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Virus Movement in Soil Columns Flooded with Secondary Sewage Effluent

J. C. LANCE,* C. P. GERBA, AND J. L. MELNICK

U.S. Water Conservation Laboratory, Agricultural Research Service, Phoenix, Arizona 85040, and Department of Virology and Epidemiology, Baylor College of Medicine, Houston, Texas 77030*

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Secondary sewage effluent containing about 3×10^4 plaque-forming units of polio virus type 1 (LSc) per ml was passed through columns 250 cm in length packed with calcareous sand from an area in the Salt River bed used for groundwater recharge of secondary sewage effluent. Viruses were not detected in 1-ml samples extracted from the columns below the 160-cm level. However, viruses were detected in 5 of 43 100-ml samples of the column drainage water. Most of the viruses were adsorbed in the top 5 cm of soil. Virus removal was not affected by the infiltration rate, which varied between 15 and 55 cm/day. Flooding a column continuously for 27 days with the sewage water virus mixture did not saturate the top few centimeters of soil with viruses and did not seem to affect virus movement. Flooding with deionized water caused virus desorption from the soil and increased their movement through the columns. Adding $CaCl_2$ to the deionized water prevented most of the virus desorption. Adding a pulse of deionized water followed by sewage water started a virus front moving through the columns, but the viruses were readsorbed and none was detected in outflow samples. Drying the soil for 1 day between applying the virus and flooding with deionized water greatly reduced desorption, and drying for 5 days prevented desorption. Large reductions (99.99% or more) of virus would be expected after passage of secondary sewage effluent through 250 cm of the calcareous sand similar to that used in our laboratory columns unless heavy rains fell within 1 day after the application of sewage stopped. Such virus movement could be minimized by the proper management of flooding and drying cycles.

High-rate land filtration of secondary sewage effluent is an effective way to remove most of the nitrogen, phosphorus, organic material, and fecal bacteria from the wastewater (2, 3, 9-12). Since wastewater may infiltrate the soil at a rate of 15 to 60 cm/day, the potential for virus movement into the groundwater could be great unless viruses were adsorbed by the soil. Gilbert et al. (8) monitored samples from four wells at the Flushing Meadows wastewater renovation project every 2 months during 1974 and detected no viruses in any well sample. These basins had been intermittently flooded with sewage water for 7 years (through 1974) at an average hydraulic loading rate of 90 m/year. These data showed that the system quite effectively removed virus from secondary effluent, but did not indicate the mechanism of removal.

Gerba et al. (7) and Bitton (1) reviewed several reports of laboratory experiments on virus adsorption by soils. However, in most of the experiments viruses were suspended in tap water, distilled water, or dilute salt solutions and applied to soil columns. In other experiments, short columns were used and flow rates were not reported. These conditions limit the use of these data for predicting virus movement under natural field conditions.

Previous studies on the removal of N, P, and organic material from secondary sewage effluent by our laboratory soil columns showed that they were good models of the Flushing Meadows field project with respect to infiltration, gases, dissolved salts, organic carbon, and fecal coliform bacteria (2, 9-12). Therefore, these columns were used in experiments designed to study adsorption, desorption, and movement of added poliovirus and to determine whether the soil could be saturated with viruses applied in secondary sewage effluent.

MATERIALS AND METHODS

Construction of soil columns. Soil columns were constructed by packing polyvinyl chloride pipe with loamy sand from basins used for rapid infiltration of secondary sewage effluent. These basins, which are located in the dry Salt River bed near Phoenix, Ariz., had been intermittently flooded for 4 years

with a total infiltration of about 350 m of secondary effluent (2, 3). The columns were intermittently flooded in the laboratory during various experiments for 3 additional years before the virus movement experiments began. Each column consisted of a 2.75-m length of 10-cm (inside diameter) polyvinyl chloride pipe filled with 6 cm of pea gravel on the bottom and 250 cm of loamy sand on the top. The air-dried soil (3% clay, 8% silt, and 89% sand) was packed so that the average bulk density of each column ranged from 1.5 to 1.6 g/cm^3. However, slight differences in packing resulted in a range of infiltration rates from 15 to 55 cm/day. Secondary sewage effluent was applied with a Mariotte siphon to maintain a 15-cm constant pressure head at the soil surface. The flow rate and cumulative flow through the columns were measured by weighing the outflow daily. Details of the flow system were described previously (9, 11).

Application of virus in sewage water. A virus suspension (poliovirus type 1 LSc) was mixed with dechlorinated secondary sewage effluent to obtain a concentration of about 3×10^4 plaque-forming units (PFU)/ml in water applied to two laboratory columns flooded on a schedule of 9 days of flooding alternated with 5 days of drying. (The columns were allowed to drain for 5 days at room temperature.) Viruses were applied during three flooding cycles, and average infiltration rates for the two columns were 55 and 15 cm/day. Samples (2 to 5 ml) were extracted daily from ceramic samplers at depths of 2, 5, 10, 20, 40, 80, 160, and 240 cm and from the outlet line (250 cm). Preliminary tests indicated that 0 to 10% virus loss occurred when samples were drawn through the ceramic samplers. A 100-ml sample of the cumulative outflow of each day was concentrated as described by Wallis et al. (15). After the third 5-day drying period, the column with a 55-cm/day infiltration rate was flooded with the virus sewage water mixture for 27 days and samples were extracted at the various depths on 8 different days during this long flooding period.

One or two drops each of fetal calf serum and a mixture of penicillin and streptomycin were added to water samples before freezing. Samples were packed in dry ice and shipped to Houston, Tex., where the virus assays were performed. Portions (1 ml) of the samples were assayed by the agar overlay technique (13). Virus assays were performed on Buffalo green monkey cells (5). The cell line was passaged, grown, and maintained as described previously (13).

Desorption of viruses. A third soil column with a 45-cm/day infiltration rate was flooded with the sewage water-virus mixture for 2 days, dried for 2 h, and flooded with a 1 mM $CaCl_2$ solution for 2 days. This experiment was repeated using a 3 mM $CaCl_2$ solution. The column was sampled at the various depths during each day that it was flooded with the $CaCl_2$ solution. The salt concentration in duplicate water samples was calculated from electrical conductivity measured with a Wheatstone bridge.

The 45-cm/day column was also used in an experiment to study virus desorption and movement when a pulse of deionized water moved through the soil. The column was flooded with the sewage water-virus mixture for 2 days and dried for 2 h. A 10-cm amount of distilled water was infiltrated and followed immediately by sewage water with no added virus. Flooding with sewage water continued for 2 days. Samples were extracted down to the 80-cm depth 1 h after the sewage water with no virus was added (e.g., 1 h after the deionized water infiltrated) and at all depths after 1 and 2 days of flooding with sewage water.

The 55-cm/day column was then used to determine the effect of various drying times between the virus application in sewage water and flooding with deionized water on virus desorption and movement. In each experiment the column was flooded with the sewage water-virus mixture for 2 days, dried, and flooded with deionized water for 2 or 3 days. The drying times were 2 h, and 1 and 3 days. The column was sampled at the various depths during each day that it was flooded with deionized water.

RESULTS AND DISCUSSION

Virus movement during flooding with sewage water. Most of the viruses were removed from the sewage as it passed through the first few centimeters of soil (Fig. 1; Tables 1 and 2). Virus removal by the two columns was similar even though the infiltration rates were quite different (55 and 15 cm/day). Viruses were detected in only 3 of the 43 1-ml samples from the 160-cm depth and none was detected in (1-ml) samples from the 240- and 250-cm depths. Viruses were detected in 5 of the 43 (100-ml) samples of the daily cumulative drainage from the columns. A few more viruses moved to the 40-cm depth in the 15-cm/day column than in the 55-cm/day column (Fig. 1). This was probably due to the higher average concentration of viruses in the sewage applied to the 15-cm/day column.

The average concentration of viable virus in the sewage reservoir declined sharply from 2.6×10^4 to 3.4×10^3 PFU/ml during day 1 of storage and then declined at a lower rate to 1.7×10^3 during day 2 and 6.1×10^2 during day 3. The sewage water-virus mixture in the reservoir was usually replaced after 1 or 2 days, except in some instances when the same mixture was used during a weekend. However, the similarity in virus removal by two columns with different infiltration rates, and thus different water detention times (40 and 160 h), suggests that the natural dying off of virus was not the dominant removal mechanism inside the soil columns. This is not surprising since poliovirus is known to survive almost 100 days in sand flooded with wastewater (6).

Movement through the soil reduced the virus concentration by about 2 logs during the first 2 cm of travel, but an additional 38 cm of travel

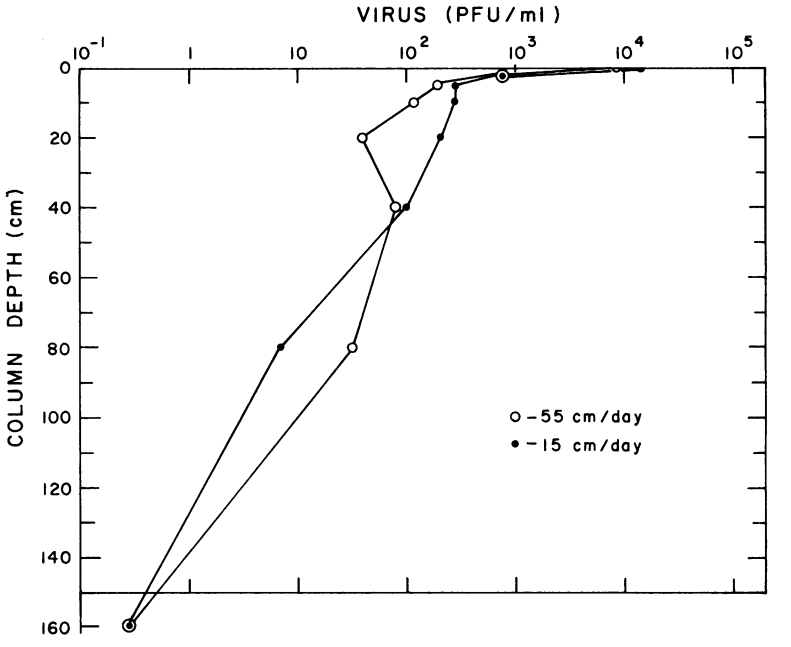

FIG. 1. *Virus concentrations at various depths in soil columns flooded with sewage seeded with poliovirus. Data are averages for three flooding cycles of 9 days of flooding and 5 days of drying.*

TABLE 1. *Virus removal by a soil column with an infiltration rate of 55 cm/day*

Depth (cm)	Range of virus remaining for all cycles (PFU/ml)	Avg[a] no. (PFU/ml) of viruses remaining during:			
		Cycle 1	Cycle 2	Cycle 3	Avg
0	125–44,000	8,600	5,525	11,329	8,485
2	15–4,350	940	475	866	760
5	0–735	97	154	320	190
10	0–720	47	86	230	121
20	0–120	24	23	71	39
40	0–472	102	61	78	80
80	0–150	44	38	13	32
160	0–5	0.5	0.5	0	0.3
240		0	0	0	0
250		0	0	0	0
250[b]	0–180	20	1	0.5	7

[a] Average for nine sample dates during each 9-day flooding period. Five-day drying periods were alternated with the flooding periods.

[b] A 100-ml sample of a 24-h cumulative outflow (PFU per 100 ml).

was required to reduce the virus concentration by another log (Fig. 1).

These data indicate that near the soil surface factors other than adsorption were involved in virus removal. If adsorption were the only factor involved, then virus removal with depth should have been directly proportional to the concentration of virus detected at any depth along the column. This removal phenomenon can be described mathematically by the following equation: $(dC_v/dx) = -kC_v$, where C_v = virus concentration detected at any depth in the column, x = column depth, and k = removal constant.

TABLE 2. *Virus removal by a soil column with an infiltration rate of 15 cm/day*

Depth (cm)	Range of virus remaining for all cycles (PFU/ml)	Avg[a] no. (PFU/ml) of viruses remaining during:			
		Cycle 1	Cycle 2	Cycle 3	Avg
0	150–57,000	11,392	18,850	9,821	13,354
2	80–2,850	713	807	856	792
5	0–1,350	304	448	105	286
10	15–1,100	242	491	105	280
20	0–690	172	348	113	211
40	5–450	138	56	103	99
80	0–35	9	6	6	7
160	0–5	0	1	0	0.3
240		0	0	0	0
250		0	0	0	0
250[b]	0–4	0	0	1.0	0.3

[a] Average for four to six sampling dates during each 9-day flooding period. Five-day drying periods were alternated with the flooding periods.

[b] A 100-ml sample of a 24-h cumulative outflow (PFU per 100 ml).

Below a depth of 5 cm the amount of virus detected at any depth corresponded closely to values obtained by the above equation, where k is equal to 0.046 cm^{-1}. This indicates that adsorption was probably the dominant factor in virus removal at these depths. Of course, it is possible that viruses adsorb more readily to soil particles near the soil surface, resulting in a different k value. As stated previously, these columns have been flooded intermittently with sewage for 3 years, and perhaps microbial activities near the surface or other factors have caused an alteration in the nature and/or composition of the soil near the surface of the column, creating more optimal conditions for virus adsorption. The removal between 0 and 2 cm was not due to straining at the soil surface because the virus concentration also decreased sharply between the 2- and 5-cm depths, where surface straining would not affect virus removal. In addition, the diameter of the virus (23 nm) is so small that straining should not be a major factor.

Flooding the 55-cm/day column with the sewage water-virus mixture for 27 days did not saturate the upper 5 cm of the soil. The virus concentrations in samples extracted near the end of the 27-day period were about the same as those found on earlier sampling days (Table 3). A total of 14.8 m of sewage water containing an average of 1.4×10^4 PFU/ml was infiltrated during the 27 days.

Desorption of viruses with deionized water or salt solution. Applying deionized water within 2 h after the sewage water-virus mixture was removed caused considerable virus desorption, and more viruses were detected at the 80- and 160-cm depths than when the column was flooded with the sewage water-virus mixture (Table 4). Evidently, most of the viruses were adsorbed again lower in the column because only a few were detected in the 100-ml samples of the cumulative outflow. The desorption and movement of viruses were reduced greatly when $CaCl_2$ was added to the deionized water. Only a few viruses were detected in the profile samples when a column with an infiltration rate of 45 cm/day was flooded with a sewage water-virus mixture for 2 days, dried for 2 h, and flooded with 1 or 3 mM $CaCl_2$ solutions (Table 4). No viruses were detected in the cumulative outflow samples collected during these two experiments.

When a soil column was flooded with the sewage water-virus mixture followed by 10 cm of deionized water and then by sewage effluent containing no added virus, a front of viruses was detected in samples extracted 1 h after the sewage without virus was applied (Fig. 2). This was 1 h after the deionized water infiltrated the column and 8 h after the deionized water was first applied. The peak concentration of 1,000 PFU/ml occurred at the 10-cm depth. Viruses were detected at only one depth (30 PFU/ml) when samples were extracted 1 day later, and no viruses were detected on the second day after the pulse of deionized water was added to the column. Also, no viruses were detected in the 100-ml samples of the cumulative outflow on either day.

TABLE 3. *Virus movement in a soil column continuously flooded with a sewage water-virus mixture for 27 days*

Depth (cm)	PFU/ml on sample day:							
	2	6	9	13	14	20	23	27
2	3,300	765	1,550	660	300	4,850	240	405
5	3,000	960	1,200	450	210	1,600	225	240
10		630	180	585	75	300	15	175
20	135	360	210	285	25	150		80
40	165	270	630	110	0	225	35	
80	0	55	30	85	0	10	5	35
160	0	0	5	0	0	0	5	30
240	0	0	0	0	0	0	0	
250	0	0	0	0	0	0	0	
250[a]	0	0	0	0	0	12	0	

[a] A 100-ml sample of a 24-h cumulative outflow (PFU/100 ml). The virus content of the sewage water samples (0 to 2 days old) ranged from 850 to 41,500 PFU/ml.

TABLE 4. *Movement of virus in soil columns flooded with deionized water or $CaCl_2$ solutions after poliovirus was applied in sewage water for several days*[a]

Depth (cm)	PFU/ml at a leaching solution $CaCl_2$ content of:					
	0		1 mM		3 mM	
	D_1[b]	D_2	D_1	D_2	D_1	D_2
2	50	75	0	0	13	0
5	8	0	0	0	165	0
10	650	113	0	5	0	0
20	128	30	0	10		0
40	328	330	0	10	10	0
80	145	705	0	0	55	0
160	0	38	0	0	0	0
240	0	3	0	0	0	0
250	0	5	0	0	0	0
250[c]	0	20	0	0	0	0

[a] The columns were drained for 2 h between the application of sewage water and leaching solutions.

[b] Number of days the column was flooded with leaching solution before samples were taken.

[c] A 100-ml sample of a 24-h cumulative outflow (PFU per 100 ml).

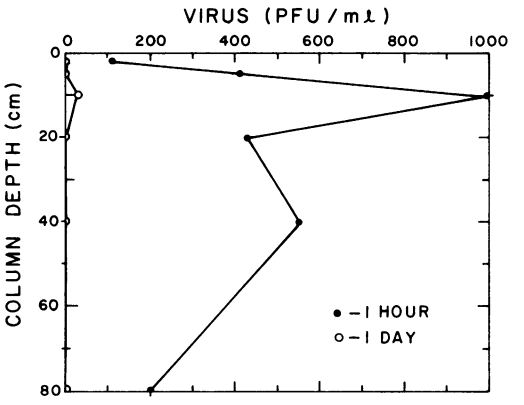

FIG. 2. *Virus concentrations at various depths in a soil column after the addition of a pulse of deionized water. The column was flooded with sewage containing poliovirus for 2 days, dried for 2 h, flooded with deionized water (10 cm infiltrated), and finally flooded with secondary sewage effluent containing no added poliovirus for 2 days. Samples were extracted after 1 h and 1 day during flooding with sewage without added virus.*

These results showed that reducing the ionic strength of the soil solution caused desorption and movement of viruses through the soil. However, most of the viruses were adsorbed again after flooding with sewage. Virus did move at least 80 cm due to the pulse of deionized water and probably would have been detected at lower depths if more samples had been extracted during day 1. The salt concentrations in the profile samples showed that the pulse of deionized water reduced salt concentrations during the first few hours, but the effect was almost gone after day 1 due to the mixing of salts from the sewage water with the pulse of deionized water by diffusion and dispersion (Fig. 3).

Effect of drying on desorption. Another experiment with the 55-cm/day column showed that virus desorption during flooding with deionized water could be prevented by drying the soil for several days between the applications of the sewage water-virus mixture and deionized water. When the soil was dried for 2 h, the peak virus concentration detected during day 1 of flooding with deionized water was 700 PFU/ml, but it was only 35 PFU/ml when the soil was dried for 1 day (Table 5). The peak virus concentration declined to 20 PFU/ml when the soil was dried for 3 days, and no viruses were detected in any sample when the soil was dried for 5 days before flooding with deionized water. No viruses were detected in the cumulative outflow samples when the soil was dried at least 1 day before flooding with deionized water (Table 5). Viruses were not detected during day 2 of flooding with deionized water when the soil had been dried for 3 days before flooding with deionized water.

The effect of the length of drying time before additions of deionized water on the movement of viruses was not due to differences in the ionic strength of the soil solutions during flooding with deionized water. Salt concentrations in samples extracted during day 1 of flooding with deionized water were greater when the deionized water was applied after 2 h of drying than when it was applied after 1 day of drying (Fig. 4). Yet many viruses moved with the deionized water in columns that were dried for 2 h, and only a few viruses moved with deionized water when the soil was dried for 1 day. Thus, the effect of drying outweighed any effect due to differences in salt concentrations. The dying off of viruses in the column probably was not an important factor because many viruses were detected in samples extracted during day 3 of flooding with deionized water (Table 5).

Also, the viruses were not inactivated by desiccation because the soil surface was still moist after 1 day of drying, and the 0- to 2-cm layer contained about 5% water by weight after 5 days of drying. Data from previous experiments with soil columns allowed to drain for 5

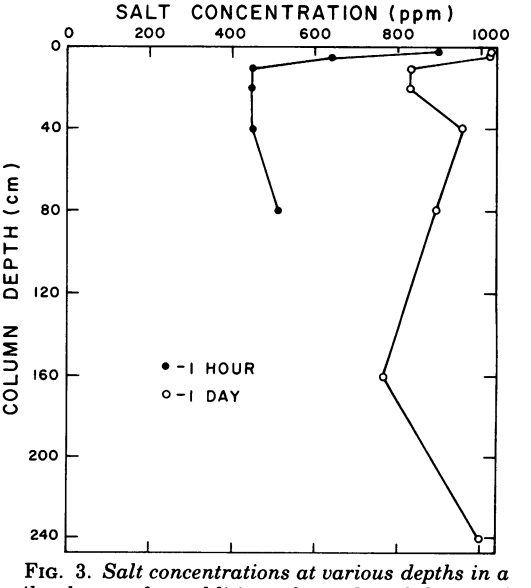

FIG. 3. *Salt concentrations at various depths in a soil column after addition of a pulse of deionized water. The column was flooded with sewage containing poliovirus for 2 days, dried for 2 h, flooded with deionized water until 10 cm infiltrated, and finally flooded with secondary sewage effluent without added virus. Samples were extracted after 1 h and 1 day of flooding with sewage without added virus.*

TABLE 5. *Movement of viruses in soil columns flooded with deionized water after poliovirus was applied in sewage water for several days*[a]

Depth (cm)	PFU/ml								
	0.1[b]			1		3		5	
	D_1[c]	D_2	D_3	D_1	D_2	D_1	D_2	$D_{0.3}$	D_1
2	50	75	0	5	0	0	0	0	0
5	8	0	0	25	0	0	0	0	0
10	650	113	300	35	10	0	0	0	0
20	128	30	75	0	10	20	0	0	0
40	328	330	120	0	20	20	0	0	0
80	145	705	128	0	10	0	0	0	0
160	0	38	8	0	0	0	0		0
240	0	3	20	0	0	0	0		0
250	0	5	8	0	0	0	0		0
250[d]	0	20		0	0	0	0		0

[a] The columns were drained for different times between applications of the virus in sewage water and deionized water.

[b] Number of days of drying before the addition of deionized water.

[c] Number of days the soil was flooded with deionized water before sampling.

[d] A 100-ml sample of a 24-h cumulative outflow (PFU per 100 ml).

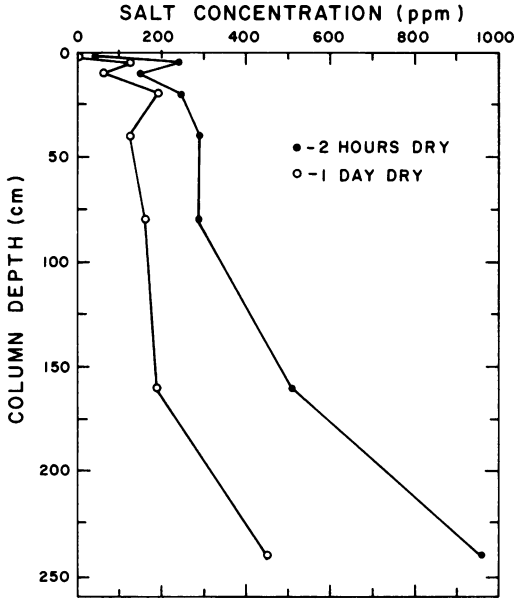

FIG. 4. *Salt concentrations in samples from columns leached with deionized water after sewage effluent applications. Different drying times separated the two applications.*

days showed that the soil moisture tension at the 2-cm depth was 200 millibars and the tension at 70 cm and below was less than 100 millibars. Instrumentation did not permit measurements above the 2-cm depth. These measurements show that soil microorganisms were not placed under much moisture stress, except perhaps very near the soil surface.

Increasing the ionic strength of the soil solution decreases the thickness of the double layer (or cloud of ions) around the soil particles and viruses and allows the viruses to move close enough to the soil particle surface to be bound by London-Van der Waals forces (1). Drying the soil allows the free water to drain from the soil pores. This may also promote virus adsorption by allowing viruses to move near the soil particles. Evidently, all of the viruses are adsorbed when the free water drains from the soil. The drainage curve from previous experiments showed that about 75% of the free water drained from the soil columns during the first day after the sewage was removed (11). This explains why most of the virus movement was eliminated by 1 day of drying before deionized water was applied. Then the viruses could not be removed merely by reducing the ionic strength of the leaching solution. Recently, Duboise et al. (6) reported virus movement in laboratory cores flooded with distilled water after poliovirus was applied. However, the columns were too short (16.5 cm) to permit much readsorption of viruses. The effect of draining the soil pores would not be noted in these short columns because the capillary fringe above the outflow end of the column probably extended to the top of the column. Thus, the column remained saturated even when the surface was not flooded.

Application of laboratory data to field systems. Several features of our laboratory soil columns facilitate application of the data to field systems. Using long columns (250 cm) allowed sampling at various depths and provided a long section of column (~200 cm) that drained when the soil surface was not flooded. Both saturated and unsaturated flow occurred during the experiments. Most of the profile samples were extracted during saturated flow. However, outflow samples were collected after water was removed from the soil surface and unsaturated flow proceeded during the drying period. Some unsaturation probably developed during the flooding period as a result of clogging at the soil surface. The poliovirus was applied in secondary sewage effluent and flow rates were accurately measured. The soils used had been flooded with secondary sewage effluent for 7 years, including 3 years after the soil was packed in the columns before the virus was applied. The columns were intermittently flooded on schedules similar to those used in groundwater recharge systems.

The virus movement in our soil columns when deionized water was applied appears to be similar to the movement through sand in a Florida field application system after heavy rains (16). However, virus movement in our columns occurred only under certain conditions described above. The application of sewage in the work reported by Wellings et al. (16) is not described in enough detail to determine whether these conditions were met in that experiment.

Shaub et al. (14) reported more virus movement in a high-rate land treatment system at Fort Devins, Mass., than we have shown. However, the soil used in that system was a coarse sand with practically no clay and a low surface area. Also, they applied primary sewage effluent rather than secondary effluent. Since Carlson et al. (4) showed that organic compounds compete with viruses for adsorption on clays, adsorption of viruses from primary effluent may be less than from secondary effluent.

Our results indicated that the absence of viruses in the wells below and adjacent to the field groundwater recharge system described by Gilbert et al. (8) was probably due to virus retention in the first few centimeters of the soil profile. The removal of practically all of the viruses applied to the soil columns for 27 days at 10^5 times the maximum concentration measured in Phoenix secondary effluent indicates that the adsorption capacity of the soil would not be saturated under these management practices. Virus movement in the field where much lower concentrations are applied in sewage may be less than that shown in the columns. The data suggest that viruses would move through 250 cm of the calcareous sand used in the laboratory columns only if heavy rains fell within 1 day after the application of sewage stopped. If this happened, virus movement could be minimized by flooding with sewage soon after the rain began. Further research is needed to determine how long the adsorbed viruses remain viable.

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