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ABSTRACT

VIRUS REDUCTION BY THE STANFORD ONSITE

WASTEWATER TREATMENT SYSTEM

A field study to examine the Stanford Onsite Wastewater Treatment System's ability to remove bacteriophage from wastewater was conducted. MS2 Coliphage was injected into the low pressure pipe (LPP) distribution system to achieve an influent concentration of 1.6×10^6 plague forming units per milliliter (PFU/ml). The bacteriophage was injected into the system three times during the day, and samples were taken from drainage tiles of the treatment system. Tile drainage was assayed on coliform bacteria host cultures for MS2 phage. The treatment system removed two to three logs (99% to 99.9%) of the phage. During the past two years, the treatment system has also reduced total organic carbon from 55 mg/l to 5 mg/l. The system also reduced the ammonium-nitrogen concentration from 41 mg/l to 1 mg/l. The nitrate-nitrogen concentration rose from less than 1 mg/l in the influent to 4 mg/l in the effluent. Over the past two years, the geometric mean fecal coliform concentration was 18 colony-forming units per ml (CFU/ml). The effluent water quality meets the Arkansas Department of Health, Standards for Outdoor Bathing Places.

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INTRODUCTION

As part of the Arkansas Individual Onsite Domestic Wastewater Renovation Project, a system has been developed to successfully treat septic tank effluent in east Arkansas soils having high seasonal water tables. In operation since August, 1987, the system uses the native silty soil (underlain by an impermeable horizon) as a filter to treat the septic tank effluent to high enough quality to meet Arkansas Department of Health standards for outdoor bathing places. Until now, no analysis had been made of the ability of the system to remove viruses from the septic tank effluent.

This study has addressed the existing wastewater treatment system's ability to remove or inactivate viruses in septic tank effluent. The application of reusable water may depend upon the water quality in terms of viruses - for example, overhead irrigation may not be acceptable if viruses could be aerosolized by the irrigation process. Subsurface or drip-trickle irrigation may be a proper application. The EPA regards a 99.9 percent (3-log) reduction in virus titer as acceptable treatment for potable water treatment systems. This study will determine the virus reduction capability of the existing onsite wastewater treatment system.

The principle objective of this project was to determine the ability or inability of an experimental individual wastewater treatment system to remove or inactivate viruses.

East Arkansas, as well as other similar regions of the United States, generally has extremely poor soils for onsite wastewater treatment and disposal. Soils vary from expansive, non-permeable clays to fine-grained silty soils. The topography is level (except for the loess ridges) and presents extremely poor drainage. Seasonal water tables rise to the surface or above during the rainy season of the year. Since communities expand by utilizing onsite wastewater treatment around the periphery until public sewer service is available, the ability of east Arkansas communities to expand is hampered by the lack of functioning individual wastewater treatment technology.

An innovative individual wastewater treatment system is in operation in east Arkansas as part of the Arkansas Onsite Domestic Wastewater Renovation Project. The system has been in operation since August, 1987, and the past 2 years' data show that the system not only disposes of wastewater efficiently, but also renovates the wastewater to a quality meeting Arkansas Department of Health criteria for public bathing places. Analyses currently performed include Total Organic Carbon, Ammonia Nitrogen, Nitrate Nitrogen, Specific Conductivity, Chloride, and Fecal Coliform. This high quality water should be

considered for reuse, and the virus study of the treatment system is instrumental in evaluating water reuse potential.

The system is in a silty soil on level terrain and was installed at a home where traditional onsite wastewater treatment technology had failed consistently. Although the new system is not viewed as a panacea for east Arkansas, it does have potential for silty soils, and data so far warrants further investigation of its potential for water reuse.

This project is related to ongoing research of the Arkansas Onsite Domestic Wastewater Renovation Project in that a virus study of the design used at the Stanford Research Site will provide information required to assess the suitability of this design as a water reuse system.

The University of Arkansas, Fayetteville, the University of Arkansas at Little Rock, the Arkansas Department of Health, the Jefferson County, Arkansas Health Unit, and the Lincoln County, Arkansas Health Unit have recently completed a three-year cooperative effort to provide data collection, analyses, engineering, and maintenance for the Stanford Research Site. Sanitarians from the Lincoln and Jefferson County Health Units collected samples and monitored water depth in the wells. The laboratory at the Arkansas Department of Health, Little Rock, provided fecal coliform analyses. The University of Arkansas, Fayetteville, Department of Agronomy, coordinated the project

and provided other chemical analyses. The University of Arkansas at Little Rock, Department of Electronics and Instrumentation, provided engineering and coordinated and performed routine maintenance for the system.

The project was performed by faculty and students at the University of Arkansas at Little Rock, but data routinely collected as part of the Stanford System Research was integrated into the project.

Virus Removal by Soil

Research has been carried out to determine the capability of various soils and of soil in general to remove viruses from a liquid suspension. These studies include batch experiments, soil column experiments, and experiments in the field. The batch experiments usually consist of stirring a virus suspension in the presence of soil, allowing the soil to settle, and measuring the amounts of virus in the settled soil and in the supernatant. The column experiments have basically been a procedure of dosing a virus suspension of known virus concentration through a soil column and measuring the virus concentration in the column effluent, and sometimes in the column itself. In the field studies, sites of sewage application are monitored for the movement of viruses through the soil.

Factors Affecting Virus Removal in Soil and Sand

Research with soil and sand has shown that the removal of viruses from water and wastewater is influenced by several measurable parameters. Since the mechanism of virus removal is adsorption, some of these influencing factors such as the ionic strength of the solution and pH would be expected since they are associated with all adsorption phenomena. Other parameters, such as temperature and the amount of organics in the soil and in the water, affect the adsorption of viruses and also affect the biochemical activity associated with destruction of the viruses. Other elements that contribute to the removal of viruses from water or wastewater are the flow conditions-saturated or unsaturated, intermittent or continuous-and flow rate.

Ionic Strength and pH

Ottawa sand used in a batch study did not retain viruses well at a pH above 9, but below pH 7 most of the viruses were bound to the sand. The high negative charge on poliovirus particles at high pH causes the virus to not be adsorbed by the similarly charged soil particles because of the repulsion of the double layers. Since Van der Waals' forces are the attractive forces, and the repulsion is due to overlap of the double layers, changing ionic strength by addition of electrolytes

alters the double layer thickness and enhances adsorption. This study showed that low pH and addition of electrolytes increased adsorption of poliovirus by Ottawa sand. Also, divalent cation addition was more effective than addition of a monovalent cation.[8] This is expected, since the Schulz-Hardy rule supports such a finding, and other studies have shown that the use of polyelectrolytes are effective in enhancing virus adsorption.[9] This effect of pH and ionic strength has been noted by several researchers,[10-14] and will not be discussed in any greater detail here.

Organics

The organic concentration of the soil, the amount of organics in the wastewater, and the amount of microbial growth on the surface of the adsorbent all affect the degree of virus adsorption from the water. As noted earlier,[15] soils with high organic content are not as effective as those with lower organic matter content in virus adsorption. Also, organics in the water compete with viruses for adsorption sites in the soil material.[16] Green and Cliver have noted that the retentiveness of sand decreases after a few weeks of operation due to the microbial growth on the sand surface, and this effect should be considered in using sand filtration for virus removal.[17]

Temperature

One effect of temperature on virus removal is the increased inactivation of viruses at higher temperatures. A batch study indicated a directly proportional relationship of inactivation of poliovirus type 1 and coxsackievirus type B1 with temperature. Temperature has been considered to be one of the most important factors affecting virus removal by soil.[11,14]

Flow Conditions and Flow Rate

Low flow rates enhance the reduction of viruses by soil,[10,12-14] and flow rates in excess of 1.6 feet per day gave erratic results in the removal of viruses.[13] However, a rate of 1 cm/hr caused most viruses to be retained in soil.[18] Unsaturated flow has been more effective for virus removal than saturated flow[13,19,20] and intermittent flow has been more effective than continuous flow.

METHODS AND PROCEDURES

The wastewater is pumped from the dose tank into the soil absorption beds. The beds are 61 cm (2 ft.) wide and 37 cm (14.5 inches) deep and receive the septic tank effluent through 0.48 cm (3/16-inch) orifi in 3.8 cm (1 1/2-inch) nominal diameter schedule 40 pvc pipe. The effluent is distributed evenly over the beds by maintaining approximately 60 cm (2 ft.)

of head. The effluent delivery is by a typical low-pressure distribution system.[3,4,5] Figure 1 is plan view of the treatment system. Beside and between the absorption beds are tile drain trenches. The drain trenches and the absorption beds are separated by 102 cm (40 inches) of undisturbed soil. The tile trenches are approximately 12.7 cm (5 inches) wide and 115 cm (45.5 inches) deep, filled with sand, and having a nominal 5 cm (2-inch) diameter Hancor "Turflow" slotted drain pipe located 10 cm (4 inches) from the trench bottom. The bottom of the drain trench corresponds to the top of a fragipan in the soil horizon. Figure 2 illustrates the relative positions of the absorption beds and drainage tiles. The tile drains presently discharge into a sump where each tile is sampled for physical, chemical, and bacteriological analyses.

Wells are located in the absorption beds, below the absorption beds (a concrete barrier exists to block cross-connection), and in the tiles. These wells are currently used for seasonal water table measurement.

A. Work Plan

For this study, MS2 bacteriophage was introduced into the distribution system to the soil absorption beds and the tile drainage was sampled and assayed for virus. The virus was pumped into the distribution system at an existing Y-strainer

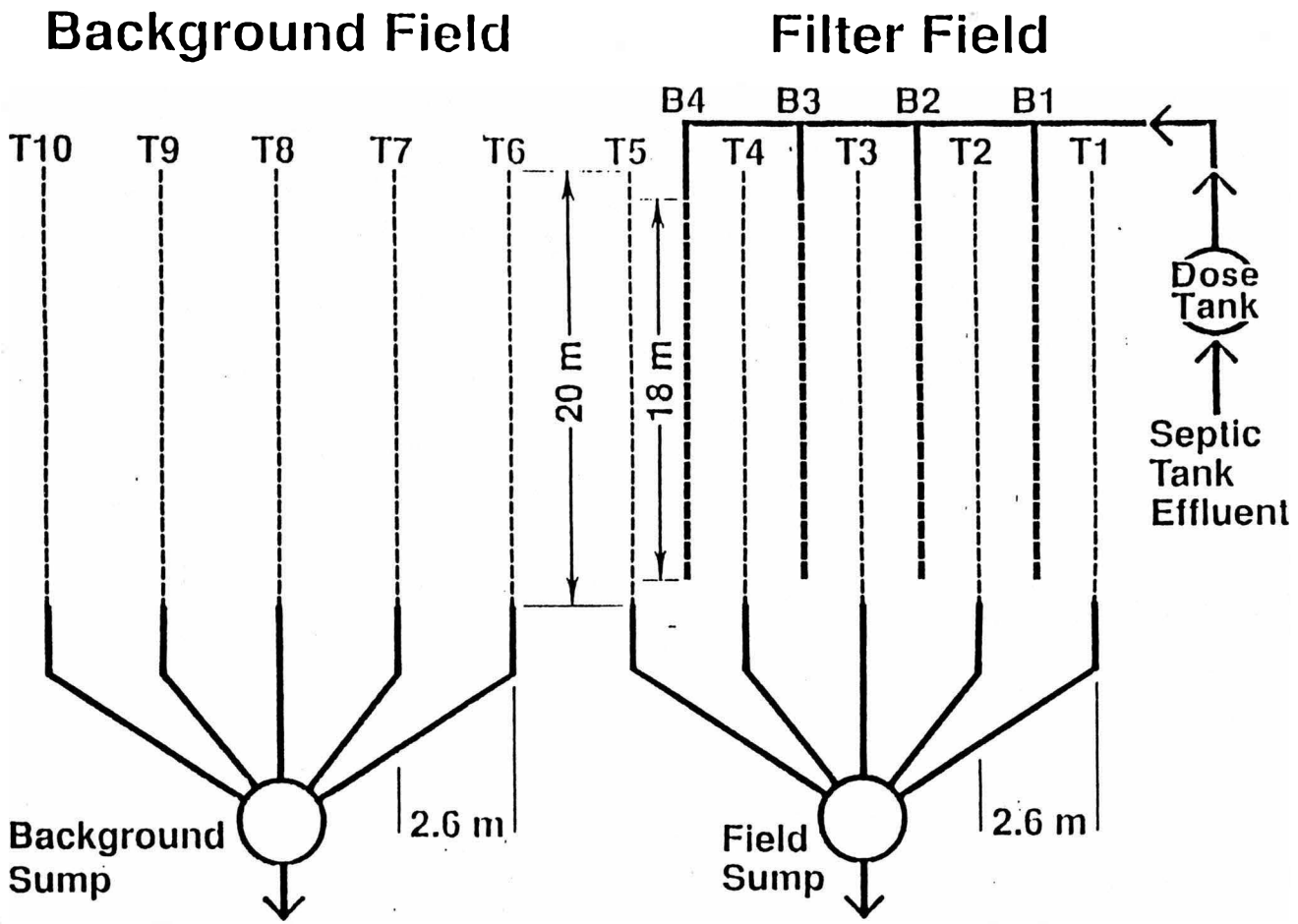

Figure 1. Plan View of Wastewater Treatment System.

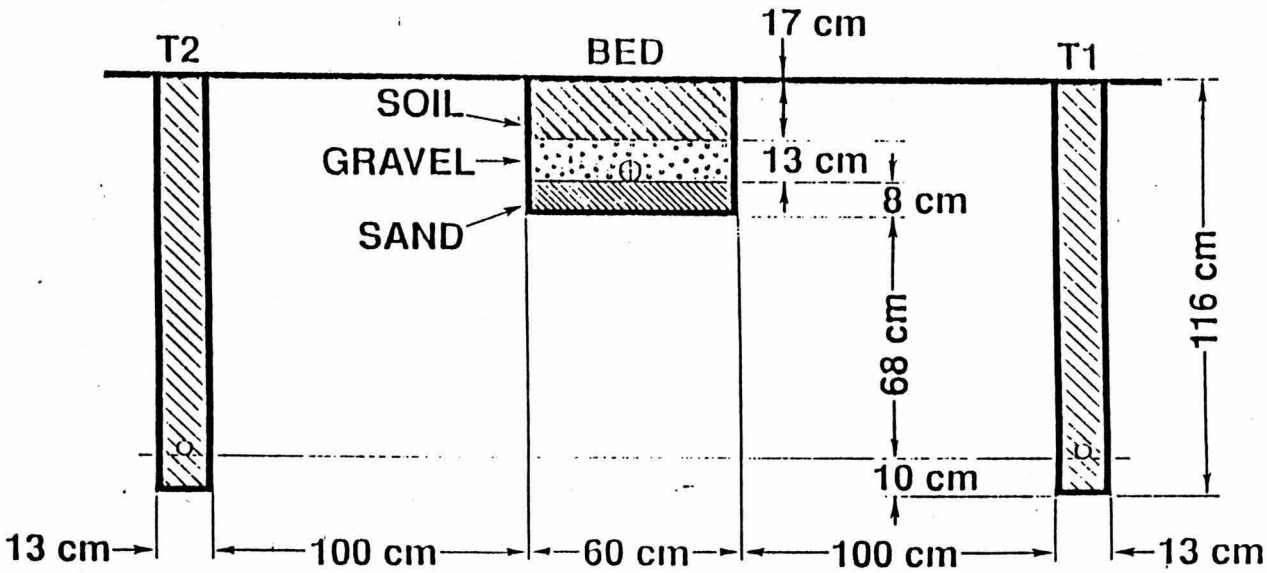

Figure 2. Typical Cross-Section Through Soil Absorption and Drainage Tiles.

downstream from the dosing pump and check valve. A high enough titer was injected to cause a final concentration in each dose to reach 10^5 PFU/l (Plague-forming units per liter). This allowed for demonstration of a 3-log (99.9%) reduction in virus concentration with a 10^2 PFU/l titer remaining in the tile line effluent.

The MS2 bacteriophage assay carried out according to Don Berman's procedure outlined in "Determining Chloramine Inactivation of Virus For the Surface Water Treatment Rule" with the corrections of November 13, 1988, on pages 7-11 as presented at the seminar "Determining Inactivation of Giardia and Viruses by Chloramines for the Surface Water Treatment Rule", AWWA 1988 Water Quality Technology Conference.[6] (See Appendix). The MS2 bacteriophage was catalog number 15597-B1, and the bacterial host was Escherichia coli catalog number 15597 from American Type Culture Collection.

The MS2 bacteriophage was be used rather than an enterovirus for several reasons. Firstly, coliphage is safe compared to poliovirus, hepatitis, or other primate-infecting viruses. Secondly, coliphage assays can be carried out in a relatively simple bacteriological laboratory rather than in a tissue culture laboratory. Thirdly, the coliform host is relatively simple to culture and maintain as compared to the BGM cell line, HEK cells, or primary tissue culture. Fourthly, the

MS2 bacteriophage assay technique to be used was developed in the EPA laboratories in Cincinnati, Ohio, and is an acceptable technique for virus studies.

B. Sampling and Data Collection

Samples were collected at two points as follows:

1. Inbed wells
2. Tile outlets

Tile drain samples were collected as grab samples by placing sample containers under each tile outlet pipe to the sump. This treatment system is unique in that each tile may be sampled individually and each sample represents an integration along the entire length of the tile.

The bacteriophage assay as previously noted, followed the method outlined by Berman in "Determining Chloramine Inactivation of Virus for the Surface Water Treatment Rule".[6] This method consists of inoculating a sample with E. Coli host in an agar suspension in the proportion of 3 ml agar, 0.5 to 1.0 ml sample, and 0.1 to 0.2 ml bacterial host per tube. This warm (45^{0} C) suspension is spread evenly over a petri dish (100 x 15 mm) containing a previously prepared and solidified bottom agar layer. The dishes are incubated overnight at 37^{0} C and the

plaques are enumerated immediately after incubation. Serial 10-fold dilutions from 10^{-1} to 10^{-4} are assayed in triplicate.

The results of the project were evaluated in terms of the reduction in virus titer as the septic tank effluent passes from the soil absorption beds into the tile drain sump. Although the reduction in titer may be caused by either inactivation or removal of viruses, the mechanism of reduction was not considered. For water treatment facilities, a 3-log (99.9%) reduction of virus concentration is required. Rose[7] has reported enteric virus concentrations in raw sewage as being in the range of 10^2 to 10^3 PFU/l.

Data collected and maintained independently of the proposed study, but valuable for the study, included Total Organic Carbon, Ammonia Nitrogen, Nitrate Nitrogen, Chloride, Specific Conductivity, and Fecal Coliform Concentrations in the treatment system influent, the tile drain discharge, and the background tile drain discharge.

PRINCIPAL FINDINGS AND SIGNIFICANCE

The results of this study show that the Stanford Onsite Domestic Wastewater Treatment System can achieve a two to three log reduction in bacteriophage titer as well as produce water that meets the Arkansas Department of Health, Standards for

Outdoor Bathing Places. Presentation of the results of the experimentation follow.

A. Virus Recovery Experiment

Before experimenting with bacteriophage in the field, a brief laboratory study was conducted to determine virus recovery efficiencies from septic tank effluent (STE) and from treated STE. MS2 bacteriophage was suspended in salt diluent made according to Berman's recipe shown in Appendix A. STE was filtered through 15.2 cm (6 inches) of course filter sand and the MS2 phage was added to the treated STE. Bacteriophage was also added to raw STE. 0.1 ml of the phage suspension was added to 100 ml each of filtered and raw STE. The STE and phage mixture was agitated gently for approximately three hours to allow the mixture to equilibrate and to let the phage adsorb to any particles suspended in the STE and filtered STE. The MS2 bacteriophage suspension, raw STE, and filtered STE were assayed for bacteriophage and recovery efficiencies were calculated. Table I illustrates these data. The recovery efficiency was calculated as follows:

$$\text{Recovery Efficiency, \%} = \frac{\text{Measured Effluent Titer}}{\text{Phage Suspension Titer}} * 100$$

TABLE I.

VIRUS RECOVERY EFFICIENCIES

Phage Suspension Titer, PFU/ml	Measured STE Titer, PFU/ml	Recovery Efficiency from STE Percent	Measured Filtered STE Titer, PFU/ml	Recovery Efficiency from Filtered STE, Percent
2.5 x 10"	1.2 x 10"	48	20 x 10"	80

B. Field Study

Bacteriophage were introduced to the wastewater treatment system by pumping them into the pressurized distribution system. The phage suspension was prevented from flowing back into the dosing tank by means of a check valve in the distribution system upstream from the point where the viruses were injected. The dosing pump was activated, and 189 liters (50 gallons) of septic tank effluent was pumped into the treatment system. The titer of the dose was 1.6×10^5 PFU/ml. The tile drain outlets were monitored and when flow began, samples were taken from the outlets until the drainage flow rate returned to a drip. This process was repeated three times during the course of the day. This produced 115 tile drainage samples.

Table II is a tabulation of virus assays over time. The assays are shown as the mean titer of the tile drainage samples taken at each time period. The system was dosed with STE and viruses at times 0, 180 minutes, and 280 minutes. From 225 minutes until 275 minutes a hard rain occured, producing 6 mm of rain in 15 minutes, and as can be seen in the assay data, the

TABLE II,

EFFLUENT VIRUS TITER

TIME FROM FIRST DOSE, MINUTES	MEAN VIRUS TITER IN EFFLUENT SAMPLES PFU/ml
18	1.2×10^2
49	7.7×10^1
121	3.0×10^0
130	1.4×10^2
138	6.4×10^1
200	2.1×10^2
215	1.4×10^2
221	3.5×10^2
230	2.2×10^2
240	2.5×10^2
250	2.5×10^2
317	1.3×10^3
322	1.2×10^3
330	8.9×10^2
335	3.7×10^2
338	1.1×10^3
343	3.2×10^3

effluent virus titer increased tenfold. Figure 3 illustrates the dosing and effluent virus concentrations over time. This figure shows clearly the effluent virus titer increase following the third dose and rain.

Over a two year average, the Stanford Onsite Wastewater Treatment System has consistently reduced the Total Organic Carbon (TOC) concentration and ammonium-nitrogen concentration to near background levels. The effluent fecal coliform concentration has a two-year geometric mean of 18 colony-forming units per 100 ml (CFU/100 ml). The background tile drainage had a two-year geometric mean fecal coliform concentration of 3 CFU/ml. Table III is a summary of the STE tile drain effluent and background water quality data over a two-year period.

TABLE III.

WATER QUALITY

Parameter	Septic Tank Effluent	Absorption Area Tile Drainage	Background Tile Drainage
Average TOC mg/l	55	5	3
Average NH_4-N Concentration mg/l	41	1	0
Average NO_3-N Concentration mg/l	<1	4	1
Average Cl Concentration mg/l	50	35	8
Geometric Mean of Fecal Coliform Concentration CFU/100 ml		18	3

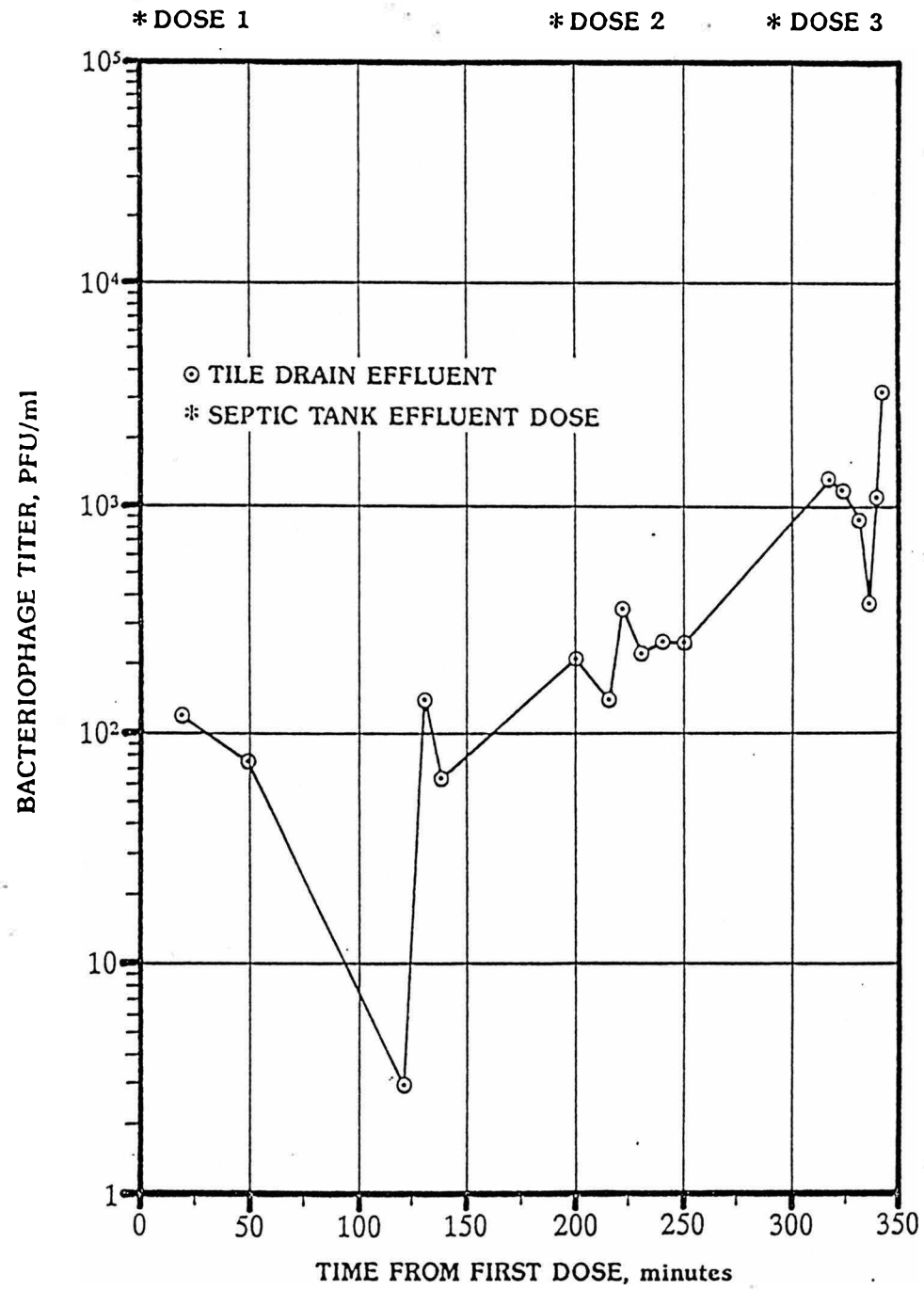

FIGURE 3
VIRUS TITER OVER TIME.

CONCLUSIONS

The Stanford Onsite Wastewater Treatment System is capable of a 2-log (99 percent) reduction in bacteriophage titer. The system has shown up to a 3 log (99.9 percent) reduction in bacteriophage titer. As more doses were applied to the system, and an intense rain fell, the system was not as effective in removing or inactivating bacteriophage. The treatment system also produces water that meets the Arkansas Department of Health Standards for Outdoor Bathing Places.

Based upon the high quality of the effluent in terms of TOC, ammonium-nitrogen, and coliform, the effluent is acceptable for nearly unlimited reuse. However, since bacteriophage did come through the system, although in relatively low concentrations, some limits upon reuse are recommended. The tile effluent is acceptable for reuse such as flushing water closets and drip-trickle irrigating onto crops producing aerial fruits such as tomatoes and fruit trees, or subsurface irrigation of trees or ornamental plants. The tile drainage is acceptable for landscaping irrigation where aerosolizing viruses is not likely. Reuse of the tile drainage in applications where body contact occurs is not recommended. The Stanford Onsite Wastewater Treatment System produces a high quality effluent with a variety of reuse possibilities.

Future work involving this system includes performing a longer-term virus study using an enteric virus model such as a Sabin-vaccine strain of poliovirus. A study of the treatment system involving modifying the dosage pattern should be undertaken. By lowering the dose volume and using more frequent intermittent doses a completely unsaturated flow condition may be maintained in the soil, even during rain events, and achieve a more complete treatment, including reduced virus numbers passing through the soil.

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