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Article

Preliminary Study on the Effect of Wastewater Storage in Septic Tank on *E. coli* Concentration in Summer

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Abstract: On-site wastewater treatment systems (OWTS) work by first storing the wastewater in a septic tank before releasing it to soils for treatment that is generally effective and sustainable. However, it is not clear how the abundance of *E. coli* changes during its passage through the tank. In this study, which was conducted under the UGA young Scholar Program in summer of 2010, we examined the change in wastewater quality parameters during the passage of the wastewater through the tank and after its release into soil. We collected wastewater samples at the inlet and outlet of an experimental septic tank in addition to obtaining water samples from lysimeters below trenches where the drainpipes were buried. We report that *E. coli* concentration was higher by 100-fold in the septic tank effluent than influent wastewater samples, indicating the growth of *E. coli* inside the tank under typical Georgian summer weather. This is contrary to the assumption that *E. coli* cells do not grow outside their host and suggests that the microbial load of the wastewater is potentially enhanced during its storage in the tank. Electrical conductivity, pH and nitrogen were similar between the influent and effluent wastewater samples. *E. coli* and total coliform concentrations were mainly below detection in lysimeter samples, indicating the effectiveness of the soil in treating the wastewater.

Keywords: on-site wastewater treatment systems; *E. coli*; total coliform; growth; septic tank; piedmont

1. Introduction

Nationally, more than a quarter of US households employ on-site wastewater systems (OWTS) to treat and dispose wastewater [1]. In Georgia, the percent use of OWTS is higher than the national average at about 37%. These systems, also known as septic systems, are commonly designed to accumulate the waste in a two-chamber tank where solids settle while the wastewater flows to a distribution box that is connected to one or more perforated drainpipes that distribute wastewater to the soil. The drainpipes are commonly installed in trenches and surrounded by a supporting material such as gravel or polystyrene to prevent clogging. Wastewater treatment occurs in the soil via biological (predation, die-off), chemical (adsorption) and physical (filtration) mechanisms before it reaches the surrounding ground or surface waters [1,2]. OWTS must, therefore, be installed in suitable soils that can accomplish the treatment processes properly [3–5].

In general, OWTS are an effective and sustainable way of treating wastewater. OWTS can negatively impact the microbial quality of surrounding water bodies. This is mainly true if OWTS are failing, which could happen due to installation of OWTS in unsuitable soils, age of the system, excessive use of water, or poor maintenance [6,7]. Properly functioning OWTS can also contaminate surrounding water bodies at times of extreme weather [8,9]. This happens due to excessive moisture in soils that decreases the depth of the unsaturated layer where the wastewater is treated before it reaches the ground water below. In the presence of excessive soil moisture, the downward movement of the wastewater is facilitated without allowing enough time for it to interact with the soil environment for treating the contaminants.

Contaminants of concern commonly associated with OWTS are microbial pathogens and nutrients (mainly nitrogen), which are the leading causes of water quality impairments in US streams and rivers [10]. There are technologies that can be retrofitted into existing OWTS to reduce the amount of contaminants in the wastewater effluent. These technologies are commonly called advanced treatment units and work by mainly manipulating the oxygen and carbon content of the wastewater [11–13]. In pre-anoxic units, for example, the wastewater is made to pass through an aerobic unit that is retrofitted between the septic tank and the drainfield to nitrify the ammonium into nitrate. The nitrified wastewater is then recycled back to the anoxic septic tank where it is denitrified in the presence of a carbon source. In another variation, the septic system is fitted with an aerobic unit before the septic tank to facilitate the processes of nitrification and denitrification to sequentially remove nitrogen. The limited field studies carried out so far to evaluate the effectiveness of these units mainly focused on nitrogen [14,15]. The impact of these units on the microbial load of the wastewater is largely unknown. The technologies are not yet popular throughout the United States as they are expensive [16].

Previous studies that looked at the impact of OWTS on water quality had mainly focused on how well wastewater is treated in soils before it joins water bodies directly by installing monitoring wells around these systems, e.g., [9,17] or indirectly by comparing water quality in areas with varying densities of OWTS, e.g., [18]. While these approaches are sound, they do not give us any information on the kind of microbial transformation the wastewater undergoes when stored in the septic tank. This is important because it might affect the microbial contaminant load of the wastewater when it leaves the tank. In this study, which was conducted under the UGA Young Scholar Program in June of 2010, we examined the change in the quality of the wastewater before it gets to and after it leaves the septic tank but before it is released to soil. Water samples were also collected from suction lysimeters installed below the trenches of the OWTS in a typical Georgian soil (red clay soil) into which the wastewater was released for treatment. We were particularly interested in investigating whether *E. coli* cells were capable of multiplying in the septic tank.

2. Materials and Methods

2.1. Study Site and On-site Wastewater Treatment System (OWTS)

The wastewater and water samples in this study were collected from an experimental on-site wastewater treatment system (OWTS) that was installed at the Westbrook Farm of the University of Georgia, Griffin Campus in 2008 [19]. Briefly, the system consisted of an above-ground dosing tank (4170 L capacity) where residential strength wastewater obtained from Cabin Creek Wastewater Treatment Plant in Griffin, GA was stored before it was dosed to a 3875 L capacity septic tank. The retention time for the wastewater in the septic tank was 6 days. The wastewater was dosed to the drainfield at a rate of 648 L per day. The dosing schedule was three times per day every 8 h, with the total dose being divided evenly over that time period. The Cabin Creek Plant served a residential area and monthly Georgia Environmental Protection Division (EPD) reports provided by the wastewater treatment plant verified that the wastewater was residential-strength as defined by five-day biochemical oxygen demand (BOD5) and total suspended solids (TSS), respectively, 45.2 and 35 mg per L [20]. The wastewater was tested for BOD5 and TSS before dosing to make sure that they were of residential-strength. The BOD5 and TSS values showed as much as 50% variation among measurements during different sampling times in June 2010. Wastewater was collected from the inlet of the wastewater treatment plant and transported to the site twice per week. The wastewater was then released from the septic tank into three drain pipes via a distribution box. The perforated drain pipes were installed in 10 m gravel trenches in a Cecil series soil (fine kaolinitic thermic typic kanhapudult) [19]. The trenches were installed in the B-horizon, which had two layers, Bt1 and Bt2. The texture of the Bt1 and Bt2 was clay and sandy clay, with saturated hydraulic conductivity (K_s) of 5.7–65 and 22–31 cm/d, respectively. The soil porosity in the Bt1 and Bt2 layers was 39 and 30%, respectively.

The septic tank was installed in the ground with approximately 15 cm protruding from the surface to allow easy access for sampling. The septic tank was dosed every 8 hours for two years. For this particular study, however, influent (SIN) and effluent (SOUT) wastewater samples from the septic tank were collected once a week for three consecutive weeks in June 2010 (3, 10 and 17 June). Water samples were also collected from ceramic suction-cup lysimeters that were installed 15 cm below the trenches, which were approximately 70 cm below the soil surface, using a hand held vacuum pump into sterile plastic bottles. The samples were stored in a cooler with ice until they were taken to the laboratory within a few hours for testing.

2.2. Water Quality Parameters

The pH and electrical conductivity (EC) of the wastewater and water samples were measured by using a hand held probe (ORION 3 Star, Thermo Scientific, Beverly, MA, USA) in duplicates. The probes were calibrated with standard solutions before every measurement according to the instructions of the manufacturer. SIN and SOUT samples collected on 3 and 24 June 2010 were also analyzed for ammonium and nitrate. Ammonium and nitrate were determined calorimetrically using the Phenate and the Cadmium Reduction methods [21], respectively. The samples were also tested for total coliform and *E. coli* by using the IDEXX Colilert-18® kit, which has a detection limit of 1 organism per 100 ml (IDEXX Laboratories, Inc., Westbrook, ME, USA) in duplicates. Based on the number of positive wells in the 97-well tray (positive was indicated by a yellow color for total coliform and UV fluorescence for *E. coli*), the corresponding most probable number (MPN) value per 100 mL sample was obtained with manufacturer supplied MPN tables [22].

2.3 Statistical Analysis

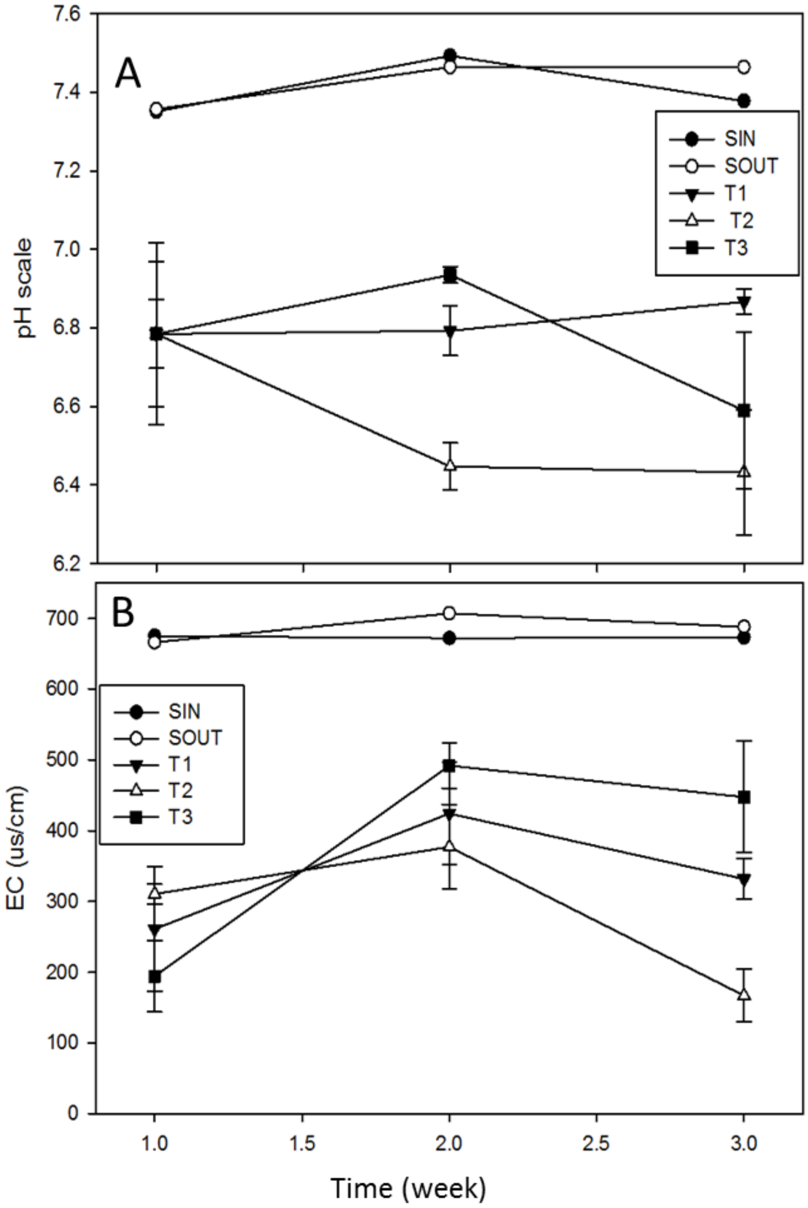
A two-way Analysis of Variance (ANOVA) was done on the pH, EC, nitrogen, total coliform and *E. coli* data to investigate the statistical significance of the effect of time (week) and location (septic tank inlet, outlet, trench 1–3) on these parameters in SAS 9.3 (SAS Institute, Inc., City, NC, USA) at significance level of $\alpha = 0.05$. One way ANOVA was also done on individual data sets to examine the significance of the effect of one of the factors (e.g., time or position) at a time. A pair-wise t-test was also done on *E. coli* data to compare the septic tank influent and effluent samples for each time period separately. The data were either log or inverse transformed to fulfill the assumptions of the models used for analysis.

3. Results

3.1. Common Water Quality Parameters

Influent and effluent wastewater samples had significantly higher pH ($P < 0.0001$) and EC ($P < 0.0002$) values than the water samples collected from lysimeters 15 cm below the trench bottoms (Figure 1). The pH values of the influent and effluent wastewater samples were similar over the three-week time, averaging about 7.4 (Figure 1A). The EC values were also similar for the two wastewater samples, with the three-week average of 673 and 678 µs cm^{-1} for the influent and effluent samples, respectively (Figure 1B). The pH values for the trench samples ranged between 6.5 and 6.8, which were 0.6 to 0.9 units below the wastewater samples. The EC values for the trench samples ranged between 285 and 378 µs cm^{-1}, which were on average 50% lower than the wastewater samples (Figure 1). The effect of time was not significant on either pH ($P = 0.1413$) or EC ($P = 0.4577$). The average ammonium concentration for two sampling times (June 3 and 24) for SIN and SOUT samples were 34.93 and 33.57 mg NH_4^+-N per L, respectively, while nitrate concentration was below detection (0.02 mg NO_3^--N per L). There was no significant difference between the SIN and SOUT samples in regards to nitrogen. We did not see any time effect either on these nitrogen forms. The dominance of ammonium in both sample types indicates that nitrification was limited in the septic tank, which is anoxic.

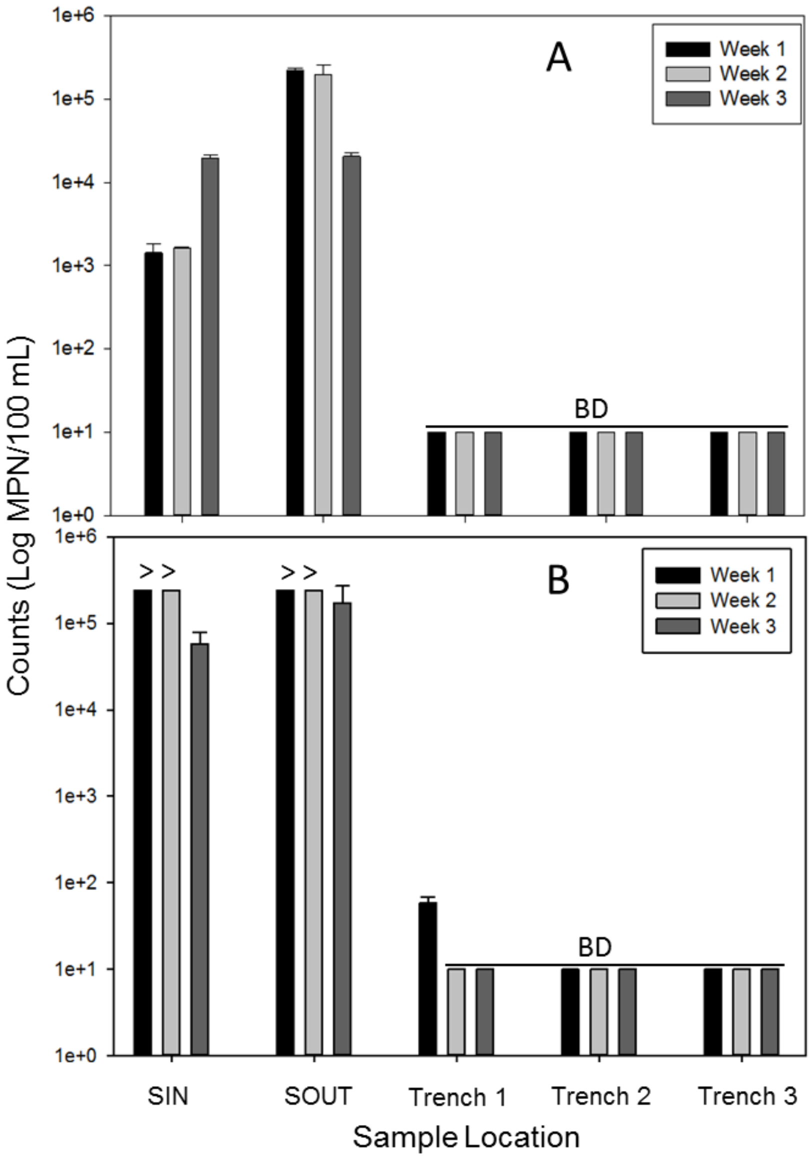
Figure 1. pH (**A**) and electrical conductivity (EC); (**B**) values of wastewater samples from septic tank inlet (SIN) and outlet (SOUT), in addition to water samples from lysimeters installed 15 cm below the drainfield trenches (T1, T2 and T3). Wastewater and water samples were collected on June 3 (week 1), June 10 (week 2) and June 17 (week 3).



3.2. Fecal Indicator Bacteria

Based on a two-way ANOVA, the main effect of location (septic tank inlet, septic tank outlet, trench 1, 2 and 3) on *E. coli* concentration was statistically significant (P = 0.0004), while the effect of time (week 1, 2 or 3) was not (P = 0.9914). *E. coli* concentrations in the septic tank influent samples were 3.1, 3.2 and 4.3 log per 100 mL while the concentrations in the septic tank effluent were 5.3, 5.3 and 4.3 log per 100 mL for the first, second and third week of sampling, respectively, indicating a 100-fold increase in *E. coli* concentration in the effluent samples in the first and second weeks (Figure 2A). A pair-wise t-test on *E. coli* concentrations of septic tank influent and effluent samples for the individual sampling week indicated that the difference was statistically significant for weeks 1 ($P = 0.0016$) and 2 ($p = 0.0016$) but not week 3 ($p = 0.6174$). *E. coli* concentrations in the trenches were below detection.

Figure 2. (**A**) *E. coli*; and total coliform (**B**) counts of wastewater samples from septic tank inlet (SIN) and outlet (SOUT), in addition to water samples from lysimeters installed 15 cm below drainfield trenches (T1, T2 and T3). Wastewater and water samples were collected on June 3 (week 1), June 10 (week 2) and June 17 (week 3).



Notes: > = greater than indicated concentration; BD = below detection.

Total coliform concentrations were above the maximum level of detection (>5.38 log MPN per 100 mL) at the dilution level that was employed during testing (1:100) for the septic tank influent and effluent samples for weeks 1 and 2 (Figure 2B). For week 3, however, total coliform concentrations were higher in the septic effluent samples (5.24 log per 100 mL) than the septic influent samples (4.76 log MPN per 100 mL). Total coliform were below detection levels (BD) in trench water samples except for water samples on the first week in trench 1.

4. Discussions

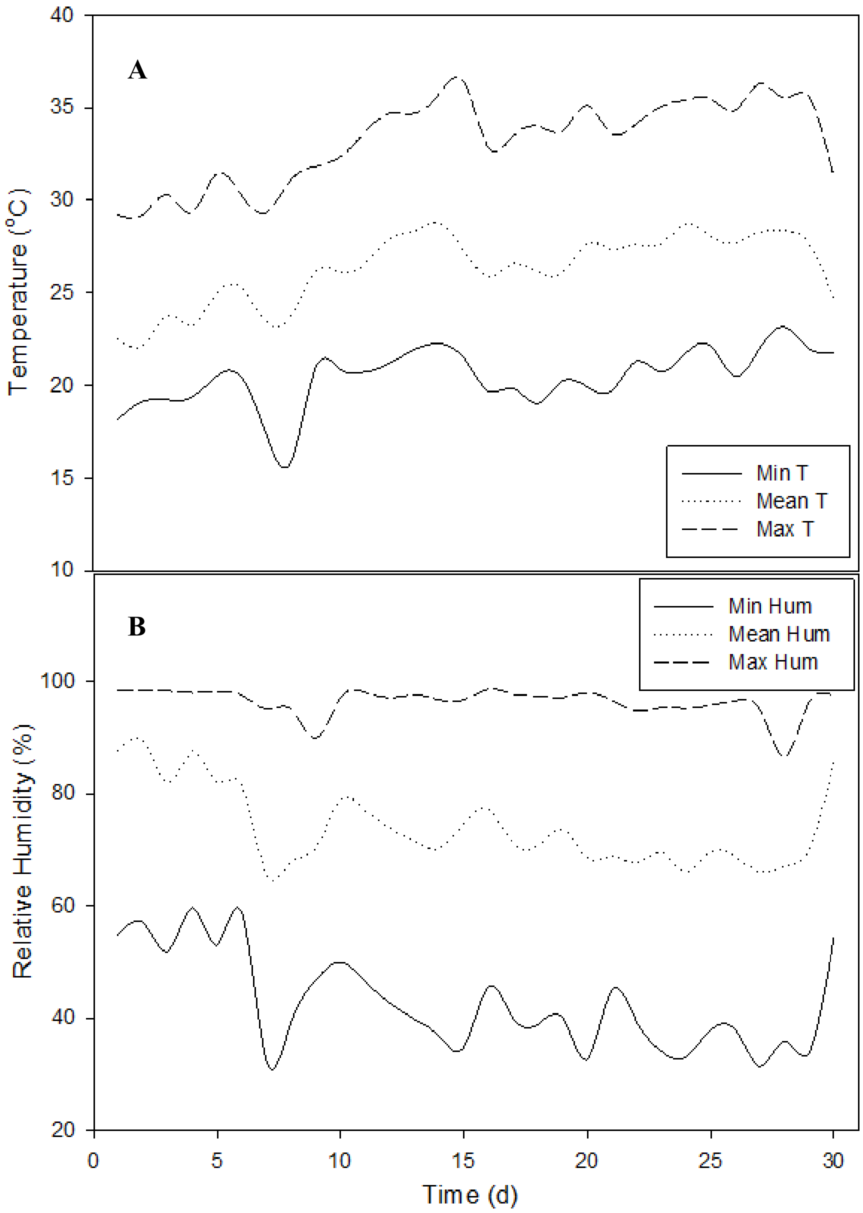
As indicated by the pH, EC, ammonium and nitrate concentrations, there was not a significant change in the chemical property of the wastewater during its passage through the septic tank. These parameters are commonly used as chemical water quality indicators. There was, however, a significant change in its microbiological property. The increase in *E. coli* concentration in the effluent samples indicated that *E. coli* was able to grow inside the septic tank under typical summer weather in GA. The maximum air temperatures during the sampling days were close to the ideal growth temperature for *E. coli,* with 29.21, 31.84 and 32.86 °C for the first, second and third sampling times, respectively (Figure 3). The maximum relative humidity was also high, with 98.5%, 89.8% and 98.6% for the first, second and third sampling times, respectively (Figure 3). Temperatures in the septic tank were not measured. Since the top 15 cm of the tank was exposed, it can be expected that the temperatures in the tank were somewhat higher than the temperatures in a typical septic tank where the top is approximately 30 cm below the surface. This pattern of *E. coli* growth can also be expected to happen in other summer months, which are warmer than June in GA (e.g., July and August) [23].

Our study supports previous findings of growth of *E. coli* in tropical soils with similar type of weather [24–26]. To our knowledge, however, growth of *E. coli* in septic tanks has not been previously reported. Ottoson and Stenstrom [27] reported the growth of *Enterococci* and *Salmonella* in sterilized sediment from a settling tank of greywater in a laboratory study in Sweden but the bacteria did not grow in unsterilized sediment. This is different from our study in that greywater does not include the solid waste from the toilet and that the growth of the bacteria happened only after removal of the indigenous microorganisms through sterilization. They also did not investigate the growth of *E. coli* in the sediment. The fact that *E. coli* is capable of multiplying in environments other than the guts of warm-blooded animals undermines the original assumption under which *E. coli* was recommended to be used as indicator of microbial water quality [28,29]. The implication of *E. coli* growth in septic tanks is that the bacterium or other pathogens could be introduced into the soil environment at enhanced concentrations. This can potentially saturate the adsorption capacity of the coarse textured soils, which are widely found in coastal areas, facilitating their downward movement to groundwater sources [4]. We observed *E. coli* growth in the septic tank in the first two weeks, but not in the third week. We are not sure why as the environmental conditions such as temperature and relative humidity were not that different among the three days (3, 10 and 17 June) the samples were collected (Figure 3).

Existing technologies to enhance the performance of OWTS involve the use of advanced treatment units that manipulate the oxygen content of the wastewater to enhance the nitrification-denitrification processes to reduce the level of nitrogen (nitrate) in the wastewater before its release to the environment [11]. This has been shown to be quite effective in reducing nitrogen load of the wastewater [15]. However, its impact on microbial contaminants is not yet clear. The aeration step in this process could potentially enhance the growth of *E. coli*, which is a facultative anaerobe.

The substantial decrease in EC of trench water samples or the non-detection of *E. coli* or total coliform in the trench water samples indicated that the soil was very effective in treating the wastewater. The textures of the B horizons where the trenches were located ranged from sandy clay to clay, indicating the dominance of the clay fraction in the soil. Clay soils are highly effective in adsorbing viruses under a number of different environmental conditions, including during rainfall [30,31]. Bacteria will also be similarly affected by clay soils due to their surface charges. The capacity of the clay soils to adsorb microorganisms even under high soil moisture conditions prevents their downward migration to the ground water. Rapid movement of microbial contaminants in the soil originating from septic systems has mainly been reported in sandy soils or karst topography that is characterized by large fissures and cracks [4,32].

Figure 3. Minimum (min), average (mean) and maximum (max) daily (**A**) air temperature; and (**B**) relatively humidity for the month of June 2010 for the study site. Wastewater and water samples were collected on June 3 (week 1), June 10 (week 2) and June 17 (week 3).



The microbial counts of the water samples from below the trenches might have been underestimated because of how they were collected. These were collected in suction samplers that have ceramic cups with an approximate pore diameter of 1.44 µm, based on the reported air-entry value of 200 kPa (Soil Moisture Equipment, Santa Barbara, CA, USA). The water samples travel through these pores into the cups because of water potential gradient between the soil and the inside of the cup. The pores are large enough to allow most bacteria to travel through, including *E. coli* whose size is about 0.55 µm in diameter [33]. However, because of the tortuous nature of the network of the pores, it is possible that the bacteria might get filtered out.

The variability of the data among the replicates for each sampling time was different for the SIN and SOUT samples. For *E. coli*, for example, the standard errors (SEs) ranged between 0.6% and 7.9% of the means for SIN samples. For the SOUT samples, the SEs ranged between 6.1% and 21.8% of the means. The variability was reasonable enough to result in significantly different means between SIN and SOUT samples for the first and second weeks. The variability increased when the three weeks *E. coli* data were pooled together. The SEs were 50% and 29% of the means for the SIN and SOUT samples, respectively, the difference mainly being between weeks 1 & 2 and week 3 (Figure 2). The large variability among the pooled data for the three-week time was probably the reason why sampling time did not have a significant effect on *E. coli* concentration (see Section 3.2). More frequent and longer sampling scheme that includes different seasons might be more appropriate to investigate the effect of time on *E. coli* growth.

5. Conclusions

The study reports the growth of *E. coli* in wastewater inside a septic tank under typical summer weather in Georgia, USA. This finding suggests that the bacterium or other pathogens could be introduced into the soil environment for treatment, as is the case for OWTS, at enhanced concentrations. This can potentially saturate the adsorption capacity of the coarse textured soils, facilitating their downward movement to groundwater sources. The growth of *E. coli* inside a septic tank is also contrary to the assumption under which *E. coli* is used as a water quality indicator. Further studies are required to identify the specific types of *E. coli* that are growing in the septic tank, in addition to the impact of retrofit technologies for enhancing OWTS performance on growth behavior of *E. coli* in septic tanks. Because of the short term and limited nature of the study, future long term studies are also needed to confirm the findings of this study over a multiple season period, preferably targeting OWTS that are in use by homeowners.

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