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Poliovirus Removal from Primary and Secondary Sewage Effluent by Soil Filtration

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Adsorption of poliovirus from primary sewage effluent was similar to that from secondary sewage effluent in both batch soil studies and experiments with soil columns 240 cm long. Virus desorption by distilled water was also similar in a soil column that had been flooded with either primary or secondary effluent seeded with virus. These results indicated that adsorption of poliovirus from primary effluent and virus movement through the soil were not affected by the higher organic content of primary sewage effluent.

Land disposal of wastewater has gained widespread interest as an alternative to more conventional high-technology treatment methods (3, 4, 19). Land filtration of secondarily treated domestic sewage is an effective way to remove most of the N, P, and organic matter and pathogenic microorganisms (3, 4, 12-16).

We have reported previously on virus removal from secondary effluent by high-rate land filtration at the Flushing Meadows wastewater renovation project (11) and in laboratory lysimeters (9). These data showed that the system effectively removed virus from secondary effluent under normal operating conditions. The laboratory studies did indicate that virus could elute near the soil surface if a heavy rainfall occurred, resulting in some virus migration. However, this could be controlled by proper management of flooding and drying cycles (11). Studies by Wellings et al. (21, 22) indicated that virus movement after periods of heavy rainfall could occur under field conditions. Thus, an understanding of the mechanisms of viral removal by soil systems is critical in the evaluation and management of land treatment systems. Adsorption is probably the predominate factor in virus removal by soils (1, 10). Thus, factors influencing adsorption phenomena will determine not only the efficiency of virus removal but also the long-term behavior of viruses in the soil. Factors that can influence viral adsorption include the presence of cations and soluble organics, pH, the nature of the adsorbent, and the type of virus (1, 8, 10). The composition of raw sewage differs significantly from that of treated sewage, in which the concentration of organics is significantly reduced. Soluble organic matter is known to interfere with virus adsorption to natural clays (5), membrane filters (20), and activated carbon (9). Organics have also been used to elute and recover viruses when the viruses were adsorbed to various nonbiological surfaces (20). Gerba et al. (9) found that poliovirus was efficiently removed initially by columns of activated carbon, but removal eventually ceased as the adsorption sites on the surface of the carbon were occupied by soluble organics. Eventually, adsorbed viruses were eluted from the carbon, and the concentration of virus in the column effluent exceeded that in the influent. In batch studies, virus adsorption was directly related to the concentration of organics in the sewage. Bitton et al. (2) found that poliovirus type 1 adsorption onto soil columns was substantially lower from secondary sewage mixed with water from a cypress dome than from tap water. In addition, viruses adsorbed to soil in tap water could be eluted with the sewage-dome water mixture.

Land filtration of raw or primary treated sewage is currently practiced in this country and abroad, but little laboratory data are available to allow an assessment of its efficiency in virus removal. Schaub and Sorber (18) recently reported the presence of indigenous enteroviruses and tracer f2 bacteriophage in the groundwater at horizontal distances of 183 m (600 feet) at a rapid infiltration site where primarily treated domestic wastewater was being discharged. Laboratory studies indicated poor virus adsorption to soil taken from the land treatment site. This work suggested that virus removal by soil from primary sewage may not be as efficient as that from treated sewage. The present study was conducted to compare viral removal by soil from both primary and secondary sewage effluents under similar conditions to determine the influ-

ence of the organic content of sewage on efficiency of virus removal.

MATERIALS AND METHODS

Construction of soil columns. Soil columns were constructed by packing a 10-cm-diameter polyvinyl chloride pipe to a depth of 250 cm with loamy sand obtained from the dry Salt River bed located near Phoenix, Ariz. Details of the soil composition, column construction, and flow system have been described elsewhere (12, 13, 15). The flow rate and cumulative flow through the column were measured by weighing the outflow daily. The average flow rate of sewage through the column was 55 cm/day.

Sewage. Primary sewage was collected from the effluent of sedimentation tanks used to hold incoming raw sewage. Secondary sewage was obtained from the effluent of an activated sludge plant.

Application of virus in sewage water. A virus suspension (poliovirus type 1, strain LSc) was mixed with dechlorinated (by addition of sodium thiosulfate) secondarily or primarily treated sewage effluent to obtain a concentration of about 2×10^4 to 3×10^4 plaque-forming units (PFU) per ml of water. This solution was applied to the soil column for 9 consecutive days, and samples (2 to 5 ml) were extracted daily from ceramic samplers at depths of 0, 2, 5, 10, 20, 40, 80, 160, and 240 cm and from the outlet line (250 cm). Previous tests (12) indicated that 0 to 10% of the virus was lost when samples were drawn through the ceramic samplers. As an added control, samples were drawn from the waterhead above the column through a ceramic sampler. The concentration of virus in this sample was used to determine the virus concentration being applied to the column. A 100-ml sample of the cumulative daily outflow was concentrated as described by Wallis et al. (20). Samples for organic carbon analysis were extracted on days 1,7, 8, and 9. After the 9-day flooding period, a 10-cm depth of distilled water (800 ml) was infiltrated, and samples were extracted for virus assay. The column was drained for 5 days and then flooded for 1 day with deionized water. Water samples for virus assay were extracted at the various depths at the end of the 1-day period.

This experiment was then repeated using secondarily treated sewage. The column was flooded for 3 consecutive days, and virus samples were removed at the various depths indicated above.

After antibiotics and 1 to 2 drops of fetal calf serum were added, the samples were frozen and shipped to Houston, Tex., where the virus assays were performed. Portions (1 ml) of the samples were assayed by the agar overlay technique (11), using buffalo green monkey cells (6). The cell line was passaged, grown, and maintained as described previously (17).

Batch studies. To determine whether there was a difference in virus adsorption between primary and secondary sewage, virus adsorption to soil in batch experiments was studied. The tests were carried out using variable soil concentrations and constant virus concentration. Primary sewage effluent used in batch studies was first filtered through a Cox filter (2-μm pore size, series AA; Cox Instrument Corp., Detroit, Mich.) to remove any large suspended matter. Virus was then added to the sewage, and 50 ml was poured into 100-ml glass centrifuge tubes containing various amounts of the same soil used in the column studies. The samples were mixed for 30 min at room temperature (about 25°C), after which the soil was pelleted by centrifugation and the supernatant was assayed for virus. The same procedure was repeated on a control centrifuge tube that did not contain soil. The difference in titer between the control and the samples containing soil was used to determine the quantity of virus adsorbed to the soil. No appreciable inactivation of the virus occurred during the batch experiments. The concentration of soil in the batch studies varied from 1 to 24 mg/100 ml.

Organic carbon analysis. In the column studies, the organic carbon content of the wastewater samples was determined with a total-carbon analyzer (Beckman Instruments, Inc., Fullerton, Calif.). The soluble organic carbon in the wastewater used in the batch studies was determined by measuring UV absorbance at 254 nm and determining total organic carbon from a standard curve as milligrams per liter (9). The correlation between total organic carbon and UV absorbance has been demonstrated previously (7).

RESULTS AND DISCUSSION

Batch studies. Adsorption of poliovirus in both primary and secondary sewage conformed to a Freundlich isotherm (Fig. 1). These results represent the average of four experiments for each type of sewage. The total organic carbon ranged from 20 to 24 mg/liter for the primary sewage and from 7 to 8 mg/liter for the secondary sewage. The slopes and y-intercepts of the Freundlich plots indicated only a slight difference in virus adsorbed by the soil from primary and secondary sewage.

Virus movement in soil columns. Most of the viruses were removed from primary and secondary sewage after passage of the wastewater through the first few centimeters of soil (Fig. 2 and Tables 1 and 2). Poliovirus removal in the column was similar for both kinds of wastewater, even though the total organic carbon content

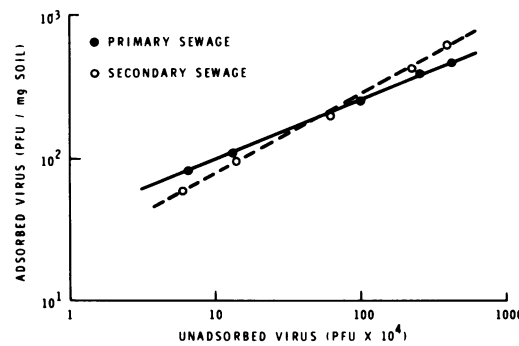

FIG. 1. *Freundlich isotherm plots of virus adsorption in primary and secondary sewage to loamy sand soil.*

was quite different in the reservoir (69.5 versus 10 mg/liters for the primary and secondary sewage, respectively) and within the soil column (Table 3) (14). When the column was flooded with primary sewage, viruses were not detected in any 1-ml samples below 40 cm, and only on one occasion was virus detected in a 100-ml sample from the 250-cm depth. Flooding the column with secondary sewage yielded similar results (Table 2), but virus was detected on one occasion at the 80-cm level.

During 1 day of storage, the average concentration of viable virus declined about 50% (from 16,300 to 8,191 PFU/ml) in the primary sewage reservoir and 47% (from 8,300 to 3,867 PFU/ml) in the secondary sewage. The sewage water-virus mixture in the reservoir was replaced every 1 to 2 days. Previous studies that we have conducted indicate that viral die-off is not a dominant removal mechanism inside the soil columns, viral die-off being greatly reduced in the soil column (12).

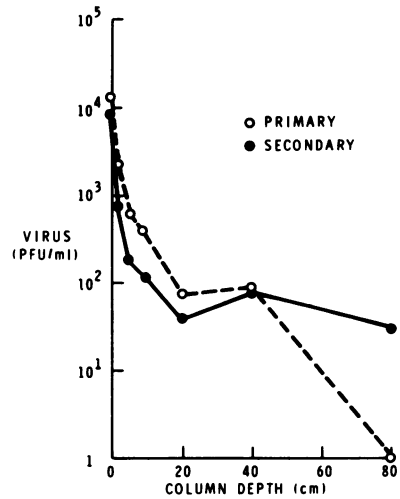

FIG. 2. *Virus concentrations at various depths in soil column flooded with primary and secondary sewage seeded with poliovirus. Data are averages for 9 days of flooding.*

Approximately 1 log of virus was removed from primary sewage during passage through the first 5 cm of soil, but an additional 35 cm of travel was necessary to reduce the virus concentration another log (Fig. 2). Similar results were obtained when the column was flooded with secondary sewage (Table 2).

Desorption of viruses with deionized water. Flooding the soil with deionized water immediately after flooding with primary effluent containing virus caused some movement of viruses, but they were not detected below the 80-cm depth (Table 1). Flooding the soil again with deionized water after allowing the soil column to drain for 5 days did not result in detection of viruses at any depth. Thus, viruses were de-

TABLE 2. *Virus concentrations at various depths in a soil column flooded with secondary effluent*

Column depth (cm)	Virus concn[a] (PFU/ml)	
	Range	Avg
0	3,600–4,200	3,867
2	900–900	900
5	400–900	733
10	150–260	207
20	130–240	170
40	100–280	210
80	0–10	5
160		0
240		0
250		0

[a] Averages for samples taken on three flooding dates.

TABLE 1. *Virus concentrations at various depths in a soil column flooded with primary sewage effluent*

Column depth (cm)	PFU/ml on day:										
	1	3	6	7	8	9	Avg of days 1–9	9.2[a]	10	14	15
0	5,950	11,815			8,500	6,500	8,191				
2	2,600	2,300	1,100	3,250	1,900	2,700	2,308	100			0
5	950	600	1,250	495	50	280	604	550			0
10	220	1,450	145	290	0	10	352	550			0
20	175	NS[b]	130	60	0	10	75	450			0
40	155	50	205	130	0	0	90	500			0
80	0	0	0	0	0	0	0	5			0
160	0	0	0	0	0	0	0	0			0
240	0	0	0	0	0	0	0		0	0	0
250	0	0	0	0	0	0	0		0	0	0
250[c]	0	0	6	0	0	0	0	NS	0	0	0

[a] Primary effluent was removed, and the column was flooded with deionized water after 9 days.
[b] NS, No sample.
[c] Samples of 100 ml.

TABLE 3. *Organic carbon content of samples extracted for virus assay from soil columns flooded with primary sewage effluent seeded with poliovirus*

Column depth (cm)	Total organic carbon (mg/liter) on day:				
	1	7	8	9	Avg
0[a]	35.0	115.0	73.0	55.0	69.5
2	34.5	101.0	47.7	57.5	60.2
5	33.7	119.5	61.0	47.5	65.4
10	31.5	119.0	45.3		65.3
20	24.3	104.0	43.0	30.5	50.5
40	20.0	97.0	43.5	51.5	53.0
80	14.0	69.0	38.0	50.7	42.9
160	6.0	57.5	38.7	49.0	37.8
240	18.7	26.5	16.0	25.0	21.5
250	15.5	36.0	13.6	32.0	24.3
Outflow[b]		16.5	8.0	4.0	9.5

[a] One-day-old primary effluent.
[b] Cumulative, 24 h.

sorbed by application of deionized water to a saturated soil but not by application of deionized water after the free water drained from the soil. This virus movement was similar to that observed with soil columns flooded with secondary effluent seeded with virus and then leached with deionized water after different drying times (12).

The results indicated that the behavior of polioviruses in soil flooded with primary sewage is markedly similar to that observed when the same soils are flooded with more highly treated wastewater. The greater concentration of organics does not appreciably affect the removal of poliovirus by the soil used in this study, and filtration through that soil can be regarded as an effective treatment method for poliovirus removal. Although elution of soil-adsorbed virus during periods of heavy rainfall can be a problem with primary sewage (if rain falls within 1 day after a flooding period has ended), we previously demonstrated that this can be controlled by reflooding the land surface with wastewater or by adding a soluble salt to the soils.

The low adsorption of virus in primary effluent to soil reported by Schaub and Sorber (18) probably reflected the soil type (unconsolidated silty sand and gravel) at the land infiltration site, although other undetermined factors could be involved. The loamy sand soil used in the present study exhibited good adsorptive properties and still allowed a high rate of infiltration of both secondary and primary sewage. The higher concentration of organics present in the primary sewage did not result in the saturation of adsorption sites after 9 days of flooding (Table 1). Thus, the nature of the soil appears to be more important in viral retention than the amount of organic matter in sewage.

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