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USING VIRUSES TO EXAMINE SOIL TREATMENT OF SEPTIC TANK EFFLUENT

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ABSTRACT

Viral contamination of drinking water supplies due to inadequate renovation of septic tank effluent (STE) is a public health concern. The purpose of this paper is to illustrate the use of a bacteriophage to evaluate virus movement in a soil treatment system. Viruses – MS2 bacteriophage – were injected into a wastewater treatment system with soil absorption trenches and drainage tiles, and the drain tile effluent was collected and assayed for the phage. The virus suspension was assayed and a measured amount of STE and virus suspension was pumped into the system allowing for calculation of the influent virus titer. Results of the virus assays showed that the wastewater treatment system generally achieves a 99.0 (2 log) to 99.9% (3 log) reduction in the concentration of viable bacteriophage after moving through one meter of silt loam soil. This paper illustrates the procedures to utilize and assay for bacteriophage in the harsh environment of a working onsite wastewater treatment system.

INTRODUCTION

Onsite wastewater treatment is used by 42% (approximately 1 million people) of Arkansas households (Ark. Statistical Abstract, 1986). Many of the households using individual wastewater treatment systems are located in East Arkansas. This region, 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 further hampering wastewater drainage.

The main source of groundwater contamination in noncommunity and individual water systems is overflow or seepage of sewage from septic systems or cesspools, chemical contamination, and surface runoff (Craun, 1985). Craun (1985) also reports that 51% of all waterborne outbreaks and 40% of all waterborne illnesses resulted from contaminated (untreated or inadequately disinfected) groundwater supplies between 1971 and 1982 in the United States.

Clearly, as seen in Craun's study, groundwater contamination is a problem in the United States and includes contamination from septic systems. Since household sewage can contain viruses, the importance of monitoring and tracing virus movement through soil and in aquifers becomes apparent. Vaughn *et al.* (1983) recovered virus particles from a subsurface wastewater disposal system at distances of 67.05 m and from aquifer depths of 18 m. The presence of viruses at these distances further stresses the importance of finding efficient and acceptable virus models to test the effectiveness of sewage treatment systems.

Yates *et al.* (1985) demonstrated that the MS2 bacteriophage has inactivation rates equal to or slower than those of poliovirus 1 and echovirus1 in most of the samples they tested for viruses. Powelson *et al.* (1990) used the MS2 bacteriophage for a test of virus transport and survival in saturated and unsaturated flow. Therefore, the MS2 phage has been shown to be an effective model and may be used for virus studies of sewage treatment systems.

The purpose of this study was to use the MS2 phage to examine virus treatment in a tile-drained onsite wastewater treatment system. This paper describes the procedures used and the modifications made to assay treated and untreated residential sewage samples.

MATERIALS AND METHODS

VIRUS

The MS2 bacteriophage was used to evaluate virus movement in a soil treatment system. The MS2 bacteriophage was catalog number 15598-B1 and was grown in *Escherichia coli* (catalog number 15597) from American Type Culture Collection (ATCC, 1990).

The MS2 phage was used in this study for several reasons. First, coliphage is relatively safe compared to poliovirus, hepatitus, or other human-infecting viruses. Second, the coliphage assay can be performed in a relatively simple bacteriological laboratory. Third, the coliform host is simple to culture and maintain in the laboratory. Finally, the MS2 bacteriophage assay technique was developed in the EPA laboratories in Cincinnati, Ohio, and is an acceptable technique for virus studies.

HOST AND VIRUS PREPARATION

American Type Culture Collection (ATCC, 1990) gives the following directions for rehydrating freeze-dried cultures of bacteria: 1) pipette 0.5 ml of appropriate broth into the vial and mix well, 2) transfer contents to a sterile test tube containing 5.0 ml of the recommended broth, 3) incubate the mixture at 37°C for a few days (2-3 days), and 4) remove the culture and store at 5°C or lower.

To recover a bacteriophage from a freeze-dried culture, American Type Culture Collection (ATCC, 1990) gives these directions: 1) prepare an actively growing broth culture of the host before opening phage specimen, 2) rehydrate the specimen aseptically with 0.5 ml of appropriate broth and mix well, 3) use 0.1 ml of this mixture for preparation of a new high-titer phage suspension, and 4) store the remaining mixture in a sterile screw-capped vial at 2-10°C.

ASSAY TECHNIQUES

The bacteriophage assay and stock suspension procedures followed the methods outlined by Berman (1988). A bacteriophage stock suspension was prepared prior to the viral assay. This method involved pipeting 0.1 ml of the rehydrated phage suspension and 0.1 ml of a Tryptone Yeast Extract (TYE) broth culture of *E. coli* to 3.0 ml warm top agar (45°C). The mixture was gently mixed and poured evenly over a previously prepared and solidified bottom agar layer. Approximately five petri dishes were prepared this way and allowed to solidify. The dishes were inverted and incubated overnight at 37°C. A sterile, rubber spatula was used to scrape the top and bottom layers into a large, sterile beaker. Enough TYE broth was added to the agar layers to make an 80 ml suspension, and 0.4 g of EDTA and 0.52 g of lysozyme were added to the mixture. The mixture was then incubated at room temperature for two hours with continuous mixing. After overnight incubation, the mixture was centrifuged at 3000 x g for 15 minutes, and the supernatant was removed, divided into aliquots, and stored at 4°C.

Once the phage stock was prepared for the assay, and a TYE broth culture of the host was incubated (about 18 hours) the night before the assay, then the bacteriophage assay could begin using the methods

described below. This method consisted of inoculating a sample with *E. coli* host in an agar suspension in the proportion of 3.0 ml agar, 0.5 to 1.0 ml sample, and 0.1 to 0.2 ml bacterial host per tube. This warm (45°C) suspension was spread evenly over a petri dish (100 x 15 mm) containing a solidified bottom agar layer. The dishes were incubated overnight at 37°C, and the plaques were enumerated immediately after incubation. Serial 10-fold dilutions from 10^{-1} to 10^{-4} were assayed.

ASSAY MODIFICATION

The freeze-dried bacterial culture was rehydrated according to ATCC 1990) directions except for the incubation time. Assay modifications were made because the suggested incubation times proved time and time again to be unsatisfactory for producing lysis in our laboratory. Prior to the field work, we attempted to assay a sample of known virus concentration and repeatedly produced no plaques. The incubation times were modified with the belief that during the prolonged incubation times the host reverted to characteristics not conducive to MS2 phage growth. Therefore, we substantially reduced the incubation times from 2-3 days to 17 hrs for rehydrating the bacterial host and from 18 hrs to 4 hrs for prepared TYE broth culture of *E. coli* for the assay procedures.

RECOVERY EFFICIENCY METHOD

Before experimenting with bacteriophage in the field, a 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 (1988). STE was filtered through 15.2 cm of coarse filter sand, and the MS2 phage was added to the treated STE. Bacteriophage was also added to untreated STE. A 0.1 ml volume of the phage suspension was added to 100 ml each of filtered and untreated STE. The STE and phage mixture was agitated gently for approximately 3 hrs 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 using the following equation:

$$\text{recovery efficiency (\%)} = \frac{\text{measured effluent titer}}{\text{phage suspension titer}} \times 100$$

The phage suspension titer, measured STE titer, and measured filtered STE titer (PFU/ml) equaled 2.5×10^{11}, 1.2×10^{11}, and 2.0×10^{11}, respectively. Therefore, the recovery efficiency from the untreated STE equaled 48% and from the filtered STE equaled 80%.

THE STANFORD SYSTEM

In the Stanford System, the wastewater is pumped from the dose tank into the soil absorption beds. The beds are 60 cm wide and 38 cm deep and receive the septic tank effluent through 0.48 cm orifi in 3.8 cm nominal diameter schedule 40 pvc pipe. The effluent is distributed evenly over the beds by maintaining approximately 60 cm of head. The effluent delivery is by a typical low-pressure distribution system (Uebler, 1982; Hargett, 1984; and Stewart and Reneau, 1988). Figure 1 is a 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 100 cm of undisturbed soil. The tile trenches are approximately 13 cm wide and 116 cm deep. Hancor "Turflow" slotted drain pipe was placed 10 cm from the trench bottom. The bottom of the drain trench corresponds to the top of a fragipan. Figure 2 illustrates the relative positions of the absorption beds and drainage tiles. The tile drains discharge into a sump where each tile is sampled for physical, chemical, and bacteriological analyses.

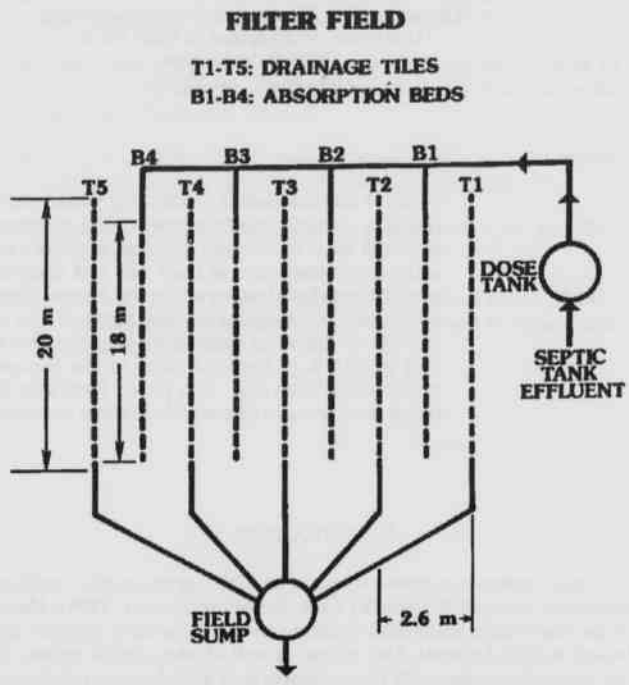

Figure 1. Plan View of Wastewater Treatment System.

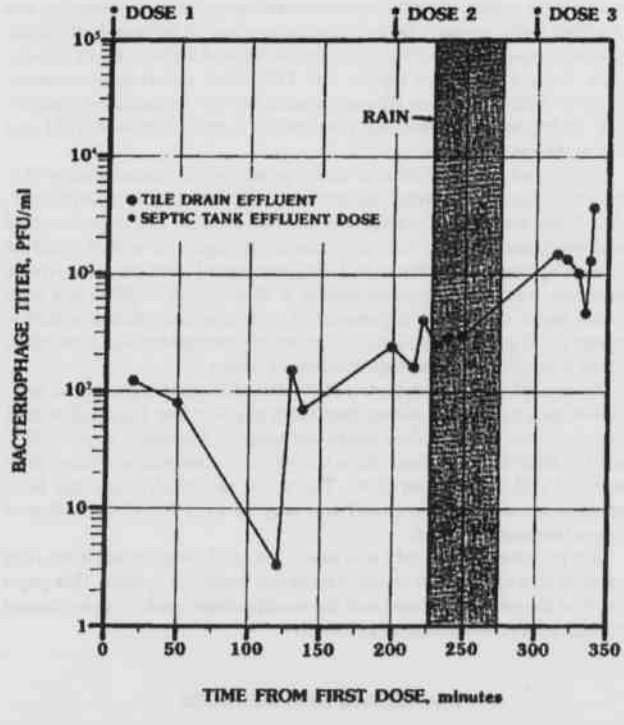

Figure 2. Typical Cross-Section Through Soil Absorption and Drainage Tiles.

FIELD STUDY

MS2 bacteriophage were introduced into 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. The virus was injected into the system at an existing Y-strainer downstream from the dosing pump and check valve. The final concentration of each dose was calculated as follows:

$$\text{Virus conc. of dose (PFU/ml)} = \frac{\text{virus concentration in suspension (PFU/ml)} * \text{vol. of suspension (ml)}}{\text{vol. of suspension (ml.)} + \text{vol. of dose (ml)}}$$

The virus titer in the suspension, volume of virus suspension, and volume of STE does were 3.9×10^8 (PFU/ml), 25 ml, 60 liters, respectively. Therefore, the final virus concentration of each dose was 1.6×10^5 PFU/ml. The system was dosed with STE and viruses at times 0, 168 minutes, and 279 minutes. Again, each dose contained 25 ml phage suspension and 60 liters STE.

The tile drain samples were taken consecutively from the outlets as soon as flow began to drain and were taken until the flow rate returned to a drip. Tile drain samples were collected as grab samples by placing 250 ml sample cups under each tile outlet pipe to the sump.

VIRAL ASSAYS

A total of 115 samples was collected from each of five tile outlets over a period of 343 minutes. Each tile sample was assayed using Berman's (1988) procedures without dilution and to dilutions of 10^{-1} and 10^{-2}. The plaques were counted immediately after overnight incubation at 37°C. The mean titer of the five tiles was calculated for each sample.

RESULTS

Figure 3 represents the MS2 virus concentrations (PFU/ml) collected after each effluent dose, and they are shown as the log mean concentration of viruses across the five drainage tiles. A hard rain fell from 209 minutes to 259 minutes. The assay data showed a slight increase in MS2 virus titer with each subsequent dose and after the rain. The mean average MS2 concentration for samples collected after dose 1 and before dose 2 equaled 8.1×10^1 PFU/ml. The average concentration after dose 2 but before dose 3 equaled 2.4×10^2 PFU/ml and after dose 3 equaled 1.3×10^3 PFU/ml. The system achieved a 99.9% (3-log) reduction for 35% of the samples, and a 99% (2 log) reduction or greater for 94% of the samples. Percent reduction in virus titer is calculated by the following algorithm:

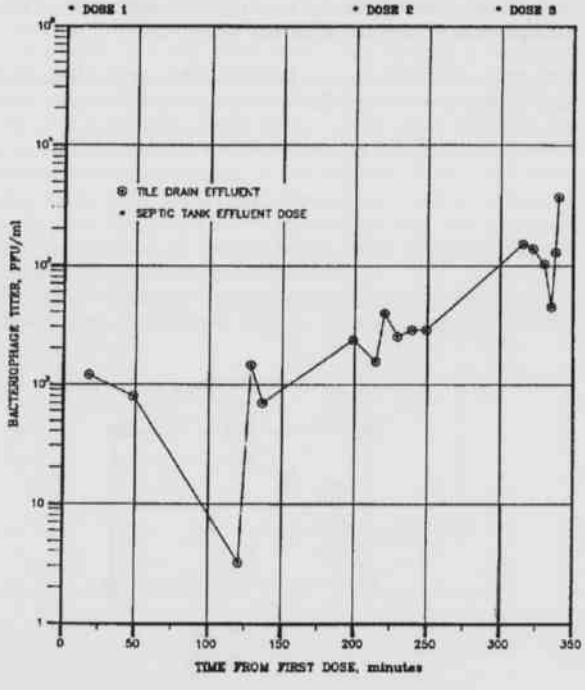

Figure 3. MS2 Virus Titer Per Sample For Each Sampling Time.

$$\text{percent reduction} = (100)\ \frac{\text{virus titer in STE(PFU/ml)} - \text{virus titer in tile effluent (PFU) ml}}{\text{virus titer in STE}}$$

Table 1 shows the numerical values of mean virus titer over the course of the sampling program.

Table 1. 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

DISCUSSION

As seen in Fig. 3, the MS2 virus concentration showed a slight increase with each subsequent dose and with rain. We believe that this general increase may be due to saturation of the system. The system was dosed with 60 L of septic tank effluent (STE) at zero minutes, again at 168 minutes, and again at 279 minutes for a total of 180 L. Powelson *et al.* (1990) demonstrated that the MS2 phage showed little adsorption or inactivation in the saturated condition compared with the unsaturated condition. We suggest that the Stanford system achieved a saturated condition; thus the system's filtration capabilities were reduced to a lower level, and more MS2 phage particles escaped with the STE.

The Stanford Onsite Wastewater Treatment system is capable of a 99.0% (2 log) reduction in virus titer and has shown up to a 99.9% (3 log) reduction in virus titer. The EPA regards a 99.99% (4 log) reduction in virus concentration as acceptable treatment for potable water treatment systems (Cave, 1990).

Rose and DuPont (1988) report that normal enteric virus concentrations in the average household are in the range of 10^2 to 10^3 PFU/L (0.1 to 1.0 PFU/ml). We injected a virus concentration (1.6×10^5 PFU/ml) that is two to three logs greater than the average concentration. Therefore, an average household virus concentration could be effectively reduced to 10^{-3} - 10^{-2} PFU/ml(3-log) with the Stanford system.

Other researchers have used the MS2 bacteriophage effectively for virus removal from septic tank effluent and suggest that the MS2 phage may be acceptable for testing soil treatment systems filtering capabilities (Yates, 1985; Powelson *et al.* 1990). Although, Goyal and Gerba (1979) concluded that no one virus may serve as the ultimate model for determining virus adsorption to soils due to a large degree of variability both between and within strains of enteroviruses. Their data show that the MS2 phage had equal to or lower percent adsorption than the poliovirus1 and echovirus 7 strains in most soil types.

CONCLUSIONS

1. The typical viral assay may have to be modified to suit the laboratory in which the assay will take place. In our case, the incubation times had to be reduced for lysis to occur on the plates.
2. Before performing a field experiment with MS2 phage, a recovery efficiency experiment should be conducted in the laboratory. This experiment will allow the researcher to determine what percentage of virus particles will adsorb in the septic tank effluent before filtration ever begins. In other words, the experiment will determine what percent of virus particles will be lost simply by introducing them to the sewage.
3. This active, tile-drained system is capable of a 99.0% (2 log) to 99.9% (3 log) removal or inactivation of MS2 phage.

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