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Virus and Bacteria Removal from Wastewater by Land Treatment

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Secondary sewage effluent and renovated water from four wells at the Flushing Meadows Wastewater Renovation Project near Phoenix, Arizona, in operation since 1967, were assayed approximately every 2 months in 1974 for viruses and enteric bacteria during flooding periods. No viruses or *Salmonella* sp. were detected in any renovated well water samples, and the numbers of fecal coliforms, fecal streptococci, and total bacteria were decreased by about 99.9% in the renovated well waters after the wastewater was filtered through about 9 m of soil.

Man has continually polluted his environment with his waste products. Only recently has he been strongly confronted with the consequences. Now, he must concern himself with his overindulgence and determine what health hazards are associated with his methods of waste disposal. He must implement management practices to assure that he does not exceed the natural ability of his environment to cleanse and recycle his wastes.

For disposing of municipal sewage effluent, land treatment is gaining interest. Certainly, large quantities of solid and liquid wastes have been applied to the soil, but the optimum amounts and application rates that the soil can handle are unknown and are dependent upon the local climate, soil type, and topography of the area. One primary concern of disposing of wastewater on land has been the fear of polluting the groundwater resources with toxic substances and other health hazards, such as pathogenic viruses and bacteria.

The fate of microorganisms in wastewater, as they contact the soil, has been an important consideration in land disposal systems. Gerba et al. (7) and Bitton (3) reviewed the subject relative to the adsorption and fate of wastewater viruses and bacteria in soil. Also, Benarde (2) assessed land disposal of sewage effluents and appraised the health effects of pathogenic organisms. However, we need more information on the movement and survival of viruses and bacteria in soil, especially under field conditions, because reuse of wastewater resource depends on removal and elimination of disease-causing organisms and other health hazards.

Most bacteria are removed in the soil surface by filtration, sedimentation, and adsorption, whereas virus removal by soil is mainly by adsorption (3, 7). However, salt concentrations, pH, organic matter, soil composition, infiltration rates, and climatic conditions may affect the degree of retention of bacteria and viruses by soil. Furthermore, the survival and movement of the retained bacteria and viruses are influenced by soil moisture, temperature, pH, nutrient availability, and antagonisms. These combinations of factors make it difficult to predict the bacteria and virus removal potential of individual soil systems; thus, each land treatment site should be evaluated individually.

Bouwer et al. (4, 5) have described the infiltration and hydraulic aspects (5) as well as the water quality improvement and economic aspects (4) of an experimental, high-rate, land treatment system that has renovated wastewater since 1967, the Flushing Meadows Project near Phoenix, Ariz. Our research was initiated in 1974 to evaluate the effective removal of viruses and bacteria from secondary sewage effluent by land treatment. Specifically, we determined the population densities of viruses, fecal coliforms, fecal streptococci, *Salmonella* sp., and total bacteria in the sewage effluent and renovated well water and evaluated their movement in soil during flooding. A brief report of a portion of this work has been published (8).

MATERIALS AND METHODS

Description of research site. The Flushing Meadows Project (Fig. 1) is located in the Salt River bed west of Phoenix and consists of six parallel horizontal soil basins that measure 6.1 by 213.4 m

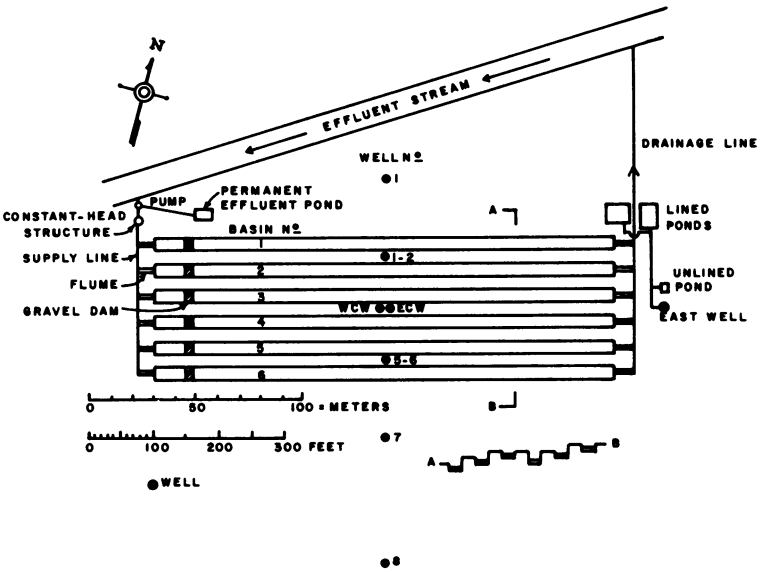

FIG. 1. *Schematic diagram of Flushing Meadows Project (from reference 4).*

each and are 6.1 m apart (5). Effluent from a secondary sewage treatment plant (activated sludge) is allowed to infiltrate into the six soil basins, which consist of 60 to 90 cm of fine, loamy sand underlaid by a succession of coarse sand and gravel layers to a 75-m depth, where a clay layer begins (Table 1). We used intermittent flooding and drying cycles of 14 days during 1974.

The static groundwater table is about 3 m deep. Observation wells for sampling the renovated sewage are installed in line midway across the basin area. The wells are 6.1 m deep, except the east center well, which is 9.1 m, and the west center well, which is 30.5 m deep. The east well is 76.2 m deep, perforated from 3 to 9 m, and plugged at the bottom with concrete. We sampled the sewage effluent and four wells (east center well, well 1-2, east well, and well 7) approximately every 2 months and assayed them for virus and bacteria populations. Only renovated wastewater samples were obtained from the wells. There was no dilution factor from the groundwater, because the native groundwater in the aquifer below the recharge area was replaced by renovated wastewater (5).

The water depth in each basin is controlled to 0.3 m by an overflow structure at the outflow end. Infiltration rates in each basin are evaluated from the difference between inflow and outflow rates measured with critical depth flumes (15). The average hydraulic loading rates were about 90 m/yr, with 122 m/yr obtained when flooding and drying cycles for maximum hydraulic loading were used (5). The suspended solids content of the secondary effluent was generally below 20 mg/liter in summer and fall but above 50 mg/liter and sometimes more than 100 mg/liter in winter and spring. Sustained high infiltration rates can be maintained if the suspended solids content of the effluent remains below 10 mg/liter (16).

The general quality of the secondary sewage effluent is shown in Table 2 and is described in detail by Bouwer et al. (4). The recharge basins are only flooded with secondary sewage effluent. Rainfall is negligible in the geographic area, where the average annual precipitation is less than 20 cm. The temperature of the sewage effluent in the basins, taken as the average between the inflow and outflow temperatures, ranged from 17°C in the winter to 32°C in the summer. The mean daily air temperature for Phoenix, Ariz., in July is about 32.1°C, with maximum and minimum temperatures of 40.4 and 23.9°C, respectively, whereas in January the mean daily temperature is about 9.8°C, with maximum and minimum temperatures of 17.8 and 1.8°C.

Virus concentration: secondary sewage effluent. To determine the concentration of naturally occurring enteric viruses in sewage effluent flowing into the infiltration basins, 4- to 20-liter sewage samples were collected from the basins during each sampling period. Virus was concentrated from the samples by a method similar to that described by Homma et al. (9). The sewage was first clarified by passing it through a Tween 80-treated cellulose cartridge filter. The clarified filtrate was then adjusted to pH 3.5, and 0.5 M $AlCl_3$ was added to obtain a final 0.0005 M concentration. The sample was filtered through a 90-mm-diameter, 5-, 1-, and 0.65-μm-porosity Cox filter series to adsorb the viruses. The adsorbed viruses were eluted from the filters with glycine buffer (pH 11.5). The final neutralized eluate volume was about 20 ml.

Virus concentration: renovated water. Samples of renovated water from each well were processed through a portable virus concentrator, using meth-

TABLE 1. *Driller's log for east well and west center well*[a]

Well	Depth (m)	Material
East well	0.9	Fine loamy sand
	0.9–8.2	Sand, gravel, and boulders
	8.2–9.1	Clean sand, gravel, and boulders
	9.1–14.9	Clean, fine sand with occasional cobbles
	14.9–24.7	Clean, fine sand with occasional thin-gravel strata
	24.7–37.5	Clean fine sand
	37.5–38.4	Fine sand with trace of clay
	38.4–41.5	Clean, fine sand
	41.5–44.5	Clean sand and gravel
	44.5–60.1	Clean, fine sand
	60.1–61.0	Fine sand and gravel
	61.0–75.3	Fine sand
	75.3	Start of clay layer
West center well	0.9	Fine loamy sand
	0.9–10.1	Sand and gravel
	10.1–13.4	Boulders and gravel
	13.4–15.2	Sand and gravel
	15.2–17.4	Sand and traces of clay
	17.4–19.2	Coarse, clean gravel
	19.2–21.9	Sand, gravel, traces of clay
	21.9–26.2	Coarse gravel and boulders
	26.2–29.9	Sand, gravel, and traces of clay
	29.9–30.5	Fine sand

[a] From Bouwer et al. (4).

ods previously described (17), with the following modifications: (i) conditions for virus adsorption were pH 3.5 with 0.0005 M $AlCl_3$; (ii) no clarifiers were used; (iii) the virus adsorber in the first stage of the concentration procedure consisted of a fiberglass, cartridge filter followed by a 142-mm-diameter, 5-, 1-, and 0.65-μm-porosity Cox filter series; and (iv) viruses in the first eluate were reconcentrated on a 90-mm-diameter, 5-, 1-, and 0.65-μm-porosity Cox filter series, yielding a final neutralized eluate volume of about 20 ml. Before freezing, 0.25 ml of fetal calf serum and 0.5 ml of a combined mixture of penicillin and streptomycin were added to the final eluate.

Each virus sampling period was about 1 week (Table 5). During this time two duplicate samples from each well of 200 to 400 liters were processed through the virus concentrator. The final volume of virus concentrate was frozen and shipped (air freight) in dry ice to Baylor College of Medicine, Houston, Tex., where they were stored at −80°C and assayed as soon as possible.

Virus isolation. Virus was isolated using primary baboon kidney cells. Kidneys obtained from immature baboons were trypsinized and grown as described by Melnick and Wenner (13).

Reconcentrated samples obtained from the virus concentrator were examined for natural virus. The concentrates were made isotonic by adding 20× saline, and the pH was adjusted to 7.5. When necessary, the samples were passed through a 0.22-μm filter (pretreated with 1% Tween 80 to prevent virus adsorption) to remove fungi and bacteria.

TABLE 2. *General chemical characteristics of the secondary sewage effluent*[a]

Component	Concn (mg/liter, except pH)
BOD_5	15
COD	50
Organic-N	1
NH_4-N	25
NO_3-N	<1
PO_4-P	13
Dissolved salts	1,020
Suspended solids	20–100
pH	7.9

[a] From Lance and Whisler (11). BOD_5, Biological oxygen demand based on 5-day incubation; COD, chemical oxygen demand.

Usually only 30 to 50% of the total sample volume was assayed at a time until the entire volume was processed. The samples were then divided into two equal volumes, and one was assayed by bottle culture and the other by the agar overlay method. Both the bottle and the overlayed cell cultures were observed daily for 14 to 21 days. In the agar overlay method, 0.1 to 0.2 ml of inocula was placed on a monolayer of cells in 30-ml glass bottles and incubated at 37°C for 1 h. The bottles were then washed with 5 ml of Eagle minimal essential medium to reduce problems with toxicity, drained of excess fluid, and overlayed with 5 ml of agar. The agar overlay medium consisted of 1× Eagle minimal essential medium without phenol red, 1.5% agar (Difco), 23 mM $MgCl_2$, 1:54,000 final concentration of neutral red, 100 U of penicillin and 100 μg of streptomycin per ml, and 0.4% $NaHCO_3$.

The second portion of 0.2- to 0.5-ml volumes of concentrate was placed in 30-ml glass bottles of drained cell cultures and incubated at 37°C for 1 h, after which 4 to 5 ml of minimal essential medium was added. The bottles were examined daily for cytopathic effects. When cytopathic effects were observed, the culture fluid was transferred to fresh bottle cultures to determine if the cytopathic effects were due to chemical toxicity or to virus infection.

Virus identification. When plaques appeared under the agar overlay, virus was removed from the plaques and transferred to fresh cultures maintained under fluid media. Progeny virus harvested from these bottles was identified by antisera pools (12) and by specific antisera.

Bacteria assay. Numbers of fecal coliforms and fecal streptococci were determined at the U.S. Water Conservation Laboratory, Phoenix, Ariz., with the standard membrane filter technique (1). *Salmonella* populations were detected and enumerated by the methods of Kenner and Clark (10). Total bacteria numbers were determined with dilution plate procedures, using plate count agar.

RESULTS AND DISCUSSION

The average number of viruses, enteric bacteria, and total bacteria in the sewage effluent

and renovated water during 1974 are summarized in Tables 3, 4, and 5. The numbers of fecal coliforms, fecal streptococci, and total bacteria were decreased about 99.9% in the renovated well water; and *Salmonella* sp. were not detected in 5-liter well water samples from the east center well at two samplings (Tables 3 and 4). The absence of any detectable viruses in the well water indicated that, with the concentration methods we used, at least 4 logs (99.99%) of the viruses were removed during the percolation of the wastewater through 9 m of sandy loam soil. Although no viruses were detected in any of the well water samples that ranged from 174 to 378 liters, there were problems with nonspecific cytotoxicity for some eluates. When this happened, several blind passages were carried out in cell cultures to confirm the absence of viruses.

Initially, well water samples at volumes less than the desired 378 liters were obtained because of problems with the filter clogging. We

TABLE 3. *Average number of viruses and bacteria detected in secondary sewage effluent and renovated wastewater from four well sites at the Flushing Meadows Project*[a]

Water sample	Virus (PFU/100 liters)[b]	Fecal coliforms (no./100 ml)	Fecal streptococci (no./100 ml)	*Salmonella* (no./100 ml)	Total bacteria (no./ml × 10^3)
Sewage effluent	2,118	244,071	28,100	21	1,811
East center well	0	73	24	0	9
Well no. 1-2	0	79	11	-	32
East well	0	112	21	-	13
Well no. 7	0	104	22	-	9

[a] The numbers are averages of results from six flooding periods analyzed from January to December 1974 at bimonthly intervals, except for *Salmonella*, which were determined twice in June and July only for the sewage effluent and the east center well.
[b] PFU, Plaque-forming units.

TABLE 4. *Average number and range of viruses, enteric bacteria, and total bacteria detected in the secondary sewage effluent and in the wastewater after renovation by filtration through soil*[a]

Type of organism	Sewage effluent		Renovated water		
	Range	Avg	Range	Avg	% Reduced
Fecal coliforms/100 ml	20,000-770,000	244,071	0-359	93	99.96
Fecal streptococci/100 ml	2,500-50,000	28,100	1-76	20	99.93
Salmonella sp./100 ml	17-26	21	0	0	100.00
Total bacteria/ml	820,000-4,410,000	1,811,400	600-41,000	10,278	99.94
Viruses (PFU/100 liters)	158-7,475	2,118	0	0	100.00

[a] Results are averages of six bimonthly water samples from four wells in 1974, except for *Salmonella*, which was determined twice in June and July in only the east center well and the sewage effluent.
[b] PFU, Plaque-forming units.

TABLE 5. *Summary of numbers and types of viral isolates from the secondary sewage effluent and the renovated wastewater wells*[a]

Sampling dates in 1974	Virus PFU/100 liters[b]		Types of viruses in sewage effluent
	Sewage effluent	Renovated water	
7 to 11 Jan.	786	0	Polio 2, echo 15
12 to 18 Mar.	2,745	0	Polio 2, echo 7
5 to 9 May	2,378	0	Polio 2 and 3
25 June to 9 July	158	0	Polio 2, coxsackie B4
27 Aug. to 12 Sept.	7,475	0	Reovirus 1 and 2[c]
19 Nov. to 11 Dec.[d]	1,142	0	Reovirus (types undetermined)[c]
Range	158-7,475	0	

[a] Numbers are averages of duplicate samples from the sewage effluent and the four well sites combined.
[b] PFU, Plaque-forming units.
[c] No plaques were noted until after 14 days under agar overlay.
[d] Data for sewage effluent were obtained during the November flood period, and data for renovated water (east well and well 7, only) were obtained during the December flood period.

overcame this problem by increasing the final filter from 0.45 to 0.65 μm in porosity. The greatest problem with clogging was during the processing of water from the east center well. Initial flow rates ranged from 4 to 6 liters/min but were usually decreased to about 2 liters/min by the time sampling was completed. Maintenance of these flow rates often required in-line pressures of 5 to 6 atm.

We also encountered some difficulty during reconcentration of initial eluates due to filter clogging, which was caused by the formation of a dark-brown precipitate when the pH was lowered before eluate was processed through the absorbing filters. The entire eluate volume could still be passed through the 90-mm filter, but this often required high pressures (as high as 6 atm).

The sewage effluent sample volumes assayed ranged from 4 to 15 liters, averaged 13 liters, and contained from 158 to 7,475 plaque-forming units/100 liters, averaging 2,118 plaque-forming units/100 liters, during 1974 (Table 4). The viruses identified in the sewage effluent included poliovirus types 2 and 3, echovirus 7 and 15, coxsackievirus B4, and reovirus types 1 and 2, which varied with the time of the year (Table 5). The number of viruses detected in the sewage effluent was greatest from August to September and smallest from June to July (Table 5). This agrees with usual trends, since concentrations of enteric viruses in sewage peak during autumn (1).

Our results indicated that human bacterial and viral pathogens are largely removed as sewage effluent percolates through the soil. After 8 years of continual operation of the Flushing Meadows Project, the viruses, enteric bacterial pathogens, and pollution indicator organisms in the renovated sewage effluent were either nondetectable or greatly decreased after filtering wastewater through soil recharge basins.

Furthermore, our results suggest that proper land treatment of municipal wastewater can be a highly satisfactory method of wastewater renovation. However, this conclusion is only based upon results from renovated well water and surface effluent samples. Tests on these water samples do not reflect the survival potential of viruses and enteric bacteria retained by the soil system. Bouwer et al. (4) reported that most fecal coliform bacteria were immobilized in the top 3 to 4 cm of soil below basins intermittently flooded with secondary sewage effluent. Others have reported similar results (6, 7, 14).

However, Wellings and associates (18, 19) in Florida emphasized that viruses retained or adsorbed by soil may constitute a potential health hazard. Apparently, the soil-retained viruses survived for long periods and under certain environmental conditions (not yet fully understood) were released and moved into the groundwater systems. Consequently, our future work will involve the establishment of procedures for detecting and assessing the survival potential of virus populations retained within the soil profile.

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