Field Evaluation of Wastewater Soil Absorption Systems with Aggregate-Free and Aggregate-Laden Infiltrative Surfaces

Final Report

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ACRONYMS AND ABBREVIATIONS

$A_{I.S.}$	-	area of the infiltrative surface
BG	-	background
bgs	-	below ground surface
BKGD	-	background
BOD_5	-	biochemical oxygen demand at 5-days
$cBOD_5$	-	carbonaceous biochemical oxygen demand at 5-days
CSM	-	Colorado School of Mines
cha	-	chamber
cfu	-	colony forming unit
CV	-	coefficient of variation
D	-	depth
dw	-	dry weight
ESE	-	Environmental Science & Engineering Division
FC	-	fecal coliforms
gra	-	gravel
HLR	-	hydraulic loading rate
ID	-	identification
I.S.	-	infiltrative surface
ISI	-	Infiltrator Systems, Inc.
MS-2	-	bacteriophage that infects *E. coli.*
nd	-	nondetectable
ND	-	no data
Ne	-	effective porosity
PBS	-	phosphate buffered saline solution
pfu	-	plague forming unit
PRD-1	-	bacteriophage that infects *Salmonella typhimurium*
SCS	-	Soil Conservation Service
STE	-	septic tank effluent
Q	-	flow rate
TBD	-	to be determined
T_r	-	travel time required
TNTC	-	too numerous to count
TOC	-	total organic carbon
USDA	-	U.S. Department of Agriculture
WSAS	-	wastewater soil absorption system

ACKNOWLEDGMENTS

This report describes the methods and results of a field study and ancillary laboratory studies completed in Colorado to evaluate the field performance of wastewater soil absorption systems including both aggregate-free (chamber) and aggregate-laden (gravel) designs. This work was made possible by the contributions and support of several individuals and organizations. The work was sponsored in large part by Infiltrator Systems, Inc. and ISI is acknowledged for their interest and support in advancing the state-of-knowledge regarding onsite wastewater system design and performance. This study would not have been possible without the participation and cooperation of the homeowners at the 16 individual residences studied. In addition, the following individuals are recognized for their contributions and valuable support:

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1.0 EXECUTIVE SUMMARY

This report discusses a field study completed at the Colorado School of Mines (CSM) that characterizes the hydraulic and purification performance of mature onsite wastewater systems (i.e., systems that have been in operation for more than one year), including systems with aggregate-free (chamber) and aggregate-laden (gravel) infiltrative surfaces. This work included a survey of 16 individual homes located near Silverthorne and Brighton, Colorado from May through December 1999. At 16 of the study sites, septic tank effluent (STE) was characterized, and at 14 of these sites, intact soil cores were successfully acquired and the soil properties were characterized with depth below the soil infiltrative surface. Effluent and soil core samples show constituent levels in the range of previously reported work. As expected, levels of nitrogen species and fecal coliform bacteria decreased with increased depth below the infiltrative surface. A large degree of variation in constituent concentrations was observed between individual systems and among duplicate cores taken within the same system. Analysis of the data revealed no significant differences between aggregate-free and aggregate-laden systems with respect to common measures used to assess hydraulic and treatment performance.

Ancillary testing was also conducted to establish a correlation between fecal coliform data collected from percolate water samples and that extracted from soil solids samples. Results in two different sand media suggest that samples of soil solids yield calculated percolate concentrations of fecal coliforms that are consistently higher than those directly measured in percolating water. This suggests that soil solids analysis can provide a conservative measure of fecal coliform bacteria in percolating soil solution.

This report also describes the methodology and results for evaluating the treatment of virus in an individual WSAS with an aggregate free infiltrative surface. During this test, two bacteriophages (MS-2 and PRD-1) and a conservative tracer (Br^-) were added to the STE before it was applied to the soil absorption system. Weekly samples of the STE were taken and characterized for the surrogate and tracer concentrations. Twenty-five days following the first addition of the surrogates and tracer, soil core samples were taken and analyzed for the added constituents. Removal of the added bacteriophages was estimated to be 3-logs between the infiltrative surface and 60-cm depth. Comparison of the concentrations of fecal coliforms to the bacteriophages measured in the same soil core samples revealed that the presence of fecal coliforms was directly and strongly correlated with the presence of MS-2 and PRD-1 virus.

The results of this study suggest that aggregate-free systems in Colorado that are sized with 50% less infiltration area for a given design flow are performing comparably to the larger aggregate-laden systems. These field results are consistent with the results of intermediate-scale sand lysimeter studies performed previously at CSM (Van Cuyk et al., 1999; Siegrist et al., 1999). The lysimeter studies showed that the treatment performance of aggregate-free systems sized at 8.4 cm/d was comparable to aggregate-laden systems sized at 5.0 cm/d during the most sensitive period, that being startup through the first year of operation, even though the hydraulic loading rate of the former is approximately 67% higher based on gross soil infiltrative surface area.

2.0 INTRODUCTION

2.1. BACKGROUND

Wastewater treatment for onsite and small community applications commonly relies on infiltration and percolation of primary effluent through soil to achieve purification prior to recharge to ground water (U.S. EPA, 1978; 1980; 1997; Jenssen and Siegrist, 1990; Crites and Tchobanoglous, 1998) (Fig. 2.1). These wastewater soil absorption systems (WSAS) can achieve high purification efficiencies due to the complex interactions of hydraulic and purification processes (Fig. 2.2) (Schwagger and Boller, 1997; Ausland, 1998; McCray et al., 2000). Extensive and lengthy contact between wastewater constituents and the soil matrix and associated biofilms occurs during unsaturated flow achieved by daily loadings limited to a small fraction of the soil's saturated hydraulic conductivity (K_{sat}) (e.g., <5 cm/d). In addition, a clogging zone evolves at the soil infiltrative surface (Fig. 2.1 and 2.2) which leads to reduced permeability and more uniform infiltration and a concomitant unsaturated flow almost regardless of hydraulic loading. Wastewater-induced clogging increases the soil biogeochemical activity and can enhance sorption, biotransformation and die-off/inactivation processes within the clogging zone itself or in the underlying unsaturated soil (Siegrist, 1987; Siegrist et al., 1991; Ausland, 1998; Van Cuyk et al., 1999; McCray et al., 2000). Clogging zone genesis has been described as a humification-like process and modeled as a function of the mass loading rates of wastewater suspended matter and bio-oxidizable substances (Siegrist, 1987; Siegrist and Boyle, 1987). In most WSAS, clogging zone genesis must occur to some degree to foster the advanced purification required before ground water recharge, but not to the point where it causes hydraulic problems.

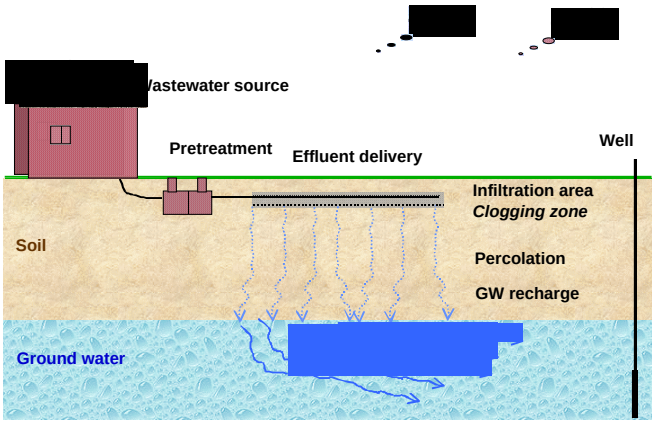

Fig. 2.1. Illustration of an onsite wastewater soil absorption system typical of the 25 million systems in operation in the U.S. today.

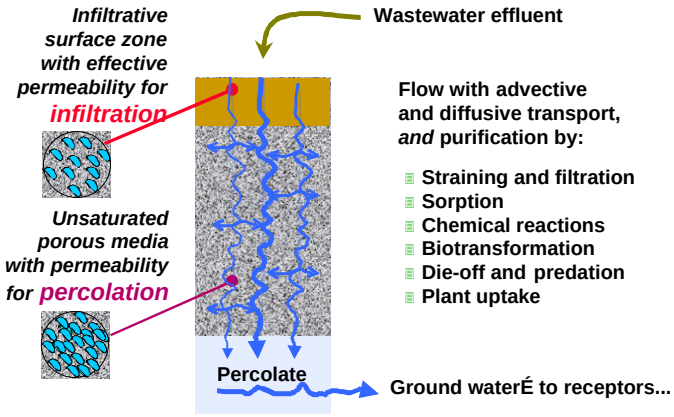

Fig. 2.2. Illustration of hydraulic and purification processes operative in a wastewater soil absorption system.

If clogging zone development is retarded or absent altogether, for example due to the application of highly pretreated effluent (e.g., sand filter effluent), purification of pathogens and other constituents of concern may be less than predicted and desired. Conversely, if soil clogging is too excessive, for example due to application of high strength effluents (e.g., restaurant wastewater), clogging can be detrimental by causing hydraulic dysfunction and soil anaerobiosis and reduced purification (e.g., slower organic matter breakdown and reduced nitrification).

System physical features, operational parameters, and environmental conditions can determine hydraulic and purification behaviors in wastewater infiltration systems. As briefly described below, the infiltrative surface character and the underlying unsaturated soil depth above a ground water table (a.k.a., vadose zone), are two system features that are commonly determined during design. The soil infiltrative surface is normally located below the original ground surface and commonly has a 15- to 30-cm thick layer of 2- to 4-cm diameter gravel placed on it to provide storage for peak wastewater flows and to support the overburden soils (Fig. 2.1). Performance data regarding the rate and extent of soil clogging in systems with gravel on the infiltrative surface (aggregate-laden) led to system designs that avoid the use of gravel aggregate (e.g., open chamber, fabric-wrapped piping, plastic media, fabric bundles). The most common type of system that provides an open or aggregate-free surface involves the use of chambers (Keys, 1996; May, 1996; Tyler et al., 1991).

Gravel on an infiltrative surface can reduce infiltration zone permeability (or infiltrability) by (1) blocking pore entries, (2) becoming embedded in the soil matrix, (3) yielding fines that are deposited in pore entries, or (4) focusing wastewater constituents as a result of the reduced permeability due to the effects of (1)-(3) (Amerson, et al., 1991; Jenssen and Siegrist, 1990; Siegrist, 1987; Siegrist and Boyle, 1987; Siegrist et al., 1991; Tyler and Converse, 1994). Based on an equivalency concept with respect to infiltrability, aggregate-free systems are being utilized with design infiltration areas (i.e., gross total area provided) on the order of 40% less than required with aggregate-laden systems. While keeping the daily loading rate onto the open or effective infiltrative area the same (i.e., that surface not masked or impacted directly by gravel), this strategy does increase the relative hydraulic loading rate on the gross infiltration area by 67%. While previous experience with aggregate-free systems has revealed satisfactory hydraulic performance (May, 1996; England and Dix, 1999), until recently, comparatively less experimental data has existed regarding purification performance (Van Cuyk et al., 1999).

The depth of the soil vadose zone to ground water can affect hydraulic function and in turn purification by influencing the soil water content, aeration status, media surface area, and hydraulic retention time. In the U.S., depths for soil infiltration systems range from 0.6 to 1.2 m and for intermittent sand filters, from 0.6 and 0.9 m (US EPA, 1980; Anderson et al., 1985; Crites and Tchobanoglous, 1998). While a high degree of treatment normally occurs in the infiltration zone as soil clogging develops, at higher hydraulic loading rates and with nonuniform distribution methods, constituents of concern that would normally be treated can be transported through the vadose zone to ground water. For example, many studies have shown that a large percentage of bacteria remain near the infiltrative surface when effluents are applied to porous media (Brown et al., 1979; Kristiansen, 1991; Smith et al., 1985; Huysman and Verstraete, 1993; Emerick et al., 1997; Stevik et al., 1999). However, if hydraulic loading rates are too high or the dosing frequency is too low, some microbes can be transported to lower regions in a soil matrix,

posing a purification concern in systems that are too shallow to ground water. Alternatively, while depth is important to hydraulic and purification behavior, at some point there is limited gain in purification by increasing vadose zone depth (Peeples et al., 1991).

In Colorado, wastewater soil absorption systems (WSAS) are designed based on long-term acceptance rates (LTAR) and a design flow estimated at 225 gpd/bedroom (based on 75 gpcd * 2 per/bedroom* 150% peaking factor). The LTAR ranges are typical of those reported in the literature and used in other state codes (e.g., fine to loamy sand w/ 6-10 minute per inch (MPI) percolation test = 1.2 gpd/ft^2, sandy loam to loam w/ 11-20 MPI = 0.72 gpd/ft^2, loam w/21-30 MPI = 0.50 gpd/ft^2, silt loam to sandy clay loam w/ 31-40 MPI = 0.40 gpd/ft^2, etc.). Thus, design application rates in Colorado are mostly in the range of 0.40 to 1.2 gpd/ft^2. The required distance between the bottom of an infiltration trench or bed and high ground water is 4 ft. Regulations allow infiltration area to include sidewall area (below the distribution pipe) and bottom area. The State allows a 50% reduction in the standard infiltration area sizing of WSAS's when Infiltrator chambers are used. As of Fall 1999, there were about 26,000 Infiltrator chamber systems installed in Colorado since the first installations occurred in 1991.

2.2. GOALS, OBJECTIVES AND SCOPE

Research was initiated in the Environmental Science & Engineering Division at the Colorado School of Mines (CSM) to study the hydraulic and purification behavior of wastewater soil infiltration systems from start-up through initial clogging zone development and to quantify the effects of infiltrative surface character and vadose zone soil depth. The entire research effort is comprised of controlled laboratory experimentation with 3-dimensional lysimeters, field monitoring of mature soil infiltration systems, and transport/fate and process modeling. The methods and results of the 3-D lysimeter studies are described in previous publications and forthcoming papers (e.g., Fischer, 1999; Masson, 1999; Van Cuyk et. al., 1999; Siegrist et al., 1999). Results of these 3-D laboratory lysimeter studies completed in 1999 revealed that the performance of aggregate-free systems was comparable to aggregate-laden systems, even though the hydraulic loading rate of the aggregate-free system is 67% higher (i.e., 8.4 vs. 5.0 cm/day, respectively, based on gross horizontal area provided). For both system types, it was shown that a 60- to 90-cm depth to groundwater provided adequate depth of unsaturated media for purification of conventional pollutants (e.g., $cBOD_5$, suspended solids, ammonia nitrogen) as well as bacteria and virus to occur.

Field studies were initiated during late 1998 and intensive sampling and analysis was completed during the fall of 1999. These studies were designed to complement the lysimeter studies by focusing on infiltration of domestic STE in more mature soil absorption systems under field conditions. At each of 14 to 16 onsite WSAS that had been in operation for one year or longer, wastewater effluent quality being applied to the soil and the corresponding constituent concentrations with soil depth below the infiltrative surface were characterized through sampling and analyses. Completion of the field studies was intended to provide insight into the comparative performance of aggregate-free versus aggregate-laden systems after maturity is reached and incipient or continuous ponding is present. The field studies described herein were completed in two parts:

(1) Monitoring of 16 systems of which 10 were Infiltrator chambers and 6 were gravel systems. Data collected included residence characteristics, system design features, STE composition, occurrence and depth of ponding of the infiltration surface, and chemical and bacterial characteristics with depth below the infiltrative surface.

(2) Evaluation of virus treatment in one of the study sites using a conservative tracer (Br^-) and two viral surrogates (MS-2 and PRD-1 bacteriophages).

This report describes the methods and results of this field work. Section 3 contains a description of the monitoring of field WSAS's for physical properties and chemical and bacterial treatment while Section 4 summarizes the virus treatment study. Section 5 presents the conclusions and recommendations derived from the work.

3.0 MONITORING OF HYDRAULIC AND TREATMENT PERFORMANCE

3.1. TECHNICAL APPROACH AND METHODS

3.1.1. Home Identification and Characteristics

Study subdivisions in Colorado were identified based on individual expressions of interest to collaborate with the CSM research team by county environmental health department staff as well as subdivision developers and homeowner's associations. The two study areas identified included the Hamilton Creek subdivision in Summit County, Colorado and the Todd Creek Farms subdivision in Adams County, Colorado. The Hamilton Creek subdivision is located approximately 60 miles west of Denver, near the town of Silverthorne and is at an elevation of ~9,000 feet. Todd Creek Farms subdivision, located 40 miles northeast of Denver, is at an elevation of ~5,000 feet.

Working with the environmental health departments in Summit County and Tri-County, Colorado, individual homes within each of the two study subdivisions were identified by letters of invitation to participate and by support from key subdivision persons (e.g., president of homeowners association). Homeowner questionnaires were used to gather information regarding (1) dwelling occupancy and water using and waste generating fixtures and (2) general soil absorption system design features. General screening criteria were established so the study would include individual wastewater systems that were: designed and installed according to modern practice; between 1 and 10 years old; and loaded at $\geq$25 to 50% of the design flow capacity. Detailed information regarding the onsite system site evaluation, design, and installation was gathered from county records, as-built construction drawings and interviews with homeowners, as well as field observations.

In the Hamilton Creek subdivision, a pool of systems were identified that had been in operation for periods of 1 to 5 years or longer and included both aggregate-free Infiltrator chamber systems and aggregate-laden gravel systems. A total of 11 systems were sampled in Hamilton Creek, including 7 with chambers and 4 with gravel at the infiltrative surface (Table 3.1). For all of the homes in Hamilton Creek, STE samples were collected and at 9 of these homes, soil cores were taken and analyzed. In the Todd Creek Farms subdivision, a total of 5 homes were monitored, including 3 with chambers and 2 with gravel at the infiltrative surface (Table 3.1). At all of these homes, STE samples and soil cores were collected.

All of the onsite systems included septic tank pretreatment with a dosed (but not uniform, pressure distribution) application of STE to WSAS trenches or narrow beds. Some characteristics of the homes and their onsite systems are summarized in Table 3.1 while a photograph of a Hamilton Creek home is shown in Figure 3.1.

Table 3.1. Selected characteristics of onsite wastewater systems monitored in this study.

Home site[1] ID	Res. size (BR)	Water use (gal/mon)	Date of system installation	Infiltrative surface (I.S.) type	Area of I.S. (ft^2)	Est. HLR[2] (cm/day)	Ponded	STE samples (no.)	Soil core
1	4	6083	1995	Chamber	600	1.4	No	2	Yes
2	3	7785	1991	Chamber	396	2.7	Yes	2	Yes
3	6	5150	1988/97[3]	Gravel	558	1.2	No	2	Yes
4	4	7625	1988	Gravel	1680	0.6	Yes	1	No
5	5	7008	1994	Chamber	528	1.8	No	2	Yes
6	4	4167	1991	Chamber	735	0.8	ND	1	No
7	3	5191	1989	Chamber	930	0.8	Yes	1	Yes
8	2	2058	1989	Gravel	651	0.4	Yes	2	Yes
9	3	2308	1994	Chamber	738	0.4	No	1	Yes
10	4	5350	1992	Gravel	864	0.8	Yes	1	Yes
11	4	8500	1990	Chamber	393	2.9	Yes	1	Yes
12*	3	7550	1997	Chamber	2244	0.4	No	2	Yes
13*	3	-	1996	Gravel	1680	-	Yes	1	Yes
14*	4	-	1997	Chamber	TBD	-	Yes	1	Yes
15*	3	9400	1997	Gravel	1536	0.8	No (wet)	1	Yes
16*	3	9880	1998	Chamber	2208	0.6	Yes	1	Yes

[1] * denotes homes located in Brighton, CO.
[2] HLR = flow (gpd)/area of infiltrative surface (ft^2) where 1 cm/d = 0.24 gpd/ft^2.
[3] After a system hydraulic failure, a new soil infiltration system was installed in 1997.

Fig. 3.1. Study home in Hamilton Creek subdivision in Summit County, Colorado.

3.1.2. Evaluation and Monitoring Methods

Soil Characteristics. General soil characteristics for the two subdivision locations were initially assessed from USDA Soil Conservation Survey (SCS) reports (USDA, 1974; 1977). Soils in the Hamilton Creek subdivision near Silverthorne were reported to consist of Anvik and Frisco soils (mixed Boralfic and mixed Typic Cryoborolls) of deep, well drained material formed in colluvium and glacial drift derived from a variety of rocks (USDA, 1977). These soils are on mountainous uplands that have slopes of 6-35%. The typical soil profile includes a brown loam surface layer (0-15 in., 0-38 cm) with a subsoil of clay loam (15-20 in., 38-51 cm). The depth to bedrock is >5 ft. (1.5 m) and depth to high ground water is > 6 ft. (1.8 m). Rock fragments (10-24 in. (25-60 cm) diameter) make up 30-80% of the solum. Soils in the Todd Creek Farms subdivision near Brighton consist of nearly level to strongly sloping, well drained Platner and Ulm loams (fine, montmorillonitic mesic soils) (USDA, 1974). The typical soil profile includes a heavy loam surface layer (0-7 in., 0-18 cm), with a subsoil of silty clay (7-13 in., 18-33 cm) and a substratum of clay (13-22 in., 33-56 cm). It is listed by SCS as having slow permeability and an average depth to bedrock of 40 to 60 in. (1-1.5 m).

Soil samples collected from the depths of the infiltrative surface in the two subdivisions were analyzed for grain size distribution and these results revealed a coarse-grained soil texture which included considerable pebble and cobble fractions. As shown in Fig. 3.2, the grain size analyses revealed <10 wt.% of the soil was in the silt and clay fraction (i.e., wt.% passing through a no. 200 sieve which is 0.074 mm diameter).

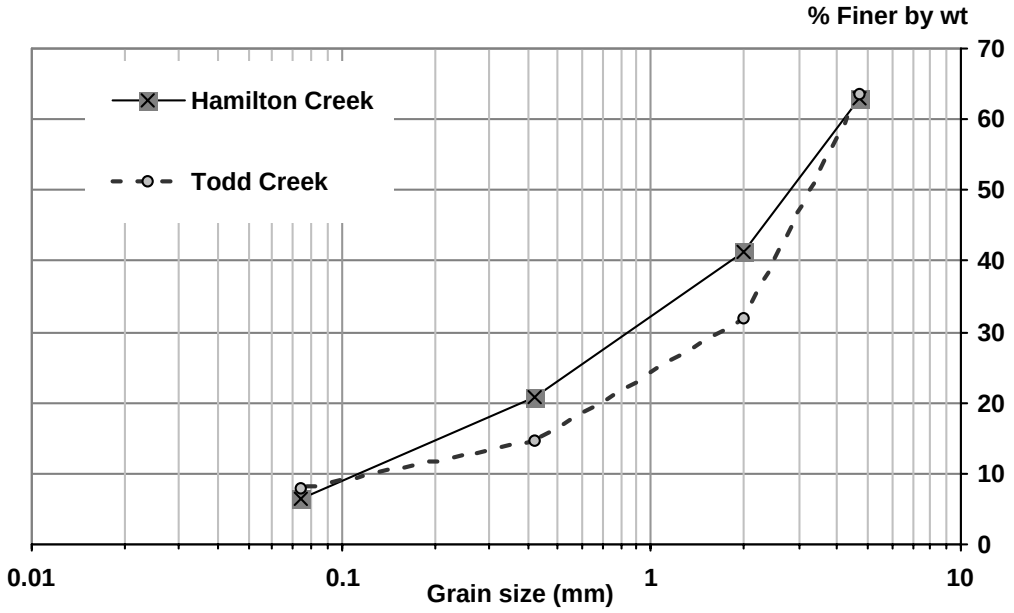

Fig. 3.2. Grain size distributions for soil samples collected from the infiltrative surface depths at study sites in two subdivisions monitored in Colorado.

Wastewater Flow and Hydraulic Loading Rate. Water use data were collected via water use records and/or periodic readings of water meters at each home. The water use data were

assumed to be representative of wastewater flow. The flow data were used along with the WSAS area determined from the as-built plans to calculate the estimated hydraulic loading rate (HLR) in gpd/ft^2 that each WSAS was actually receiving (see Table 3.1).

Septic Tank Effluent Sampling and Analysis. Septic tank effluent was collected from the dosing chamber or from within the baffle in the last compartment of the septic tank. Grab samples were taken and placed in sterile polypropylene bottles and stored at 4C until brought to the laboratory for analysis. Two STE samples were collected from most sites at least 7 days apart. All laboratory analysis of the STE was performed within 24 hours of sample collection. The following characteristics of the STE were determined following standard methods (APHA, 1998).

- pH was measured electrometrically.
- Alkalinity was measured (total alkalinity) via titration with sulfuric acid according to APHA method 2320B.
- $cBOD_5$ (carbonaceous biochemical oxygen demand) was measured according to APHA method 5210B.
- COD analysis was performed using a Hach reactor digestion, colorimetric method (Hach 1992, U.S. EPA-approved).
- Total solids and total suspended solids were measured according to APHA methods 2540B and 2540D.
- Total nitrogen (TN) was measured by persulfate digestion, nitrate nitrogen by chromotropic acid method and ammonium by salicylate method (Hach 1992, U.S. EPA-approved).
- Total phosphorus (TP) was measured according to EPA acid persulfate method (U.S. EPA 365.2).
- Fecal coliform analysis was performed by membrane filtration according to APHA method 9222D. All dilutions plated in duplicate.

WSAS Soil Coring, Sampling and Analysis. The WSAS was probed at two spatially separate locations using double casing and thin-tube sampling methods. This entailed hand excavation from ground surface to the top of a chamber or gravel trench (see Figs. 3.3 and 3.4). In the case of a chamber, an access hole was cut in the top of the chamber. With a gravel system, hand excavation was completed to the top of the gravel. In either case, when any ponding was present, a steel stove-pipe or PVC plastic pipe was used to case the hand excavation hole. To enable penetration through the gravel, the casing was gradually driven down and aggregate was removed with a post-hole digging tool. The occurrence and magnitude of ponding within the system was manually determined.

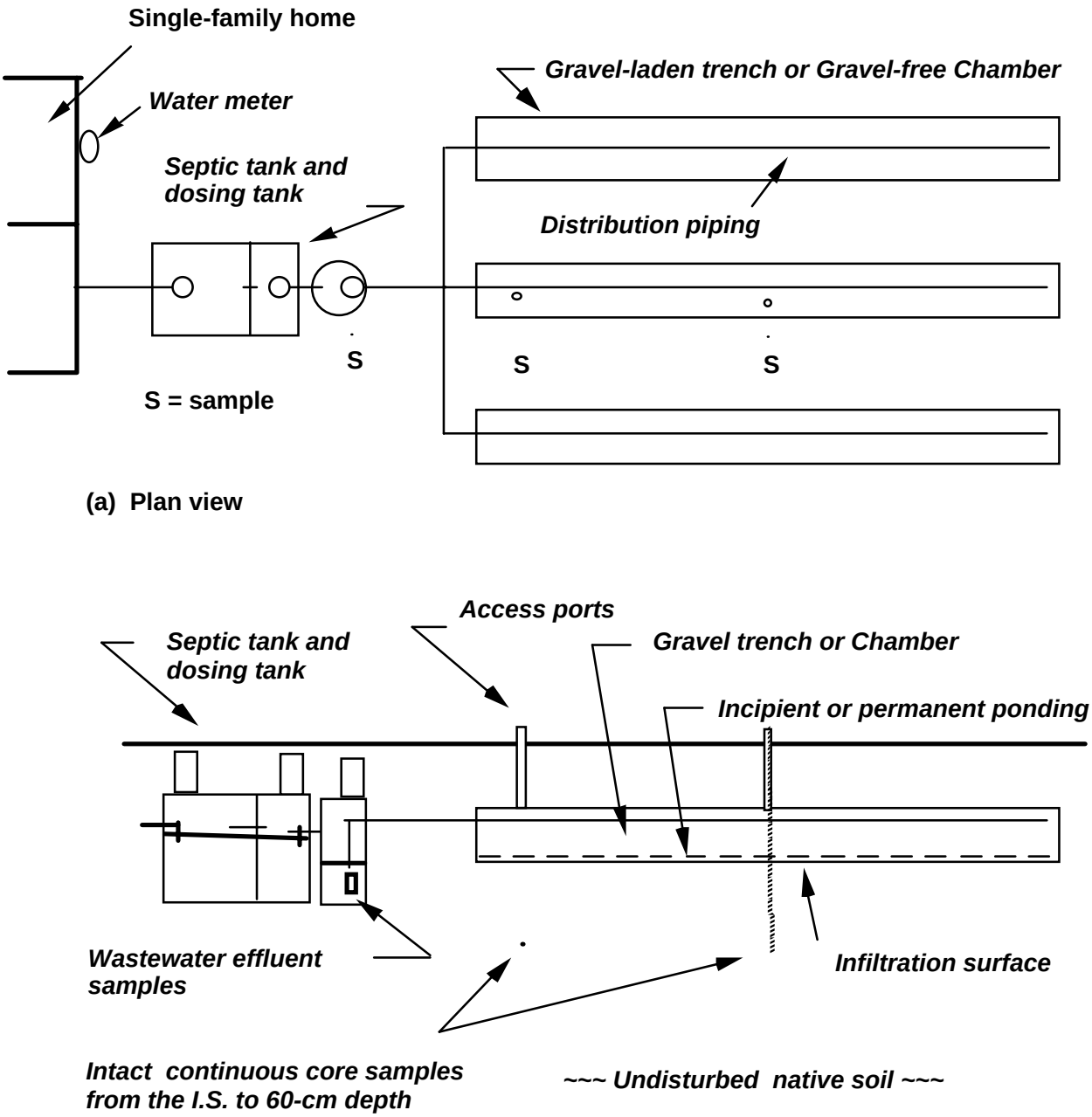

Fig. 3.3. Illustration of the general field site monitoring locations.

Fig. 3.4. Hand excavation from ground surface to the top of a chamber system in Summit County.

Care was taken upon encountering the soil infiltrative surface to avoid unnecessary disruption of the surface soil and any clogging layer. Then, a thin-tube sampling probe with a precleaned stainless steel or acetate liner (2 in. diam. by 6 in. long) was driven into the undisturbed soil and an intact core was retrieved within the sleeve. The core was capped with plastic end caps, labeled, and placed in a cooler containing blue ice. The casing was driven further into the probe hole and then the push probe (after cleaning with 90% v/v ethanol in water followed by a deionized water rinse) was inserted into the probe hole and driven another depth interval (nominally 16 cm or 6 in.). This process was repeated until a depth of 24 to 30 in. (60 to 75 cm) was reached or cobbles and dense soil prevented further penetration.

As just described, relatively intact core samples were aseptically collected from the WSAS infiltrative surface vertically downward to a depth of 60 to 75 cm below it. In addition, a background location outside of the infiltration area was also cored. Samples were then stored at 4C until laboratory analysis was performed at CSM. In the lab, the cores were carefully opened and the outer-most soil media was removed and wasted. Then, subsamples of the interior of the core were taken with sterile utensils at up to 4 intervals that corresponded approximately to those used in the CSM laboratory lysimeter study (Van Cuyk et al., 1999) (e.g., 0-5 cm, 10-15 cm, 25-30 cm, and 55-60 cm below the infiltrative surface). All laboratory analyses for water content and fecal coliform bacteria were performed within 24 hours of sample collection. After drying, soil samples were stored at 4C until analyses were made for organic matter and nutrients.

Analyses of field core subsamples were made for the following characteristics:

- Soil color was recorded using the Munsell Color Chart.
- Soil pH was measured on a 1:1 (solids:solution) extract using a calibrated pH electrode.
- Water content was measured gravimetrically and recorded as percent dry weight.
- Dried soil samples were also analyzed for organic matter, total nitrogen, ammonium, nitrate, available phosphorus. Results were expressed on a dry weight basis.
- Fecal coliform analysis on soil core samples was performed aseptically in duplicate by taking a known weight (~4 grams) of moist soil and adding 40 mL of 1.5% beef extract solution to a yield a final dilution of ~1:10 (sand:beef extract). APHA method 9221A suggests extraction for coliform bacteria in sediments and sludges using 10% phosphate buffered saline (PBS). However, a comparison of extraction methods conducted at the bench scale at the CSM microbiology laboratory using 6 different extractants (including PBS) proved beef extract to be the most efficient method for removing the coliform bacteria (Masson, 1999). Following the addition of beef extract, samples were shaken for 2 minutes at 350 rpm and then allowed to settle for 1 minute at which time the liquid sample was analyzed. Early in the study, an aliquot of liquid (typically 1 mL) was withdrawn from mid-depth of a sterile 50 ml conical (Masson, 1999) and analyzed directly (for low levels) or diluted as needed (for high levels). Analyses for fecal coliform bacteria were made according to the membrane filtration method (APHA method 9222D). To reduce the method quantitation limit, all 40 mL of the extraction broth were filtered and analyzed for homes 8 to 16. All sample dilutions were plated in duplicate. Results are expressed as org./g soil, based on the dry weight of the soil.

3.1.3. Ancillary Study of Soil Solid vs. Soil Percolate Fecal Coliform Measurements

Controlled laboratory experiments were conducted using known concentrations of *E.coli* bacteria applied to sand or silty sand to determine the relationship between microbial densities estimated in percolating soil water based on analysis of soil solids (e.g., from a soil core) as compared to those measured directly in collected percolate water (e.g., as measured in a pan lysimeter). This information was deemed necessary for an understanding of how results obtained from soil cores correlate to levels of bacteria being transported in soil water. It was hypothesized that solids samples should yield calculated percolate concentrations of fecal coliforms that are always equal to or higher than those measured directly in percolating water. Tyler and Converse (1998) acknowledged that there were no criteria established for soil systems and no current method for equating soil water values (org/mL) with soil-solids extracted values.

Experiments were conducted in mini-columns (50-mL polypropylene syringe barrels) filled with low organic content (TOC= 0.017% dry weight) clean medium sand (d_{10} =0.22 mm, d_{60} =0.60 mm) with *E.coli* added at 10^5 cfu/ml in sterile phosphate buffered saline (PBS) solution. The columns were dosed four times daily (every 6 hours) in an automated fashion at total hydraulic loadings of approximately 5 cm/day. A second column experiment was run under the same conditions using a different sand media that contained a higher organic carbon content (TOC= 0.225% dry weight).

3.2. RESULTS

3.2.1. WSAS Characteristics and Performance

WSAS Age and HLR. The chamber systems varied in age from 1 to 10 yr. while the gravel systems were 2 to 11 yr. old (Fig. 3.5). The estimated hydraulic loading rates averaged 1.31 cm/d for the chamber systems compared to 0.76 cm/d for the gravel systems (Fig. 3.6). For the chamber systems, 5 of the 10 exhibited some degree of effluent ponding while for the gravel 4 of 6 exhibited ponding. These data suggest comparable hydraulic performance with the chamber systems receiving a higher loading rate on average that was 70% higher (1.31 vs. 0.76 cm/d based on gross horizontal infiltrative surface area).

Septic Tank Effluent Composition. A total of 16 individual onsite systems were monitored including 10 with chambers and 6 with gravel. Descriptive statistics for each subdivision are presented in Tables 3.2 and 3.3 while results for individual homes may be found in the Appendix (see Table A.1). The STE composition at the individual study homes was typical of residential STE containing appreciable concentrations of pollutants. In the Hamilton Creek development, the average concentrations were: BOD_5 = 175 mg/L, TSS = 258 mg/L, total N = 62 mg-N/L, total P = 7.7 mg-P/L, and fecal coliform bacteria = 4×10^6 to 6.3×10^6 cfu/100mL. Table 3.4 presents a synopsis of some literature values, allowing a comparison of the STE composition determined in this study to previously reported values.

WSAS Soil Coring and Analyses. A total of 14 WSAS (9 chamber and 5 gravel systems) were successfully sampled wherein a set of soil cores were taken at each site. Some of the systems were continuously ponded while others were not. In general, coring was difficult and time consuming due to common problems with field monitoring (e.g., locating subsurface system boundaries, system depth, rocky soil). Detailed results for each WSAS may be found in the Appendix (Tables A.2 and A.3) while a summary of the results follow.

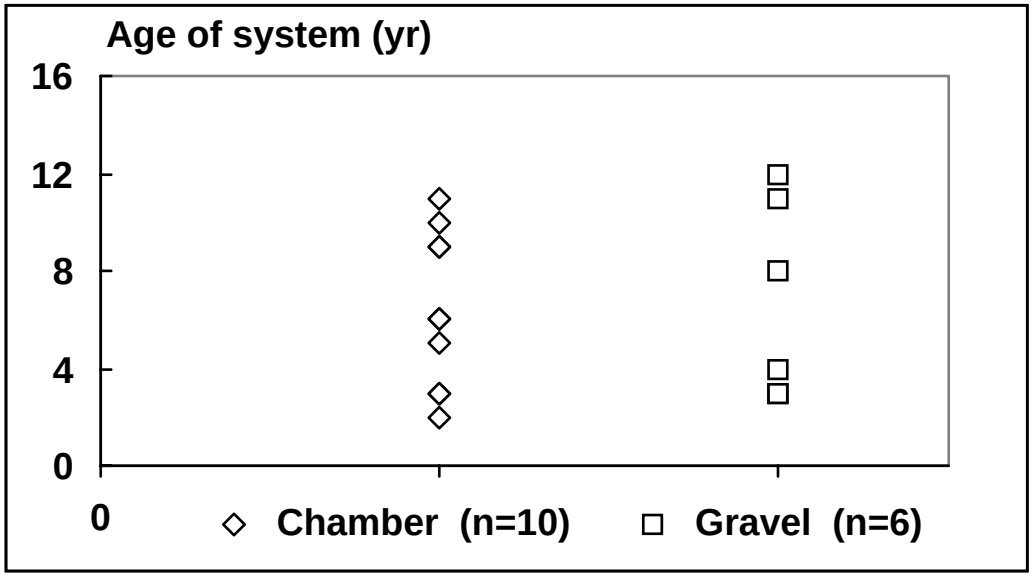

Fig. 3.5. Comparison of system age (yr.) for the wastewater systems studied.
Note that each symbol may represent more than one sample result.

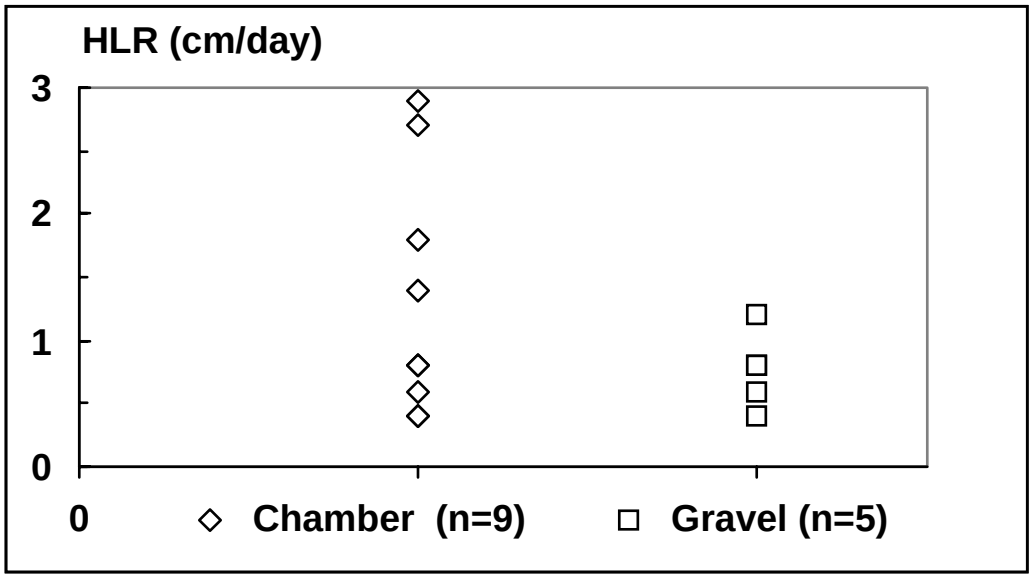

Fig. 3.6. Comparison of hydraulic loading rates for the WSAS's studied (1 cm/d = 0.24 gpd/ft^2).
Note that each symbol may represent more than one sample result.

Table 3.2. Descriptive statistics for septic tank effluent composition in Hamilton Creek.[1]

Parameter	Units	Average	Std. dev.	CV	No.	Minimum	Maximum
pH	-	-	-	-	16	6.95	7.94
Alkalinity	mg-$CaCO_3$/L	528	142	0.27	16	288	860
BOD_5	mg/L	175	52	0.30	14	98	358
COD	mg/L	260	165	0.63	16	109	990
TSS	mg/L	251	246	0.98	16	20	958
TN	mg-N/L	62	23	0.37	16	41	102
NH_3-N	mg-N/L	43	17	0.40	16	3	64
NO_3-N	mg-N/L	1.3	0.7	0.54	16	0.5	2.4
Total P	mg-P/L	7.7	2.0	0.26	9	5.7	11.1
Fecal coli.	cfu/100mL				16	4.00E+06	6.30E+06

[1] See Appendix Table A.1 for detailed results for each WSAS.

Table 3.3. Descriptive statistics for septic tank effluent composition in Todd Creek Farms.[1]

Parameter	Units	Average	Std.dev.	CV	No.	Minimum	Maximum
pH	-	-	-	-	5	7.05	8.04
Alkalinity	mg-$CaCO_3$/L	676	25	0.37	5	658	726
BOD_5	mg/L	332	46	0.14	3	385	301
COD	mg/L	496	303	0.61	5	170	825
TSS	mg/L	102	58	0.67	5	0	143
TN	mg-N/L	69	10	0.14	5	56	84
NH_3-N	mg-N/L	66	9.8	0.15	5	54	75
NO_3-N	mg-N/L	2	0.7	0.35	5	0.9	2.6
Total P	mg-P/L	10	2.4	0.24	5	6.25	11.95
Fecal Coli.	cfu/100mL				5	2.5E+05	1.3E+07

[1] See Appendix Table A.1 for detailed results for each WSAS.

Table 3.4. Comparison of STE composition in Hamilton Creek with literature values. [1]

Parameter (units)	Average	Std. dev.	Range	Reference
BOD_5 (mg/L)	175 81 132	52 31	98 to 358 29 to 140 150 to 250	This CSM study Tyler et al., 1991 Crites & Tchobanoglous, 1998 Harkin et al., 1979
COD (mg/L)	260 157 445	164 46	109 to 990 49 to 244 250 to 500	This CSM study Tyler et al., 1991 Crites & Tchobanoglous, 1998 Harkin et al., 1979
TSS (mg/L)	251 87	245	20 to 958 40 to 140	This CSM study Crites & Tchobanoglous, 1998 Harkin et al., 1979
NH_4-N (mg-N/L)	43 50 41	17 11	3 to 64 10 to 69 30 to 50	This CSM study Tyler et al., 1991 Crites & Tchobanoglous, 1998
NO_3-N (mg-N/L)	1.3 0	0.74 0	0.5 to 2.4 0 to 2	This CSM study Tyler et al., 1991
Total P (mg-P/L)	7.7 17.3 7.3	2.0	5.7 to 11.1 12 to 20	This CSM study Crites & Tchobanoglous, 1998 Harkin et al., 1979
Fecal coli. (org/100mL)	2.14E+06 1.00E+06		4.0E+04 to 5.1E+06 1.0E+06 to 1.0E+08	This CSM study Crites & Tchobanoglous, 1998 Harkin et al., 1979

[1] A blank cell indicates information not available.

Water content versus depth for the soil cores collected from all of the study homes is shown in Fig. 3.7. Water content is generally highest near the infiltrative surface and declines with increasing depth below it. Figure 3.8 presents ammonium-nitrogen and Fig. 3.9 presents nitrate-nitrogen data from soil cores samples by interval and by type of infiltrative surface (see Appendix Fig. A.1 for detailed nitrogen data for homes 2, 3 and 7). Fig. 3.10 presents available phosphorus data for the soil core samples. Ammonium-nitrogen and nitrate-nitrogen appear to be present throughout the sampling depths at each site, with highest levels at depths closest to the infiltrative surface. Tables 3.5 and 3.6 illustrate that the nitrogen data collected in this study are generally similar to the results reported in recent work of Tyler and Converse (1998).

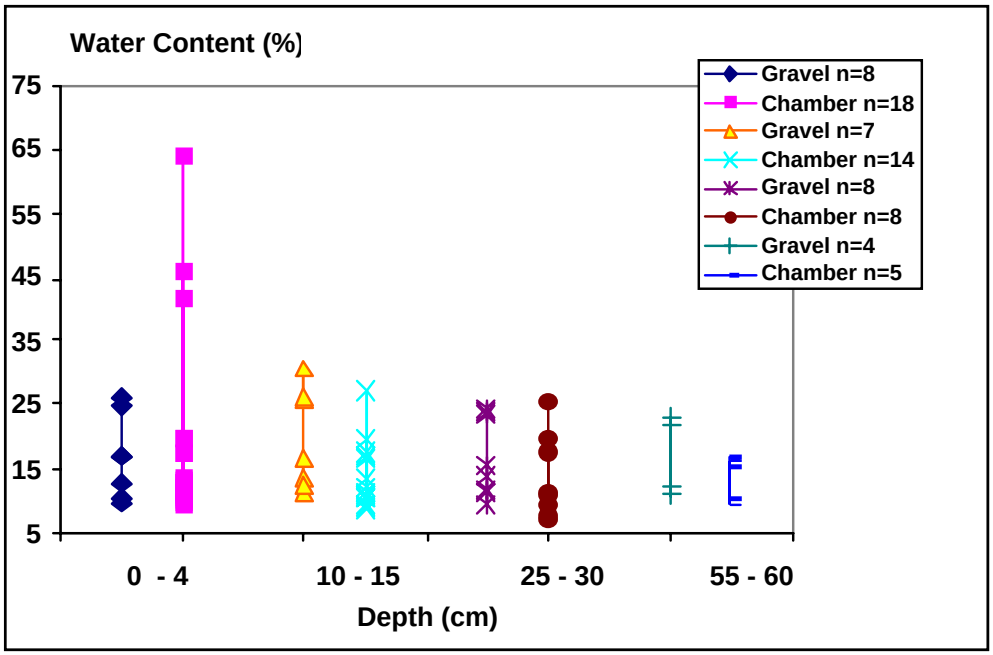

Fig. 3.7. Water content in soil core samples by depth below the infiltrative surface. Note that each symbol may represent more than one sample result and background soil core data are excluded.

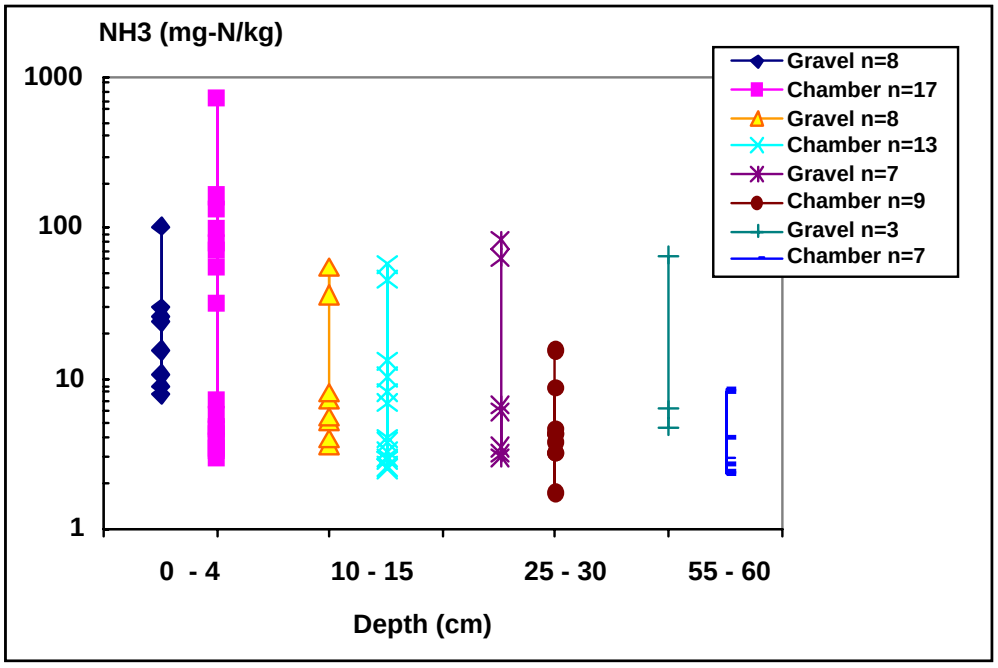

Fig. 3.8. Ammonium-nitrogen in soil core samples by depth below the infiltrative surface. Note that each symbol may represent more than one sample result and background soil core data are excluded.

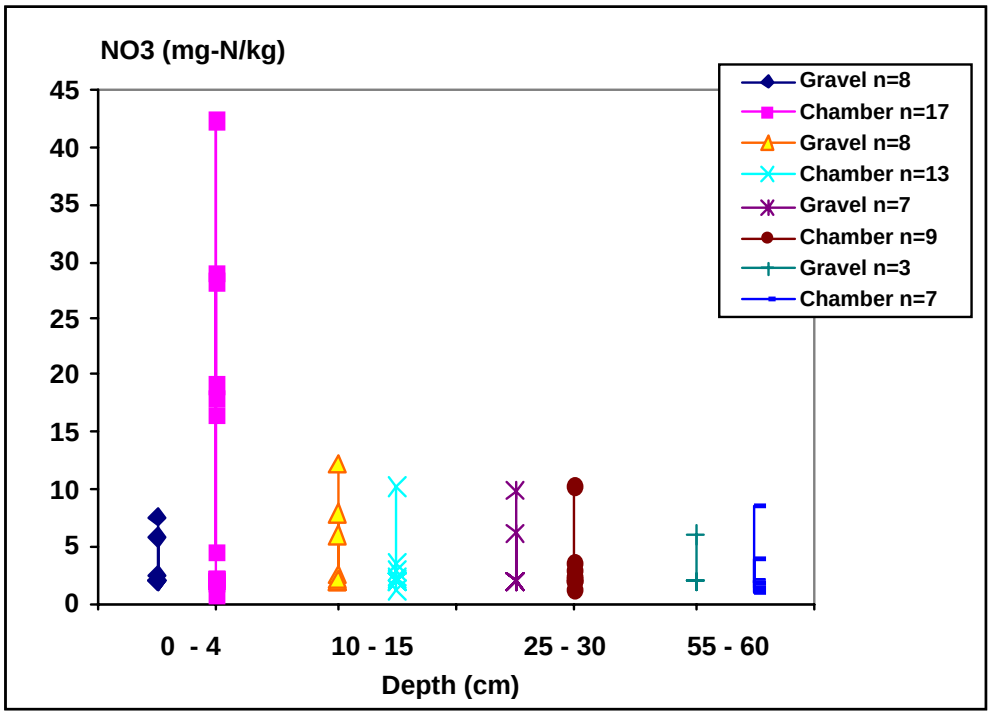

Fig. 3.9. Nitrate-nitrogen in soil core samples by depth below the infiltrative surface. Note that each symbol may represent more than one sample result and background soil core data are excluded.

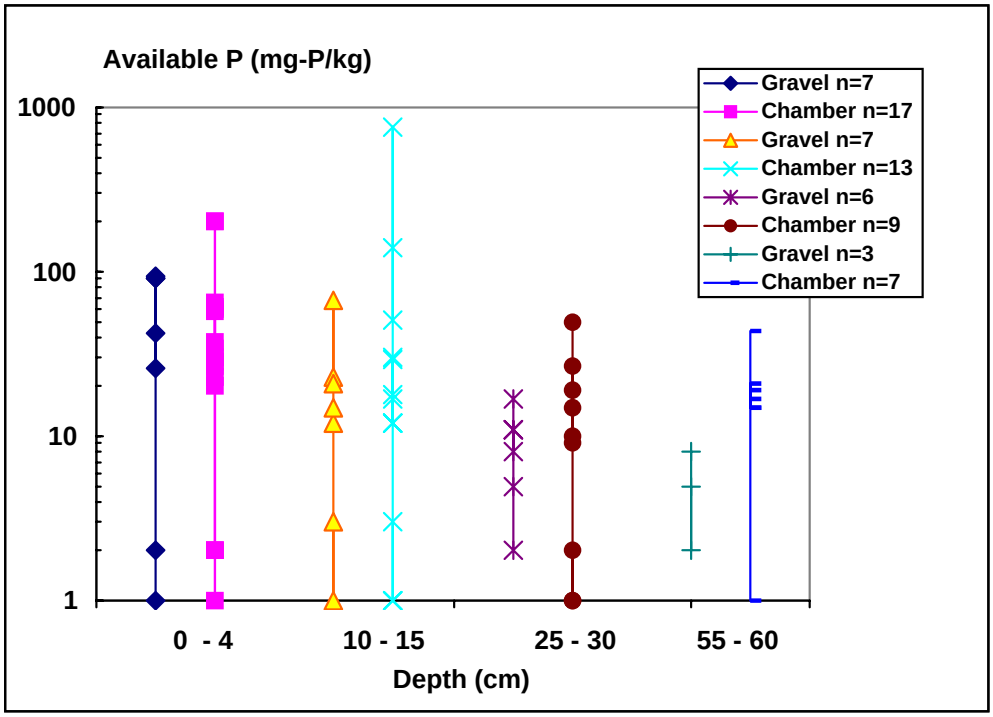

Fig. 3.10. Available phosphorus in soil core samples by depth below the infiltrative surface. Note that each symbol may represent more than one sample result and background soil core data are excluded.

Table 3.5. Summary of water content and nutrient concentrations with depth below soil infiltration systems receiving effluent during this study.

Soil depth (cm)	Ammonia (mg-N/kg dw) Average (range)	Nitrate (mg-N/kg dw) Average (range)	Water content (dry wt.%) Average (range)
0 – 5	64 (3 to 721)	8 (1 to 42)	18.4 (10 to 26)
10 – 15	14 (2 to 57)	5 (1 to 17)	16 (9 to 31)
25 – 30	13.5 (2 to 82)	3.3 (1 to 10)	15 (7 to 25)
55 - 60	11 (2 to 66)	3 (1 to 8)	15 (9 to 22)
BKGD 0 - 4	13.8	1 (0 to 2)	13 (8 to 18)
BKGD 10 - 15	8.4 (6 to 14)	0.8 (0.6 to 0.9)	10 (5 to 14)
BKGD Batch[1]	5.6 (3 to 9)	3 (2 to 8)	8 (2 to 21)

[1] Batch samples were collected at same depth as infiltrative surface and a 0-10 cm interval was mixed for sampling.

Table 3.6. Water content and nutrient concentrations with depth below soil infiltration systems receiving aerobically treated effluent (Converse and Tyler, 1998).

Soil depth (cm)	Ammonia (mg-N/kg dw) Average (range)	Nitrate (mg-N/kg dw) Average (range)	Water content (dry wt.%) Average (range)
0 – 15	4 (0 to 35)	7 (0 to 33)	12 (4 to 45)
15 – 30	13 (0 to 96)	9 (1 to 29)	22 (4 to 40)
30 – 45	11 (1 to 112)	8 (1 to 23)	22 (4 to 48)
45 – 60	7 (1 to 38)	8 (0 to 22)	21 (7 to 33)

The results for fecal coliform bacteria levels with depth are summarized in Table 3.7. Figure 3.11 provides a graphical summary of fecal coliform levels at soil coring depths for chamber versus gravel systems. As shown, the results for the chamber systems are comparable to those for the gravel systems. At most sites, fecal coliform concentrations declined with depth and by 30 to 60 cm depth, fecal coliform bacteria were very low or not detected (detection level of 1 org. per g soil) (see Table 3.7). A statistical comparison of the fecal coliform bacteria levels at 30-cm and 60-cm below the infiltrative surface in chamber systems versus gravel systems was made following a Mann-Whitney nonparametric test procedure (Minitab, Inc., 1995). This

analysis revealed that the fecal coliform levels at both depths were not significantly different at 95% confidence (p=0.05).

Table 3.8 presents literature comparison values for soil core fecal coliform levels with depth below an infiltrative surface. Compared to the results of Converse and Tyler (1998) as summarized in Table 3.8, the fecal coliform results observed in this study are similar (see Tables 3.7 and 3.8). It is noted that the STE concentrations applied to the soil absorption systems in the CSM study were considerably higher, ranging from 250,000 to 1,300,000 org./100 mL compared to a maximum of 150,000 org./100 mL reported by Converse and Tyler (1998).

Table 3.7. Summary of fecal coliform concentrations with soil depth during this study.

Source	Units	Median	Min.	Max.	Samples
Wastewater	org/100 mL	-	250000	1300000	21
Soil @ depth (cm)					
0-4	org/g dw	53	4582	15000	22
10-15	org/g dw	27	1261	4028	19
25-30	org/g dw	< 1	3377	14029	17
55-60	org/g dw	19	925	2853	10
BKGD 0-4	org/g dw	< 1	< 1	< 1	4
BKGD 10-15	org/g dw	< 1	< 1	< 1	3
BKGD 25-30	org/g dw	< 1	< 1	< 1	1
BKGD 55-60	org/g dw	< 1	< 1	< 1	0
BKGD Batch [1]	org/g dw	< 1	< 1	< 1	17

[1] Batch samples were collected at same depth as infiltrative surface and a 0-10 cm interval was mixed for analysis.

Table 3.8. Fecal coliform concentrations with soil depth in sands/sandy loam soils reported by Converse and Tyler (1998).

Source	Units	Median	Average	Max.	Samples
Wastewater	org/100mL	1850	33778	150000	14
Soil @ depth (cm)					
0 – 2	org/g dw	2	83	798	20
2 – 15	org/g dw	5	40	482	28
15 – 30	org/g dw	2	22	216	28
30 – 45	org/g dw	<1	6	70	28
45 – 60	org/g dw	1	19	318	27
60 – 75	org/g dw	<1	7	120	25
75 – 90	org/g dw	<1	1	9	23
90 – 105	org/g dw	<1	1	5	22

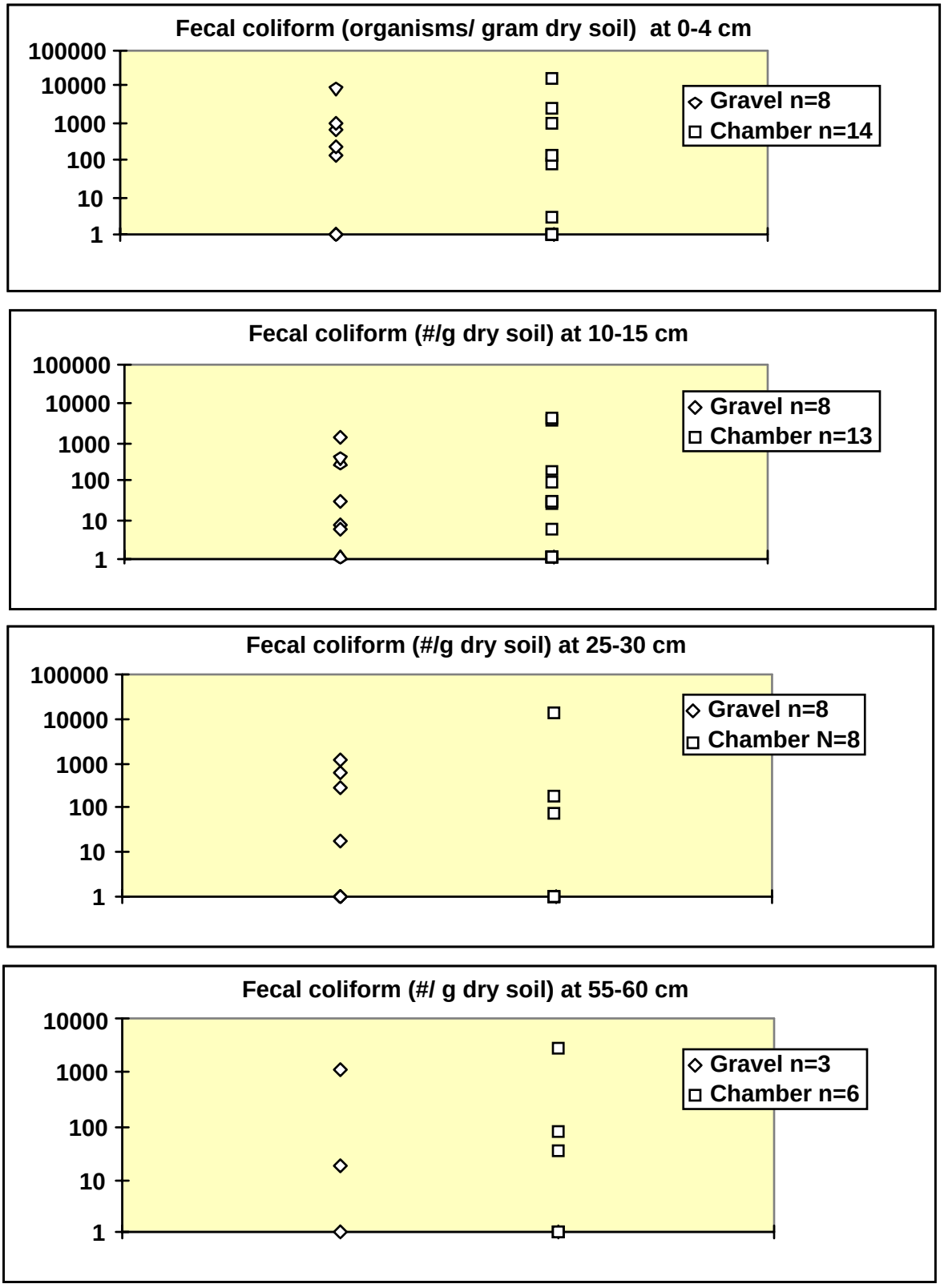

Fig. 3.11. Fecal coliform data in soil samples from all sampled sites.
Note that each symbol may represent more than one sample result and background soil core data are excluded.

3.2.2. Comparison of Fecal Coliforms in Soil Solid vs. Percolate Samples

The results for medium sand with 0.017 wt.% total organic carbon (TOC) and a silty sand with 0.225 wt.% TOC, are presented in Fig. 3.12 and 3.13, respectively. These data suggest that in both types of sand media, at the dose of bacteria used, values for *E.coli* obtained from soil core extracts would be higher (therefore a more conservative measure) compared to the concentrations actually contained in the percolate/soil water. This relationship is reasonable and likely due to the retention of bacteria on soil solids. These bacteria may not be mobile in the soil water, but they are measured in the extract made from the bulk solids. Further testing on various laboratory and field soil samples at different levels of influent bacteria, as well as virus, will enable correlations to be developed between soil core and pore water concentrations at different environmental conditions.

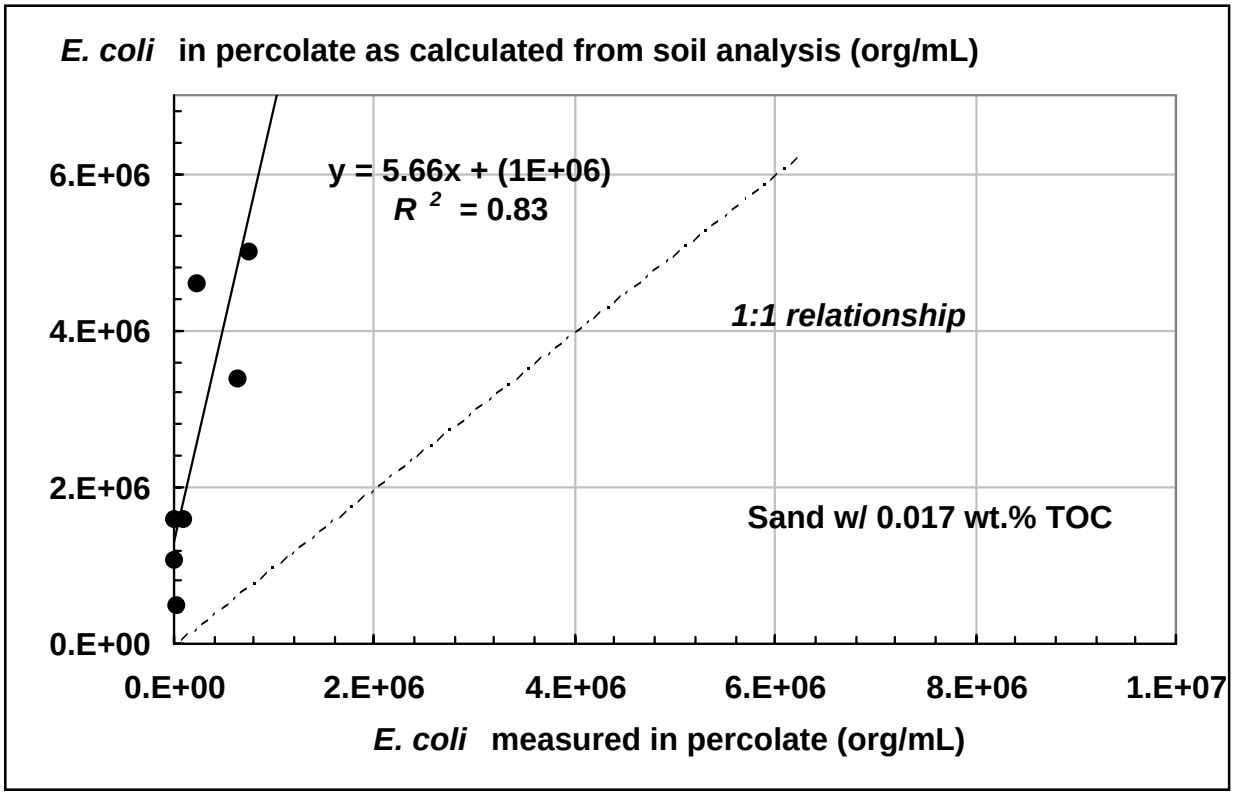

Fig. 3.12. Relationship of *E. coli* determined by analyses of extracts from sand versus direct analysis of percolate water.
Note: Each point represents the average of duplicate columns.

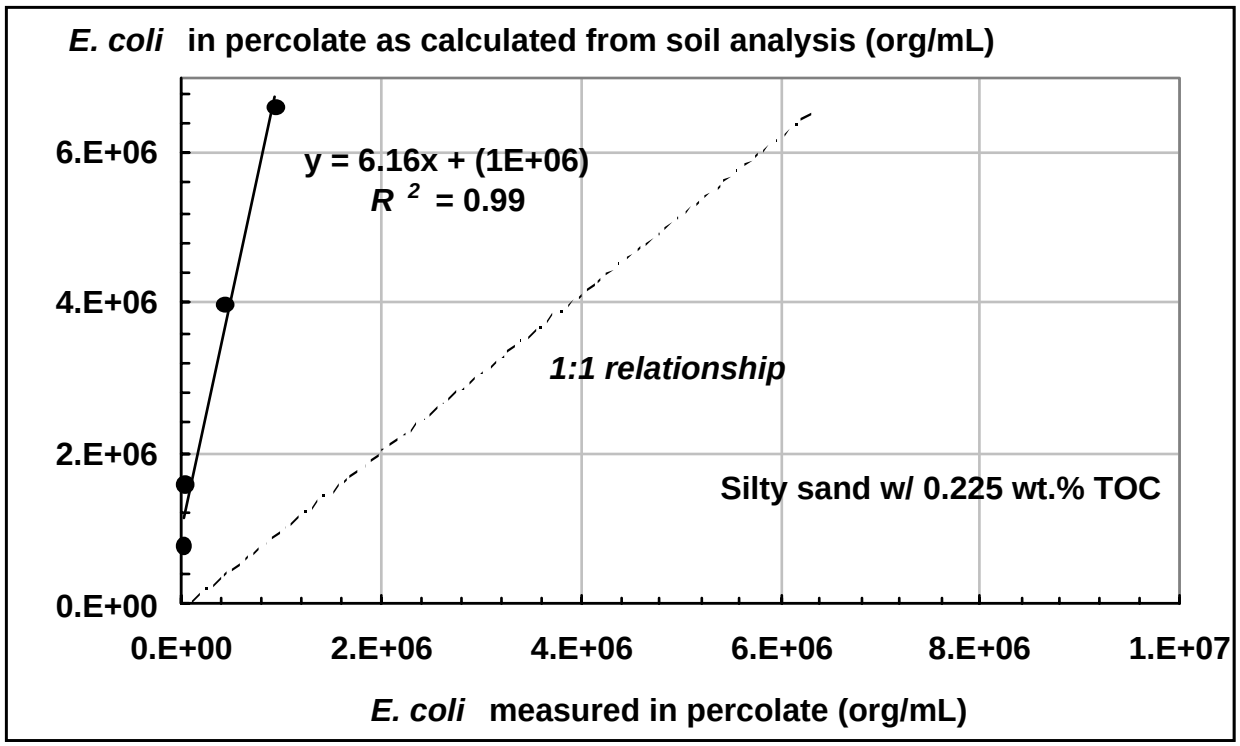

Fig. 3.13. Relationship of *E. coli* determined by analyses of extracts from silty sand versus direct analysis of percolate water.
Note: Each point represents the average of duplicate columns.

3.3. DISCUSSION

The interpretation of soil purification efficiency requires determination that wastewater did in fact reach the infiltrative surface location at which soil cores have been collected. Such an assessment can be made by integrated consideration of several factors, including: ponding (or wetness), soil color, water content and nutrient levels in the soil core profile, as well as the presence of fecal coliform bacteria near the infiltrative surface. Based on these parameters, most soil cores were collected at locations where STE had infiltrated.

Ammonium-nitrogen and nitrate-nitrogen appear to be present throughout the sampling depths and in background samples collected at each site, with the highest levels at depths closest to the infiltrative surface. These data are consistent with results found in laboratory lysimeters, where ammonium-nitrogen and nitrate-nitrogen levels were highest from 0 to 8 cm below the infiltrative surface (Van Cuyk 1999; Fischer, 1999) and with recent published field research (e.g., Tyler and Converse, 1998). In the CSM lysimeters, nitrification rate measurements indicated that nitrification was greatest at 3 cm, was less at 8 cm, and essentially did not occur at the 30 cm depth (Fischer, 1999). Values for nitrogen species (Figs. 3.8 and 3.9) and fecal coliform (Fig. 3.11) at soil coring depths for the chamber versus gravel systems show these systems to be performing comparably.

Tables 3.7 and 3.8 enable literature comparisons for nitrogen and fecal coliform and demonstrate that the data collected in this study is generally consistent with previously reported results. While a large degree of variation in constituent concentrations was observed between individual systems, and even among duplicate cores taken within the same system, the values measured for both system types were for all practical purposes, comparable.

4.0 MONITORING OF VIRUS TREATMENT EFFICIENCY

4.1. TECHNICAL APPROACH AND METHODS

4.1.1. Evaluation of a WSAS under Field Conditions

From the pool of 16 WSAS monitored for chemical and bacterial characteristics (as described in Section 3), one system was chosen for a field-scale evaluation of virus treatment efficiency using a multicomponent surrogate and tracer methodology. This effort was viewed as experimental and a means to refine a methodology employed during controlled laboratory experiments (Van Cuyk et al., 1999) and apply it under field conditions to a mature operating WSAS. Conservative tracers and viral surrogates had been used previously in studying flow and transport in ground water systems (both native bacteriophage and spiked phage) (Harvey, 1997a; 1997b) and appeared quite suitable for evaluation of WSAS under field conditions. However, multicomponent surrogates and tracers had received relatively limited use for evaluation of WSAS under field conditions. Field studies reported in the literature included studies in Florida with virus surrogate spiking of a research site near Tampa by Anderson et al. (1991, 1994) and spiking of cesspools near the Florida Keys by Rose et al. (1999). Field studies completed in California by Oakely et al. (1999) and in Massachusetts by Higgins et al. (1999) relied on indigenous bacteriophage. Most of these studies (all but the study by Anderson et al. (1994)) did not employ multicomponent mixtures containing a conservative tracer plus two contrasting viral surrogates. Thus the field testing completed in this study was viewed as a methods development and evaluation effort.

In this study, a multicomponent surrogate and tracer mixture was used to confirm that a mature WSAS, designed with Infiltrator chambers and a typical reduced infiltrative surface area, can remove virus such that the concentrations are reduced by $\geq$ 3 logs between the infiltrative surface and 60 to 90 cm depth below it. For this evaluation, two viral surrogates and a conservative tracer were to be added to the STE being applied to a soil absorption system (Fig. 4.1). Then after a period of time, during which application of STE and the surrogates/tracer continued, duplicate soil cores were collected from the infiltrative surface vertically downward to 60 to 75 cm depth below it. From each core, duplicate soil samples were aseptically collected and analyzed to quantify the concentrations of the viral surrogates and the tracer as well as the soil water content and fecal coliform concentrations.

For this test, a mature onsite system was selected for study. This system had been in operation for approximately eight years, was estimated to have a current HLR of ~ 0.7 gpd/ft^2 (~2.7 cm/d), and exhibited some STE ponding of the infiltrative surface (Table 3.1, home site 2). In addition, this site provided easy access to the septic tank and chamber soil absorption system which facilitated the required sampling activities.

The multicomponent mixture was comprised of two viral surrogates, MS-2 and PRD-1 bacteriophages (not infectious to humans)(Van Duin, 1988), in addition to the conservative tracer, bromide. MS-2 and PRD-1 had been previously used as viral surrogates in ground water transport studies (Harvey, 1997a,b). MS-2 is an icosahedral phage with a diameter of 26 nm

(VanDuin, 1988) and a pH_{iep} of 3.9 (Bales et al., 1991) while PRD-1 is an icosahedral lipid phage with a diameter of 62 nm (Olsen et al., 1974). MS-2 and PRD-1 bacteriophage assays were made following the plaque-forming-unit technique (*Escherichia coli* and *Salmonella typhimurium* host, respectively) described by Adams (1959).

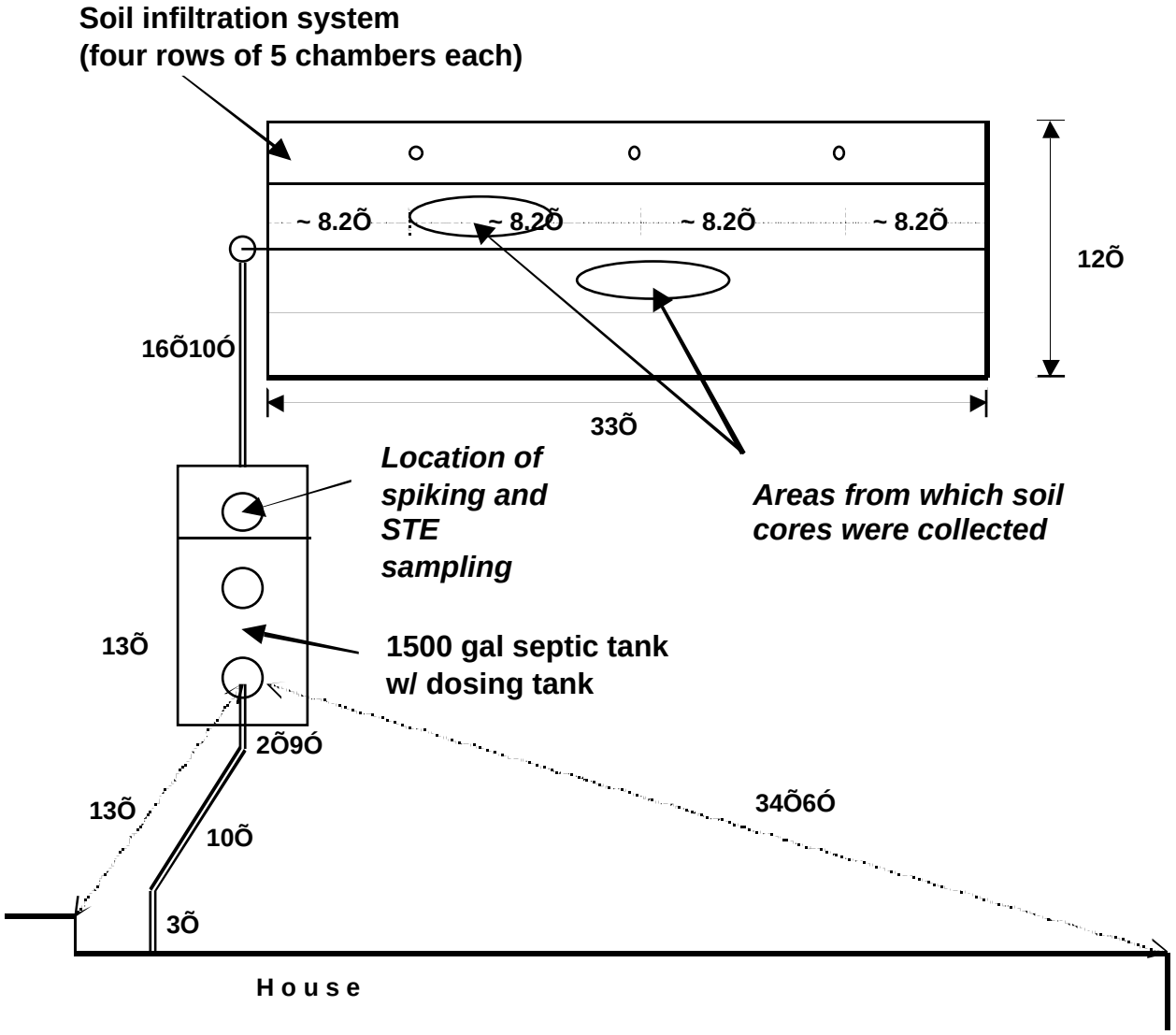

Fig. 4.1. Site layout for the onsite wastewater system studied during the virus treatment test at Site 2.

The volume of wastewater in the STE dosing tank at the study site was estimated to be approximately 250 gallons. Prior to the addition of the surrogates and tracer mixture, STE samples were collected to quantify pretreatment levels of bromide, MS-2 and PRD-1. There was no detection of any of these surrogates or tracers in the untreated STE. Stock solutions of bromide (added as KBr), MS-2 and PRD-1 were added to the STE dosing tank to obtain final concentrations targeted at 500 mg-Br/L of bromide tracer and 100,000 pfu/mL of both MS-2 and PRD-1. The bacteriophage concentrations were selected to be representative of those in a home STE during or soon after a viral infection within the household.

Following surrogate/tracer addition to the STE in the dosing tank, it was mixed using a submersible pump which recirculated STE within the tank for approximately 10 minutes. After this period of mixing, five (5) grab samples of the STE, amended with the surrogates and tracer, were collected to characterize the time zero conditions. Subsequently, STE samples were collected from the dosing tank weekly in order to characterize the concentrations of surrogates and tracers being dosed into the soil infiltration system over time.

An estimate of the time required for effluent to infiltrate and percolate to a depth of 60 cm was made based on the daily flow, area of infiltrative surface, and an effective porosity for the soil based on the following relationship:

$$T_r = \frac{(A_{I.S.})(D)(Ne)}{Q} \qquad [4.1]$$

where, T_r = travel time required for effluent to reach the depth of interest (days), D = depth of interest (m), $A_{I.S.}$ = infiltrative surface area (m^2), Q = daily flow (m^3/day), and Ne = effective porosity (v/v). This relationship assumes uniform application and infiltration into the absorption system and is thus a first approximation of travel times. For the study site, the $A_{I.S.}$ was determined from the as-built drawings to be 36.8 m^2, the depth of interest to evaluate was 0.60 m, the average daily flow was 1.0 m^3/d, and the effective porosity was estimated at Ne=0.20 v/v. For these conditions, the time required for applied effluent to percolate to 60 cm depth below the infiltrative surface was 4.4 days. To ensure that adequate time was allowed for the surrogates/tracers to be distributed in the system and infiltrate/percolate into the soil, soil core sampling was not commenced for a few weeks after the initial addition of the surrogates/tracers to the STE. During this time, samples were collected from the STE being applied to the WSAS to identify changes in concentrations over time.

Twenty-five days after introduction of the viral surrogates and tracer, coring of the subsurface beneath the infiltrative surface commenced. Soil cores were taken at two spatially separate locations and at each location, soil subsamples were collected in duplicate at depths of 0-5, 10-15, 25-30 and 55-60 cm below the infiltrative surface. Extraction and analysis were conducted for Br-, MS-2, PRD-1 concentrations, in addition to fecal coliform concentrations and water content.

4.1.2. Ancillary Study of Bacteriophage Inactivation in STE

An ancillary study was completed to determine the rate and extent of inactivation of the bacteriophages (MS-2 and PRD-1) in the STE over time. In this bench-scale test, two of the samples collected from the dosing tank following addition of the multicomponent mixture were saved and stored in the dark at either 4C or 20C for a period of 26 days. At five timepoints (time 0 corresponds to the initial dosing concentration in the chamber) during this 26-day period, the concentrations of MS-2 and PRD-1 remaining in the samples were measured.

4.2. RESULTS

The concentrations of conservative tracer (Br-) and viral surrogates (MS-2 and PRD-1) measured in the STE at Site 2 over time are presented in Table 4.1 and Figure 4.2. These results show a decline in the bromide concentration in the dosing tank that is consistent with the decline in concentration expected based on dilution due to incoming STE with no tracer in it. After five days, the Br- concentration had declined by 99%. Similarly, the levels of MS-2 and PRD-1 declined during the initial 5-day period (Figure 4.3). However, between 12 and 20 days, the bromide concentration continued to drop toward zero (nondetectable at < 1 mg/L), but the MS-2 and PRD-1 levels remained relatively unchanged. These apparently unchanging virus levels are not due to analytical error since the variability between duplicate analyses was only 11% compared to the variability in concentrations from time-point to time-point which was nearly 1 log. Rather, these data suggest the potential for growth of the bacteriophages in the STE. If this in fact did occur, then the applied dose of viral surrogates to the soil absorption system could have been higher than that anticipated based on the bacteriophage concentrations that were spiked into the STE.

Table 4.1. Br-, MS-2, and PRD-1 concentrations in the STE dosing tank at Site 2 with time.

Elapsed time after spiking (days)	Bromide			MS-2			PRD-1		
	Average (mg/L)	Std. dev. (mg/L)	CV	Average (pfu/mL)	Std. dev. (pfu/mL)	CV	Average (pfu/mL)	Std. dev. (pfu/mL)	CV
0	570.00	9.54	0.02	75000	35355	0.47	153333	128582	0.83
5	8.58	7.03	0.82	1125	760	0.68	2250	957	0.43
12	<1	-	-	275	50	0.18	463	250	0.54
19	<1	-	-	225	87	0.38	1113	595	0.52
25	<1	-	-	72	10	0.15	8	10	1.25

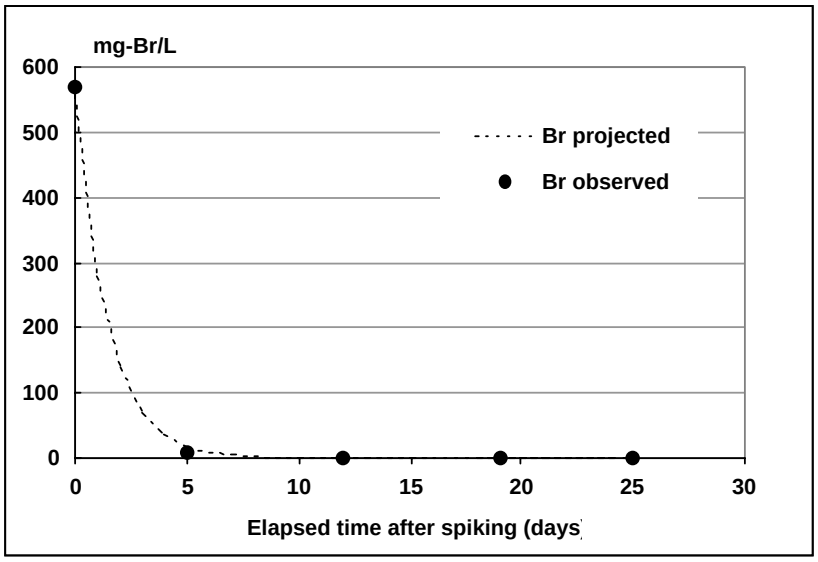

Fig. 4.2. Observed and predicted concentrations of Br^- tracer in the STE at Site 2 over time. Note the dashed line represents the projected Br^- concentration change due to dilution alone.

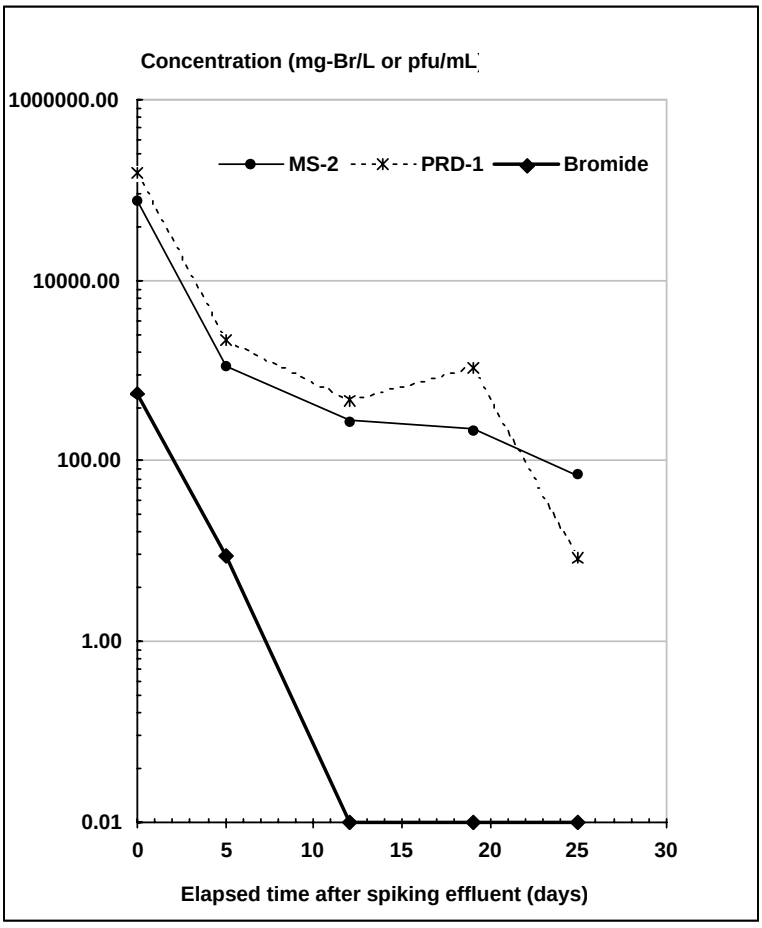

Fig. 4.3. Concentrations of MS-2 and PRD-1 bacteriophages and the conservative tracer, Br-, in the STE dosing tank at Site 2 over time.

Results of the bench-scale inactivation test are shown in Fig. 4.4. These data indicate that there can indeed be some apparent growth of bacteriophage in the STE at 4C and 20C. In the same sample over time, the PRD-1 levels increase at both temperatures while those of MS-2 increased at 4C but not at 20C. After approximately 20 days, all samples began to show signs of inactivation. The results of the dosing tank measurements and the bench-scale test with STE from the study site strongly suggest that there is growth of the added MS-2 and PRD-1 occurring in the STE dosing chamber at Site 2 (temperature of the chamber was measured at ~8C). These results show the need for more intensive monitoring of the temporal changes in surrogate/tracer concentrations where unexpected growth could be occurring.

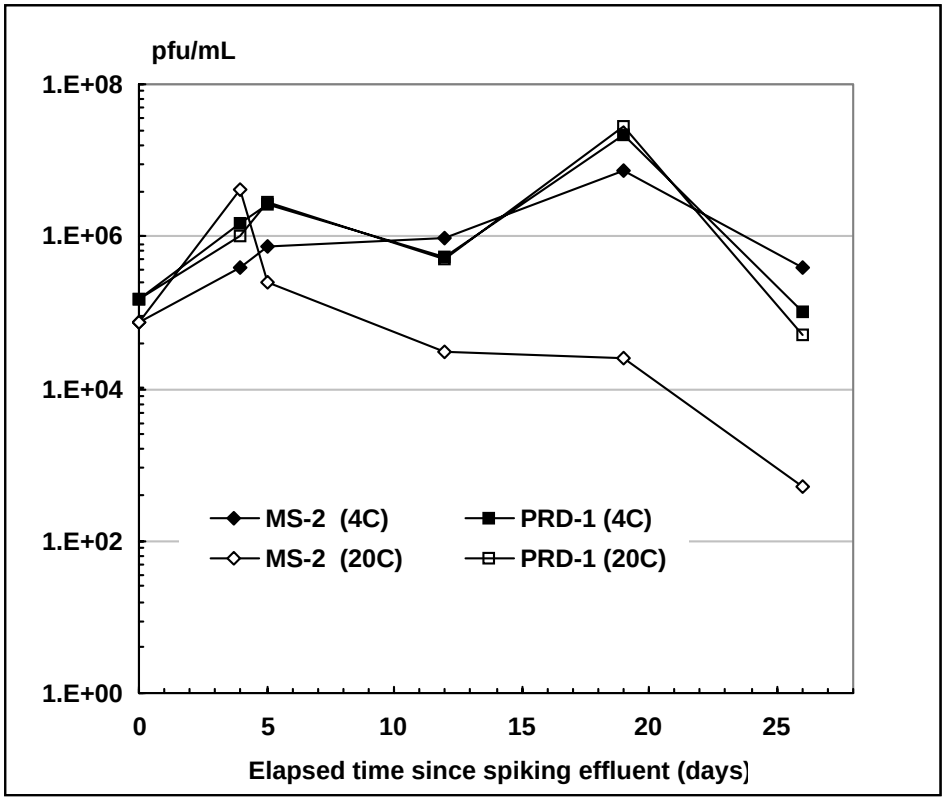

Fig. 4.4. Inactivation of MS-2 and PRD-1 in STE during incubation at 20C and 4C.
Note that all samples were run in duplicate and the average percent difference was 11%.

Results of soil coring showed no bromide in any of the extracted soil cores. This is likely the result of the bromide concentrations decline with time due to dilution in the dosing tank (Fig. 4.2, 4.3). In addition, the fast travel time in the soil infiltration system may have resulted in the added bromide migrating into and through the depth interval of interest prior to collection of the soil cores.

Soil core values for MS-2 and PRD-1 are graphically depicted in Figure 4.5 along with fecal coliform densities at each coring interval. Detailed results may be found in Appendix Table A.4. An overall trend of lower levels of virus and bacteria with increasing depth below the infiltrative

surface was observed, although, one core at 25-30 cm depth did show the highest number of fecal coliform bacteria.

The relationship of MS-2 and PRD-1 to fecal coliform concentrations is of interest, since fecal coliforms are often used as indicators of microbial contamination. As shown in Fig. 4.6, the concentrations of fecal coliforms (cfu/g) measured in soil core samples exceeded that of the MS-2 all of the time (26 of 26 pairs or 100%) and was higher than that of the PRD-1 most of the time (23 of 26 pairs or 88%) (see Appendix Table A.5 for detailed results). These data suggest that, under the conditions examined, fecal coliforms in soil extracts may be a reasonable indicator for the presence virus at the same location.

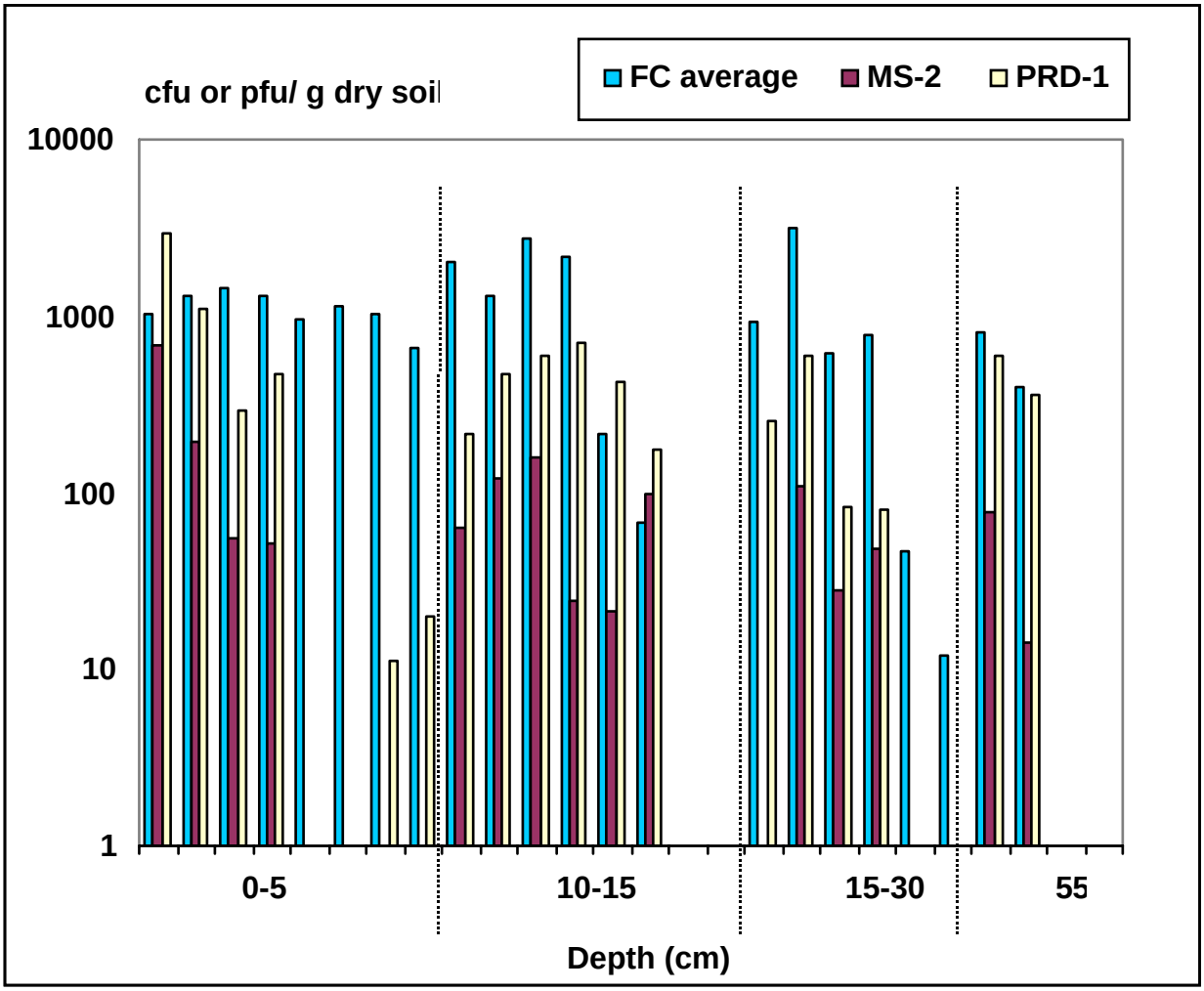

Fig. 4.5. Fecal coliform, MS-2 and PRD-1 levels in soil core extracts collected from Site 2, 25 days following addition of surrogates and tracer to the STE being applied. Note: Initial influent levels were 570 mg/L of bromide, 75,000 pfu/mL of MS-2 and 153,000 pfu/mL of PRD-1. Zero values (blank bars) represent non-detects.

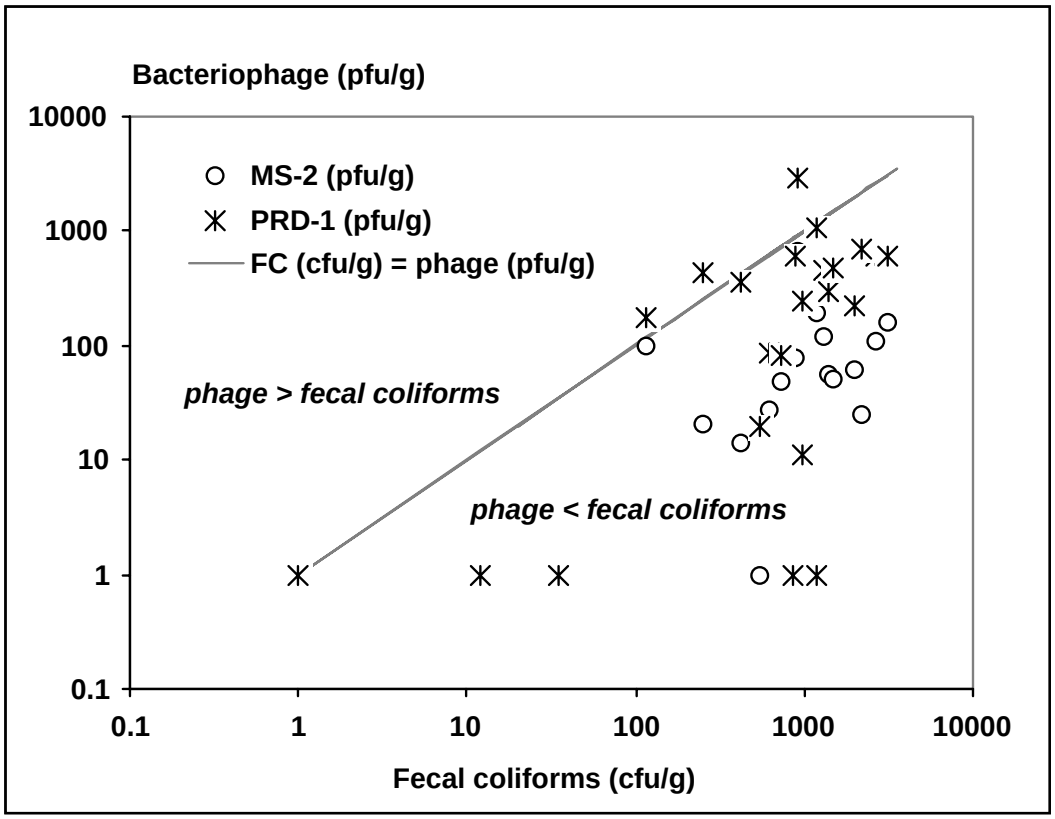

Fig. 4.6. Relationship of bacteriophages to fecal coliforms as determined in the same soil samples at depths of 0 to 60 cm below the infiltrative surface of a WSAS.
Note: 26 samples were analyzed for all three constituents (see Table A.5 for details).

The removal of bacteriophages was conservatively estimated based on the assumption that all bacteriophage detected in the extraction of soil solids were mobile in the soil pore water. Based on a 15 dry wt.% water content, the pfu/g dry soil values were converted to pfu/mL of pore water. These estimated pore water values were then compared to the dose concentration of MS-2 or PRD-1. As presented in Table A.4, at a depth of 30 cm below the WSAS infiltrative surface, the concentrations of MS-2 detected were <1, <1, <1, 28, 48 and 108 pfu/g for the various core samples. The concentrations of PRD-1 were <1, <1, 80, 84, 250, and 594 pfu/g. Using the median value detected for each bacteriophage (14 pfu/g for MS-2 and 82 pfu/g for PRD-1), the pore water concentrations were estimated to be 97 pfu/mL for MS-2 and 547 pfu/mL for PRD-1. Compared to the dose concentrations of 75,000 pfu/mL for MS-2 and 153,300 pfu/mL for PRD-1, these estimated pore water concentrations correspond to a removal efficiency of 99.9% for MS-2 and 99.6% for PRD-1. Considering that the extracted bacteriophages may not have all been mobile in the pore water (as noted for the fecal coliforms, see Figs. 3.12 and 3.13) and some growth of the spiked bacteriophage may have occurred, it is reasonable to conclude that a 3-log removal of the applied viral surrogates was achieved. Achievement of 3-log removal of virus after STE infiltration at 1 to 3 cm/d and percolation through 60 to 90 cm of natural soil is reasonable to achieve as shown in this and previous field studies (see Table 4.2).

Table 4.2. Results of field studies of virus treatment in wastewater soil absorption systems.

Investigator	Study characteristics/ *location*	Virus and concentrations applied	Method of assessment	Findings
This CSM study, 2000	2.7 cm/d HLR of STE to a mature chamber soil absorption system *Colorado*	Spiking of STE with MS-2 at $7.5x10^4$ and PRD-1 at $1.5x10^5$ pfu/mL	Soil core collection and extraction	99.9% removal after 60 cm
Higgins et al., 1999	3cm/day HLR of STE to a buried sand filter constructed of medium sand *Massachusetts*	Indigenous MS-2 at $3x10^4$ pfu/mL in raw wastewater and $7.8x10^3$ in STE applied to sand	Pressure-free pan lysimeters placed during sand placement in buried lined cells	74.44% removal in septic tank 99.17% removal in 30cm[1] 98.45% removal in 60 cm[1] 99.79% removal in 152cm[1]
Oakley et al., 1999	Variable loading (0.81-6.5 cm/day) of STE to a soil absorption system in clay loam *California*	Indigenous ϕX174 at $1x10^0$ to $1x10^4$ pfu/mL in STE	Suction-lysimeters augured and driven into intact natural soil	1-log removal in recirculating gravel filter 100% removal in 60 cm soil
Anderson et al., 1991	Onsite soil absorption systems and subdivisions on fine sandy soils *Florida*	Indigenous virus present in STE at 0.06 to 43.7 MPN of infectious units per L	Soil cores and extraction plus ground water samples	No Enterovirus were detected in soil samples below the soil infiltration area at four homes At one home, virus was detected in shallow ground water at 0.6 to 0.9 m depth right under the system but not 3 m downgradient from it
Gilbert et al., 1976	Secondary effluent land applied at 100 m/year with cyclic flooding onto fine loamy sand *Arizona*	Indigenous Enterovirus at $1x10^3$ to $7x10^3$ pfu/100L in municipal effluent	Ground water sampling and analysis	99.99% removal in 3 to 9 m soil

[1] %removals shown in soil are based on the STE levels applied to the soil.

4.3. DISCUSSION

The results of the evaluation completed revealed that under the conditions examined, a 3-log treatment efficiency for virus was achieved by 60-cm depth below the STE soil infiltratve surface. Removal of $\geq$ 3 logs of virus during soil absorption of STE through 60-cm depth is consistent with the results of the CSM laboratory studies (Van Cuyk et al., 1999) as well as the results of previous field studies reported by other investigators (see Table 4.2).

In this study, a strong correlation was observed between the concentrations of fecal coliforms in soil samples and the concentrations of the MS-2 and PRD-1 virus. These data suggest that under the conditions examined, fecal coliform bacteria in soil extracts may be an indicator of the presence of virus at the same location. This is in contrast to previous work conducted where indigenous bacteria and virus were isolated in ground water below municipal wastewater rapid infiltration basins. In these ground water systems, virus occurrence could not be correlated to the occurrence of total or fecal coliforms, indicating the limitation of microbial water quality indicators for predicting their virological quality (Vaughn et al., 1983; Keswick and Gerba, 1980). However, the correlation in the ground water beneath and away from systems receiving higher rate application of more highly pretreated wastewater may be different than that in the vadose zone immediately under a WSAS. In the ground water at a given point in time and space where comparative analyses were made, the processes controlling the transport/fate of the wastewater effluent fecal coliforms versus the virus might have either affected them differently and/or had sufficient time to yield differences that caused the poor correlation. Such differences might not arise in the vadose zone immediately beneath a WSAS, either due to the absence of the same transport/fate processes or lack of adequate time for the processes to yield erratic differences in concentrations. If a strong correlation between fecal coliforms and virus does exist immediately below a WSAS, this could provide an indicator of virus treatment. For example, given that fecal coliforms were very low or not detected in soil samples collected at 30 to 60 cm depth below the infiltrative surface in the pool of 16 systems examined (see Section 3), it might be plausible to assume that if virus surrogates had been added in the STE at those sites, the virus might not have been present at or past the 30 to 60 cm depths.

The virus testing completed in this study was viewed as a methods development and evaluation effort. We had originally envisioned testing up to five soil absorption systems for virus treatment efficiency, but we were unable to accomplish this due to the extensive effort required to conduct this type of field testing *in situ*. From the experience gained through this test, a few changes would be recommended. The first suggestion would be to monitor bromide concentrations in the STE dosing chamber and add necessary stock solution of bromide in order to ensure continued high dosing of the tracer. This would help sustain conservative tracer addition during the entire period of study and aid in the assessment of virus treatment, especially in cores where no virus surrogates are detected. In this experiment we were fortunate to have high levels of the virus detected in some of the surface (0-4 cm) cores collected, ensuring that some virus-amended STE had indeed reached the location from which soil cores were collected and analyzed. Since STE samples collected just prior to addition of the multicomponent mixture showed no MS-2 or PRD-1 in the system it was assumed that surrogates detected in soil cores were those intentionally added. The possibility that measured virus surrogates could be "native" to the STE should be tested for in any system prior to initiating such a test. In addition, the

possibility that growth of the added virus surrogates could be occurring should be taken into consideration when deciding on sampling times and locations.

5.0 CONCLUSIONS

A field study was completed to monitor the performance of mature wastewater soil absorption systems in Colorado to gain insight into the comparative performance of aggregate-free (chamber) and aggregate-laden (gravel) infiltration systems. A total of 16 individual onsite wastewater systems were monitored including both aggregate-free (10 chamber systems) and aggregate-laden systems (6 gravel systems). Data collected at each site included residence characteristics, system design features, STE composition, occurrence and depth of ponding of the soil infiltrative surface, and pollutant concentrations with depth below the infiltrative surface for parameters such as nutrients and fecal coliform bacteria. A laboratory study was completed to determine the relationship between fecal coliform bacteria concentrations measured directly in percolating water versus analyses of bulk soil samples. Finally, virus treatment efficiency was evaluated using a multicomponent mixture of MS-2 and PRD-1 bacteriophages and a conservative bromide tracer.

Based on the work completed and with due consideration of the related 3-D lysimeter research and previously reported findings (Van Cuyk et al., 1999; Siegrist et al., 1999), the following conclusions have been drawn and several recommendations can be made.

1. The STE composition at the individual study homes was typical of residential STE containing appreciable concentrations of pollutants. In the Hamilton Creek subdivision where 11 homes were monitored, the average concentrations were: BOD_5 = 175 mg/L, TSS = 258 mg/L, total N = 62 mg-N/L, total P = 7.7 mg-P/L, and fecal coliform bacteria = 4 x 10^6 to 6.3 x 10^6 cfu/100mL.

2. A total of 16 individual onsite systems were monitored including 10 with chambers and 6 with gravel. The chamber systems varied in age from 1 to 10 yr. while the gravel systems were 2 to 11 yr. old. The estimated hydraulic loading rates averaged 0.32 gpd/ft^2 (1.31 cm/d) for the chamber systems compared to 0.18 gpd/ft^2 (0.76 cm/d) for the gravel systems. For the chamber systems, 5 of the 10 exhibited some degree of effluent ponding while for the gravel, 4 of 6 exhibited ponding. These data suggest comparable hydraulic performance with the chamber systems receiving a higher loading rate as compared to the gravel systems (based on the normal 50% reduction allowed in the gross infiltration area for a chamber system).

3. Monitoring of soil properties and pollutant concentrations with depth beneath the infiltrative surfaces of 14 homes revealed spatially variable concentrations. At most sites, pollutant concentrations declined with depth and by 60 cm depth, fecal coliform bacteria were not detected. A Mann-Whitney nonparametric analysis revealed that the fecal coliform levels at both 30- and 60-cm depths were not significantly different between chamber and gravel systems at 95% confidence (p=0.05).

4. Based on bench-scale analyses completed with mini-columns and two soil media (clean sand with low TOC and silty sand with higher TOC), the estimated concentrations of fecal coliforms in percolating water can be conservatively estimated based on analysis of bulk soil solids. Further experimentation is warranted under a wider range of environmental and process conditions and for other constituents of interest such as nutrients and virus.

5. A methodology for using a multicomponent mixture of virus surrogates and a conservative tracer to assess virus purification in a wastewater soil absorption system was successfully applied under field conditions. In this study, 3-log reductions in the applied MS-2 and PRD-1 viral surrogate concentrations were achieve at 30 cm below the infiltration surface. The results of this effort also revealed a strong correlation between fecal coliform concentrations measured in soil core samples to MS-2 and PRD-1 virus concentrations. It was observed that the bacteriophage may exhibit apparent growth in residential STE and this must be accounted for in test design and execution.

6. Under the conditions examined in this study, the performance measurements made for the chamber systems were comparable to those determined for gravel systems, even though the chambers were estimated to be receiving a hydraulic loading rate of 0.32 gpd/ft^2 (1.31 cm/d) as compared to 0.18 gpd/ft^2 (0.76 cm/d) for the gravel systems. The performance observations made under field conditions are consistent with the findings derived from 3-D lysimeter studies carried out under controlled laboratory conditions at CSM (see Van Cuyk et al., 1999; Siegrist et al., 1999).

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7.0 APPENDIX

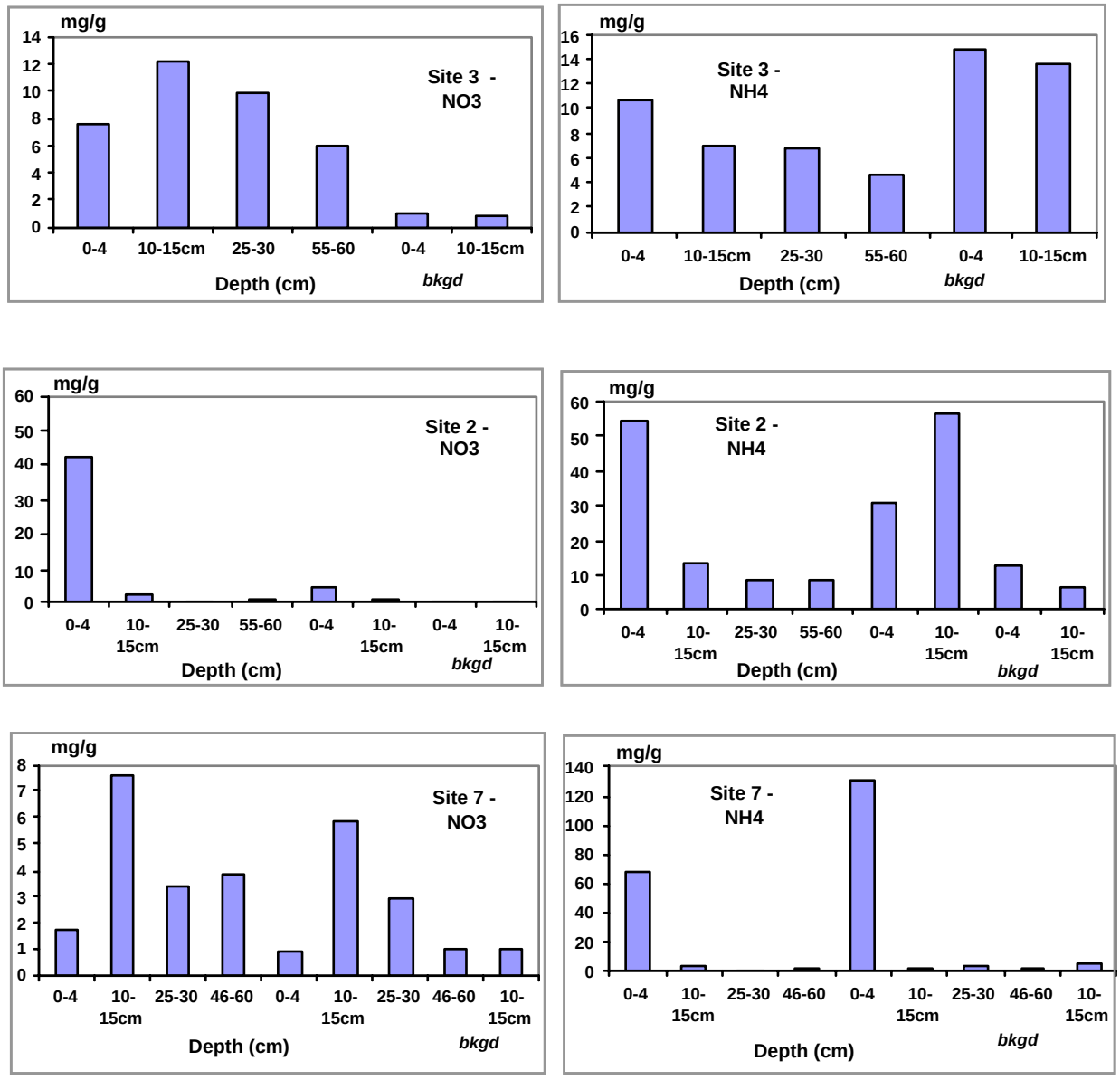

Fig. A.1. Nitrate- and ammonium-nitrogen in soil cores from homes 2, 3 and 7.

Table A.1. Characteristics of septic tank effluent samples collected from homes in the Hamilton Creek subdivision near Silverthorne, Colorado and the Todd Creek Farms subdivision near Brighton, Colorado.

Site ID	Type	pH	Alk. (mg/L)	$cBOD_5$ (mg/L)	COD (mg/L)	TSS (mg/L)	TN (mg/L)	NH_3 (mg/L)	NO_3 (mg/L)	TP (mg/L)	FC (cfu/100mL)
1	Cha[1]	7.83 7.58	460 452	113 128	109 110	55 98	41 44	25 35	1.2 0.6	6.83 6.9	5.10E+06 3.30E+06
2	Cha	7.10 7.74	420 390	173 143	124 134	20 80	47 44	37 31	0.7 0.5	5.68 6.15	4.00E+04 1.30E+05
3	Gra[1]	7.41 7.53	510 480	171 149	244 260	72 125	46 68	54 62	0.6 1.5	7.33 8.8	1.50E+06 6.20E+06
4	Gra	7.26	486	134	325	185	64	47	1.2	11.1	3.00E+06
5	Cha	7.94 7.8	524 416	112 98	162 207	118 270	68 64	50 56	0.7 0.8	10.6 ND	2.80E+05 2.40E+05
6	Cha	7.5	288	250	330	512	18	3	1.8	ND	6.30E+06
7	Cha	7.5	860	250	675	958	96	42	2.4	ND	3.00E+06
8	Gra	7.3 7.6	634 620	170 ND[2]	137 120	275 305	102 102	63 57	1.8 1.2	ND ND	3.20E+05 1.80E+05
9	Cha	6.95	594	358	990	370	74	27	3.2	ND	6.00E+06
10	Gra	7.53	616	205	425	515	58	32	1.3	ND	4.70E+05
11	Cha	7.5	692	ND	540	60	64	64	0.8	6.23	6.00E+05
12*[3]	Cha	8.04 7.95	726 658	ND ND	350 320	128 0	84 70	74.6 70.2	1.5 0.9	11.95 11.85	6.30E+05 ND
13*	Gra	7.29	678	300	815	143	56	54.4	1.7	9.25	6.30E+05
14*	Cha	7.05	674	310	825	125	72	73.4	1.1	6.25	1.30E+07
15*	Gra	8.17	320	184	270	ND	ND	ND	ND	ND	ND
16*	Cha	7.32	692	385	170	115	64	55.6	2.6	8.90	2.50E+05

[1] For Type, Cha = chamber system and Gra = gravel system.
[2] ND = no data.
[3] Denotes homes located in Brighton, CO.

Table A.2. Summary of soil core data for onsite wastewater systems in Hamilton Creek. [1]

Site ID [2]	Soil core loc.	Depth below I.S. (cm)	Soil color	Water content (wt.%)	Fecal coli. (org./g)	NH_3 (mg-N/kg)	NO_3 (mg-N/kg)	P (mg-P/kg)	Org. matter (mg/kg)
1 *cha No*	A	0-4	10YR4/6	13.2	2548	7.02	18.04	65	1.2
		10-15	10YR3/6	10.6	3801	8.15	8.35	51	1.6
	BG[3]	0-4	10YR2/2	18.6	<1	12.47	1.84	38	5.1
		25-30	7.5YR5/2	13.0	<1	6.39	1.84	25	2.1
2 *cha Yes*	A	0-4	10YR3/4	64.2	126	54.6	42.2	200	3.2
		10-15	10YR3/4	16.6	85	13.1	2.9	75	1
		25-30	10YR4/6	13.7	83	8.54	1.1	50	0.9
		55-60	10YR4/4	17.0	79	8.45	1.3	44	0.8
	B	0-4	10YR4/4	42.0	79	31.14	4.4	200	1.6
		10-15	10YR4/3	10.6	30	56.8	1.3	140	.06
	BG	0-4	10YR3/4	8.7	<1	12.47	0.5	42	1.2
		10-15	10YR4/4	5.1	<1	6.12	0.6	37	1
3 *gra No*	A	0-4	10YR2/1	9.7	<1	10.7	7.5	26	1.7
		10-15	10YR2/1	16.7	7	7.0	12.2	23	1.3
		25-30	10YR2/2	15.6	17	6.7	9.9	17	1.4
		55-60	10YR3/2	11.3	19	4.7	6.0	8	0.6
	BG	0-4	10YR2/1	13.6	<1	14.9	1.1	27	2
		10-15	10YR2/1	12.4	<1	13.6	0.8	28	2
5 *cha No*	A	0-4	10YR3/2	12.7	<1	4.75	28.2	38	2.3
		10-15	10YR4/4	9.6	<1	2.56	17.37	29	1.3
	B	0–12	10YR3/4	11.5	<1	3.84	16.48	28	1.2
		dupe.	10YR3/4	10.7	<1	4.54	29.02	34	2.3
		trip.	10YR3/4	10.1	<1	5.69	19.08	30	1.6
7 *cha Yes*	A	0-4	10YR4/4	19.8	<1	69.3	1.7	58	0.7
		10-15	10YR5/6	19.8	<1	3.3	7.5	12	0.9
		25-30	10YR4/4	17.5	<1	1.7	3.4	9	0.7
		46-60	10YR4/4	16.9	<1	2.3	3.8	15	0.8
	B	0-4	10YR4/2	19.6	3	130.4	0.8	61	0.7
		10-15	10YR4/6	17.8	<1	2.5	5.9	12	1
		25-30	10YR5/6	19.6	<1	3.1	2.9	10	0.7
		46-60	10YR3/3	15.4	<1	2.7	0.9	21	0.4
	BG	0-4	10YR2/1	-	<1				
		10-15	10YR3/2	14.3	<1	5.7	0.9	36	0.7

[1] Results expressed on dry weight basis.

[2] Site ID *cha* = chamber and *gra* = gravel. *No* = no ponding and *Yes* = ponding.

[3] BG = background cores taken at stated distance below ground surface (bgs) and analyzed in duplicate (dupl.).

Table A.2 cont. Summary of soil core data for onsite wastewater systems in Hamilton Creek. [1]

Site ID [2]	Soil core loc.	Depth below I.S. (cm)	Soil color	Water content (wt.%)	Fecal coli. (org./g)	NH_3 (mg-N/kg)	NO_3 (mg-N/kg)	P (mg-P/kg)	Org. matter (mg/kg)
8	A	0-4	10YR2/1	12.7	<1	15.75	1.91	90	0.8
gra		10-15	10YR2/1	11.4	31	3.63	2.01	67	0.5
Yes		25-30	10YR3/2	11.5	<1	3.45	1.94	11	0.8
	B	0-4	10YR3/1	10.5	131	8.86	1.85	90	0.6
		10-15	10YR3/1	13.6	6	3.95	2.57	21	0.7
		25-30	10YR3/1	9.5	<1	3.18	1.89	8	0.7
	BG[3]	105 cm bgs	10YR4/3	4.6	<1	5.16	1.84	25	1.3
		Dupl.	10YR4/3	4.7	<1	4.76	1.89	24	1.3
9	A	0-4	10YR3/6	9.8	<1	3.29	2.10	23	0.9
cha		10-15	10YR4/6	8.7	<1	2.87	2.00	17	0.8
No		25-30	10YR4/6	7.2	<1	4.32	1.93	15	0.7
		55-60	10YR4/6	9.3	<1	2.40	2.08	17	0.6
	B	0-4	10YR4/6	9.6	<1	2.91	2.09	20	0.9
		10-15	10YR4/6	9.2	<1	2.92	1.96	18	0.8
		25-30	10YR4/6	7.8	<1	4.32	1.94	19	0.8
		55-60	10YR5/6	16.2	<1	2.94	1.82	19	1.1
	BG	60 cm bgs	10YR4/3	4.2	<1	4.39	2.08	51	1.5
		Dupl.	10YR4/3	4.4	<1	4.17	2.06	50	1.6
10	A	0-4	10YR3/1	16.9	8730	102.77	1.92	93	1.3
gra		10-15	10YR4/4	12.4	293	5.3	7.89	12	0.8
Yes		25-30	10YR4/3	11.7	245	2.9	6.18	11	1.0
	B	0-4	10YR3/2	16.9	661	29.29	5.74	42	1.0
		25-30	10YR4/3	13.8	639	8.11	6.08	15	0.9
	BG	75 cm bgs	10YR5/3	3.2	<1	5.53	1.98	30	0.8
		Dupl.	10YR5/3	3.1	<1	3.69	1.92	30	0.6
11	A	0-4	10YR3/1	17.3	918	99.03	1.97	59	1.1
cha		10-15	10YR3/2	12.3	27	6.75	2.00	30	1.4
Yes		25-30	10YR3/1	10.8	191	4.48	2.03	27	1.1
		55-60	10YR3/1	10.4	34	4.04	1.97	15	0.9
	BG	75 cm bgs	10YR3/2	2.7	<1	8.44	2.09	22	3.9
		Dupl.	10YR3/2	(18.6)	<1	8.48	2.01	23	3.6

[1] Results expressed on dry weight basis.

[2] Site ID *cha* = chamber and *gra* = gravel. *No* = no ponding and *Yes* = ponding.

[3] BG = background cores taken at stated distance below ground surface (bgs) and analyzed in duplicate (dupl.).

Table A.3. Summary of soil core data for onsite wastewater systems in Todd Creek Farms. [1]

Site ID [2]	Soil core loc.	Depth below I.S. (cm)	Soil color	Water content (wt.%)	Fecal coli. (org./g)	NH_3 (mg-N/kg)	NO_3 (mg-N/kg)	P (mg-P/kg)	Org. matter (mg/kg)
12	A	0-4	10YR2/1	12.7	<1	4.26	1.92	2	1.4
cha		10-15	10YR2/1	11.4	31	3.96	1.99	1	1.3
No		25-30	10YR3/2	11.5	<1	3.73	2.00	1	1.1
	B	0-4	10YR3/1	10.5	131	3.36	2.13	1	0.9
		10-15	10YR3/1	13.6	6	3.76	2.00	1	0.9
		25-30	10YR3/1	9.5	<1	3.15	2.34	2	0.3
	BG[3]	105 cm bgs	10YR4/3	4.6	<1	4.11	2.12	1	1.4
		Dupl.	10YR4/3	4.7	<1	4.23	1.85	2	0.3
13	A	0-4	10YR5/4	26.3	227	7.78	2.52	2	0.5
gra		10-15	10YR5/4	31.1	<1	5.59	1.89	1	0.5
Yes		25-30	10YR5/4	23.3	<1	5.99	1.90	5	0.6
		55-60	10YR5/4	21.6	<1	6.26	1.85	2	0.6
	BG	50 cm bgs	10YR4/3	19.2	<1	6.19	3.27	6	0.7
		Dupl.	10YR4/3	18.7	<1	5.82	1.97	5	0.8
14	A	0-4	10YR3/1	46.0	TNTC[4]	721.99	1.97	27	1.7
Cha		10-15	10YR4/3	27.1	4208	10.13	10.49	1	1.1
Yes		25-30	10YR4/3	25.4	14029	15.21	10.19	1	0.8
		55-60	10YR5/4	ND	2853	8.19	8.44	1	0.8
	BG	70 cm bgs	10YR4/4	5.4	<1	3.17	7.97	8	0.1
		Dupl.	10YR4/4	5.8	<1	3.27	1.55	8	0.1
15	A	0-4	10YR5/4	24.9	1465	24.06	2.05	1	0.7
Gra		10-15	10YR5/4	25.9	1428	55.16	1.89	3	0.6
No		25-30	10YR4/4	23.9	1277	62.62	2.01	2	0.6
		55-60	10YR4/3	23.0	1108	65.75	1.95	5	0.6
	B	0-4	10YR4/3	25.2	TNTC	25.68	1.96	6	1.0
		10-15	10YR5/4	26.1	424	35.63	2.21	2	1.1
		25-30	10YR5/4	24.4	310	82.52	1.92	6	1.1
	BG	60cm bgs	10YR5/4	12.1	<1	9.2	5.17	4	1.8
16	A	0-4	10YR3/1	17.3	918	159.85	1.92	2	1.2
cha	B	0-4	10YR3/2	12.3	27	80.44	2.18	2	1.1
Yes		10-15	10YR3/1	10.8	191	45.20	2.50	2	1.1
	BG	45 cm bgs	10YR3/2	21.5	<1	8.76	1..85	3	2.0
		Dupl.	10YR3/2	21.5	<1				

[1] Results expressed on dry weight basis.
[2] Site ID *cha* = chamber and *gra* = gravel. *No* = no ponding and *Yes* = ponding.
[3] BG = background cores taken at stated distance below ground surface (bgs) and analyzed in duplicate (dupl.).
[4] TNTC = too numerous to count (>15,000/g). Results are express on a dry weight basis.

Table A.4. Fecal coliforms and MS-2 and PRD-1 bacteriophages with soil depth at Site 2.

Soil core sample	Depth (cm)	Fecal coliforms (cfu/g dry soil)	MS-2 (pfu/g dry soil)	PRD-1 (pfu/g dry soil)
1a(1)	5	928	673	2922
1a(2)	5	1091		
1b(1)	5	1181	194	1084
1b(2)	5	1394		
2a(1)	15	1994	62	218
2a(2)	15	2088		
2b(1)	15	1287	120	464
2b(2)	15	1347		
3a(1)	30	966	<1	250
3a(2)	30	858		
3b(1)	30	2646	108	594
3b(2)	30	3564		
4a(1)	60	895	77	602
4a(2)	60	741		
4b(1)	60	418	14	360
4b(2)	60	367		
5a(1)	5	1387	55	292
5a(2)	5	1533		
5b(1)	5	1487	52	471
5b(2)	5	1152		
6a(1)	15	3073	158	591
6a(2)	15	2364		
6b(1)	15	2161	25	712
6b(2)	15	2112		
7a(1)	30	625	28	84
7a(2)	30	616		
7b(1)	30	717	48	80
7b(2)	30	845		
9a(1)	5	864	<1	<1
9a(2)	5	1030		
9b(1)	5	1175	<1	<1
9b(2)	5	1125		
10a(1)	15	254	21	423
10a(2)	15	169		
10b(1)	15	117	98	176
10b(2)	15	20		
11a(1)	30	35	<1	<1
11a(2)	30	58		
11b(1)	30	12	<1	<1
11b(2)	30	12		
12a(1)	60	<1	<1	<1
12a(2)	60	<1		
12b(1)	60	<1	<1	<1
12b(2)	60	<1		

Table A.4. cont. Fecal coliforms and MS-2 and PRD-1 bacteriophages with soil depth at Site 2.

Soil core sample[1]	Depth (cm)	Fecal coliforms (cfu/g dry soil)	MS-2 (pfu/g dry soil)	PRD-1 (pfu/g dry soil)
13a(1)	5	969	<1	11
13a(2)	5	1079		
13b(1)	5	540	<1	20
13b(2)	5	761		
14a(1)	15	<1	<1	<1
14a(2)	15	<1		
14b(1)	15	<1	<1	<1
14b(2)	15	<1		

[1] Core segment code conveys the following: the number gives the segment number, the letter gives the duplicate subsample, and the (number) gives the duplicate analysis. For example, 1a(1) = segment 1 (location 1 at 5 cm depth), core subsample (a), and duplicate analysis (1) versus 14b(2) = segment 14 (location 4 at 15 cm depth), core subsample (b), and duplicate analysis (2).

Table A.5. Relationship of fecal coliforms and bacteriophage in soil core samples collected below the infiltrative surface of a mature WSAS.

Depth	Fecal coliforms	MS-2	PRD-1	Ratios	
(cm)	(cfu/g dry soil)	(pfu/g dry soil)	(pfu/g dry soil)	FC/MS-2	FC/PRD-1
5	928	673	2922	1.4	0.3
5	1091				
5	1181	194	1084	6.1	1.1
5	1394				
15	1994	62	218	32.2	9.1
15	2088				
15	1287	120	464	10.7	2.8
15	1347				
30	966	1	250	966.0	3.9
30	858				
30	2646	108	594	24.5	4.5
30	3564				
60	895	77	602	11.6	1.5
60	741				
60	418	14	360	29.9	1.2
60	367				
5	1387	55	292	25.2	4.8
5	1533				
5	1487	52	471	28.6	3.2
5	1152				
15	3073	158	591	19.4	5.2
15	2364				
15	2161	25	712	86.4	3.0
15	2112				
30	625	28	84	22.3	7.4
30	616				
30	717	48	80	14.9	9.0
30	845				
5	864	1	1	864.0	864.0
5	1030				
5	1175	1	1	1175.0	1175.0
5	1125				
15	254	21	423	12.1	0.6
15	169				
15	117	98	176	1.2	0.7
15	20				
30	35	1	1	35.0	35.0
30	58				
30	12	1	1	12.0	12.0
30	12				
60	1	1	1	1.0	1.0
60	1				
60	1	1	1	1.0	1.0
60	1				
5	969	1	11	969.0	88.1
5	1079				
5	540	1	20	540.0	27.0
5	761				
15	1	1	1	1.0	1.0
15	1				
15	1	1	1	1.0	1.0
15	1				
Count =	52	26	26	26	26
Min =	1	1	1	1	0.32
Max =	3564	673	2922	1175	1175

Median =	861	23	197	20.9	3.51

Note: values of 1 = nondetect at <1