Efficacy of Bacterial Reduction

by

Onsite Wastewater Treatments

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Abstract:

Effective onsite wastewater treatment is critical for maintaining the safety of environmental water supplies and is typically composed of a septic tank releasing effluent into a leach field. A traditional septic tank, an aerobic chamber, and a submerged-bed wetland used in conjunction with the traditional septic tank were compared in their ability to reduce the enteric bacterial load in treated efflux. Triplicate soil columns containing either sandy loam or clay soils were used to mimic leach fields and determine the effect of soil type on the efficiency of removing fecal bacteria removal from septic tank effluent. Wastewater samples were collected before and after treatment by each of the three methods. *E. coli* and total coliform (TC) levels were assessed using USEPA method 1604. *E. coli* and TC removal by soil columns containing low or high levels of clay were also compared. Each soil type was used for three columns and effluent from those three columns was combined for analysis. Effluent from the septic tank serves as the influent for the soil columns.

During winter and early spring, aerobic treatment resulted in the greatest reduction of total coliforms and *E. coli*; however, during late spring and summer, the trend was reversed with the septic tank + submerged-bed wetland providing greater reduction of total coliforms and *E. coli*. Mean total coliform and *E. coli* concentrations over the entire study period were not statistically different between the aerobic treatment and the septic tank + submerged-bed wetland. Concentrations of *E. coli* and total coliforms were significantly higher when the septic tank was used alone. During winter and early spring, type 3 soil significantly reduced the concentrations of total coliforms and *E. coli* more than did type1b soil. The septic tank + submerged-flow wetland in winter was as effective in removing enteric bacteria from wastewater as the aerobic treatment. Aerobic treatment provides a greater reduction of enteric bacteria than does a traditional septic tank alone. Leach fields containing high levels of clay were more effective in treating wastewater than leach field with low concentrations of clay.

Introduction:

In the United States, roughly 25% of households employ some type of onsite wastewater treatment system (USEPA staff, 1980; Neralla et al., 2000), with approximately 60 million Americans living in homes served by such systems (Crites and Tchobanoglous, 1998). The most commonly used onsite system is a conventional septic tank that releases treated effluent into a leach field. The aqueous effluent continues to move horizontally and vertically through the leach field, providing potential opportunity for fecal wastes to contaminate groundwater (Ogden et al., 2001). While moving through the leach field, pathogens in the wastewater degrade over time or are minimized through soil biota (Steer et al., 2002).

At least an estimated 100 million people in the United States rely on groundwater wells as their source of drinking water (USEPA, 2000; Vega et al., 2003). Over half of all disease outbreaks in the U.S. have been blamed on pathogens contaminating groundwater (USEPA, 2000; Vega et al., 2003). Pathogens may enter groundwater supplies by a number of routes, including leaky sewer pipes, leachate from poorly designed or malfunctioning septic tanks, and infiltration from other environmental sources (Vega et al., 2003; Scandura and Sobsey, 1997). In the U.S., malfunctioning septic systems are the third most cited source of groundwater contamination (USEPA, 1998).

Soil conditions and water table depth limit the effectiveness of leach fields, and system failure poses a threat to environmental and human health (Neralla et al., 2000). Constructed wetlands have been suggested as a possible inexpensive addition to conventional septic systems that would enable greater nutrient reduction, though large reductions of fecal coliforms and pathogens are not expected (Neralla et al., 2000). Neralla and colleagues (2000) report an approximately ninety percent reduction in fecal coliforms through the use of constructed wetlands. Mbuligwe (2005) tested a full-scale septic tank/constructed wetland system in which total coliforms were reduced by 37.4% by the septic tank alone and by an additional 99.99% in the constructed wetland, yielding an overall reduction of total coliforms of nearly 100%. Similar, though slightly greater, reductions were reported with respect to fecal coliform concentrations (Mbuligwe, 2005). Kaseva (2004) reports a 43-60% reduction in total coliforms when using constructed wetlands to further treat effluent from upflow anaerobic sludge blanket, an alternative to conventional septic tanks used in tropical areas.

The Texas Commission on Environmental Quality (TCEQ) permits three soil classifications (types) to be used in septic leach fields. Those "types" include type Ib (sandy) soils, Type II (sand/silt) soils and Type III (silt/clay) soils. According to the TCEQ gravely soil (type Ia) is too permeable to be allowed for use in septic leach fields and the low hydraulic conductivity of clay soil (type IV) makes it unsuitable as well.

The efficacy of constructed wetlands in reducing microbiota present in wastewater can vary significantly based on local climatalogical and soil conditions (Vega et al., 2003), illustrating the need for regionally-specific data. The treatment of wastewater in constructed wetlands is a result of a number of interacting features, including plants, soil, and wastewater, that form a complex set of physical, chemical, and biological processes (Hagendorf et al., 2005). A recent constructed wetland study conducted in Texas showed an approximate 1-log reduction in S*almonella sp.* during winter months. Greater reduction occurred during summer months, but viable *Salmonella* cells were present in effluent from the constructed wetland during both summer and winter conditions. Phages were reduced by 2.5 logs during summer months and an 8-log reduction in winter months due to treatment within the constructed wetland (Vega et al., 2003).

Constructed wetlands are usually used in tandem with a conventional septic tank responsible for pre-treatment of the wastewater. The wetland is composed of a soil filter, with both horizontal and vertical components, containing various plant species and sometimes utilizes a polishing pond or small reservoir just prior to discharge of treated effluent (Hagendorf et al., 2005). The wetland used in this study follows this basic design, without a final polishing pond.

E. coli is commonly used as an indicator organism for fecal pollution because it is not able to reproduce in environmental waters. A recent study showed that *E. coli* in sterile well or tap water decreased by approximately 1 log unit in 49 days, while the decay was increase to approximately 3 log units over the same time duration in nonsterile well or tap water (Foppen et al., 2007). Based on this information, we expected the concentrations of *E. coli* to decrease over time with each wastewater treatment. Foppen et al. (2007) also showed that *E. coli* was reduced by all sediments tested by 2 to >5 log units over sediment distances ranging from 0.4-0.9 cm. Similarly, we expected greater reduction in concentrations of *E. coli* in the effluents from the soil columns. In spite of the reduction in numbers of *E. coli* with increasing soil depth, it is still possible for *E. coli* to be transported to groundwater relatively quickly due to the presence of large-diameter pores in the soil (Foppen et al., 2007).

Aerobic treatment of wastewater is used as an alternative to a traditional septic tank in areas where soils do not meet the TCEQ standards for leach fields. These systems require additional maintenance relative to septic tanks; however, they do not rely on leach field soils to aid the treatment process. Potts et. al. (2004) reported a 98-100% reduction of fecal coliforms using aeration treatment, which was significantly greater than the reduction seen by venting directly to a leach field. These researchers found aeration to significantly impact the removal of nitrogen, biological oxygen demand, fecal coliforms, and *E. coli*.

Standard methods of determining treatment efficacy in the wastewater industry are based upon measures of total suspended solids and biological oxygen demand. Nutrient reduction, especially with respect to the reduction of nitrates to nitrites and ammonia, is also considered. The numbers and types of microorganisms surviving treatment are largely unknown in the area of onsite wastewater treatment. Because traditional onsite systems involve release effluent into a leach field that provides the opportunity for microorganisms in the effluent to enter additional environmental media, it is critically important that the organisms capable of surviving treatment be identified.

The purpose of this study was to examine the comparative effectiveness of bacterial reduction by three primary onsite wastewater treatment systems in reducing fecal bacteria loads. A conventional septic tank, a constructed wetland in tandem with a conventional septic tank, and an aerobic waste treatment unit were compared. Bacterial reduction by two soil types, type Ib and type II, were compared in an effort to quantify the additional treatment offered by leach fields and to determine if coarser soil poses and increased risk of environmental contamination from leach fields.

Materials and Methods:

Raw wastewater is supplied by the Waco Municipal Area Regional Sewerage System (WMARSS). The systems is dosed during three time periods designed to mimic typical household use with 35% of the total daily influent entering the septic tank between 6:00am and 9:00am, 25% between 11:00am and 2:00pm, and 40% between 5:00pm and 8:00 pm, following the NSF/ANSII Standard 40 protocol. The aerobic unit does at a 3-hour offset relative to septic dosing times. Effluent from the septic tank enters a submerged-bed constructed wetland that is 10 ft x 50 ft and has approximately 6 inches of gravel above the water level. Effluent from the constructed wetland is returned to the WMARSS plant for treatment prior to release into environmental waters. A portion of the septic tank effluent is pumped directly into soil columns designed to mimic leach fields. The columns contain either type Ib sandy loam soil or type III fine grain soil, with each soil type in triplicate columns. Effluents from the triplicate columns are pooled prior to analysis. Excess effluent is disposed of according to EPA regulations. Treated effluent from the aerobic unit is also returned to WMARSS for treatment (see Figure 1).

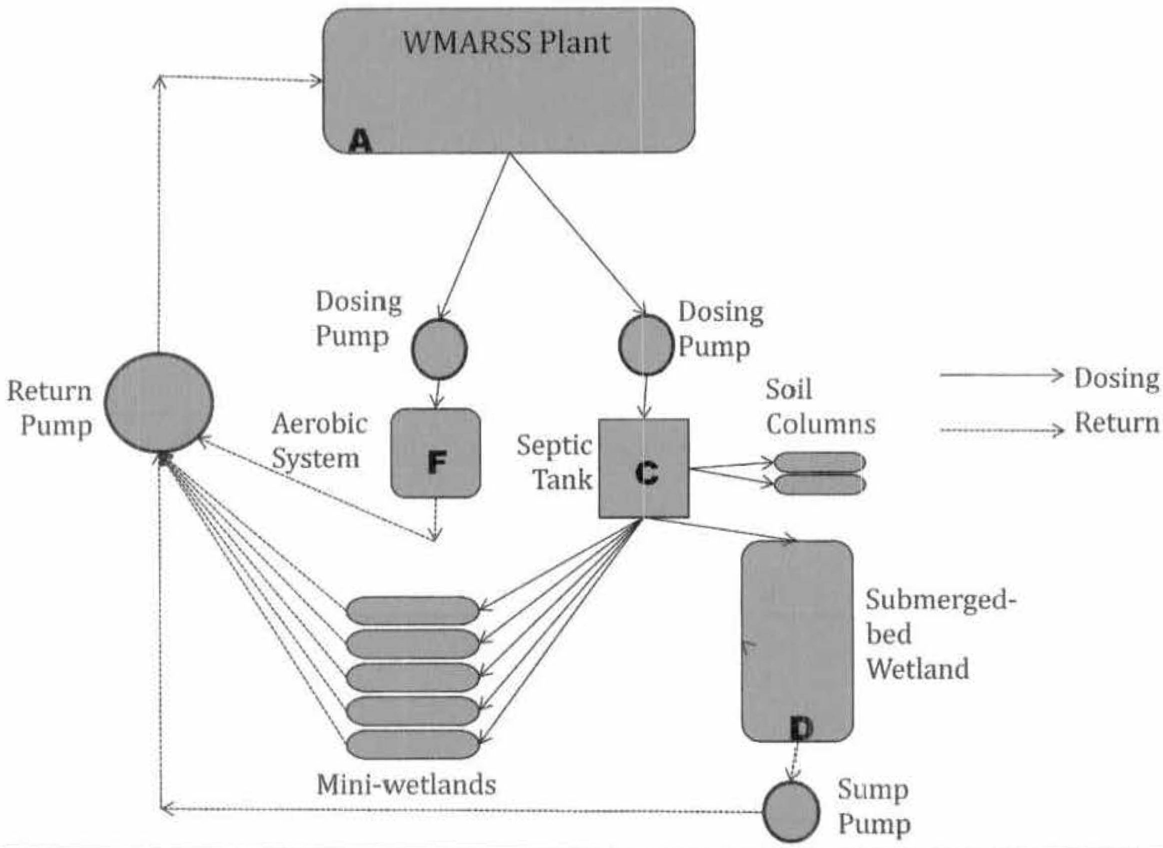


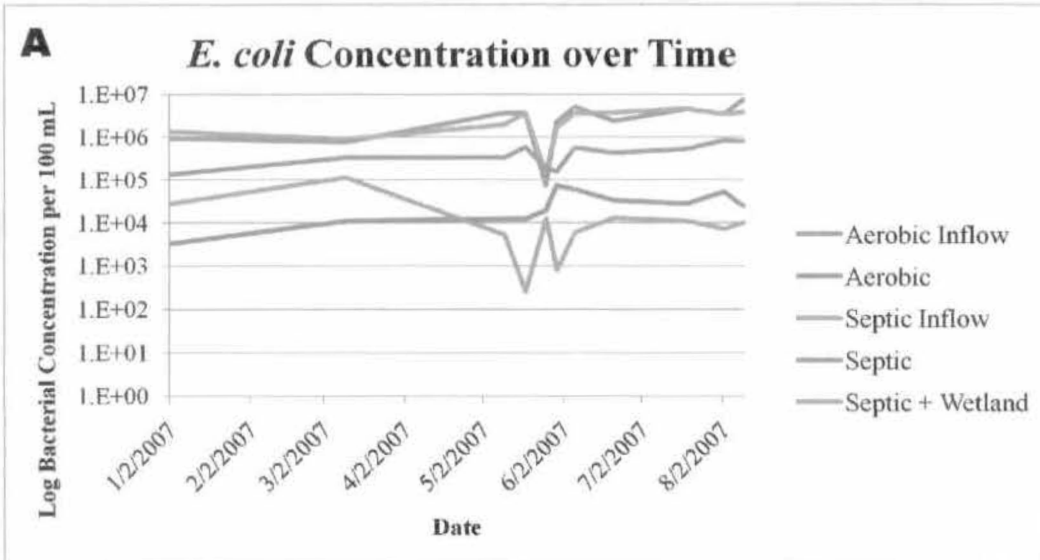
Figure 1: **Schematic of the Baylor Wastewater Research Program site.** Arrows indicate direction of flow. Dotted arrows indicate return flow to WMARSS treatment plant.

Water samples were collected directly from effluent from each treatment system in sterile polypropylene (VWR International, West Chester, PA) or PETG (Nalgene Nunc International, Rochester, NY) containers and were processed according to EPA protocol 1604. Briefly, water samples were filtered using filter funnels (Pall Corporation, East Hills, NY) containing 0.45 µm filters (Millipore, Billerica, MA). Membranes were incubated overnight at 37°C on MI agar (Difco). Blue colonies were enumerated as *E. coli* isolates while colonies fluorescing under UV irradiation were counted as total coliform isolates. Statistical analyses were completed using either Microsoft Excel or JMP.

Results:

Wastewater treatment efficacy was compared for a traditional septic tank alone, a septic tank in conjunction with a submerged-bed wetland, and an aerobic waste treatment unit. Efficacy was determined based on reduction of fecal indicator bacteria as determined by membrane filtration.

In all sites other than the constructed wetland, the concentrations of both *E. coli* and total coliforms generally increased over time (Figure 2). The bacterial concentrations in the constructed wetland effluent decreased in the spring and summer months. A storm event occurred 26 May 2007, resulting in a drop in concentration of total coliforms and *E. coli* in all sites other than the aerobic unit. The constructed wetland experienced a drop in *E. coli* concentrations due to the May 26 rain event; however, the concentration of total coliforms in the wetland effluent increased during this same time period (Figure 2).



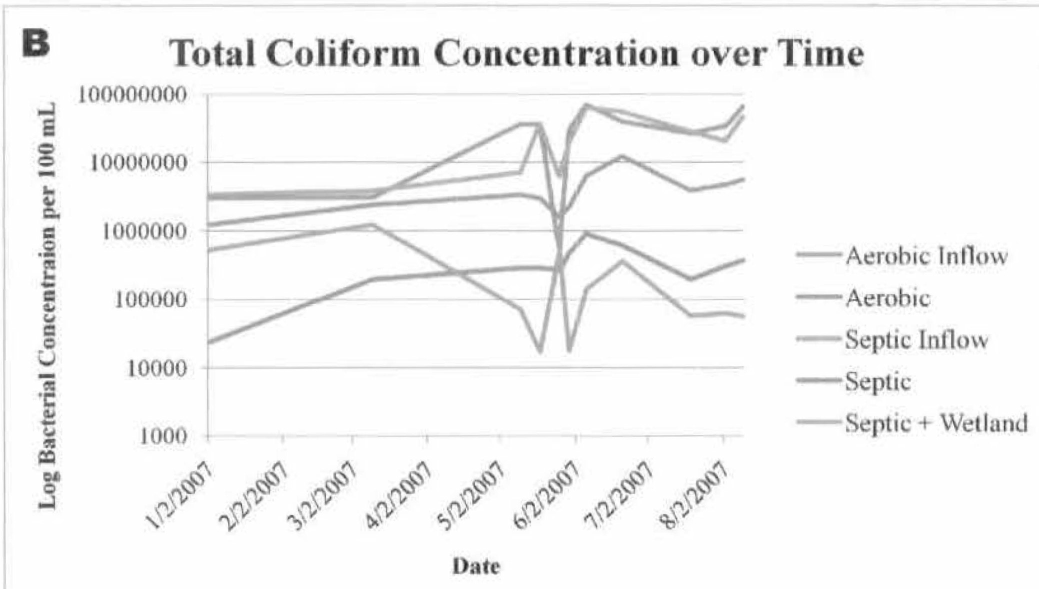


Figure 2: **Concentration of fecal bacteria.** Lines depict concentration (CFU/100 mL) of *E. coli* (A) and total coliforms (B) for five test sites over the study period. Sites include the raw influent into the septic tank (green), effluent from the septic tank (purple), effluent from the submerged-bed wetland (light blue), effluent from the aerobic unit (red), and raw influent into the aerobic unit (dark blue).

Though some variation exists in the bacterial concentrations present in the influents into the septic tank and aerobic unit due to dosing schedule, these variations are not statistically significant (Figure 3). Mean concentrations of total coliforms and *E. coli* over all sample dates (January – August, 2007) are not statistically different between the aerobic unit and the constructed wetland effluents; however, the septic tank effluent was significantly greater than both the aerobic and wetland effluents (Figure 4).

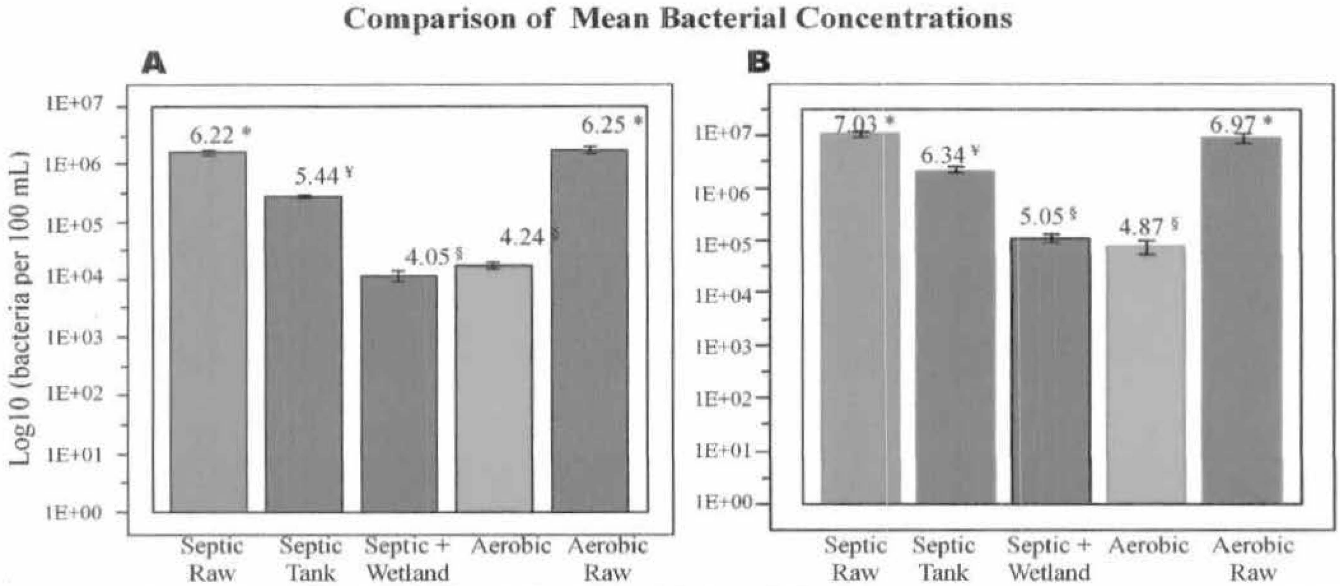


Figure 3: **Mean concentrations of fecal bacteria.** Bars indicate mean concentration of *E. coli* (A) of total coliforms (B) for the raw influent into the septic tank (red), effluent from the septic tank (green), effluent from the submerged-bed wetland (blue), effluent from the aerobic unit (orange), and raw influent into the aerobic unit (purple). Standard error base are shown. Actual means are listed above the bars. *, ¥, § indicate statistical groups.

The septic tank alone achieved a mean reduction of 84.22% in *E. coli* concentrations. Addition of the constructed wetland increased the *E. coli* reduction to 98.34%, almost equal to the 98.91% achieved by the aerobic system (Figure 4). Similar reductions were seen in total coliform concentrations (data not shown). Comparatively, the WMARSS plant achieved a mean *E. coli* reduction of 100% (Figure 4).

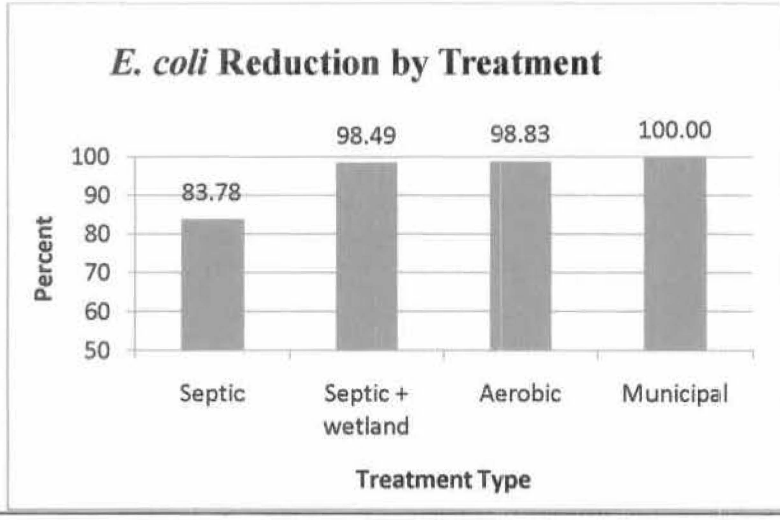


Figure 4: **Percent reduction of *E. coli* by treatment.** Bars indicate the percent reduction of *E. coli* by the septic tank, the submerged-bed wetland, the aerobic unit, and the municipal wastewater treatment plant (WMARSS).

No statistical significance was found between the type Ib and type III soils in the mean concentrations of *E. coli* and total coliforms in their effluents (Figure 5) when all sample dates were considered. During cooler months, January – March, the *E. coli* concentrations were significantly lower in the effluent from the type III soils than in the effluent from the type Ib soils (Figure 6). Similar differences were seen in concentration of total coliforms in the effluents from type Ib and type II soils, with type III soils generating statistically lower concentrations of total coliforms (data not shown).

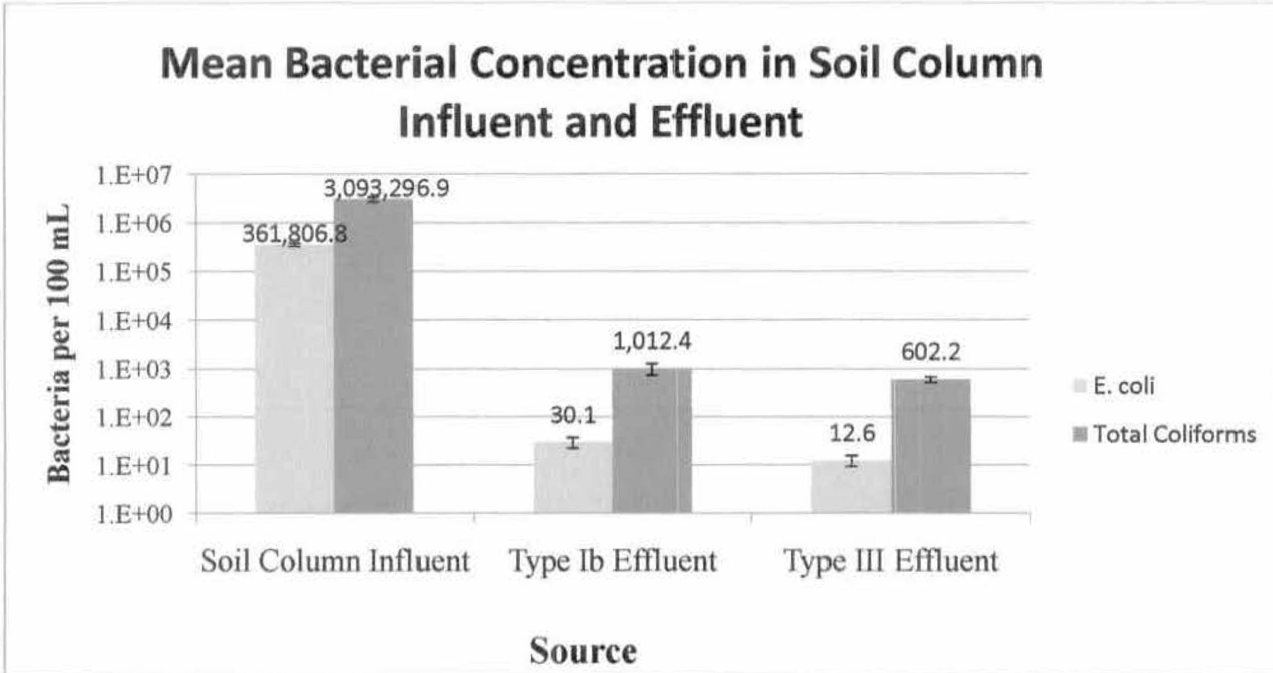


Figure 5: **Effect of soil type on fecal bacteria within wastewater.** Mean concentrations of *E. coli* (light grey) and total coliforms (dark grey) shown with standard error bars. Means are listed above the bars. Soil column influent is the effluent from the septic tank. Concentrations of *E. coli* and total coliforms in effluents from both soil types are statistically less than the corresponding concentrations in the influent.

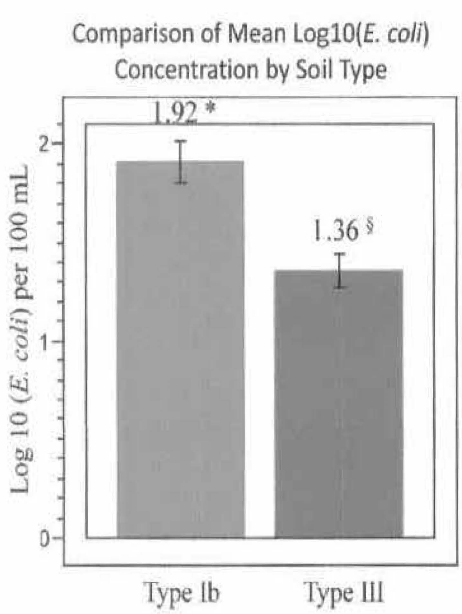


Figure 6: **Mean log10(*E. coli*) concentrations in type Ib and type III for Jan.-Mar.** Bars indicate the mean concentration of *E. coli* per 100 mL by each soil type. Standard error bars are included and means are listed above each bar. * and § are statistically different.

Discussion:

As temperatures warmed in the spring months, the concentrations of both total coliforms and *E. coli* decreased in the effluent from the constructed wetland. During this same period of time, all other sites experienced an overall increase in total coliform and *E. coli* concentrations (Figure 2), likely due to ambient temperatures being more conducive to bacterial survival. The trends resulted in the submerged-bed wetland, when used in tandem with a conventional septic tank, outperforming the aerobic unit in terms of bacterial reduction during warmer months. This trend held throughout the summer, from May through August; however, there is no statistical difference in the overall mean bacterial concentration in the effluent from the constructed wetland and the aerobic unit (Figure 3). The ability of the submerged-bed wetland to produce effluent with low loads of total coliforms and *E. coli* indicate the potential addition of such wetlands to be used in warm semi-arid regions as an alternative to the conventional septic tank system, as was suggested by Neralla et. al. (2000).

The May 26 storm event dramatically lowered concentrations of total coliforms and *E. coli* in all sample sites (Figure 2). This is most likely due to the dilution of the inflowing untreated sewage with storm runoff. Not surprisingly, lower loading rates led to lower concentrations in the treated effluents. The submerged-bed wetland experienced a slow decrease in *E. coli* and coliform levels preceding the May 26 storm event. This is likely due to a series of rainfalls throughout the month of May, which would dilute the contents of the wetland.

Treatment by the septic tank resulted in an 83.78% reduction in *E. coli* in the wastewater (Figure 4), leaving 5.44×10^5 *E. coli* in the treated effluent as it entered either the soil columns or the constructed wetland (Figure 3). The wetland further treated the wastewater for a total reduction in *E. coli* of 98.49% (Figure 4) leaving approximately 4×10^4 *E. coli* per 100 mL remaining in the treated effluent (Figure 3). Both type Ib and type III soils resulted in greater than 99% reduction in *E. coli* with a 30.1 and 12.6 mean *E. coli* concentration per 100 mL respectively (Figure 5). Though no significant difference was detected in mean *E. coli* concentrations in soil column effluents over the entire study period (Figure 5), the type III soils resulted in significantly lower *E. coli* concentrations during the January – March time period (Figure 6). Higher temperatures during summer months likely resulted in the increased bacterial reduction seen by soil columns during this time period. During extremely hot dry periods, especially in July and August, increased evaoptranspiration resulted in minimal or no effluent from the soil columns. Both the septic tank + submerged-bed wetland and the aerobic unit produced a better than 98% reduction in *E. coli*, resulting in more than 4×10^4 *E. coli* per 100 mL remaining in the treated effluent (Figure 4). The wetland continues to produce effluent with mean total coliform concentrations above the 1000 CFU/100 mL recommended by the EPA, unlike another study reporting similar percent reductions in total coliforms (Steer et al., 2002).

Interestingly, the aerobic unit appears to reduce the total coliform concentration slightly more than does the submerged-bed wetland; however, the trend is the opposite for *E. coli* concentrations with the aerobic unit producing effluent with slightly higher concentrations than that of the submerged-bed wetland (Figure 3). Though these trends are not statistically different they raise an interesting possibility that reduction of total coliforms may not occur at the same rate as the reduction of *E. coli* under some conditions. This indicates a need for greater understanding of the ability of onsite systems to reduce concentrations of microorganisms of interest, specifically pathogenic microbes, instead of relying on the reduction of indicator organisms as a measure of effective wastewater treatment. If treatments affect different species of microorganisms differently, then the possibility of differential treatment exists and should be examined more closely.

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