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Virus and Bacteria Removal from Wastewater by Rapid Infiltration Through Soil

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A rapid infiltration land wastewater application site, composed of unconsolidated silty sand and gravel, which has been in continuous operation for over 30 years was examined for the accumulation and/or migration of a tracer virus (coliphage f2), indigenous enteroviruses, and enteric indicator bacteria in the soils and underlying groundwater. Tracer f2 penetrated into groundwater together with the front of percolating primary effluent and was not observed to concentrate on the upper soil layers. The tracer virus concentration in a 60-foot (about 18.3-m)-deep observation well directly beneath the wastewater application area began to increase within 48 h after application to the soil. The tracer level in this well stabilized after 72 h at a level of approximately 47% of the average applied concentration. Indigenous enteroviruses and tracer f2 were sporadically detected in the groundwater at horizontal distances of 600 feet (about 183 m) from the application zone. Laboratory soil adsorption studies confirmed the poor virus adsorption observed at the site. This was especially true on surface soils when contained in wastewater. Enteric indicator bacteria were readily concentrated on the soil surface by filtration on the soil surface mat. However, during tracer f2 virus tests, comparison studies with fecal *Streptococcus* revealed that bacteria capable of penetrating the surface were able to migrate into the groundwater. They were detected at the same locations as tracer and enteric viruses.

Land application has been used as a wastewater disposal method for many years. Wastewater can be applied by spray irrigation, overland flow, or rapid infiltration. Treatment effectiveness is a function of the physical, chemical, and biological properties of the soil as well as wastewater application practices.

There are several major advantages to wastewater disposal by land application, including relatively low system capital and operating costs, unsophisticated facility requirements, the value of the nutrients imparted to the land, and recharge of groundwater by infiltration-percolation. Unfortunately, there are several potential problem areas which need more definition. For example, the actual effectiveness of land application of wastewater in removing enteric microorganisms is not fully understood.

A number of studies have attempted to evaluate the enteric microbial removal potential of soils. McGauhey and Krone (9) summarized existing information and concluded that coliforms and other bacteria move only a few feet in an unsaturated zone and several hundred feet in a saturated zone. These investigators indicated that bacteria behave like other particulates in soil and are removed by straining, sedimentation, entrapment, and adsorption, and are influenced adversely by antagonistic environmental factors. They concluded that soil systems are quite efficient in removing bacteria and hypothesized that viruses can be removed with equal effectiveness, principally by adsorption.

Driver and co-workers (4) reviewed previous studies and supported McGauhey and Krone's conclusions. They suggested that the rapid infiltration and spray irrigation modes of wastewater treatment should provide the most efficient removal of bacteria and viruses of any land wastewater treatment method. Further, they concluded that, depending on the nature of the soil, soil depths ranging from 1 cm to 2.1 m are sufficient to remove bacteria and viruses from wastewater, and that the mechanisms of removal are similar in most soil systems, except that the relative effectiveness of the various mechanisms differs according to the mode of land application. They suggested that, for rapid infiltration and spray irrigation systems, the most important microbial removal mechanism is filtration, followed by fixation or adsorption.

A recent review of the fate of wastewater microorganisms in soil by Gerba et al. (6) summarizes the known mechanisms of their removal in soils. They indicate that whereas filtration on the soil is the principal mechanism of bacterial removal, virus removal is predominantly by adsorption to soils, especially clays. They cite recent research which indicates that changes in the water or soil characteristics may allow virus de-adsorption and subsequently penetration to deeper soil layers.

The requirement for research into potential health and hygiene problems associated with land application of wastewater was recognized and discussed by Sorber and co-workers (13). They pointed out that, during land application of wastewater, particularly when rapid infiltration (RI) was the mode of application, enteric viruses and bacteria, as well as potentially toxic chemicals (e.g., heavy metals and pesticides), may reach the groundwater.

The purpose of this study was to determine the effectiveness of removal of enteric bacteria and viruses by the rapid infiltration mode of wastewater treatment. This study was divided into two parts. The first part was designed to provide base-line data on selected microbial parameters which involved laboratory investigations with soil and wastewater samples collected at the site. Soil and well sampling techniques were developed during two 1-day sampling periods.

The second part of the investigation was designed to determine the capability of the rapid infiltration system to treat or remove enteric bacteria and viruses over a total treatment cycle. A virus tracer study was performed. Indicator organisms were assayed in soil and groundwater samples. In addition, enteric viruses were concentrated and assayed from groundwater samples.

MATERIALS AND METHODS

Study site. The site selected for study was located at Fort Devens, Mass., a U.S. Army installation situated northwest of Boston. During this study the installation had a permanent resident population of approximately 10,000 in addition to a daily work force of 5,000.

The sewage treatment facility has been in continuous operation since 1942. Collected sewage is comminuted and pumped from a central pump station to Imhoff tanks which have a retention time of approximately 6 h. Generally, the wastewater flow is between 1.0 and 1.5 million gallons/day (3,785 to 5,678 m^3/day).

The Imhoff tank effluent is distributed through a dosing tank to the RI cells without chlorination. There are 22 RI cells, each with an average area of about 0.8 acre (0.324 hectare), constructed on a steep-sided kame. The soil beneath the RI cells is principally unconsolidated silty sands and gravel of glacial origin, underlaid with bedrock.

Under normal operating conditions, three RI cells receive wastewater for a 2- to 3-day period. A subsequent period of 2 days is required for complete effluent infiltration. At that time, the cells are allowed a resting period of 10 days. During the year prior to this study, a 7-day application period followed by a 14-day resting period was used. The RI cells are cycled on a regular basis and receive effluent for approximately 50 days each year. Winter conditions, although reducing infiltration somewhat, do not hamper normal operations.

During the past 30 years, the major operational problem has been clogging of the soil on the rapid infiltration beds. To alleviate this problem, the beds are renovated by removal of 1 to 4 feet (0.305 to 1.22 m) of the surface soils and replacing them with bank run sand and gravel after loosening the exposed subsurface layers. The most recent renovation occurred in 1968. During this renovation, a black asphaltic-appearing layer was observed at a soil depth of between 18 and 24 inches (44 to 59 cm).

Numerous observation wells were installed at the study site. They were wash-drilled with diamond core drills and they were cased during the drilling operation. Tap water was used for substrate removal from the casing. Fine sediments were removed by pumping. Observation wells no. 3, 7, and 8 have metal riser pipe and no. 10 metal screens. All other wells were installed with PVC riser pipes and no. 20 PVC screens. Protective well casings to a depth of at least 4 feet (1.22 m) below the ground surface were provided to all of the wells except no. 3 and 8. In all cases, riser pipes were extended 36 inches (92 cm) above the surface and metal caps were installed to prevent contamination. Specifications of the observation wells used during this study can be found in Table 1.

Virus stock preparation and assay. Encephalomyocarditis virus (EMC) and poliovirus I (Chat strain) were utilized for laboratory studies of enterovirus adsorption to soils. The EMC virus was grown and assayed on mouse L continuous culture cell line whereas poliovirus I was grown and assayed on BGM and KB continuous cell lines. Procedures used in the growth and maintenance of cell lines as well as cell culture techniques, virus filtration, and virus plaque assay have been detailed elsewhere (11).

The f2 bacteriophage was grown as described by Loeb and Sinder (8), as modified by Kruse et al. (7). Eleven- to 23-liter stocks were grown in a laboratory fermentor where a concentration of over 10^{12} plaque-forming units (PFU)/ml was attained. Chloroform was added as a preservative and the stocks were temporarily stored under refrigeration. The f2 were assayed by the soft agar overlay method (tryptone yeast extract) utilizing a lawn of male *Escherichia coli*, strain K-13 (American Type Culture Collection no. 15766). Incubation was at 35°C for 18 h. Field samples were heated to 37°C for 5 min prior to assay to eliminate the chloroform preservative added in the field.

Virus adsorption studies. The two enteric viruses

TABLE 1. *Observation well specifications*[a]

Observation well no.	Ground surface elevation (feet)	Well depth (feet)	Well screen length (feet)	Well diam (inches)
3	209.30	9.5	3.0	1.25
7	223.18	28.4	10.0	1.25
8	219.91	19.0	3.0	1.25
10	218.91	22.8	10.0	1.25
11	212.39	14.9	10.0	1.25
15	269.31	94.5	40.0	2.50
17	213.41	18.0	10.0	1.25
19	211.56	22.5	10.0	1.25
20	210.89	16.6	10.0	1.25

[a] One foot = 0.348 m; 1 inch = 2.54 cm.

and f2 bacteriophage were used to evaluate the adsorptive capacity of the various soils. Autoclaved, weighed samples (10 g) of soil from unrenovated or renovated (1968 renovation) cells were mixed with 40 ml of autoclaved primary treated sewage or deionized water containing 10^{-3} M $CaCl_2$. The mixture was stirred vigorously for 5 min with a magnetic stirrer. At that time, virus to a concentration of 10^4 PFU/ml was added and the samples were stirred slowly for a 30-min adsorption period. Aliquants (10 ml) were centrifuged at 12,000 × *g* for 10 min to separate the solids and associated virions from the liquid phase. The supernatant portion was assayed for infectious virus on the appropriate cell culture. Virus titers were expressed as PFU per gram of sample.

Virus field studies. The tracer study at the site was performed by continuous addition of stock f2 bacteriophage into the wastewater at the dosing tank with a peristaltic pump over the 7-day application period. The pump flow was adjusted daily to provide a virus concentration of 10^5 PFU/ml of wastewater applied to the RI cells. Cells no. 10, 15, 16, and 17 were utilized for the tracer tests. Effluent, soil, and groundwater samples were analyzed for f2 bacteriophage prior to, during, and after dosing of the wastewater.

The migration of indigenous enteric viruses from the wastewater applied to the RI cells was evaluated during the f2 tracer virus field study. One-liter composite samples of wastewater standing on the beds and 4-liter samples of groundwater obtained from observation wells were concentrated for enteric viruses by the bentonite adsorption procedure. A 70-mg amount of powdered bentonite clay per liter followed by $CaCl_2$ to provide a concentration of 10^{-2} M was added to the samples. Samples were intermittently shaken for 20 min to allow the virions to adsorb to the clay. The samples were then filtered through a 142-mm-diameter AP25 fiber glass (Millipore Corp.) prefilter to collect the bentonite and adsorbed virions. The adsorbed virions were eluted from the clay on the prefilters by the addition of 10 ml of tryptose phosphate broth, and eluates were drawn through the filter by suction and collected in a small vacuum flask. The samples were placed in 10 ml of polypropylene snap-lock tubes and frozen until assay. Upon thawing, ethylenediaminetetraacetic acid (0.1 M) was added to dissolve a precipitate which formed during freezing, and 0.25 ml of sample was placed upon replicate sets of BGM and KB cell monolayer tissue culture plates. After a 60-min virus attachment period, the residual sample was aspirated from the plates to lessen bacterial overgrowth and the solid overlay was added. Four days of incubation were allowed to enhance detection of enterovirus plaques.

Grab samples of primary effluent, ponded wastewater in RI cells and the Nashua River, were taken with a dip spoon. The RI wastewater samples were a composite of equal portions from each test cell. Samples for microbiological analysis were placed in 1-gallon (3.785-liter) plastic containers or 32-ounce (0.95-liter) sterile prescription bottles and iced until assay. Samples collected during the f2 bacteriophage tracer study were placed in 5-ml polypropylene snap-lock tubes. Chloroform and 0.5 ml of 5× nutrient broth were added, and the tubes were frozen until analysis.

Sampling of observation wells was conducted by placing a weighted plastic tube into the wells to a depth of 6 inches (15.2 cm) beneath the groundwater surface. Suction was applied by a hand pump which delivered the samples to a 1-liter fleaker bottle. The first liter of sample was discarded, and subsequent portions were collected for analysis. In observation wells no. 2 and 15, the depth to groundwater was too great to obtain the samples by suction; thus, these wells had to be sampled with a 400-ml-capacity, Kemmerer-type water sampler (7). Again, the first sample volume collected was discarded. Samples were placed in containers as previously described.

Water table measurements were made routinely at the observation wells during the bacteriophage tracer study. A measured line connected to a conductivity probe and meter was utilized for these determinations. Measurements were taken from the surface of the groundwater table to the top of the well stand pipes.

RESULTS

Virus adsorption to soils. Poliovirus I, EMC virus, and f2 bacteriophage were utilized to compare the capacity of renovated and unrenovated RI cell soil layers to adsorb the virions.

Enteric indicator bacteria studies. Indigenous indicator bacteria concentrations of the wastewater, soil, and groundwater were determined. Total coliform, fecal coliform, and fecal *Streptococcus* analyses were performed utilizing membrane filter techniques as described previously (1). Results were expressed as colony-forming units (CFU) per volume of liquid or dry weight of soil.

Microbiological and hydrological sampling and preparation. Soil sampling for microbiological analysis was performed on five visibly distinct soil layers extending to approximately 30 inches (73.5 cm) beneath the surface. Shovel and pick were used to dig the proper depth, and then one side of the hole was carefully cut to

provide a vertical face for sampling. Samples from each soil layer were taken from the midpoint of the layer by scooping into the vertical cut face with a Teflon spoon. The first several scoops of each layer were discarded to minimize contamination from other soil layers. All soil samples were sealed in doubled plastic bags, and placed on ice until analysis. Samples for microbiological analyses were processed within 8 h of collection.

Total coliform, fecal coliform, fecal *Streptococcus*, and f2 bacteriophage were eluted from the soils by the addition of nutrient broth at pH 7.2 (5 g of soil and 10 ml of eluent) followed by vigorous mixing with a magnetic stirrer for 5 min. Heavy particles were allowed to settle for 1 min, and the supernatant portions were assayed as described previously.

Separate aliquants of the soil samples were weighed into petri dishes, dried overnight at 105°C, and reweighed. The soil dry weight was used in the calculation of virus and indicator bacteria.

Figure 1A illustrates EMC virus adsorption in deionized water containing 10^{-3} $CaCl_2$ for soils from renovated and unrenovated cells. Neither surface soil was capable of extensive adsorption. Below the surface layer, 60 to 80% of the virus was adsorbed by the soil from the renovated cell. The soil from the unrenovated cell, on the other hand, did not retain significant virus in the upper subsurface layers but began to approach the adsorptive capacity of renovated cell soil at the 18-inch (45.7-cm) depth. At the 30-inch (76.2-cm) layer, the soil adsorptive capacity of renovated and unrenovated cells was equal.

Replacement of the suspending fluid with domestic, primary effluent resulted in a significantly different relationship between both soils and EMC virus. Figure 1B shows that, with sewage, the adsorptive capabilities of soils observed in the distilled water tests were not attained. A maximum of 7.5% adsorption was observed. Comparative studies utilizing poliovirus I also reveal poor virus adsorption capabilities for the soils saturated with primary effluent (Fig. 1C). Virus adsorption reached 24% at the mid-depths, but overall adsorption was about 10% for soils from both cells. Studies utilizing f2 bacteriophage with primary effluent indicated that there was essentially no adsorption of this virus to any of the soil layers (Fig. 1D).

f2 Bacteriophage adsorption at the field site. For comparison of the laboratory results to actual site conditions, the soils in renovated cell no. 16 and unrenovated cell no. 17 were analyzed prior to and during the f2 bacteriophage tracer study. Samples from soil layers similar to those analyzed in laboratory virus adsorption studies were treated with tryptose phosphate broth for elution of f2 bacteriophage. Table 2 shows the soil concentrations of f2 bac-

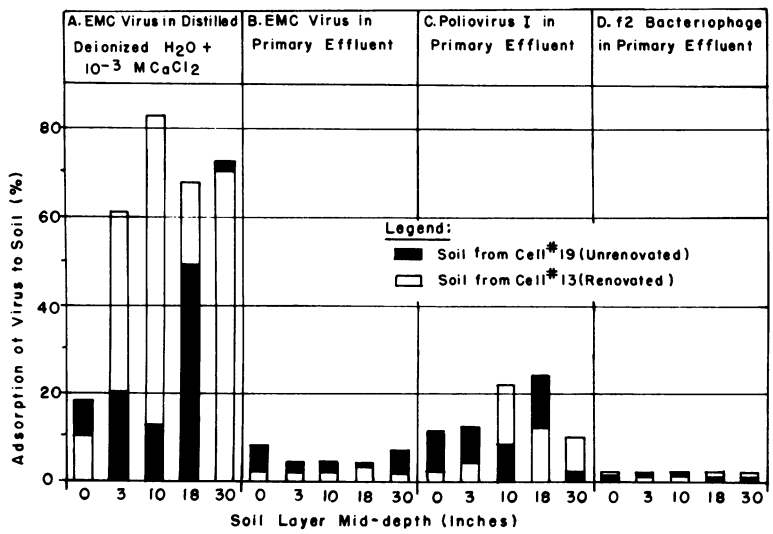

FIG. 1. *Virus adsorption to soils from various depths. The selected liquids and soils were mixed together at a 5:1 ratio. Virus was added and allowed to adsorb to the soils for 30 min. The mixture was centrifuged to pellet the soils and associated virions. Unadsorbed virions in the supernatant portion were assayed. The percent virus adsorption was determined from the supernatant virus as a fraction of virus assayed in the mixture prior to centrifugation.*

teriophage in terms of soil dry weight. A surprisingly high background concentration of indigenous bacteriophage was observed at the soil surface in both cells on day zero. Once past the surface, the concentration of virus was much lower. On day 13 of the test, the concentration of virus on cell no. 16 was uniform over the entire soil profile. At this time, all the effluent had infiltrated past the soil surface and the soil had a high water content. The unrenovated cell no. 17 still retained 6 to 8 inches (15.2 to 20.3 cm) of water on this sampling day. On day 21, a uniform distribution of virus was observed in cell no. 16, albeit somewhat lower than on day 13. Cell no. 17 had a dry surface at this time, and comparable but somewhat less uniform virus concentrations with depth were observed. It is important to note that, after infiltration was complete in each cell, the observed bacteriophage concentration was approximately that which could have been predicted from laboratory test results based on the average concentration of virus dosed into the system (2.5×10^5 PFU/ml of sewage). Since only minor amounts of virus were retained at the surface, it would appear that the virus passed through these upper soil layers with ease.

Indicator bacteria association with soils at the field site. The soils obtained for enterovirus association experiments also were examined for enteric indicator bacteria. Bacteria were eluted from separate soil samples as previously described. The eluates were assayed for total coliform, fecal coliform, and fecal *Streptococcus*. Table 3 shows that the concentration of microorganisms at the soil surface was significantly greater than it was in the subsurface soils. Once past the soil surface, there was a more gradual decline in the numbers of organisms with RI cell depth. In all samples there were higher concentrations of total coliform than fecal coliform and there were more fecal coliform than fecal *Streptococcus*. The ratio of both fecal coliform and fecal *Streptococcus* to total coliform increased once past the soil surface.

During the f2 tracer study, indigenous fecal *Streptococcus* analyses were conducted to compare the movements of indicator bacteria to those of the bacteriophage. The average fecal *Streptococcus* concentration found in the effluent applied to the cells was 10^5 CFU/100 ml. Table 4 shows that the zero time samples contained relatively few fecal *Streptococcus* in either cell no. 16 or 17, and those detected were principally in the surface layer. However, neither cell had received effluent for at least 4 weeks. After flooding and infiltration/percolation, there were appreciable bacterial concentrations on the soil surface, but the concentrations diminished once past the surface. Comparison of the day 13 and day 21 sampling pe-

TABLE 2. *f2 Bacteriophage tracer at various soil depths*

Sample[a]	PFU/g of dry soil				
	Day 0		Day 13, cell 16	Day 21	
	Cell 16	Cell 17		Cell 16	Cell 17
Surface	8.7×10^3	1.8×10^3	8.6×10^2	4.1×10^2	9.9×10^2
3 inches	7.4×10^2	5.6×10^1	7.8×10^2	2.1×10^2	2.6×10^2
10 inches	1.1×10^1	1.4×10^1	9.7×10^2	1.3×10^2	6.8×10^1
18 inches	8.9×10^1	4.5×10^1	1.1×10^3	3.7×10^2	2.9×10^1
30 inches	4.7×10^1		2.1×10^2	2.7×10^2	9.0×10^1

[a] One inch = 2.54 cm.

TABLE 3. *Preliminary analysis for indigenous indicator organisms*

Sample[b]	CFU/g (dry wt) of soil or CFU/ml of effluent					
	Total coliform		Fecal coliform		Fecal *Streptococcus*	
	Mar. 1974	June 1974	Mar. 1974	June 1974	Mar. 1974	June 1974
Effluent on RI cells		4.2×10^5		4.2×10^4 (0.1)[a]		8.1×10^2 (0.002)
Soil surface	9.6×10^6	2.5×10^7	3.5×10^4 (0.004)	2.5×10^6 (0.1)	1.8×10^4 (0.002)	6.2×10^4 (0.003)
6-inch depth	2.5×10^5	7.8×10^4	1.8×10^4 (0.07)	2.5×10^4 (0.3)	1.4×10^3 (0.006)	6.6×10^2 (0.009)
12-inch depth		2.3×10^4		7.5×10^3 (0.3)		1.5×10^2 (0.007)

[a] Numbers in parentheses indicate the ratio of the indicator organism concentration to total coliform concentration for the same test period and sample.

[b] One inch = 2.54 cm.

riods on cell no. 16 suggest that the 7-day drying period did not severely affect fecal *Streptococcus* levels, especially in the upper soil layers.

Groundwater migration. Groundwater levels in the observation wells were measured daily during the tracer study from day 0 through day 7 and again on days 10, 13, and 20. Groundwater contours were developed from these data. Of particular significance are the various stages of groundwater mounding during the infiltration period. At day zero no mound existed below the beds being flooded and the groundwater flow was generally in the direction of the Nashua River (Fig. 2). A groundwater mound began to build within 2 days and it became relatively stable after 5 days of infiltration (Fig. 3). After flooding of the beds ceased, the mound began to decay and by day 20 only a small portion of the mound remained.

TABLE 4. *Indigenous fecal Streptococcus at various soil depths*

Soil depth (inches)[a]	CFU/g of dry soil				
	Day 0		Day 13, cell 16	Day 21	
	Cell 16	Cell 17		Cell 16	Cell 17
0	1.6	1.0×10^2	2.9×10^3	1.5×10^3	1.3×10^2
3	3.0	3.0	4.9×10^2	5.3×10^2	1.7×10^1
10	<3.0	<3.0	1.9×10^2	1.3×10^2	1.3×10^1
18	<3.0	<3.0	8.5×10^1	1.6×10^1	<3.0
30	<3.0		3.0×10^1	<3.0	<3.0

[a] One inch = 2.54 cm.

Hydraulic conductivity, void ratio, and grain size analyses were conducted on soil samples from the RI site. Hydraulic conductivity was approximately 10^{-2} cm/s under unit head conditions. This would result in a field penetration time of 48 to 72 h for the wastewater to reach the groundwater table. Since hydraulic conductivity would change slightly with changes in void ratio and grain size, the laboratory data cannot predict lateral movement within the saturated aquifer.

Figure 4 illustrates the elevation of the groundwater mound directly beneath the flooded RI cells as observed in observation well no. 15. It can be seen that the mound decayed at a linear rate upon cessation of flooding of the beds. Through extrapolation it would appear that the water table would return to its initial elevation within 17 days after termination of wastewater application.

Tracer movement. The f2 bacteriophage was introduced in the primary effluent to RI cells no. 10, 15, 16, and 17 to determine migration into the groundwater. An effective concentration of 10^5 PFU/ml was maintained over the 7-day wastewater application cycle.

The standing wastewater from all four beds was composited every 8 h during the bacteriophage application period. Samples from obser-

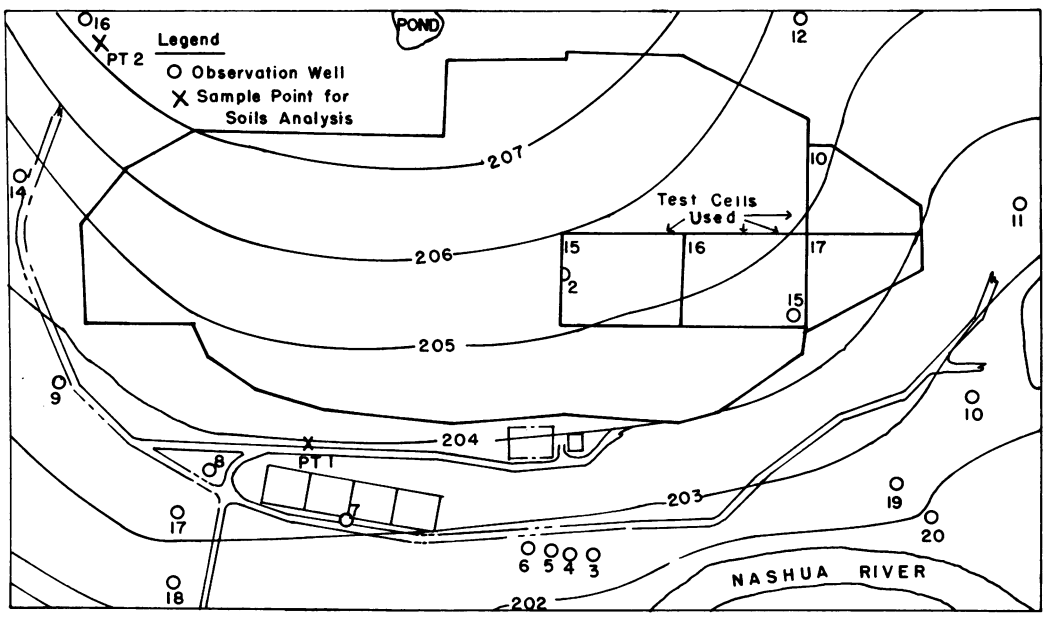

FIG. 2. *Groundwater contour prior to wastewater application to study cells. The groundwater level was determined with a measured conductivity probe at the observation wells 1 h before wastewater application to the cells commenced.*

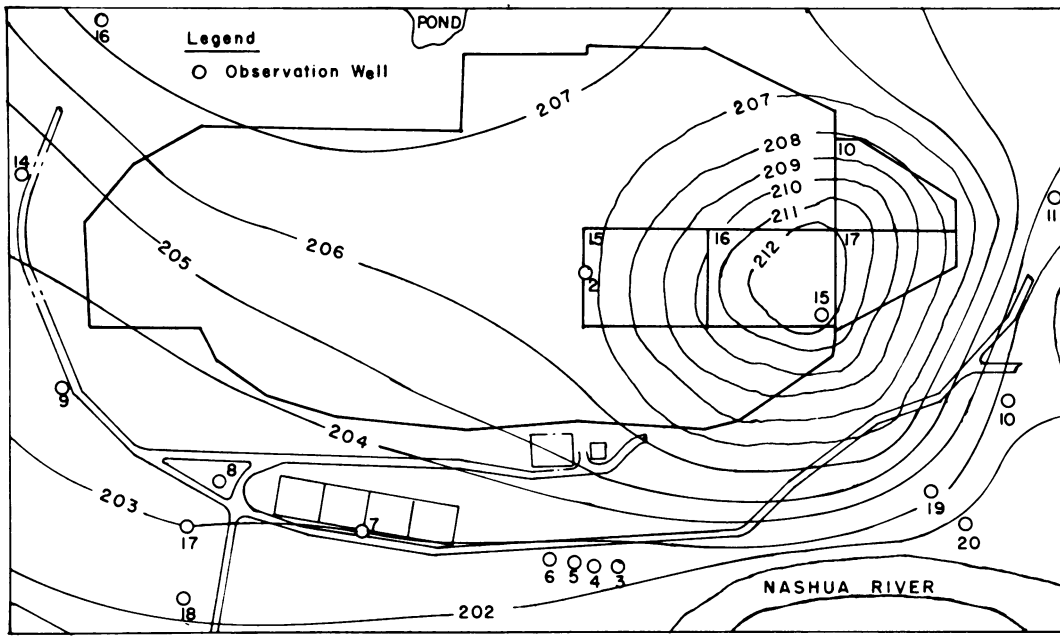

FIG. 3. *Groundwater contour on the fifth wastewater application day. The groundwater level was determined with a measured conductivity probe at the observation wells after 120 h of wastewater application.*

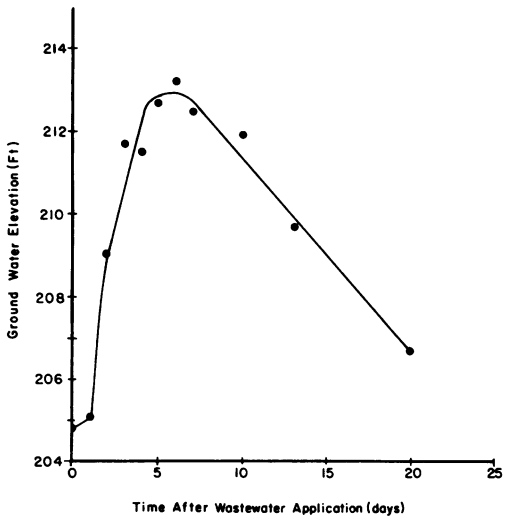

FIG. 4. *Groundwater mound at observation well directly beneath wastewater application zone. Observation well 15 was observed for groundwater mounding with a measured conductivity probe for 21 days after testing was initiated.*

vation wells no. 10, 11, 15, 19, and 20 were taken at the same times. Observation wells no. 3, 7, 8, 17, and 21 were sampled every 24 h. Also, samples from all wells were obtained on days 10, 13, and 21. Results can be found in Table 5. Figure 5 illustrates bacteriophage movement into the groundwater beneath the RI beds as observed at well no. 15. The virus reached the well after approximately 48 h, the same time as the groundwater mound began to form. The virus levels peaked and stabilized between days 3 and 6. The average bacteriophage concentration in well no. 15 during this peak period was 1.17×10^5 PFU/ml, which was approximately 47% of the composite average on the RI cells. After application to the cells ceased, the tracer concentration declined steadily. By day 20, the level was only 2% of the peak concentration.

A comparison of tracer bacteriophage in the outlying observation wells with well no. 15 and the composite is provided in Table 5. It is interesting to note that frequent "spikes" of f2 were observed in the perimeter wells most directly in the path of groundwater flow. High concentrations were noted in well no. 20 at 72 h (100% of the composite) and in well no. 10 at 156 h (7.2% of the composite). The tracer concentrations in observation wells no. 3, 7, 8, 17, and 21 during the test period were relatively low.

Indigenous enteric virus movement. During the f2 tracer study, samples from observation wells no. 10, 11, 15, 19, and 20 plus the composited wastewater on the cells were concentrated for indigenous enteric virus analyses. Table 6 shows that the average indigenous enteric virus concentration for the composite effluent

TABLE 5. *Concentrations of tracer f2 bacteriophage on RI cells and in observation wells*

Sample	PFU/ml							
	0.1[a]	1.0	1.3	1.7	2.0	2.3	2.7	3.0
RI cells[b]	3.9×10^5	3.6×10^5	2.7×10^5	2.3×10^5	1.6×10^5	2.4×10^5	2.1×10^5	1.4×10^5
Well 15	1.3×10^2	3.3×10^0	1.9×10^1	2.3×10^1	7.3×10^2	2.6×10^3	5.8×10^4	1.3×10^5
Well 10	4.2×10^0	1.0×10^0	2.3×10^0	5.6×10^0	4.3×10^0	5.2×10^1	2.3×10^1	1.0×10^0
Well 11	4.0×10^0	6.0×10^0	4.8×10^1	1.0×10^1	1.7×10^3	4.6×10^0	4.0×10^0	2.5×10^3
Well 19	6.5×10^0	4.0×10^0	4.6×10^0	1.3×10^0	1.8×10^2	0.3×10^0	2.3×10^1	9.8×10^1
Well 20	$<1.0 \times 10^0$		4.0×10^0	4.0×10^0	1.5×10^2	0.3×10^0	$<1.0 \times 10^0$	2.7×10^5

Sample	3.3[a]	3.7	4.0	4.3	4.7	5.0	5.3
RI cells	2.1×10^5	1.4×10^5	2.6×10^5	3.2×10^5	3.5×10^5	2.3×10^5	3.0×10^5
Well 15	1.4×10^5	6.1×10^4	1.5×10^5	8.4×10^4	7.5×10^4	1.6×10^5	8.7×10^5
Well 10	1.3×10^0	1.2×10^1	2.2×10^1	1.3×10^0	7.5×10^4	1.6×10^5	8.7×10^4
Well 11	3.6×10^0	5.0×10^0	2.3×10^0	1.0×10^0	9.3×10^0	3.3×10^0	8.0×10^0
Well 19	1.6×10^0	$<1.0 \times 10^0$	9.0×10^0	2.0×10^0	3.0×10^1	1.0×10^0	0.3×10^0
Well 20	1.1×10^1	2.0×10^1	1.7×10^0	3.6×10^0	1.0×10^3	2.5×10^3	1.0×10^0

Sample	5.7[a]	6	6.5	6.9	11	14	21
RI cells	1.6×10^5	3.7×10^5	1.7×10^5	2.0×10^5			
Well 15	1.3×10^5	1.3×10^5	1.2×10^5	3.8×10^4	1.9×10^4	2.3×10^4	2.4×10^3
Well 10	8.5×10^4	1.7×10^0	1.8×10^4		$>3.0 \times 10^2$	1.2×10^1	$<1.0 \times 10^0$
Well 11	0.6×10^0	5.0×10^1	0.6×10^0	$<1.0 \times 10^0$	5.6×10^1	$<1.0 \times 10^0$	1.0×10^0
Well 19	$<1.0 \times 10^0$	2.6×10^0		2.0×10^0	1.6×10^2	3.0×10^0	$<1.0 \times 10^0$
Well 20	$<1.0 \times 10^0$	0.3×10^0	0.6×10^0	$<1.0 \times 10^0$	8.3×10^0	$<1.0 \times 10^0$	$<1.0 \times 10^0$

[a] Time after wastewater application (days).
[b] Composite of wastewater after applied to RI cells.

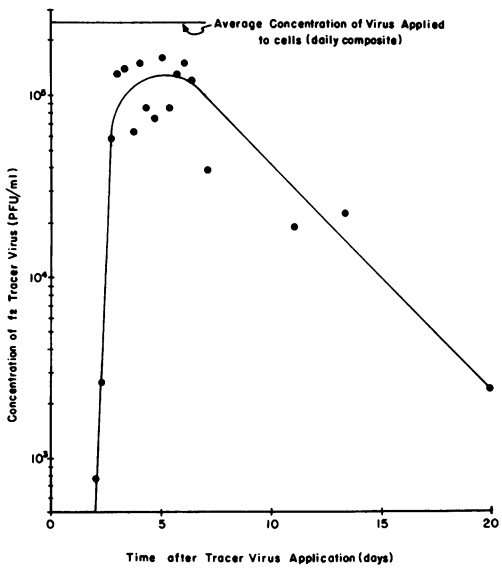

FIG. 5. *Tracer virus (f2) in observation well 15. Tracer virus f2 was sampled in observation well 15 directly beneath the wastewater application zone with a Kemmerer water sampler. Samples were taken every 8 h for the first 7 test days and once each on days 10, 13, and 21.*

samples was 276 PFU/liter. The virus isolations from well samples were sporadic, although at times concentrations were as high as 10% of the composite average. Values observed for well no. 15 were thought to be lower than anticipated because of difficulties encountered in eliminating a precipitate from the sample prior to assay.

TABLE 6. *Indigenous enteric virus concentrations*

Sampling day	PFU/liter					
	Effluent composite	Observation well				
		15	10	11	19	20
1	420	10		38	12.5	
2	60	0	5		20	1.3
3	230	0	30	0	1.3	5
4	255	25	10	0	0	7.3
5	245		0	5	7.6	
6		15	7.8	7	15	
7	445				3.8	
11					0	3.2
21		0		0		
Avg	276	8.3	10.6	8.3	7.5	4.2

Fecal *Streptococcus* movement. Composite wastewater samples and samples from observation wells no. 10, 11, 15, 19, and 20 were evaluated. Figure 6 illustrates that the fecal *Streptococcus* concentrations declined as the wastewater rested on the RI cells. This reduction could be due to die-off or to settling. It is interesting to note that *Streptococcus* reached observation well no. 15 in 48 h despite their considerably larger size. The peak concentration in well no. 15 was reached at day 5 and declined constantly

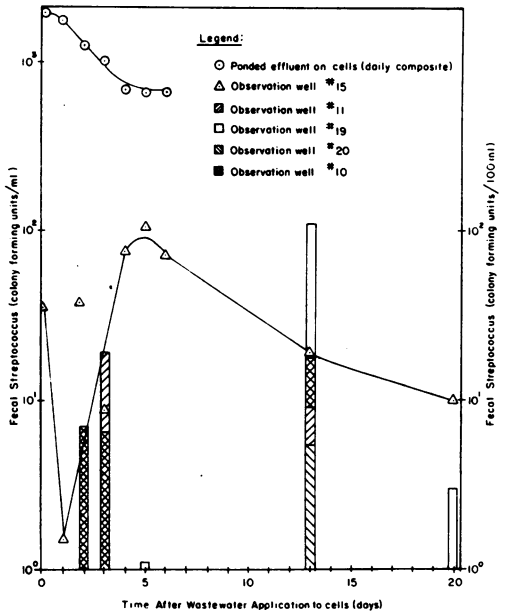

FIG. 6. *Fecal Streptococcus concentrations during tracer virus test. Fecal Streptococcus levels were determined in the ponded primary wastewater effluent and in observation wells by membrane filter techniques. The bars are read on the right margin. The symbols △ and ⊙ are read on the left margin.*

thereafter. Samples from the other observation wells in line with the groundwater movement indicated some sporatic "spikes."

DISCUSSION

Direct laboratory comparison of enterovirus and f2 bacteriophage adsorption to renovated and unrenovated RI cell soil layers indicated that viruses suspended in primary effluent were poorly held by all soil layers. Bacteriophage adsorption was minimal. When contained in deionized water with metal cation to enhance adsorption, the soils from the renovated cells adsorbed significant numbers of virus except in the high organic content surface layer. The soils from the unrenovated cell were not able to adsorb significant numbers of virus in the upper subsurface layers, indicating some depletion of adsorptive sites. Previous experimentation has indicated that at low cation and high soluble organic concentrations and moderate pH (such as would be found in wastewater), clays, soils, and other inorganic minerals do not adsorb virions extensively (2, 3, 12). Mutually exclusive electronegative surface charges compete with short-range attractive forces on the virions and solids. Additionally, small-molecular-weight organics effectively compete with virus for soil adsorption sites. It is apparent that, even in the absence of sewage, the adsorptive sites in the upper soil layers of unrenovated cells were depleted. Studies by Moore and coworkers (10) would indicate that the f2 bacteriophage could not be expected to adsorb to either soils or organic solids in the upper soil layers at the pH used in the present study. In their studies they observed that, in almost all instances, poliovirus was more readily adsorbed to clays and sewage solids than was f2 bacteriophage.

Field studies utilizing tracer f2 bacteriophage and indigenous enteroviruses demonstrated that viruses move past the upper soil layers as was predicted by the laboratory adsorption studies. The field studies demonstrated that, in addition to poor adsorption, other soil phenomena such as filtration or straining were not a factor, mainly because of the grain size of the sandy, silty, gravelly soils in relation to the extremely small virus particles. Even the finer surface mat provided by filtration and deposition of sewage-suspended solids had little capability to retain virions. Thus, virus penetration and groundwater mound formation at observation well no. 15 occurred in the same time frame (approximately 48 h after wastewater application was initiated). Importantly, the f2 bacteriophage stabilized in the groundwater beneath the cells at almost 50% of the applied virus concentration. Indigenous enteric viruses were also observed in the wastewater, but their relative recovery in observation well no. 15 was sporadic and was approximately 10% of the virus concentration in the wastewater. Part of this lower recovery may be due to the greater soil adsorption of enterovirus as observed with poliovirus in laboratory studies. If indeed this was the principal operating factor in the field, then the observed results could have been anticipated. However, the procedural difficulty in eliminating flocs which had formed in the virus concentrates after frozen storage may have resulted in a blocking of virus attachment and infection of cell monolayers during assay.

At times enteric viruses and f2 bacteriophage were observed in relatively high numbers at observation wells located in the general path of the groundwater movement. However, it would appear that at this site there is some heterogeneity in the nature of the aquifer. The irregularities may be important influences on the groundwater flow patterns resulting in differential flow conditions. Thus, viruses dispersed in the wastewater would be subjected to the conditions and would occur in sporadic high concentrations over considerable distances. The farther the water travels, the greater the ef-

fects of differential flow on virus migration and, therefore, the greater the divergence of observed virus levels. It is also possible that, over the years, channelization has occurred in the aquifer. Such conditions would sporadically short circuit the flow in the aquifer until a new equilibrium in the groundwater system is achieved. These probable phenomena need verification through more detailed aquifer characterization by the selective installation of additional groundwater monitoring points.

It is important to realize that, in the RI system studied, viruses are capable of horizontal migration in the groundwater to distances greater than 600 feet (183 m) from their source. Also, the results emphasize the importance of frequent or continuous composite sampling to ascertain the potential for virus contamination of the groundwater. The fact that peak indigenous enteric virus concentrations observed in the perimeter wells did not have the same temporal relationships as did the f2 bacteriophage may be due to the interaction of groundwater contaminated by previous wastewater applications to other RI cells.

The bacteriological indicator organisms behaved somewhat differently than did the viruses at the RI site. The soil surface retained a very large concentration of total coliform, fecal coliform, and fecal *Streptococcus* organisms after the wastewater had completely infiltrated the soil surface. Once past the surface zone, the bacterial concentration dropped significantly but tended to decline at a slower rate at successive sampling depths. Unlike the viruses, the bacteria were filtered or strained at the soil surface, most likely because of their much larger relative size. It is doubtful that adsorption played an important role in retaining the bacteria in the soil. Fecal *Streptococcus* determinations revealed that the number of viable organisms declined in the standing wastewater on the RI cells. Also, it was observed that there was a reduction of fecal *Streptococcus* on the soils with drying, although it was not determined if this was due to die-off or the continued migration of the organisms through the soil.

The fecal *Streptococcus* organisms were detected in significant numbers in the groundwater mound under observation well no. 15 in the same time frame as were the viruses. However, unlike the tracer virus, previous applications to the site could have resulted in the maintenance of residual organisms in the groundwater. Organism regrowth is a further possibility. Consequently, an accurate assessment of their movement is difficult. On occasion, fecal *Streptococcus* organisms were observed in the observation wells in line with the groundwater flow. Their occurrences did not correspond with tracer bacteriophage occurrence in the wells; however, this could be explained by different migration times and also the fact the indigenous microorganims like the enteric viruses are always in the wastewater and their presence may result from previous wastewater applications to other RI cells.

The results of this study can be compared with a tritium tracer study conducted at the Fort Devens rapid infiltration site by Stewart (14). The tritium tracer test utilized 75 Ci of tritium applied to the same RI cells as were used in the study reported herein. Tritium was applied over a 4-day period. The investigator experienced various problems which resulted in unexpectedly high tritium dilution on the beds. The tritium was detected mainly in observation wells no. 10, 11, 19, and 20 and, to a lesser extent, in wells no. 5, 6, 7, 8. The tritium showed up in the same wells as did the bacteriophage used as a tracer in this study. It was interesting to note that in the perimeter wells the tritium tracer was detected in sporadic "spikes" much like the bacteriophage, enterovirus, and fecal *Streptococcus* organisms were in this study. Stewart calculated that a higher than anticipated dilution existed in the observation wells. The greatest tritium recovery revealed a dilution of at least 75-fold which indicated that either the wells are not completely in the main stream of groundwater flow or that the main pulse had not arrived within 10 days of application. The latter possibility would seem unlikely in light of the observed infiltration rates and the relatively low affinity of these soils for tritium. The present study supports his observation that the hydraulic conductivities of the soil layers in the unsaturated zone beneath the beds were highly variable. It is possible that in some wells at the site the stratified layers within the depth of the well screens would conduct water at various rates. Also, the water table may have exceeded the elevation of the well screen and therefore stagnant water in the pipe above the well screen may have been sampled.

Wellings et al. (15) demonstrated circumstances similar to those in the present study. They investigated enterovirus movement in a cypress dome after the spray irrigation of a packaged treatment plant effluent. They observed sporadic "spikes" of natural enteric viruses in groundwater beneath the application area which may be similar in nature to the "spikes" noted in the present study. They suggested that the virus migration observed in

their study may have been the result of changes in the soil/water ratio (such as by rainfall), causing selective virus elution from the soils. Significant movement of poliovirus contained in distilled water and treated wastewater was observed in sandy soil cores by Duboise et al. (5). Simulated rainfall enhanced elution of virus from soil particles although the movement was transient with each water application and reassociation was observed at greater soil depths. High soil pH (9.0) also enhanced virus migration through the soil columns.

Presently, studies are being conducted on operational spray irrigation and overland runoff land wastewater application sites to determine the capabilities of various soil classes and wastewater applications to remove enteric and seeded tracer viruses during treatment.

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