GROWTH KINETICS OF WILDLIFE *E. COLI* ISOLATES IN SOIL AND WATER

A Thesis

by

MEGHAN ANNE GALLAGHER

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2012

Major Subject: Biological and Agricultural Engineering

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ABSTRACT

Growth Kinetics of Wildlife *E. coli* Isolates in Soil and Water.

(May 2012)

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Chair of Advisory Committee: Dr. Raghupathy Karthikeyan

Bacteria are the major cause of surface water contamination in the United States. US Environmental Protection Agency (USEPA) uses the Total Maximum Daily Load (TMDL) process to regulate the *E. coli* loads from fecal sources in a watershed. Different point and non-point sources can contribute to the fecal contamination of a waterbody including municipal and on-site wastewater treatment plants, livestock, birds, and wildlife. Unfortunately, wildlife sources in many rural watersheds are poorly characterized. *E. coli* is also known to persist in waterbodies when no known fecal sources are present. In this study, *E. coli* from wildlife fecal material was enumerated. It was found that *E. coli* concentrations varied with the season the fecal samples were collected. When studying the fate of *E. coli* under different environmental factors, no growth was observed in soil at 4% moisture content and in water at 10˚C. The highest *E. coli* growth was recorded in water at 30˚C. It can be seen from these results that there was variation in the fate of *E. coli* under different environmental conditions. The fate of *E. coli* in the environment is a complex process and is influenced by many factors and their interactions, making it difficult to predict. The findings from this study along with

additional studies can be used to improve the accuracy of model predictions to estimate the *E. coli* loads in watersheds.

ACKNOWLEDGEMENTS

I would like to express my gratitude to my committee chair, Dr. Karthikeyan, for his patience in training and guiding me in the research process. The professional growth and lessons I have learned under his supervision are invaluable. I am thankful for his dedication to support and challenge while conducting this research. I would also like to thank my committee members, Dr. Gentry, Dr. Mukhtar, and Dr. Huang, for providing direction and assistance throughout the course of this research. I am grateful for their encouragement as I have worked towards my Master's degree. I would like to acknowledge the Texas State Soil and Water Conservation Board (TSSWCB) for funding this project through a USEPA CWA §319 (h) NPS grant.

I would like to acknowledge the Department of Biological and Agricultural Engineering for the friendly and supportive environment during my graduate studies. I would specifically like to thank Ms. Bailey Sullivan for training me in the laboratory and for problem solving with me when complications in my research arose. I also want to express my gratitude to Ms. Kyna Borel for providing the GIS map for the Cedar Creek watershed and for being supportive during research complications. Thanks go to Ms. Reema Parikh, Mr. Kyle McKee, and Ms. Aubrey Harris for their assistance in the laboratory, as well as Dr. Israel Parker for collecting and providing the samples from the field.

I am grateful for my friends who have supported and encouraged me to press on and finish. Thanks go to my boyfriend who has helped keep me focused in the midst of stress. I am especially grateful to my mom, dad, and sister who have been supportive through the whole process, freely giving wisdom, love, and encouragement to persevere through times of struggle.

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CHAPTER I

INTRODUCTION

1.1. Introduction

The leading cause of impairment for waterbodies in the United States is from bacteria (USEPA, 2008a). Bacterial impairment of rivers and streams originates from fecal contamination. Wastewater effluents and fecal material from both livestock and wildlife are potential sources of fecal contamination in a watershed. Warm-blooded mammals shed pathogenic bacteria in their feces. Pathogenic bacteria such as *Salmonella typhi*, *Shigella*, *Campylobacter jejuni*, and *Escherichia coli* (*E. coli*) O157:H7 are responsible for waterborne diseases that include typhoid fever, dysentery, campylobacteriosis, and *E. coli* 0157:H7 infection, respectively. These illnesses can include symptoms of diarrhea, fever, nausea, vomiting, and abdominal cramps. Few of these symptoms can last for days and even lead to death in immune-compromised individuals (USEPA, 2008b).

Testing for the presence of each enteric pathogen in a waterbody is time consuming and costly. Instead, an indicator organism is used to monitor fecal contamination in a water body. The presence of an indicator organism in a water sample suggests pathogenic microorganisms may be present as well. A good indicator organism for fecal contamination has the following characteristics: it is a part of warm-blooded mammals'

This thesis follows the style of *Transactions of ASABE*.

micro flora, its presence in water is associated with the occurrence of waterborne disease, it is easy to enumerate in the laboratory, and it is able to outlast the presence of enteric pathogens, yet not sustained in the environment outside of fecal material. *E. coli* is the current indicator organism for fecal contamination and used by the Environmental Protection Agency (EPA) to assess bacterial impairment in waterbodies. Bacterial impairment in Texas waterbodies is defined by *E. coli* concentrations higher than a geometric mean of 126 CFU /100 mL or exceeding 394 CFU/100 mL in a single grab sample. There are 405 streams in Texas that are bacterially impaired according to the 303(d) list of impaired waterbodies in the United States (TCEQ, 2008).

The Clean Water Act requires all waters listed on the 303(d) list of impaired waterbodies to have Total Maximum Daily Loads (TMDLs) developed (USEPA, 2012). A TMDL determines the maximum amount of a pollutant able to enter a waterbody while meeting the water quality standards. TMDLs are developed for each pollutant in a waterbody. After identifying the pollutant of concern, the loading capacity of the waterbody is estimated. Next, the sources of the pollutant are identified and the amount of pollutant entering the waterbody from each source is estimated. The current pollutant load is calculated and reductions needed to meet the loading capacity of the waterbody are determined. The allowable pollutant load is then allocated among the different sources to meet the water quality standards, while including a margin of safety that accounts for seasonal variation. Once the TMDL is developed, a watershed plan is enacted to restore the water quality of impaired waterbodies (USEPA, 2012).

Water quality/watershed modeling tools such as Spatially Explicit Load Enrichment Calculation Tool (SELECT), Hydrological Simulation Program-Fortran (HSPF), and Soil and Water Assessment Tool (SWAT) are used in the bacterial TMDL process to characterize *E. coli* sources in a watershed and estimate the required reductions in *E. coli* loads (Bicknell et al., 1997; Pachepsky et al., 2006; Sadeghi and Arnold, 2002; Teague et al., 2009). *E. coli* sources in the watershed include both point and non-point sources. Point sources are specific locations that discharge the pollutant, typically effluent from wastewater treatment plants/facilities. Non-point sources are from non-specific locations across a watershed, carried by runoff to the waterbody. The amount of *E. coli* released from the point source can be quantified through the effluent discharge regulations, whereas *E. coli* loads from non-point sources are estimated. *E. coli* loads from non-point sources are determined through estimating the *E. coli* concentration of cattle, wildlife, and avian fecal material. In rural watersheds, wildlife can contribute a majority of the fecal pollution and should be considered (Harmel et al., 2010). Unfortunately, *E. coli* concentrations present in wildlife fecal material are not well documented.

Recent studies have shown that *E. coli* can survive and regrow in the environment even after fecal waste is removed (Byappanahalli et al., 2003; Ishii et al., 2006; Sherer et al., 1992; Stephenson and Rychert, 1982). Enteric bacteria can survive in sediment for months (An et al., 2002; Anderson et al., 2005; Byanppanahalli et al., 2003; Desmarais et al., 2002; Franz et al., 2005; Ishii et al., 2006; Sherer et al., 1992; Solo-Gabriele et al.,

2000; Stephenson and Rychert, 1982). Franz et al. (2005) recorded *E. coli* O157:H7 survival in soil between 56 and 133 days at 10˚C in their laboratory setup. In a comprehensive literature review by Crane and Moore (1984), the trend observed was that temperature, pH, moisture content, and nutrient supply affect the survival of enteric bacteria in soil. Additionally, seasonal change affects the die-off rates of indicator organisms in soil (Crane and Moore, 1984).

Soil moisture is one of the parameters that regulates growth and survival of *E. coli* (Chandler et al., 1980; Crane and Moore, 1984; Habteselassie et al., 2008; Sjogren, 1994). Changing moisture content due to tidal environment or drying out from droughts can promote the growth of *E. coli* in the terrestrial environment (Desmarais et al., 2002; Solo-Gabriele et al., 2000). Studies have found that antibiotic resistant *E. coli* survives in soil when temperature is lower, 5˚C to 10˚C, and with saturated moisture conditions (Crane and Moore, 1984; Sjogren, 1994). Sjogren (1994) observed soil amended with *E. coli* survived at 5˚C with saturated moisture conditions for 21 to 23 months depending on the type of soil used. Padia (2010) observed the growth of *E. coli* isolates from cattle and raccoon fecal material was higher at 25% soil moisture content than at 83% soil moisture content. Under certain soil moisture conditions, *E. coli* growth in soil can be a potential non-point source if transported with runoff to local waterbodies.

The trends of *E. coli* survival in variety of waterbodies including lake, river, sea, and creek have been studied (Carlucci et al., 1961; Faust et al., 1975; Filip et al., 1988; Flint,

1987; Hendricks, 1972; Jamieson et al., 2005; McFeters et al., 1974; Padia, 2010). Temperature is suggested to be the most important factor that affects bacterial survival in water (Faust et al., 1975). Growth of *E. coli* lab strain, ATCC 11775, in river water was studied to determine growth characteristics at different temperatures (Hendricks, 1972). Hendricks (1972) observed the highest growth rate of *E. coli* at 30˚C with a generation time of 34.5 hours, compared to the generation times of 333.3 hours and 1,000 hours at 20˚C and 5˚C, respectively. Padia (2010) studied the survival of *E. coli* from fecal material in creek water at different temperatures (0, 10, 20, and 50˚C) over one-week time span. It was found that *E. coli* from raccoon and cattle feces had a sustained growth over time at 20˚C, yet at 50˚C there was no growth from any species' fecal material after 24 hours (Padia, 2010).

Further understanding of how *E. coli* responds to environmental factors is needed to improve the accuracy of modeling tools to estimates *E. coli* loads from different non-point sources entering a waterbody. *E. coli* concentrations of fecal material from potential sources and the fate of *E. coli* from fecal material are both needed in estimating *E. coli* loads. In this research project, fate of *E. coli* isolated from wildlife feces under different environmental factors was studied under laboratory conditions.

1.2. Objectives

The main objective of this research was to study the fate of *E. coli* isolates from wildlife fecal material in water at different temperatures and in soil at different moisture conditions.

The specific objectives were to

1) enumerate and obtain isolates from wildlife fecal material,
2) determine kinetic characteristics of *E. coli* isolates enumerated from feral hog and deer fecal material in water at different temperatures, and
3) determine kinetic characteristics of *E. coli* isolates enumerated from feral hog and deer fecal material in soil at different soil moisture conditions.

CHAPTER II

GROWTH KINETICS OF WILDLIFE *E. coli* ISOLATES IN SOIL AND WATER

2.1. Introduction

Fecal contamination from point and nonpoint sources has bacterially impaired 405 waterbodies in Texas (TCEQ, 2008). Point sources including effluent from wastewater treatment plants (WWTP) are known direct sources. Non-point sources include feces from wildlife, avian, domestic animals, and on-site wastewater treatment systems. Point sources can be regulated directly, whereas nonpoint sources need to be characterized and best management practices (BMPs) need to be established to regulate the amount of fecal contamination that occurs. Indicator organisms have been used to estimate the amount of fecal contamination that has occurred in a waterbody. At present, *E. coli* has been used to indicate the potential of fecal contamination in a waterbody (USEPA, 2002).

E. coli is a part of the intestinal micro flora of warm-blooded mammals, can survive longer than other enteric pathogens, and is easy to enumerate in the lab, making it a good indicator organism for estimating fecal contamination. The presence of *E. coli* in a waterbody is not an indication that waterborne diseases will occur but rather an indication that pathogenic bacteria could be present as well. If the pathogenic bacteria are present, then there is a risk of waterborne diseases. Under sub-tropical and temperate environments *E. coli* has been observed to persist (Anderson et al., 2005;

Byanppanahalli et al., 2003; Desmarais et al., 2002; Ishii et al., 2006; Solo-Gabriele et al., 2000). Sources of *E. coli* in a waterbody are not only external sources but also *in situ*. Specifically, sediments have been found to be reservoirs of enteric bacteria, including *E. coli,* and a potential source in waterbodies (An et al., 2002; Anderson et al., 2005; Byanppanahalli et al., 2003; Desmarais et al., 2002; Ishii et al., 2006; Solo-Gabriele et al., 2000; Sherer et al., 1992; Stephenson and Rychert, 1982). Environmental controls have been shown to have a role in sustaining *E. coli* populations in the environment (Chandler and Craven, 1980; Crane and Moore, 1984; Habteslelassie et al., 2008).

Temperature affects the *E. coli* concentration in waterbodies (Carlucci et al., 1961; Faust et al., 1975; Filip et al., 1988; Flint, 1987; Hendricks, 1972; McFeters et al., 1974; Padia, 2010). The survival and growth rate of *E. coli* in river water can be affected by temperature differently. Hendricks (1972) observed a higher growth rate of *E. coli* in river water at 30˚C than at lower temperatures. However, Flint (1987) observed the survival of *E. coli* in river water was less at 30˚C than at 4˚C and 25˚C. Filip et al. (1988) also observed longer survival of *E. coli* at lower temperatures. They reported *E. coli* survived for 100 days in groundwater at 10˚C (Filip et al., 1988). Additionally, Padia (2010) found that *E. coli* survival in creek water was the highest at 20˚C compared to *E. coli* survival at 0˚C, 10˚C, and 50˚C over one-week.

Growth and survival of *E. coli* in soil is affected by moisture content (Crane and Moore, 1984; Desmarais et al., 2002; Ogden et al., 2001; Solo-Gabriele et al., 2000; Sjogren, 1994). Change in soil moisture content from dry to saturated conditions was found to promote the growth of *E. coli* in soil (Desmarais et al., 2002; Solo-Gabriele et al., 2000). At dry soil conditions, the *E. coli* die-off was observed to be faster than saturated soil moisture conditions (Ogden et al., 2001). Sjogren (1994) set-up laboratory soil microcosms and observed that the survival of *E. coli* was the longest when soil was under saturated moisture conditions, lasting up to 23.3 months.

Watershed modeling tools incorporate environmental factors to estimate *E. coli* loads in a watershed but more data is needed (Benham et al., 2006). *E. coli* load estimation tools are used to determine sources of *E. coli* in a watershed and the amount that each source is contributing to a waterbody. A Total Maximum Daily Load (TMDL) for *E. coli*, maximum amount of *E. coli* able to enter a waterbody and still meet bacterial water quality standards, is determined using modeling tools. The survival and growth of *E. coli* in both the terrestrial and aquatic environment affect the amount of *E. coli* that enters a waterbody. Further research is needed to characterize the effect of water temperature and soil moisture content on the growth of *E. coli*.

The main objective of this research was to study the fate of *E. coli* isolates from wildlife fecal material in water at different temperatures and in soil at different moisture conditions. The specific objectives were to (1) enumerate and obtain isolates from

wildlife species' fecal material, (2) determine kinetic characteristics of *E. coli* isolates enumerated from feral hog and deer fecal material in water at different temperatures, and (3) determine kinetic characteristics of *E. coli* isolates enumerated from feral hog and deer fecal material in soil at different soil moisture conditions.

2.2. Study Area Description

Cedar Creek watershed is located in East Central Texas within both Brazos and Robertson County (Figure 2.1).

Table 2.1. Cedar Creek Watershed Characteristics.

Total Area	340.54 km^2
Land Use	95.3% undeveloped forest 3.9% developed area 0.82% open waters
Climate	subtropical
Rainfall (Annual)	810-1220 mm
Soil*	sandy loam (66% sand, 18% silt, and 16% clay) 1.2% organic matter strongly acidic (pH 5.2)

***Tested at the Soil, Water and Forage Testing Laboratory, Texas A&M University

Cedar Creek is one of the 405 impaired water bodies in Texas that does not meet the bacteria criteria for the state (TCEQ, 2008). It also is categorized as 5c which requires additional data and information for a TMDL to be scheduled by the Texas Commission on Environmental Quality (TCEQ). There is very little urban influence in Cedar Creek. The land use is mainly rangelands and forested areas (Table 2.1). Direct fecal deposition from cattle, wildlife, and birds along with other non-point sources contribute to the fecal contamination of the creek.

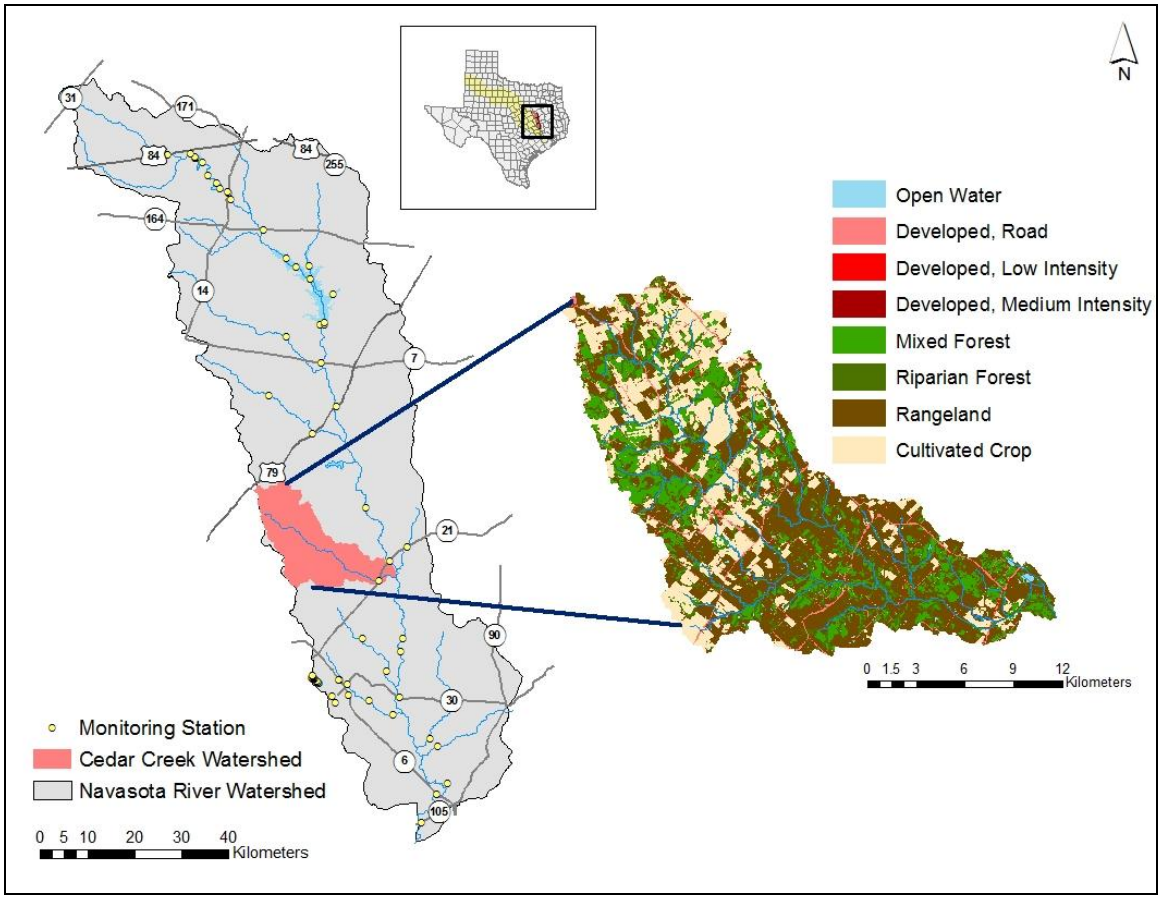

Figure 2.1. Location of Cedar Creek Watershed in central Texas.

2.3. Methods and Materials

2.3.1. Sampling Protocol

Two sub-watersheds within Cedar Creek watershed were used for sampling with landowner co-operation. The land use of the sub-watersheds is mainly rangeland. The sampling protocol for obtaining the fecal material included a grid-design for trapping the wildlife. A wildlife expert designed the protocol for trapping and collecting the fecal material. The wildlife species trapped included raccoon, opossum, feral hog, deer, skunk, and armadillo. A more detailed description of sampling protocol is discussed in Padia (2010). Briefly, once the wildlife species were trapped the fecal material was collected using sterile Whirl-Pak® bags while wearing latex gloves. The sex, age, date of trapping, and location were recorded. The samples were transported in an insulated cooler on ice at 5˚C to the Water Quality Engineering Laboratory at Texas A&M University.

2.3.2. Enumerating *E. coli* from Fecal Samples

The fecal samples were brought to the lab and kept frozen at -20˚C until processed. When processed, the fecal material was defrosted and one gram was measured out with sterile scoop. The samples were serially diluted in de-ionized (DI) water. The diluted samples were run through a membrane filtration system, following the EPA method 1603 (USEPA, 2002). The membrane of 0.45 μm pore size was removed from the filtration system with sterile forceps and placed on modified Thermo-tolerant *E. coli*, mTEC, (Difco®) agar plates. The plates were inverted and placed in an incubator at

35.5 ± 0.5˚C for two hours to revive the cells. Then the plates were sealed in Whirl-Pak® bags and placed in a water bath at 44.5 ± 0.5˚C for 22 hrs to select for thermo-tolerant *E. coli*. The plates were counted and values of 30 to 300 CFUs (colony forming units) were recorded.

Randomly selected isolates from each plate were streaked on Nutrient agar with, 4-methylumbelliferyl-β-D-glucuronide (MUG), (Difco®) and placed inverted in an incubator at 35.5˚C for 24 hrs. MUG is a colorless substrate that is hydrolyzed by an enzyme present in *E. coli*, to a fluorescent product, 4-methylumberlliferone. *E. coli* was confirmed if the MUG plates fluoresced. Confirmed isolates were stored in labeled centrifuge tubes with 1 mL of Luria-Bertani (LB) broth (Difco®) and 10% glycerol in -20˚C freezer.

2.3.3. Growth Kinetics of *E. coli* in Water under Different Temperatures

Three *E. coli* isolates from each feral hog (H1-3, H4-1, and H7-1) and deer (D1-c, D2-c, and D2-d) fecal sample were enriched in 100 mL of LB broth at 35.5˚C for 24 hrs. The enriched LB broth was diluted to 10^{-4} by adding 1 mL of LB broth into 100 mL sterile DI water, stirred, and then 1 mL of the diluted LB broth was added to 100 mL of sterile DI water. Finally, creek water microcosms were made with 1 mL of the 10^{-4} dilution of LB broth is added to 100 mL of sterile Cedar Creek water (autoclaved three times at 121˚C for 15 min). The creek water microcosms were triplicated and kept in an incubator set according to the experimental temperatures (10, 25, and 30˚C). Over

30 hrs, 0.1 mL of each water microcosm were spread plated onto MacConkey agar (Difco®) plate at different sampling times. The *E. coli* concentrations (CFU/mL) were recorded at each sampling time.

The kinetic characteristics of *E. coli* strains in water at different temperatures were determined using first order kinetics. The natural log of the bacterial counts was plotted against time to obtain the rate constant, k. A trend line was fitted to the data to determine the k-value from the slope of the line. If the k-value was positive, then the doubling time (t_d) was calculated. If the k-value was negative, then the half-life ($t_{1/2}$) was calculated.

2.3.4. Growth Kinetics of *E. coli* in Soil under Different Moisture Conditions

One *E. coli* isolate from each feral hog (H1-3) and deer (D2-c) fecal sample was enriched in 100 mL of LB broth. Cedar Creek soil (Table 2.1; autoclaved three times at 121˚C for 15 min and dried in oven for 10 hrs) was used in soil microcosms with 1 mL of enriched LB broth. An estimated amount of sterile DI water (0, 6, and 15 mL) was added to 30 g of soil to obtain experimental moisture contents (4, 25, and 57%). Three replicated soil microcosms were placed on a rotary shaker at 200 rpm at room temperature (22˚C). Over one week, *E. coli* was enumerated by serially diluting one gram soil from each microcosm in DI water and 0.1 mL of the last three dilutions was spread-plated onto MacConkey agar plates. The *E. coli* bacterial counts (CFU/g) were recorded at five to seven different sampling times for the duration of a week. One

isolate from each MacConkey plate with growth was randomly selected and streaked onto nutrient agar with MUG and grown at 35.5˚C for 24 hrs to confirm the isolate was still *E. coli* and not contamination.

The kinetic characteristics of *E. coli* strains in soil at different soil moisture conditions were determined in the same manner as described in Section 2.3.3.

2.3.6. Statistical Analysis

The medians and ranges of *E. coli* concentrations (CFU/g_{wet}) were calculated for wildlife species' fecal material. Design Expert 8.0 was used to analyze the *E. coli* concentration data. *E. coli* concentration data of wildlife fecal material was analyzed using a two-way ANOVA model with species and season as factors with a *p*-value of 0.05. The assumption of normal distribution was not met when checking the normal plot of the residuals but the data was normalized by applying base 10 log transformations. Any interaction between season and species was broken up through applying two one-way ANOVA models with a *p*-value of 0.05 to season and species separately. Differences in *E. coli* concentrations among different species' fecal material were determined using Least Square (LS) Means. Difference between *E. coli* concentrations from fecal samples collected in summer and winter was tested using a two-way factorial model.

2.4. Results and Discussion

2.4.1. *E. coli* Concentration in Wildlife Fecal Samples

Various wildlife fecal samples, collected from Cedar Creek watershed, were enumerated for *E. coli*. The *E. coli* concentrations were reported in CFUs per g of wet fecal material. The medians and ranges of *E. coli* concentrations from different wildlife species are presented in Table 2.2. *E. coli* was enumerated from fresh fecal material in few cases and from frozen fecal samples in other cases. In general, *E. coli* concentrations of the frozen samples are less than the fresh samples (Gentry, 2012). So, the values reported here may be lower than the concentration of *E. coli* in the fecal samples. Armadillo fecal material had the highest median *E. coli* concentration of 1.01×10^{7} CFU/g_{wet} and skunk fecal material had the lowest *E. coli* concentration of 7.83×10^{3} CFU/g_{wet} (Table 2.2). Cox et al. (2005) reported median concentrations of fecal coliform for deer (2.2×10^{6} CFU/g_{wet}) and feral hog (4.1×10^{4} CFU/g_{wet}) fecal material in Sydney within the ranges observed in this study for *E. coli* concentration of deer (4.60×10^{4} - 2.69×10^{7} CFU/g_{wet}) and feral hog (7.95×10^{4} - 4.16×10^{7} CFU/g_{wet}) fecal material.

A study of *E. coli* occurrence in cattle feces conducted in Scotland reported higher prevalence in winter months than in summer months (Ogden et al., 2004). However, studies in the United States reported *E. coli* occurrence in cattle feces was highest in summer months (Barkocy-Gallagher et al., 2003; Hancock et al., 1997; Van Donkersgoed et al., 2001). Both the studies in Scotland and in the United States acknowledge a difference in the occurrence of *E. coli* in cattle fecal material during

different seasons. The effect of seasonal difference in fecal sample collection on the *E. coli* concentration of wildlife species' fecal material was tested in this study by applying a two-way ANOVA model with fecal material wildlife species' type and season of fecal sample collection ($p < 0.05$). The difference in *E. coli* concentration of samples collected in different seasons was statistically significant in the model; difference in *E. coli* concentration of different wildlife species' fecal material was not statistically significant. The interaction effect between the season of fecal sample collection and the wildlife species type of fecal sample on *E. coli* concentration was statistically significant. This interaction was further investigated through running two one-way ANOVA models with the *E. coli* concentration data sorted by season and then by species ($p < 0.05$).

The one-way ANOVA model with wildlife species type of fecal sample as the factor was applied to the *E. coli* concentration data. A graphical representation that compares *E. coli* concentration from each species' fecal material is shown in Figure 2.2. The ANOVA showed the type of wildlife feces was statistically significant in the model. The LS means were determined and the *E. coli* concentration from skunk feces was significantly different than the feces from all other wildlife species sampled. These differences could be due to the difference in the physiological difference of digestive systems and the corresponding dietary habits. All of the wildlife species sampled are mono-gastric omnivorous scavengers except deer which are ruminant herbivorous grazers. Ruminant species have four-chamber stomachs and completely depend on

microbial flora to break down food, limiting the diet to mainly grass and vegetation. Whereas, mono-gastric species have single-chambered stomachs and use enzymes in saliva to assist in breaking down food, allowing for a more diverse diet. Raccoon and opossum both had large variability of *E. coli* concentrations within their species fecal material. This large variability of both raccoons and opossum could be accounted for by the diversity in their dietary habits. If the number of fecal samples for skunk, armadillo, and hog (omnivorous scavengers) increased then a large variability in *E. coli* concentration might have been observed because of variation in their dietary habits.

The one-way ANOVA model with season as the factor showed a statically significant difference in *E. coli* concentrations of fecal material collected in the summer and winter ($p < 0.05$). The median and range of *E. coli* concentrations for fecal samples collected in summer and winter are shown in Table 2.3. In summer, opossum fecal material had the highest median *E. coli* concentration of 1.45×10^7 CFU/g_{wet} and deer fecal material had the lowest median *E. coli* concentration of 4.30×10^5 CFU/g_{wet} (Table 2.3). During winter, the highest median *E. coli* concentration was from deer fecal material (9.44×10^5 CFU/g_{wet}) and the lowest median *E. coli* concentration was from opossum fecal material (6.55×10^3 CFU/g_{wet}).

E. coli concentration data was further analyzed to test statistically significant difference in season for each wildlife species' fecal material. Median *E. coli* concentrations of fecal samples collected in the summer and winter are compared in a bar graph shown in

Figure 2.3. Median *E. coli* concentrations from deer fecal samples collected in the summer and winter were not significantly different ($p < 0.05$). However, median *E. coli* concentrations were significantly higher for both raccoon and opossum fecal samples collected during summer than winter ($p < 0.05$).

The data from this research can be used to improve the accuracy of *E. coli* load estimates determined from watershed models. Current watershed models do not incorporate differences in *E. coli* concentration due to changes in season. In general, significantly higher *E. coli* concentrations of fecal material in the summer than in the winter were observed ($p < 0.05$). This translates to higher *E. coli* loads during summer months than in the winter. According to *E. coli* concentrations of wildlife fecal material presented here and previous studies on prevalence of *E. coli* in cattle feces, allocations of *E. coli* loads from potential sources are underestimated in the summer when seasonal difference in *E. coli* concentration is not incorporated.

Table 2.2. *E. coli* concentration (CFU/g_{wet}) in feces of different species collected throughout the year.

Species	Number of samples analyzed	CFU/ g of wet fecal material	
		Median	**Range**
Feral hog	11	1.06×10^{6}	7.95×10^{4} - 4.16×10^{7}
Raccoon	69	4.50×10^{6}	8.95×10^{3} - 3.16×10^{9}
Opossum	71	7.70×10^{6}	9.78×10^{1} - 2.78×10^{9}
Skunk	4	7.83×10^{3}	5.01×10^{2} - 7.62×10^{4}
Deer	10	5.90×10^{5}	4.60×10^{4} - 2.69×10^{7}
Armadillo	5	1.01×10^{7}	2.95×10^{5} - 4.98×10^{8}

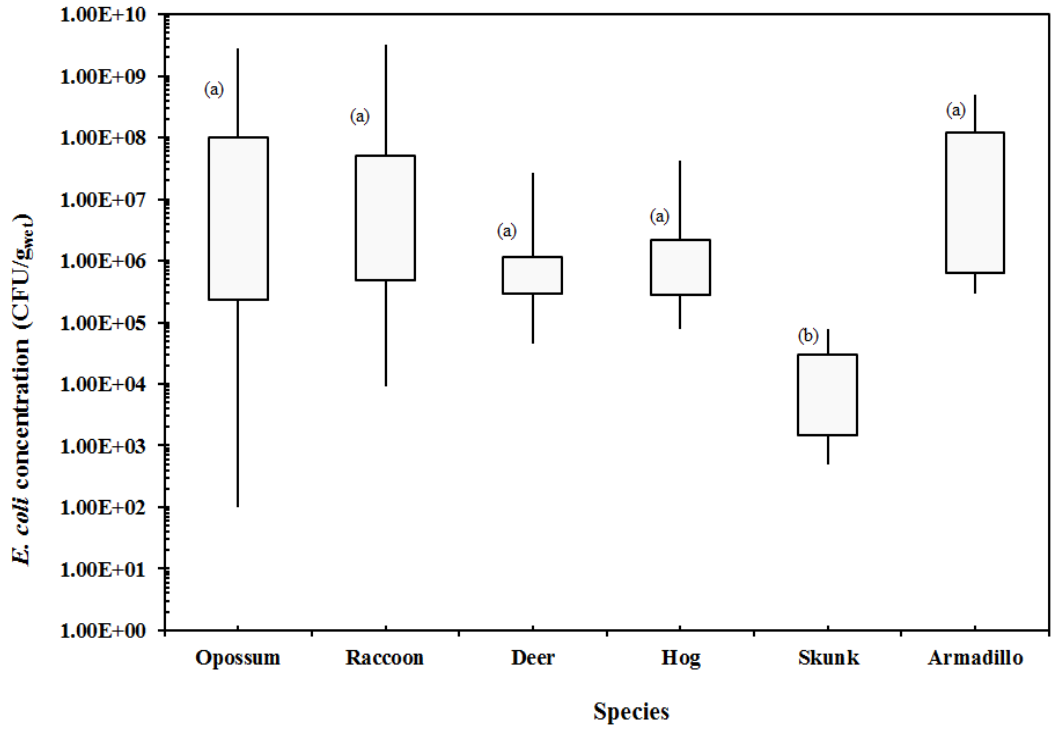

Figure 2.2. Comparison of *E. coli* concentrations (CFU/g_{wet}) from different species fecal material collected throughout the year. (b) – significant difference at a *p*-value less than 0.05.

Table 2.3. *E. coli* concentration (CFU/g_{wet}) in feces of different species collected during summer and winter.

Species	Statistic	Summer	Winter
Opossum	n	61	10
	Range	1.00×10^{4} - 2.78×10^{9}	9.78×10^{1} - 2.39×10^{5}
	Median	(a) 1.45×10^{7}	(b) 6.55×10^{3}
Raccoon	n	54	15
	Range	9.93×10^{3} - 3.16×10^{9}	8.95×10^{3} - 1.27×10^{7}
	Median	(a) 9.59×10^{6}	(b) 5.91×10^{4}
Deer	n	6	4
	Range	4.60×10^{4} - 1.28×10^{6}	2.19×10^{5} - 2.69×10^{7}
	Median	(a) 4.30×10^{5}	(a) 9.44×10^{5}
Feral hog	n	11	N/A
	Range	7.95×10^{4} - 4.16×10^{7}	N/A
	Median	1.06×10^{6}	N/A
Armadillo	n	5	N/A
	Range	2.95×10^{5} - 4.98×10^{8}	N/A
	Median	1.01×10^{7}	N/A
Skunk	n	N/A	4
	Range	N/A	5.01×10^{2} - 7.62×10^{4}
	Median	N/A	7.83×10^{3}

N/A – not available; (b) – significant difference at a *p*-value less than 0.05

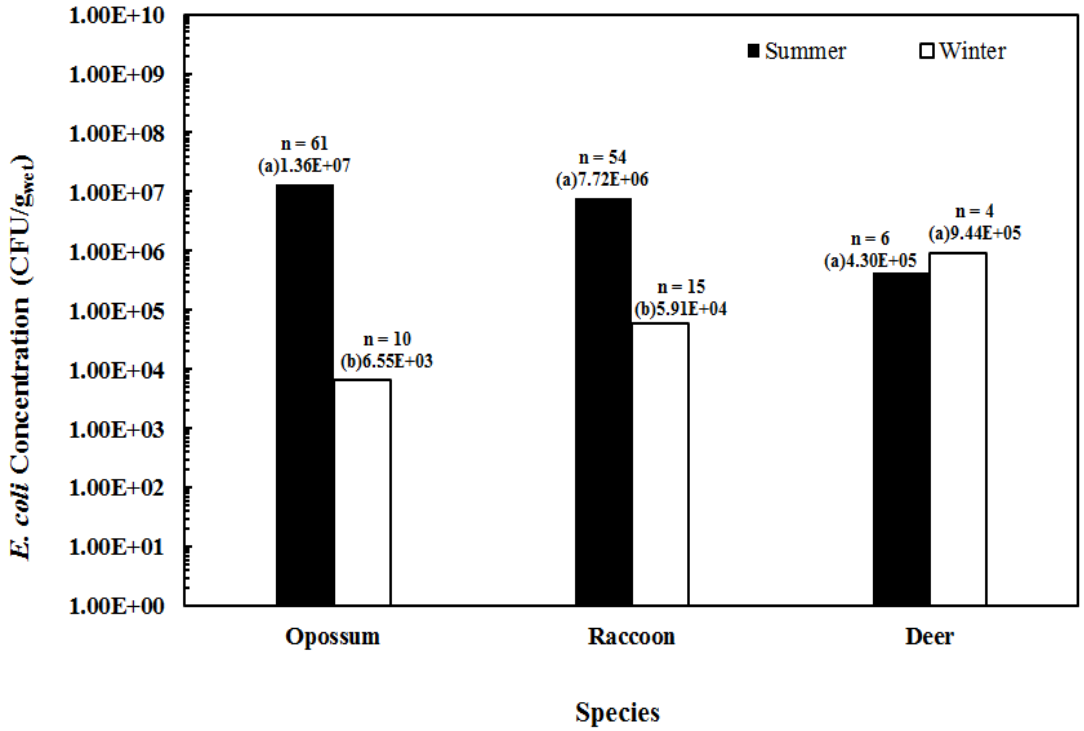

Figure 2.3. Comparison of *E. coli* concentrations (CFU/g_{wet}) from different species fecal material collected in summer and winter. (b) – significant difference at a *p*-value less than 0.05

2.4.2. Growth of *E. coli* in Water at Different Temperatures

The change in concentration of *E. coli* isolates from feral hog and deer fecal material in sterilized Cedar Creek water was observed at different temperatures. Preliminary eight hour studies were conducted and it was found that water temperature affected the *E. coli* concentration over time. Concentration of *E. coli* increased in water at 25˚C and at 30˚C over time. At 10˚C, decrease in *E. coli* concentration was observed (data not shown).

These findings were further tested with one isolate from feral hog fecal material (H1-3) and one isolate from deer fecal material (D2-c) over 30 hours. *E. coli* concentrations for H1-3 decreased in water at 10˚C and increased in water at 25˚C and 30˚C (Figures 2.4 – 2.6). The change in *E. coli* concentrations for D2-c was the same for all three temperatures (Figures 2.7 – 2.9). Padia (2010) and Hendricks et al. (1972) observed increase in *E. coli* concentration in water at 30˚C over time. However, Crane and Moore (1986) and Reddy et al. (1981) reported *E. coli* concentrations in water decreased at higher temperatures, such as 30˚C.

These contradicting observations in the fate of *E. coli* in water at 30˚C might be due to the difference in *E. coli* isolates used in these studies. In this study and the study by Padia (2010), thermo-tolerant *E. coli* was enumerated directly from the fecal material of potential sources, whereas laboratory *E. coli* strains that did not originate from fecal material were used in other studies (Crane and Moore, 1980; Reddy et al., 1981). The high temperature of the intestinal tract of mammals (~30˚C) promotes growth of thermo-

tolerant enteric bacteria, such as *E. coli*. For this reason, the EPA method to enumerate *E. coli* in water uses a temperature of 44.5˚C to select for thermo-tolerant *E. coli* (USEPA, 2002). The results from this study using thermo-tolerant *E. coli* give a more accurate depiction of the fate of *E. coli* from fecal deposition compared to studies using laboratory *E. coli* strains.

The kinetic constant (k), doubling time (t_d), and half-life ($t_{1/2}$) were determined assuming first order kinetics. The kinetic characteristics for *E. coli* isolates, H1-3 and D2-c, at each water temperature are shown in Table 2.4. The kinetic constants for both H1-3 and D2-c were the lowest at 10˚C and the highest at 30˚C in water (Table 2.4). The kinetic constants for H1-3 and D2-c were negative, indicating the k-value was a decay rate (Table 2.4). The growth rate of *E. coli* varies with temperature and is crucial in determining total maximum daily loads (TMDLs) for *E. coli*. The duration of this study was 30 hours in the laboratory but considered a day, 24 hours, in the aquatic environment. The doubling time/half-life, $t_d/t_{1/2}$, along with the k-value, can be used to quantify the amount of *E. coli* present after a period of time.

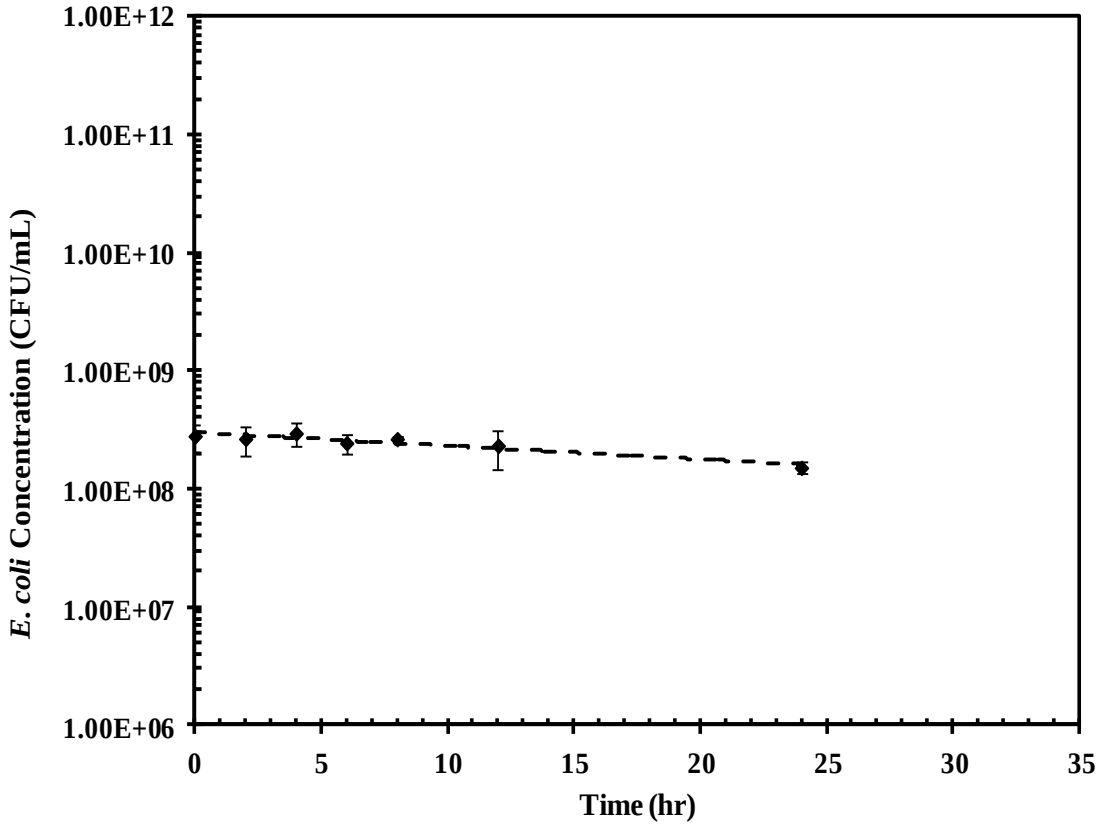

Figure 2.4. Concentrations (CFU/mL) of *E. coli* isolate, H1-3, in sterilized Cedar Creek water at 10˚C over time.

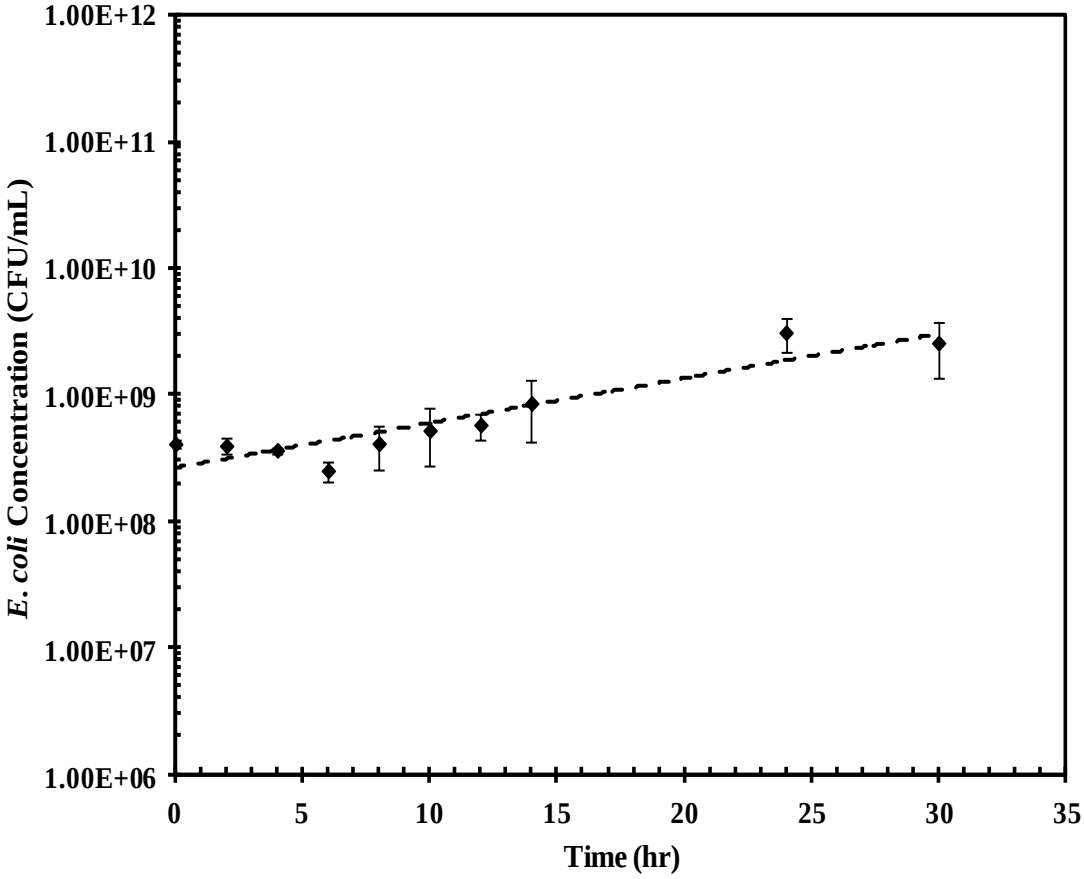

Figure 2.5. Concentrations (CFU/mL) of *E. coli* isolate, H1-3, in sterilized Cedar Creek water at 25˚C over time.

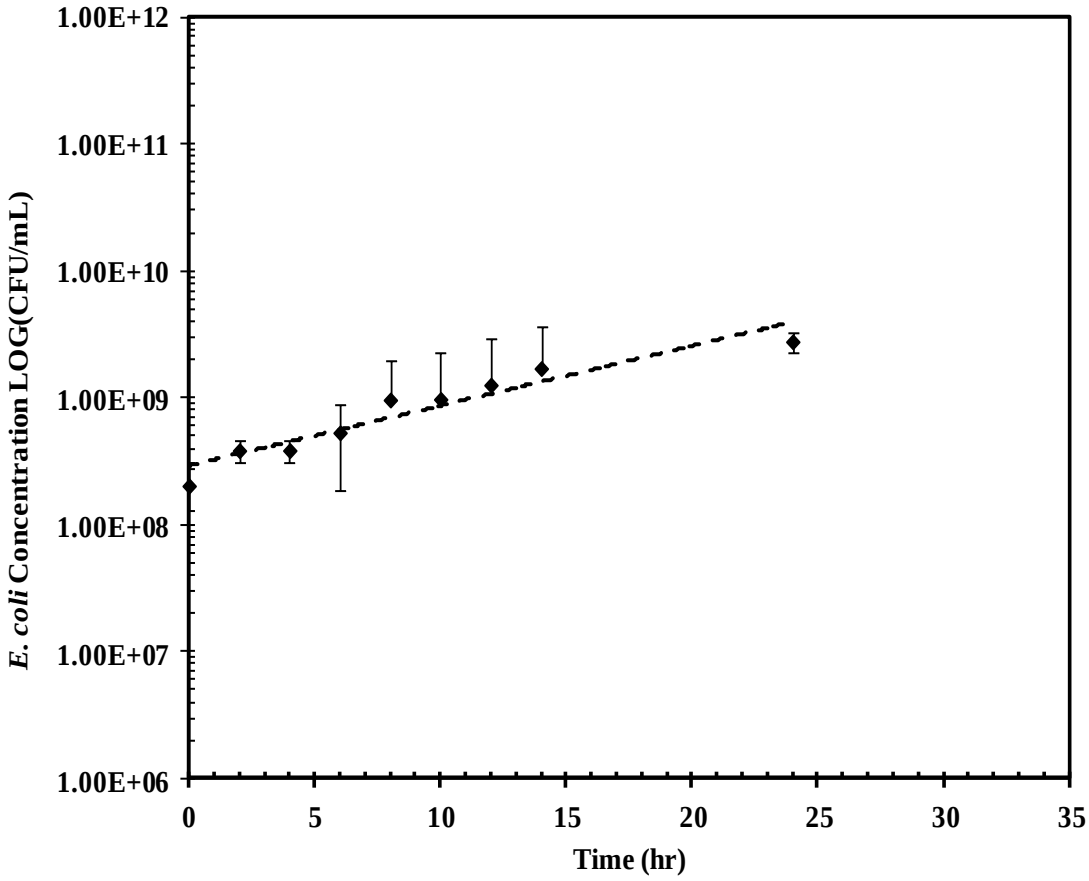

Figure 2.6. Concentrations (CFU/mL) of *E. coli* isolate, H1-3, in sterilized Cedar Creek water at 30˚C over time.

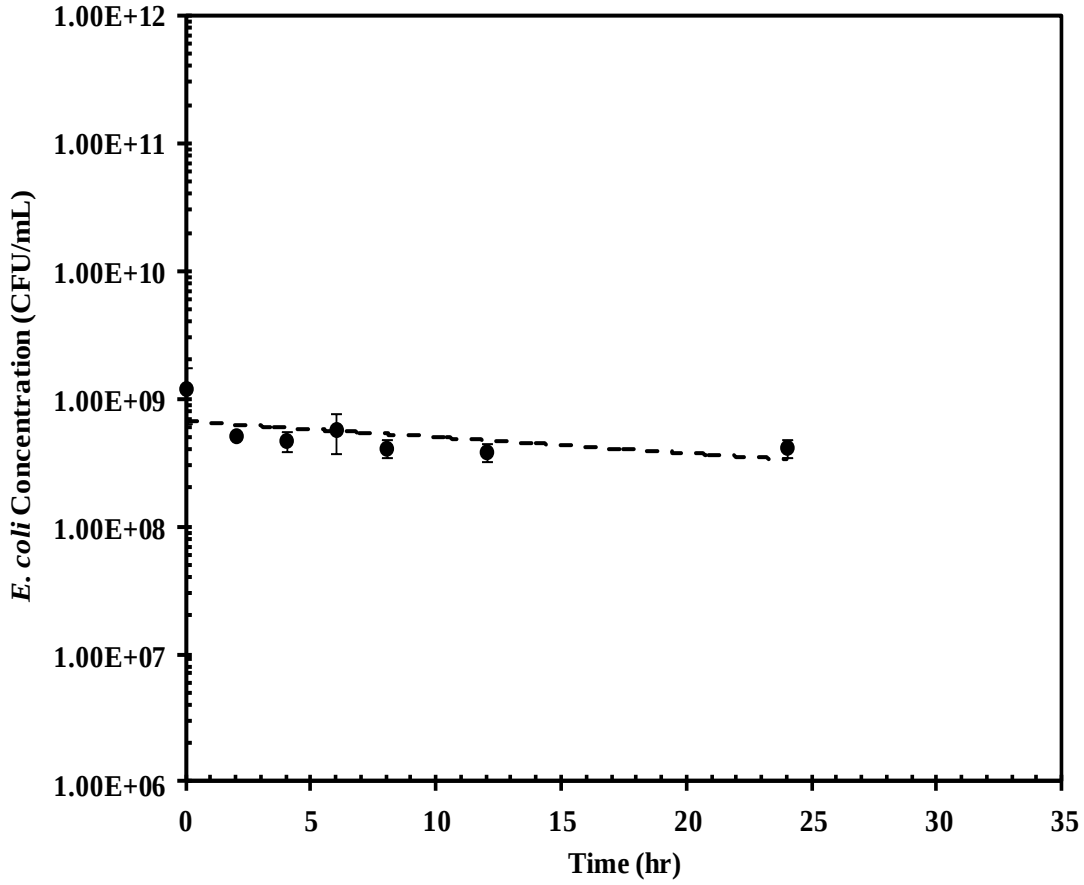

Figure 2.7. Concentrations (CFU/mL) of *E. coli* isolate, D2-c, in sterilized Cedar Creek water at 10˚C over time.

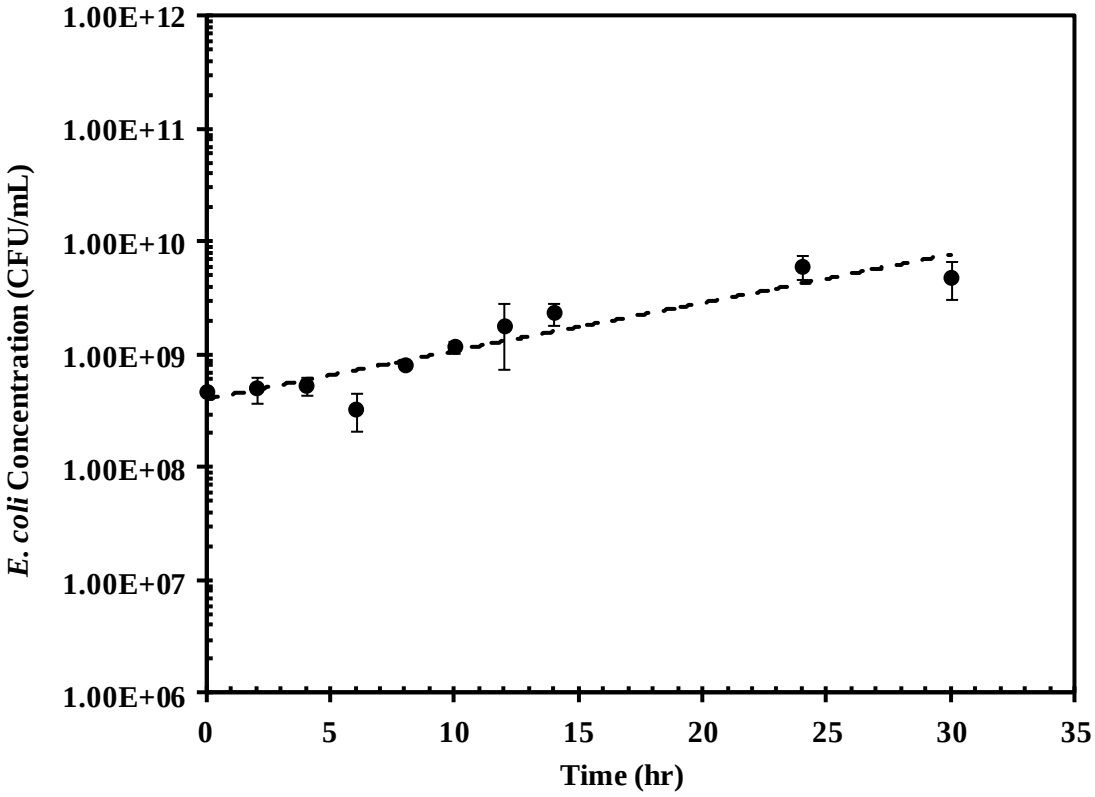

Figure 2.8. Concentrations (CFU/mL) of *E. coli* isolate, D2-c, in sterilized Cedar Creek water at 25˚C over time.

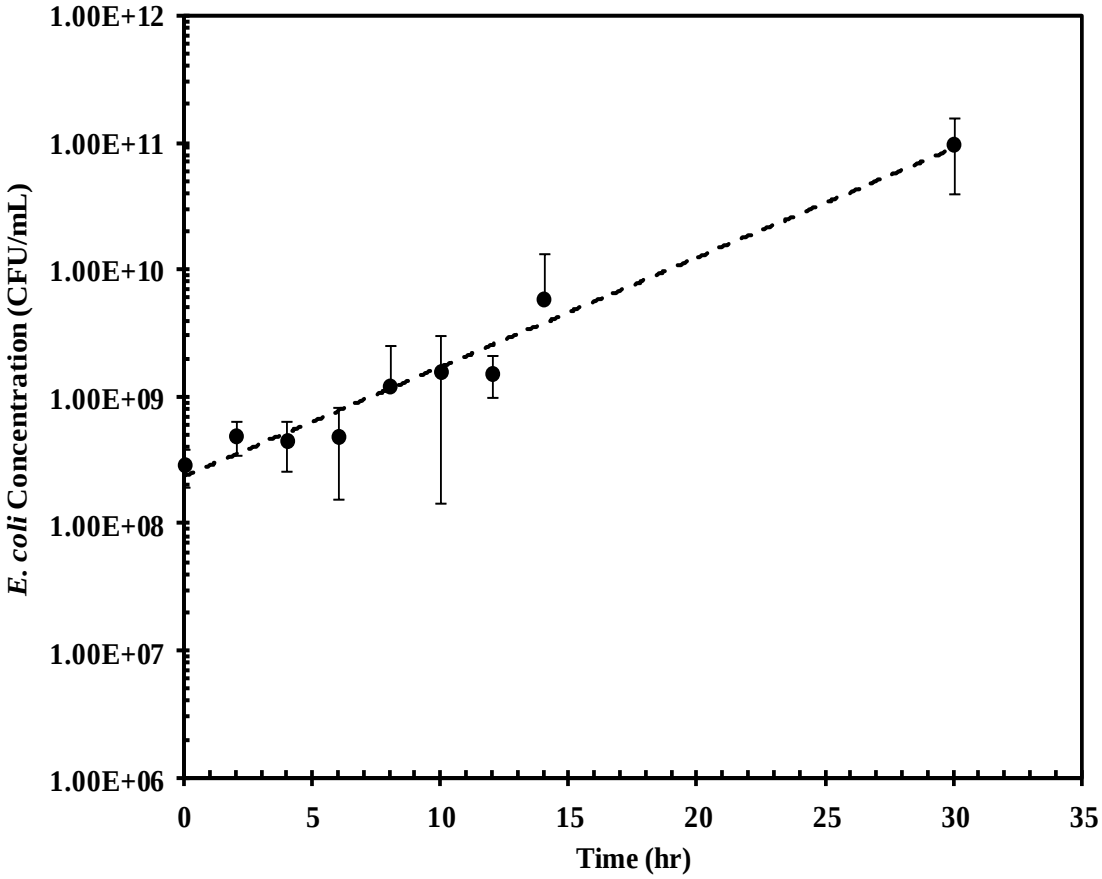

Figure 2.9. Concentrations (CFU/mL) of *E. coli* isolate, D2-c, in sterilized Cedar Creek water at 30˚C over time.

Table 2.4. Kinetic characteristics of *E. coli* isolates from feral hog and deer feces in sterilized Cedar Creek water at different temperatures.

Temperature	Feral hog (H1-3)			Deer (D2-c)		
	k (hr^{-1})	$t_{1/2}$ (hr)	t_d (hr)	k (hr^{-1})	$t_{1/2}$ (hr)	t_d (hr)
10˚C	-0.0261 ($R^2 = 0.89$)	26.6	-	-0.0286 ($R^2 = 0.35$)	24.2	-
25˚C	0.0812 ($R^2 = 0.86$)	-	8.5	0.0985 ($R^2 = 0.86$)	-	7.0
30˚C	0.109 ($R^2 = 0.91$)	-	6.4	0.198 ($R^2 = 0.97$)	-	3.5

2.4.2. Growth of *E. coli* in Soil under Different Moisture Conditions

Concentrations of *E. coli* isolates from feral hog and deer fecal material in sterilized Cedar Creek soil (Table 2.1) at different moisture conditions were measured over time (Figures 2.10 – 2.15). Both *E. coli* isolates from feral hog (H1-3) and deer (D2-c) decreased in concentrations over 168 hours for 4%, 25%, and 57% moisture content, with the exception of H1-3 at 57% with a slight increase in concentration. At 4% moisture content, the *E. coli* concentrations for both isolates (H1-3 and D2-c) decreased to zero after only 50 hours. These observations were explained by first order kinetic constant (k), doubling time (t_d), and half-life ($t_{1/2}$). Kinetic study results for *E. coli* isolates, H1-3 and D2-c, in soil at different moisture contents are presented in Table 2.5.

In this study, a greater rate of decay was observed for *E. coli* in soil at 4% moisture content compared to soil at 25% or 57% moisture content (Table 2.5). Previous studies reported higher die-off rate for *E. coli* in dry soil than in saturated soil (Berry and Miller, 2005; Chandler and Craven, 1980; Crane and Moore, 1984; Ogden et al., 2001). Based on the results from this study, *E. coli* concentration in dry soil with 4% moisture content will decrease to half the initial concentration after approximately three to four hours (Table 2.5). This rapid decay of *E. coli* concentration in dry soil would continue to occur until the *E. coli* concentrations becomes zero.

Several previous studies showed *E. coli* growth in saturated soils (Chandler and Craven, 1980; Crane and Moore, 1984; Ogden et al., 2001). At 57% soil moisture content,

growth was observed in *E. coli* isolated from feral hog feces (H1-3) while decay was observed in *E. coli* isolated from deer feces (D2-c). This difference may be due to the difference in moisture content of feral hog and deer fecal material. While processing the samples, it was observed that feral hog feces had more moisture than deer feces. Oliver et al. (2006) showed the die-off of *E. coli* varied with the moisture condition of the fecal material.

It should be noted that in this study the effect of soil moisture content on the growth of *E. coli* was conducted in sterile soil microcosms, keeping all other environmental variables constant. Non-sterile soil might have resulted in lower *E. coli* concentrations due to competition for nutrients by the other microorganisms present in the soil (LaLiberte and Grimes, 1981; Tate, 1978). The level of competition from other microorganisms is variable from soil to soil and would be difficult to predict. This study was designed to gain understanding of the fate of *E. coli* under different moisture contents with little to no competition within the soil.

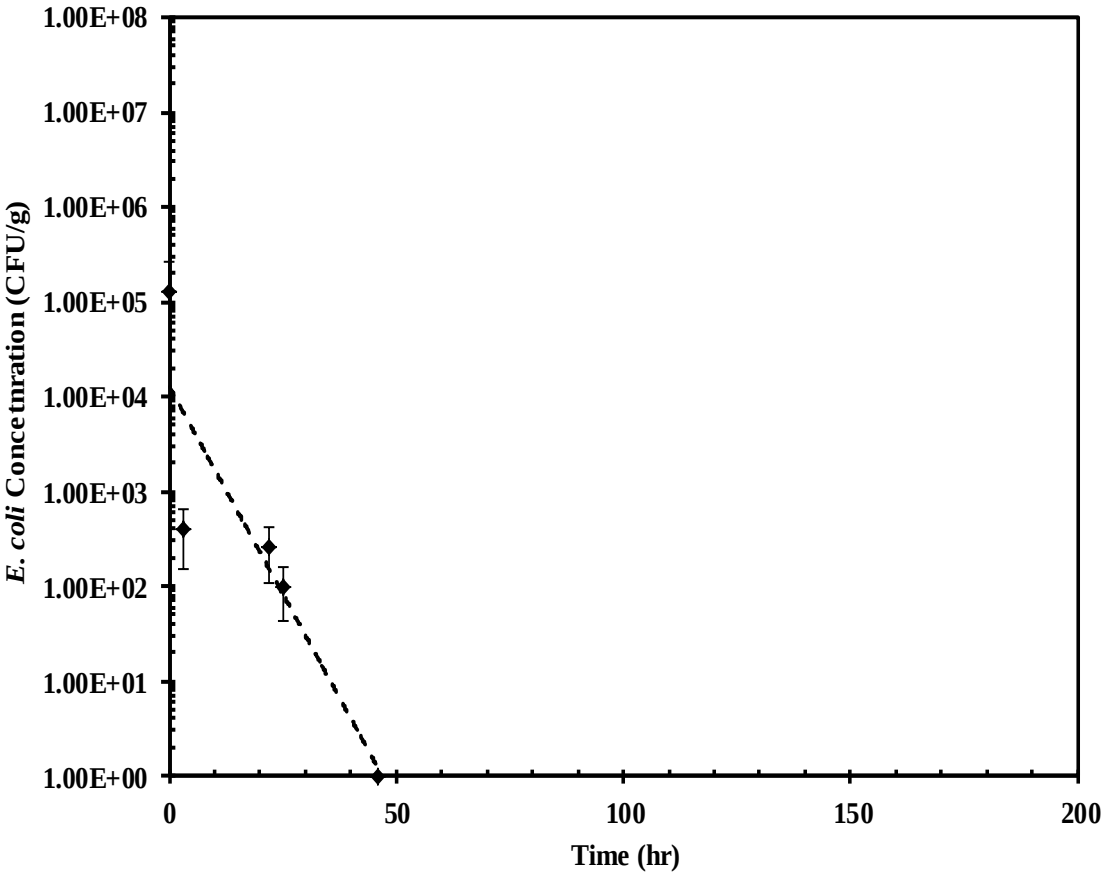

Figure 2.10. Concentrations (CFU/g) of *E. coli* isolate, H1-3, in sterilized Cedar Creek soil at 4% moisture content over time.

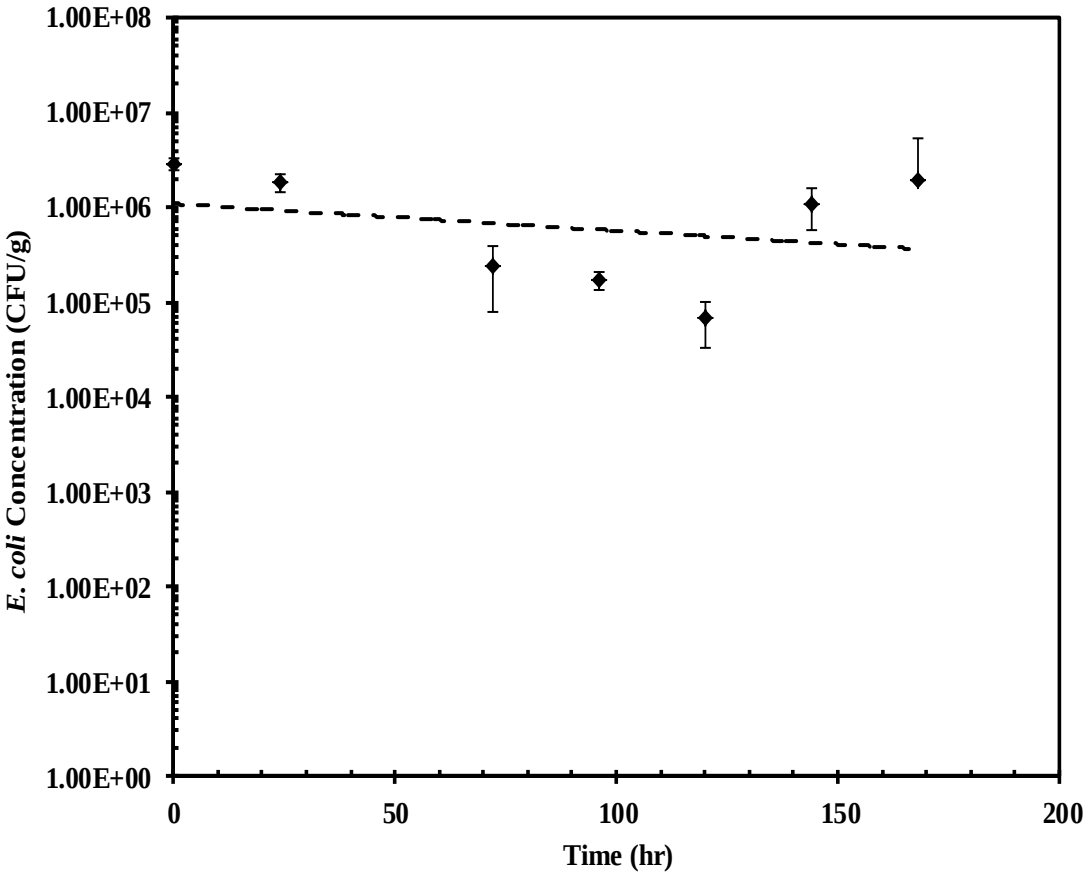

Figure 2.11. Concentrations (CFU/g) of *E. coli* isolate, H1-3, in sterilized Cedar Creek soil at 25% moisture content over time.

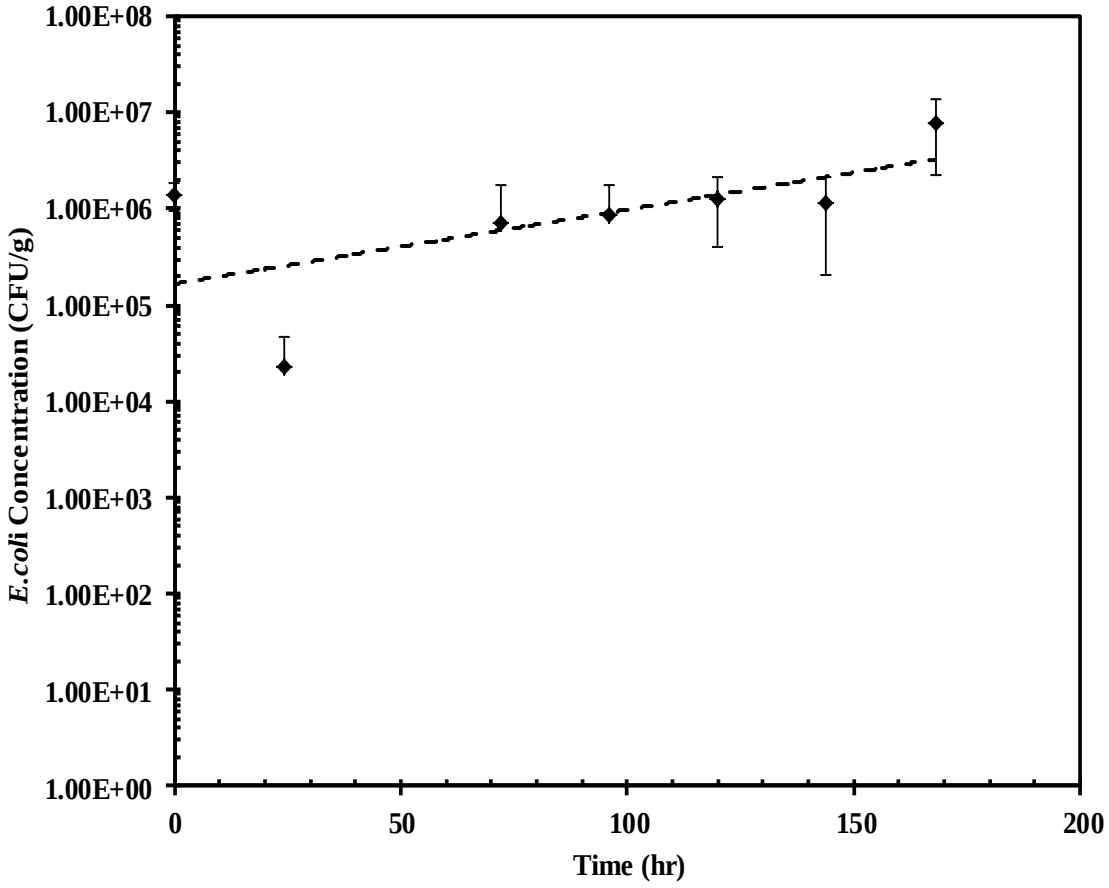

Figure 2.12. Concentrations (CFU/g) of *E. coli* isolate, H1-3, in sterilized Cedar Creek soil at 57% moisture content over time.

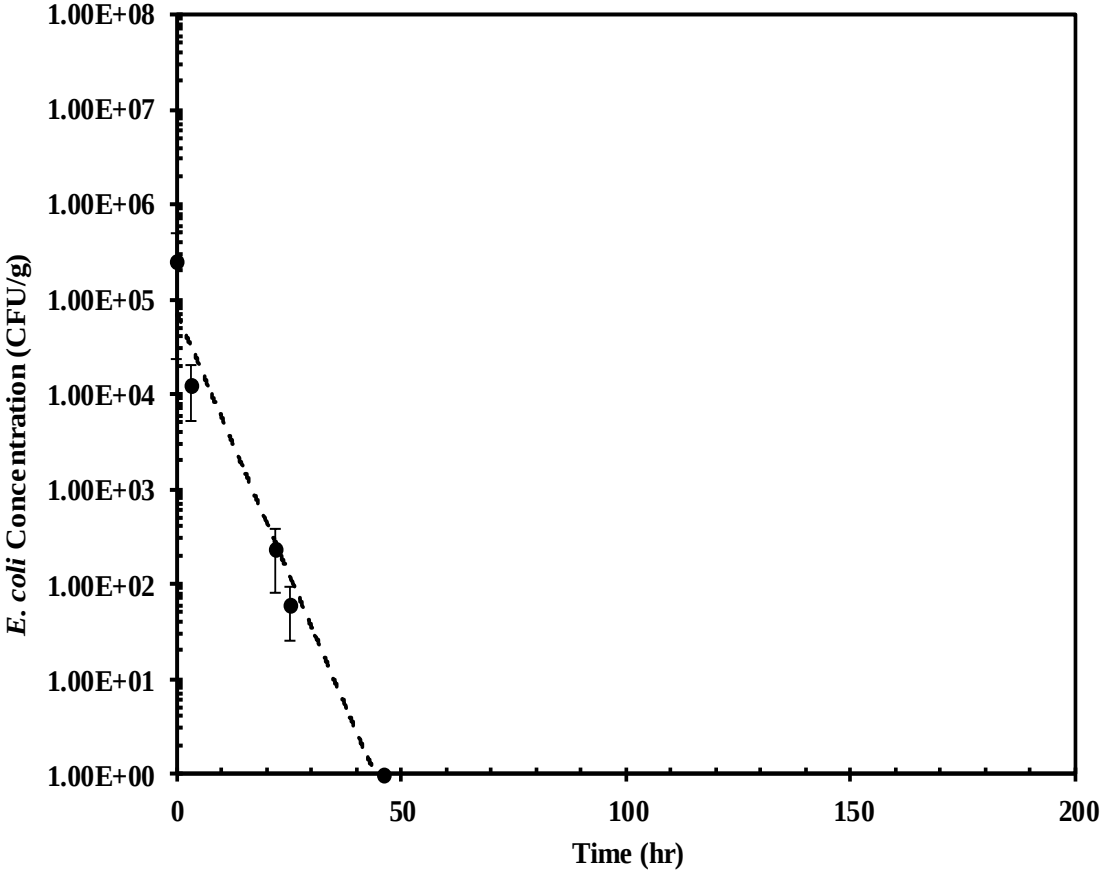

Figure 2.13. Concentrations (CFU/g) of *E. coli* isolate, D2-c, in sterilized Cedar Creek soil at 4% moisture content over time.

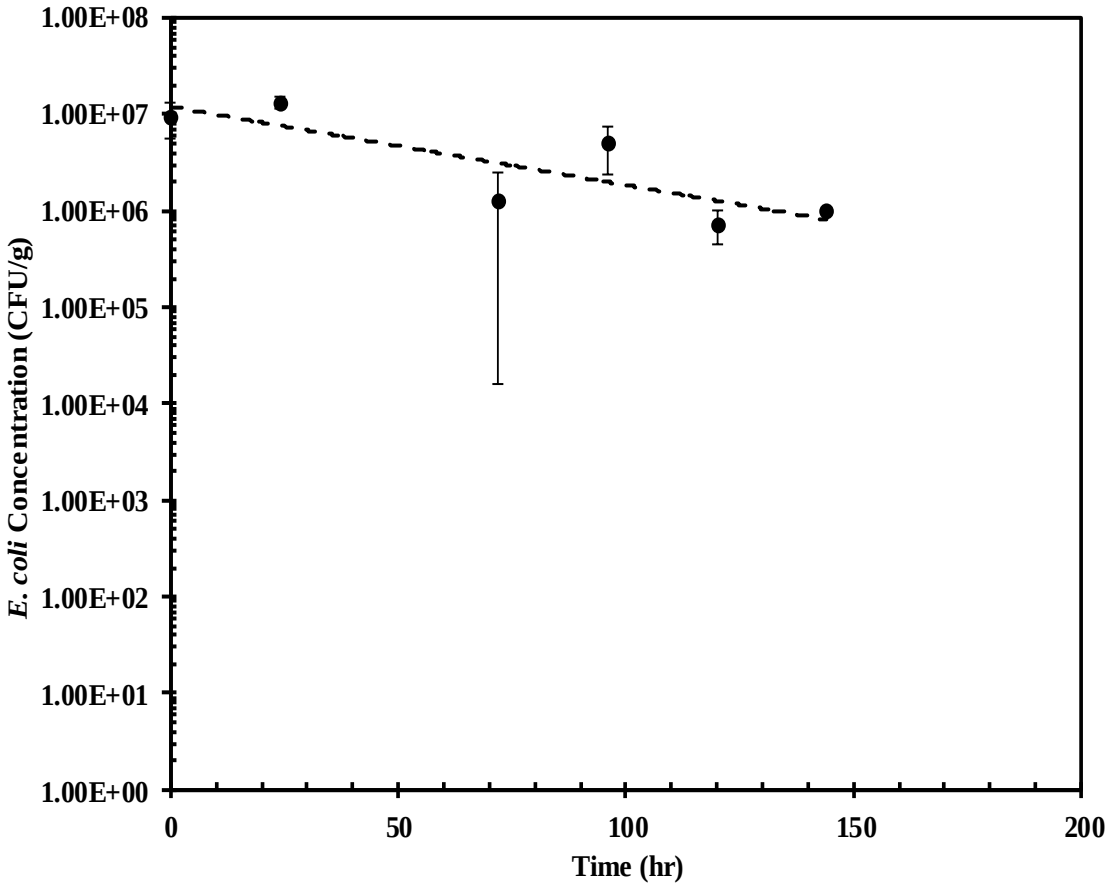

Figure 2.14. Concentrations (CFU/g) of *E. coli* isolate, D2-c, in sterilized Cedar Creek soil at 25% moisture content over time.

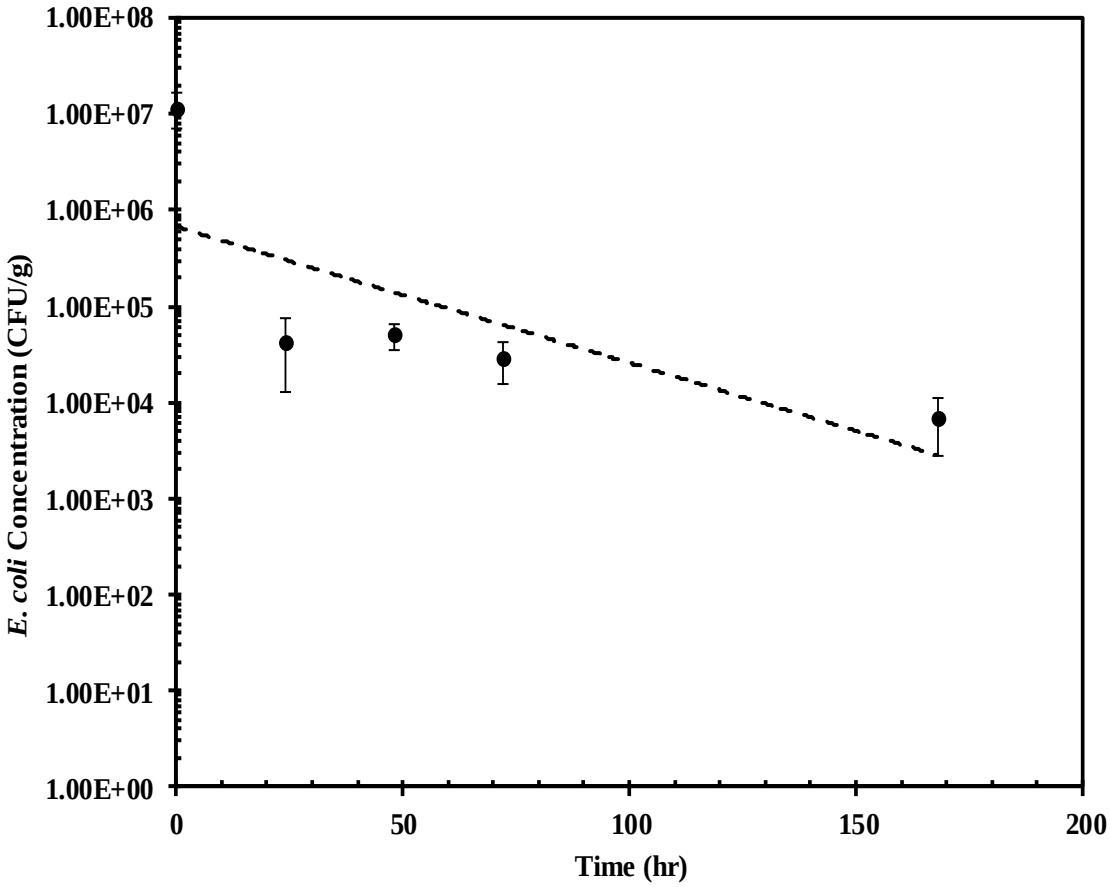

Figure 2.15. Concentrations (CFU/g) of *E. coli* isolate, D2-c, in sterilized Cedar Creek soil at 57% moisture content over time.

Table 2.5. Kinetic characteristics of *E. coli* isolates from feral hog and deer feces in sterilized Cedar Creek soil at different moisture contents.

Volumetric Moisture Content	Feral hog (H1-3)			Deer (D2-c)		
	k (hr^{-1})	$t_{1/2}$ (hr)	t_d (hr)	k (hr^{-1})	$t_{1/2}$ (hr)	t_d (hr)
4%	-0.2016 ($R^2 = 0.81$)	3.4	-	-0.2539 ($R^2 = 0.96$)	2.7	-
25%	-0.0065 ($R^2 = 0.08$)	106.6	-	-0.0187 ($R^2 = 0.69$)	37.1	-
57%	0.0177 ($R^2 = 0.38$)	-	39.2	-0.0326 ($R^2 = 0.55$)	21.3	-

2.5. Conclusions

Six different wildlife sources that could potentially contribute to *E. coli* contamination in Cedar Creek watershed, Texas were identified. The wildlife sources included feces from raccoon, opossum, feral hog, deer, skunk, and armadillo. The fecal material of each wildlife species was enumerated for *E. coli* and reported as CFU per g wet basis. The large range and variability in *E. coli* concentrations among few of the wildlife feces could be because of diverse dietary habits. There was a statistically significant difference in *E. coli* concentration in fecal samples collected in the summer and winter.

The growth of *E. coli* in sterilized Cedar Creek water at different temperatures varied depending on water temperature. Both deer and feral hog fecal *E. coli* isolates had the highest growth rate in water at 30°C and both isolates died off in water at 10°C. At 4% soil moisture content, both isolates died off rapidly. This suggests that under dry soil conditions *E. coli* will not survive for a longer duration and will not pose a threat to nearby waterbodies. The *E. coli* isolate from feral hog fecal sample persisted in soil at 25% moisture content and slightly grew at 57% moisture content. However it should be noted that the correlation between *E. coli* concentration and these moisture contents was very low. Results from this study show the high variability in *E. coli* persistence, survival, and decay in terrestrial environment. It is evident that fate of *E. coli* in the environment is a complex process and governed by various factors. The interacting effects of these environmental factors add varying degrees of complexity to model and predict fecal contamination in watersheds.

CHAPTER III

SUMMARY AND FUTURE RECOMMENDATIONS

3.1. Summary

1. Wildlife sources of the fecal contamination in Cedar Creek watershed were identified. *E. coli* concentrations of six different wildlife species' fecal material were quantified and reported in CFU per g wet basis. Statistically significant difference was observed for *E. coli* concentrations of fecal samples collected between summer and winter. Watershed modeling and load estimation tools such as SELECT, SWAT, and HSPF should include the summer and winter *E. coli* concentrations from the different wildlife species' feces while estimating temporal *E. coli* loads resulting from wildlife sources. The results from this study emphasize that *E. coli* load estimates to waterbodies in the watershed will be more representative with direct source characterization and identification of sources.

2. Kinetic constants were obtained for *E. coli* isolates from feral hog and deer fecal material in water at 10˚C, 25˚C, and 30˚C over 30 hour study duration. From this study it was found that *E. coli* growth was optimum at 30˚C in water. There was a slight growth observed for *E. coli* in water at 25˚C while a slight decay was observed for *E. coli* in water at 10˚C. These results point out that *E. coli* can persist in the aquatic systems even after excreted from animals.

3. Kinetic study was conducted for *E. coli* isolates from feral hog and deer fecal material in soil at 4%, 25%, and 57% over one week study duration. *E. coli* had the fastest die-off rate under dry moisture condition (4%) in soil. At 25% soil moisture content, the *E. coli* concentration in soil decreased for both *E. coli* isolates. Under saturated soil moisture conditions (57%), the *E. coli* isolated from feral hog fecal material had a slight growth while *E. coli* isolated from deer fecal material decayed.

3.2. Future Recommendations

The data presented from this research is a beginning of cataloging *E. coli* concentration in wildlife fecal material. In this study, fecal sample sizes for feral hog, deer, skunk, and armadillo were much lower than raccoon and opossum. Additional fecal samples should be collected and analyzed to capture the variability in *E. coli* concentrations for those species. Further research should be conducted to study the seasonal variability in *E. coli* concentration in fecal material.

Modeling the fate of *E. coli* in the environment is a complex process. There are many environmental controls that can affect the fate of *E. coli* but only two were considered in this study. Other environmental controls such as amount of carbon and soil type should be included while studying the fate of *E. coli*. This study focused on differences in the kinetic characteristics of *E. coli* isolates from feral hog and deer fecal material. Differences in kinetic characteristics for *E. coli* isolates from other potential sources' fecal material, such as other wildlife species and birds should also be studied.

Additionally, this study was conducted under controlled laboratory conditions. Repetitive studies of the growth rate of *E. coli* in soil and water will verify the reproducibility of the results with higher R^2. Further studies should be built upon the understanding of the fate of *E. coli* under controlled conditions, incorporating more variables to better represent field conditions.

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