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THE OCCURRENCE OF HUMAN ENTEROVIRUSES IN A GROUNDWATER AQUIFER RECHARGED WITH TERTIARY WASTEWATER EFFLUENTS

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ABSTRACT

The occurrence of human enteroviruses in groundwater aquifer recharged with tertiary wastewater effluents.

A two-year study of the impact of human viruses on a tertiary treatment-groundwater recharge system located on Long Island is currently nearing completion. Raw influents, chlorinated tertiary effluents, and groundwater from beneath a uniquely designed recharge basin were assayed on a weekly basis for the presence of indigenous human enteroviruses and coliform bacteria. While high concentrations of viruses were routinely isolated from sewage influents, the chlorinated tertiary effluents were positive for virus in only 3 of 20 samples. In spite of the high quality effluent being recharged, viruses were detected in the groundwater aquifer on several occasions indicating their ability to percolate through the recharge basin. This finding was confirmed by the results of two poliovirus seeding experiments carried out at the field installation. At both high (75-100 cm/hr) and low (6 cm/hr) infiltration rates seeded polioviruses were detected at all sampling levels as well as in the groundwater aquifer, 7.62 m below the recharge basin. It would appear that lower infiltration rates promote better virus removal in the specific type of soil used in this study.

1. Introduction

Increased demands for additional sources of potable water have resulted in the need to supplement groundwater reserves. Among methods proposed to augment groundwater supplies are those involving recharge with renovated domestic wastewater including: spray irrigation; overland flow; deep well injection; and basin recharge. The use of wastewater in any of the above schemes of aquifer supplementation has often met with opposition because of the potential hazard posed by the presence of the human viruses which commonly occur in sewage (Bernard, 1973; Gerba et al., 1975).

Laboratory studies have identified several factors which affect virus removal during the passage of wastewater through soil. Drewry and Eliassen (1968) indicated that adsorption rather than filtration was the probable mechanism of virus removal during sand or soil percolation. Gerba et al. (1975) reported that the adsorption process was strongly influenced by a number of factors including the pH of recharged water, the chemical composition and moisture content of the soil, and infiltration rate through the soil. The ionic strength of the adsorbing environment has also been shown to be important in the attachment of virus to soil particles (Duboise et al., 1976; Lance et al., 1976; Wellings et al., 1975). Clean, dry sand was shown to have little removal capabilities (Berg, 1973), while moistened sand demonstrated an improved removal efficiency (Nestor and Costin, 1971). Drewry and Eliassen (1968) reported that soils containing high concentrations of clay and silt were extremely effective virus adsorbants. Bitton

(1975) indicated that this efficiency was due primarily to their large surface area.

While adsorption mechanisms have been the subject of a number of laboratory studies, comparatively few have addressed the presence of naturally occurring viruses at operational recharge installations. Wellings et al., (1975) reported the isolation of poliovirus and coxsackievirus from groundwater beneath a cypress dome used for the recharge of secondarily-treated effluent. Schaub and Sorber (1977) also demonstrated the sporadic occurrence of enteroviruses in recharged groundwater. Gilbert et al., (1976) were unable to detect viruses in groundwater samples taken at the Flushing Meadows recharge project. Recently, Vaughn et al., (in press) demonstrated the presence of a variety of enteroviruses in groundwater aquifers adjacent to wastewater recharge basins at three separate recharge sites located on Long Island.

A major portion of the above studies were conducted with sewage effluents which received no more than secondary treatment. The present report describes the results of routine viral monitoring and field experimentation at a uniquely designed recharge installation which uses tertiary treated effluents.

2. Methods and Materials

A. Test Site

The site selected was located at the 12-Pines treatment facility in Medford, New York. The plant combines conventional primary and secondary treatment processes with tertiary treatment (denitrification-filtration) and chlorination. Treated sewage is then discharged into nearby recharge

basins, or a portion diverted to the test recharge basin. The physical and chemical characteristics of the tertiary effluent and the renovated wastewater may be seen in Table 1. Thirty yards northeast of the recharge basins is the study facility built and operated by the U.S. Geological Survey (Figure 1). The structure is a miniaturized version of the adjacent recharge basins, consisting of a circular test basin 6.09 m in diameter (25 m^2) whose surface is approximately 7.62 m above the static water level. A manhole has been carefully constructed through the center of the basin to a depth of 6.4 m. Within the manhole, gravity samplers (38.7 cm^2 capture area) have been constructed at depths of 0.75, 2.25 and 5.34 m which extend 0.9 m into the surrounding recharge basin. At the bottom of the manhole, a well has been sunk into the water table to allow testing of waters which have percolated through the system. The test basin has been equipped with instrumentation for measuring water level, infiltration rate, temperature and conductivity. The soil in the basin consists primarily of coarse sand and fine gravel and contains an average of 1.12% silt and clay (Table 2). The study facility operates at a normal loading rate of 40,000 l per day and combines the advantage of a sufficiently large operating surface with the ability to control variations which might be experimentally applied to the system.

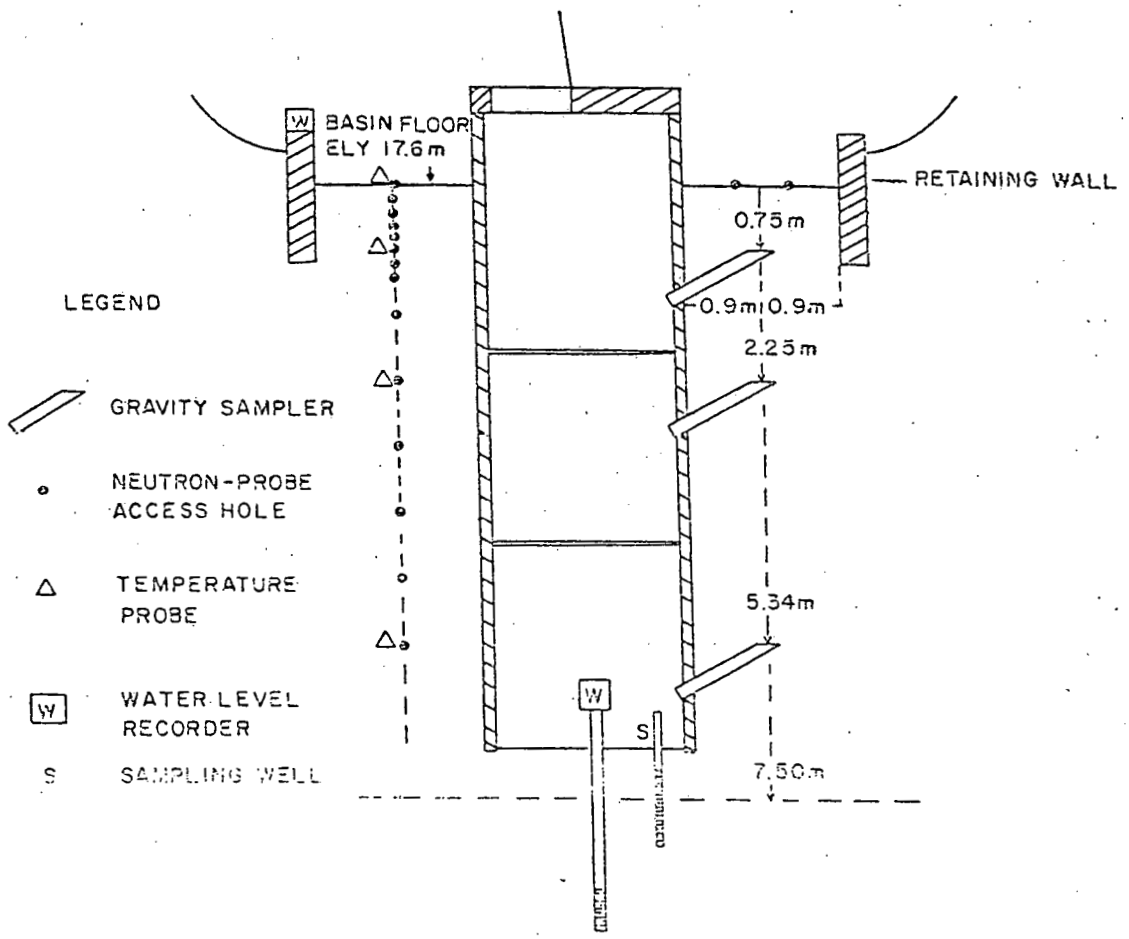
B. Sample Collection

Samples of raw sewage (influent), tertiary effluent, and groundwater from beneath the test basin were collected at weekly intervals. Raw sewage grab samples were collected in sterile 4 l containers. Tertiary effluent (100 l) and

Table 1. Chemical and Physical Characteristics of 12 Pines Tertiary-Treated Sewage Effluent and Renovated Well Water.

	Mean Values	
	Effluent	Well Water
Turbidity (NTU)	4.58 (1.2-9.0)[a/]	7.2 (2.5-15)
Conductivity (μmho)	439.50 (393-500)	459 (389-500)
pH	6.61 (6.10-7.20)	6.11 (5.2-7.1)
Total alkalinity (mg/l)	95.38 (48-115)	67.12 (31-98)
Chloride (mg/l)	54.13 (47-64)	53.5 (50-56)
Sulfate (mg/l)	35.63 (28-54)	36 (29-56)
Nitrate-nitrogen (mg/l)	3.38 (0.07-16.0)	7.54 (.09-17)
Nitrite-nitrogen (mg/l)	0.11 (0.006-0.49)	.026 (.003-.083)
Ammonia-nitrogen (mg/l)	5.34 (0.06-15)	.38 (.16-1.1)
Total Kjeldahl nitrogen (mg/l)	6.63 (0.9-17)	2.27 (.6-10)
Ortho phosphate (mg/l)	6.13 (4.8-6.8)	6.07 (4.8-7.0)
Fe^{+++} (mg/l)	<0.05 (<0.05)	0.05 (<0.05-0.1)
Mn^{++} (mg/l)	0.02 (0.01-0.03)	.078 (.02-.15)
Mg^{++} (mg/l)	4.53 (2.7-7.8)	4.26 (2.6-7.0)
Ca^{++} (mg/l)	19.0 (15-24)	21.37 (17-33)
Na^{+} (mg/l)	58.25 (54-68)	59.12 (54-68)
K^{+} (mg/l)	12.25 (11-13)	12.87 (12-14)
Total suspended solids (mg/l)	5.00 (1-14)	----
Total organic carbon (mg/l)	15.13 (10-29)	----

[a/] Number in parentheses represent the range of values.



Schematic of the test basin facility.

Figure 1

Table 2. Percentage of particles in each size range in soil samples from unsaturated zone, below test basin.

(all values in percent)

Particle diameter, in millimeters	Depth below basin surface (m) 0.75	2.25	5.34
Silt and clay <0.0625	0.30	0.75	1.2
Sand			
0.0625-0.125	0.4	1.8	1.6
0.125-0.250	3.3	23.5	10.9
0.250-0.5	14.1	27.5	25.2
0.5-1	36.4	25.8	36.3
1-2	13.4	4.1	6.2
Gravel			
2-4	6.9	4.2	4.3
4-8	6.4	4.1	4.3
8-16	9.6	8.0	6.2
16-32	9.3	0.0	3.5
32-64	0.0	0.0	0.0

groundwater (400 l) samples were collected in sterile 220 l tanks (Plast-i-cube, Greif Brothers Corp.). Between collections, tanks were thoroughly rinsed with tap water, sanitized with 0.12 N hydrochloric acid (thirty minutes), and rinsed once again with tap water. Immediately before collection at each site, tanks and pumping equipment were rinsed with 40 to 80 l of water to be sampled. These precautions were taken in order to obviate the chance of cross-contamination between samples.

C. Virus Concentration Procedures

Viruses in raw sewage samples were concentrated by an inorganic flocculation procedure (Farrah et al., 1976). The resulting concentrates (20-50 ml) were supplemented with 10% fetal calf serum and stored at -72 C.

Viruses in large volume water samples were initially concentrated by means of an Aquella Virus Concentrator (Carborundum Corporation) using a series of prefilters to remove debris. Viruses were adsorbed to fiberglass depth cartridge filters (K27), and an epoxy-fiber glass-asbestos filters (1.0 and 0.45 um Cox) at a pH of 3.5 and 0.5 mM $AlCl_3$ (Farrah et al., 1976). Elution of adsorbed virus was carried out with 2 l volumes 0.1 M glycine at pH 11.5. Eluates were then neutralized to pH 7.5 in an equal volume of pH 2.0 glycine. The concentration procedures routinely yielded a 4 l volume which was reconcentrated in the laboratory by means of an inorganic flocculation procedure (Farrah et al., 1976) to a final volume of 50-100 ml. After the addition of 10% fetal calf serum, samples were stored at -72 C to await assay.

D. Isolation and Identification

Viral enumerations from field samples were carried out on monolayers of low-passaged Buffalo Green Monkey Kidney Cells (BGM - Microbiological Associates), which were grown on minimum essential medium with Hanks balanced salt solution supplemented with 10% fetal calf serum. Five-tenths ml sample volumes were placed on cell monolayers in 25 cm^2 flasks, and incubated for 1 hr to facilitate virus attachment. After decanting excess sample material, cells were overlain with 4 ml neutral red agar medium (Melnick and Wenner, 1964), and incubated at 36 C under 5% CO_2 for a period of 10 days. Daily readings were taken to determine the presence of viruses which appeared as plaques. Following the incubation period, plaques were picked and enriched on monolayers of BGM cells propagated in twenty-four well cluster dishes (Costar). Isolates were identified in microtiter plates by serum neutralization techniques (Melnick et al., 1973) using enterovirus typing pools.

E. Coliform Studies

In order to correlate virus data with a recognizable biological pollution indicator, total and fecal coliform numbers were determined for all samples collected. Coliform enumerations were carried out using standard "most probable number" methods (Standard Methods, 1976).

F. Basin Seeding Experiments

On two occasions, effluents entering the test basin were seeded with poliovirus type 1 (LSc) in order to assess the virus-removing capacity of the basin under the stress of high virus concentrations. The procedures for each experiment

differed and will be described separately.

a) Experiment #1

Monodispersed poliovirus (LsC) stocks were propagated on monolayers of Buffalo Green Monkey Kidney cells (BGM) in Blake bottles according to the procedures described by Jakubowski et al., (1975). Poliovirus was added to 8500 l of unchlorinated tertiary-treated wastewater entering the previously drained basin. Viruses were inoculated through an injection pipe which assured uniform distribution of virus particles. The final concentration of poliovirus in the seeded effluent was 7.0×10^4 PFU/l.

The seeded effluent was allowed to drain (infiltration rate = 6 cm/hr) to a depth of 4-6 cm above the basin floor before resumption of normal recharge procedures with unseeded tertiary effluent. The intention of this manipulation was the production of a "band" of water containing high numbers of virus whose progress through the basin could be traced.

Samples for virus assay taken during the experiment included: tertiary sewage (40 l) and observation well water (400 l) samples taken prior to virus seeding in order to supply background information; 2 l composite samples collected at intervals from the gravity samplers located at 0.75, 2.25 and 5.34 m depths in the basin; observation well samples (400 l each) taken at intervals for a period of several days. Large volume samples (40-400 l) were processed using previously described virus concentrator methods. Virus particles in 2 l gravity samples were concentrated via an inorganic flocculation method (Farrah et al. 1976).

All samples were assayed on monolayers of BGM cells using a plaque overlay technique. Because of the low numbers expected, 9-10 ml volumes of each sample reconcentrate were analyzed for virus content.

b) Experiment #2

The initial seeding experiment was carried out at an infiltration rate of 6 cm/hr. The second experiment was designed to assess the effects an increased infiltration rate would have on virus removal in the basin. For several weeks preceding the experiment, the basin received no effluent which promoted moderate drying. One week before the experiment, the previously clogged top 2.5 cm of basin bottom were removed and replaced with clean sand. As a result of these measures, an infiltration rate of 75-100 cm/hr was realized. A 4000 l unchlorinated effluent sample was seeded with poliovirus (LSc) and allowed to drain through the basin as before. Normal recharge operations were then resumed. Because of the rapid infiltration rate, 1 l gravity samples were taken at intervals which were determined according to the movement of the seeded "front" through the basin. Within an hour of the passage of the "front" through each of the sampling levels, interval sampling was curtailed in lieu of large volume composite samples, which were collected at each level over a period of 2-3 hours. Viruses contained within these samples were concentrated by an organic flocculation method (Katzenelson et al., 1976). Groundwater samples (380 l) were also collected during the experiment, and processed through an Aquella Virus Concentrator using a modified 3% beef extract elution method (Landry et al., in press).

Table 3. Enterovirus and Coliform Isolations from 12-Pines Raw Sewage Influent.

Sample No.	Date Collected	Virus PFU[a]/gal	Coliforms/100 ml Total	Coliforms/100 ml Fecal
A1	11/10/76	1,800	4.6×10^6	--
A8	12/2/76	1,720	1.1×10^7	4.6×10^6
A11	12/6/76	9,000	1.1×10^7	4.6×10^6
A14	1/21/77	20,400	1.2×10^7	5.0×10^6
A16	5/6/77	180	---	---
A20	5/11/77	1,920	4.3×10^7	1.5×10^7
A23	5/18/77	590	---	---
A24	5/24/77	432	9.3×10^7	9.3×10^7
A29	5/26/77	450	4.3×10^8	4.3×10^8
A32	6/2/77	880	---	---
A35	6/7/77	2,688	---	---
A36	6/20/77	76	1.1×10^8	4.3×10^5
A41	6/27/77	10,192	2.4×10^8	2.3×10^7
A42	7/13/77	522	2.3×10^7	2.3×10^7
A45	7/18/77	1,500	4.3×10^7	2.3×10^7
A48	7/27/77	1,863	7.5×10^7	4.0×10^5
A53	8/2/77	1,728	1.1×10^9	1.5×10^7
A56	8/8/77	40.5	1.5×10^8	9.3×10^7
A59	10/11/77	682	1.1×10^9	1.1×10^9

[a]PFU-plaque forming units.

Table 4. Enterovirus and Coliform Isolations from 12 Pines Tertiary Sewage Effluent.

Sample No.	Date Collected	Virus PFU[a]/gal	Coliforms/100 ml Total	Coliforms/100 ml Fecal
A2	11/10/76	308	3.8×10^6	---
A9	12/2/76	59	2.3×10^1	<3
A3	11/10/76	0	2.3×10^5	---
A7	11/29/76	0	<3	<3
A12	12/6/76	0	1.5×10^1	1.5×10^1
A17	5/6/77	4	---	---
A19	5/11/77	0	1.7×10^1	8
A22	5/18/77	0	---	---
A25	5/24/77	0	4.6×10^2	2.3×10^1
A28	5/26/77	0	2.4×10^3	3
A31	6/2/77	0	---	---
A34	6/7/77	0	---	---
A38	6/20/77	0	4.6×10^4	4.6×10^4
A40	6/27/77	0	2.4×10^4	4.3×10^3
A44	7/13/77	0	<3	<3
A46	7/18/77	0	<3	<3
A49	7/27/77	0	4	<3
A52	8/2/77	0	<3	<3
A55	8/8/77	0	4	<3
A58	10/11/77	0	<3	<3

[a]PFU-plaque forming units.

Table 5. Enterovirus and Coliform Isolations for 12 Pines Renovated Wastewater (Groundwater Observation Well).

Sample No.	Date Collected	Virus PFU[a]/gal	Coliforms/100 ml Total	Coliforms/100 ml Fecal
A4	11/10/76	2.5	9.3×10^2	---
A5	11/18/76	0	4.3×10^2	9.6×10^1
A6	11/29/76	0	9.3×10^3	4.3×10^3
A10	12/2/76	0	4.3×10^2	4.3×10^2
A13	12/6/76	0	1.2×10^2	4
A15	5/6/77	2	---	---
A18	5/11/77	0	4.9×10^1	2
A21	5/18/77	0	---	---
A26	5/24/77	0	7.5×10^1	9
A27	5/26/77	0	4.6×10^2	2.3×10^1
A30	6/2/77	0.8	---	---
A33	6/7/77	0	---	---
A37	6/20/77	0	7.5×10^2	3.9×10^2
A39	6/27/77	0	2.4×10^3	4.3×10^2
A43	7/13/77	0	4.3×10^1	4
A47	7/18/77	0	1.1×10^4	4.3×10^2
A50	7/27/77	0	2.4×10^3	2.3×10^1
A51	8/2/77	2.3	2.5×10^1	<3
A54	8/8/77	2.5	2.1×10^3	9
A57	10/11/77	0	2.8×10^3	3×10^2
A62	10/18/77	0.52	4.3×10^2	7.0×10^1
A64	10/26/77	0	<3	<3
A67	10/31/77	0	<3	<3

[a]PFU-plaque forming units.

Tertiary effluent was applied to the basin for a period of 5 hr. All samples were analyzed for virus content as previously described.

3. Results

A. Enumerations from field samples

The treatment system under study was operating at one-quarter to one-half capacity during the sampling period described below. It was therefore often necessary to supplement the raw sewage entering the treatment plant with fresh water. This process introduced a dilution which was reflected in virus and coliform counts from influent and effluent samples.

As would be expected, raw influent samples routinely yielded large numbers of viruses and coliform bacteria (Table 3). A significant reduction in isolation frequency was noted in the treated effluent (Table 4), where viruses were detected on only three occasions. In spite of the infrequency of isolations from effluents, viruses were detected in the groundwater beneath the recharge basin on six occasions (Table 5) indicating the ability of viruses to penetrate the basin. Little correlation was observed between virus and coliform occurrences in most of the samples analyzed.

Viruses identified from raw influent and tertiary effluent included: Poliovirus types 1 and 2; Coxsackievirus types A16, B2, B3, B5, and B6; and ECHO virus types 18, 21, 25, and 27. To date, only one of the groundwater isolates has been positively identified as being Poliovirus type 2.

B. Basin Seeding Experiments

Experiment #1

Table 6 summarizes the data resulting from the first seeding experiment which was carried out at a low infiltration rate (6 cm/hr). Significant reduction in viral numbers resulted from passage through the recharge basin, the greatest removals occurring between the 2.25 and 5.34 m levels. In spite of these encouraging removal rates, some virus particles were apparently able to penetrate the entire length of the basin. Peaks in viral numbers were noted between 6 and 10 hours after seeding in the 0.75 m level (level 1); between 11 and 24 hours in the 2.25 m level (level 2); between 23 and 25 hours in the 5.34 m level (level 3); and at 24 hours in the observation well (level 4). The almost simultaneous appearance of virus in the first two levels within three hours of seeding suggested that the virus-laden band of wastewater did not move uniformly between these levels. There was evidence of consistent virus movement from level 1 to level 2, but only a brief period of movement from level 2 to level 3 (occurring between hour 23 and 25). Few viruses were shown to have successfully moved from level 3 to the groundwater aquifer (level 4).

Experiment #2

The second experiment indicated that decreased viral retention occurred as a result of high infiltration rate (Table 7). Large numbers of virus were detected at all sampling levels. The seeded effluent apparently moved through the first sampling level in a sharply defined band. By the time the band reached levels 2 and 3, it had been rendered less compact by diffusion within the soil column.

Table 6. The recovery of poliovirus at various depths during sewage recharge at a low infiltration rate (6 cm/hr). Initial Seeded Virus Concentration = 7×10^4.

Depth Below Surface of Basin Floor (m)	Sample #	Time of Collection after Seeding (hr)	Virus PFU/ℓ
0.75 (Level 1)	1	1.45-2.25	0
"	2	2.25-3.00	0
"	3	3.00-4.63	7.90
"	4	4.63-6.66	16.90
"	5	6.66-8.53	20.00
"	6	8.53-9.25	17.70
"	7	9.25-10.08	25.25
"	8	23.63-24.25	1.90
"	9	28.81-29.33	3.70
2.25 (Level 2)	1	2.26-2.91	5.45
"	2	2.91-3.61	0
"	3	3.61-5.26	3.18
"	4	5.26-7.66	3.60
"	5	7.66-10.08	11.60
"	6	10.08-11.16	2.20
"	7	11.16-11.91	23.30
"	8	23.68-24.33	1.59
"	9	28.78-29.41	19.50
5.34 (Level 3)	1	6.83-9.41	0
"	2	9.41-11.91	0
"	3	23.83-25.08	38.20
"	4	28.75-29.83	0
7.62 (Level 4)	1	0	0
"	2	12	0
"	3	24	0.35
"	4	30	0
"	5	48	0
"	6	54	0.08
"	7	72	0.07

Table 7. The recovery of poliovirus at various depths during sewage recharge at a high infiltration rate (75-100 cm/hr). Initial Seeded Virus Concentrations = $1.84 \times 10^5/\ell$.

Depth Below Surface of Basin Floor (m)	Sample #	Time of Collection after Seeding (hr)	Virus PFU/ℓ ($\times 10^4$)
0.75 (Level 1)	1	0.60-0.81	78.00
"	2	0.86-0.91	97.50
"	3	1.03-1.06	26.50
"	4	1.20-1.23	1.46
"	5	1.41-1.45	1.94
"	6	1.58-1.61	1.22
"	7	1.75-1.78	0.79
"	8	2.00-2.03	0.90
"	9	1.41-2.45	0.03
2.25 (Level 2)	1	1.20-1.30	2.44
"	2	1.41-1.50	8.58
"	3	1.58-1.63	6.70
"	4	1.75-1.81	5.40
"	5	2.00-2.08	1.81
"	6	2.25-2.31	1.38
"	7	2.50-2.56	0.31
"	8	2.58-3.86	0.05
5.34 (Level 3)	1	1.78-2.15	8.82
"	2	2.16-2.28	0.54
"	3	2.33-2.41	0.27
"	4	2.50-2.55	1.81
"	5	2.81-2.85	10.10
"	6	3.00-3.03	9.80
"	7	3.28-3.31	3.32
"	8	3.50-3.53	1.96
"	9	3.53-4.03	0.12
7.62 (Level 4)	1	2.50-2.66	0.11
"	2	4.66-4.83	0.001

from level 3 may have resulted from channeling through portions of the basin. Reduced numbers of virus were recovered from the aquifer (level 4). The reduction was likely caused by virus dilution following entrance into the groundwater table.

4. Discussion

The recent development of improved virus-concentrating methods has greatly facilitated the routine isolation of human viruses from large volumes of water. Such methods, however, cannot guarantee a 100% efficiency of virus concentration. The field data presented in the preceding section must therefore be considered to be representative of the minimum numbers of virus in each sample. The inability to detect viruses within the constraints of our testing system cannot preclude the possiblity of virus occurrence in very low concentrations.

Currently practiced sewage treatment methods cannot insure the removal of all human viruses, and their isolation from treated wastewater effluents has been the subject of numerous report (Buras, 1976; Clarke et al., 1951; Metcalf et al., 1972). The presence of these organisms has been viewed as a potential health hazard to wastewater reuse operations, especially those involving groundwater recharge (Bernard, 1973; Berg, 1973; Hori et al., 1970). To date, relatively few field studies have been carried out which addressed the question of naturally occurring viruses in wastewater recharge systems (Gilbert et al., 1976; Schaub and Sorber, 1977; Wellings et al., 1975; Vaughn et al., in press).

The present study provides information concerning virus removal in a basin recharged with tertiary effluent. Viruses were detected in recharged groundwater on several occasions during field sampling in spite of the fact that tertiary effluents were shown to contain few virus particles. The progress of aquifer-entrained viruses in the study area cannot be commented upon, but two previous reports have indicated the possible horizontal movement of viruses through groundwater aquifers (Wellings et al., 1975; Vaughn et al., in press).

Data from field studies were verified by the results of the basin seeding experiments. In both instances, poliovirus type 1 was shown to be capable of penetrating the groundwater aquifer (Note: Preliminary field data from this laboratory indicates that poliovirus type 1 may adsorb more readily to soil surfaces than other members of the enterovirus group [Vaughn et al., in press]. Basin seeding data may therefore represent a conservative model.) A comparison of the two experiments indicated that lower infiltration rates resulted in a greater efficiency of virus removal during basin percolation of tertiary effluent. The result is in accord with those of Lance et al. (1976) who reported efficient virus removal during the low rate (15-55 cm/day) passage of sewage through soils which contained slightly higher levels of clay and silt than those described in this report (3% clay, 8% silt).

The results of the present study indicate that infiltration rate should be an important consideration for basin recharge operation.

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LITERATURE CITED

Bernard, M.A. 1973. Land disposal and sewage effluent: Appraisal of health effects of pathogenic organisms. JAWWA. 65:432-440.

Berg, G. 1973. Reassessment of the virus problem in sewage and in surface and renovated waters. S.H. Jenkins, ed. In Progress in Water Technology, Vol. 3. Pergamon Press, New York.

Bitton, G. 1975. Adsorption of viruses onto surfaces in soil and water. Water Res. 9:473-484.

Buras, N. 1976. Concentration of enteric viruses in wastewater and effluent: a two-year survey. Water Res. 10:295-298.

Clarke, E.M., O.S. Knowles, F.T. Shimada, A.J. Rhodes, R.C. Ritchie and W.L. Donahue. 1951. Coxsackie virus in urban sewage. Can. Jour. Pub. Hlth. 42:103.

Drewry, W.A. and R. Eliassen. 1968. Virus movement in groundwater. Jour. Water Poll. Control Fed. 40:R257-R271.

Duboise, S.M., B.E. Moore and B.P. Saqik. 1976. Poliovirus survival and movement in a sandy forest soil. Appl. Environ. Microbiol. 31:536-543.

Farrah, S.R., C.P. Gerba, C. Wallis and J.L. Melnick. 1976. Concentration of viruses from large volumes of tap water using pleated membrane filters. Appl. Microbiol. 31: 221-226.

Gerba, C.P., C. Wallis and J.L. Melnick. 1975. Fate of wastewater bacteria and viruses in soil. Jour. Irrig. Drain Div. of Amer. Soc. Civ. Eng. 101:157-174.

Gilbert, R.S., C.P. Gerba, R.C. Rice, H. Bouwer, C. Wallis and J.L. Melnick. 1976. Virus and bacteria removal from wastewater by land treatment. Appl. Environ. Microbiol. 32:333-338.

Hori, D.H., N.C. Burbank, R.H. Young, L.S. Law and H.W. Klemmer. 1970. Migration of Poliovirus type 2 in percolating water through selected Oahu soils. Tech. Rep. No. 36, Water Resources Center, University of Hawaii, Honolulu.

Jakubowski, W., W.F. Hill, Jr., and N.A. Clarke. 1975. Comparative study of four microporous filters for concentrating viruses from drinking water. Appl. Microbiol. 30:58-65.

Katzenelson, E.B., B. Fattal, and T. Hostovesky. 1976. Organic flocculation: an efficient second-step concentration method for the detection of viruses in tap water. Appl. Environ. Microbiol. 32:638-639.

Lance, J.C., C.P. Gerba and J.L. Melnick. 1976. Virus movement in soil columns flooded with secondary sewage effluent. Appl. Environ. Microbiol. 32:520-526.

Landry, E.F., J.M. Vaughn, M.Z. Thomas, and T.J. Vicale. Elution efficiency of beef extract for the recovery of poliovirus from wastewater effluents. In Press. Appl. Environ. Microbiol.

Metcalf, T.G., J.M. Vaughn and W.C. Stiles. 1972. The occurrence of human viruses and coliphage in marine waters and shellfish. M. Ruivo, ed. In Marine Pollution and Sea Life. Fishing News, London.

Melnick, J.L., V. Rennick, B. Humpil, N.J. Schmidt and H.H. Ho. 1973. Lypholized combination pools of enterovirus equine antisera: preparation and test procedures for the identification of field strains of 42 enteroviruses. Bull. W.H.O. 48:263-268.

Melnick, J.L., and H.A. Wenner. 1964. Enteroviruses, p. 529-602. In E.H. Lennette and N.J. Schmidt (ed.), Diagnostic procedures for viral and rickettsial infections, 4th ed. American Public Health Association, New York.

Nestor, I. and L. Costin. 1971. The removal of Coxsackie virus from water by sand obtained from the rapid sand filters of water plants. Jour. Hyg. Epidem. Microbiol. Immuno. 15:129-136.

Schaub, S.A. and C.A. Sorber. 1977. Virus and bacteria: removal from wastewater by rapid infiltration through soil. Appl. Environ. Microbiol. 33:609-619.

Standard Methods for Examination of Water and Wastewater 14th ed. 1976. American Public Health Association, New York.

Vaughn, J.M., E.F. Landry, L.J. Baranosky, C.A. Beckwith, M.C. Dahl, and N.C. Delihas. A survey of human virus occurrence in wastewater recharged groundwater on Long Island. In Press. Appl. and Environ. Microbiol.

Wellings, F.M., A.L. Lewis, C.W. Mountain and L.V. Pierce. 1975. Demonstration of virus in groundwater after effluent discharge onto soil. Appl. Microbiol. 29:751-757.