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Original Article

Occurrence and Fate of *E. coli* from Various Non-point Sources in a Subtropical Watershed

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Abstract

Bacteria of fecal origin are the primary cause of surface water contamination in the US. *E. coli* is used as an indicator of fecal contamination and detection of *E. coli* in a water body above regulatory standards poses a potential health hazard. Various sources contribute to the bacterial contamination of a water body, and these sources need to be identified and quantified to estimate bacteria loads in the waterbody accurately. In-situ re-growth is also believed to be a considerable source of *E. coli* in many cases. Also re-growth of *E. coli* in landscapes due to favorable environmental conditions (e.g., rainfall after dry weather conditions) is one of the major phenomena affecting *E. coli* concentration in streams. The objective of this study was to identify, characterize, and quantify *E. coli* concentration from feces of four different animal species, and monitor survival, growth and re-growth at four different temperatures and moisture contents over a period of seven days. Wildlife and range cattle fecal samples from the Cedar Creek watershed in East Central TX, USA were identified and feces from four species out of those were quantified for the *E. coli* concentrations. No significant difference was found while comparing the *E. coli* concentration for each species between the genders. Sub-adult cattle feces had significantly higher *E. coli* concentrations than those from adult cattle. Growth and die-off rates of *E. coli* were measured at different temperatures (0°C, 10°C, 25°C, and 50°C) in creek water and moisture conditions (4%, 25% 56.5% and 83%; volumetric basis) in soil. *E. coli* concentrations in cattle and raccoon feces showed the highest survivability and growth at 20°C. There was no survival of *E. coli* from either species at 50°C after 24 h. *E. coli* in cattle and raccoon fecal samples exhibited greater growth at lower, nearly aerobic soil moisture content (25%) for all days compared to nearly anaerobic soil moisture content (83%).

Key words*: Fecal bacteria, Water quality*

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INTRODUCTION

Bacteria are the leading cause of impairment of surface waters, including rivers, lakes, and streams in the US (USEPA 2008). As of January 2007, 197 water bodies in the State of Texas were impaired because they did not meet the bacteria concentration criteria established by the state to protect contact recreation use. A geometric mean of 126 colony-forming units (CFU)/100 mL and a single maximum of 394 CFU/100 mL for *Escherichia coli* (*E. coli*) are the criteria used to determine the impairment for freshwater contact recreation use (TSSWCB 2007). Fecal contamination of a waterbody is commonly determined by detecting the presence of indicator organisms. Fecal contamination is the pollution caused due to microorganisms like bacteria, protozoa, virus and fungi present in the intestine of humans and animals. *E. coli* is used as an indicator organism to identify fecal contamination of water bodies (Byappanahalli et al. 2003; Chin et al. 2009). Presence of indicator organisms suggests potential occurrence of pathogenic strains of the

bacteria, protozoa, virus, and fungi (Bolster et al. 2009).

To meet the criteria set by the regulatory agencies, watershed models are often applied to study the current status of water quality and the impacts of various management plans. Watershed models such as Soil Water Assessment Tool (SWAT) and Hydrologic Simulation Program in Fortran (HSPF) or the load-duration curve method are typically used in Total Maximum Daily Load (TMDL) and Watershed Protection Plan (WPP) development (Benham et al. 2007). Most of the models are limited in their ability to simulate bacteria concentrations during varying environmental conditions. These models use literature values for the concentration of *E. coli* in various fecal sources. It is necessary to accurately identify and characterize the sources and also quantify them to accurately predict the bacterial loads using watershed models. Studying the survival and growth of *E. coli* under variable environmental conditions will help in modeling their fate and transport processes more accurately (Riebschleager 2008).

The growth of *E. coli* in the environment is not completely understood or documented (Ishii et al. 2006). It has become progressively clearer that given the right conditions, such as availability of nutrients, temperature, moisture, etc., these bacteria can survive and possibly replicate in soil and water (Byappanahalli et al. 2003; Ishii et al. 2006; Sherer et al. 1992; Stephenson and Rychert 1982; Wang et al. 2004). The fate and transport of *E. coli* has been investigated by several studies (Bolster et al. 2009; Habteselassie et al. 2007; Ishii et al. 2006; Sherer et al. 1992; Wang et al. 1996) but still better understanding is required to improve the modeling of fate and transport processes.

In this study *E. coli* concentrations of various fecal sources were determined. We also examined the survival and growth of *E. coli* at four different temperatures in water and at four different moisture contents in soil at a constant temperature. The water temperatures selected for this study were 0°C, 10°C, 20°C, and 50°C to represent the actual seasonal temperatures found in the study area and waste treatment practices such as composting. The four soil moistures contents selected were 4%, 25%, 56.5%, and 83% (volumetric basis) with the purpose of studying growth and survival under dry, damp, wet and saturated soil environment.

MATERIAL AND METHODS

Study Area Description

Cedar Creek Watershed, located in Brazos County and Robertson County in East Central Texas, has a total area of 340.54 km^2, of which about 95.3% is undeveloped forest land, 3.9% developed area and 0.82% open waters (Figure 1). The local climate is subtropical and temperate. Summers are warm and hot with occasional showers. Winters are mild with periods of low temperatures usually lasting less than two months. The annual rainfall in this area is from 810 to 1220 mm. The dominant soil type is clayey loam soil.

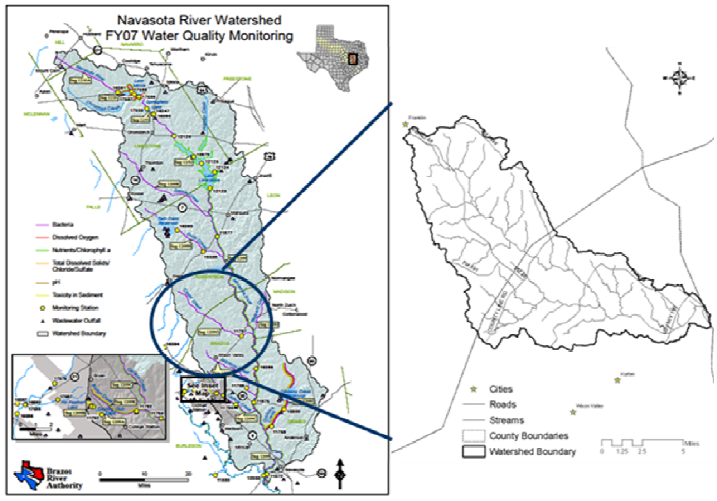

Figure 1 Study Area: Cedar Creek Watershed, Texas, USA

Cedar Creek is one of the several water bodies deemed impaired because it does not meet bacteria criteria (TSSWCB 2007; TCEQ 2008). It falls under 5c category, which means additional data and information needs to be collected before a TMDL is scheduled by the Texas Commission on Environmental Quality (TCEQ 2008). Cedar Creek has little or no urban influence. Except for direct deposition from animals there is no other evidence of point source

contamination into this Creek. Bacterial contamination is mainly resulting from agricultural and rural sources such as cattle and wildlife.

Sampling Protocol

Two sub-watersheds were selected for the study based on the watershed survey and co-operation of land owners. These sub-watersheds were located on the southwest of Cedar Creek watershed. Various non-point sources of *E. coli* (wildlife and cattle) were identified in the study area. Out of those sources,fecal samples were collected specifically from Armadillos, Opossums, Raccoons, and Cattle. The fecal material was collected by trapping the wild animals from the two sub-watersheds during summer for three months. Trapping of animals and collection of fecal material described below was conducted according to a standard protocol established by a wildlife expert (Lopez 2008). During the same sampling period, fecal material was collected from grazing cows in the watershed right after deposition.

A grid-design was used for 42 traps per sub-watershed, each measuring 81 cm × 25 cm × 30 cm. (raccoons/feral cat Tomahawk Live Trap, Tomahawk, WI). The traps were spaced at 150 m. This spacing distance has shown adequate sampling of animals that are highly attracted to aromatic baits (e.g., raccoons and opossums). Randomly located trap arrays were used in order to capture armadillos, rabbits, and skunks (i.e., species less attracted to bait). Variable array setups were designed to take advantage of the local vegetative community and topography. The arrays were fabricated out of 61 cm tall chicken fencing with 61 cm long wooden stakes. Each array had 8-12 armadillo/rabbit traps (43 traps total for each sub-watershed; 48 cm × 15 cm × 15 cm; Tomahawk Live Trap, Tomahawk, WI).

The traps were laid in the evening and kept there till next morning. The trapped animals were released next day early morning and fecal material was collected in Whirl-Pak Bags ® (Nasco, WI). Date of trapping, species information, trap number, tag number (in case of cattle), age and gender of the animal were labeled on each sampling bag. The Whirl-Pak Bags® were kept in coolers with ice and transported to the laboratory.

Enumerating *E. coli* from Fecal Samples

All fecal samples were brought to the laboratory, kept frozen until analyzed and enumerated for *E. coli* using a method used by Byappanahalli et al. (2003). All the samples were analyzed between 24 and 72 h after they were brought to the laboratory. Fecal samples were first thawed to room temperature and then a 1 g sub-sample was taken from each fecal sample and added to 9.5 mL of sterile de-ionized water in a test tube. The test tube with its contents was vortexed for two minutes to elutriate bacteria from the fecal sub-sample. The suspension was serially diluted and filtered using Millipore® 0.45 µm membrane filters. A standard membrane-filtration method (EPA Method 1603) to enumerate *E. coli* in water was used to estimate *E. coli* concentrations. Briefly, vortexed aqueous solution was filtered through a membrane filter placed on a filter base using sterilized forcep to retain the bacteria and then direct count of *E. coli* was obtained based on the development of colonies that grew on the surface of the membrane filter placed on a selective nutrient medium(USEPA, 2002).

The nutrient medium for analyses was prepared by adding 45.6 g of dehydrated modified membrane-Thermotolerant*Escherichia coli* (modified mTEC) agar powder (Becton-Dickinson, NJ) to 1 L of de-ionized water and then boiling the mixture for one minute. Modified mTEC agar is a selective and differential medium used for chromomeric detection of *E. coli*. The agar was autoclaved at 121°C for 15 minutes, poured into 9 × 50 mm Petri plates, and allowed to solidify at room temperature. Petri plates with membranes were incubated in inverted position for 2 h at 35± 0.5°C to resuscitate the stressed cells. After two hours of incubation, Petri plates were transferred into a Whirl-Pak® bag. The bag was sealed and incubated in a water bath at 44.5 ± 0.2°C for 22 to 24 h. The Petri plates were removed from the water bath and the number of red/magenta colonies developed on the membrane were counted and recorded. Aseptic techniques were followed throughout experiments and if any growth observed on a control plate then that counting was rejected. Only the plates having colonies between 30 and 300 were used to report *E. coli* concentrations as CFUs per g of wet fecal material. The gravimetric moisture content of all fecal samples were determined simultaneously by drying 1 g of the wet sample at 100°C for 24 h. Moisture content was calculated as (Wet weight of fecal sample – Dry weight of fecal sample) × 100 ÷ Wet weight of fecal sample.

Once colonies were obtained on mTEC agar, one randomly selected colony from each sample was isolated by streaking on Luria-Bertani (LB) Agar (Becton-Dickinson, NJ) and incubated at 35°C for 24 h. After the colonies were obtained on LB Agar, one randomly selected colony was again streaked on MacConkey agar (Becton-Dickinson, NJ) to confirm the presence of *E. coli* in the samples. If colonies were obtained on both media then it positively confirmed that the bacteria isolated were *E. coli*.

Fate of *E. coli* under Different Environmental Conditions

Three fecal samples were randomly selected from each of the two species: cattle and raccoons. Each sample was exposed to different temperatures and moisture conditions. The experiments for testing the growth and survival of *E. coli* under different temperature conditions were done by mixing

the fecal samples with sterilized water collected from Cedar Creek. To study the effect of moisture conditions, isolates of *E. coli* of the same samples were inoculated with soil from Cedar Creek watershed. Glassware and supplies used in the experiments were sterilized by autoclaving at 121°C for 15 minutes.

E. coli Survival in Water at Different Temperatures

To mimic direct fecal deposition in streams and to study the effects of temperature on *E. coli* survival in the streams, ten grams of fecal sample was mixed with 95 mL of sterilized (autoclaved three times at 121°C for 15 minutes) Cedar Creek water. The mixture was then divided into four equal volumes in sterilized bottles and stored at 0°C, 10°C, 25°C and 50°C. *E. coli* in water was enumerated after 1, 24, 72, 120, and 168 h using EPA Method 1603. The enumeration for each time sampling was in triplicates and median *E. coli*concentrationswere reported as CFU per 100 mL.

E. coli Survival in Soil at Different Moisture Conditions

Isolates of the same samples used to study *E. coli* survival in water at different temperatures were used to study the survival of *E. coli* at different moisture contents in soil. This would simulate *E. coli* fate within the soil matrix after fecal matter is deposited on soil surface and *E. coli* survival after isolated from feces at varying soil water contents. *E. coli* isolates were streaked on LB agar and allowed to grow for 24 h at 35°C. Out of the colonies obtained after 24 h one randomly selected colony was cultured in LB broth at 35°C for 24 h (Bolster et al. 2009). A sterilized bottle was filled with 30 g of sterilized (autoclaved three times at 121°C for 15 minutes) soil from Cedar Creek and 1 mL of the inoculated broth was added to the soil in each bottle. Then, 0, 6, 15, and 22.5 mL of sterile de-ionized water was added to the soil with inoculum to obtain 4%, 25%, 56.5% and 83% moisture content (on volumetric basis; dry bulk density of soil was 1.06 g/mL), respectively. Soil samples were incubated at room temperature. *E. coli* in soil was enumerated after 1, 24, 72, 120, and 168 h. The soil samples were enumerated for *E. coli* the same way as the fecal materials were enumerated. The enumeration for each time sampling was in triplicates and the median *E. coli*concentrationswere reported as CFU per g wet weight of soil. First order rate constants for *E. coli* survival in water at different temperatures and in soil at different moisture conditions were determined by calculating the slope of the linear regression line of log *E. coli* concentration (y axis) vs. time (x axis) plot.

Statistical Analysis

Results from the experimental study were analyzed using SPSS Statistics17.0 software (SPSS Inc. 2008). Based on preliminary statistical analysis, *E. coli* concentrations of fecal samples resulting from all species were not normally distributed. So a non-parametric test was performed to analyze *E. coli* concentrations. Kruskal-Wallis test was used to find if there was any significant difference in *E. coli* concentrations resulting from the four species (McDonald 2009).To find whether there was difference in a particular species either based on gender or age Mann–Whitney test was performed. Mann–Whitney test was used only when two variables were compared. It is the non-parametric equivalent to Student's t-test (McDonald 2009).

During the *E. coli* survival and growth experiments the temperature and moisture treatments were exclusive of each other; i.e., the moisture conditions were not changed while measuring the survival and growth at different temperatures and the temperature was not changed while studying the survival and growth at different moisture conditions. For the survival and growth of *E. coli* under different temperature and moisture conditions, *E. coli*concentrationswere analyzed using SPSS Statistics17.0 software (SPSS Inc., Chicago). Upon checking the normality of the *E. coli* concentrations obtained for both the treatments (temperature and moisture conditions) it was found that the data was skewed. Therefore to find whether there was a difference in *E. coli*concentrationson different days and within treatments the Kruskal-Wallis test was performed. The research hypotheses that were statistically tested were: (1) *E. coli* concentration from feces of a species subjected to different temperatures on any particular day will be different for different temperatures in water, (2) *E. coli* concentration from feces of a species subjected to different moisture conditions on any particular day will be different for different moisture conditions in soil, (3) *E. coli* concentration from feces of a species at a particular temperature in water will be different on different days, and (4) *E. coli* concentration from feces of a species at a particular moisture condition in soil will be different on different days.

RESULTS AND DISCUSSION

E. coli Concentration in Feces of Different Species

The *E. coli* concentrations from cattle and wildlife feces samples collected from the Cedar Creek watershed were reported in CFU/g of wet fecal material and then converted in CFU/g of dry fecal material based on corresponding moisture content of the feces. Various samples from four different species were used to analyze for their *E. coli* concentrations. Table 1 presents the fecal *E. coli* concentration of different species collected during summer. All samples were analyzed under similar temperature conditions and collected independent of each other.

Table 1 *E. coli* concentration in feces of different species

Species	Number of samples		CFU/ g of dry fecal material	
	Collected	Analyzed	Median	Range
Armadillo	7	5	1.09×10^{7}	4.32×10^{5} - 6.83×10^{8}
Raccoons	86	43	2.29×10^{7}	3.06×10^{5} - 5.46×10^{9}
Opossum	76	57	3.71×10^{7}	3.31×10^{4} - 3.24×10^{9}
Cattle	26	17	1.61×10^{5}	3.35×10^{2} - 1.74×10^{7}

The four species exhibited a lot of variability in the concentration of *E. coli* in their feces. Out of the four species analyzed, median *E. coli* concentrations from opossum (3.71×107 CFU/g) and raccoons (2.29×107 CFU/g) feces were higher than cattle (1.61×105 CFU/g) and armadillo (1.09×107 CFU/g). The *E. coli* concentrations from cattle feces were found to be the lowest of all the species analyzed.

Figure 2 shows the distribution of *E. coli* in four different species. It was observed that data for all the species were highly skewed with a number of outliers shown as asterisks and dots above the box plots. A non-parametric analysis of all *E. coli* concentrations of all four species showed a significant difference among the *E. coli* concentrations of the four species ($p < 0.05$). *E. coli* concentrations from feces of different animals were different possibly due to their feed types. The omnivorous nature of armadillo, opossum, and raccoons could be attributed to higher *E. coli* counts than herbivorous cattle.

Additionally, data showed that median *E. coli* concentrations in the feces of wildlife and cattle varied with age and gender (Table 2).

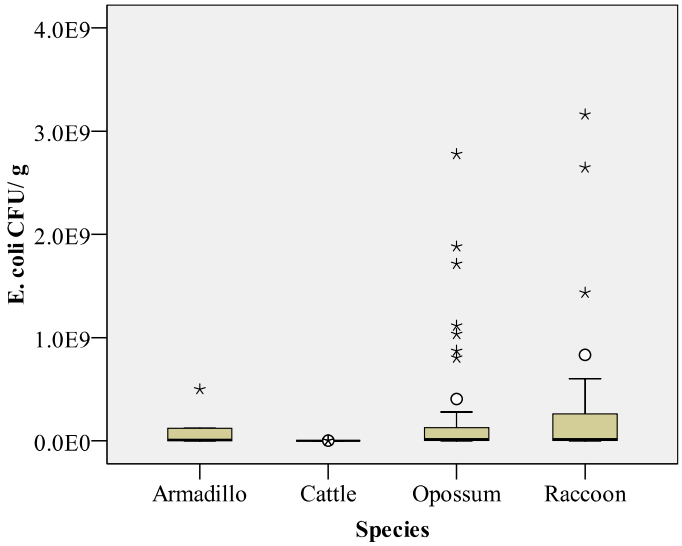

Figure 2 *E. coli* concentration in feces of different species

Table 2 *E. coli* concentration in feces resulting from species of different age and gender.

Species		CFU/g of dry fecal material			
		Male	Female	Adult	Sub-adult
Opossum	n	32	25	47	10
	Range	1.41×10^{5} - 3.13×10^{9}	3.31×10^{4} - 3.24×10^{9}	3.31×E04 - 3.24×10^{9}	3.23×10^{5} - 2.87×10^{9}
	Median	4.91×10^{6}	4.29×10^{7}	2.71×10^{7}	2.37×10^{8}
Raccoons	n	29	14	40	3
	Range	3.06×10^{5} - 5.46×10^{9}	6.95×10^{5} - 3.21×10^{9}	6.46×10^{5} - 5.46×10^{9}	3.06×10^{5} - 4.51×10^{7}
	Median	1.93×10^{7}	1.92×10^{7}	2.54×10^{7}	8.68×10^{5}
Armadillos	n	4	1	5	0
	Range	4.32×10^{5} - 1.52×10^{8}	NA	4.32×10^{5} - 6.83×10^{8}	NA
	Median	5.91×10^{6}	6.83×10^{8}(*)	1.09×10^{7}	NA
Cattle	n	5	12	13	4
	Range	2.67×10^{4} - 9.18×10^{5}	3.35×10^{2} - 1.74×10^{7}	3.35×10^{2} - 1.74×10^{7}	1.61×10^{5} - 9.18×10^{5}
	Median	1.61×10^{5}	1.03×10^{5}	1.82×10^{5}(a)	5.40×10^{4} (b)

*** note that this is only one value and not really a median.**

(a) & (b) indicates statistically significant values at ($p < 0.05$)

As shown in Table 2, sub-adult opossums shed more bacteria than adults, but the difference was not statistically significant ($p > 0.05$). Also, there was no statistical difference between the *E. coli* concentrations in the feces of male and female opossums (Table 2). Adults and female raccoons demonstrated higher median *E. coli* concentration than their male and sub-adult counterparts, respectively, but the difference was not statistically significant ($p > 0.05$) (Table 2). Data for only adult animals wereavailable for armadillos. While a difference in the *E. coli* concentration from males and females can be observed in the box plots (graphs not shown here), it was not statistically significant according to the Mann-Whitney test ($p > 0.05$) (Table 2).

Feces from calves had a significantly higher ($p < 0.05$) *E. coli* concentration than adult cows (Table 2). Even though it seemed from the median values that *E. coli* concentration from male cattle was higher than females, the difference was not statistically significant ($p > 0.05$) (Figure 4b). To our knowledge, this is the first study which examined the variation of *E. coli* count with respect to age and gender of animals, particularly wild animals.

Of all the species studied, only cattle showed a statistically significant difference in the *E. coli* concentration between adults and sub-adults. There are several studies which have examined cattle gut microflora. Cary and Moon (1995), Wells et al. (1991) and Zhao et al. (1995) had observed that *E. coli* O157:H7 concentration was significantly higher in feces of calves compared to adult feces. Rasmussen et al. (1993) and Fenlon and Wilson (2000) explained that adult cattle have a fully developed rumen where the combination of a highly volatile fatty acid concentration and a low pH inhibits the growth of *E. coli* O157:H7. No statistically significant difference in *E. coli* concentration was observed between genders of all four species studied. This may be mainly because there is no reported difference in digestion patterns or enteric bacteria occurrence between males and females of the same species.

Fate of *E. coli* under Different Environmental Conditions

The results of analysis of *E. coli* concentration from cattle and raccoons at different temperature and moisture conditions over a period of seven days showed different trends and variability. Median *E. coli* concentrations in CFU per 100 mL of water or g of dry soil were plotted in Figures 3 through 6.

Effects of Temperature on *E. coli* Survival in Water

The survival of *E. coli* from cattle and raccoon in water at different temperatures over a period of seven days showed highly variable *E. coli* counts (Figures 3 and 4). *E. coli* concentrations in cattle and raccoon fecal samples at the beginning of the experiments were determined after one hour. These background concentrations are presented for comparison with the bacterial concentrations from subsequent days. For both species maximum survival and growth of *E. coli* was observed at 20°C and no growth was seen at 50°C.

At 0°C, there was a slight decrease in cattle *E. coli* growth after 24 h. The concentration increased after 72 h and then decreased until the end of the incubation period (Figure 3). *E. coli* from raccoon feces at 0°C (Figure 4) showed a decrease after 24 h, increased at 72 h, decreased at 120 h, and increased again after168 h.

A gradual increase in the cattle *E. coli* concentration was observed at 10°C until the fifth day (120 h) and then there was a decline by one order of magnitude after 168 h (Figure 3). While the *E. coli* from raccoon feces decreased after 24 h, increased at 72 h, decreased at 120 h, and increased again after168 h. This survival trend of *E. coli* from raccoon feces was similar to that of 0°C (Figure 4).

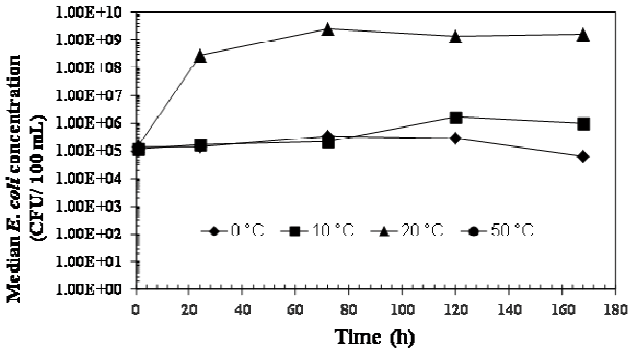

Figure 3 Survival of *E. coli* (in cattle feces) in water at different temperatures.

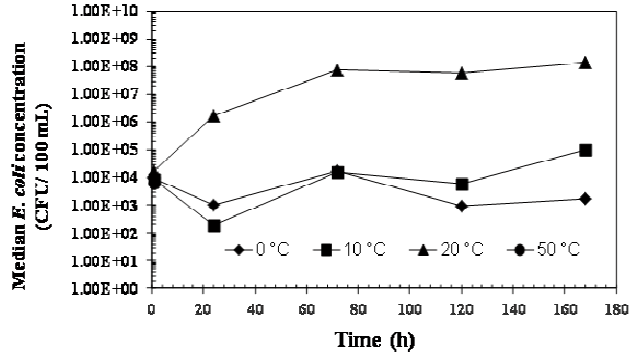

Figure 4 Survival of *E. coli* (in raccoon feces) in water at different temperatures.

The highest cattle *E. coli* growth was observed at 72 h at 20°C. The concentration dropped at 120 h and again increased after 168 h (Figure 3). At 20°C, *E. coli* from raccoons showed a similar trend as cattle with the only difference being that the highest counts for this temperature were observed at 168 h as opposed to 72 h in cattle (Figure 3 and 4). The decline on any given day might be due to the depletion of nutrients over time and increased competition for nutrients within bacterial population. The re-growth could possibly have occurred due to the nutrition available from the organic matter of the dead bacterial cells.

There was no significant difference in cattle *E. coli* concentrations at 0° and 10°C ($p > 0.05$) for any given incubation temperature over the 7-day study period . However, at 20°C the *E. coli* concentrations were significantly different for different days ($p < 0.05$) (Figure 3). The Kruskal-Wallis test statistics for *E. coli* in raccoon feces showed that there was a significant difference ($p < 0.05$) in *E. coli* concentrations among different days at all temperatures except at 0°C ($p > 0.05$) (Figure 4). Since no survival was observed at 50°C after 24 h, the results obtained for that temperature were excluded from statistical analysis.

Kruskal-Wallis test for *E. coli* concentration obtained after 1 h from both species did not support the hypothesis that the concentrations were different from each other at different incubation temperatures ($p > 0.05$) (Figure 3 and 4). This result just reinforced the laboratory analysis as *E. coli* concentrations after 1 h were not expected to be different for different temperatures since they were background numbers. *E. coli* concentrations among temperatures were significantly different at all other days. The results show that *E. coli* concentrations observed at 20°C were significantly higher on any day compared to the other incubation temperatures studied. For both the species studied, it was observed that at 50°C there was no survival of *E. coli* after 1 h.

Habteselassie et al. (2007) found that *E. coli* survived better at lower temperatures in soil, whereas in our study the survival of *E. coli* resulting from cattle and raccoon was the highest at 20°C compared to the survival at 0°C and 10°C (Figure 3 and 4). Similar results for growth of *E. coli* at a temperature of about 19°C in manure rich soils were found by Berry and Miller (2005). Wang et al. (2004) also found greater growth and survival of *E. coli* in dairy cow fecal material at 27°C compared to 4°C or 41°C. Our study with different temperatures was conducted with water but considering the amount of organic matter available from the feces mixed with water this situation can be compared to findings of Berry and Miller (2005).

In the results described above, it can also be observed that at 0°C both the species did not show a statistically significant difference in the *E. coli* concentrations between different days. It was possible because *E. coli* needs at least 7.5°C temperature for growth and is not able to continue protein synthesis below 7.5°C (Shaw et al. 1971). As a result, *E. coli* is growing inconsistently at 0°C showing no significant trends. The *E. coli* concentration in raccoon feces at 10°C showed that *E. coli* can survive for a long time at 10°C. Considering the fact that *E. coli* is a mesophilic organism it was not unexpected for it to show no growth at 50°C, which is too high a temperature for a mesophile to survive. In this study, *E. coli* growth was measured at different temperature conditions using fecal material directly added to water. If *E. coli* isolates from the feces were used instead, different growth results might have been observed. This may be due to the fact that *E. coli* would not have to compete with other bacteria in fecal material. Also, the organic matter availability, as food for bacteria, would have been different under such conditions.

Effects of Soil Moisture on Survival of *E. coli*

The growth and survival of *E. coli* under different moisture conditions for cattle and raccoon species showed a similar trend to each other. The maximum survival and growth was observed at 25% moisture content of the soil sample followed by 56.5% moisture content. *E. coli* are facultative anaerobes, which was reaffirmed from the results obtained that the bacteria had the highest growth and survival at 25% moisture content, indicating that among all soil moisture contents selected for this experiment, soil at 25% moisture content provided the most suitable conditions for their survival and growth.

Under dry conditions (4%), bacteria did not totally dieoff, but by 168 h the concentrations reduced considerably: by two orders of magnitude for cattle samples (Figure 5) and by one-half for raccoon (Figure 6) samples. At 56.5% soil moisture content, *E. coli* concentration in cattle showed a gradual increase until 120 h followed by a reduction at 168 h whereas raccoon *E. coli* concentration declined on the fifth day (120 h) and increased on seventh day (168 h). At 83% moisture content, *E. coli* from cattle (Figure 5) reduced after a gradual growth until the fifth day whereas the *E. coli* concentrations in raccoon samples (Figure 6) continued to rise from 1 h to 168 h.

Upon performing the Kruskal-Wallis test on the *E. coli*concentrations obtained in cattle for different moisture contents on different days, it showed that at all four moisture conditions the *E. coli* concentration on each day were different from one another other ($p < 0.05$). The 1 h old samples showed the background concentration of *E. coli* for each moisture condition. A statistical difference in concentration of *E. coli* between different days indicates significant growth or decline. It can be observed that in cattle the 25% moisture condition had the highest *E. coli* concentration on any given day followed by 56.5%, 83%, and

4% moisture contents. The *E. coli* concentration from raccoons samples (Figure 6) showed a similar trend as cattle at 4%, 25% and 83% moisture content ($p < 0.05$) but at 56.5% moisture condition there were no statistical differences in concentrations among different days.

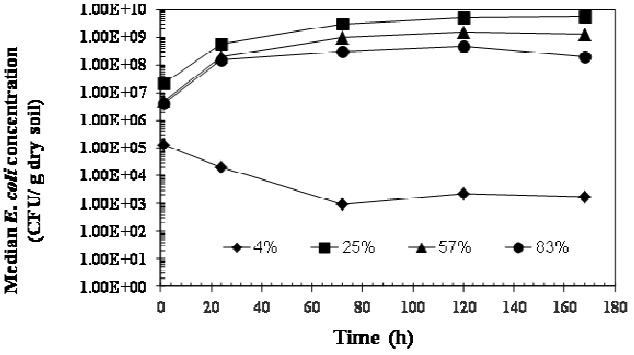

Figure 5 Survival of *E. coli* (isolated from cattle feces) in soil at different moisture contents.

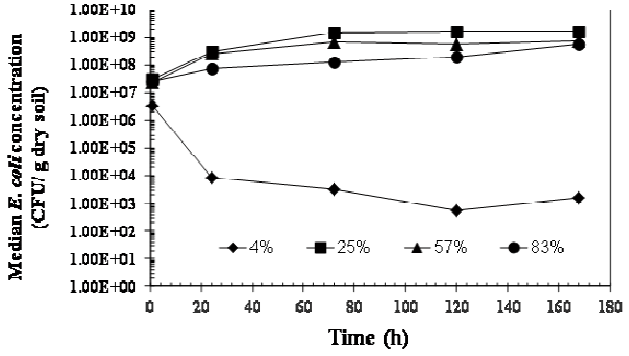

Figure 6 Survival of *E. coli* (isolated from raccoon feces) in soil at different moisture contents

The test statistics for growth and survival of cattle and raccoons show that *E. coli* concentrations on all days except 1 h ($p > 0.05$) were different for different moisture contents ($p < 0.05$). It was expected for 1 h concentrations to be not significantly different from each other for the different moisture conditions since they are background concentrations. Also, the difference in *E. coli* concentration at different moisture conditions between all the days ($p < 0.05$) suggests growth or decline of *E. coli* after one hour.

Though there is lack of quantitative information on survival rates of enteric bacteria under different soil moisture conditions, numerous studies have suggested that soil moisture is the principal factor affecting the survival of enteric bacteria in soil (Jamieson et al. 2002; Islam et al. 2004; Muirhead et al. 2004, 2006). Chandler and Craven (1978) and Ogden et al. (2001) found a rapid decline in *E. coli* concentration under dry conditions due to desiccation. A study by Jiang et al. (2002) discovered that *E. coli* can continue to exist for extended periods of time at less than 4% moisture condition in soil. We observed that the concentration of *E. coli* from both cattle and raccoons did not die-off at 4% soil moisture within seven days, but the concentrations reduced considerably after 24 h.

In a study by Sjogren (1994) using soil microcosms under controlled conditions in a laboratory, it was found that *E. coli* survived for longer periods under saturated conditions. Hagedorn et al. (1978) and Tate (1978) also found the *E. coli* populations to be greatest under very high moisture conditions in soil. Wang et al. (2004) found interactions between temperature and moisture content. At 27°C, *E. coli* concentrations were greater in dairy cow fecal material at very high (83%) moisture for the first two weeks after excretion, but greater at lower (55% and 30%) moisture thereafter until 15 weeks; however, at 4°C and 41°C, *E. coli* concentrations were consistently greater at very high moisture content for the entire 15-week period. This study found that the survival and growth of *E. coli* peaked at 25% moisture conditions. Chandler and Craven (1978) on the contrary indicated the survival of *E. coli* to be less in soil under cool and moist weather conditions.

The concentration of *E. coli* in this study did increase at 55.6% and 83% moisture but it was less than the concentration found at 25% on any given day. This study was conducted under room temperature conditions. The bacteria possibly found most favorable environment to survive and grow at the particular temperature (room temperature) and 25% soil moisture. Given the facultatively anaerobic nature of these bacteria, it can be assumed that *E. coli* chose to be facultative at 25% soil moisture condition and room temperature as it provided optimum conditions for their survival and growth.

It should be noted that *E. coli* survival and growth in the environment can be influenced by the interacting effects of moisture conditions and temperatures (Wang et al. 2004). Other important physical chemical properties, such as pH,affecting the survival of microorganisms should be taken into account to the study the growth and survival of bacteria. In this study, under different environmental conditions, the temperature and moisture studies were independent of each other. In future studies, interaction of different temperatures and moisture conditions should be considered to study the effect of environment on survival and growth of bacteria. While modeling the fate and transport of *E. coli* in the environment, these complex effects should be considered.

At 20°C temperature and 25% soil moisture content, *E. coli* from both the species seem to show trends similar to each

other, even though there was difference between *E. coli* concentrations of the two species. The kinetic constants for cattle and raccoon *E. coli* concentrations at 20°C temperature (Table 3) and 25% moisture content (Table 4) were similar to each other. It can be observed from the graphs and kinetic constants that at 0°C temperature and at 4% moisture content there was decay (i.e., decline) of *E. coli*concentrations over time. At all other temperatures (except 50°C) and moisture contents, growth was observed. Wang et al. (2004) found *E. coli* in dairy cow fecal material at 83% moisture followed the first-order model only from day 3 to day 20 after excretion. Over this period, *E. coli* exhibited decay with rate coefficients that increased with temperature (-0.0046/h at 4°C, -0.0083 at 27°C, and -0.013 at 41°C). In this study, except at 0˚C and 4% moisture content, *E. coli* growth was observed in water and soil. This may be attributed to shorter incubation periods and different *E. coli* isolates used in this study as compared to the study by Wang et al. (2004).Morever, in this study the isolates were directly added to soil or fecal pellets were added to water. Wang et al. (2004) studied the *E. coli* fate directly in cow manure.

The kinetics analyses in this study show that *E. coli* isolated from cattle feces doubled every 45 hours in water at 10°C while doubling every 16 hours at 20°C. *E. coli* isolates from raccoon feces doubled at a slightly faster rate in water at the same temperature conditions. It would take 38 hours for *E. coli* isolates from cattle to double at 83% moisture condition in soil while it would take roughly 24 hours to double in 25% and 57% moisture condition. It should be noted that *E. coli* isolates from raccoon would double slightly at a slower rate in soil at the same moisture conditions.

Table 3 First order rate constant for *E. coli* survival in water.

	k_T (hr^{-1})	
	Cattle	**Raccoon**
0 °C	-0.0025	-0.0073
10 °C	0.0151	0.0218
20 °C	0.0425	0.0472

Table 4 First order rate constant for *E. coli* survival in soil.

	k_{MC}(hr^{-1})	
	Cattle	**Raccoon**
4%	-0.0237	-0.0385
25%	0.0289	0.0207
55.6%	0.0281	0.0162
83%	0.0182	0.0162

CONCLUSIONS

Four different non-point sources of *E. coli* were identified in Cedar Creek watershed. The sources were quantified for their *E. coli* content. *E. coli* concentrations were reported as CFU/g of dry fecal material. Cattle showed variability in *E. coli* concentrations between adult and calves, with calves having higher *E. coli* concentration in their feces than adults. No statistical differences in fecal *E. coli* concentrations were detected between males and female for any species.

The growth and survival of *E. coli* subjected to different temperature conditions showed high variability in results over time.*E. coli* concentrations in cattle and raccoon feces showed the highest survivability and growth at 20°C out of all the temperatures studied. There was no survival of *E. coli* from either species at 50°C after 24 h. *E. coli* in cattle and raccoons samples exhibited greater growth at lower, nearly aerobic soil moisture content (25%) for all days compared to nearly anaerobic soil moisture content (83%).

This study verified the facultative behavior of *E. coli* contributing to accelerated growth levels at cooler temperature and nearly aerobic conditions. Future studies should consider the effect of the interaction of different temperatures and moisture conditions on the survival and growth of *E. coli* in animal feces.

Watershed modeling tools generally lack the capacity to simulatebacteria life cycle and behavior under different environmental conditions. The growth trends observed under different environmental conditions in this study would improve prediction of *E. coli* loads in a waterbody during different times of a year, thus helping to addressseasonal variation, which is one of the major factors governing the bacterial loadings in a water body. Understanding the behavior of bacteria under different environmental conditions also helps to develop proper manure management techniques before land application of manure.

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