, University Medical Center, Tucson, Ariz.) was propagated in the same manner as MS2, with the exception that *Salmonella typhimurium* Lt2 (also provided by J. Ito) was used as the host bacterium.

Sludge. Anaerobically digested sewage sludge (mean retention time, 15 days) was collected in sterile 1-liter plastic bottles (Nalgene; Nalge Co., Rochester, N.Y.) at the Ina Road Wastewater Treatment Facility in Tucson, Ariz. The sludge was then transported back to the laboratory and stored at 4°C until needed.

TABLE 1. Characteristics of soil used in this study

Characteristic	Pima Clay Loam	Brazito Sandy Loam
Texture analysis		
% Sand	45.1	74.7
% Silt	30.6	14.6
% Clay	24.3	10.7
pH (saturated paste)	8.14	8.03
Electrical conductivity (mS/cm) (saturated paste)	1.60	8.20
Soluble basic cations (mg/liter) (in saturated paste)		
Na	112	24.6
K	45.4	71.2
Ca	220	109
Mg	17.7	8.77
Total organic C (%, wt/wt)	0.456	0.304
Total N (%, wt/wt)	0.052	0.034
Extractable PO_4-P (mg/kg)	14.7	20.2

Soil collection. Two soils common to southern Arizona were collected during the hot, arid summer season. An agricultural production soil, Pima Clay Loam (fine-silty, mixed, thermic Typic Torrifluvent) was collected in a field near the Marana Agricultural Center of the University of Arizona. The soil had not received sludge for at least 2 years and was collected from 10 to 15 cm below the surface. To avoid salts that tend to accumulate on the top of the irrigation furrow, the soil was taken from the bottom of the furrow. After collection, the soil was air dried at room temperature for 1 day. It was then sieved (pore size, <2 mm) to remove small stones and plant debris and subsequently stored at 4°C until needed.

The other soil type was Brazito Sandy Loam, which is characterized as a mixed, thermic Typic Torripsamment. The collection site was 100 m south of the Rillito River at the Campbell Avenue Farms of the University of Arizona, Tucson. Surface soil samples were dried at room temperature for 24 h, sieved (pore size, <2 mm), and stored at 4°C until needed. Characteristics of both soil types are listed in Table 1.

Experimental design. For each treatment, three temperatures, based on average soil temperatures measured 10 cm below the soil surface in southern Arizona, were chosen to determine the inactivation rates of the three viruses. These temperatures were 15, 27, and 40°C, corresponding to the average soil temperatures for winter, spring or fall, and summer field conditions, respectively, in southern Arizona. The respective temperatures were maintained constant by incubation in dry air. Duplicate experiments were conducted for each application method at each temperature.

In application method 1, virus stocks of MS2, PRD-1, and poliovirus 1 (titers, ca. 1.0×10^{10}, 1.5×10^{11}, and 1.4×10^7 PFU/ml for MS2, PRD-1, and poliovirus, respectively) were seeded into anaerobically digested sewage sludge (ca. 1.5% solids) to stimulate virus contamination from sludge. The virus was mixed with the sludge by using a magnetic stirrer

for 30 min at room temperature. Duplicate samples of Pima Clay Loam and Brazito Sandy Loam soils (250 g of dry soil) were amended with 75 ml of this sludge. This amendment corresponds to a soil moisture content of 30% and was determined on the basis of observations of soil moisture content taken before and immediately after sludge application to agricultural lands. Each of the amended soils was thoroughly mixed to evenly distribute the sludge throughout the soil, and the container was then sealed to prevent moisture loss. The containers were then incubated at the appropriate temperature. Moisture was monitored daily, and, when necessary, distilled water was added to maintain consistency in moisture content.

In application method 2, sludge (67.5 ml) was added to duplicate containers of 250 g (dry weight basis) of each soil type. The sludge was thoroughly mixed with the soil and allowed to stand for 15 min at room temperature. Each virus from the same stock (2.5 ml) was then added to each container and thoroughly mixed to distribute the viruses throughout the amended medium. The containers were sealed, incubated, and monitored for moisture loss as described for treatment method 1. Distilled water was added to these soils when necessary to maintain constant moisture conditions.

Application method 3 was essentially the same as method 1. Soil moisture, however, was allowed to evaporate to more closely simulate field conditions and to permit determination of the significance of this factor on virus survival. Each incubator used provided sufficiently dry airflow to simulate the evaporation rates observed in the field. Moisture content was determined on each sample day throughout the experiment or until virus was no longer detectable.

Sample collection. Selection of sampling days depended on the temperature at which the containers were held. For the temperature of 15°C, the samples were processed on days 0, 1, 2, 7, 11, 15, 21, and 28; for 27°C, they were processed on days 0, 1, 2, 3, 7, 10, and 16; and for 40°C, they were processed on days 0, 1, 2, 3, 5, and 7. No virus was detected after 7 days in the evaporation studies regardless of temperature, so the experiment was terminated at that point.

Sample processing. Viruses were eluted from the sludge-amended soil in the ratio of 5 g (wet weight) of soil to 50 ml of 3% beef extract (Beef Extract V; BBL Microbiology Systems, Cockeysville, Md.) buffered with 7.3 g of Na_2HPO_4 per liter (EM Science, EM Industries Inc., Cherry Hill, N.J.) and 1.10 g of citric acid per liter (Mallinckrodt, St. Louis, Mo.). The pH of the suspension was adjusted to 9.4 with 5 N NaOH and stirred continuously for 30 min on a magnetic stirrer, and then the soil was separated from the eluent by centrifugation at 15,300 × *g* for 30 min (JA-14 rotor; Spinco Division, Beckman Instruments). The supernatant was decanted into a 50-ml sterile plastic centrifuge tube (Costar, Cambridge, Mass.), and the pH of the solution was adjusted to neutrality. A fraction (ca. 7 ml) was collected in a sterile 15-ml plastic vial (Falcon, Oxnard, Calif.) for the bacteriophage assays, while the remainder was frozen (−10 to 60°C) until cells for the poliovirus assay were ready.

Assay of poliovirus. Samples were kept frozen (−10 to −60°C) until BGM cell monolayers, grown for 5 days at 37°C in 5% CO_2 in six-well plastic plates (multidish; Nunc, Roskilde, Denmark), were ready for use. The tissue culture growth medium was removed from the plates, and the cells were infected with 0.1 ml of 1:10 serial dilutions of the sample. After adsorption was allowed for 45 min (with agitation every 15 min) at 37°C in 5% CO_2, 3 ml of overlay medium (final concentrations, 1× MEM and 0.5% agar) was added to each well. The plates were incubated for 48 h at 37°C in 5% CO_2, and then the medium was removed and the cells were stained with 0.1% aqueous crystal violet to visualize plaques. The plaques were enumerated, and the inactivation rates were calculated.

TABLE 2. Inactivation rate equations of MS2 in sludge-amended soil at different temperatures

Temp (°C)	Soil type	Method	Regression equation[a]	r^2 (%)	Significance[b]
15	Clay loam	1	$y = -0.366x - 0.165$	99.1	a
		2	$y = -0.300x - 0.123$	98.7	a
	Sandy loam	1	$y = -0.394x - 0.340$	98.5	a
		2	$y = -0.362x - 0.602$	95.5	a
27	Clay loam	1	$y = -0.629x - 0.703$	80.9	b
		2	$y = -0.699x - 0.325$	92.7	b
	Sandy loam	1	$y = -0.652x - 0.515$	91.2	b
		2	$y = -0.688x - 0.514$	92.2	b
40	Clay loam		—[c]	ND[d]	ND
	Sandy loam		—	ND	ND

[a] Regression equation is $y = mx + b$, where $(-m)$ is the k value, x is the time in days, and y is the $\log_{10} N_t/N_0$.

[b] Treatments followed by the same letter were not significantly different ($P \leq 0.05$).

[c] —, after 24 h, no virus was recovered either in the method 1 or method 2 treatment, and this corresponded to a minimum 7 $\log_{10}$ reduction.

[d] ND, not determined.

Bacteriophage assays. Samples were kept at 4°C until assayed for both bacteriophages. Serial dilutions (1:10) were made from samples in Tris buffer, added to test tubes containing 3 ml of molten 1% TSB top agar (46°C) and 1 ml of a 4- to 6-h culture of *E. coli* ATCC 15597 or *S. typhimurium* for MS2 and PRD-1, respectively, and poured onto solid TSA (1.5% agar) plates. The plates were incubated for 18 to 24 h at 37°C, after which time the plaques were enumerated and the $\log_{10}$ reduction and the inactivation rates for both viruses were calculated.

Data analysis. To determine virus inactivation rates, the numbers of virus per gram (dry weight) of soil were transformed into $\log_{10}$ PFU/gram (dry weight) of soil. Linear regression was used to determine the k value ($\log_{10}$ reduction per day) of virus by the following equation: $\log_{10} N_t/N_0 = -mx + b$, where $\log_{10} N_t/N_0$ is the ratio of the $\log_{10}$ value at time t (measured in days) to the initial $\log_{10}$ value ($\log N_0$), x is the time in days, b is the intercept value, and m is the slope. Minitab statistical software (15) was used to determine the regression equation, and the k value was defined as −m. Factorial analysis was used to determine if significant differences in the k value for the first two methods of application ($P \leq 0.05$) were due to soil texture, method of virus addition, or both. One-way analysis of variance was used to determine significant differences ($P \leq 0.05$) due to increasing temperature. In the drying studies, values were reported as $\log_{10} N_t/N_0$ only because the data were not linearly correlated.

RESULTS

The inactivation rate equations for MS2 (application methods 1 and 2) in the two sludge-amended soils are shown in Table 2. At 15 and 27°C, sludge-amended clay loam soils afforded more protection against inactivation, but this difference was not significant ($P \leq 0.05$). The inactivation rate of

TABLE 3. Inactivation rate equations of poliovirus in sludge-amended soil at different temperatures

Temp (°C)	Soil type	Method	Regression equation[a]	r^2 (%)	Significance[b]
15	Clay loam	1	$y = -0.064x - 0.186$	90.4	a
		2	$y = -0.066x - 0.068$	93.0	a
	Sandy loam	1	$y = -0.095x - 0.310$	94.2	b
		2	$y = -0.100x - 0.163$	97.1	b
27	Clay loam	1	$y = -0.113x - 0.107$	94.6	c
		2	$y = -0.138x - 0.021$	83.3	c
	Sandy loam	1	$y = -0.154x - 0.112$	95.2	d
		2	$y = -0.149x - 0.230$	88.9	d
40	Clay loam		—[c]	ND[d]	ND
	Sandy loam		—	ND	ND

[a] Regression equation is $y = mx + b$, where $(-m)$ is the k value, x is the time in days, and y is the $\log_{10} N_t/N_0$.
[b] Treatments followed by the same letter were not significantly different ($P \leq 0.05$).
[c] —, after 24 h, no virus was recovered either in method 1 or method 2 treatment, and this corresponded to a minimum 5 $\log_{10}$ reduction.
[d] ND, not determined.

TABLE 4. Inactivation rate equations of PRD-1 in sludge-amended soil at different temperatures

Temp (°C)	Soil type	Method	Regression equation[a]	r^2 (%)	Significance[b]
15	Clay loam	1	No inactivation	ND[c]	ND
		2	No inactivation	ND	ND
	Sandy loan	1	No inactivation	ND	ND
		2	No inactivation	ND	ND
27	Clay loam	1	No inactivation	ND	ND
		2	No inactivation	ND	ND
	Sandy loam	1	No inactivation	ND	ND
		2	No inactivation	ND	ND
40	Clay loam	1	$y = -0.208x - 0.070$	85.9	a
		2	$y = -0.151x + 0.222$	79.2	b
	Sandy loam	1	$y = -0.282x + 0.140$	90.1	c
		2	$y = -0.162x + 0.020$	87.2	d

[a] Regression equation is $y = mx + b$, where $(-m)$ is the k value, x is the time in days, and y is the $\log_{10} N_t/N_0$.
[b] Treatments followed by the same letter were not significantly different ($P \leq 0.05$).
[c] ND, not determined.

MS2 in both soils nearly doubled as the temperature was increased from 15 to 27°C, and this was statistically significant ($P \leq 0.05$). At 40°C, no MS2 was recovered after 24 h, and this corresponded to a minimum 7 $\log_{10}$ reduction. The results of the different methods of virus application (method 1 versus method 2) were not statistically significant ($P \leq 0.05$).

Poliovirus was more resistant to inactivation than MS2 was at 15 and 27°C (Table 3). In the case of poliovirus, sludge-amended clay loam soils afforded more protection against inactivation than sandy loam soils and this was significant both at 15 and 27°C ($P \leq 0.05$). Temperature was also a significant factor in the inactivation of this virus. At 40°C, no poliovirus was recovered after 24 h, and this corresponded to a minimum of a 5 $\log_{10}$ reduction. The results of the different methods of virus application were not statistically significant ($P \leq 0.05$).

Of the three viruses tested, coliphage PRD-1 was the most resistant to inactivation under the test conditions. No reduction of this virus was observed after 28 days at 15°C or after 16 days at 27°C. After 7 days at 40°C, less than a 2 $\log_{10}$ reduction of PRD-1 had occurred (Fig. 1 and Table 4). Sludge-amended clay loam soils afforded more protection against viral inactivation than sludge-amended sandy loam soils (Fig. 2). Significantly less inactivation was observed when viruses were added after sludge amendment (method 2) than when viruses were seeded in sludge before soil amendment (method 1).

For all three viruses, drying to less than 5% soil moisture resulted in a more rapid loss of infectivity (Table 5) than when the viruses were held in amended soil at a constant moisture. Only MS2 in Brazito Sandy Loam was recovered after 7 days at 15°C. At 27°C, no viruses were recovered beyond 3 days, and, at 40°C, no viruses were recovered

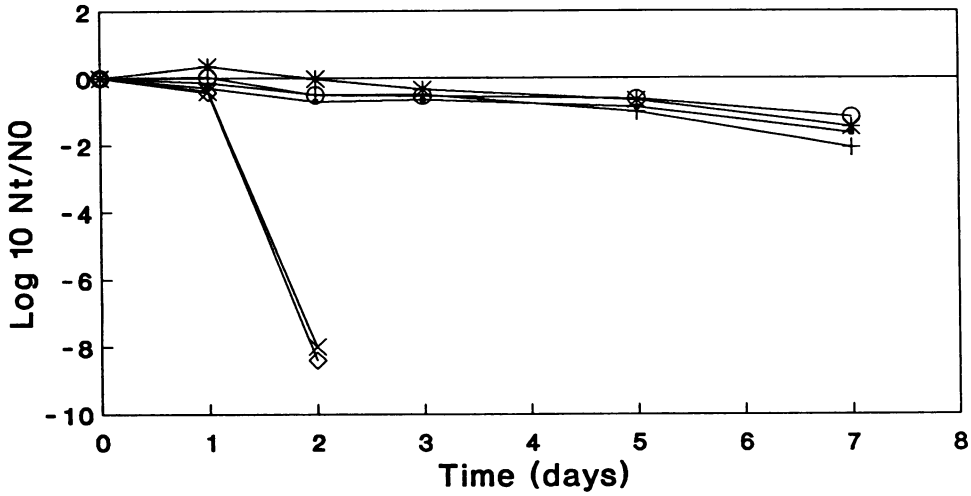

FIG. 1. Average $\log_{10}$ reduction of bacteriophage PRD-1 in sludge-amended soil at 40°C. Symbols: —•—, Pima Clay Loam, method 1; —+—, Brazito Sandy Loam, method 1; —✳—, Pima Clay Loam, method 2; —⊖—, Brazito Sandy Loam, method 2; —✳—, Pima Clay Loam, evaporation; —◇—, Brazito Sandy Loam, evaporation.

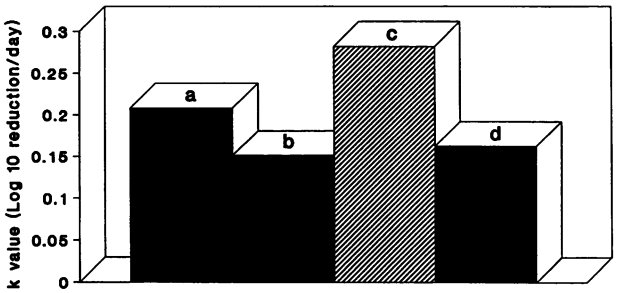

FIG. 2. Inactivation rates (k) of bacteriophage PRD-1 in sludge-amended soil maintained at 30% moisture at 40°C. Values with different letters were significantly different ($P \leq 0.05$). Symbols: ■, Pima Clay Loam, method 1; ■, Pima Clay Loam, method 2; ▨, Brazito Sandy Loam, method 1; ■, Brazito Sandy Loam, method 2.

beyond 24 h except PRD-1 (Table 5 and Fig. 1). At a low moisture content (1 to 2%), more inactivation of MS2 was observed in Pima Clay Loam than in Brazito Sandy Loam both at 15 and 27°C. A similar situation was observed in the poliovirus drying study at 27°C.

DISCUSSION

In desert soils, evaporation of soil moisture is perhaps the most important factor affecting virus survival. The rate of evaporation is directly related to temperature and relative humidity. Yeager and O'Brien (21) studied the survival of poliovirus added to soil in New Mexico. The virus was seeded in groundwater, river water, and septic tank liquor and then applied to sandy loam soils. It was found that, when the soil moisture was evaporated to 2.9% (wt/wt), rapid loss of virus infectivity occurred regardless of soil temperature or the medium in which the virus was applied. This study concurs with the results of Yeager and O'Brien (21). In comparison to the results of application methods where the moisture content was maintained, the most dramatic losses of titer were observed when the moisture content was reduced from 20 to 1% in the 15°C study, from 15 to 5% in the 27°C study, and from 7 to 1% (PRD-1 only) in the 40°C study.

If soil moisture is maintained at 10% or greater, temperature becomes the most important factor affecting the inactivation of viruses in soils (21). The survival of viruses is enhanced by a combination of low soil temperature and sufficient moisture (2, 6). Bagdasar'yan (1) reported that poliovirus type 1 could persist for up to 170 days at temperatures of 3 to 10°C and that a significant decrease of viability occurred when the soil temperature was raised to 18 to 23°C. Yeager and O'Brien (21) reported similar results in soils amended with septic tank liquor. At 4°C and with constant moisture, viruses persisted for 180 days, while at 37°C no viruses persisted after 12 days. When moisture was maintained, this study with three different viruses produced similar results. Significant increases were seen in the inactivation rates of MS2 and poliovirus as the temperature was raised from 15 to 40°C. PRD-1 was exceptional in that a noticeable reduction (less than 2 $\log_{10}$ in 11 days) of the virus was only observed in the 40°C study.

Certain soil characteristics also influence virus survival. Hurst et al. (10) reported that virus survival decreased as a function of increasing soil pH and resin-extractable phosphorus. It was also reported that virus survival increases with increasing levels of exchangeable aluminum. The relative amounts of clay and humic minerals may also enhance survival (3). In general, both clay minerials and viruses are negatively charged. However, viruses do adsorb readily to clay minerals by electrostatic interactions, and the percentage of viral adsorption increases with increasing clay mineral content (7). It is speculated that this adsorption protects the viral genome from attack by nucleases or other antagonistic factors in soil (3).

However, in rapidly drying soils, virus survival may decrease more rapidly in clay soils than in sandy soils as was reported in this study. This phenomenon might be explained by the water-holding capacity of soil (8). Clay soils can hold more water than sandy soils, but when water is evaporating from both soils, the clay soil, because of its mineral content, will bind the remaining water more tightly than the sandy soil at the identical soil moisture content, making it less available for biological activity. Our study seemed to agree with

TABLE 5. Average $\log_{10}$ reduction of MS2, poliovirus, and PRD-1 in sludge-amended soils evaporated to dryness (method) at 15, 27, and 40°C

Temp (°C)	Day	MS2		Poliovirus		PRD-1		Avg soil moisture[a]	
		Pima	Brazito	Pima	Brazito	Pima	Brazito	Pima	Brazito
15	0	0	0	0	0	0	0	0.35	0.28
	1	−0.47	−0.49	−0.37	−0.23	−0.24	+0.02	0.29	0.27
	2	−1.12	−1.17	−0.32	−0.16	−0.36	+0.01	0.19	0.20
	7	−7.73	−5.07	−4.36	−4.97	−7.11	−7.52	0.02	0.01
	11	—[b]	−7.73	—	—	—	—	—	0.01
27	0	0	0	0	0	0	0	0.35	0.30
	1	−1.04	−1.04	−0.68	−0.30	−0.25	−0.17	0.12	0.15
	2	−6.23	−4.05	−4.97	−1.68	−5.53	−1.52	0.05	0.03
	3	—	−6.08	—	−5.01	−7.43	−8.05	0.00	0.01
40	0	0	0	0	0	0	0	0.35	0.31
	1	−7.00	−7.00	−5.00	−5.00	−0.40	−0.43	0.10	0.07
	2	—	—	—	—	−8.00	−8.40	0.01	0.01

[a] Soil moisture was reported as a gravimetric soil moisture content (weight/weight).
[b] —, no virus was recovered for these sample days and soil types.

this, especially for MS2 in the 15 and 27°C studies and for poliovirus in the 27°C study.

Bacteriophages MS2 and PRD-1 are often used to model the fate of other enteric viruses on the basis of size and structural similarities to other enteric viruses. These viruses are used extensively in the field because they pose no threat to human health (14). Poliovirus was used in this experiment because it is a typical virus isolated from sewage (9) and to provide a comparison for the assumptions made in using MS2 phage as a model for poliovirus. In the case of constant moisture studies, each virus had its own unique inactivation curve. If MS2 was used as a model for an enteric virus such as poliovirus, the survival of the pathogen would be grossly underestimated. However, PRD-1 might be a much better model to use with its extremely long survival time even at high soil temperatures (40°C).

In the arid Southwest, agricultural land is irrigated. The moisture content of these soils is maintained between field capacity (15%) and saturation throughout the life cycle of the crop. Thus, it is important that sludge treatment processes at the wastewater treatment plant are set to inactivate the most resistant potential pathogenic viruses. In Arizona, sludge drying basins would be a feasible and effective alternative. Treated sewage effluent could be used to rehydrate the sludge if it is desired to apply the product as a liquid. For temperate regions of the country, thermophilic treatment, perhaps by composting, may achieve the desired destruction of viruses before disposal of the sludge on land.

In summary, our studies of sludge-amended soils agree with previous work where virus survival in soil was studied as a result of application of sewage effluent. Low temperatures and moist soil conditions favored the longest survival of the virus. In contrast, hot and dry soil conditions led to the greatest inactivation of all of the viruses tested. In sludge-amended soils where moisture was maintained, less inactivation was observed in the fine-textured Pima Clay Loam than in the Brazito Sandy Loam for MS2 and poliovirus at 15 and 27°C and for PRD-1 at 40°C.

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