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Virus Transport and Survival After Land Application of Sewage Sludge†

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The survial and transport patterns of poliovirus 1 and echovirus 1 were studied in undisturbed soil cores which were treated with digested sludge and exposed to natural weather conditions prevailing in north central Florida. It was shown that, under those experimental conditions, enteroviruses are relatively rapidly inactivated in the soil. A more rapid virus decline was observed during the warm and dry fall season than during the warm and wet summer season. The monitoring of soil core leachates has shown that both viruses were effectively retained by the sludge-treated soil.

Recent legislation requires acceptable methods for disposing of sewage sludges. Land application of this cumbersome material appears to be an acceptable alternative. Sludge harbors, however, a wide range of human pathogens and parasites, and it is thus essential to examine the practice of sludge application to land from a public health standpoint. Environmental Protection Agency investigators have shown that virus concentrations range from 3.8×10^3 to 11.6×10^3 PFU/liter of sludge (1). Viruses are not completely destroyed after anaerobic (1, 2, 8, 20) and aerobic (Scheuerman et al., unpublished data) digestion, composting (21), or irradiation (19). It is therefore important to gain some knowledge of the transport and survival patterns of viruses in sludge-amended soils. Numerous studies of the fate of viruses after wastewater application to soils have been undertaken (for a review, see references 3, 7, and 11). Comparatively little is known about the viral aspects of sludge application to land (6, 14, 17, 18). Viruses may survive for relatively long periods in sludge-amended soils under cold winter temperatures. Using lysimeters, Damgaard-Larsen et al. (6) also showed that viruses were completely removed by sludge-treated soils.

In this study, the survival and transport of viruses in sludge-treated soils were evaluated under natural conditions prevailing in north central Florida. Undisturbed soil cores were used, and environmental parameters (i.e., soil temperature, soil moisture, and rainfall) were monitored. The protocol of sludge disposal onto soil was similar to that practiced at sludge disposal sites.

MATERIALS AND METHODS

Viruses and viral assays. Poliovirus 1 (LSc strain) and echovirus 1 (Farouk strain) were both used in this study. Virus stocks were prepared and kept at −70°C until used. They were assayed by the plaque technique with AV3 (human amnion) or MA104 (simian kidney) cell monolayers. Each viral count shown represents the average of triplicate runs. Virus numbers were expressed as PFU.

Sludges. A lagooned sludge was sampled at the West Florida Agricultural Experiment Station, Jay. This station, where sludge application to land was carried out for many years, was also the site for our field study (9). The lagooned sludge was a mixture of aerobically (1/3) and anaerobically (2/3) digested sludges from the Montclair and Main Street wastewater treatment plants in Pensacola, Fla.

Virus association with sludge solids. Before sludge application to soil cores, it was desirable to know the extent of virus association with sludge solids. Poliovirus 1 and echovirus 1 suspensions were added to sludge samples, and the mixtures were magnetically stirred for 60 min. Samples were subsequently centrifuged at $1,400 \times g$ for 10 min at 4°C, and the supernatants were assayed for viruses to determine the percentage of sludge-associated viruses.

Soil. The soil used in this study was Eustis fine sand typical of Florida soils. It was classified as a Psammentic Paleudult. The top horizon contained 94.8% sand, 2.4% silt, and 2.8% clay, and less than 1% organic matter (5).

Sludge application to soil cores. Undisturbed soil cores were obtained by driving pipes made of polyvinyl chloride into the soil. The cores were 33 cm long and had a diameter of 15.5 cm. Porous ceramic cups were installed at the bottom of the soil columns. These cups had a pore diameter of 1.4 to 2.1 μm (no. 2131, Soil Moisture Equipment Corp., Santa Barbara, Calif.).

Two soil cores were used for each virus under study. The cores were insulated by duct insulation and exposed to natural weather conditions outside the Environmental Engineering Sciences Building at the University of Florida. All the cores rested on a wooden box so that soil leachates generated during natural rainfall could be collected.

Each soil core was treated with 2.5 cm of virus-seeded liquid sludge. The applied sludge was allowed to soak in and dry on top of the soil for 1 to 4 days. During this period, the drying sludge solids on the soil surface were monitored for the presence of viruses. After the drying period, the sludge was mixed with the top 2.5 cm of soil, which was then monitored for the presence of viruses. Soil monitoring was continued until viruses could no longer be detected. Leachates from the sludge-treated cores were collected after each natural rainfall event, concentrated by the method of Farrah et al. (10), and assayed for viruses. The volume, conductivity, and pH of each leachate sample were also recorded.

Virus was detected in sludge and soil by methods described by Hurst et al. (13) and by Bitton et al. (4), respectively.

Measurement of environmental parameters. Soil temperature, soil moisture, and rainfall were monitored. The soil temperature was monitored every hour with thermocouples placed at the soil surface and at depths of 2.5, 10, and 20 cm

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TABLE 1. Virus association with lagoon sludge solids[a]

Virus	Virus input (total PFU)	Virus in sludge supernatant (total PFU)	Solid-associated virus (%)
Poliovirus 1	8.6×10^8	4.1×10^7	95.2
Echovirus 1	2.9×10^6	2.3×10^6	20.7

[a] Lagoon sludge was a mixture of aerobically digested sludge (1/3) and anaerobically digested sludge (2/3). The mixture was kept in a lagoon at the West Florida Agricultural Experiment Station before ultimately being disposed of on land. The pH, conductivity, and percent solids of the sludge were 7.0, 1,525 μS/cm, and 7, respectively.

in one of the soil cores. The thermocouples were connected to an Esterline Angus Key Programmable Data Acquisition System (model PD-2064, Esterline Angus Instrument Corp., Indianapolis, Ind.) which printed voltage (millivolts) at each thermocouple every hour. The voltages measured were later converted to temperature readings with the use of a computer. The soil moisture was monitored only when a sample of soil was obtained for viral assay. A rain gauge attached to the wooden box supporting the soil cores allowed the measurement of rainfall after each rain event.

RESULTS

Virus transport and survival were studied in soil cores which were treated with sludge and exposed to natural weather conditions. The protocol of sludge disposal onto soil was similar to that practiced at sludge disposal sites. Virus transport and survival were monitored during two different runs (summer and fall in Gainesville, Fla.) with the same cores. Monitoring for surviving viruses were terminated when no virus was detectable in soil samples.

Virus association with sludge solids. Before virus transport through soil cores was studied, it was necessary to assess the extent of virus association with sludge solids. Poliovirus 1 and echovirus 1 were added to lagooned sludge (2/3 anaerobic and 1/3 aerobic sludge). After magnetic stirring for 60 min, the fraction of sludge solid-associated virus was determined. As shown in Table 1, 95.2% of poliovirus was found associated with sludge solids, whereas only 20.7% of echovirus was observed to be associated with lagooned sludge solids. The virus-seeded sludge was then applied to the undisturbed soil cores.

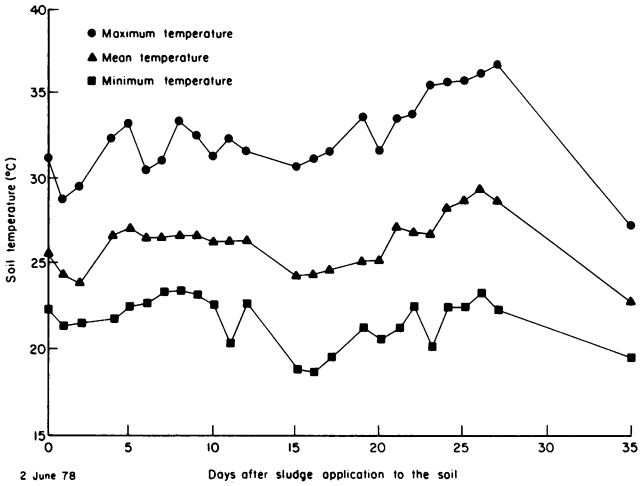

FIG. 1. Daily soil temperature (2.5 cm below the soil surface of a large core of Eustis fine sand) for the duration of the survival experiment that began 2 June 1978.

Fate of viruses in sludge-treated soil cores during summer (2 June to 24 August 1978). The experiment on the fate of viruses in sludge-treated soil cores was initiated in the summer when the weather is generally warm and wet in the Gainesville area. Temperature data were collected with thermocouples placed at the surface of the soil and at the 2.5-, 10-, and 20-cm depths. Datum analysis showed that there was no significant difference between soil temperature readings at these different depths. Therefore, only the soil temperature at the 2.5-cm depth is shown in Fig. 1. The average temperature ranged from 23.5 to 29°C during a 35-day period beginning on 2 June 1978. With regard to rainfall, the study period was very wet, with 13.6 cm of cumulative rainfall from 2 June to 7 July 1978. Poliovirus survival was monitored in two soil cores which had been treated with 1 in. (2.5 cm) of virus-seeded sludge. There was no drastic decline in virus numbers in the drying sludge before the sludge was mixed with the top 2.5 cm of soil (Table 2). Soil monitoring revealed that poliovirus could be detected for up to 35 days in both soil cores. It is difficult to correlate virus survival

TABLE 2. Survival of poliovirus 1 after suspension in liquid sludge and subsequent application to soil cores of Eustis fine sand exposed to natural conditions (2 June 1978 to 7 July 1978)

Sampling date (1978)	Days after the beginning of expt	Cumulative rainfall (cm)	Sludge solid content (%, wt/wt)	Soil moisture (%, wt/wt)	No. of viruses (PFU/g [dry wt] of sludge or soil)	
					LC3[a]	LC4
Liquid sludge sample (2 June)	0	0	2.9		4.4×10^7	4.4×10^7
Drying sludge samples						
3 June	1	1.80	23.1		2.5×10^6	4.9×10^6
6 June	4[b]	6.03			1.0×10^6	5.0×10^5
Soil samples (top 2.5 cm)						
6 June	4	6.03		14.7	3.3×10^4	4.1×10^4
7 June	5	7.03		0.9	8.7×10^4	7.4×10^4
9 June	7	7.28		9.9	2.5×10^3	2.4×10^3
23 June	21	10.45		0.2	4.7	17.1
7 July	35	13.63		0.2	1.3	0.8

[a] Soil core number.
[b] Sludge was mixed with the top 2.5 cm of soil on day 4.

TABLE 3. Analysis for the presence of poliovirus 1 (Sabin) in soil leachates collected after natural rainfall from cores of Eustis fine sand which had been treated with 2.5 cm of seeded liquid sludge (2 June 1978 to 24 August 1978)

Dates of leachate collection (1978)	Cumulative rainfall (cm)	Soil core[a] no.	Cumulative leachate vol (ml)	Cumulative no. of pore volumes[b]	Cumulative virus breakthrough (total PFU)	Cumulative % of total virus applied	Range of conductivity values of leachates collected (μS/cm at 25°C)	Range of pH values of leachates collected
5 June–24 August	51.05	C1	1,544 (8.2)[c]	0.7	0	0	114–1,200	6.3–7.8
5 June–24 August	51.05	C2	1,135 (6.0)	0.5	0	0	106–1,360	5.7–7.2

[a] One inch (or 2.5 cm) of lagooned sludge seeded with a total of 6.1×10^8 PFU of poliovirus was applied on top of the soil cores. The soil cores were exposed to natural conditions.
[b] One pore volume for the soil cores equals 2,178 ml.
[c] Values in parentheses represent the number of centimeters of cumulative leachate volume.

with soil moisture since this parameter was not continuously monitored. Heavy rainfall, however, did not allow the soil (or sludge on the soil surface) to dry for an extended period, and this probably contributed to longer virus survival.

Viruses in soil leachates were monitored for a period of 3 months (2 June to 24 August 1978). Despite leachate concentration via membrane filtration and despite a 51-cm rainfall during the study period, no virus was detected in any of the leachates (Table 3).

Fate of viruses in sludge-treated soil cores during fall-winter seasons (11 October 1978 to 20 January 1979). We have shown earlier (Table 1) that echovirus 1 had less affinity for sludge solids than did poliovirus 1. Moreover, Goyal and Gerba (12) have reported that echovirus 1 (Farouk) adsorbed poorly to soils when compared with poliovirus 1. It was thus decided to compare these two virus types regarding their transport pattern and their persistence in sludge-treated soil cores. This experiment was started on 11 October 1978 and terminated on 20 January 1979.

During the study period the average soil temperature, as monitored with thermocouples placed in a soil core, ranged from 18 to 27°C. Only 0.13 cm of rain fell from 11 October 1978 to 1 November 1978 (Table 4). This was the period during which virus survival was monitored. Neither poliovirus nor echovirus was detectable in soil after 8 days of exposure to natural conditions in the dry fall season. The two enteroviruses were completely inactivated sometime between the days 8 and 21 (Table 4). Soil leachates were also monitored (Table 5). Neither poliovirus nor echovirus was detected in the leachates from all the soil cores.

DISCUSSION

Compared with sewage effluents, sludge offers an advantage regarding viruses during land disposal: sludge solids may bind viruses, thus immobilizing them at the upper layer of the soil profile. We have found that virus association with sludge solids may be substantial, as shown for poliovirus 1. The degree of association is, however, dependent on virus type, since only 20% of echovirus 1 became associated with lagoon sludge. Virus-sludge association also depends on sludge type. For example, viruses have more affinity for aerobically digested sludge than for anaerobically digested sludge (16). Viruses are embedded within sludge flocs (22) and, to a lesser degree, adsorbed to floc surfaces. It is postulated that this embedding process may substantially help in virus retention during sludge application to land.

Important aspects of sludge application to land are virus survival and transport through soils. We have conducted two experimental runs during the summer and fall-winter seasons in north central Florida. During the warm and wet summer season, viruses survived for up to 35 days. During the warm and dry fall season, a more rapid decline (no virus detected after 8 days) was observed in the sludge drying on the soil surface and in the top 2.5 cm of soil. It appears, therefore, that virus survival in sludge-amended soils is mainly affected by desiccation and temperature. It is not then surprising that Danish workers (6, 15) have found that virus survival in sludge-amended soils is prolonged by low temperatures (0 to 10°C). Our data is supported by those we found under field conditions (9): indigenous viruses survived for up to 9 days

TABLE 4. Survival of poliovirus 1 and echovirus 1 after suspension in liquid sludge and subsequent application to soil cores of Eustis fine sand exposed to natural conditions (11 October 1978 to 1 November 1978)

Sampling date (1978)	Days after beginning of expt	Cumulative rainfall (cm)	Sludge solid content (%, wt/wt)	Soil moisture (%, wt/wt)	No. of viruses (PFU/g [dry wt] of sludge or soil)			
					Echovirus 1		Poliovirus 1	
					C1[a]	C2	C3	C4
Liquid sludge sample (11 October)	0	0	7.0		8.6×10^4	8.6×10^4	2.6×10^7	2.6×10^7
Drying sludge sample (14 October)	3[b]	0	38.0		1.6×10^4	1.3×10^4	1.3×10^6	2.9×10^6
Soil samples (top 2.5 cm)								
14 October	3	0		7.5	2.8×10^2	1.9×10^2	4.3×10^4	1.9×10^4
16 October	5	0		3.0	6.9×10^1	1.6×10^1	3.5×10^3	2.1×10^2
19 October	8	0		1.0	5.6×10^1	6.3×10^1	3.6×10^3	2.1×10^2
1 November	21	0.13		1.0	0	0	0	0

[a] Soil core number.
[b] Sludge was mixed with the top 2.5 cm of soil on day 3.

TABLE 5. Analysis for the presence of poliovirus 1 (Sabin) and echovirus 1 (Farouk) in soil leachates collected after natural rainfall from soil cores which had been treated with 2.5 cm of seeded liquid sludge (11 October 1978 to 20 January 1979)

Dates of leachates collection	Cumulative rainfall (cm)[a]	Virus type[b]	Cumulative leachate vol (ml)[c]	Cumulative no. of pore volumes[c]	Cumulative virus breakthrough (total PFU)	Cumulative % of total virus applied	Range of conductivity values of leachates collected (μS/cm)	Range of pH values of leachates collected
1 December 78–20 January 79	24.95	E1	750 (4.0)[d]	0.3	0	0	375–800	6.3–6.8
1 December 78–20 January 79	24.95	E1	980 (5.2)	0.5	0	0	190–975	6.1–6.3
1 December 78–20 January 79	24.95	P1	920 (4.9)	0.4	0	0	280–1,200	5.9–6.9
28 December 78–20 January 79	24.95	P1	410 (2.2)	0.2	0	0	560–875	6.0–6.9

[a] The cumulative rainfall values represent the total rainfall from the beginning of the experiment on 11 October 1978.
[b] One inch (2.5 cm) of lagooned sludges seeded with a total of 8.6×10^8 PFU of poliovirus 1 or 2.9×10^6 PFU of echovirus 1 was applied on top of the soil cores. The soil cores were exposed to natural conditions.
[c] One pore volume represents 2,178 ml.
[d] Values in parentheses represent the number of centimeters of cumulative leachate volume.

during the fall at a sludge disposal site in Jay, Fla. The lagooned sludge applied at the Jay site was the same as the one used in our core experiments.

Virus transport after sludge application was also studied through continuous monitoring of soil leachates for viruses. No virus breakthrough was detected during the summer or the fall-winter seasons. The summer season was typical of Florida, since 51 cm of rain fell between 2 June and 24 August 1978. Our results support those of Damgaard-Larsen et al. (6), who showed that viruses are well retained in sludge-amended soils. It is worth noting that echovirus 1, despite its poor capacity for adsorption to soils (12), was not detected in any of the soil leachates.

The degree of saturation of the soil matrix is an important consideration with regard to virus movement through soils. In most of the studies dealing with this topic, the soil columns were operated under saturated flow conditions, in which the bulk of water flows through macropores. Exposed to natural conditions, our soil cores, were, however, under unsaturated flow conditions, which promote the flow of water through micropores or its retention as a film around soil particles. This may in turn promote virus retention on the soil matrix.

On the basis of this study, it can be concluded that, with respect to sludge application to land, enteroviruses are efficiently retained by the sludge-soil matrix and their association with sludge solids would play a significant role in the restriction of their movement through soils. With respect to their survival in soils, viruses are significantly inactivated in soils under warm and dry conditions.

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