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Standridge, Jon H.; Olstadt, Jeremy; Sonzogni, William C.
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# Passage of Microorganisms In Septic Tank Effluents Through Mound Sand In a Controlled Laboratory Environment

Jon Standridge, Jeremy Olstadt, William Sonzogni
Wisconsin State Laboratory of Hygiene
2601 Agriculture Dr.
P.O. Box 7996
Madison, WI 53707-7996

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## Introduction

Pristine groundwater is a greatly treasured Wisconsin resource. Protection of this resource in part relies on removal of organisms from anthropogenic activities that are likely to contaminate ground water. While there is a fairly large literature on the passage of microbes through soil, the specific question of the passage of organisms from relatively clean wastewater effluents through mound sand has received little attention. The basic question to be answered by the research described in this report is: "Do microorganisms suspended in wastewaters from private onsite wastewater treatment systems pass through mound sand in laboratory scale column experiments" This is a complex, multi-faceted question that could be answered with many approaches. The intent of this report is to capture all experimental data with minimal interpretation and discussion.

## Experimental Design

As water passes through a mound sand matrix, organisms are removed in three ways, filtration, adsorption or die-off. These mechanisms of removal are complex. Filtration relies on depth, preferential flow paths, saturation, biofilms, surface matting, temperature, flow rate, and dosing frequency. Adsorption depends on microbial flora, surface matting, cation concentration, temperature and pH. Die-off depends on microbial predation, temperature, saturation, drying, organic matrix, microbe multiplication and time. This leads to the following list of experimental variables which could be evaluated.

- Depth
- Saturation
- Time
- Dosing rate
- pH
- Cation concentration
- Dosing schedule
- Temperature
- Dosing effluent quality

This list yields thousands of possible experimental variable combinations. In order to reduce the number of variables to a manageable number, we chose to standardize them as follows.

-Depth- Columns were prepared in a variety of depths from 12 inches to 60 inches (see table 5. below)

-Dosing rate - For the first six months of the experiment the mound sand columns were dosed at two gallons per square foot per day for high quality effluents, and one gallon per day for the lower quality effluents. The actual daily dose volumes for the three inch (.25 ft) inside diameter columns were calculated by determining the surface area of the column, as pi $R^2$ [3.14 x 0.125 ft x 0.125 ft = 0.049 $ft^2$], which means 0.049 gallons (186 mL) would be added daily to each column for a one gallon per square foot per day dose and 0.098 gallons (372 mL) for a two gallon per square foot per day dose. The effluent was administered in five equal doses (37 and 74 mL respectively) per day with four doses spaced four hours apart and the last dose of the day administered after an eight hour interval. An exception to this dosing schedule was that one 24" column was dosed once per day only. During the final ten weeks of the experiment, the dosing volumes were doubled for eight weeks, and then doubled again (four times the original volume) for the final two weeks.

-Saturation - Saturation was never achieved. Some drying occurred between each dosing.

-pH- For this study pH of the effluent was unaltered.

-Temperature - All tests were run at $20^0$ C.

-Time - The leachate from each column was tested for organism pass through every three weeks during the first 30 weeks of the study and then with increasing frequency during the last weeks, peaking at three times per week at the end of the experiment.

-Cation concentrations were unaltered.

-Dosing effluent quality - Two small scale on-site waste water facilities were chosen for collection of septic tank effluent to be used for dosing the experimental sand columns.

**Site one.** This system consisted of a septic tank vault followed by a single pass sand filter, preceding the infiltration field. Samples were collected from the pumping tank just after the sand filter. This site was chosen to represent a high quality effluent with biological oxygen demand (BOD) and suspended solids (SS) values of less than 30/30 mg/L.

**Site two.** Samples were collected from a small scale on-site treatment system serving a mobile home park. The system consisted of three septic tank vaults in series followed by a recirculating sand filter in line prior to the infiltration beds. The samples were collected directly from the surface of the third septic vault. Site two was chosen to represent a typical effluent from a conventional septic tank with BOD/SS levels of approximately 220/150 mg/L.

Effluents from both systems were collected once per week and stored at 2-4$^{\circ}$ C prior to being dosed to the columns. Each weekly collection of effluents was tested for BOD and SS. These results are summarized in tables 1 and 2 below.

**Table 1.**

Site one - single pass sand filter effluent

| Date | Source | BOD mg/L | SS mg/L |
|---|---|---|---|
| 10/9/00 | sand filter | <6 | <5 |
| 11/20/00 | sand filter | <2 | <5 |
| 11/29/00 | sand filter | <2 | <5 |
| 12/7/00 | sand filter | <2 | <5 |
| 12/14/00 | sand filter | <2 | <5 |
| 1/10/01 | sand filter | <2 | <5 |
| 1/17/01 | sand filter | <6 | <5 |
| 1/24/01 | sand filter | <3 | <5 |
| 1/31/01 | sand filter | <3 | <5 |
| 2/7/01 | sand filter | <3 | <5 |
| 2/14/01 | sand filter | <3 | ND |
| 2/21/01 | sand filter | <2 | ND |
| 2/28/01 | sand filter | <3 | ND |
| 3/7/01 | sand filter | <2 | ND |
| 3/16/01 | sand filter | <2 | <2.5 |
| 3/22/01 | sand filter | <2 | ND |
| 3/28/01 | sand filter | <2 | ND |
| 4/4/01 | sand filter | <3 | ND |
| 4/12/01 | sand filter | | |
| 4/19/01 | sand filter | <3 | ND |
| 4/25/01 | sand filter | <3 | ND |
| 5/2/01 | sand filter | <3 | ND |
| 5/10/01 | sand filter | <2 | ND |
| 5/17/01 | sand filter | <2 | ND |
| 5/24/01 | sand filter | <2 | ND |
| 5/31/01 | sand filter | >40.3 | ND |
| 6/6/01 | sand filter | <3 | ND |
| 6/11/01 | sand filter | <2 | ND |
| 6/14/01 | sand filter | <2 | ND |
| 6/18/01 | sand filter | <2 | ND |
| 6/20/01 | sand filter | <2 | ND |
| 6/25/01 | sand filter | <2 | 17 |
| 6/27/01 | sand filter | 2.9 | 6 |
| 7/2/01 | sand filter | <2 | <2.2 |

**Table 2.**

Conventional septic tank vault effluent

| Date | Source | BOD mg/L | SS mg/L |
|---|---|---|---|
| 10/17/00 | septic vault | 162 | 276 |
| 11/11/00 | septic vault | 220 | 196 |
| 11/20/00 | septic vault | 151 | 96 |
| 11/29/00 | septic vault | 166 | 79 |
| 12/7/00 | septic vault | 212 | 72 |
| 12/14/00 | septic vault | 217 | 50 |
| 1/10/01 | septic vault | 240.8 | <5 |
| 1/17/01 | septic vault | 301 | 160 |
| 1/24/01 | septic vault | 255 | 176 |
| 1/31/01 | septic vault | 361 | 204 |
| 2/7/01 | septic vault | 324 | 148 |
| 2/14/01 | septic vault | 226 | 41 |
| 2/21/01 | septic vault | 232 | 255 |
| 2/28/01 | septic vault | 268 | 168 |
| 3/7/01 | septic vault | 244 | 54 |
| 3/16/01 | septic vault | 239 | 34 |
| 3/22/01 | septic vault | 179 | 35 |
| 3/28/01 | septic vault | 252 | 136 |
| 4/4/01 | septic vault | 289 | 128 |
| 4/19/01 | septic vault | 341 | 120 |
| 4/25/01 | septic vault | 210 | 120 |
| 5/2/01 | septic vault | 266 | 200 |
| 5/10/01 | septic vault | 305 | 630 |
| 5/17/01 | septic vault | 217 | 185 |
| 5/24/01 | septic vault | 267 | 146 |
| 5/31/01 | septic vault | 241 | 184 |
| 6/6/01 | septic vault | 275 | 60 |
| 6/11/01 | septic vault | 214 | 40 |
| 6/14/01 | septic vault | 361 | 332 |
| 6/18/01 | septic vault | 217 | 50 |
| 6/20/01 | septic vault | 279 | 94 |
| 6/25/01 | septic vault | 360 | 175 |
| 6/27/01 | septic vault | 314 | 206 |
| 7/2/01 | septic vault | 271 | 94 |

In addition to the traditional BOD/SS measures of effluent strength, the microbial makeup of the spiking effluents is also important. There are virtually thousands of pathogenic and non pathogenic organisms that could be present in septage and therefore enumerated as part of this project. To keep the project costs within reason, we chose to use the traditional total coliform, fecal coliform and *E. coli* indicators of fecal pollution as well as two innovative indicators, enterococci and male specific coliphages.

Total coliform and *E. coli* counts were performed using the standard minimal media ONPG-MUG enzyme based test system (Colilert$_{TM}$) with the Quantitray$_{TM}$ enumeration system. Total coliform is the indicator that has been traditionally used by water microbiologists since 1920 to detect microbial contamination of wells. While total coliform does, in fact, detect outside contamination of a water well system, its ability to accurately predict the presence of fecal material is limited. None the less, it is important to include this indicator since Wisconsin groundwater standards are still based on total coliform occurrence. *E. coli* is included since it does accurately predict the presence of fecal contamination in a water sample, and thus, is considered an excellent predictor of imminent health risk from consuming water from a positive well.

Fecal coliform is a traditionally used indicator of fecal contamination in water. While it has generally been replaced by *E. coli*, we are including it in this study to tie the data to previously published studies. The detection method used was the standard membrane filter fecal coliform test (MFFCC).

While *E. coli* has generally been proven to be an excellent indicator organism and public health tool, its utility can be challenged in situations where contaminated water is filtered through soil. The surface characteristics and shape of *E. coli* allow it to be easily removed as it moves through a soil column, unlike the small round virus often associated with waterborne disease outbreaks, that might move through more readily. For this proposal we chose to include two additional indicators, enterococci and coliphage, that behave more like the viral pathogens in their ability to move through soil.

Enterococci are small round bacteria found in the intestinal track of warm blooded animals. For fifteen years, they have been suggested by the USEPA as the best indicator organism to be used in testing of recreational waters, because of the strong correlations with their presence in water with the occurrence of illness in swimmers. Enterococci counts were performed using a proprietary defined substrate enzyme based test system called Enterolert$_{TM}$ combined with the Quantitray$_{TM}$ enumeration system.

Male specific coliphages have been suggested by several researchers as the fecal indicator of choice because of their increased likelihood of behaving like a pathogenic virus in ground water contamination situations. Coliphage are viruses that infect *E. coli* bacteria. Male specific coliphages are viruses of *E. coli* that infect the bacterial cells through sex pili appendages that are only found on the bacteria when they are at or near body temperatures. In other words, the detection of male specific coliphages is strong evidence of fecal contamination from a warm blooded animal. Additionally, since the coliphages are in fact viruses, they behave in the environment much as human pathogenic virus do. For this study, samples were extracted with freon or chloroform. Recovered phage were then detected using the double agar layer plating method using a host organism vulnerable to infection by male specific coliphages.

Since the main focus of the research was to characterize the passage of microbes through mound sand, the microbiological indicator flora of the effluents was determined and altered when necessary. The high BOD/SS septic tank effluent was tested on five occasions to determine the concentrations of the five chosen indicators. These data are presented below in table 3.

**Table 3.** Naturally occurring flora for high strength septic tank effluent

| total coliform /100mL | *E. coli* /100mL | fecal coliform /100mL | *Enterococcus* / 100mL | male-specific coliphage pfu/mL |
|---|---|---|---|---|
| 4,245,000 | 120,330 | 117,000 | | |
| 1,789,000 | 197,000 | 170,000 | 5290 | 340 |
| 4,352,000 | 256,000 | 320,000 | 43,520 | 5700 |
| 5,475,000 | 158,000 | 480,000 | 30,760 | 15,850 |
| 12,997,000 | 1,153,000 | 930,000 | 7760 | 5600 |

Due to the variability it was determined that the effluent should be always be spiked with additional organisms in order to be sure the columns were being challenged with a large number of organisms. The additional organism spike levels were based on the approximate ratios of the organisms to the levels detected in the actual septic effluent. The target spike levels for the high BOD/SS septic tank effluent for each of the indicators were: Fecal coliform (510,000 cfu/100mL), total coliform (6,100,000 cfu/100mL), *E.coli* (490,000 cfu/100mL), *Enterococci* (24,000 cfu/100mL) and male-specific coliphage (7600 pfu/100mL).

Known concentration bacteria spikes were prepared by growing bacterial suspensions of the indicators from stock cultures to a 0.5 McFarland Standard ($1.5x10^8$) optical density measured using a Mini 20$^{tm}$ Spectrophotometer. The male-specific coliphage stock supply was prepared by viral extraction followed by culture confirmation titering to produce a ($9.3x10^9$) suspension. Each known concentration suspension was then diluted to the pre-calculated target number of organisms used to spike the effluent.

For the clean sand filtered septic effluent, two spiking effluents representing fecal coliform levels of both 1,000 and 10,000 were required. Once again the background levels of naturally occurring total coliform, fecal coliform, *E.coli*, enterococci and coliphage were determined by testing the first five septic effluent collections. These results are presented below in Table 4. The levels were generally so low that organisms needed to be added to achieve the 1000 and 10,000 fecal coliform levels needed. The spiking solutions were prepared as described above. The actual target spike levels for the clean sand filtered septic tank effluent for each of the indicators was: Fecal coliform (1,000) total coliform (36,000 cfu/100mL), *E.coli* (1,500 cfu/100mL), *Enterococci* (1,400 cfu/100mL) and male-specific coliphage (1,000pfu/100mL)for the 1000 fecal coliform spike and fecal coliform (10,000) total coliform (360,000 cfu/100mL), *E.coli* (15,000 cfu/100mL), *Enterococci* (14,000 cfu/100mL) and male-specific coliphage (10,000pfu/100mL) for the 10,000 fecal coliform level spike.

**Table 4.** Natural microbial levels from the high quality sand filtered septic effluent

| total coliform /100mL | *E. coli* /100mL | fecal coliform /100mL | *Enterococcus* / 100mL | coliphage pfu/mL |
|---|---|---|---|---|
| | 118.7 | 190 | | |
| 88.2 | 11 | 5 | 47.9 | <5 |
| 1119.9 | 17.1 | 19 | 6.3 | <5 |
| 365.4 | 33.1 | 18 | 3.1 | <5 |
| 11 | 3.1 | 2 | 1 | <5 |

A matrix chart of the septic effluent dosing to the columns is summarized in table 5. Table 6 presents the summarized information with numbers assigned to each column that will be used when referring to the data generated from the columns.

**Table 5.** Summary of column dosing

| Column depth | BOD/SS 30/30<br>Five doses/day<br>2 gpd/ft$^2$<br>$10^3$ organisms/100ml | BOD/SS 30/30<br>Five doses/day<br>2 gpd/ft$^2$<br>$10^4$ organisms/100ml | BOD/SS 220/150<br>Five doses/day<br>1 gpd/ft$^2$<br>$10^{6-7}$ organisms/100ml | BOD/SS 30/30<br>one dose/day<br>2 gpd/ft$^2$<br>$10^4$ organisms/100ml |
|---|---|---|---|---|
| 12" | Column 1 | | | |
| 18" | Column 2 | Column 4 | | |
| 24" | Column 3 | Column 5 | Column 8 | Column 10 |
| 30" | | Column 6 | | |
| 36" | | Column 7 | Column 9 | |
| 48" | | | Column 11 | |
| 60" | | | Column 12 | |

**Table 6.** Summary of column dosing

| Column number | Column length | BOD/SS target | Fecal coliform target | Dosing volume | dose/day |
|---|---|---|---|---|---|
| 1 | 12 inches | 30/30 | 1000 | 2 gpd/ft$^2$ | 5 |
| 2 | 18 inches | 30/30 | 1000 | 2 gpd/ft$^2$ | 5 |
| 3 | 24 inches | 30/30 | 1000 | 2 gpd/ft$^2$ | 5 |
| 4 | 18 inches | 30/30 | 10,000 | 2 gpd/ft$^2$ | 5 |
| 5 | 24 inches | 30/30 | 10,000 | 2 gpd/ft$^2$ | 5 |
| 6 | 30 inches | 30/30 | 10,000 | 2 gpd/ft$^2$ | 5 |
| 7 | 36 inches | 30/30 | 10,000 | 2 gpd/ft$^2$ | 5 |
| 8 | 24 inches | 220/150 | 1,000,000 - 10,000,000 | 1 gpd/ft$^2$ | 5 |
| 9 | 36 inches | 220/150 | 1,000,000 - 10,000,000 | 1 gpd/ft$^2$ | 5 |
| 10 | 24 inches | 30/30 | 10,000 | 2 gpd/ft$^2$ | 1 |
| 11 | 48 inches | 220/150 | 1,000,000 - 10,000,000 | 1 gpd/ft$^2$ | 5 |
| 12 | 60 inches | 220/150 | 1,000,000 - 10,000,000 | 1 gpd/ft$^2$ | 5 |

Column construction was based on the recommendations found in the review paper published by Bitton et al (1979). This paper reviews and evaluates column construction protocols outlined in the literature. Columns were constructed of 3 inch PVC pipe with the pipe sections mounted vertically in a bench top rack. The bottom of each pipe section was fitted with a glazed ceramic buechner funnel lined with nylon screening. The funnel was sealed in place with silicone caulk. The mound sand was purchased from a Dane County sand and gravel dealer and was tested by the University of Wisconsin Soil and Plant Analysis Lab. This analysis was determined suitable by Leroy Jansky, Department of Commerce Wastewater Specialist as meeting the ASTM C-33 standard for use in Wisconsin mound systems. The dry mound sand was scooped into the columns and compacted by tapping the sides of the column. Each column was topped off with six inches of 3/4 inch washed gravel. The spiked septic tank effluent was delivered to the top of the columns through 1/8$^{th}$ inch inside diameter Tygon$_{TM}$ tubing. (See figures 1a/b below).

**Figure 1a (drawing).**

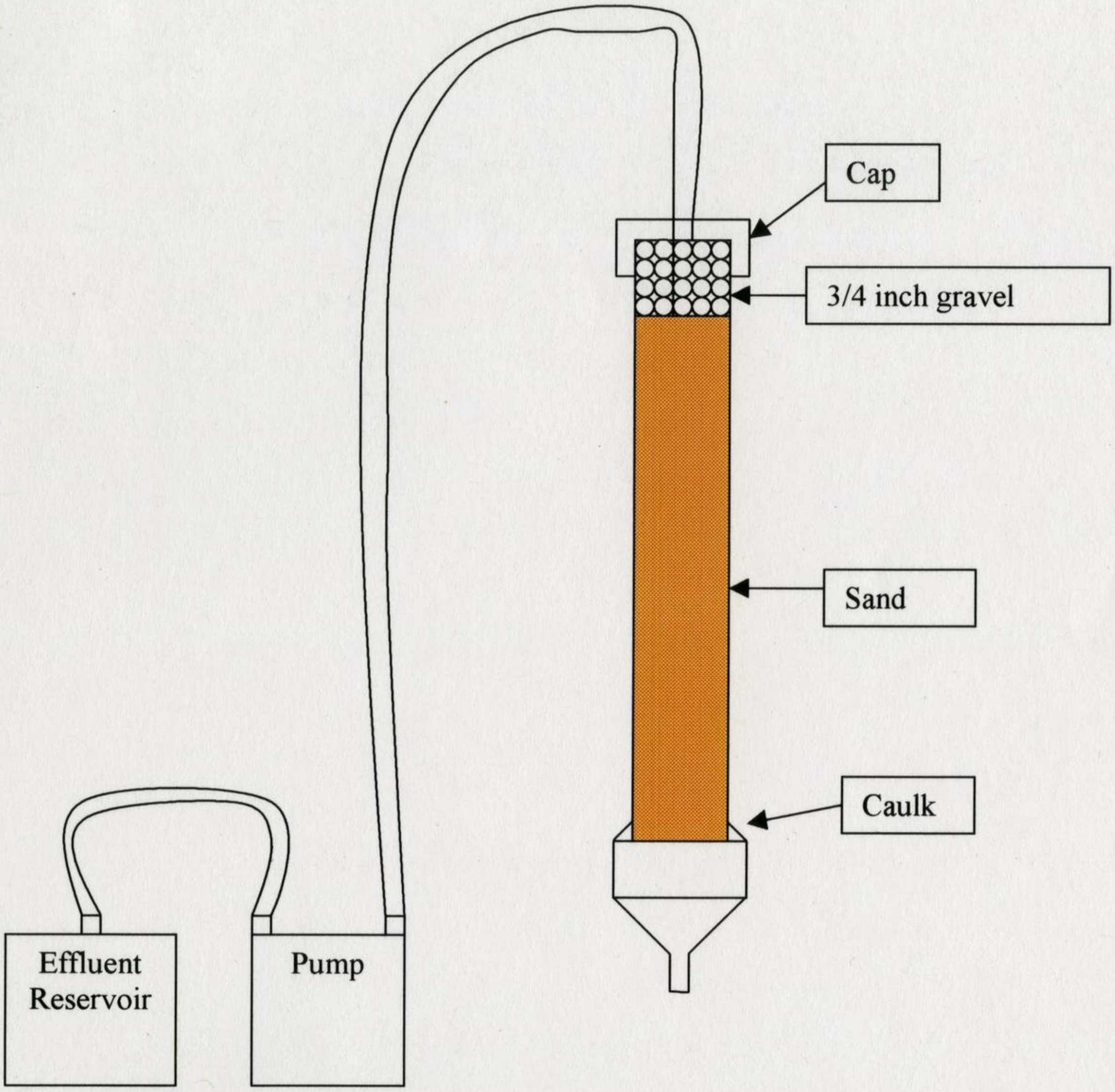
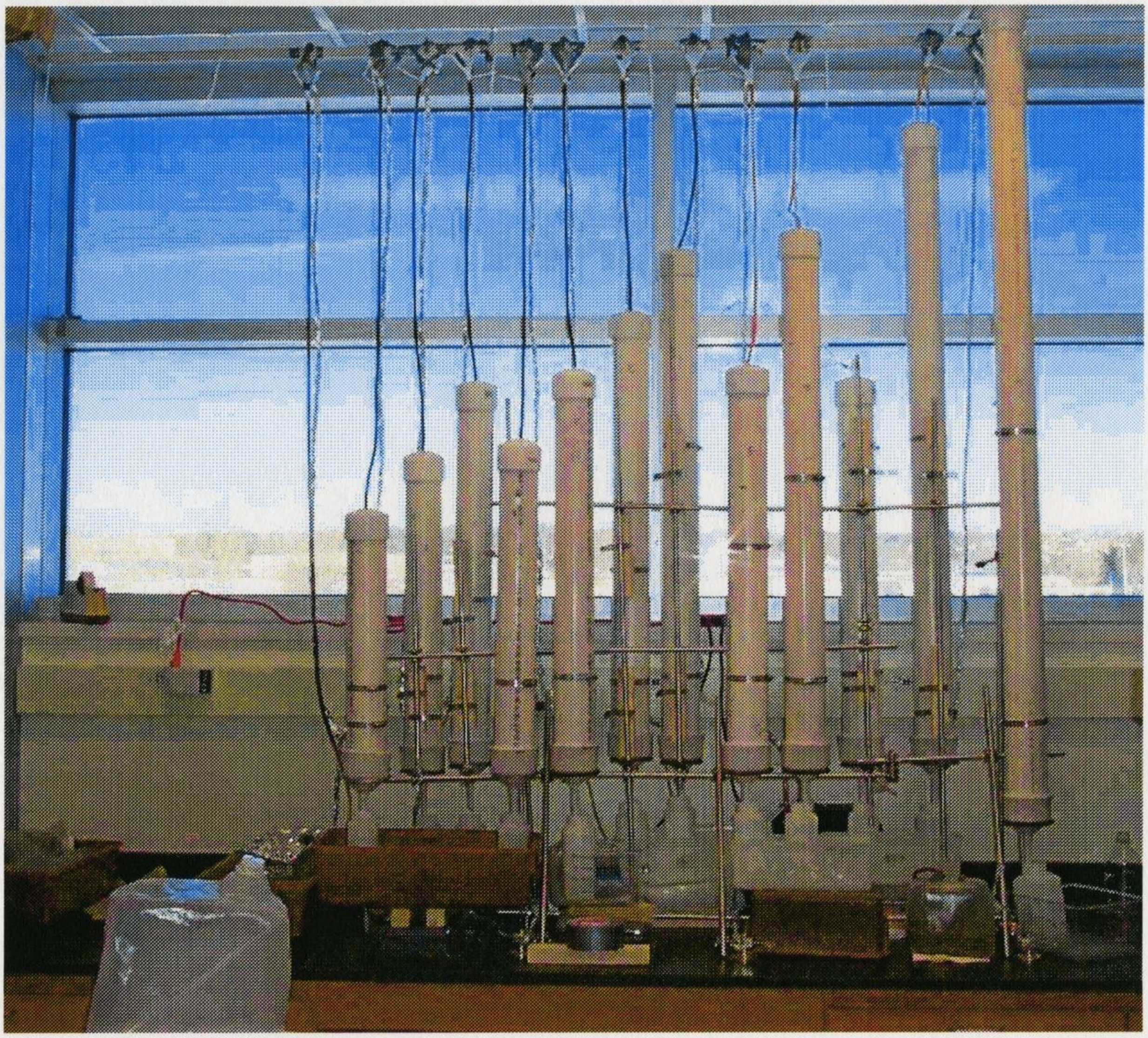


Figure 1.b (photo)



Since the microbe counts of the spiking solutions were measured in the spiking reservoirs, there was some concern as to whether the microbe levels actually dripping from the 1/8th inch tubing would be the same as in the reservoirs. Simultaneous determinations of microbial content of the reservoirs compared to the microbial content at the column end of the spike delivery tubing were made. The results are summarized in table 7. Analysis of this data indicates that there is no difference between the microbial content of the microbial reservoir and the microbial content of the effluent being delivered through the tubing to the columns.

**Table 7.**

| Effluent straight from spiking reservoir | | Effluent at column input | |
|---|---|---|---|
| | **Column 1** | | **Column 1** |
| total coliform | 64,400 | total coliform | 52,000 |
| *E. coli* | 1440 | *E. coli* | 3100 |
| fecal coliform | 1000 | fecal coliform | 240 |
| *Enterococci faecalis* | 1350 | *Enterococci faecalis* | >2419.2 |
| male-specific coliphage | 1000 | male-specific coliphage | <5 |
| | **Column 7** | | **Column 7** |
| total coliform | 644,000 | total coliform | 644,000 |
| *E. coli* | 14,528 | *E. coli* | 19,180 |
| fecal coliform | 10,000 | fecal coliform | 15,500 |
| *Enterococci faecalis* | 13,620 | *Enterococci faecalis* | 7490 |
| male-specific coliphage | 10,000 | male-specific coliphage | 10,000 |
| | **Column 12** | | **Column 12** |
| total coliform | 32,200,000 | total coliform | 10,900,000 |
| *E. coli* | 1,313,000 | *E. coli* | 906,000 |
| fecal coliform | 1,160,000 | fecal coliform | 1,130,000 |
| *Enterococci faecalis* | 31,000 | *Enterococci faecalis* | 52,000 |
| male-specific coliphage | 410,000 | male-specific coliphage | 160,000 |

Flows through the tubing were periodically monitored and adjusted to specifications. Clogged lines were occasionally observed and immediately corrected. Generally we were able to keep the flow rates within 10% of the target throughout the duration of the experiment.

To further characterize column function, dissolved oxygen and conductivity determinations were made on the both the septage being dosed to the column top and the effluent collected from the bottom of the column. These results are presented in table 8. below. The conductivity values remained unchanged as the septage passed through the columns. The dissolved oxygen values remained unchanged for the low strength wastes, but increased significantly for the high strength wastes as the septage passed through the columns.

**Table 8.**

Dissolved oxygen and conductivity at inlet and outlet of columns

| | *Pre-Column Effluent* | | *Post-Column Effluent* | |
|---|---|---|---|---|
| Column | DO mg/L | Conductivity umho's/cm | DO mg/L | Conductivity umho's/cm |
| 1 | 8 | 1340 | 8.2 | 1340 |
| 2 | 7.8 | 1290 | 8.3 | 1330 |
| 3 | 7.5 | 1320 | 8.3 | 1290 |
| 4 | 7.1 | 1310 | 8.3 | 1340 |
| 5 | 7.3 | 1390 | 8.3 | 1300 |
| 6 | 7.3 | 1300 | 8.3 | 1290 |
| 7 | 6.8 | 1370 | 8.3 | 1300 |
| 8 | 6.5 | 3670 | 8.2 | 3630 |
| 9 | 4.1 | 3460 | 8.2 | 3470 |
| 10 | 8.5 | 1360 | 8 | 1340 |
| 11 | 5.6 | 3280 | 8.05 | 3490 |
| 12 | 4.8 | 3610 | 8.1 | 3500 |

Preliminary dosing of the columns was begun onto dry sand. The longer columns were dosed many times before the sand was saturated enough so that effluent would drip from the bottom of the column in response to the dosing. At week 22, data was collected to characterize the flow volumes from the bottom of each column immediately after each dosing. Since the columns were dosed at four hour intervals, the effluent was captured for various time intervals from the bottom of the columns and measured. These data are presented graphically for representative column lengths in figures 2-12 below. Generally, the drip rate of effluent from the bottom of the columns was related to the depth of the sand. The twelve inch column would begin dripping almost immediately after dosing and the longer columns would have a slower response with the longest columns dripping fairly steadily at all times. The strength of the effluent also affected the drip rate. Figures 4 and 5 present data from 24 inch columns both receiving low strength wastes. The graphs are very similar. Almost all of the 74 mL dose passed through the column during the four hour observation period. On both columns there was an immediate flush of about 10mL followed by a steady increase in flow peaking at 60 to 90 minutes. Figure 6 contains the data for the 24 inch column dosed with the high strength waste. Once again there is an immediate flush but the flow is steady for the four hour observation period. Only 24 of the 34 mLs applied had passed through by the end of the period. This phenomenon could be explained either by the lower hydraulic loading or by biofilm formation interfering with flows. The same observations can be made in the 36 inch columns presented in figures 8 and 9.

**Figure 2.** 12 inch column receiving 2 gallons/day low strength effluent (column 1)

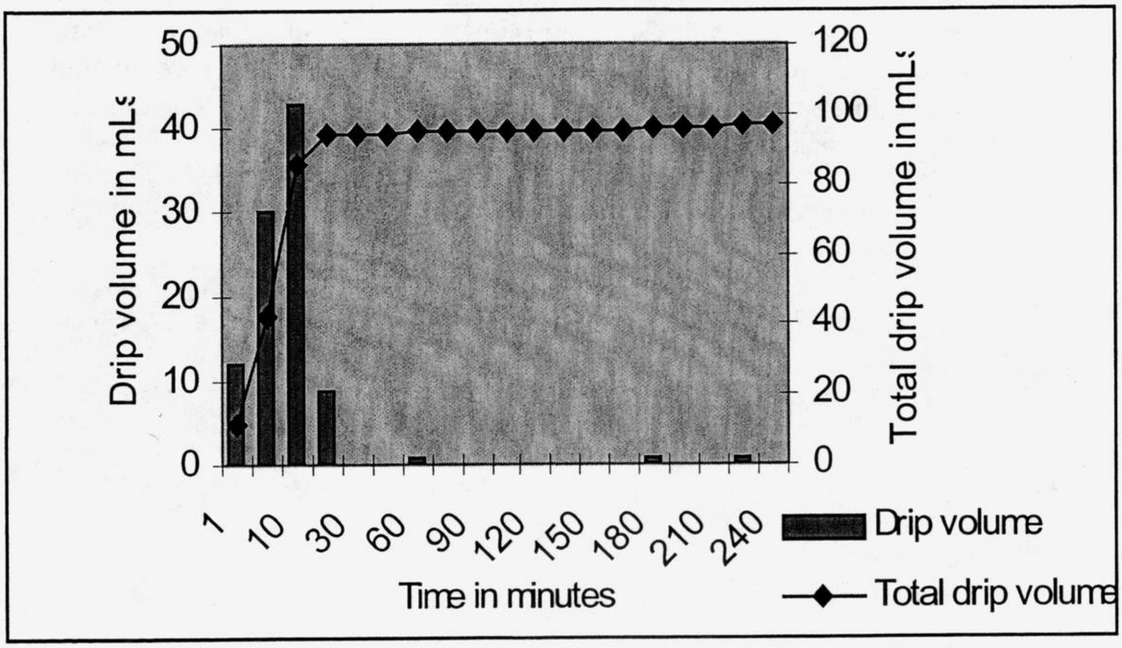



**Figure 3.** 18 inch column receiving 2 gallons/day low strength effluent (column 2)

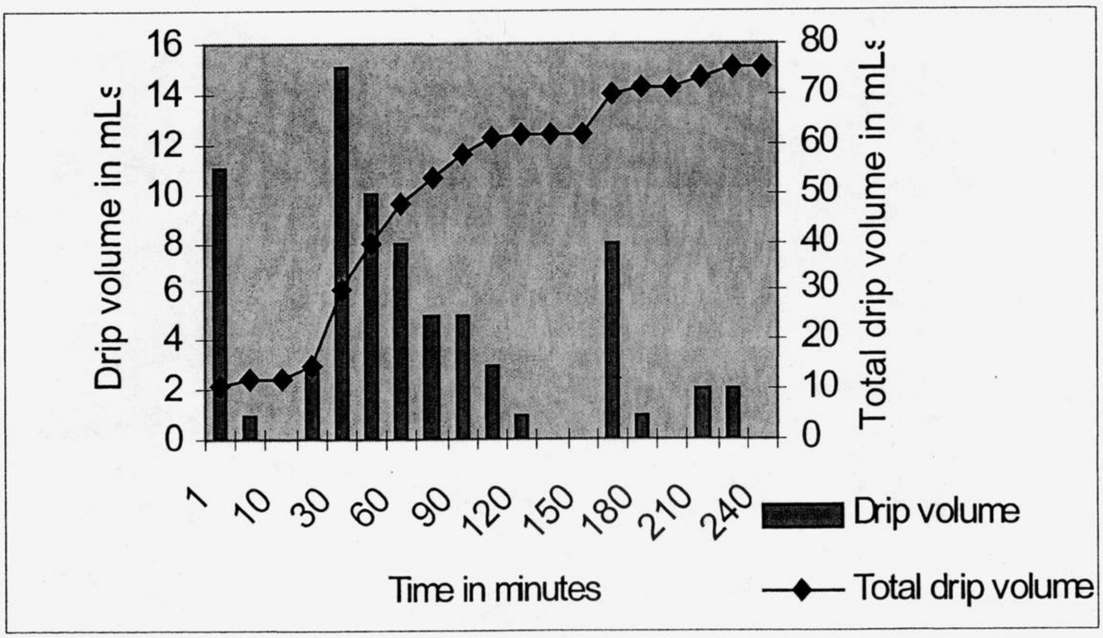


**Figure 4.** 24 inch column receiving 2 gallons/day low strength effluent (column 3)

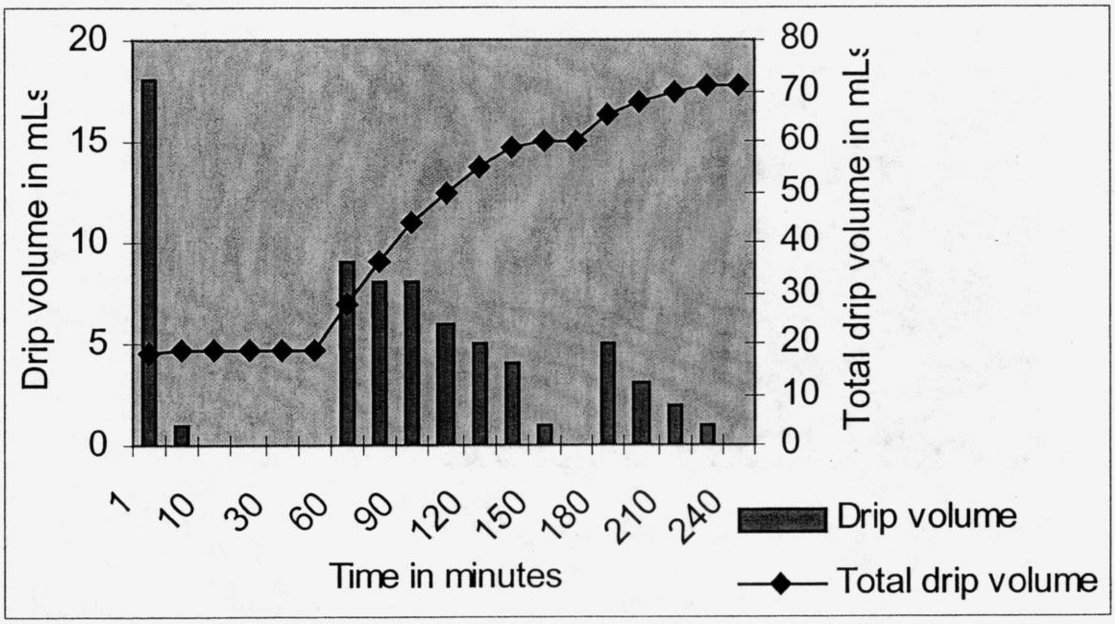



**Figure 5.** 24 inch column receiving 2 gallons/day low strength effluent (column 5)

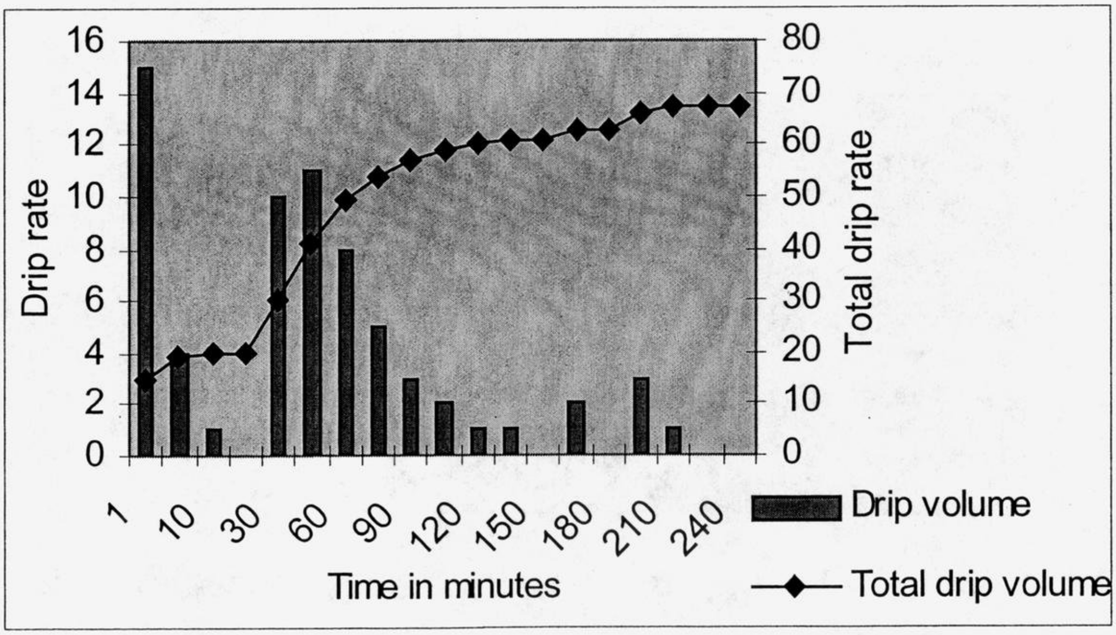


**Figure 6.** 24 inch column receiving 1 gallon/day high strength effluent (column 8)

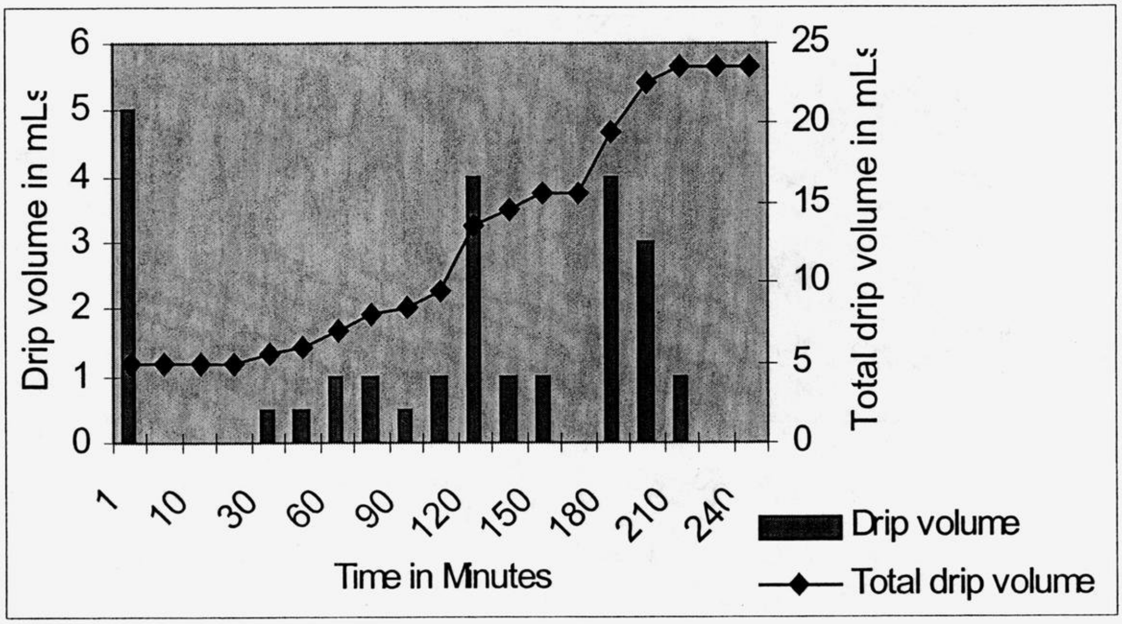



**Figure 7.** 30 inch column receiving 2 gallon/day low strength effluent (column 6)

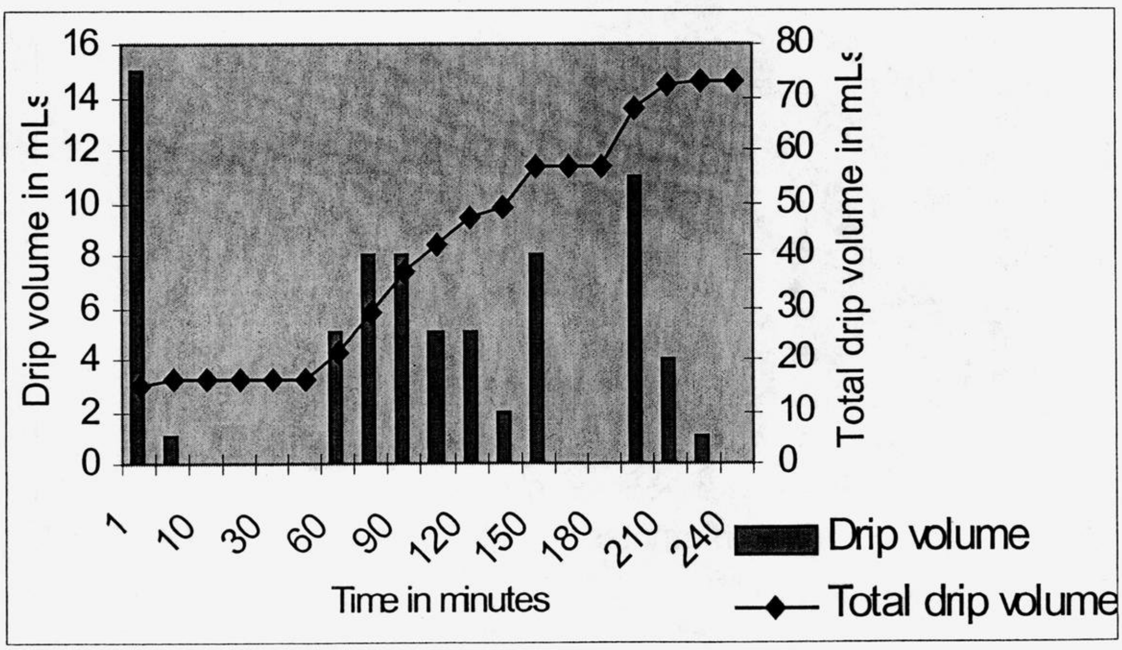


**Figure 8.** 36 inch column receiving 2 gallon/day low strength effluent (column 7)

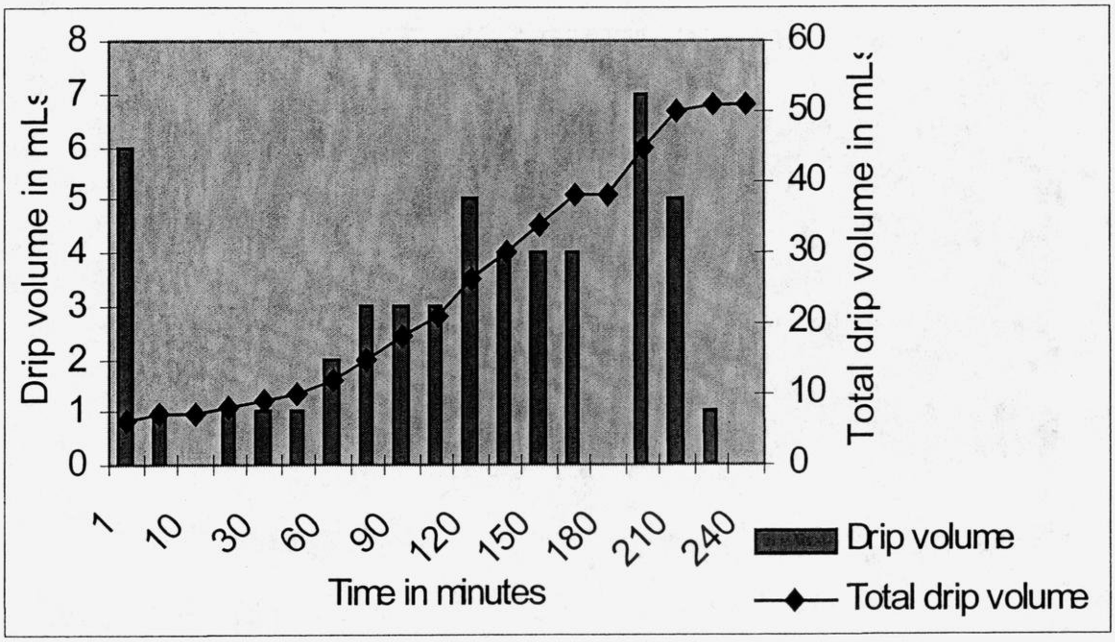



**Figure 9.** 36 inch column receiving 1 gallon/day high strength effluent (column 9)

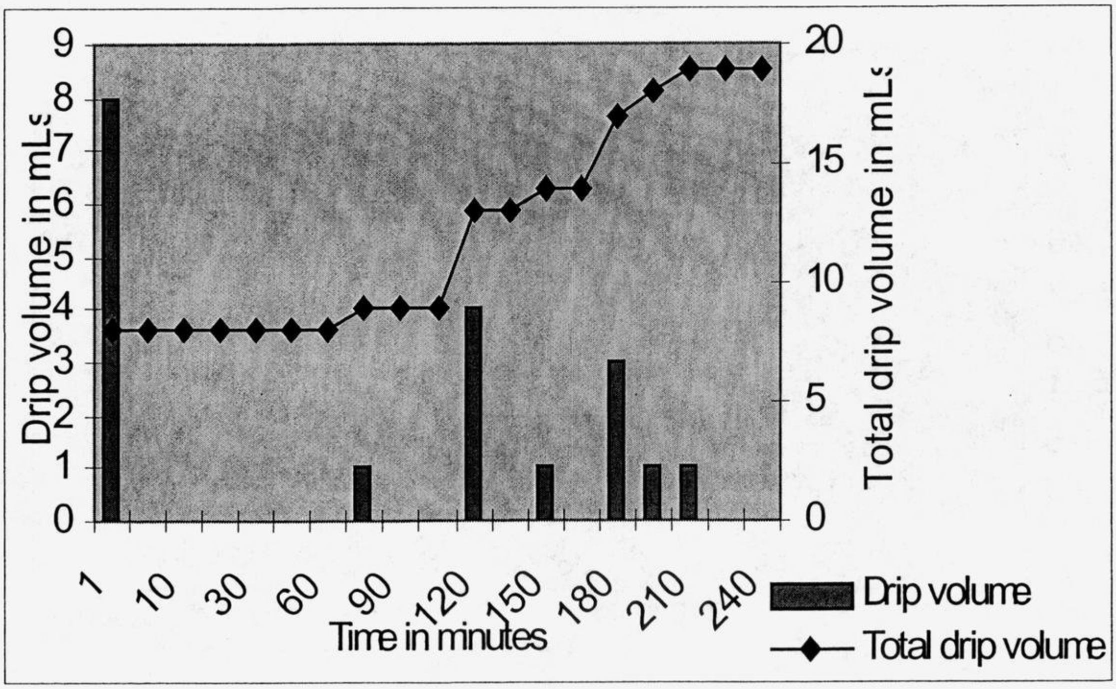


**Figure 10.** 48 inch column receiving 1 gallon/day high strength effluent (column 11)

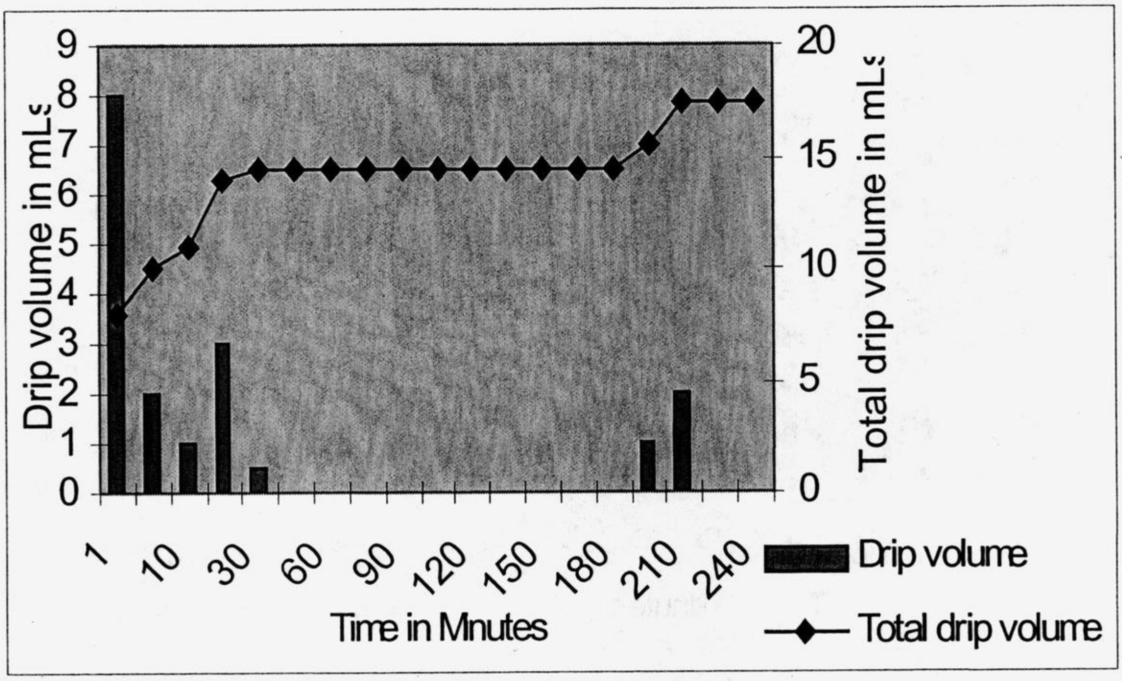



**Figure 11.** 60 inch column receiving 1 gallon/day high strength effluent (column 12)

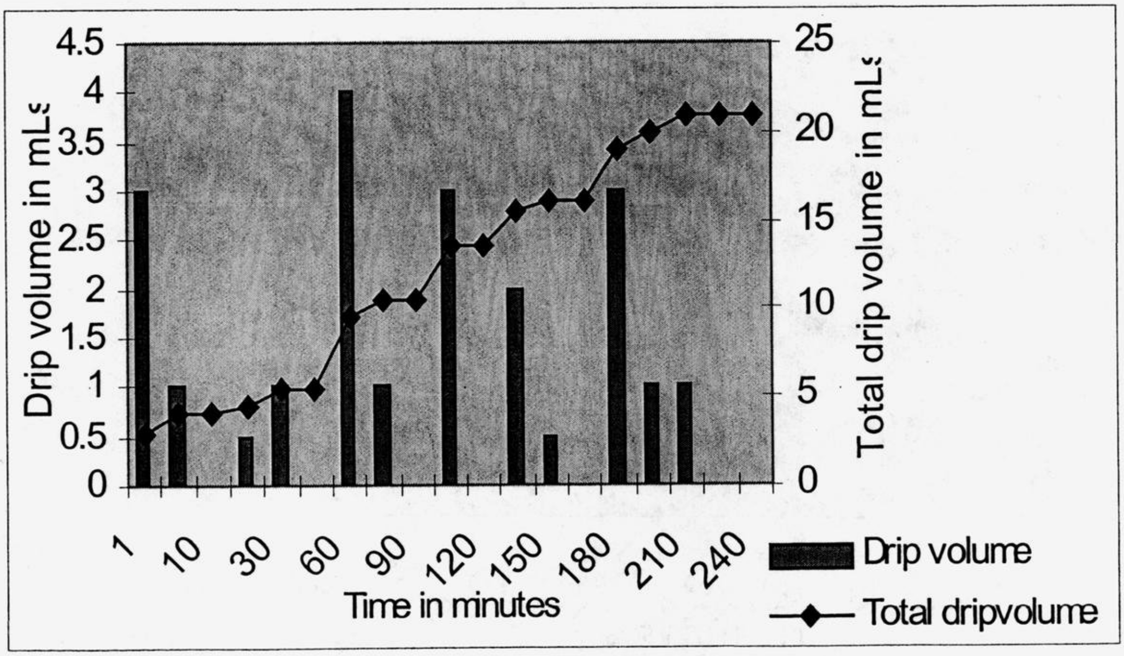
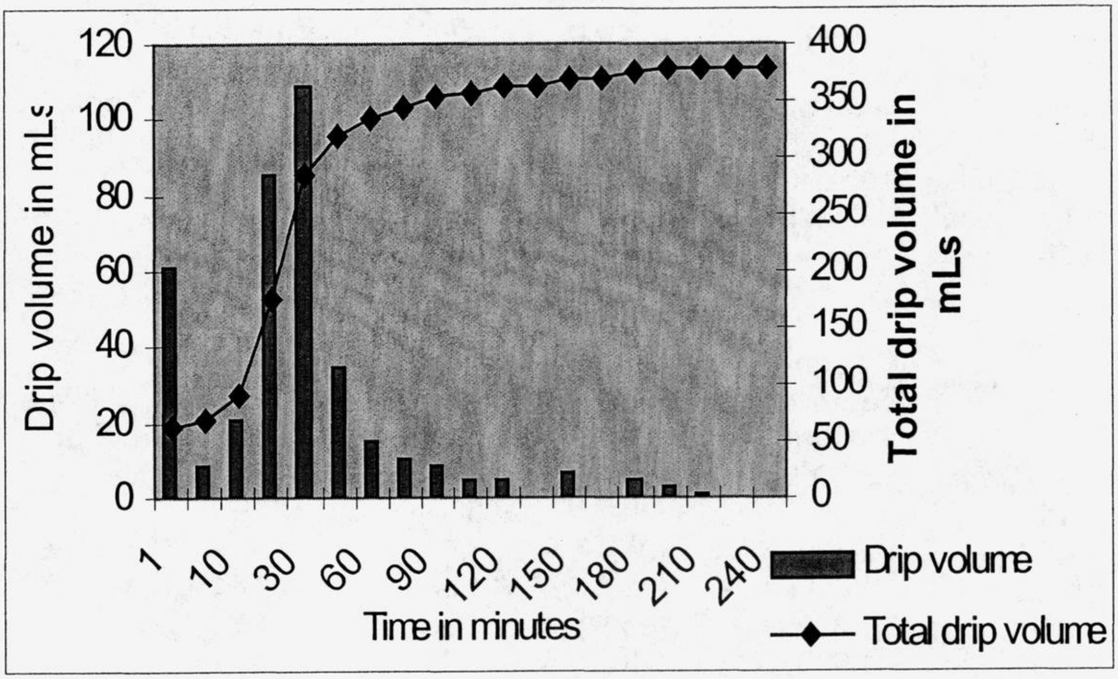


**Figure 12.** 24 inch column receiving 1 gallon/day high strength effluent, dosed once per day (column 10)




Counts of the microbial indicators for all five target organisms were determined for each batch of spike material prepared. This data is presented in tables 9-11. Preparing consistent spikes over the duration of the experiment was one of the most difficult aspects of this study. Although there was some week to week variability in the spiking doses, we are generally very pleased with the results. The spiked septic tank effluents always contained sufficient numbers of organisms to be representative of organism levels that could be expected in actual systems. Tables 9-11 are divided into two portions where the top portion summarizes the target levels for each organism followed by the geometric mean(average) of all the counts for each organism. The second portion of each table contains the raw data from the analysis of each week's spike preparation used to calculate the geometric means.

**Table 9.** Organism spike concentrations for columns 1-3.

| Columns 1-3 | total coliform / 100mL | E. coli / 100mL | fecal coliform / 100mL | Enterococci / 100mL | male-specific coliphage / 100mL |
|---|---|---|---|---|---|
| Target #/100mL | 35,640 | 1440 | 1000 | 1350 | 1000 |
| geometric mean Of all data | 33,035 | 3,002 | 1,247 | 520 | 459 |

| Columns 1-3 | Total coliform / 100mL | E. coli / 100mL | fecal coliform / 100mL | Enterococci /100mL | Male-specific coliphage / 100ml |
|---|---|---|---|---|---|
| *Target #/100mL* | *35,640* | *1440* | *1000* | *1350* | *1000* |
| **12/14/00** | 727 | 548 | 300) | 119 | <5 |
| **1/4/01** | 6370 | 2010 | 270 | 200 | 6500 |
| **1/5/01** | 4410 | 1300 | 260 | 77.1 | 1000 |
| **1/7/01** | 4640 | 1850 | 230 | 54.7 | <5 |
| **1/8/01** | 3680 | 940 | 220 | 77.1 | <5 |
| **1/9/01** | 3,730 | 1414 | 168 | 79.4 | 500 |
| **1/10/01** | 2,210 | 921 | 120 | 63.7 | <5 |
| **1/11/01** | 129,970 | 1,320 | 160 | 107 | 26,000 |
| **1/18/01** | 54,750 | 1,730 | 900 | 145 | 1500 |
| **1/25/01** | 43,500 | 1,565 | 730 | 81.6 | 200 |
| **2/1/01** | 36,900 | 6,867 | 6000 | 1313 | 500 |
| **2/8/01** | 37,300 | 13,200 | 9600 | 813 | 300 |
| **2/15/01** | 51,200 | 2,602 | 1400 | 749 | 100 |
| **2/22/01** | 41,000 | 9,700 | 5300 | 1233 | 100 |
| **3/1/01** | 239,200 | 17,500 | 11,200 | 1565 | 200 |
| **3/9/01** | 52,900 | 14,136 | 3,200 | 1565 | 100 |
| **3/16/01** | 24,000 | 12,997 | 1,400 | 2000 | 100 |
| **3/23/01** | 29,900 | 450 | 390 | 201 | 100 |
| **3/29/01** | 13,400 | 1,000 | 17 | 2000 | 500 |
| **4/5/01** | 12,000 | 98 | 11 | 74 | 1200 |
| **4/12/01** | 13,500 | 1,000 | LA | 107 | 1000 |
| **4/19/01** | 64,400 | 3,448 | 2,200 | 2000 | 1000 |
| **4/26/01** | 23,100 | 1,565 | 660 | 1000 | 600 |
| **5/3/01** | 106,700 | 5,172 | 4,000 | 1112 | 1500 |
| **5/10/01** | 17,500 | 1,669 | 1,900 | 1000 | 1000 |
| **5/17/01** | 31,300 | 5,200 | 760 | 1354 | 900 |
| **5/24/01** | >2,419,200 | 9,800 | 14,300 | 2000 | 1000 |
| **5/31/01** | 57,600 | 8,600 | 6,000 | 1669 | 1100 |

**Table 9. continued**

| Columns 1-3 | Total coliform / 100mL | E. coli / 100mL | fecal coliform / 100mL | Enterococci /100mL | Male-specific coliphage / 100ml |
|---|---|---|---|---|---|
| *Target #/100mL* | *35,640* | *1440* | *1000* | *1350* | *1000* |
| **6/6/01** | 29,900 | 28,200 | 19,800 | 1956 | 300 |
| **6/11/01** | 75,400 | 7,400 | 9,900 | 884 | 200 |
| **6/14/01** | 75,400 | 9,700 | 8,200 | 836 | 500 |
| **6/18/01** | 129,100 | 8,500 | 7,000 | 4100 | 200 |
| **6/20/01** | 117,800 | 3,000 | 1,200 | 860 | 300 |
| **6/25/01** | 49,600 | 3,100 | 770 | 733 | 100 |
| **6/27/01** | 24,600 | 1,145 | 1,300 | 677 | 100 |
| **7/3/01** | 54,800 | 2,000 | 16,000 | 703 | 200 |

**Table 10.** Organism spike concentrations for columns 4,5,6,7, and 10.

| Columns 4-7,10 | total coliform / 100mL | E. coli / 100mL | fecal coliform / 100mL | Enterococci / 100mL | male-specific coliphage / 100mL |
|---|---|---|---|---|---|
| Target #/100mL | 359,568 | 14,528 | 10,000 | 13,620 | 10,000 |
| Geometric mean all data | 295663 | 27075 | 9952 | 5160 | 3516 |
| **12/14/00** | 2,400 | 2,400 | 2500 | 1733 | 1000 |
| **1/4/01** | 81,640 | 15,410 | 1,000 | 1,210 | 12000 |
| **1/5/01** | 57,940 | 12,590 | 1000 | 860 | 8500 |
| **1/7/01** | 98,040 | 8,050 | 900 | 866 | 5500 |
| **1/8/01** | 48,840 | 13,330 | 900 | 740 | 500 |
| **1/9/01** | 30,760 | 10,810 | 690 | 980 | 2000 |
| **1/10/01** | 32,550 | 8650 | 460 | 866.4 | 500 |
| **1/11/01** | 677,000 | 13,170 | 1900 | 1414 | 140,000 |
| **1/18/01** | 496,000 | 14,970 | 3900 | 1450 | 10,000 |
| **1/25/01** | 435,000 | 15,650 | 7300 | 816.4 | 2000 |
| **2/1/01** | 369,000 | 68,670 | 60,000 | 13,130 | 5000 |
| **2/8/01** | 373,000 | 132,000 | 96,000 | 8,130 | 3000 |
| **2/15/01** | 512,000 | 26,020 | 14,000 | 7,490 | 1000 |
| **2/22/01** | 410,000 | 97,000 | 53,000 | 12,330 | 1000 |
| **3/1/01** | 2,392,000 | 175,000 | 112,000 | 15,650 | 2000 |
| **3/9/01** | 529,000 | 141,360 | 32,000 | 15,650 | 1000 |
| **3/16/01** | 240,000 | 129,970 | 14,000 | 20,000 | 1000 |
| **3/23/01** | 299,000 | 4,500 | 3,900 | 2,010 | 1000 |

**Table 10. continued**

| Columns 4-7,10 | Total coliform / 100mL | E. coli / 100mL | fecal coliform / 100mL | Enterococci /100mL | Male-specific coliphage / 100ml |
|---|---|---|---|---|---|
| Target #/100mL | 359,568 | 14,528 | 10,000 | 13,620 | 10,000 |
| **3/29/01** | 134,000 | 10,000 | 170 | 20,000 | 5000 |
| **4/5/01** | 120,000 | 1,046 | 110 | 740 | 12,000 |
| **4/12/01** | 135,000 | 10,000 | | 1,070 | 10,000 |
| **4/19/01** | 644,000 | 34,480 | 22,000 | 20,000 | 10,000 |
| **4/26/01** | 231,000 | 15,650 | 6,600 | 10,000 | 6000 |
| **5/3/01** | 1,067,000 | 51,720 | 40,000 | 11,120 | 15,000 |
| **5/10/01** | 175,000 | 16,690 | 19,000 | 10,000 | 10,000 |
| **5/17/01** | 313,00 | 52,000 | 7,600 | 13,540 | 9,000 |
| **5/24/01** | 24,000,000 | 98,000 | 143,000 | 20,000 | 10,000 |
| **5/31/01** | 576,000 | 86,000 | 60,000 | 16,690 | 11,000 |
| **6/6/01** | 299,000 | 282,000 | 198,000 | 19,560 | 3,000 |
| **6/11/01** | 754,000 | 74,000 | 99,000 | 8,840 | 2,000 |
| **6/14/01** | 754,000 | 97,000 | 82,000 | 8,360 | 5,000 |
| **6/18/01** | 1,291,000 | 85,000 | 70,000 | 41,000 | 2,000 |
| **6/20/01** | 1,178,000 | 30,000 | 12,000 | 8,600 | 3,000 |
| **6/25/01** | 496,000 | 31,000 | 7,700 | 7,330 | 1,000 |
| **6/27/01** | 246,000 | 11,450 | 13,000 | 6,770 | 1,000 |
| **7/3/01** | 548,000 | 20,000 | 160,000 | 7,030 | 2,000 |

**Table 11.** Organism spike concentrations for columns 8,9,11 and 12

| Columns 8,9,11,12 | Total coliform / 100mL | E. coli / 100mL | Fecal coliform / 100mL | Enterococci / 100mL | Male-specific coliphage / 100mL |
|---|---|---|---|---|---|
| Target #/100mL | 6,768,575 | 485,100 | 475,000 | 24,015 | 7559 |
| Geometric mean All data | 16,517,310 | 595,418 | 525,223 | 45,931 | 194,446 |
| **12/14/00** | | | 3,100,000 | | 310,000 |
| **1/4/01** | 19,863,000 | 487,000 | 200,000 | 14,390 | 229,500 |
| **1/5/01** | 24,192,000 | 189,000 | 200,000 | 18,500 | 290500 |
| **1/7/01** | 17,329,000 | 74,000 | 100,000 | 5,880 | 290,000 |
| **1/8/01** | 10,462,000 | 86,640 | 95,000 | 7630 | 88,500 |
| **1/9/01** | 12,033,000 | 104,620 | 108,000 | 7,980 | 95,000 |
| **1/10/01** | 7,701,000 | 81,640 | 86,000 | 4500 | 18,000 |
| **1/11/01** | 48,700,000 | 1,850,000 | 1,060,000 | 29,090 | 28,000 |
| **1/18/01** | 1,000,000 | 985,000 | 470,000 | 46,110 | 160,000 |
| **1/25/01** | 19,900,000 | 637,000 | 400,000 | 18,500 | 64,500 |
| **2/1/01** | 29,800,000 | 473,000 | 430,000 | 20,000 | 350,000 |
| **2/8/01** | 21,800,000 | 1,145,000 | 1,000,000 | 63,000 | 440,000 |

**Table 11, continued**

| Columns 8,9,11,12 | Total coliform / 100mL | E. coli / 100mL | fecal coliform / 100mL | Enterococci /100mL | Male-specific coliphage / 100ml |
|---|---|---|---|---|---|
| Target #/100mL | 6,768,575 | 485,100 | 475,000 | 24,015 | 7559 |
| **2/15/01** | 17,500,000 | 496,000 | 200,000 | 38,730 | 96,000 |
| **2/22/01** | 3,000,000 | 318,000 | 140,000 | 30,760 | 41,000 |
| **3/1/01** | 24,192,000 | 408,000 | 400,000 | 2,000,000 | 140,000 |
| **3/9/01** | 5,475,000 | 278,000 | 92,000 | 3,255,000 | 62,000 |
| **3/16/01** | 17,329,000 | 908,000 | 140,000 | 910,000 | 100,000 |
| **3/23/01** | 14,800,000 | 324,000 | 610,000 | 246,000 | 20,000 |
| **3/29/01** | 12,000,000 | 594,000 | 640,000 | 213,000 | 30,000 |
| **4/5/01** | 24,192,000 | 285,000 | 200,000 | 31,000 | 400,000 |
| **4/12/01** | 7,701,000 | 173,000 | 30,000 | 96,000 | 570,000 |
| **4/19/01** | 32,200,000 | 1,313,000 | 1,160,000 | 31,000 | 410,000 |
| **4/26/01** | 6,300,000 | 613,000 | 200,000 | 54,750 | 240,000 |
| **5/3/01** | 39,900,000 | 455,000 | 440,000 | 54,750 | 970,000 |
| **5/10/01** | 51,200,000 | 780,000 | 5,400,000 | 20,000 | 2,300,000 |
| **5/17/01** | 25,900,000 | 313,000 | 2,600,000 | 20,000 | 440,000 |
| **5/24/01** | 27,200,000 | 862,000 | 820,000 | 86,640 | 450,000 |
| **5/31/01** | 22,800,000 | 1,529,000 | 2,300,000 | 30,000 | 200,000 |
| **6/6/01** | 14,600,000 | 1,000,000 | 1,030,000 | 31,000 | 300,000 |
| **6/11/01** | 13,400,000 | 1,259,000 | 1,430,000 | 52,000 | 140,000 |
| **6/14/01** | 15,531,000 | 1,789,000 | 1,800,000 | 97,000 | 340,000 |
| **6/18/01** | 47,300,000 | 5,172,000 | 3,100,000 | 40,000 | 370,000 |
| **6/20/01** | 24,900,000 | 2,187,000 | 1,600,000 | 30,760 | 490,000 |
| **6/25/01** | 24,196,000 | 2,603,000 | 2,000,000 | 98,000 | 290,000 |
| **6/27/01** | 14,600,000 | 2,000,000 | 1,520,000 | 30,000 | 750,000 |
| **7/3/01** | 24,900,000 | 1,722,000 | 2,500,000 | 18,500 | 270,000 |

## Results and Discussion

The overall objective of this study was to determine if a variety of microbes in septic tank effluents of varying qualities could in fact be removed by passage through mound sand in a controlled laboratory environment. The results are summarized in a series of tables below. All of the data is presented in pairs of tables where the eight columns dosed with the high quality effluent (BOD and suspended solids <30 mg/L) are separated from the four columns that received the low quality septic tank effluent (BOD and suspended solids>100).

In addition to the microbial occurrence data collected on the column effluents, pH values were also determined. Table 12 presents the low, high and average pH values for the weekly effluent samples collected from the bottom of the experimental columns. In all experiments the pH was extremely stable.

**Table 12.**
Summary of column effluent pH's for columns dosed with high quality septic system effluent.

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Column number** | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 10 |
| **Column Length** | **12 inch** | **18 inch** | **24 inch** | **18 inch** | **24 inch** | **30 inch** | **36 inch** | **24 inch** |
| **Doses per day** | **5/day** | **5/day** | **5/day** | **5/day** | **5/day** | **5/day** | **5/day** | **1/day** |
| **Dosing Rate** | 2 gpd/ft$^2$ | 2 gpd/ft$^2$ | 2 gpd/ft$^2$ | 2 gpd/ft$^2$ | 2 gpd/ft$^2$ | 2 gpd/ft$^2$ | 2 gpd/ft$^2$ | 2 gpd/ft$^2$ |
| **Target microbe Dose/ 100 mL** | 1,000 | 1,000 | 1,000 | 10,000 | 10,000 | 10,000 | 10,000 | 10,000 |
| **Low pH** | 7.9 | 8.1 | 8.3 | 8.3 | 8.3 | 8.4 | 8.4 | 8.2 |
| **High pH** | 8.6 | 8.6 | 8.6 | 8.6 | 8.7 | 8.6 | 8.6 | 8.7 |
| **Average pH** | 8.3 | 8.4 | 8.5 | 8.4 | 8.5 | 8.5 | 8.5 | 8.4 |

**Table 13.**
Summary of column effluent pH's for columns dosed with low quality septic system effluent.

| | | | | |
|---|---|---|---|---|
| **Column number** | 8 | 9 | 11 | 12 |
| **Column Length** | **24 inch** | **36 inch** | **48 inch** | **60 inch** |
| **Doses per day** | **5/day** | **5/day** | **5/day** | **5/day** |
| **Dosing Rate** | 1 gpd/ft$^2$ | 1 gpd/ft$^2$ | 1 gpd/ft$^2$ | 1 gpd/ft$^2$ |
| **Target microbe dose/100mL** | 1,000,000 | 1,000,000 | 1,000,000 | 1,000,000 |
| **Low pH** | 7.8 | 7.6 | 8.0 | 7.8 |
| **High pH** | 8.5 | 8.4 | 8.5 | 8.4 |
| **Average pH** | 8.1 | 8.1 | 8.3 | 8.0 |

Total coliform is the indicator that has been traditionally used by water microbiologists since 1920 to detect microbial contamination of wells. While total coliform does, in fact, detect outside contamination of a water well system, its ability to accurately predict the presence of fecal material is limited. The total coliform data is presented in tables 14 and 15. The data collected through week 18 on the columns dosed with the high quality effluent 5 times per day at 2 gpd/ft$^2$ conclusively demonstrates the ability of mound sand to remove almost all of the total coliform organisms introduced to the tops of the columns. The occasional low level detections were most likely due to the difficulty in maintaining sterile conditions during sample collection. The removal efficiency could be characterized as four log reduction, or 99.99% removal.

The 24 inch column dosed once per day with high quality effluent spiked with 10,000 organisms/ 100 mL, presents a different picture. Total coliforms appeared in the effluent of this column at week three and continued to increase in numbers over time. This once per day dosing scenario resulted in a removal efficiency of 3 logs or 99.9%. Additionally when increases in dosing volume were begun on the remainder of the columns at week 19, with a doubling of the flow rate, passage of total coliform can be observed in the 18 inch column dosed with $3x10^5$ organisms but not in the longer columns, or in the columns dosed with $3.3x10^4$ organisms. At week 29 the flow rate was again doubled, resulting in total coliform breakthrough in all of the columns. For the 24, 36,48 and 60 inch columns dosed with the low quality effluent and $1.7x10^7$ organisms, breakthrough also didn't occur until the second phase of dose increases was begun. Once the columns failed, removal efficiencies on these columns dropped to less than 2 logs and was similar for all three dose levels. There is one quirk in the data that we were unable to explain. Of the four columns dosed with the low quality effluent and the highest microbial counts, the shortest (24 inch) performed the best.

It should be noted that the 48 inch column became bio-fouled two weeks after the dosing rate was increased. A microbial mat formed at the surface of the column resulting in an over flow from the top of the column which in turn dripped down the outside of the column contaminating the effluent. The counts for this column from weeks 24 and 27 are most likely from the dripping contamination. Testing on this column was terminated at this point.

**Table 14.** Total coliform levels/ 100 mLs for columns dosed with high quality septic system effluent.

| *Column number* | *1* | *2* | *3* | *4* | *5* | *6* | *7* | *10* |
|---|---|---|---|---|---|---|---|---|
| **Column Length** | 12 inch | 18 inch | 24 inch | 18 inch | 24 inch | 30 inch | 36 inch | 24 inch |
| **Doses per day** | 5/day | 5/day | 5/day | 5/day | 5/day | 5/day | 5/day | 1/day |
| **Dosing Rate** | 2 gpd/ft$^2$ | 2 gpd/ft$^2$ | 2 gpd/ft$^2$ | 2 gpd/ft$^2$ | 2 gpd/ft$^2$ | 2 gpd/ft$^2$ | 2 gpd/ft$^2$ | 2 gpd/ft$^2$ |
| **Volume/dose** | 74 mLs | 74 mLs | 74 mLs | 74 mLs | 74 mLs | 74 mLs | 74 mLs | 372mLs |
| **Microbe dose level/100mL** | 33,000 | 33,000 | 33,000 | 300,000 | 300,000 | 300,000 | 300,000 | 300,000 |
| **Week 3 12/15/00** | 2 | 1 | 0 | 0 | 0 | 2 | 0 | 47.2 |
| **Week 6 1/04/01** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 410.6 |
| **Week 9 1/25/01** | 2 | 0 | 0 | 6.3 | 0 | 0 | 0 | 129.6 |
| **Week 12 2/15/01** | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 16,160 |
| **Week 15 3/7/01** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 24,810 |
| **Week 18 3/28/01** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3270 |
| **Week 21 4/19/01*** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| **Volume/dose** | 148 mL | 148 mL | 148 mL | 148 mL | 148 mL | 148 mL | 148 mL | 744 mLs |
| **Week 23 5/3/01** | 7.4 | | | 200 | | | | 816.4 |
| **Week 24 5/9/01** | 0 | 1 | 0 | 53.8 | 14.6 | sacrificed | 0 | 81,640 |
| **Week 27 6/1/01** | 0 | 0 | 0 | 365.4 | 28.2 | | 0 | |
| **Week 29 6/14/01**** | 57.6 | 310 | 13.5 | 13,960 | 114.5 | | 310 | |
| **Volume/dose** | 296 mL | 296 mL | 296 mL | 296 mL | 296 mL | | 296 mL | |
| **Week 30 6/19/01** | 58.3 | 116.2 | 8.4 | 3,950 | 0 | | 630 | |
| **Week 30 6/21/01** | 27.9 | 66.3 | 6.3 | 3,310 | 95.9 | | 410 | |
| **Week 30 6/22/01** | 18.5 | 29.5 | 8.6 | 2430 | 113.7 | | 285.1 | |
| **Week 31 6/26/01** | 17.3 | 56.3 | 12.2 | 1732.9 | 107.1 | | 261.3 | |
| **Week 31 6/28/01** | 23.1 | 45.5 | 9.8 | 3360 | 435.2 | | 78.9 | |

- *Flow rate doubled at end of week 19
- **Flow rate quadrupled at end of week 28

**Table 15.** Total coliform levels/100mLs for columns dosed with low quality septic system effluent.

| *Column number* | *8* | *9* | *11* | *12* |
|---|---|---|---|---|
| **Column Length** | 24 inch | 36 inch | 48 inch | 60 inch |
| **Dosing Rate** | 1 gpd/ft$^2$ | 1 gpd/ft$^2$ | 1 gpd/ft$^2$ | 1 gpd/ft$^2$ |
| **Dosing volume** | 37 mLs | 37 mLs | 37 mLs | 37 mLs |
| **Doses per day** | 5/day | 5/day | 5/day | 5/day |
| **Microbe dose level/100mL** | 17,000,000 | 17,000,000 | 17,000,000 | 17,000,000 |
| **Week 3 12/15/00** | 0 | 0 | 0 | 0 |
| **Week 6 1/04/01** | 0 | 0 | 0 | 0 |
| **Week 9 1/25/01** | 0 | 0 | 0 | 0 |
| **Week 12 2/15/01** | 0 | 0 | 0 | 0 |
| **Week 15 3/7/01** | 0 | 0 | 0 | 0 |
| **Week 18 3/28/01*** | 0 | 0 | 0 | 0 |
| **Dosing volume** | 74mLs | 74mLs | 74mLs | 74mLs |
| **Week 21 4/19/01** | 0 | 0 | 0 | 12.1 |
| **Week 23 5/3/01** | 0 | | | |
| **Week 24 5/9/01** | 0 | 0 | 20.3 | 3.1 |
| **Week 27 6/1/01** | 0 | 0 | >2419.2 | 0 |
| **Week 29 6/14/01**** | >241,920 | 310 | biofouled | >241,920 |
| **Dosing volume** | 148mLs | 148mLs | 148mLs | 148mLs |
| **Week 30 6/19/01** | | | | |
| **Week 30 6/21/01** | 3590 | >241,960 | | >241,960 |
| **Week 30 6/22/01** | 2750 | 6050 | | >241,960 |
| **Week 31 6/26/01** | 23,590 | 241,960 | | >241,960 |
| **Week 31 6/28/01** | 98,040 | >241,960 | | >241,960 |
| | 141,360 | >241,960 | | >241,960 |

*Flow rate doubled at end of week 19
**Flow rate quadrupled at end of week 28

The fecal coliform data is presented in tables 16 and 17. Once again, the total absence of this indicator in the effluents from all columns before the dose rates were increased, supports the observation that the mound sand is very capable of removing enteric bacteria. With one exception, the conclusions and observations that can be drawn from this data are the same as those outlined above related to the total coliform data set. The exception is that the columns dosed with the high quality effluent with the lowest concentrations of organisms showed very little passage of fecal coliform, even after dosing rates were increased.

**Table 16.** Fecal coliform levels/100 mLs for columns dosed with high quality septic system effluent.

| *Column number* | *1* | *2* | *3* | *4* | *5* | *6* | *7* | *10* |
|---|---|---|---|---|---|---|---|---|
| **Column Length** | 12 inch | 18 inch | 24 inch | 18 inch | 24 inch | 30 inch | 36 inch | 24 inch |
| **Doses per day** | 5/day | 5/day | 5/day | 5/day | 5/day | 5/day | 5/day | 1/day |
| **Dosing Rate** | 2 gpd/ft$^2$ | 2 gpd/ft$^2$ | 2 gpd/ft$^2$ | 2 gpd/ft$^2$ | 2 gpd/ft$^2$ | 2 gpd/ft$^2$ | 2 gpd/ft$^2$ | 2 gpd/ft$^2$ |
| **Volume/dose** | 74 mLs | 74 mLs | 74 mLs | 74 mLs | 74 mLs | 74 mLs | 74 mLs | 372mLs |
| **Microbe dose level/100mL** | 1,200 | 1,200 | 1,200 | 10,000 | 10,000 | 10,000 | 10,000 | 10,000 |
| **Week 3 12/15/00** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 17 |
| **Week 6 1/04/01** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 |
| **Week 9 1/25/01** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 60 |
| **Week 12 2/15/01** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5600 |
| **Week 15 3/7/01** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1000 |
| **Week 18 3/28/01*** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
| **Volume/dose** | 148 mL | 148 mL | 148 mL | 148 mL | 148 mL | 148 mL | 148 mL | 744 mLs |
| **Week 21 4/19/01** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Week 23 5/3/01** | 0 | | | 1 | | | | |
| **Week 24 5/9/01** | 0 | 0 | 0 | 0 | 0 | sacrificed | 0 | 3900 |
| **Week 27 6/1/01** | 0 | 0 | 0 | 10 | 2 | | 0 | 810 |
| **Week 29 6/14/01**** | 10 | 40 | <10 | 4500 | 50 | | 100 | 11,500 |
| **Volume/dose** | 296 mL | 296 mL | 296 mL | 296 mL | 296 mL | | 296 mL | |
| **Week 30 6/19/01** | 10 | 20 | 0 | 360 | 40 | | 230 | |
| **Week 30 6/21/01** | 0 | 0 | 0 | 400 | 20 | | 80 | |
| **Week 30 6/22/01** | 0 | 0 | 0 | 300 | 0 | | 30 | |
| **Week 31 6/26/01** | 0 | 0 | 0 | 40 | 30 | | 40 | |
| **Week 31 6/28/01** | 0 | 0 | 0 | 50 | 0 | | 0 | |

*Flow rate doubled at end of week 19

**Flow rate quadrupled at end of week 28

**Table 17.** Fecal coliform levels/100 mLs for columns dosed with low quality septic system effluent.

| *Column number* | *8* | *9* | *11* | *12* |
|---|---|---|---|---|
| **Column Length** | 24 inch | 36 inch | 48 inch | 60 inch |
| **Dosing Rate** | 1 gpd/ft$^2$ | 1 gpd/ft$^2$ | 1 gpd/ft$^2$ | 1 gpd/ft$^2$ |
| **Dosing volume** | 37 mLs | 37 mLs | 37 mLs | 37 mLs |
| **Doses per day** | 5/day | 5/day | 5/day | 5/day |
| **Average microbe dose level/100mL** | 530,000 | 530,000 | 530,000 | 530,000 |
| **Week 3 12/15/00** | 0 | 0 | 0 | 0 |
| **Week 6 1/04/01** | 0 | 0 | 0 | 0 |
| **Week 9 1/25/01** | 0 | 0 | 0 | 0 |
| **Week 12 2/15/01** | 0 | 0 | 0 | 0 |
| **Week 15 3/7/01** | 0 | 0 | 0 | 0 |
| **Week 18 3/28/01** | 0 | 0 | 0 | 0 |
| **Week 21 4/19/01*** | 0 | 0 | 0 | 0 |
| **Dosing volume** | 74mLs | 74mLs | 74mLs | 74mLs |
| **Week 23 5/3/01** | 0 | | | |
| **Week 24 5/9/01** | 0 | 0 | 0 | 0 |
| **Week 27 6/1/01** | 0 | 0 | 15,000 | 0 |
| **Week 29 6/14/01**** | 2500 | 20 | biofouled | 800 |
| **Dosing volume** | 148mLs | 148mLs | 148mLs | 148mLs |
| **Week 30 6/19/01** | 470 | 190,000 | | 190,000 |
| **Week 30 6/21/01** | 90 | 500 | | 270,000 |
| **Week 30 6/22/01** | 330 | 18,000 | | 220,000 |
| **Week 31 6/26/01** | 4700 | 120,000 | | 0 |
| **Week 31 6/28/01** | 5100 | 238,000 | | 8,600 |

*Flow rate doubled at end of week 19
**Flow rate quadrupled at end of week 28

The data from the *E. coli* testing is presented in tables 18 and 19. The data and subsequent conclusions and observations tracks well with the fecal coliform data with one exception. The *E. coli* counts tend to be slightly higher and there are a few more detections in columns 1-3. This is most likely due to the testing methodology. It has been well documented that the Colilert™ test system is more effective at recovering stressed organisms and thus is expected to have higher counts and more detections at low levels than the fecal coliform (MFFCC) test.

**Table 18.** *E. coli* levels/100 mLs for columns dosed with high quality septic system effluent

| *Column number* | *1* | *2* | *3* | *4* | *5* | *6* | *7* | *10* |
|---|---|---|---|---|---|---|---|---|
| **Column Length** | 12 inch | 18 inch | 24 inch | 18 inch | 24 inch | 30 inch | 36 inch | 24 inch |
| **Doses per day** | 5/day | 5/day | 5/day | 5/day | 5/day | 5/day | 5/day | 1/day |
| **Dosing Rate** | 2 gpd/ft$^2$ | 2 gpd/ft$^2$ | 2 gpd/ft$^2$ | 2 gpd/ft$^2$ | 2 gpd/ft$^2$ | 2 gpd/ft$^2$ | 2 gpd/ft$^2$ | 2 gpd/ft$^2$ |
| **Volume/dose** | 74 mLs | 74 mLs | 74 mLs | 74 mLs | 74 mLs | 74 mLs | 74 mLs | 372mLs |
| **Microbe dose level/100mL** | 3,000 | 3,000 | 3,000 | 27,000 | 27,000 | 27,000 | 27,000 | 27,000 |
| **Week 3 12/15/00** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 39.3 |
| **Week 6 1/04/01** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 172.3 |
| **Week 9 1/25/01** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 58.1 |
| **Week 12 2/15/01** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5280 |
| **Week 15 3/7/01** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6760 |
| **Week 18 3/28/01** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 40.8 |
| **Week 21 4/19/01*** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3.1 |
| **Volume/dose** | 148 mL | 148 mL | 148 mL | 148 mL | 148 mL | 148 mL | 148 mL | 744 mLs |
| **Week 23 5/3/01** | 0 | | | 6.3 | | | | |
| **Week 24 5/9/01** | 0 | 0 | 0 | 6.3 | 2 | sacrificed | 0 | 5280 |
| **Week 27 6/1/01** | 0 | 0 | 0 | 11.9 | 1 | | 0 | 9330 |
| **Week 29 6/14/01**** | 100 | 100 | 3.1 | 6050 | 17.3 | | 200 | 31,000 |
| **Volume/dose** | 296 mL | 296 mL | 296 mL | 296 mL | 296 mL | | 296 mL | |
| **Week 30 6/19/01** | 14.5 | 14.5 | 3.1 | 860 | 0 | | 410 | |
| **Week 30 6/21/01** | 1 | 3.1 | 1 | 410 | 12 | | 100 | |
| **Week 30 6/22/01** | 2 | 5.2 | 3.1 | 300 | 11 | | 103.9 | |
| **Week 31 6/26/01** | 0 | 1 | 0 | 85.5 | 2 | | 38.4 | |
| **Week 31 6/28/01** | 0 | 0 | 0 | 210 | 4.1 | | 13.4 | |

**Table 19.** *E. coli* levels/100 mLs for columns dosed with low quality septic system effluent

| *Column number* | *8* | *9* | *11* | *12* |
|---|---|---|---|---|
| **Column Length** | 24 inch | 36 inch | 48 inch | 60 inch |
| **Doses per day** | 5/day | 5/day | 5/day | 5/day |
| **Dosing volume** | 37 mLs | 37 mLs | 37 mLs | 37 mLs |
| **Dosing Rate** | 1 gpd/ft$^2$ | 1 gpd/ft$^2$ | 1 gpd/ft$^2$ | 1 gpd/ft$^2$ |
| **Microbe dose level/100mL** | 600,000 | 600,000 | 600,000 | 600,000 |
| **Week 3 12/15/00** | 0 | 0 | 0 | 0 |
| **Week 6 1/04/01** | 0 | 0 | 0 | 0 |
| **Week 9 1/25/01** | 0 | 0 | 0 | 0 |
| **Week 12 2/15/01** | 0 | 0 | 0 | 0 |
| **Week 15 3/7/01** | 0 | 0 | 0 | 0 |
| **Week 18 3/28/01** | 0 | 0 | 0 | 0 |
| **Week 21 4/19/01*** | 0 | 0 | 0 | 0 |
| **Dosing volume** | 74mLs | 74mLs | 74mLs | 74mLs |
| **Week 23 5/3/01** | 0 | | | |
| **Week 24 5/9/01** | 0 | 0 | 0 | 0 |
| **Week 27 6/1/01** | 0 | 0 | >2419.2 | 0 |
| **Week 29 6/14/01**** | 2090 | 1 | biofouled | 173,290 |
| **Dosing volume** | 148mLs | 148mLs | 148mLs | 148mLs |
| **Week 30 6/19/01** | 410 | 241,960 | | 241,960 |
| **Week 30 6/21/01** | 310 | 980 | | >241,960 |
| **Week 30 6/22/01** | 630 | 26,130 | | >241,960 |
| **Week 31 6/26/01** | 4040 | >241,960 | | 16,070 |
| **Week 31 6/28/01** | 6700 | >241,960 | | 48,840 |

*Flow rate doubled at end of week 19
**Flow rate quadrupled at end of week 28

The data from the enterococci testing is presented in tables 20 and 21. This bacteria was included in the study because its physical characteristics allow it to pass through a soil matrix more readily than coliforms. Once again the mound sand was capable of removing the organism as long as the columns were not hydraulically overloaded.

**Table 20.** Enterococci levels/100 mLs for columns dosed with high quality septic system effluent.

| *Column number* | *1* | *2* | *3* | *4* | *5* | *6* | *7* | *10* |
|---|---|---|---|---|---|---|---|---|
| Column Length | 12 inch | 18 inch | 24 inch | 18 inch | 24 inch | 30 inch | 36 inch | 24 inch |
| Doses per day | 5/day | 5/day | 5/day | 5/day | 5/day | 5/day | 5/day | 1/day |
| Dosing Rate | 2 gpd/ft$^2$ | 2 gpd/ft$^2$ | 2 gpd/ft$^2$ | 2 gpd/ft$^2$ | 2 gpd/ft$^2$ | 2 gpd/ft$^2$ | 2 gpd/ft$^2$ | 2 gpd/ft$^2$ |
| Volume/dose | 74 mLs | 74 mLs | 74 mLs | 74 mLs | 74 mLs | 74 mLs | 74 mLs | 372 mLs |
| Microbe dose level/100mL | 460 | 460 | 460 | 3,500 | 3,500 | 3,500 | 3,500 | 3,500 |
| Week 6 1/04/01 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Week 9 1/25/01 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4.1 |
| Week 12 2/15/01 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 16.1 |
| Week 15 3/7/01 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 122.3 |
| Week 18 3/28/01 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 310 |
| Week 21 4/19/01* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 21.8 |
| Volume/dose | 148 mL | 148 mL | 148 mL | 148 mL | 148 mL | 148 mL | 148 mL | 744 mLs |
| Week 23 5/3/01 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 14.8 |
| Week 24 | 0 | | | 0 | | | | |
| Week 27 6/1/01 | 0 | 0 | 0 | 0 | 0 | sacrificed | 0 | 344.8 |
| Week 29 6/14/01** | 1 | 0 | 0 | 1 | 0 | | 0 | 648.8 |
| Volume/dose | 296 mL | 296 mL | 296 mL | 296 mL | 296 mL | | 296 mL | 1488mL |
| Week 30 6/19/01 | 0 | 0 | 0 | 7980 | 0 | | 0 | 410 |
| Week 30 6/21/01 | 0 | 0 | 0 | 200 | 0 | | 3.1 | |
| Week 30 6/22/01 | 3.1 | 1 | 0 | 30.9 | 1 | | 2 | |
| Week 31 6/26/01 | 2 | 1 | 0 | 21.6 | 0 | | 0 | |
| Week 31 6/28/01 | 0 | 0 | 0 | 16 | 0 | | 0 | |

*Flow rate doubled at end of week 19

**Flow rate quadrupled at end of week 28

**Table 21.** Enterococci levels/100 mLs for columns dosed with low quality septic system effluent.

| *Column number* | *8* | *9* | *11* | *12* |
|---|---|---|---|---|
| **Column Length** | 24 inch | 36 inch | 48 inch | 60 inch |
| **Doses per day** | 5/day | 5/day | 5/day | 5/day |
| **Dosing volume** | 37 mLs | 37 mLs | 37 mLs | 37 mLs |
| **Dosing Rate** | 1 gpd/ft$^2$ | 1 gpd/ft$^2$ | 1 gpd/ft$^2$ | 1 gpd/ft$^2$ |
| **Microbe dose level/100mL** | 46,000 | 46,000 | 46,000 | 46,000 |
| **Week 3 12/15/00** | 0 | 0 | 0 | 0 |
| **Week 6 1/04/01** | 0 | 0 | 0 | 0 |
| **Week 9 1/25/01** | 0 | 0 | 0 | 0 |
| **Week 12 2/15/01** | 0 | 0 | 0 | 0 |
| **Week 15 3/7/01** | 0 | 0 | 0 | 0 |
| **Week 18 3/28/01** | 0 | 0 | 0 | 0 |
| **Week 21 4/19/01*** | 0 | 0 | 0 | 0 |
| **Dosing volume** | 74mLs | 74mLs | 74mLs | 74mLs |
| **Week 23 5/3/01** | 0 | | | |
| **Week 24** | 0 | 0 | 0 | 0 |
| **Week 27 6/1/01** | 0 | 0 | 324.1 | 0 |
| **Week 29 6/14/01**** | 410 | 6.3 | biofouled | 12,100 |
| **Dosing volume** | 148mLs | 148mLs | 148mLs | 148mLs |
| **Week 30 6/19/01** | 35.9 | 3590 | | 0 |
| **Week 30 6/21/01** | 32.3 | 100 | | 5,290 |
| **Week 30 6/22/01** | 58.3 | 410 | | 100 |
| **Week 31 6/26/01** | 200 | 1320 | | 620 |
| **Week 31 6/28/01** | 410 | 4640 | | 850 |

*Flow rate doubled at end of week 19

**Flow rate quadrupled at end of week 28

Male specific coliphage were included in this study as a surrogate for enteric virus. The extremely small size of virus particles as well as their surface charge characteristics make them the most likely organisms to pass through mound sand. Virus passage is particularly worrisome since the majority of gastro-intestinal disease in the U.S. is caused by viruses. The data collected to determine the passage of coliphage through the columns is presented in tables 22 and 23. For almost all of the columns dosed with the high quality effluent, even those spiked with the higher organism levels of organisms, all of the coliphage were removed. The exception was one occasion where a low level was detected from the 24 inch column dosed once per day. The organism was detected in each of the columns receiving the low quality effluent with the highest organism doses after the dosing rates were increased.

**Table 22.** Male Specific Coliphage/100 mLs levels for columns dosed with high quality septic system effluent.

| *Column number* | *1* | *2* | *3* | *4* | *5* | *6* | *7* | *10* |
|---|---|---|---|---|---|---|---|---|
| Column Length | 12 inch | 18 inch | 24 inch | 18 inch | 24 inch | 30 inch | 36 inch | 24 inch |
| Doses per day | 5/day | 5/day | 5/day | 5/day | 5/day | 5/day | 5/day | 1/day |
| Dosing Rate | 2 gpd/ft$^2$ | 2 gpd/ft$^2$ | 2 gpd/ft$^2$ | 2 gpd/ft$^2$ | 2 gpd/ft$^2$ | 2 gpd/ft$^2$ | 2 gpd/ft$^2$ | 2 gpd/ft$^2$ |
| Volume/dose | 74 mLs | 74 mLs | 74 mLs | 74 mLs | 74 mLs | 74 mLs | 74 mLs | 372 mLs |
| Microbe dose Level/100 mL | 460 | 460 | 460 | 3,500 | 3,500 | 3,500 | 3,500 | 3,500 |
| Week 3 12/15/00 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Week 6 1/04/01 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Week 9 1/25/01 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Week 12 2/15/01 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Week 15 3/7/01 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Week 18 3/28/01 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Week 21 4/20/01* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Volume/dose | 148 mL | 148 mL | 148 mL | 148 mL | 148 mL | 148 mL | 148 mL | 744 mLs |
| Week 24 5/9/01 | 0 | 0 | 0 | 0 | 0 | sacrificed | 0 | 10 |
| Week 27 6/1/01 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 |
| Week 29 6/14/01** | 0 | 0 | 0 | 0 | 0 | | 0 | 0 |
| Volume/dose | 296 mL | 296 mL | 296 mL | 296 mL | 296 mL | | 296 mL | |
| Week 30 6/19/01 | 0 | 0 | 0 | 0 | 0 | | 0 | |
| Week 30 6/21/01 | 0 | 0 | 0 | 0 | 0 | | 0 | |
| Week 30 6/22/01 | 0 | 0 | 0 | 0 | 0 | | 0 | |
| Week 31 6/26/01 | 0 | 0 | 0 | 0 | 0 | | 0 | |
| Week 31 6/28/01 | 0 | 0 | 0 | 0 | 0 | | 0 | |

*Flow rate doubled at end of week 19

**Flow rate quadrupled at end of week 28

**Table 23.** Male Specific coliphage/100mLs levels for columns dosed with low quality septic system effluent.

| *Column number* | *8* | *9* | *11* | *12* |
|---|---|---|---|---|
| **Column Length** | 24 inch | 36 inch | 48 inch | 60 inch |
| **Doses per day** | 5/day | 5/day | 5/day | 5/day |
| **Dosing volume** | 37 mLs | 37 mLs | 37 mLs | 37 mLs |
| **Dosing Rate** | 1 gpd/ft$^2$ | 1 gpd/ft$^2$ | 1 gpd/ft$^2$ | 1 gpd/ft$^2$ |
| **Microbe dose Level/100 mL** | 190,000 | 190,000 | 190,000 | 190,000 |
| **Week 3 12/15/00** | 0 | 0 | 0 | 0 |
| **Week 6 1/04/01** | 0 | 0 | 0 | 0 |
| **Week 9 1/25/01** | 30 | 0 | 0 | 100 |
| **Week 12 2/15/01** | 0 | 0 | 0 | 0 |
| **Week 15 3/7/01** | 0 | 0 | 0 | 0 |
| **Week 18 3/28/01** | 0 | 0 | 0 | 0 |
| **Week 21 4/20/01*** | 0 | 0 | 0 | 0 |
| **Dosing volume** | 74mLs | 74mLs | 74mLs | 74mLs |
| **Week 23 5/3/01** | 0 | | | |
| **Week 24 5/9/01** | 0 | 0 | 0 | 0 |
| **Week 27 6/1/01** | 0 | 0 | 1170 | 0 |
| **Week 29 6/14/01**** | 20 | 0 | Bio-fouled | 1350 |
| **Dosing volume** | 148mLs | 148mLs | 148mLs | 148mLs |
| **Week 30 6/19/01** | 40 | 30 | | 3270 |
| **Week 30 6/21/01** | 140 | 0 | | 20 |
| **Week 30 6/22/01** | 120 | 0 | | 0 |
| **Week 31 6/26/01** | 250 | 280 | | 120 |
| **Week 31 6/28/01** | 190 | 390 | | 180 |

*Flow rate doubled at end of week 19

**Flow rate quadrupled at end of week 28

Five months into the study the researchers were surprised that very few organisms were passing through the columns. At week number 23 of the study it was decided to sacrifice the 30 inch column being dosed with the high quality effluent with the middle microbe dose to determine how far into the column the organisms had traveled. The column was removed from the rack and the sand was removed by pushing it up from the bottom using a piston device. Two inch sand segments from the first 12 inches were collected into separate sterile sampling bags as were four inch segments from the remainder of the column. Organisms from these sand samples were then extracted into sterile buffer which was subsequently tested for each of the study organisms. Interpretation of the data from the examination of sand samples for the organisms must be done with the understanding that the sample size tested is only 1% of what can be tested for a water sample. Additionally, the extraction of the organisms from the sand matrix may result in substantial loss. It was beyond the scope of this study to characterize this loss. The results are presented in table 24. None of the coliphage or enterococci were able to penetrate or survive in any part of the column. The total coliform, and to some extent the *E. coli* and fecal coliform were detected in decreasing amounts as you moved down the column.

**Table 24.** Organism counts in sand from sacrificed 30 inch column - high quality effluent, 1000 FC/100mL dose rate, dosed 5 times per day, 2gpd/ft$^2$

| | *total coliform /gm* | *E. coli /gm* | *Enterococci /gm* | *fecal coliform /gm* | *coliphage /gm* |
|---|---|---|---|---|---|
| gravel / sand interface | 91 | 4 | <1 | 10 | <300 |
| 0 - 2 inches | 74 | 1 | <1 | <10 | <300 |
| 2 - 4 inches | 85 | 3 | <1 | <10 | <300 |
| 4 - 6 inches | 18 | <1 | <1 | <10 | <300 |
| 6 - 8 inches | 11 | 1 | 1 | <10 | <300 |
| 8 - 10 inches | 10 | 1 | <1 | <10 | <300 |
| 10 - 12 inches | 3 | <1 | <1 | <10 | <300 |
| 12 - 16 inches | 5 | 1 | <1 | <10 | <300 |
| 16 - 20 inches | 5 | <1 | <1 | <10 | <300 |
| 20 - 24 inches | 5 | <1 | <1 | <10 | <300 |
| 24 - 28 inches | 4 | <1 | <1 | <10 | <300 |
| 28 - 30 inches | <1 | <1 | <1 | <10 | <300 |
| Residual | <1 | <1 | <1 | <10 | <300 |

At the completion of all dosing, the sand from various depths in four additional columns was collected and analyzed as described above. The data is presented in tables 25-28. For column 3, a 24 inch column dosed with high quality effluent with the lowest organism spike dose, decreasing levels of total coliforms were detected as the sampling moved down the column. While few sporadic low level enterococci and *E. coli* detections occurred, no coliphage or fecal coliforms were observed. For column 5, a 24 inch column dosed with high quality effluent with the middle organism spike dose, the findings were the same as for column 3 with the exception that the organism levels were somewhat higher. Column 8, a 24 inch column dosed with the low quality septic tank effluent with the highest organism dose, the trends are the same as columns 3 and 5 but with higher levels and significant amounts of enterococci found. Fecal coliform also shows up in this column, while no coliphage was detected. In this column the fecal coliform levels are significantly higher than the *E. coli* levels. A possible explanation for this would be that *Klebsiella*, which is the organism detected in the fecal coliform test in addition to *E. coli* is not only passing through the column, but multiplying. This phenomenon has been reported in other studies and is the main reason the fecal coliform has been replaced by the *E. coli* test as the preferred monitoring tool. The data from column12, the 60 inch column dosed with the low quality effluent and high microbe concentration, is similar to the column 8 data.

**Table 25.** Column 3- 24 inches, high quality effluent, 1000 FC/100mL dose rate, dosed 5 times per day, 2gpd/$ft^2$

| | total coliform / gm | *E. coli* /gm | Enterococci /gm | fecal coliform /gm | coliphage /gm |
|---|---|---|---|---|---|
| 0-1 inches | 137.6 | 2 | <1 | <10 | <300 |
| 1-2 inches | 101.9 | <1 | 1 | <10 | <300 |
| 2 - 4 inches | 65 | <1 | 2 | <10 | <300 |
| 4 - 6 inches | 23.1 | 4.1 | 3.1 | <10 | <300 |
| 6 - 8 inches | 10.8 | <1 | <1 | <10 | <300 |
| 8 - 10 inches | 12.2 | <1 | 1 | <10 | <300 |
| 10 - 12 inches | 2 | <1 | <1 | <10 | <300 |
| 12-14 inches | 1 | <1 | <1 | <10 | <300 |
| 14-16 inches | 1 | <1 | <1 | <10 | <300 |
| 16-18 inches | 1 | <1 | <1 | <10 | <300 |
| 18-20 inches | <1 | <1 | <1 | <10 | <300 |
| 20-22 inches | <1 | <1 | <1 | <10 | <300 |
| 22-24 inches | 2 | <1 | <1 | <10 | <300 |
| Residual | 1 | <1 | <1 | <10 | <300 |

**Table 26.** Column 5 -24 inches, high quality effluent, 10,000 FC/100mL dose rate, dosed 5 times per day, 2gpd/$ft^2$

| | total coliform /gm | *E. coli* /gm | Enterococci /gm | fecal coliform /gm | male-specific coliphage /gm |
|---|---|---|---|---|---|
| 0-1 inches | 1732.9 | 6.3 | 26.5 | <10 | <300 |
| 1-2 inches | 387.3 | 13.4 | 27.9 | <10 | <300 |
| 2 - 4 inches | 344.8 | 12.2 | 13.4 | <10 | <300 |
| 4 - 6 inches | 105.4 | 8.6 | 3.1 | <10 | <300 |
| 6 - 8 inches | 67 | 3.1 | <1 | <10 | <300 |
| 8 - 10 inches | 27.5 | 2 | 2 | <10 | <300 |
| 10 - 12 inches | 14.8 | 1 | 1 | <10 | <300 |
| 12-14 inches | 8.5 | 2 | 1 | <10 | <300 |
| 14-16 inches | 10.9 | <1 | 1 | <10 | <300 |
| 16-18 inches | 12 | 3.1 | 1 | <10 | <300 |
| 18-20 inches | 9.7 | <1 | <1 | <10 | <300 |
| 20-22 inches | 8.6 | <1 | <1 | <10 | <300 |
| 22-24 inches | 10.6 | <1 | <1 | <10 | <300 |
| Residual | 6.3 | <1 | <1 | <10 | <300 |

**Table 27.** Column 8 - 24 inches, low quality effluent, 1,000,000 FC/100mL dose rate, dosed 5 times per day, 1gpd/$ft^2$

| | total coliform /gm | *E. coli* /gm | Enterococci /gm | fecal coliform /gm | male-specific coliphage /gm |
|---|---|---|---|---|---|
| 0-1 inches | >2419.6 | 488.4 | 70.8 | 10,000 | <300 |
| 1-2 inches | >2419.6 | 435.2 | 98.7 | 9000 | <300 |
| 2 - 4 inches | >2419.6 | 260.3 | 24.3 | 12,000 | <300 |
| 4 - 6 inches | >2419.6 | 72.3 | 52.1 | 2000 | <300 |
| 6 - 8 inches | >2419.6 | 40.4 | 24.6 | 1000 | <300 |
| 8 - 10 inches | >2419.6 | 4.1 | 5.2 | 240 | <300 |
| 10 - 12 inches | >2419.6 | 5.2 | 4.1 | 170 | <300 |
| 12-14 inches | >2419.6 | 12.1 | 7.4 | 150 | <300 |
| 14-16 inches | >2419.6 | 4.1 | 1 | 100 | <300 |
| 16-18 inches | >2419.6 | 9.7 | 2 | 20 | <300 |
| 18-20 inches | 2419.6 | 9.6 | 4.1 | 70 | <300 |
| 20-22 inches | 1119.9 | 7.4 | 1 | 40 | <300 |
| 22-24 inches | >2419.6 | 12.1 | 3.1 | 20 | <300 |
| Residual | 325.5 | 4.1 | 2 | 10 | <300 |

**Table 28.** Column 12- 60 inches, low quality effluent, 1,000,000 FC/100mL dose rate, dosed 5 times per day, 2gpd/ft$^2$

| | total coliform /gm | *E. coli* /gm | Enterococci /gm | fecal coliform /gm | coliphage /gm |
|---|---|---|---|---|---|
| 0-1 inches | >2419.6 | 613.1 | 344.8 | 7300 | <300 |
| 1-2 inches | >2419.6 | 770.1 | 117.8 | 5000 | <300 |
| 2 - 4 inches | >2419.6 | 206.4 | 30.1 | 1900 | <300 |
| 4 - 6 inches | >2419.6 | 35.5 | 6.2 | 450 | <300 |
| 6 - 8 inches | >2419.6 | 5.2 | 2 | 70 | <300 |
| 8 - 10 inches | >2419.6 | 9.8 | 3 | 110 | <300 |
| 10 - 12 inches | 579.4 | <1 | 4.1 | 50 | <300 |
| 12-14 inches | 547.5 | <1 | 1 | <10 | <300 |
| 14-16 inches | 344.8 | 2 | <1 | 20 | <300 |
| 16-18 inches | 1732.9 | 13.4 | 6.3 | <10 | <300 |
| 18-20 inches | 770.1 | 7.4 | 4 | 30 | <300 |
| 20-22 inches | 224.7 | <1 | <1 | 10 | <300 |
| 22-24 inches | 125.9 | <1 | <1 | 10 | <300 |
| 24-30 inches | 37.9 | <1 | 1 | <10 | <300 |
| 30-36 inches | 5.2 | <1 | <1 | <10 | <300 |
| 36-42 inches | 9.8 | <1 | <1 | <10 | <300 |
| 42-48 inches | 12.2 | <1 | <1 | <10 | <300 |
| 48-54 inches | 1 | <1 | <1 | <10 | <300 |
| 54-60 inches | <1 | <1 | <1 | <10 | <300 |

One of the goals of this project was to compare various microbial indicators regarding their ability to pass through the mound sand. The researchers expected that enterococci and coliphages would pass through the sand matrix more readily than coliforms and *E. coli*, and thus prove to be better indicators of septic system failure. Interestingly this was not the case.

## Conclusions

The objective of this project was to determine if microbes can be removed as they pass through a mound sand matrix in a controlled laboratory environment. It must be noted that all of the conclusions drawn below are based on a laboratory controlled environment may or may not represent what actually happens in "real world" systems. The study looked at a matrix of several variables which could influence passage; sand depth, general quality of effluent (strength as measured by biological oxygen demand and suspended solids), hydraulic dosing rate, and microbe dose level. The results can be summarized as follows

1. Organisms in reasonably clean effluents do not pass through mound sand in columns 12 inches or longer when dosed evenly throughout the day at the rate of two gallons per day per square foot of surface area.

2. Organisms in low quality septic tank effluents do not pass through mound sand in columns 24 inches or longer when dosed evenly throughout the day at the rate of one gallon per day per square foot of surface area.
3. If mound sand columns are loaded with either poorly spaced dosing or excessive flows, organisms will pass through the sand, even when the column length is 60 inches.
4. Mound sand removes all of the organisms tested with equal efficiency.