APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Jan. 1988, p. 118–123
0099-2240/88/010118-06$02.00/0

Vol. 54, No. 1

Poliovirus Retention in Soil Columns after Application of Chemical- and Polyelectrolyte-Conditioned Dewatered Sludges

OSCAR C. PANCORBO,[1]† GABRIEL BITTON,[1]* SAMUEL R. FARRAH,[2] GEORGE E. GIFFORD,[3] AND ALLEN R. OVERMAN[4]

Departments of Environmental Engineering Sciences,[1] Microbiology and Cell Sciences,[2] Immunology and Medical Microbiology,[3] and Agricultural Engineering,[4] University of Florida, Gainesville, Florida 32611

Received 20 July 1987/Accepted 15 October 1987

The transport of poliovirus type 1 (strain LSc) was studied in Red Bay sandy loam columns that were treated with chemical- or polyelectrolyte-conditioned dewatered sludges and then leached with natural rainwater under saturated flow conditions. Poliovirus was concentrated in the alum and ferric chloride sludges that were produced following the flocculation of virus-seeded raw sewage. Virtually complete inactivation of the virus was observed following the flocculation of raw sewage or the stabilization of alum and ferric chloride sludges with lime at pH 11.5. Poliovirus was also concentrated in polyelectrolyte-conditioned dewatered sludge that was produced from virus-seeded, anaerobically digested sludge. Despite the saturated flow conditions for a sustained period, no viruses were detected in the leachates of the soil columns that were treated with these chemical and chemically treated sludges. Since the viruses were mostly associated with the solids in these sludge samples, it is believed that they were immobilized along with the sludge solids in the top portion of the soil columns.

Concern over the possible contamination of groundwater with pathogenic viruses following the disposal of sludge on land has prompted research activity in this area in recent years. Results of laboratory (3, 4, 6, 11, 17) and field (8, 12) studies have demonstrated that viruses in digested sludges are effectively retained in the soil and do not readily migrate into groundwater. However, additional research is needed in which other sludge types that are routinely applied to the soil are used.

Chemical flocculation of wastewater at municipal treatment facilities produces large quantities of chemical sludge which are often disposed of by direct application to land (24). This treatment is intended to remove phosphates and other chemical constituents with the coagulants alum, ferric chloride, or lime (24); and it has also been shown to remove seeded poliovirus type 1 (1, 5, 19, 20) and indigenous enteric viruses (16) from wastewater. The chemical sludges that are produced, however, have been found to contain significant concentrations of viruses, which makes land disposal of these sludges a potential health hazard (16, 19, 20). To reduce their pathogen content and odor, alum and ferric chloride sludges precipitated from raw wastewater are often treated with lime at a pH of 11 or greater before disposal on land (9, 24, 25). Although the treatment of sludge with lime has been shown to destroy the *Salmonella* sp. and *Pseudomonas aeruginosa* that are present in sludge (9), little information is available with regard to the effect of such treatment on viruses in sludge. In a study done in the United Kingdom, Goddard et al. (10) were generally unable to detect indigenous enteroviruses in sludge that was conditioned with lime at pH 11 and then concentrated in a filter plate press. These investigators attributed their results to the virucidal action of the high pH, but indicated that the high solids content (39%) of the concentrated sludge may have interfered with virus recovery. Goddard et al. (10) also found higher titers of indigenous enteroviruses in polyelectrolyte-conditioned dewatered sludge than in the untreated sludge. High concentrations of cationic polyelectrolytes were believed to have released the viruses that were bound to sludge solids, which led to their detection. Despite the viral hazards that are present in chemical and chemically treated sludges, there are currently no data on the fate of viruses in soils treated with these sludge types.

In this study, the transport of poliovirus type 1 was investigated in soil columns that were treated with chemical- or polyelectrolyte-conditioned dewatered sludges and then leached with natural rainwater. The chemical sludges were precipitated from poliovirus-seeded raw sewage with alum, ferric chloride, or lime. Selected sludge samples containing alum and ferric chloride were also stabilized by treatment with lime at pH 11.5 before they were applied to the soil columns. The polyelectrolyte-conditioned dewatered sludge was produced in the laboratory from poliovirus-seeded, anaerobically digested sludge.

MATERIALS AND METHODS

Virus and viral assays. Poliovirus type 1 (strain LSc) was used in this study. Stocks were prepared as described previously (2) and were kept at −70°C until use. Virus was assayed by the plaque technique on AV3 (human amnion) or MA104 (simian kidney) cell monolayers as described previously (2, 18). Each viral count given in this report represents the average of triplicate assays and is expressed as PFU.

Polyelectrolyte-conditioned dewatered sludge. Anaerobically digested (60 days) sludge from the Main Street Wastewater Treatment Plant (Gainesville, Fla.) was conditioned and dewatered in our laboratory in a manner similar to that routinely employed at that wastewater treatment plant. Prior to conditioning and dewatering, the pH and conductivity of

* Corresponding author.
† Present address: Environmental Health Science Program, Department of Food Science and Technology, The University of Georgia, Athens, GA 30602.

the digested sludge were 6.0 and 3,950 S/cm, respectively, as measured with a digital pH meter (Corning Glass Works, Corning, N.Y.) and a conductivity meter (Beckman Instruments, Inc., Fullerton, Calif.), respectively. The solids content of the sludge sample was 2.0% on a weight (grams) to volume (milliliters) basis, and was determined from the dry weight of a measured volume of sludge after 24 h in an oven at 105°C. The digested sludge (1,000 ml) was seeded with poliovirus (1 ml of stock in phosphate-buffered saline [PBS] containing 2% fetal calf serum [FCS]), while the suspension was mixed on a magnetic stirrer. After a 1-min mixing period, a portion of the unfractionated sludge (i.e., a sludge sample without the solids separated out) was diluted in PBS containing 2% FCS and was assayed directly for seeded viruses by the plaque technique. We have previously reported (18) that there is greater than 98% recovery of seeded poliovirus in unfractionated sludge by this dilution and subsequent direct assay on cell cultures. In this manner, it is believed that the amount of initial virus added to the sludge was accurately determined. After the poliovirus was seeded, the sludge was conditioned with 1,200 mg (final concentration in the sludge) of the cationic polyelectrolyte per liter (Hercofloc no. 871; Hercules Co., Atlanta, Ga.) as the sludge was rapidly mixed on a magnetic stirrer. Mixing was continued slowly for an additional 5 min. The entire sludge sample was then centrifuged at 320 × *g* for 10 min at 25°C (all centrifugation in this study was performed with an RC5-B centrifuge [Ivan Sorvall, Inc., Norwalk, Conn.]). The supernatant was decanted, assayed for viruses as described above, and discarded. The conditioned dewatered sludge volume and solids content (i.e., as a percent, by the method described above) were measured. The conditioned dewatered sludge that was produced was then assayed for viruses as described below.

Chemical sludges. The chemical sludges were precipitated from poliovirus-seeded raw sewage by the general procedure described by Sattar et al. (20) and with the coagulants alum, ferric chloride, or lime. The raw sewage was obtained from the University of Florida Campus Wastewater Treatment Plant (pH 9.0; conductivity, 520 S/cm) or from the Main Street Wastewater Treatment Plant (pH 8.9; conductivity, 770 S/cm), both of which are located in Gainesville, Fla. The pH and conductivity of raw sewage samples were measured as described above for digested sludge. Raw sewage (1,000 ml) was seeded with poliovirus (1 ml of stock in PBS containing 2% FCS) while it was mixed on a magnetic stirrer. A portion of this mixture was immediately diluted in PBS containing 2% FCS, adjusted to neutral pH by the addition of 1 M glycine buffer (pH 2.0 or 11.5, if necessary), and then assayed for viruses. The viral assay was again performed after the addition of coagulant. The final concentration of alum in sewage was 300 mg/liter [as $Al_2(SO_4)_3 \cdot 18H_2O$], and the final concentration of ferric chloride was 50 mg/liter (as $FeCl_3$). Similar concentrations of these coagulants were used by Wolf et al. (26) when they precipitated an activated sludge effluent as a tertiary treatment process. Following the addition of alum or ferric chloride, the pH of the solution was adjusted with 0.2 N HCl to 6.0 or 5.0, respectively, to achieve maximum flocculation (7). Lime was added until a pH of 11.5 was achieved, as recommended by Sattar et al. (20); a final pH of 11.1 to 11.3 and a final concentration in sewage of 150 to 250 mg of $Ca(OH)_2$ per liter were achieved. Following the addition of the coagulants, the sewage samples were mixed on a magnetic stirrer rapidly for 10 min and slowly for 5 min. The flocculated sewage samples were then transferred to Imhoff cones, and 60 min was allowed for the formation and settling of the chemical sludges. The supernatants in the Imhoff cones were siphoned off, assayed for viruses as described above, and discarded. The sludge volume and solids content (i.e., as a percent, by the method described above) were measured for each chemical sludge sample. The chemical sludges produced were then assayed for viruses as described below.

Lime-stabilized chemical sludges. A sample of alum sludge and a sample of ferric chloride sludge, which were produced and assayed for viruses as described above, were stabilized with lime by the procedure described by Farrell et al. (9). The sludges were treated with a 5% (wt/vol) aqueous slurry of $Ca(OH)_2$ until a pH of 11.5 was achieved and were maintained for 5 min. The final concentrations of lime in the alum and ferric chloride sludges were 1,390 and 625 mg/liter (as $Ca(OH)_2$), respectively. After 30 min of mixing on a magnetic stirrer, the pHs of the alum and ferric chloride sludges were 11.3 and 11.1, respectively. The lime-stabilized chemical sludges were then assayed for viruses as described below.

Assay of seeded poliovirus in sludges. The conditioned dewatered chemical and lime-stabilized chemical sludges produced were assayed for poliovirus by previously described methods (18). Briefly, a portion of well-mixed, unfractionated sludge was diluted in PBS containing 2% FCS, adjusted to neutral pH by the addition of 1 M glycine buffer (pH 2.0 or 11.5, if necessary), and then assayed directly for viruses by the plaque technique. This assay was performed to determine the total amount of virus present in the sludge sample. A portion of the unfractionated sludge was subsequently centrifuged at 1,400 × *g* for 10 min at 4°C, and the sludge supernatant that was produced was assayed for viruses. This assay allowed the calculation of viable unadsorbed virus (in the sludge supernatant) as a percentage of the total virus in unfractionated sludge (18). The sludge solids-associated virus fraction (as a percentage of the viral titer in unfractionated sludge) was also determined by the difference in virus PFU between unfractionated sludge and the sludge supernatant (18).

Rainwater. The rainwater used in soil transport studies was collected from an area adjacent to the Environmental Engineering Sciences Building at the University of Florida, Gainesville. The average weighted pH and conductivity for the rainwater were 4.46 and 23.9 S/cm, respectively. Additional chemical characteristics of the rainwater have been published previously (4).

Soil and soil columns. The soil used was a Red Bay sandy loam taken from the West Florida Agricultural Experiment Station, Jay, Fla., and was classified as a Rhodic Paleudult, fine-loamy, siliceous, thermic. The subsoil sample used (A2 and B1t horizons) displayed the following characteristics: sand, 58%; silt, 18%; clay, 24%; pH (in 1:1 water) 5.5; bulk density, 1.46 g/ml; saturated hydraulic conductivity, 18 cm/h (4). With gentle tapping, the air-dried soil was packed into acrylic plastic columns (length, 29 cm [filled to 27 cm with soil, leaving 2 cm at the top for the sludge]; internal diameter, 4.8 cm). This reproduced the field bulk density (1.46 g/ml). A polypropylene screen (105-μm pore size) was used to support the soil in the columns, but it allowed the free movement of virus. The soil columns were then placed in column holders (Soil Moisture Equipment Corp., Santa Barbara, Calif.), and the air in the columns was displaced by flushing the columns with CO_2 for 60 min to ensure the subsequent uniform wetting of the soil. The soil columns were then conditioned by passing two pore volumes (450 ml) of rainwater through the columns with a peristaltic pump

TABLE 1. Association of poliovirus type 1 with polyelectrolyte conditioned dewatered sludge

Expt no.	Virus in sludge (10^6 PFU)[a]	Virus in sludge supernatant[b]		Virus in conditioned dewatered sludge			Conditioned dewatered sludge parameters	
		10^6 PFU	Recovery (%)[c]	10^6 PFU[d]	Recovery (%)[c]	Solids-associated virus (%)[e]	Vol (ml)	Solids content (%; wt/vol)
1	28	2.4	8.6	25	89	>99	320	5.1
2	36	2.4	6.7	26	72	>99	310	4.7

[a] Unfractionated, anaerobically digested sludge (1.0 liters; 2% solids content) before the addition of polyelectrolyte.
[b] After the addition of polyelectrolyte (1,200 mg/liter; Hercofloc no. 871) and subsequent centrifugation at 320 × *g* for 10 min at 25°C.
[c] Percentage of viral PFU in the sludge before the addition of polyelectrolyte.
[d] In unfractionated sludge.
[e] Percentage of viral PFU in unfractionated conditioned dewatered sludge.

(Buchler Instruments Div., Nuclear-Chicago Corp., Fort Lee, N.J.).

Sludge application to soil columns. The conditioned, dewatered chemical and lime-stabilized chemical sludges were separately applied on top of the soil columns; were allowed to soak in; and were then worked 2.5 cm under the soil. The columns were then leached with rainwater, which was applied continuously to the columns at 5 ml/min with a peristaltic pump. Soil leachates were collected in 0.5-pore-volume fractions (113 ml), and the pH and conductivity of each fraction were measured as described above for digested sludge. A portion of each leachate fraction was diluted in PBS containing 2% FCS and was assayed directly for viruses. To detect small numbers of viruses, leachate pore volumes 0.5 through 5.0 (and 5.5 through the final pore volume) for a given soil column were combined and concentrated 160-fold as follows. Each leachate sample (i.e., combined fractions) was adjusted to pH 3.5 by the addition of 1 M glycine buffer (pH 2.0) and adjusted to a final concentration of 0.0005 M aluminum chloride. The treated sample was then passed through a series of 3.0- and 0.45-μm-pore-size filters (Filterite Corp., Timonium, Md.) in a holder (diameter, 47 mm). Adsorbed viruses were eluted from the filters with 7 ml of PBS containing 10% FCS (pH 9.0). The filter eluate was adjusted to neutral pH by the addition of 1 M glycine buffer (pH 2.0) and assayed for viruses.

RESULTS

Polyelectrolyte-conditioned dewatered sludge. The fate of poliovirus type 1 seeded in anaerobically digested sludge was determined after the sludge was conditioned with a cationic polyelectrolyte and was dewatered by centrifugation. In two experiments, 72 and 89% of the poliovirus initially added to the sludge was detected in the unfractionated (i.e., without solids separated) conditioned dewatered sludge (Table 1). In both experiments, more than 99% of the viruses found in the conditioned dewatered sludge were associated with the sludge solids (Table 1). The conditioned dewatered sludge that was produced had a solids content that was 2.5-fold greater than that of the untreated sludge (Table 1). Conditioned dewatered sludge samples from the two experiments were applied to two separate columns of Red Bay sandy loam (Table 2). Despite the application of 10 pore volumes of rainwater, viruses were not detected in the soil column leachates. The increased conductivity and pH of some leachate samples, however, indicated that sludge constituents migrated through the soil (Table 2).

Chemical sludges. Chemical sludges were precipitated from poliovirus-seeded raw sewage with alum, ferric chloride, or lime. All of the poliovirus that was initially added to the raw sewage was detected in the unfractionated sewage sample after the addition of alum, while only 16 to 25% of poliovirus was detected after the addition of ferric chloride, and no viruses were detected after the addition of lime at pH 11.3 (Table 3). Variable fractions of seeded polioviruses were detected in the unfractionated chemical sludges that were produced; these ranged from 23 to 96% for alum sludges, 9.8 to 13% for ferric chloride sludges, and 0 to 0.13% for lime sludges (Table 3). In most experiments, particularly those involving lime, a large fraction of the poliovirus titer initially added to the raw sewage could not be detected either in the sewage supernatant after flocculation or in the chemical sludge that was produced. Between 97 and 100% of the viruses detected in the chemical sludges were associated with the sludge solids (Table 3).

Selected alum and ferric chloride sludge samples were stabilized by treatment with lime at pH 11.5. Twice as much lime was required to raise the pH to 11.5 for the alum sludge than for the ferric chloride sludge (Table 4). At 30 min after treatment with lime, no viruses were detected in the unfractionated alum sludge, whereas 0.12% of initial poliovirus titer was detected in the unfractionated ferric chloride sludge, 100% of which was associated with the sludge solids (Table 4).

Chemical and lime-stabilized chemical sludge samples were applied to separate columns of Red Bay sandy loam. Viruses were not detected in the soil column leachates after up to 10 pore volumes of natural rainwater was applied (Table 5). Increased conductivity and pH of some leachate samples indicated, however, that sludge constituents migrated through the soil (Table 5).

DISCUSSION

Although wastewater sludges undergo a variety of treatment processes, little is known about the effects of many of

TABLE 2. Retention of poliovirus type 1 in Red Bay sandy loam columns treated with conditioned dewatered sludge and then leached with rainwater

Column no.[a] (total no. of pore vol eluted)	Total viral titer contained in the sludge applied (10^6 PFU)[b]	Range of conductivity values of leachates (μS/cm at 25°C)[c]	Range of pH of leachates[c]
1	1.3	41–148	5.5–6.7
2	1.4	37–175	5.6–6.5

[a] A total of 10 pore volumes were eluted from each column. One pore volume for the soil columns is equal to 225 ml.
[b] Conditioned dewatered sludges (17 ml) from experiments 1 and 2 shown in Table 1 were applied to soil columns 1 and 2, respectively. The total viral titer detected in the soil leachates was 0 PFU for both columns. Soil leachates were collected in 0.5-pore-volume fractions.
[c] Soil leachates were collected in 0.5-pore-volume fractions.

TABLE 3. Association of poliovirus type 1 with chemical sludges

Chemical sludge type and expt no.	Virus in sewage			Virus in sewage supernatant[b]		Virus in chemical sludge			Chemical sludge parameters	
	Before addition of coagulant (10^6 PFU)	After addition of coagulant[a]								
		10^6 PFU	Recovery (%)[c]	10^6 PFU	Recovery (%)[c]	10^6 PFU[d]	Recovery (%)[c]	Solids-associated virus (%)[e]	Vol (ml)	Solids content (%; wt/vol)
Alum										
1	53	ND[f]	ND	9.4	18	12	23	97	20	ND
2	45	45	100	1.6	3.6	43	96	>99	50	0.3
3	39	ND	ND	0.47	1.2	11	28	>99	21	1.3
Ferric chloride										
1	43	7.7	18	0.40	0.93	4.7	11	99	31	0.5
2	45	7.3	16	0.93	2.1	4.4	9.8	97	33	0.4
3	39	9.8	25	0.93	2.4	5.2	13	99	31	0.7
Lime										
1	58	ND	ND	0.10	0.17	0.075	0.13	100	30	ND
2	45	0	0	0	0	0	0	0	37	1.0

[a] Final concentrations in raw sewage (1.0 liter) of the coagulants (and pHs) were 300 mg of $Al_2(SO_4)_3 \cdot 18H_2O$ (pH 6.0) per liter, 50 mg of $FeCl_3$ (pH 5.0) per liter, and 150 mg (pH 11.1; lime experiment 1) or 250 mg (pH 11.3; lime experiment 2) of $Ca(OH)_2$ per liter.
[b] After the addition of coagulant and subsequent sedimentation of sludge in an Imhoff cone for 60 min.
[c] Percentage of viral PFU in the sewage before the addition of coagulant.
[d] In unfractionated sludge.
[e] Percentage of viral PFU in unfractionated chemical sludge.
[f] ND, Not done.

these treatments on viral pathogens (25). In the experiments described here, poliovirus type 1 was concentrated in sludge after the sludge was conditioned with a cationic polyelectrolyte and dewatered by centrifugation. Moreover, greater than 99% of the viruses in the conditioned dewatered sludge were associated with the sludge solids. The concentration of viruses demonstrated here following the conditioning and dewatering of sludge was consistent with the results of the study by Goddard et al. (10), in which they found higher titers of indigenous enteroviruses in polyelectrolyte-conditioned dewatered sludge than in untreated sludge. Cationic polyelectrolytes have long been known to aid in the removal of viruses from water during coagulation (23). The association of poliovirus with the solids in the conditioned dewatered sludge is an important factor in the complete retention of the virus in the Red Bay sandy loam columns that were treated with this sludge. The viruses were probably immobilized along with the sludge solids in the top portion of the soil columns, and rainwater was unable to transport the viruses through the soil. In a previous study (4), we demonstrated that columns of the Red Bay sandy loam retained 98.8% of the poliovirus that was applied in anaerobically digested sludge and leached with rainwater. Other investigators (3, 6, 11, 17) have also shown that viruses are effectively retained in soil columns treated with digested sludges. Greater transport of poliovirus type 1 in sludge-treated soil columns has occurred, however, during leaching with deionized water under saturated flow conditions (17). It is well known that the artificially low ionic strength of deionized water (13, 21) and saturated flow conditions (14) promote viral movement in soil. In the present study, despite the saturated flow conditions with natural rainwater for a sustained period, it was remarkable that no viruses were detected in the soil leachates.

During chemical flocculation of raw sewage, particularly with ferric chloride or lime, it was found that a large fraction of the poliovirus titer initially added to the sewage could not be detected in any of the samples, including the chemical sludges that were produced. Since all samples were assayed without the separation of solids (i.e., unfractionated), it follows that the viral titer reductions must be attributed to either direct viral inactivation or inhibition of viral infection of cells once the viruses are plated onto the cell monolayer, perhaps because of the viral association with solids. Sobsey et al. (22) have also reported difficulties in recovering poliovirus type 1 from a ferric chloride precipitate. In the lime flocculation experiments, the dramatic reductions in viral titer were probably due to viral inactivation resulting from

TABLE 4. Inactivation of poliovirus type 1 following stabilization of chemical sludges with lime

Chemical sludge type[a]	$Ca(OH)_2$ concn (mg/liter) used	pH[b]	Virus in sludge before liming (10^6 PFU)[c]	Virus in sludge 30 min after treatment with lime		
				10^6 PFU[c]	Recovery (%)[d]	Solids-associated virus (%)[e]
Alum	1,390	11.3	11	0	0	0
Ferric chloride	625	11.1	5.2	0.0062	0.12	100

[a] Alum and ferric chloride sludge samples were from the alum experiment 3 and the ferric chloride experiment 3, respectively, shown in Table 3.
[b] pH was determined 30 min after treatment with lime.
[c] In unfractionated sludge.
[d] Percentage of viral PFU in unfractionated sludge before liming.
[e] Percentage of viral PFU in unfractionated sludge 30 min after liming.

TABLE 5. Retention of poliovirus type 1 in Red Bay sandy loam columns treated with chemical or lime-stabilized chemical sludges and then leached with rainwater

Chemical sludge type and column no.[a]	Total no. of pore volumes eluted[b]	Sludge vol (ml) applied	Total viral titer contained in the sludge applied (10^6 PFU)[c]	Range of conductivity values of leachates (μS/cm at 25°C)[d]	Range of pH values of leachates[d]
Alum					
Column 1	5.0	15	9.0	43–142	5.4–5.5
Column 2	10	43	37	30–240	5.0–5.6
Ferric chloride					
Column 1	10	22	3.3	32–175	5.5–5.7
Column 2	9.0	25	3.3	31–187	4.8–5.1
Lime					
Column 1	2.0	24	0.060	95–130	5.4–5.6
Column 2	10	32	0	27–145	5.6–6.9
Alum (lime-stabilized)					
Column 1	10	12	0	25–118	5.7–6.9
Ferric chloride (lime-stabilized)					
Column 1	10	22	0.0043	30–255	5.4–5.7

[a] Chemical sludges from experiments 1 and 2 shown in Table 3 for each sludge type (alum, ferric chloride, or lime) were applied to soil columns 1 and 2, respectively. The lime-stabilized chemical sludges (Table 4) were also applied to soil columns.
[b] One pore volume for the soil columns is equal to 225 ml.
[c] The total viral titer detected in the soil leachates was 0 PFU for all samples. Soil leachates were collected in 0.5-pore-volume fractions.
[d] Soil leachates were collected in 0.5-pore-volume fractions.

the high pH (greater than 11). Other investigators (1, 19, 20) have similarly shown poliovirus type 1 inactivation during lime flocculation of wastewater at high pHs. In the present study, poliovirus was concentrated in the alum and ferric chloride sludges that were produced, and 97 to 100% of the viruses detected in these samples were associated with the sludge solids. Following the coagulation of raw sewage in laboratory-scale pilot plants, Lund and Ronne (16) also demonstrated the accumulation of indigenous enteric viruses in alum, ferric chloride, and lime (pH 10.5) sludges. These investigators did not observe any viral inactivation in the chemical sludges that were produced. Sattar and Ramia (19) also readily detected indigenous enteric viruses in all lime sludges obtained from a Canadian wastewater treatment plant in which flocculation of raw sewage with lime at pH 10 was used. Similar to the results of this study, however, Sattar et al. (20) showed that poliovirus type 1 was completely removed from raw sewage following flocculation with lime at a higher pH (11.5) and recovery in the lime sludge of only 0.005% of the total viral input.

Because of the large quantities of chemical sludges usually produced, lime treatment is particularly suited for the stabilization of these sludge types (9). At a relatively low cost, lime stabilization reduces the odor and bacterial pathogen content of sludges (9, 25). The effectiveness of this treatment, however, is dependent on the pH that is achieved and maintained. Goddard et al. (10) have demonstrated the virtually complete destruction of indigenous enteroviruses in sludge conditioned with lime at pH 11. In this study we have also shown the complete loss of poliovirus type 1 titer in alum sludge treated with lime at pH 11.5. As reported previously by other investigators (9), the alum sludge requires more lime to achieve a pH of 11.5 than does the ferric chloride sludge. At 30 min after treatment with lime, the alum sludge had a slightly higher pH than did the ferric chloride sludge. All of the 0.12% of the initial poliovirus titer detected in the ferric chloride sludge was associated with the solids.

All viruses in chemical and lime-stabilized chemical sludges were retained in the Red Bay sandy loam columns that were treated with sludge and then leached with natural rainwater under saturated flow conditions. The viruses were probably embedded within the sludge solids during the flocculation process and were subsequently immobilized, along with the sludge solids, in the top portion of the soil columns. It is likely that the ample level of cations (particularly aluminum, iron, and calcium) that was present in these sludges further promoted the adsorption of viruses to sludge and soil surfaces (13). It should be noted, however, that poliovirus type 1 (strain LSc) has been shown to adsorb readily to soils that have been treated with wastewater (15, 21), and to not be significantly mobilized in soil columns by rainwater (15). Other viruses have been shown to behave differently in the soil in that they migrate more readily through the soil profile (15). Therefore, the results with poliovirus type 1 (strain LSc) presented here that indicate that viruses are completely retained in soil columns treated with chemical or chemically treated sludges should be confirmed with other enteric viruses.

ACKNOWLEDGMENTS

This study was supported by grant R804570 from the U.S. Environmental Protection Agency.

LITERATURE CITED

1. **Berg, G., R. B. Dean, and D. R. Dahling.** 1968. Removal of poliovirus 1 from secondary effluents by lime flocculation and rapid sand filtration. J. Am. Water Works Assoc. **60**:193–198.
2. **Bitton, G., N. Masterson, and G. E. Gifford.** 1976. Effect of secondary treated effluent on the movement of viruses through a cypress dome soil. J. Env. Quality **5**:370–375.
3. **Bitton, G., O. C. Pancorbo, and S. R. Farrah.** 1984. Virus transport and survival after land application of sewage sludge. Appl. Environ. Microbiol. **47**:905–909.
4. **Bitton, G., O. C. Pancorbo, A. R. Overman, and G. E. Gifford.** 1978. Retention of viruses during sludge application to soils.

Prog. Water Technol. **10**:597–606.
5. **Brunner, D. R., and O. J. Sproul.** 1970. Virus inactivation during phosphate precipitation. J. Sanit. Eng. Div. **96**:365–379.
6. **Dangaard-Larsen, S., K. O. Jensen, E. Lund, and B. Nissen.** 1977. Survival and movement of enterovirus in connection with land disposal of sludges. Water Res. **11**:503–508.
7. **Fair, G. M., J. C. Geyer, and D. A. Okun.** 1968. Water and wastewater engineering, vol. 2. Water purification and wastewater treatment and disposal. John Wiley & Sons, Inc., New York.
8. **Farrah, S. R., G. Bitton, E. M. Hoffmann, O. Lanni, O. C. Pancorbo, M. C. Lutrick, and J. E. Bertrand.** 1981. Survival of enteroviruses and coliform bacteria in a sludge lagoon. Appl. Environ. Microbiol. **41**:459–465.
9. **Farrell, J. B., J. E. Smith, Jr., S. W. Hathaway, and R. B. Dean.** 1974. Lime stabilization of primary sludges. J. Am. Water Works Assoc. **46**:113–122.
10. **Goddard, M. R., J. Bates, and M. Butler.** 1982. Isolation of indigenous enteroviruses from chemically treated and dewatered sludge samples. Appl. Environ. Microbiol. **44**:1042–1046.
11. **Jorgensen, P. H.** 1985. Examination of the penetration of enteric viruses in soils under simulated conditions in the laboratory. Water Sci. Technol. **17**:197–199.
12. **Jorgensen, P. H., and E. Lund.** 1985. Detection and stability of enteric viruses in sludge, soil and ground water. Water Sci. Technol. **17**:185–195.
13. **Lance, J. C., and C. P. Gerba.** 1984. Effect of ionic composition of suspending solution on virus adsorption by a soil column. Appl. Environ. Microbiol. **47**:484–488.
14. **Lance, J. C., and C. P. Gerba.** 1984. Virus movement in soil during saturated and unsaturated flow. Appl. Environ. Microbiol. **47**:335–337.
15. **Landry, E. F., J. M. Vaughn, M. Z. Thomas, and C. A. Beckwith.** 1979. Adsorption of enteroviruses to soil cores and their subsequent elution by artificial rainwater. Appl. Environ. Microbiol. **38**:680–687.
16. **Lund, E., and V. Ronne.** 1973. On the isolation of virus from sewage treatment plant sludges. Water Res. **7**:863–871.
17. **Moore, B. E., B. P. Sagik, and C. A. Sorber.** 1978. Land application of sludges: minimizing the impact of viruses on water resources, p. 154–167. *In* B. P. Sagik and C. A. Sorber (ed.), Risk assessment and health effects of land application of municipal wastewater and sludges. Center for Applied Research and Technology, The University of Texas at San Antonio, San Antonio, Tex.
18. **Pancorbo, O. C., P. R. Scheuerman, S. R. Farrah, and G. Bitton.** 1981. Effect of sludge type on poliovirus association with and recovery from sludge solids. Can. J. Microbiol. **27**:279–287.
19. **Sattar, S. A., and S. Ramia.** 1978. Viruses in sewage: effect of phosphate removal with calcium hydroxide (lime). Can. J. Microbiol. **24**:1004–1006.
20. **Sattar, S. A., S. Ramia, and J. C. N. Westwood.** 1976. Calcium hydroxide (lime) and the elimination of human pathogenic viruses from sewage: studies with experimentally contaminated (poliovirus type 1, Sabin) and pilot plant samples. Can. J. Public Health **67**:221–226.
21. **Sobsey, M. D., C. H. Dean, M. E. Knuckles, and R. A. Wagner.** 1980. Interactions and survival of enteric viruses in soil materials. Appl. Environ. Microbiol. **40**:92–101.
22. **Sobsey, M. D., C. P. Gerba, C. Wallis, and J. L. Melnick.** 1977. Concentration of enteroviruses from large volumes of turbid estuary water. Can. J. Microbiol. **23**:770–778.
23. **Thorup, R. T., F. P. Nixon, D. F. Wentworth, and O. J. Sproul.** 1970. Virus removal by coagulation with polyelectrolytes. J. Am. Water Works Assoc. **62**:97–101.
24. **U.S. Environmental Protection Agency.** 1973. Physical-chemical wastewater treatment plant design. EPA-625/4-73-002a. U.S. Environmental Protection Agency, Environmental Research Information Center, Cincinnati, Ohio.
25. **U.S. Environmental Protection Agency.** 1978. Sludge treatment and disposal, vol. 1. Sludge treatment. EPA-625/4-78-012. U.S. Environmental Protection Agency, Environmental Research Information Center, Cincinnati, Ohio.
26. **Wolf, H. W., R. S. Safferman, A. R. Mixson, and C. E. Stringer.** 1974. Virus inactivation during tertiary treatment, p. 145–157. *In* J. F. Malina, Jr., and B. P. Sagik (ed.), Virus survival in water and wastewater systems. Center for Research in Water Resources, University of Texas, Austin, Tex.