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Survival of Enteroviruses in Rapid-Infiltration Basins During the Land Application of Wastewater

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The downward migration through soil of seeded poliovirus type 1 and echovirus type 1 and of naturally occurring enteroviruses during infiltration of sewage effluent through rapid-infiltration basins was investigated. After 5 days of flooding, the amount of seeded poliovirus type 1 that had migrated 5 to 10 cm downward through the soil profile was found to be 11% of that remaining at the initial burial depth. The amount of echovirus type 1 determined to have moved an equal distance was at least 100-fold less. Migration of naturally occurring enteroviruses during infiltration of sewage effluent through soil could not be measured with accuracy because of the possibility of virus survival from previous applications of effluent. The rate of inactivation for seeded poliovirus 1 and echovirus 1 buried in the infiltration basins ranged between 0.04 and 0.15 $\log_{10}$ units per day during the time when the basins were flooded. Inactivation of these same seeded virus types and of indigenous enterovirus populations in the infiltration basins during the drying portion of the sewage application cycle ranged between 0.11 and 0.52 $\log_{10}$ units per day. The rate of virus inactivation was dependent upon the rate of soil moisture loss. These results indicate that drying cycles during the land application of wastewater enhance virus inactivation in the soil.

Over 100 types of human viruses are known to be excreted in the feces of humans, and studies have shown that enteric viruses easily survive present methods of sewage treatment (15). Once released into the environment, these viruses may persist for several months in natural waters (16). It has been established that low levels of enteroviruses are probably introduced into soil systems by land application of wastewater and sludge (19).

Enteroviruses have been isolated from soil of rapid-infiltration basins receiving primary and secondary sewage effluents (12). 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 (24). Seeded polioviruses were shown by Tierney et al. (22) to survive in effluent-irrigated soil for 11 days during the summer and 96 days during the winter. Sadovski and co-workers (18), in two separate experiments performed at different sites, found the rates of poliovirus inactivation in sewage effluent-irrigated plots to be 1 $\log_{10}$ loss in 8 days (0.12 $\log_{10}$ units/day) and reduction from 9.1×10^3 plaque-forming units (PFU) to 47 PFU in 10 days (0.25 $\log_{10}$ units/day).

Enteroviruses have been detected at the soil surface in fields irrigated with raw sewage (10). Enteric viruses have also been found on market fruits and vegetables harvested from sewage-irrigated fields (14), and it has been demonstrated that enteroviruses are capable of surviving on the surface of vegetables for 10 to 15 days at refrigerator temperatures (1). Thus, the land disposal of wastewater can pose a potential risk of human illness.

The objectives of this study were measurement of enterovirus downward migration through soil during rapid infiltration of sewage effluent and measurement of enterovirus inactivation rates in soil of rapid-infiltration basins during periods of flooding and drying. The infiltration basins used in this study were part of a pilot system designed to study the high-rate land treatment of wastewater for water quality improvement and groundwater recharge. Two field studies were performed. The first was conducted during the winter of 1977 and was intended to determine whether viruses from sewage water could be found in the soil of infiltration basins at quantities great enough to allow determination of inactivation rates. The second field study was conducted during the fall of 1978 in order to compare the rates of inactivation of seeded laboratory virus and viruses from sewage water under parallel environmental conditions.

MATERIALS AND METHODS

Cells and cell cultures. Buffalo green monkey (BGM) kidney cells, a continuous cell line, were used

for growth of virus stocks and for isolation and assay of viruses from the environment. These cells were cultured in Eagle minimal essential medium (21) 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 but with a reduction in fetal bovine serum to 2% and an increase in sodium bicarbonate to 0.25%.

Virus and virus assays. The enteroviruses used for experiments involving seeded virus were plaque-purified strains of poliovirus 1 (strain LSc-2ab) and echovirus 1 (strain Farouk). Enterovirus stocks were grown in BGM cells from plaque-purified virus as follows. Thirty-two-ounce (ca. 960-ml) bottles of BGM cells were inoculated at a multiplicity of infection of 0.2 to 1.0 PFU and incubated for 1 h at 37°C, and maintenance medium without serum was added 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 at low speed (3,000 rpm for 15 min). Virus stock was then stored at −20°C until use. Enterovirus assays were done by the PFU method as previously described (17).

Samples processed for determination of indigenous virus were assayed by the same procedure. When necessary, enterovirus samples were diluted in tris(hydroxymethyl)aminomethane (Tris) buffer containing (per liter): 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, plus 100 U of penicillin and 100 μg of streptomycin per ml.

Virus recovery technique. The method used for elution and concentration of enteroviruses from soil samples has been described (12). Briefly, this method involves mixing the soil with 0.25 M glycine plus 0.05 M ethylenediaminetetraacetic acid buffer, pH 11.5, on a magnetic stirrer. The elution mixture is then centrifuged and the viruses in the supernatant are concentrated with a combined low-pH aluminum-organic flocculation and membrane filter concentration procedure.

Natural isolates. For measuring the survival of viruses from sewage in field soils, samples were collected from different depths in infiltration basins that had been flooded with either primary or secondary (activated sludge) sewage effluent. The secondary effluent infiltration basins were 6 by 100 m in size, and the primary effluent basin was 3.3 by 10 m in size. The flooding schedule was 9 days flooding alternated with 12 days drying for the secondary effluent basins, and 5 days flooding followed by 9 days of drying for the primary effluent basin. Soil samples were collected on various days after termination of a flooding cycle. The soil samples collected during both the fall and winter studies were taken on the same number of days after initiation of the drying portion of the cycle and at the same soil depths.

There was no standing water on the surface of the basins when the samples were collected. The soil samples (200 g) were placed in plastic bags and frozen on dry ice immediately after collection. Viruses adsorbed to the soil were eluted and concentrated by the method previously described. A sample size of 200 g was chosen since that amount of soil was expected to yield an accurate estimate of the amount of virus in the basin and could be readily handled during the virus elution and concentration procedures.

Studies with seeded virus. To measure the extent of downward migration of virus during infiltration of sewage effluent through soil, laboratory stocks of poliovirus 1 and echovirus 1 were buried in open-ended sections of a polyvinyl chloride water pipe. The pipe sections were 30 cm long by 15 cm internal diameter and were buried in a vertical position such that the openings at the ends of the pipe sections would allow vertical movement of infiltrating effluent through the soil within the pipe. The sections of pipe were buried in holes 30 and 60 cm deep. For each seeded virus type, two sections of pipe were buried at a depth of 60 cm and three sections were buried at a depth of 30 cm. The pipe sections were then filled with sand from the basins to within 5 cm of the upper edge of the pipe. At this location in each pipe section, 50 g of sand containing 2×10^8 PFU of virus was added. The remaining upper portion of each pipe section was then filled with sand from the basin.

The holes into which the pipe sections were placed were made with a post-hole digging tool. In filling the pipe sections, the soil removed from the hole during digging was replaced in inverted order so that the basin soil profile was reestablished within the pipe sections. Soil was added to the pipe sections in increments of approximately 5 cm to allow even packing of the soil. After the addition of each soil increment, the soil in the pipe section was packed with a tamping tool. The uppermost surface of the newly packed soil was scraped with the end of a wooden dowel before further addition of soil to prevent formation of interfaces between the layers of soil that might alter water infiltration patterns. The purpose of having the virus buried 5 cm below the soil surface was to prevent dispersion of the seeded virus into the effluent of the entire basin and also to eliminate any possible effects of wind and ultraviolet irradiation on the virus in the upper portion of the soil during the drying cycle.

Extraction of samples from the pipe sections was done by excavating around the pipe and then inserting the blade of a hoe underneath the bottom of the pipe. The pipe section and its soil contents were then lifted out of the hole intact, and a plunger with the same diameter as the inside of the pipe section was used to push the soil column out of the pipe. Samples of soil corresponding to the bottom, middle, and upper 5 cm of the pipe section were collected for later analysis. Virus determinations of soil in the upper 5 cm were used to determine how much of the original virus seeded into the pipe remained in its initial position. Measurement of the relative virus levels in soil from the midpoint and bottom of the pipe section was used to provide an indication of vertical virus migration during the infiltration of sewage effluent, as well as the rate of virus survival at these different depths. For virus detection, 200-g samples of soil were collected from the different positions in the pipe section. These samples were then frozen and subsequently eluted as described for the natural isolate samples.

Poliovirus 1 and echovirus 1 were also seeded into 50-ml plastic centrifuge tubes containing soil. A series

of tubes was buried at each of four depths in the basin (5, 15, 30, and 60 cm) below the surface. A soil auger was used to make holes for their emplacement, and soil was repacked firmly around the tubes. Before burial, the tubes were filled approximately 40% by addition of 25 g of air-dried soil. Distilled water (8 ml) containing 5×10^7 PFU of either poliovirus or echovirus was then added to the soil, and the contents of the tubes were then mixed thoroughly by Vortex mixing. The remainder of the tube volume was then filled with soil from the basin. One group of tubes was closed watertight before burial, and one group was buried in a vertical position with both ends open (Fig. 1).

The final filling of the tubes with soil from the infiltration basin was done at the land treatment site. The conical bottom ends of the open tubes were removed in such a manner that a small lip of the cone remained to hold the contents of the tube in place during the burial operation.

The centrifuge tubes were buried in a circular array around the outside of the pipe sections so that no tube was placed directly above another. This arrangement was used to reduce the possibility of vertical virus migration from one open tube into another placed at a lower depth.

On sampling dates, a set of open and closed tubes of each virus type from each soil depth was removed from the basin. The tubes were frozen intact and shipped to Houston for analysis. Soil from these tubes was then assayed for infectivity by direct inoculation after dilution in Tris buffer. The relative subsurface positions of the pipe sections and centrifuge tubes are illustrated in Fig. 2.

Virus added to effluent percolating through open soil. Two small primary effluent infiltration basins, 1 m square and 30 cm deep, were dug. Each of the basins was flooded with 75 cm of sewage effluent over a 24-h period. The effluent was placed into the basins in three portions of 25 cm each. Since the rate of effluent infiltration into the large basins used for the fall field study was 15 cm/day, a 75-cm application of sewage water was equal to the amount percolated through the soil in the large basins over the 5-day flooding period. Also, the final 25-cm portion of effluent placed into one of the basins was seeded with 10^9 PFU of poliovirus type 1. Since the basins each held 150 liters of liquid, the titer of virus in the effluent applied to the seeded basin contained a concentration of 6.7×10^6 PFU of added virus per liter. After complete infiltration of the final 25 cm of effluent into the small basins, 200-g soil samples were taken for virus determinations. The samples were collected at the same depths as samples taken for natural isolate virus determinations in the other portions of the field studies. Virus determinations for the samples from the small basins were performed by the method described for natural isolates.

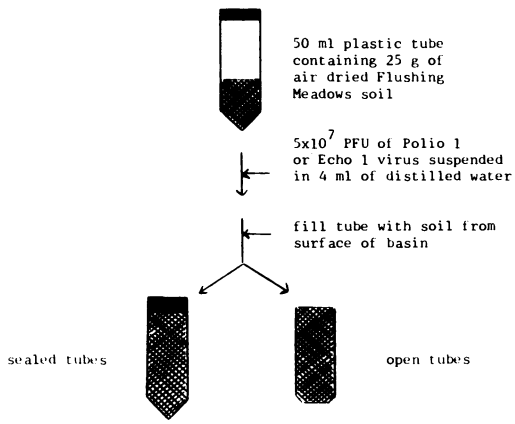

FIG. 1. *Technique used for measuring virus survival in soil, using plastic centrifuge tubes.*

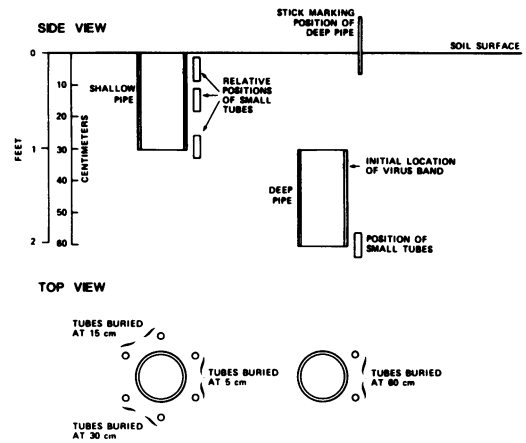

FIG. 2. *Relative positioning of buried virus samples.*

RESULTS

Detection and survival of enteroviruses originating from sewage under winter conditions in Phoenix, Ariz. This study was designed to determine the occurrence of animal viruses in a soil profile beneath two rapid-infiltration basins that regularly receive primarily and secondarily treated sewage effluents. Soil samples (200 g) were collected from shallow basins that had been flooded with either primary or secondary (activated sludge) sewage. The basins were part of pilot systems designed to study the high-rate land treatment of wastewater for water quality improvement. Those receiving secondary sewage were part of the Flushing Meadows project, which has been described in detail (8). The Flushing Meadows soil is a loamy sand containing 89% sand, 8% silt, and 3% clay. The soil beneath the site receiving primary sewage effluent had a slightly higher silt and clay content. This soil contains 73% sand, 22% silt, and 5% clay and is classified as a sandy loam.

Virus was recovered from all soil depths tested (Tables 1 and 2). The greatest amount of virus was in the 0- to 2.5-cm region of the soil profile. Deeper regions in the rapid-infiltration basin soil

TABLE 1. *Naturally occurring virus in primary sewage effluent basin on day 1 during winter*

Soil depth (cm)	PFU/200 g of soil (dry wt)	Moisture content of soil (% of wet wt)
0.0–2.5	101	22.3
2.5–10.0	6	15.4
10.0–25.0[a]	5	25.8
25.0–30.0	0	12.1

[a] This sample consisted mostly of clay.

TABLE 2. *Naturally occurring virus in secondary sewage effluent basin during winter*[a]

Soil depth (cm)	PFU/200 g of soil (dry wt)		
	40 m[b]	100 m[b]	Avg
Day 1			
Sludge layer[c]			
0–2.5	54	16	35
2.5–10.0	3	3	3
10.0–25.0	2	0	1
Day 3			
Sludge layer	16	23	19.5
0–2.5	7	5	6
2.5–10.0	2	1	1.5
10.0–25.0	1	0	0.5

[a] Soil moisture content of all samples averaged approximately 16%.

[b] Indicates horizontal distance of sampling site from effluent inlet.

[c] Too fluid to be collected.

contained much lower amounts of virus. Titer reductions were sharp between the 0- to 2.5-cm and the 2.5- to 10-cm regions. Reductions in virus titer between zones below 2.5 cm were, in general, more gradual. The sludge layer (composed of fine suspended matter that collected at the soil surface during infiltration of sewage) was too fluid to be collected on day 1 and was scraped away before collecting the soil samples. The sludge layer was collected on day 3 and was found to contain an average 3.25-fold-greater amount of virus than the surface soil layer beneath it. Thus, a great deal of virus appears either to have been associated with particulate solids in the sewage effluent or to have adsorbed to the sludge layer during infiltration. The amount of virus found at a distance of 40 m from the wastewater inlet was slightly greater than that found at a distance of 100 m. Such a result might be caused by a partial settling of fine suspended solids and associated adsorbed virus from the effluent during its flow down the length of the basin. The resultant effect of such settling would be less virus reaching the position farthest from the sewage inlet.

The moisture content of the soil sample at the 0- to 2.5-cm depth was greater than that of the deeper soil samples (with the exception of the 10- to 25-cm sample from the primary effluent basin). This difference in soil moisture content was less appreciable on day 3. The amount of virus inactivated over the 2-day period (day 1 to day 3) in the soil at the secondary effluent infiltration basin was 83% for the 0- to 2.5-cm depth and 50% for the 2.5- to 10-cm and 10- to 25-cm depths.

Detection and survival of seeded poliovirus, echovirus, and naturally occurring enteroviruses from sewage water in soil during fall conditions in Phoenix, Ariz. Virus titer decreased more rapidly for open tubes than for closed tubes (Table 3). Viruses could not be detected in open-tube or closed-tube samples at 11 days. When the soil was flooded again between 11 and 19 days, virus was detected on day 19 in two open-tube samples. This may have been due to addition of viruses to the soil from sewage during the flooding or to experimental error. A summary of the difference in rate of virus inactivation in the closed and open buried tubes is presented in Table 4. The values shown

TABLE 3. *Seeded virus survival and soil moisture levels in small tubes buried in a sewage effluent rapid-infiltration basin*[a]

Tube depth (cm)	Virus survival after no. of days drying[b]				
	Day 1[c]	Day 3	Day 5	Day 11	Day 19
Polio 1, open tubes					
5	7.0×10^{3}	5.4×10^{2}	1.9×10^{1}	<1	
15	3.1×10^{3}	3.5×10^{3}	4.0×10^{3}		
30	1.2×10^{4}	1.4×10^{4}	4.0×10^{3}	<1	2.1
60	1.1×10^{4}		1.9×10^{3}		
Polio 1, closed tubes					
5	1.1×10^{4}	6.0×10^{4}	9.3×10^{3}	<1	
15	1.7×10^{4}	2.7×10^{4}	1.2×10^{4}		
30	2.7×10^{4}	1.2×10^{4}	2.6×10^{4}	<1	
60	1.2×10^{4}		1.3×10^{4}		
Echo 1, open tubes					
5	7.3×10^{1}	1.4×10^{1}	2.2	<1	
15	3.5×10^{1}	3.4×10^{1}	1.3		
30	1.9×10^{1}	2.6×10^{1}	5.7	<1	6.3
60	1.6×10^{2}		2.6		
Echo 1, closed tubes					
5	2.2×10^{3}	9.9×10^{2}	<1	<1	
15	1.7×10^{3}	8.6×10^{2}	<1		
30	7.5×10^{2}	1.1×10^{3}	3.3×10^{2}	3.6×10^{2}	
60	4.9×10^{2}		8.5×10^{2}		

[a] Soil moisture in open tubes decreased from about 20 to 25% to about 15 to 18% during the day period. Closed tubes remained fairly constant at around 10 to 12%.

[b] Virus titers are given as number of PFU/gram (dry weight) of soil sample. Initial titer of poliovirus 1 was 2.4×10^{4}. Initial titer of echovirus 1 was 9.1×10^{3}.

[c] First day after a 5-day flooding period. Basins were flooded again between days 11 and 19.

TABLE 4. *Rates of inactivation for virus in small tubes buried in a sewage infiltration basin*[a]

Determination	Poliovirus type 1		Echovirus type 1	
	Flooding cycle	Drying cycle	Flooding cycle	Drying cycle
Closed tubes	−0.02	0.04	−0.10	−0.07
Open tubes	−0.06	−0.11	−0.25	−0.28
Difference	0.04	0.15	0.15	0.21
Statistical significance of the difference (*P*)	<0.02	<0.01	<0.01	<0.07

[a] Survival rates expressed as $\log_{10}$ changes in virus titer per day.

for the rate of virus inactivation are the mean rate obtained from the four different depths at which tubes were buried. For echovirus 1 the laboratory inactivation value was 0.08 $\log_{10}$ units/day. The indicated increase in poliovirus 1 titer in closed tubes during the drying cycle is presumed to represent the result of random error in soil sampling and assay. In general, the rate of virus inactivation during drying was greater than during flooding. It also appears that echovirus 1 was generally inactivated more rapidly than poliovirus 1 during both the flooding and drying cycles (Table 4). Values obtained in laboratory studies for virus inactivation rates in this same soil, at constant moisture, for poliovirus 1 were 0.05 to 0.10 $\log_{10}$ units/day (data not shown).

The amount of poliovirus seeded into the pipe sections which migrated downward a distance of 10 cm to the 15-cm depth during the 5-day flooding period was 18% of that remaining at the initial position (Table 5). No poliovirus was detected 25 cm below the initial position. The observed migration of echovirus was less than that of poliovirus, with the amount having moved to the 15-cm position being less than 0.05% of that found remaining at the initial position. These percentage values are based on the average virus titers on day 1 of both the deep and shallow pipes seeded with the type of virus in question. The downward migration of virus in unseeded areas in this same basin appeared to be greater than the migration of seeded virus. This apparent greater migration, however, could be deceptive and attributable to the possibility that some of the subsurface viruses had survived from the previous sewage application cycle.

On the 1st, 3rd, and 5th days of basin flooding, samples of between 1.5 and 2.0 liters of the sewage effluent entering the basin were collected for virus analysis. Viruses were concentrated from the sewage samples by the technique of Gerba et al. (6). Indigenous titers of enterovirus in the sewage samples on the sampling dates were 738, 953, and 614 PFU/liter of sewage effluent, respectively. From these data the average concentration of indigenous enterovirus in the sewage effluent during flooding of the basin was estimated to have been 768 PFU/liter.

Seeded poliovirus did not migrate very far into the soil in the small basins (Table 6). On day 0, the viral titer at the 5-cm depth was 11% of that found at the surface. No virus could be detected at the 15-cm or 30-cm depths. No virus was recovered from soil of the unseeded basin.

The average percentage change in soil moisture and virus titer (averages per day between sampling dates), along with the reduction in virus titer in the samples calculated as the rate of $\log_{10}$ decrease in PFU per day (averages per day between sampling dates), for all of the field experiments are listed in Table 7. The listed values represent the average moisture and infectivity rates that occurred at all depths. The rates of virus titer and moisture change in the closed tubes were not included in this table since drying was not involved in virus survival in these tubes.

TABLE 5. *Virus and soil moisture levels in soil samples from seeded pipe sections and in samples of unseeded soil from the same basin*[a]

Sample depth (cm)	Virus survival after no. of days drying[b]		
	Day 1	Day 3	Day 5
Polio 1, shallow pipe			
5	1.2×10^3	5.6×10^1	4
15	8.0×10^1	2.2×10^1	<1
30	<1	<1	ND[c]
Polio 1, deep pipe			
5	8.4×10^3		4.5×10^1
15	9.6×10^1		<1
30	<1		ND
Echo 1, shallow pipe			
5	9.4×10^2	4.3×10^1	<1
15	<1	<1	ND
30	<1	<1	ND
Echo 1, deep pipe			
5	1.1×10^3		1.2×10^2
15	<1		ND
30	<1		ND
Natural isolates			
Surface	3.2×10^2	1.2×10^1	<1
5	5.0×10^1	<1	<1
15	2.6×10^1	6	<1
30	<1	<1	<1
45	<1	<1	<1
60	<1	<1	<1

[a] Soil moisture in the pipe sections decreased from about 20 to 15% during the drying period.

[b] Virus titers are given as number of PFU/200 g (dry weight) of samples.

[c] ND, Not quantitated because no virus was anticipated to be found.

TABLE 6. *Survival of seeded poliovirus 1 and virus from primary sewage effluent in the small infiltration basins*[a]

Sample depth (cm)	Virus survival after no. of days drying[b]			
	Day 0	Day 1	Day 4	Day 8
Surface	5.0×10^4	1.6×10^4	2.1×10^2	<1
5	5.4×10^3	7.6×10^2	6.3×10^1	<1
15	<1	<1		
30	<1	<1		

[a] Soil moisture at the depths tested decreased from 19 to 6% during the drying period. Two basins, one seeded with virus and one unseeded, were examined for this portion of the study. However, no virus was recovered from soil of the unseeded basins.

[b] Virus titers given as number of PFU/200 g (dry weight) of sample.

TABLE 7. *Reduction in soil moisture and virus titer during the drying portion of the sewage application cycle in field study*

Soil samples containing virus	Avg % reductions/day, between sampling dates		Rate of reduction in virus titer (log_{10} units/day)
	Moisture content	Virus titer	
Fall weather conditions			
Polio 1 in small basin	34.7	33	0.52
Polio 1 buried in pipe sections	8.4	31	0.49
Echo 1 buried in pipe sections	10.5	28	0.45
Indigenous virus in large basin	13.8	30	0.48
Polio 1 buried in small open tubes	7.8	13	0.11
Echo 1 buried in small open tubes	6.4	19	0.28
Winter weather conditions, indigenous virus	24.7	30	0.23

The rate of virus inactivation in both open basins and pipe sections was similar and independent of virus type. The rate of virus inactivation in the small open tubes was much less than that occurring in the pipe sections or in the open basins. The fact that all of these values plot on the same curve (Fig. 3) indicated that the lower rate of virus inactivation which had occurred in the small open tubes was due to a generally slower rate of drying in the tubes relative to the pipe sections and open basin. The probable reason for this slower rate of drying in the tubes is that proportionately more of the soil volume in the small tubes was surrounded by impermeable material, in this case plastic. Thus, the differences between inactivation rates of viruses buried in the soil in the various containers were related to differences in the rates of moisture loss. The plot of moisture loss in soil versus virus inactivation in soil does not parallel the plot obtained for virus inactivation in sludge solids (11).

The inactivation rate of indigenous viruses during the winter study was less than that which occurred during the fall study, possibly because of differences in weather conditions (primarily temperature) between the two studies.

DISCUSSION

A great deal of information has been generated concerning the retention of enteroviruses by soil columns and adsorption to soil in batch experiments. These reports have been reviewed by Bitton (2), Burge and Marsh (4), Elliott and Ellis (5), and Gerba et al. (7). A few researchers have also attempted to correlate laboratory results concerning the capacity of specific soils to remove viruses applied in sewage effluents with field data (3, 7, 20). No information is currently available, however, on the occurrence of unseeded viruses in soil beneath a wastewater land treatment site.

The sludge layer that formed on the surface of the infiltration basins contained a much greater amount of virus than did the uppermost soil region. Two possible explanations could account for this finding. First, many of the viruses in the wastewater effluent applied to the basin were solid associated and were removed by filtration of the sewage solids. Second, the viruses in the effluent were initially not solid associated and adsorbed to the sludge layer during the filtration process. Supportive evidence for the first proposal, that a large portion of the viruses in the sewage effluent was associated with solid particulates, comes from the winter field study. In general, during the winter study the levels of

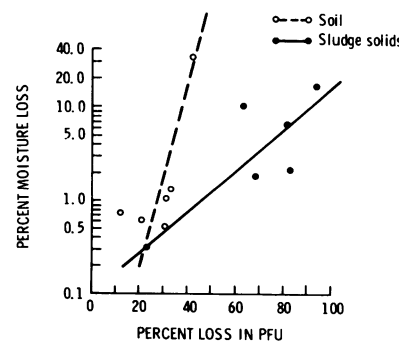

FIG. 3. *Comparison between moisture loss and virus inactivation in disposed sewage sludge solids and in soil.*

virus found at a distance of 100 m from the sewage inlet were lower than the levels of virus found at a distance of 40 m from the sewage inlet. Presumably, this difference in virus concentration at the two distances from the inlet was due to a settling out of sewage particulates and their associated virus particles during flow of the effluent down the length of the basin.

The finding in the present study that virus concentrations in the upper surface of the soil profile were approximately 1 $\log_{10}$ higher than those found between the 2.5- and 25-cm levels closely corresponds with the data of Lance et al. (13) concerning the removal of enteroviruses from wastewater applied to laboratory columns of the same soil. Lance et al. (13) found that 1 $\log_{10}$ of virus was removed by passage of effluent through the first few centimeters of the soil column, with removal being less at greater depths in the column. Thus, studies with the long soil columns may provide good model systems for virus removal under field conditions.

Goyal and Gerba (9) found in batch studies with small soil samples that whereas poliovirus type 1 adsorbs well to many different soils, echovirus type 1 does not. Because of these findings it was thought that echovirus type 1 might migrate through the soil profile during infiltration of wastewater to a greater extent than poliovirus type 1. Examining the movement of these two virus types through the soil contained in the pipe sections (Table 5) indicated that echovirus 1 did not move through the soil further than poliovirus 1. In fact, echovirus 1 appeared to move to a lesser degree than poliovirus 1. It appears that conditions under which viruses are adsorbed to soil previous to infiltration of sewage effluent result in little virus elution or migration. Thus, the adsorptive behavior of viruses in laboratory batch studies may not be totally reflective of their behavior under field conditions.

Virus did not persist for extended periods of time in the soil. Virus buried in the small open tubes could be detected on day 19 and appeared to survive a second flooding period (Table 3), but these might have originated from sewage applied during the second flooding. The titers of virus found on day 19 were much lower than those found on day 1. As shown in Table 4, the rate of virus inactivation appeared to be greater in the open buried tubes than in the closed buried tubes during both the flooding and drying portions of the effluent application cycle. In general, echovirus 1 was inactivated more rapidly in the buried tubes than was poliovirus 2. Unseeded virus in the basin soil could not be detected on the 5th day of drying. Seeded poliovirus in the open soil of the small basin was detectable on day 4 but not on day 8. The average percentage reductions in virus titer (per day, between sampling dates) and the rates of reduction in virus titer were similar for both poliovirus 1 and echovirus 1 in the buried pipe sections. The percentage reductions and rates of reduction in virus titer for poliovirus 1 and unseeded virus in open soil were likewise similar and were nearly identical to the reductions in virus titer which occurred for the seeded virus in pipe sections. The laboratory-derived value for rate of virus titer reduction at a constant 15% moisture and at the temperature of the fall field study (average 25°C) was 0.235 $\log_{10}$ reduction in virus titer per day (data not shown). This laboratory study-derived value is approximately one-half of the actual field study inactivation rate, indicating that survival of viruses under constant environmental conditions in the laboratory is not identical to survival under field conditions. This difference in virus survival was hardly unexpected, due to the constantly changing temperature and moisture level of soil in the field. The rate was similar to that in buried open tubes where drainage was somewhat impaired.

Conditions in the open soil, pipe sections, and open plastic tubes were thought to have been similar during the course of this study with respect to environmental factors. The percentage reductions and rates of reduction in virus titers in the small plastic tubes were, however, less than those found in the pipe sections or open soil. The possible difference in conditions between virus buried in the different manners may have been the rate at which moisture passed from soil in the pipe sections and small tubes into the surrounding soil. The reason for this difference in rate of moisture transmission could be that a higher ratio of plastic surface-to-soil content volume existed for the contents of the small tubes in comparison with the pipe sections. Such a difference in ratio of plastic surface-to-soil content volume would be even greater between the tubes and open soil.

Drying of the soil had a major influence on the rate at which virus was inactivated (Fig. 3). These results also indicated that the observed differences in viral inactivation rates between the small tubes, pipe sections, and open soil were due to differences in soil drying rates. It is important to note that poliovirus 1, echovirus 1, and the indigenous enterovirus population in the soil were all inactivated similarly with respect to drying.

It was of interest that the curves representing virus loss in soil and disposed sludge solids were not parallel. Enteroviruses in soil seemed to be slightly more resistant to drying. However, the

results of the two separate studies may not be fully supportive of this conclusion since they were done at different times and there is a great deal of scatter between the points. Nonetheless, these findings do confirm the addition of a second important factor, that of aeration, to the already well-known environmental factor of temperature which influences virus survival in sludge and soil (23, 25).

In summary, the conclusions of this study are as follows. Under field conditions the downward migration through soil of seeded poliovirus type 1 was found to be greater than that of echovirus type 1. Migration of naturally occurring enteroviruses during infiltration of sewage effluent through soil could not be measured with accuracy because of the possibility of virus survival from previous applications of effluent. Inactivation of these same seeded virus types and indigenous enterovirus populations in soil during drying of the infiltration basins appeared to be similarly related to the loss of soil moisture content. Allowing the soil in rapid infiltration basins to dry periodically between effluent application cycles would thus be advantageous to help prevent build-up of high virus levels in the soil and thereby aid in lessening potential virus contamination of groundwater. The maximum depth at which virus survival was measured during this study was 60 cm. It is likely that with increasing depth below the ground surface, the extent of virus exposure to desiccation during a given basin drying period will be reduced. In the portion of this study concerned with survival of virus in buried pipe sections (Table 5), it appeared that higher amounts of virus were detectable in the deeply buried pipe sections than in the shallow buried pipe sections. It is uncertain whether this phenomenon was due to a more rapid drying of soil near the surface or to lower oxygen levels and smaller populations of aerobic bacteria at the deeper depths. Studies conducted in our laboratory have indicated that the presence of aerobic microorganisms is detrimental to enterovirus survival (C. J. Hurst, C. P. Gerba, and I. Cech, manuscript submitted for publication).

The wastewater flooding and drying cycle used in this study is that currently used at the land application sites studied to optimize nitrogen removal and to prevent soil clogging. Thus, it appears that allowing drying periods for virus die-off would involve no added trouble in terms of effluent application management.

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