OCCURRENCE AND FATE OF *Escherichia coli* FROM NON-POINT SOURCES IN CEDAR CREEK WATERSHED, TEXAS

A Thesis

by

REEMA PADIA

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 2010

Major Subject: Biological and Agricultural Engineering

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Approved by:

Co-Chairs of Committee,	Saqib Mukhtar
	Raghupathy Karthikeyan
Committee Members,	Terry Gentry
Head of Department,	Gerald Riskowski

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ABSTRACT

Occurrence and Fate of *Escherichia coli* from Non-Point Sources in Cedar Creek Watershed, Texas. (May 2010)

Reema Padia, B.Tech, Centre for Environmental Planning and Technology

Co-Chairs of Advisory Committee: Dr. Saqib Mukhtar
Dr. Raghupathy Karthikeyan

Fecal contamination is the pollution caused by the microorganisms residing in the intestine of warm blooded animals and humans. Bacteria are the prime cause of contamination of surface waters in the US. The transport of microorganisms into waterways can have detrimental effects on water quality and human health especially if the pathogenic strains are ingested. *E. coli* is used as an indicator of fecal contamination. Detection of these bacteria in a water body above set limits poses a potential health hazard. Various sources contribute to the bacterial contamination of a water body. The sources need to be identified and quantified for their *E. coli* content to measure bacteria loads in the waterbody accurately. In many cases, in-situ re-growth is also believed to be a considerable source of *E. coli.* 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. Thus the environmental factors like temperature and soil moisture that influence transport, persistence, re-growth, and survival of *E. coli* in landscapes were studied. The objective of this study was to identify, characterize and quantify *E. coli* loads from feces of four different animals and monitor survival, growth and re-growth at four different temperatures and moisture contents over a period of seven days. Findings of this research will aid in Watershed

Protection Plan (WPP) development and Total Maximum Daily Load (TMDL) development to address impairment from point and non-point source pollution of *E. coli.* Wildlife and range cattle manure samples responsible for fecal contamination of Cedar Creek were identified and four fecal sources out of those were quantified for the *E. coli* concentrations. No significant difference was found upon comparing the *E. coli* concentration for each species between the genders. Sub-adult cattle demonstrated significantly higher *E. coli* concentrations than adult cattle.

Growth and die-off rates were measured at different temperatures (0°C, 10°C, 25°C, and 50°C) and moisture conditions (1%, 25% 56.5% and 83%). *E. coli* concentrations in cattle and raccoons feces showed 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%).

To my family and friends …

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Lastly, I offer my regards to all of those who supported me in any respect during the successful completion of my thesis.

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CHAPTER I

INTRODUCTION: THE IMPORTANCE OF RESEARCH

1.1. Introduction

The term water body generally refers to a stream, reservoir, or estuary. Water bodies are designated according to their appropriate use such as aquatic life, public water supply, contact recreation, or oyster waters. The designated use of a water body could be affected because of contamination. The state authorities set water quality standards and criteria to evaluate and manage point and non-point contaminant loadings into its water bodies (TCEQ, 2008). Failure to meet with the criteria may lead to the impairment of the water body. The contaminants could be chemical or bacterial. Bacteria are the leading cause of impairment of surface waters, including rivers, lakes, and streams in the U.S. (USEPA, 2008).

Presence of indicator organisms is generally used to determine the fecal contamination of a water body. Fecal coliforms were a common indicator of fecal contamination until it was found that they can survive outside the gut of warm blooded organism (Sherer et al., 1992). Henceforth, *Escherichia coli* (*E. coli*) bacteria are used as indicator organisms in identifying fecal contamination of water bodies (Byappanahalli et al., 2003; Chin et al., 2009). If indicator organisms are found in a water body then it suggests the presence of fecal contamination and thus possible occurrence of pathogenic strains of the bacteria (Bolster et al., 2009). It is important to be aware that presence of *E. coli* in water does not mean drinking it or coming in contact with it will make a person ill. It merely points toward the likelihood of existence of pathogenic strains. *E. coli* is a single species of bacteria from the vast majority of bacteria called fecal coliforms. It is normally found in

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This thesis follows the style of Biological Engineering.

the intestines of animals and humans. Only some strains of *E. coli* are pathogenic which include Enteropathogenic, Enteroinvasive, Enterotoxigenic, and Enterohemmorrhagic. The symptoms of illness associated with these strains are diarrhea, bloody diarrhea, urinary tract infection and incase of Enterohemmorrhagic strains like O157:H7 hemolytic uremic syndrome (Anderson, & Davidson, 1997).

All the states require monitoring and evaluating public water bodies within their boundary, according to federal regulations. The states need to identify the water bodies that are impaired and determine the maximum amount of contaminant it can receive per day to achieve and maintain applicable water quality standards. These limiting loadings are called Total Maximum Daily Loads (TMDLs). The next step is to identify the sources contributing to the total contaminant loading that involves intensive data collection and analysis. Once the sources are identified allowable loads and required load reductions are assigned. Modeling tools like Soil Water Assessment Tool (SWAT) and Hydrologic Simulation Program in Fortran (HSPF) or the load-duration curve method are often used to aid in the TMDL development process (Chin et al, 2009).

The Texas Commission on Environmental Quality (TCEQ) evaluates water bodies in the state and identifies those that do not meet uses and criteria defined in the Texas Surface Water Quality Standards (TSWQS). Once a water body is identified it is assigned one of the five categories assigned to each impairment parameter in each segment that affects the use of the water body as defined in TSWQS (TCEQ, 2008) Since January 2007, 197 water bodies in Texas were impaired because they did not meet the bacteria criteria established by the state to protect contact recreation use. A geometric mean of 126 CFU/100 mL and a single maximum of 394 CFU/100 mL for *E. coli* were the criteria

used to determine the impairment for freshwater contact recreation use (TSSWCB, 2007).Cedar Creek located partly in Brazos County and partly in Robertson County in East Central Texas is one of the several water bodies deemed impaired because it did not meet bacteria criteria. It falls under 5c category which means additional data and information needs to be collected before a TMDL is scheduled. Since there is insufficient information to determine the best course of action it is necessary to identify and quantify the potential sources to fill in the data gaps (TCEQ, 2008).

Cedar Creek has little or no urban influence. Except for direct deposition from animals there is no other evidence for point source contamination into this Creek. The main sources of bacteria are assumed to be agricultural, rural, cattle and wildlife animals. In majority of the rural and agricultural stream impairments due to bacteria, specifically *E. coli*, the specific sources and accurate quantities from each source have not been accurately determined. It is necessary to determine the *E. coli* loads resulting from cattle and wildlife animals in Cedar Creek watershed.

Numerous reports used in TMDL development give information about the *E. coli* content of feces for some domestic and wildlife species. But since this information has not undergone extensive peer review it has not been the focus of reported research. As a result there is a high level of uncertainty in identifying *E. coli* loads and sources for use in watershed modeling and *E. coli* load estimation tools (TSSWCB, 2007).

Several studies (Bolster et al., 2009; Habteselassie et al., 2007; Ishii et al., 2006; Sherer et al., 1992; Wang et al., 1996) have been conducted to investigate the fate and transport of *E. coli* but still better understanding is required to improve the modeling of these

processes. A number of environmental factors and management practices affect the fate and transport of *E. coli* in rural and agricultural landscapes. There is a need to identify the dominant environmental factors that affect the fate and transport of *E. coli* in rural landscapes.

The fate of *E. coli* needs to be studied under different environmental factors such as temperature, moisture content, and pH to be familiar with the phenomenon of re-growth. Generally it is assumed that the population of these bacteria declines rapidly once they are emitted of the intestines and as they enter soil or water . However, studies (Fenlon, & Wilson, 2000; Islam et al., 2004; Jamieson et al., 2002; Jiang et al., 2002; Muirhead et al., 2006) suggest that under certain favorable environmental conditions including optimum temperature, moisture, pH, nutrient levels, and competition with other common bacteria, *E. coli* survive for a much longer period of time.

1.2. Objectives

- The first objective of this study was to identify, characterize, and quantify the *E. coli* loads resulting from cattle and wildlife animals in Cedar Creek water shed.
- The second objective of this study was to monitor survival, growth, and re-growth of *E. coli* under different environmental (temperature and moisture) conditions.

1.3. Rationale

This study will help to quantify the *E. coli* concentrations from the fecal material of various different species. The concentrations obtained will be useful information for watershed modeling and predictions of *E. coli* loads in a watershed. Accurate

identification, characterization, and quantification of *E. coli* sources in the impaired watershed will help improve *E. coli* load estimation tools like SELECT (Spatially Explicit Load Enrichment Calculation Tool). Load estimation tool estimates *E. coli* loads from different sources from literature values. Literature does not provide *E. coli* concentrations for several species and also they have not gone through extensive peer review since they were not the focus of reported research (TSSWCB, 2007).

Studying the survival and growth of bacteria at different temperature and moisture conditions will provide further understanding into long term water quality conditions. Several studies have examined the presence and growth of *E. coli* in water and soil/ stream sediment and also concluded ubiquity and persistence of the bacteria for long periods of time (Bolster et al., 2009; Habteselassie et al 2007; Ishii et al., 2006; Jamieson et al., 2002; Muirhead et al., 2004; Sherer et al., 1992; Wang et al., 1996).

This study looked at the survival and growth of *E. coli* at four different temperatures in water and at four different moisture contents in soil with a constant temperature (room temperature). The temperatures selected for this study were 0°C, 10°C, 20°C and 50°C. The four moisture contents selected were 1%, 25%, 56.5% and 83% with the purpose of studying growth and survival under dry, damp, wet, and saturated environment.

The results and findings of this study would help protect and restore the water quality from point and non-point source pollution in Cedar Creek watershed. The ultimate outcome of this research would aid in Watershed Protection Plan (WPP) development and Total Maximum Daily Load (TMDL) development to address impairment due to bacteria in the State of Texas.

CHAPTER II

OCCURRENCE AND FATE OF *E. coli* FROM VARIOUS NON-POINT SOURCES IN A SUBTROPICAL WATERSHED

2.1. Introduction

Bacteria are the leading cause of impairment of surface waters, including rivers, lakes, and streams in the U.S. (USEPA, 2008). Water bodies are designated according to their appropriate use to support aquatic life, supply drinking water, and provide contact recreation. The designated use of a water body could be affected because of biological and chemical contamination. The regulatory authorities set water quality standards and criteria to evaluate and manage point and non-point contaminant loadings into water bodies that cause contamination (TCEQ, 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 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). The transport of microorganisms from land into waterways can have detrimental effects on water quality and human health especially if the pathogenic strains of *E. coli* are ingested. In many cases, in-situ re-growth is believed to be a considerable source of *E. coli*. (Byappanahalli et al., 2003; Muirhead et al., 2004*)*.

Fecal contamination of a water body 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 occurrence of pathogenic strains of the bacteria, protozoa, virus, and fungi (Bolster et al., 2009).

The first step in developing either a Watershed Protection Plan (WPP) or a Total Maximum Daily Load (TMDL) plan is to identify the sources contributing to the total contaminant loading in a watershed. This involves intensive data collection and analysis. Once the sources are identified allowable loads and required load reductions are calculated based Load Reduction Curve analysis (Chin et al., 2009). Sources can be point, non-point or the growth of bacteria surviving in either soil or water. Thus accurate sources of pollutant can never be exactly determined. Generally, watershed models are applied to study the impacts of various management plans and current state of water quality.

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 TMDL development. Most of the models are limited in their ability to simulate bacteria concentrations during varying climatic conditions and they also do not take into the account of bacterial life cycles. 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 the fate and transport processes more accurately as well (Riebschleager, 2008).

The growth of *E. coli* in the environment is not properly understood or documented (Ishii et al., 2006). On the other hand, 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 (Byanppanahalli et al., 2003; Ishii et al., 2006; Sherer et al., 1992; Stephenson and Rychert 1982). 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.

A number of environmental factors and management practices affect the fate and transport of *E. coli* in rural and agricultural landscapes. There is a need to identify the dominant environmental factors and physical and chemical properties of soil and water that affect the fate and transport of *E. coli* in rural landscapes. To understand re-growth phenomenon better, the fate of *E. coli* needs to be monitored under different environmental factors such as temperature, moisture content, and pH. It is sometimes assumed that once these bacteria are shed and as they enter soil or water, their population declines rapidly. However, studies suggest that under certain favorable environmental conditions including optimum temperature, moisture, pH, nutrient levels, and competition with other common bacteria, *E. coli* survive for a much longer period of time (Fenlon and Wilson, 2000; Islam et al., 2004; Jamieson et al., 2002; Jiang et al., 2002; Muirhead et al., 2006). Studying the survival and growth of bacteria at different

temperature and moisture conditions will provide further understanding of long term water quality conditions. Several studies have examined the presence and growth of *E. coli* in water and soil/ stream sediment and concluded ubiquity and persistence of the bacteria for long periods of time (Bolster et al., 2009; Habteselassie et al., 2007; Ishii et al., 2006; Jamieson et al., 2002; Muirhead et al., 2004; Sherer et al., 1992; Wang et al., 1996).

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. The four soil moistures contents selected were 1%, 25%, 56.5%, and 83% with the purpose of studying growth and survival under dry, damp, wet and saturated soil environment. The results from this study are presented in this paper.

2.2. Study Area Description

The study area for this research was Cedar Creek watershed, located in Brazos County & Robertson County in East Central Texas (Figure 2.1.). It 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. 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.

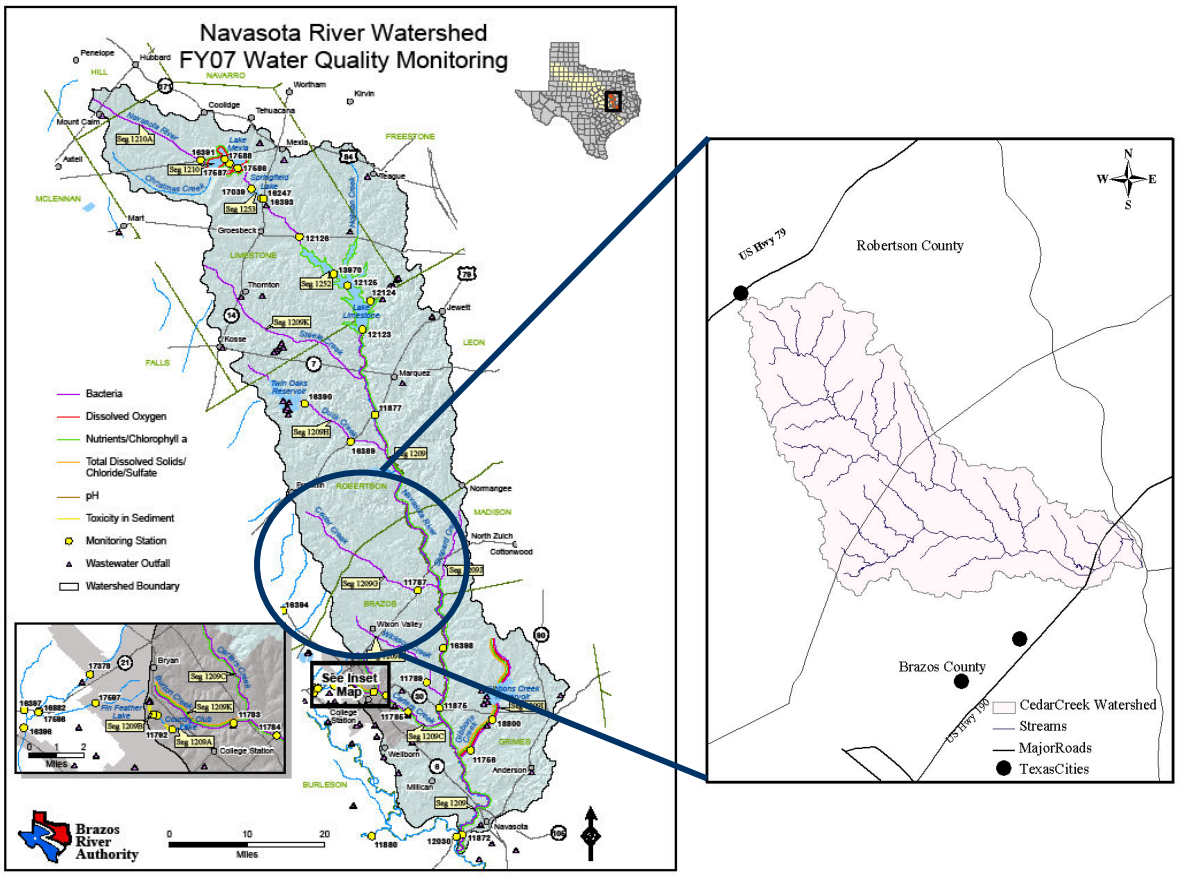

Figure 2.1. Study Area: Cedar Creek watershed, Texas.

Cedar Creek, located partly in Brazos County and partly in Robertson County in East Central Texas, is one of the several water bodies deemed impaired because it does not meet bacteria criteria (TSSWCB, 2007). It falls under 5c category which means additional data and information needs to be collected before a TMDL is scheduled by the TCEQ. Cedar Creek has little or no urban influence. Except for direct deposition from animals there is no other evidence for point source contamination into this Creek. Bacterial contamination is mainly resulting from agricultural and rural sources such as cattle and wildlife.

2.3. Methods and Materials

2.3.1. Collection of Fecal Samples from the Watershed

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 south–west of Cedar Creek watershed. Various non-point sources of *E. coli* (wildlife and cattle) were identified in the study area and fecal samples from those sources were collected. The fecal material was collected by trapping the animals from the two sub-watersheds during summer for three months. Trapping of animals and collection of fecal material was conducted according to a standard protocol by a wild life expert.

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. Age of the animal was broadly categorized by observing the animal into two groups, namely adult and sub-adult. The Whirl-Pak Bags® were kept in coolers with ice and transported to the laboratory.

2.3.2. 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. One g of sub-sample was taken from each fecal sample and added to 9.5 mL of sterile de-ionized water in a test tube. Then, the test tube was vortexed for two minutes to elutriate bacteria from the fecal 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-ionzed water and then boiling the mixture for one minute. Modified mTEC agar is a selective and differential medium used for chromogenic 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 through the 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 colony forming units (**CFU**s) per g of wet fecal material. The gravimetric moisture content of all fecal samples were determined simultaneously by drying one g of the wet sample at 100°C for 24 h. Moisture content was calculated on wet basis [(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*.

2.3.3. Survival, Growth, and Re-growth 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.

2.3.3.1. E. coli *survival in Water at Different Temperatures*

Ten g 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* numbers were reported as CFU per 100 mL.

2.3.3.2. E. coli *survival in Soil at Different Moisture Conditions*

Isolates of the same samples used in 2.3.3.1 were used to study the survival of *E. coli* at different soil moisture 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 1%, 25%, 56.5% and 83% moisture content, respectively. Soil samples were incubated at room temperature. *E. coli* in soil was enumerated after 1, 24,

72, 120, and 168 h. The enumeration for each time sampling was in triplicates and the median *E. coli* numbers were reported as CFU per g wet weight of soil.

2.3.4. 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 is any significant difference in *E. coli* concentrations resulting from the four species. Kruskal-Wallis test is a non-parametric counterpart of ANOVA. ANOVA tests the equality of means of data belonging different categories (McDonald, 2009) while Kruskal-Wallis tests whether k (more than two) independent samples that are defined by a grouping variable are from the same population (SPSS Statistics 17.0 Command Syntax Reference). For each species, two tests were performed: one to check the difference in *E. coli* concentration in a species based on gender and another based on age. 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 are to be 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* numbers were 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. Typically non- parametric tests for statistical analysis are used in such cases where data is skewed or asymmetric. Therefore to find whether there was a difference in *E. coli* numbers on 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 and moisture conditions measured on any particular day are different for different temperature and moisture conditions and (2) *E. coli* concentration from feces of a species at a particular temperature or moisture condition will be different on different days.

2.4. Results and Discussion

2.4.1. *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 per g of wet fecal material. Various samples from four different species were used to analyze for their *E. coli* content. Table 2.1 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 2.1. *E. coli* concentration in feces of different species

Species	Number of samples		CFU/ g of wet fecal material	
	Collected	Analyzed	**Median**	**Range**
Armadillo	7	5	1.01×10^{7}	2.95×10^{5} - 4.98×10^{8}
Raccoons	86	43	1.59×10^{7}	1.88×10^{5} - 3.16×10^{9}
Opossum	76	57	1.60×10^{7}	1.82×10^{4} - 2.78×10^{9}
Cattle	26	20	2.17×10^{5}	9.42×10^{1} - 1.92×10^{6}

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 (1.60×10^{7} CFU/g) and raccoons (1.59×10^{7} CFU/g) feces were higher than cattle (2.17×10^{5} CFU/g) and armadillo (1.01×10^{7} CFU/g). The *E. coli* count from cattle feces was found to be the lowest of all the species analyzed.

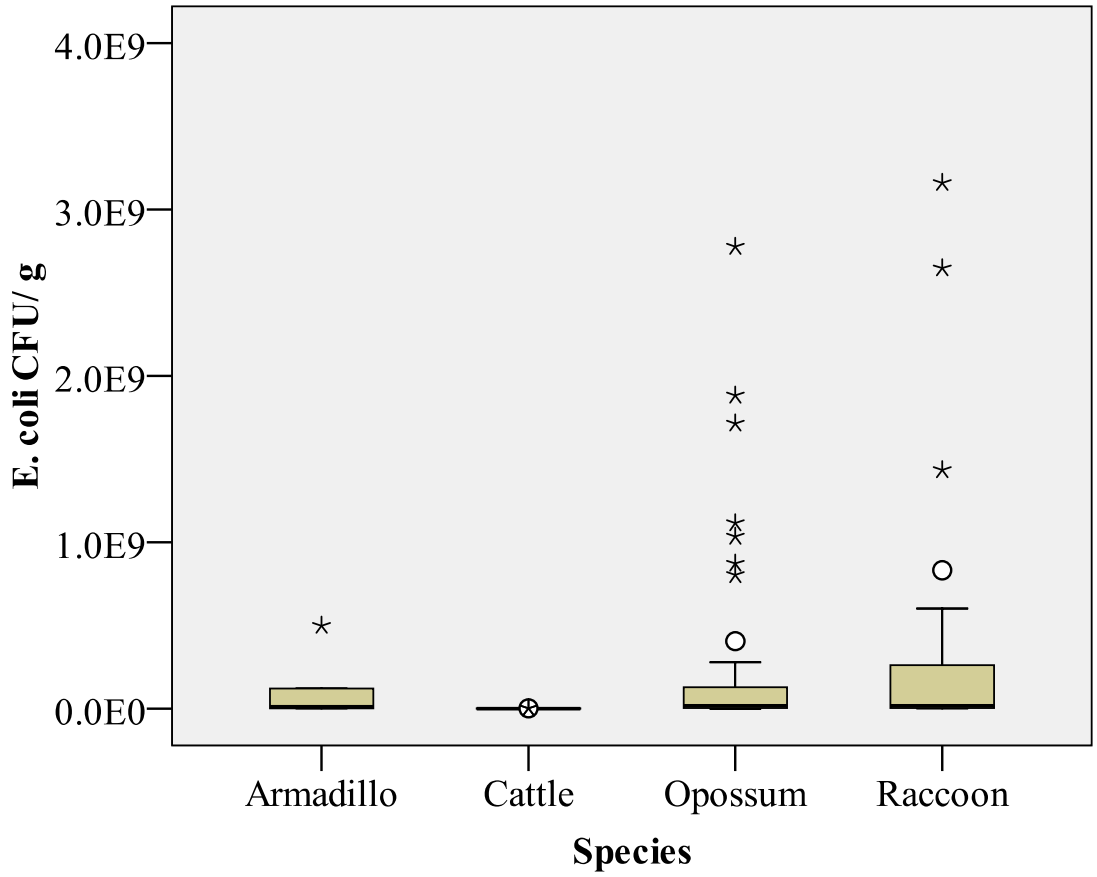

Figure 2.2. *E. coli* concentrations in feces of different species.

Figure 2.2 shows the distribution of *E. coli* in four different species. It was observed that data for all the species were severely 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 ($p < 0.05$). 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 (Figures 2.3-2.9). It is also observed that *E. coli* concentration data are severely skewed with a number of outliers (Figures 2.3-2.9).

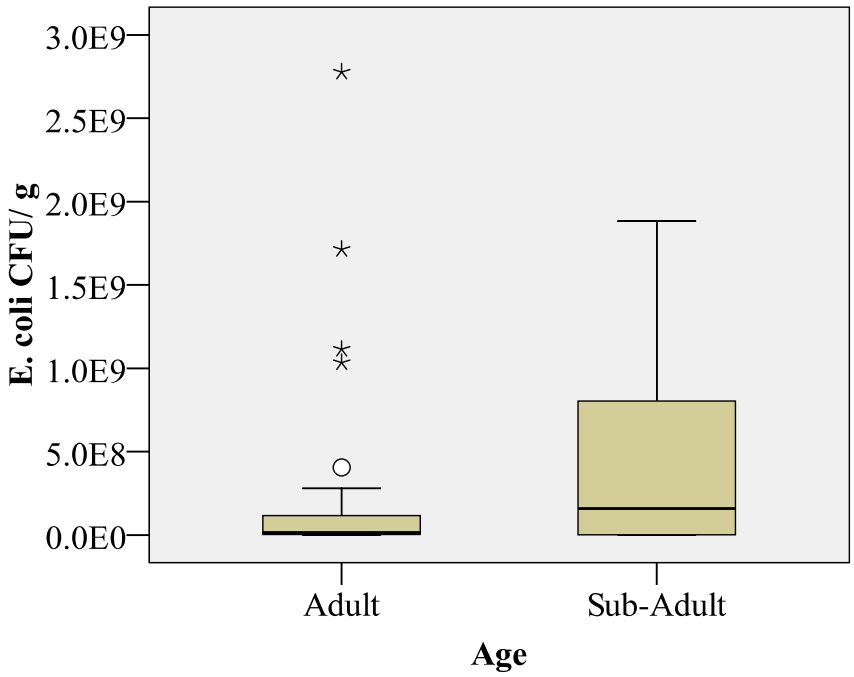

Figure 2.3. *E. coli* concentration in opossums based on age.

As shown in Figure 2.3, 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 (Figure 2.4).

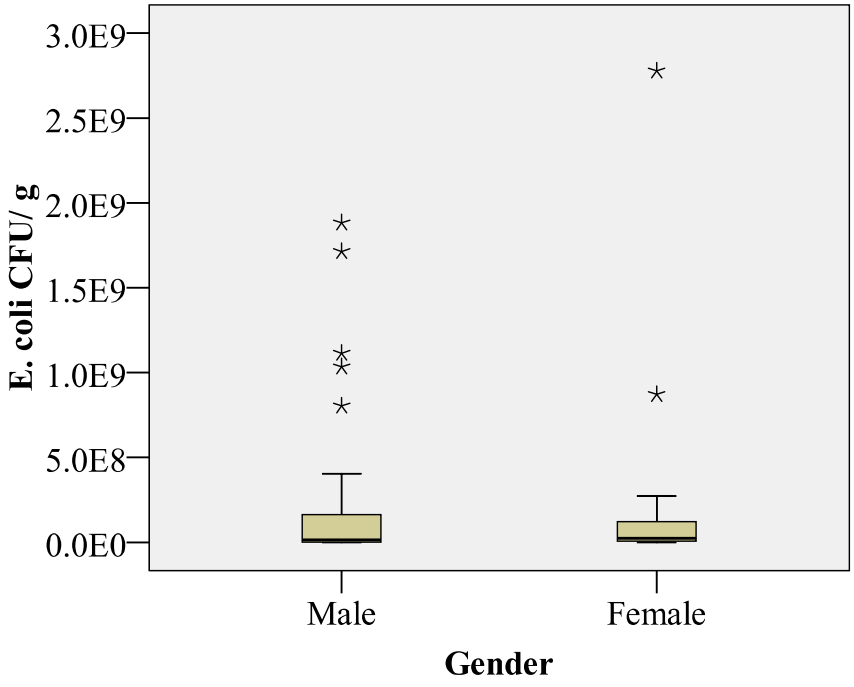

Figure 2.4. *E. coli* concentration in opossums based on gender.

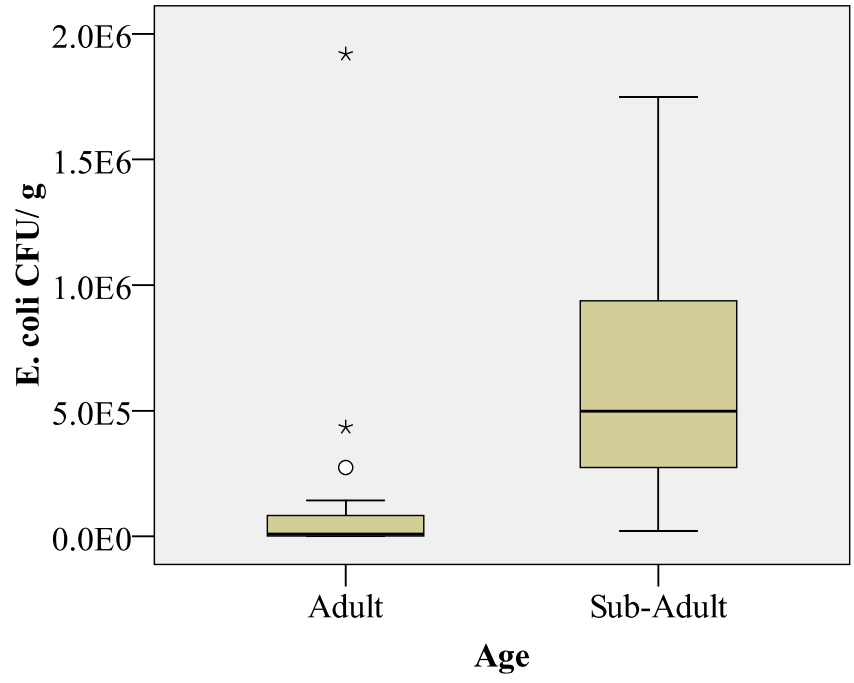

Figure 2.5. *E. coli* concentration in cattle based on age.

Calves showed a significantly higher ($p < 0.05$) *E. coli* concentration than adult cows (Figure 2.5). 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 2.6).

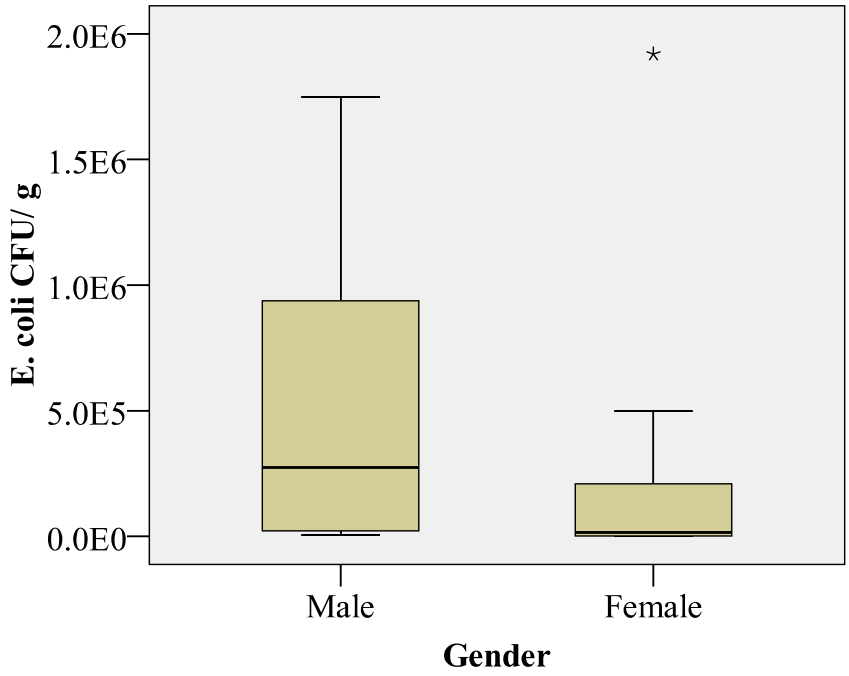

Figure 2.6: *E. coli* concentration in cattle based on gender.

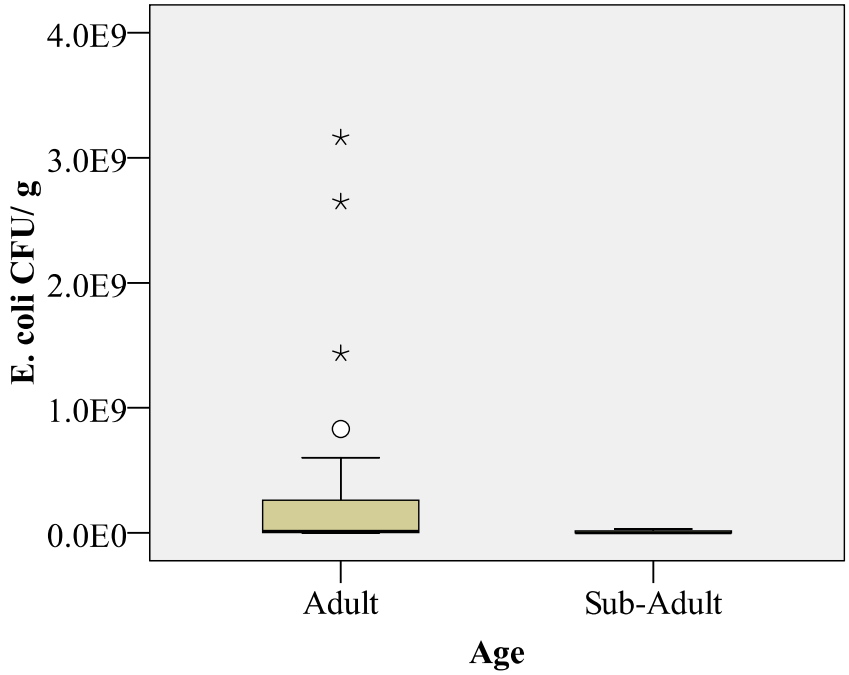

Figure 2.7. *E. coli* concentration in raccoons based on age.

Adults and female raccoons demonstrated higher median *E. coli* concentration than their male and sub-adult counterparts respectively (Figure 2.7 and 2.8), but the difference was not statistically significant ($p > 0.05$).

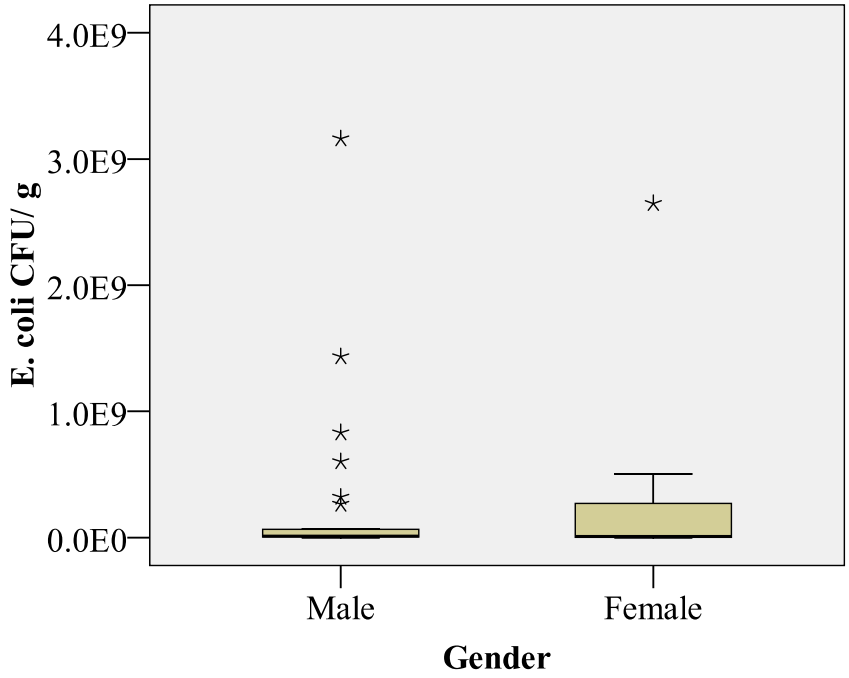

Figure 2.8. *E. coli* concentration in raccoons based on gender.

Data for only adult animals was available for armadillos. While a difference in the *E. coli* concentration from males and females can be observed in the box plots it was not statistically significant according to the Mann-Whitney test ($p > 0.05$) (Figure 2.9).

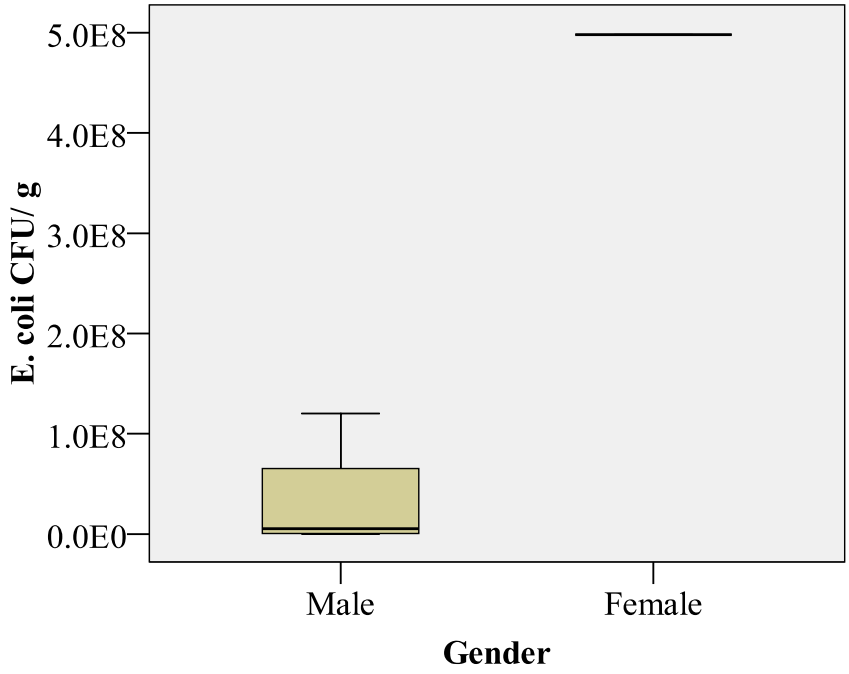

Figure 2.9. *E. coli* concentration in armadillos based on gender.

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) have observed that *E. coli* O157:H7 concentration was significantly higher in feces of calves compared to adult feces. The reason for this observation has been provided by Rasmussen et al. (1993) that adult cattle have a fully developed rumen where the combination of a high 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.

2.4.2 Survival, Growth, and Re-Growth 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.

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

The concentrations measured within and among different temperatures over a period of seven days showed highly variable *E. coli* counts (Figures 2.10 – 2.13). *E. coli* concentrations in the cattle and raccoon fecal samples at the beginning of the experiments were determined after one h. 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 is a slight decrease in *E. coli* growth after 24 h. The concentration increased after 72 h and then decreased until the end of the incubation period (Figure 2.10). *E. coli* from raccoon feces at 0°C (Figure 2.11) showed a decrease after 24 h, increased at 72 h, decreased at 120 h, and increased again after168 h.

Gradual increase in 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 2.10).

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 2.11).

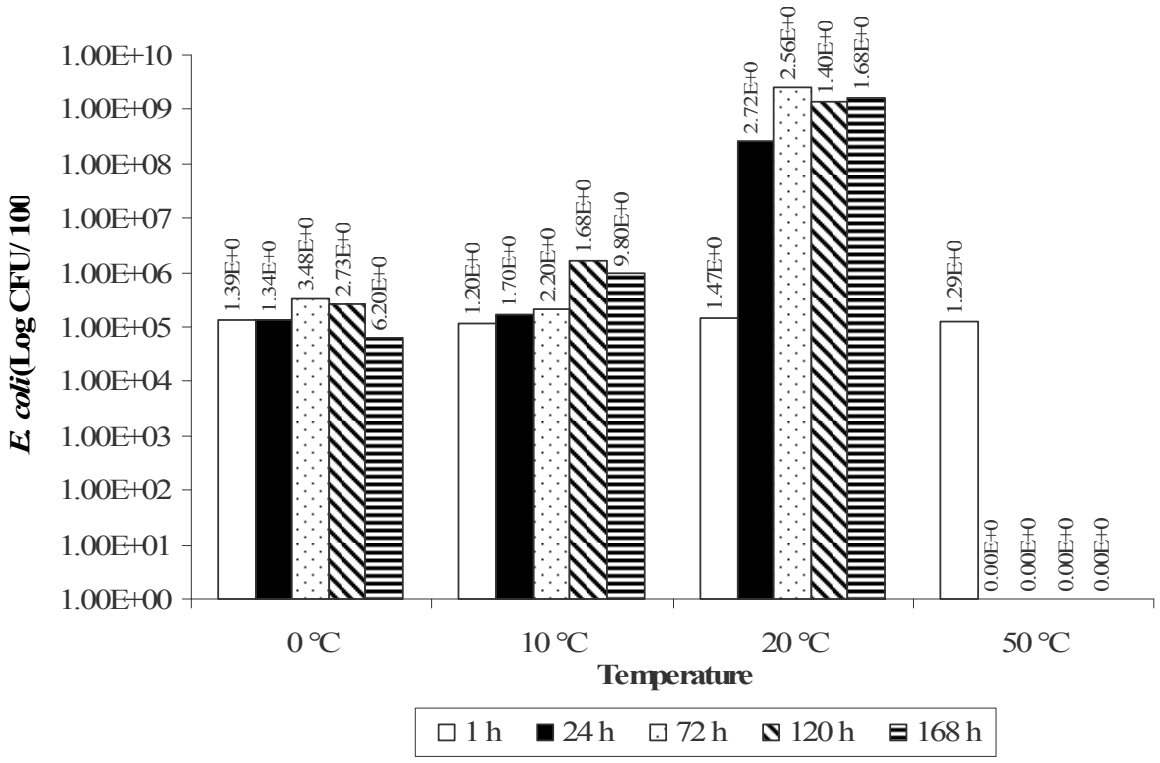

Figure 2.10. Survival of *E. coli* from cattle feces in water at different temperatures.

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 2.10). 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 2.10 and 2.11).

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.

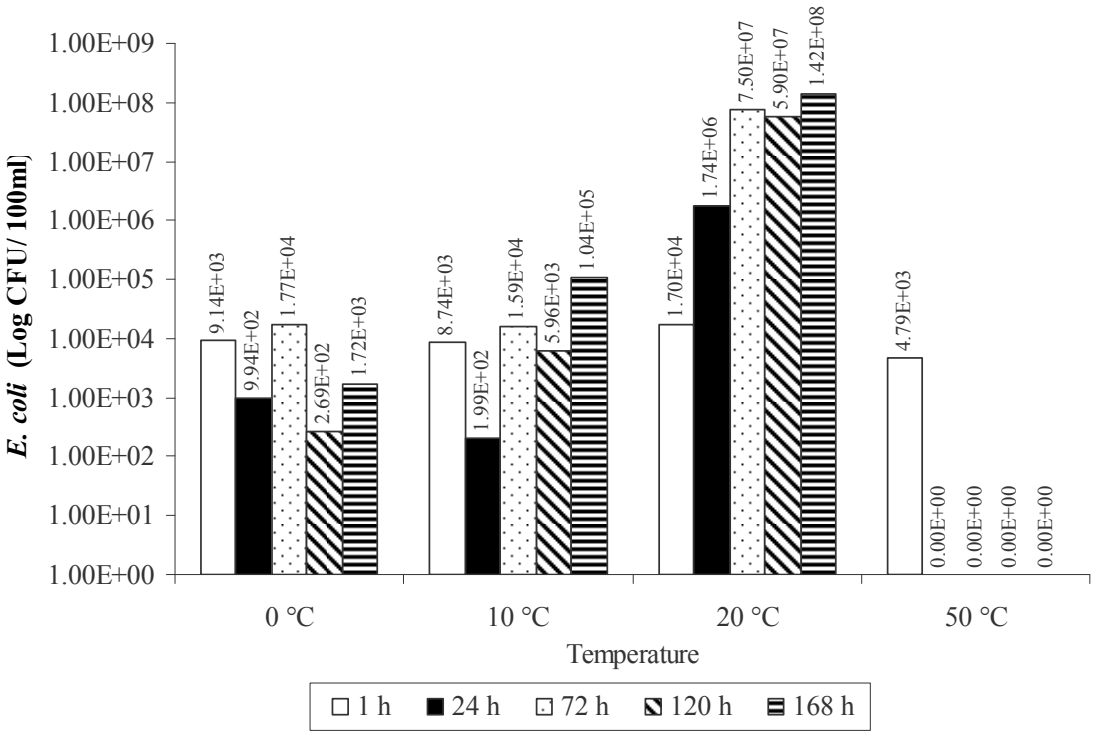

Figure 2.11. Survival of *E. coli* from raccoon in water feces at different temperatures.

There was no significant difference in cattle *E. coli* concentrations at 0° and 10°C ($p > 0.05$) for any given incubation temperature. However, at 20°C the *E. coli* concentrations are significantly different for different days ($p < 0.05$) (Figure 2.10). 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 2.11). Since no survival was observed at 50°C after 24 h, the results obtained for that temperature were excluded from statistical analysis.

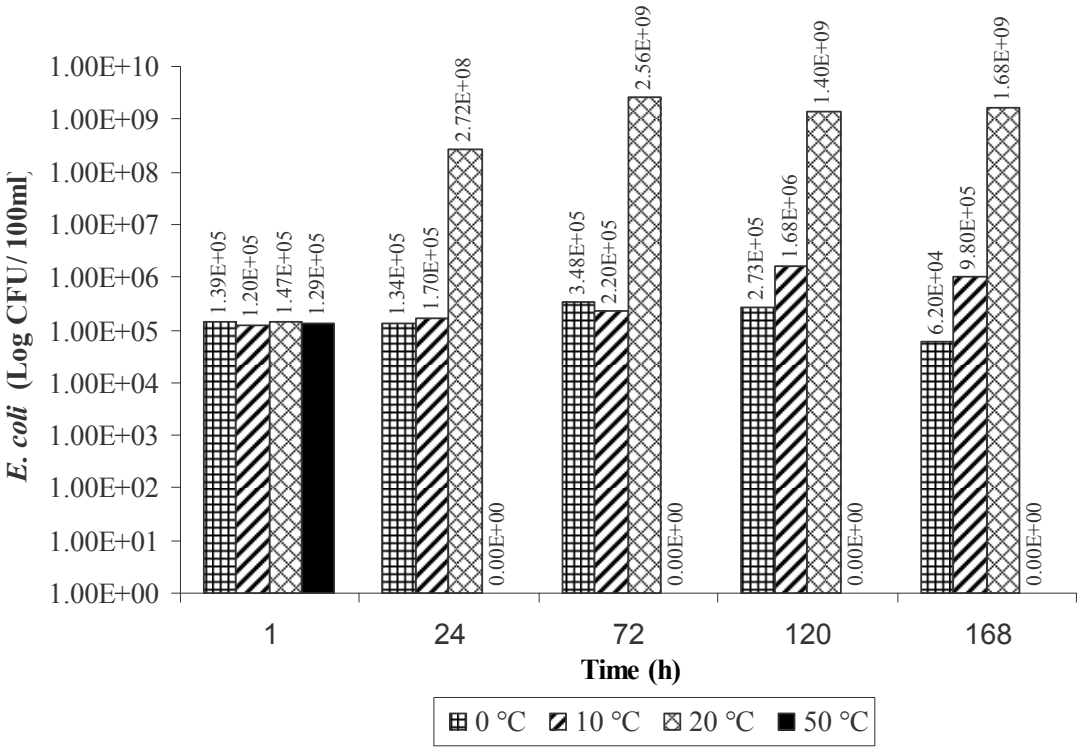

Figure 2.12. *E. coli* concentrations from cattle feces in water during seven day incubation period.

Kruskal-Wallis test for *E. coli* concentration obtained after one 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 2.11 and 2.13). This result just reinforced the laboratory analysis as *E. coli* concentrations after one 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. Figures 2.12 and 2.13 clearly show that *E. coli* concentration 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 one h.

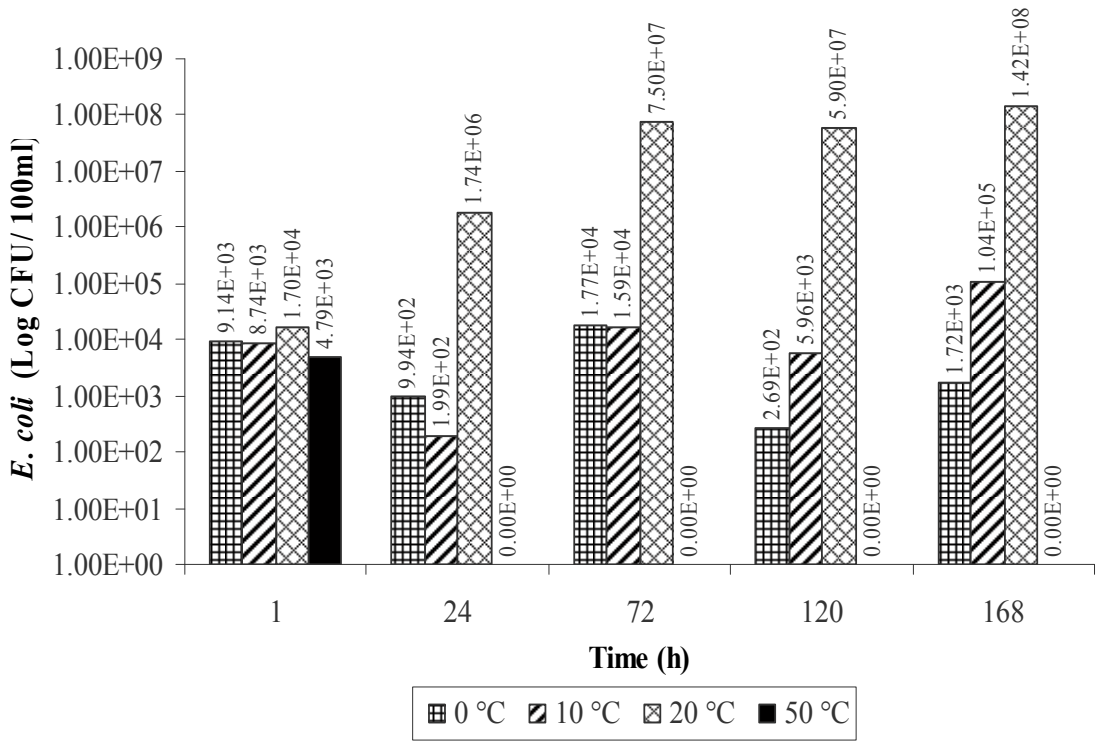

Figure 2.13. *E. coli* concentrations from raccoon feces in water during seven day incubation period.

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 2.10 and 2.11). 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). 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 do not show a statistically significant difference in the *E. coli* concentrations between different days. It could be possibly because of the fact that *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. *E. coli* concentration in raccoon feces at 10°C show consistency with findings of Filip et al. (1978) that *E. coli* can survive for a long time at 10°C. Considering the fact that *E. coli* is a mesophillic 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. This finding suggests that merely composting of manure would destroy the pathogenic organisms before land application because of typical composting temperatures of at least 55°C. This will reduce the possibility of transport of bacteria through surface runoff during rainfall events.

2.4.2.2 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 25% moisture content provided the most suitable conditions for their survival and growth.

Under dry conditions (1%), bacteria did not totally die-off but by 168 h the concentrations reduced considerably; by two orders of magnitude for cattle samples (Figure 2.14) and by one half for raccoon (Figure 2.15) 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 showed a drop on the fifth day (120 h) and growth on seventh day (168 h). At 83% moisture content, *E. coli* from cattle (Figure 2.14) reduced after a gradual growth until the fifth day whereas the *E. coli* concentrations in raccoon samples (Figure 2.15) continued to rise from one h to 168 h.

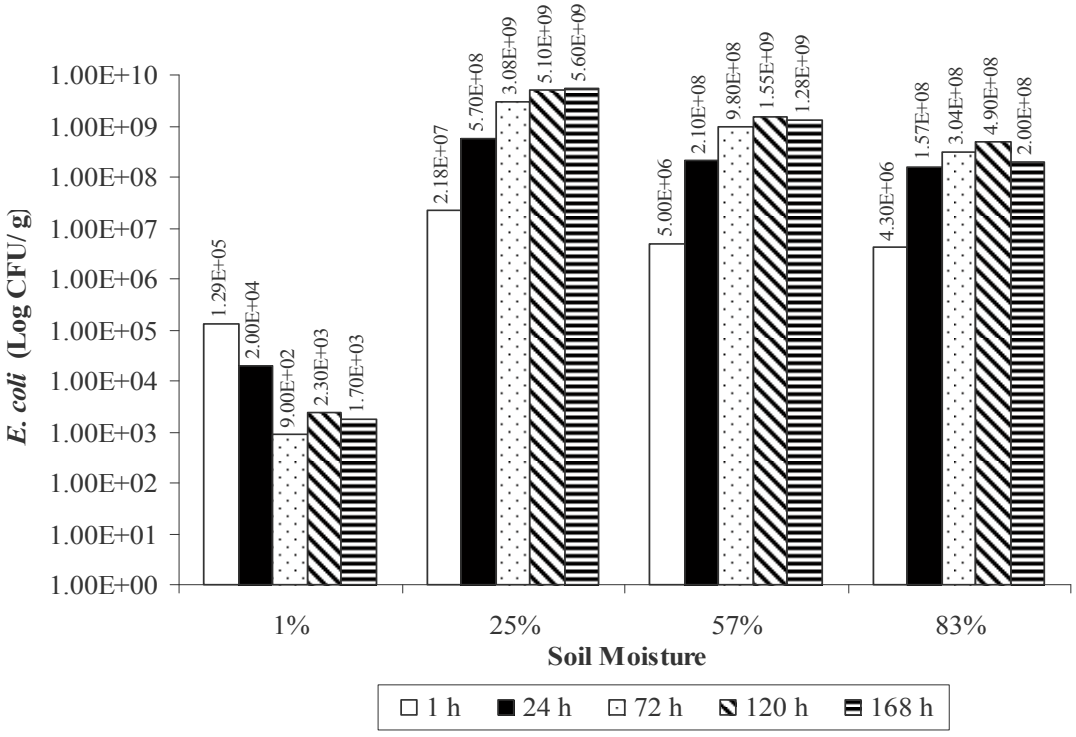

Figure 2.14. Survival of *E. coli* from cattle feces in soil at different moisture contents.

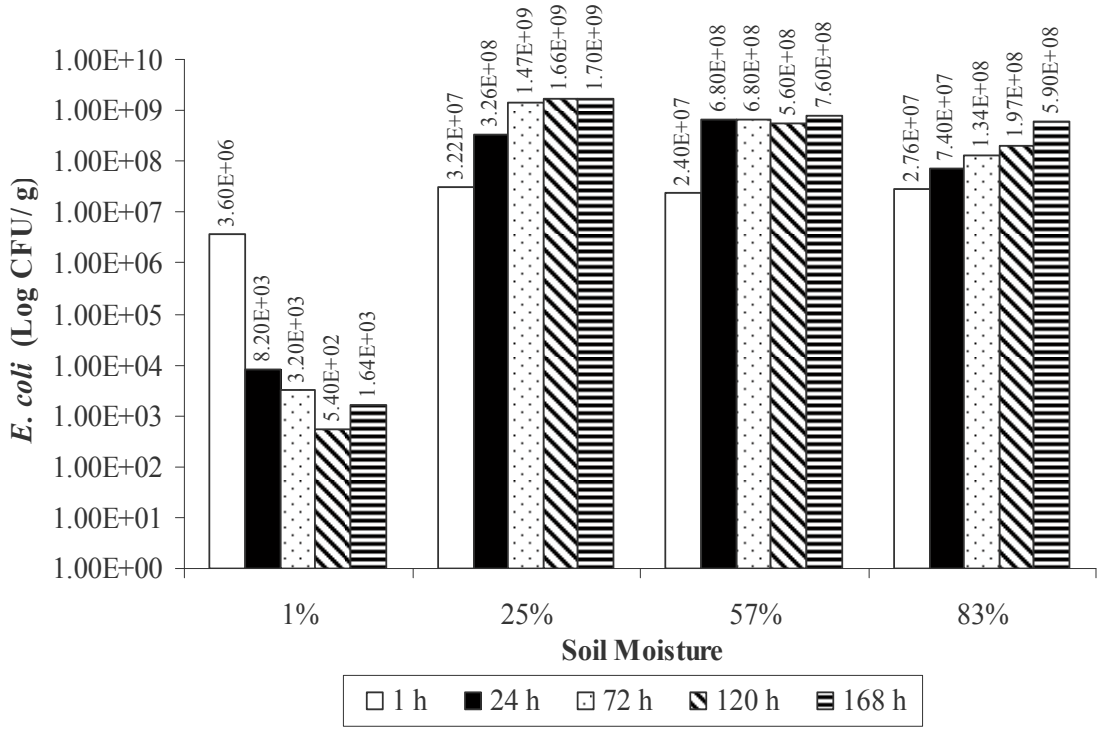

Figure 2.16. Survival of *E. coli* from raccoon feces in soil at different moisture contents.

Upon performing the Kruskal Wallis test on the *E. coli* numbers 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 ($p < 0.05$). One h 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. Figure 2.16 shows the *E. coli* concentration in cattle at all moisture conditions on a particular day. It can be observed that in cattle 25% moisture condition has the highest *E. coli* concentration on any given day followed by 56.5%, 83%, and 1% moisture contents.

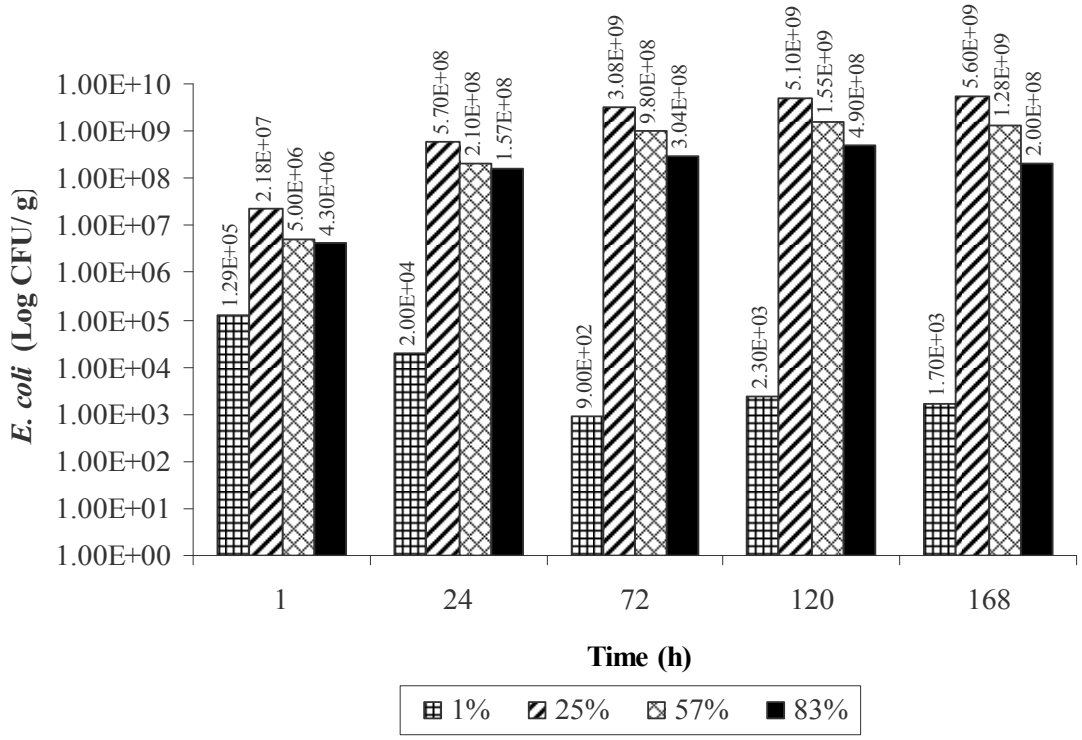

Figure 2.15. ***E. coli*** **concentrations from cattle feces in soil during seven day incubation period.**

E. coli concentration from raccoons samples (Figure 2.16) showed a similar trend as cattle at 1%, 25% and 83% moisture content ($p < 0.05$) but there is not enough evidence at 56.5% moisture condition to conclude that there is a significant difference in concentration between different days.

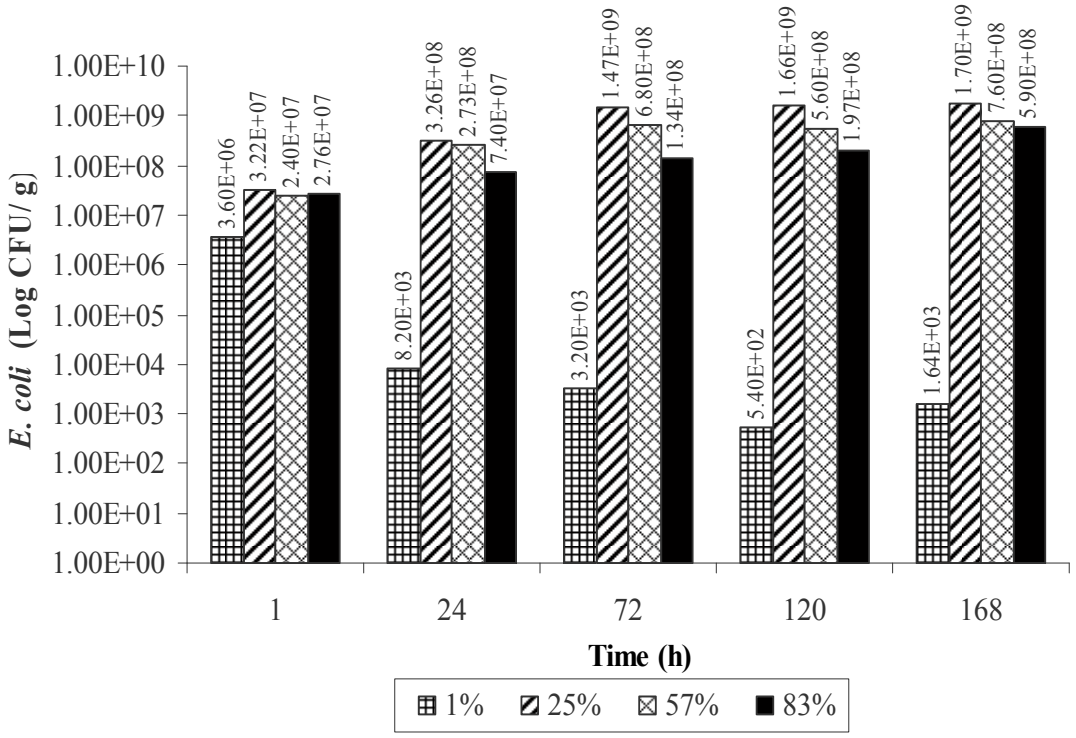

Figure 2.17. *E. coli* concentrations from raccoon feces in soil during seven day incubation period.

The test statistics for growth and survival of cattle and raccoons show that *E. coli* concentration on all days except one h ($p > 0.05$) were different for different moisture contents ($p < 0.05$). It was expected for one 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 h. The graphs in Figure 2.16 and 2.17 show the amount of difference between the moisture treatments on each day.

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). 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 1% moisture condition in soil. We found that the concentration of *E. coli* from both cattle and raccoons did not die-off within seven days but the numbers 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. This study found that the survival and growth of *E. coli* is at the peak 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 56.5% 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 this particular temperature and 25% moisture conditions. 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. Different results could have been obtained if the same moisture conditions were studied at some other temperature.

At 20°C temperature and 25% moisture content both the species seem to show trends similar to each other, even though there is difference between *E. coli* concentrations of the two species. The kinetic constants for cattle and raccoon *E. coli* concentrations at 20°C temperature (Table 2.2.) and 25% moisture content (Table 2.3.) are similar to each other. It can be observed from the graphs and kinetic constants that at 0°C temperature and at 1% moisture content there is decay i.e. decline of *E. coli* numbers over time. At all other temperatures (except 50°C) and moisture contents growth can be observed.

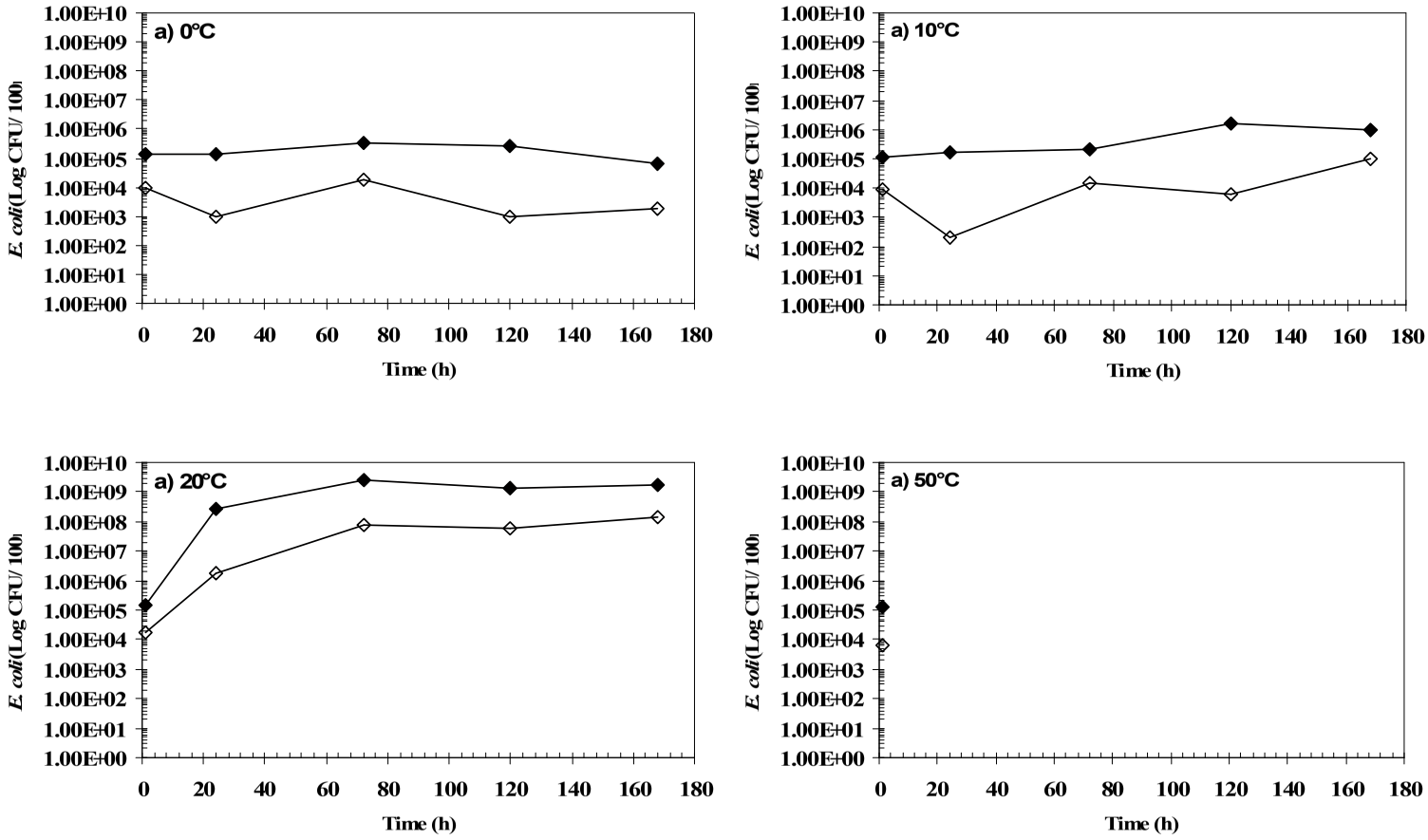

Figure 2.18. ***E. coli*** **survival in cattle (-♦-) and raccoon (-◊-) feces in water under different temperatures over the period of seven days.**

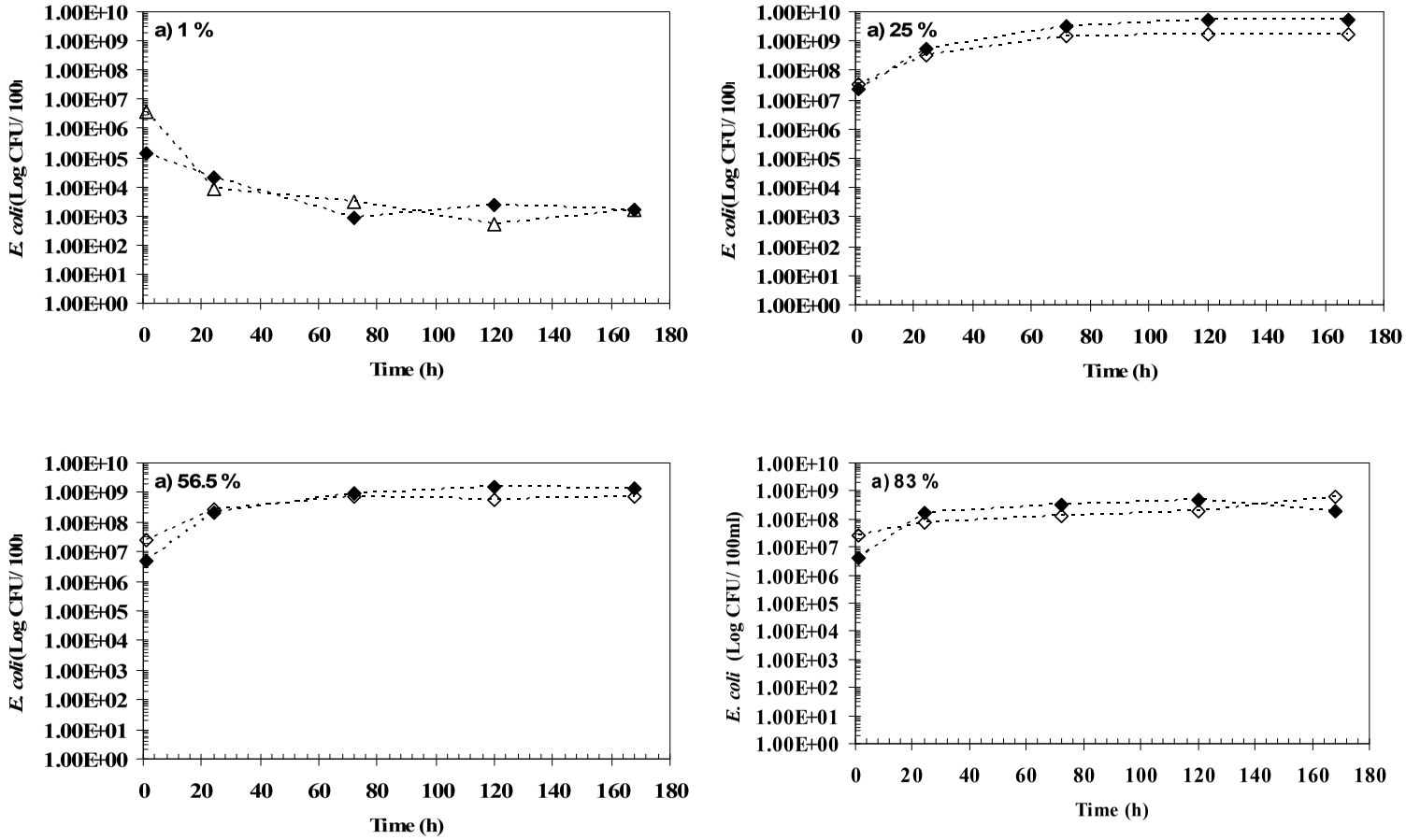

Figure 2.19. *E. coli* survival in cattle (-♦-) and raccoon (-◊-) feces in soil under different moisture conditions over the period of seven days.

Table 2.2. First order rate constant for *E. coli* concentration in cattle and raccoons at different temperatures

	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 2.3. First order rate constant for *E. coli* concentration in cattle and raccoons at different moisture conditions

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

2.5. 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. *E. coli* concentrations from feces of different animals were different possibly due to their feed types. Cattle showed variability in *E. coli* concentrations

between adult and calves, with calves having higher *E. coli* concentration in their feces than adults. No variability between males and females was observed for any species.

The growth and survival of *E. coli* subjected to different temperature conditions showed high variability in results over time. Freeze and thaw cycles of feces also affected the survivability of the bacteria. *E. coli* concentrations in cattle and raccoons feces showed 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. This suggests that composting of manure before land application may be a good option to ensure that it does not contain any potential bacterial contaminant. *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%). 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.

This finding 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.

CHAPTER III

SUMMARY AND FUTURE RECOMMENDATIONS

3.1. Summary

- Several non-point sources responsible for fecal contamination of Cedar Creek were identified. Out of the identified sources, four fecal sources were quantified for the *E. coli* concentrations.
- *E. coli* concentration for each species was compared between the genders. It was found that *E. coli* concentration do not significantly differ between genders.
- *E. coli* concentration for each species was compared between ages, broadly categorized into adult and sub-adult. Only cattle showed a significant difference with *E. coli* concentration of sub-adults (calves) higher than adults.
- *E. coli* concentrations obtained from different species can be incorporated into watershed modeling tools like SELECT, SWAT, and HSPF which so use literature values. Accurate source characterization and identification will help such tools to predict the *E. coli* loads better in a stream.
- Watershed modeling tools generally lack in their capacity to incorporate the bacteria life cycle and their behavior under different environmental conditions. The growth trends observed under different environmental conditions in this study would help in better prediction of *E. coli* loads in a waterbody during different times of a year, thus involving seasonal 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.

3.2. Future Recommendations

Other important physical chemical properties such as pH affecting the survival of microorganisms which can be taken into account for future studies related to the study of 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 can be considered to study the effect of environment on survival and growth of bacteria.

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APPENDIX A

Table A.1 – Data for *E. coli* concentrations from four species

Date of Trapping	Species	Age	Sex	Wt. (g)	Moisture Content			Plate Count	Dry Weight	*E. coli*/ g of fecal material	
					Wet Wt. (g)	Dry Wt. (g)	%			Wet Basis	Dry Basis
7/16/08	Armadillo	Adult	M	1.0178	2.6622	2.0214	31.701	3.00E+05	0.695	2.95E+05	4.32E+05
7/17/08	Armadillo	Adult	M	1.0814	2.5366	1.9664	28.997	7.00E+05	0.768	6.47E+05	9.12E+05
8/30/08	Armadillo	Adult	M	1.0316	2.4641	2.04	20.789	1.24E+08	0.817	1.20E+08	1.52E+08
9/1/08	Armadillo	Adult	F	1.0236	2.4297	1.9125	27.043	5.10E+08	0.747	4.98E+08	6.83E+08
9/4/08	Armadillo	Adult	M	1.005	2.4883	2.3246	7.0421	1.02E+07	0.934	1.01E+07	1.09E+07
7/21/08	Cow	Adult	F	1.0599	2.5811	1.4587	76.945	5.00E+02	0.244	4.72E+02	2.05E+03
7/21/08	Cow	Adult	M	1.0379	2.6456	1.4804	78.708	5.90E+03	0.221	5.68E+03	2.67E+04
7/25/08	Cow	Adult	F	1.0662	2.394	1.4328	67.085	8.60E+03	0.351	8.07E+03	2.45E+04
7/25/08	Cow	Adult	F	1.0913	2.5358	1.4518	74.665	1.56E+05	0.276	1.43E+05	5.64E+05
7/26/08	Cow	Adult	F	1.0528	2.5831	1.4857	73.864	2.89E+05	0.275	2.75E+05	1.05E+06
7/31/08	Cow	Adult	F	1.0612	2.525	1.4693	71.850	1.00E+02	0.299	9.42E+01	3.35E+02
9/3/08	Cow	Sub-Adult	M	1.0683	2.5319	1.4882	70.131	2.93E+05	0.319	2.74E+05	9.18E+05
9/4/08	Cow	Adult	F	1.0925	2.6953	1.4352	87.799	2.43E+04	0.133	2.22E+04	1.82E+05
9/4/08	Cow	Adult	F	1.0703	2.6413	1.3538	95.102	2.54E+04	0.052	2.37E+04	4.85E+05
9/4/08	Cow	Adult	F	1.0887	2.6003	1.4099	84.431	1.19E+04	0.169	1.09E+04	7.02E+04
9/4/08	Cow	Adult	F	1.0819	2.6052	1.4023	85.780	4.70E+05	0.154	4.34E+05	3.06E+06
9/5/08	Cow	Adult	F	1.063	2.6736	1.4148	88.973	2.04E+06	0.117	1.92E+06	1.74E+07
9/7/08	Cow	Adult	F	1.0167	2.7148	1.3809	96.596	8.70E+03	0.035	8.56E+03	2.51E+05

Date of Trapping	Species	Age	Sex	Wt. (g)	Moisture Content			Plate Count	Dry Weight	*E. coli*/ g of fecal material	
					Wet Wt. (g)	Dry Wt. (g)	%			Wet Basis	Dry Basis
9/8/08	Cow	Adult	Unknow	1.0772	2.6071	1.3872	87.939	3.50E+03	0.130	3.25E+03	2.69E+04
9/10/08	Cow	Sub-Adult	M	1.0363	2.7038	1.4488	86.623	2.23E+04	0.139	2.15E+04	1.61E+05
10/1/08	Cow	Adult	F	1.0683	2.479	1.4428	71.818	3.00E+02	0.301	2.81E+02	9.96E+02
10/1/08	Cow	Adult	F	1.0494	2.7129	1.4305	89.646	1.00E+02	0.109	9.53E+01	9.20E+02
6/15/09	Cow	Sub-Adult	M	1.013	5.026	2.147	134.094	9.50E+05	0.345	9.38E+05	9.38E+05
6/16/09	Cow	Sub-Adult	F	1.0027	5.008	2.161	131.744	5.00E+05	0.318	4.99E+05	1.57E+06
6/17/09	Cow	Sub-Adult	M	1.001	5.006	1.152	334.548	1.75E+06	2.348	1.75E+06	7.45E+05
6/30/08	Opossum	Adult	F	1.0431	2.3334	1.7824	30.913	8.10E+06	0.721	7.77E+06	1.12E+07
6/30/08	Opossum	Adult	M	1.0195	2.3579	1.7411	35.426	1.40E+07	0.658	1.37E+07	2.13E+07
7/2/08	Opossum	Adult	F	1.0519	2.4081	1.7623	36.645	1.70E+06	0.666	1.62E+06	2.55E+06
7/2/08	Opossum	Sub-adult	M	1.0802	2.4198	1.6531	46.38	9.00E+05	0.579	8.33E+05	1.55E+06
7/2/08	Opossum	Adult	F	1.1002	2.4083	1.6594	45.131	2.00E+04	0.604	1.82E+04	3.31E+04
7/2/08	Opossum	Sub-adult	F	1.0864	2.4545	1.7052	43.942	2.61E+07	0.609	2.40E+07	4.29E+07
7/3/08	Opossum	Adult	M	1.0467	2.373	1.6842	40.898	2.11E+07	0.619	2.02E+07	3.41E+07
7/3/08	Opossum	Adult	M	1.0312	2.5706	1.7312	48.487	3.10E+06	0.531	3.01E+06	5.84E+06
7/3/08	Opossum	Sub-adult	M	1.0551	2.3526	1.7766	32.421	2.30E+05	0.713	2.18E+05	3.23E+05
7/3/08	Opossum	Adult	F	1.0406	2.5036	1.5948	56.985	1.66E+07	0.448	1.60E+07	3.71E+07
7/4/08	Opossum	Adult	F	1.0336	2.3427	1.6995	37.846	2.81E+08	0.642	2.72E+08	4.37E+08
7/5/08	Opossum	Adult	M	1.0328	2.3447	1.7203	36.296	2.88E+08	0.658	2.79E+08	4.38E+08
7/5/08	Opossum	Sub-adult	M	1.0629	2.5116	1.6503	52.191	2.02E+08	0.508	1.90E+08	3.98E+08
7/5/08	Opossum	Sub-adult	F	1.0461	2.3923	1.7518	36.562	1.33E+08	0.664	1.27E+08	2.00E+08
7/7/08	Opossum	Adult	F	1.0698	2.4721	1.7422	41.895	2.58E+08	0.622	2.41E+08	4.15E+08

Date of Trapping	Species	Age	Sex	Wt. (g)	Moisture Content			Plate Count	Dry Weight	*E. coli*/ g of fecal material	
					Wet Wt. (g)	Dry Wt. (g)	%			Wet Basis	Dry Basis
7/7/08	Opossum	Adult	M	1.0199	2.3691	1.7247	37.363	1.31E+08	0.639	1.28E+08	2.05E+08
7/7/08	Opossum	Sub-adult	F	1.0316	2.4337	1.8676	30.312	1.96E+08	0.719	1.90E+08	2.73E+08
7/8/08	Opossum	Adult	F	1.0469	2.4358	1.7238	41.304	2.30E+07	0.614	2.20E+07	3.74E+07
7/8/08	Opossum	Adult	F	1.0513	2.4783	1.6936	46.333	6.00E+06	0.564	5.71E+06	1.06E+07
7/15/08	Opossum	Adult	M	1.1232	2.4017	1.7565	36.732	1.00E+06	0.711	8.90E+05	1.41E+06
7/15/08	Opossum	Adult	F	1.0457	2.5318	1.7741	42.709	5.70E+07	0.599	5.45E+07	9.51E+07
7/15/08	Opossum	Adult	M	1.0548	2.5651	1.7838	43.8	1.53E+07	0.593	1.45E+07	2.58E+07
7/16/08	Opossum	Adult	M	1.0866	2.469	1.8395	34.221	4.80E+07	0.715	4.42E+07	6.72E+07
7/16/08	Opossum	Adult	F	1.0513	2.2407	1.9629	14.153	2.92E+09	0.903	2.78E+09	3.24E+09
7/16/08	Opossum	Adult	M	1.0283	2.5126	1.8775	33.827	6.80E+07	0.680	6.61E+07	9.99E+07
7/17/08	Opossum	Adult	M	1.0588	2.3595	1.6669	41.55	1.18E+09	0.619	1.11E+09	1.91E+09
7/17/08	Opossum	Adult	M	1.0523	2.5988	1.7315	50.09	8.10E+06	0.525	7.70E+06	1.54E+07
7/18/08	Opossum	Adult	M	1.0506	2.3805	1.8191	30.861	3.00E+05	0.726	2.86E+05	4.13E+05
7/19/08	Opossum	Adult	M	1.0074	2.3482	1.8077	29.9	1.22E+08	0.706	1.21E+08	1.73E+08
7/19/08	Opossum	Sub-adult	M	1.07	2.4408	1.6332	49.449	8.60E+08	0.541	8.04E+08	1.59E+09
7/20/08	Opossum	Adult	F	1.07	2.3758	1.8481	28.554	7.80E+07	0.764	7.29E+07	1.02E+08
7/20/08	Opossum	Sub-adult	M	1.02	2.3828	1.7734	34.363	1.92E+09	0.669	1.88E+09	2.87E+09
7/20/08	Opossum	Adult	M	1.05	2.5091	1.8718	34.047	7.90E+06	0.693	7.52E+06	1.14E+07
7/21/08	Opossum	Adult	F	1.02	2.4392	1.7109	42.568	1.18E+08	0.586	1.16E+08	2.01E+08
7/21/08	Opossum	Adult	M	1.0927	2.5245	1.9436	29.888	1.13E+09	0.766	1.03E+09	1.47E+09
7/21/08	Opossum	Adult	F	1.0578	2.4998	1.7782	40.58	6.10E+06	0.629	5.77E+06	9.71E+06
7/22/08	Opossum	Adult	M	1.0953	2.4938	1.8437	35.261	1.00E+05	0.709	9.13E+04	1.41E+05

Date of Trapping	Species	Age	Sex	Wt. (g)	Moisture Content			Plate Count	Dry Weight	*E. coli*/ g of fecal material	
					Wet Wt. (g)	Dry Wt. (g)	%			Wet Basis	Dry Basis
7/22/08	Opossum	Adult	M	1.0476	2.5798	1.7233	49.701	1.40E+06	0.527	1.34E+06	2.66E+06
7/23/08	Opossum	Adult	M	1.0593	2.5189	1.8891	33.339	8.30E+07	0.706	7.84E+07	1.18E+08
7/23/08	Opossum	Adult	F	1.0894	2.496	1.9886	25.515	7.90E+07	0.811	7.25E+07	9.74E+07
7/25/08	Opossum	Adult	M	1.0194	2.4994	1.7418	43.495	3.20E+06	0.576	3.14E+06	5.56E+06
7/25/08	Opossum	Adult	M	1.0549	2.5552	1.6781	52.267	1.00E+05	0.504	9.48E+04	1.99E+05
7/25/08	Opossum	Adult	F	1.0467	2.5915	1.7094	51.603	1.44E+07	0.507	1.38E+07	2.84E+07
7/25/08	Opossum	Adult	M	1.0575	2.4805	1.7038	45.586	4.00E+05	0.575	3.78E+05	6.95E+05
7/26/08	Opossum	Adult	M	1.0455	2.489	1.7476	42.424	2.50E+06	0.602	2.39E+06	4.15E+06
7/26/08	Opossum	Adult	M	1.0561	2.5407	1.7497	45.208	1.81E+09	0.579	1.71E+09	3.13E+09
7/26/08	Opossum	Adult	F	1.1219	2.6258	1.6288	61.211	3.00E+06	0.435	2.67E+06	6.89E+06
7/27/08	Opossum	Adult	M	1.0956	2.5784	1.7105	50.74	2.30E+06	0.540	2.10E+06	4.26E+06
7/27/08	Opossum	Adult	M	1.0319	2.5814	1.8709	37.976	1.41E+08	0.640	1.37E+08	2.20E+08
7/27/08	Opossum	Sub-adult	M	1.0263	2.5261	1.7536	44.052	1.62E+06	0.574	1.58E+06	2.82E+06
8/31/08	Opossum	Adult	M	1.0382	2.5309	1.6291	55.356	4.20E+08	0.463	4.05E+08	9.06E+08
8/31/08	Opossum	Adult	X	1.0739	2.6599	1.5378	72.968	1.46E+07	0.290	1.36E+07	5.03E+07
9/2/08	Opossum	Adult	F	1.027	2.5861	1.8207	42.039	1.51E+07	0.595	1.47E+07	2.54E+07
9/2/08	Opossum	Sub-Adult	F	1.0211	2.5184	1.4916	68.839	8.90E+08	0.318	8.72E+08	2.80E+09
9/2/08	Opossum	Adult	F	1.0314	2.5186	1.4831	69.82	1.00E+06	0.311	9.70E+05	3.21E+06
9/2/08	Opossum	Adult	F	1.0199	2.5354	1.7462	45.195	8.60E+07	0.559	8.43E+07	1.54E+08
9/6/08	Opossum	Adult	X	1.0783	2.6199	1.6115	62.575	2.00E+05	0.404	1.85E+05	4.96E+05
6/30/08	Racoon	Adult	M	1.0236	2.4468	1.9732	24.002	1.63E+07	0.778	1.59E+07	2.10E+07
6/30/08	Racoon	Adult	F	1.0183	2.371	1.8135	30.742	4.90E+05	0.705	4.81E+05	6.95E+05

Date of Trapping	Species	Age	Sex	Wt. (g)	Moisture Content			Plate Count	Dry Weight	*E. coli*/ g of fecal material	
					Wet Wt. (g)	Dry Wt. (g)	%			Wet Basis	Dry Basis
6/30/08	Racoon	Adult	M	1.1389	2.4079	1.9227	25.235	5.50E+05	0.851	4.83E+05	6.46E+05
7/3/08	Raccoon	Adult	F	1.0256	2.4563	1.8119	35.565	6.30E+06	0.661	6.14E+06	9.53E+06
7/3/08	Raccoon	Adult	M	1.0214	2.3561	1.741	35.33	3.30E+06	0.661	3.23E+06	5.00E+06
7/5/08	Raccoon	Adult	M	1.0328	2.4261	1.9176	26.518	2.74E+08	0.759	2.65E+08	3.61E+08
7/6/08	Raccoon	Adult	M	1.0227	2.5993	2.0243	28.405	3.28E+08	0.732	3.21E+08	4.48E+08
7/7/08	Raccoon	Adult	M	1.0504	2.5367	1.7358	46.14	5.20E+07	0.566	4.95E+07	9.19E+07
7/7/08	Raccoon	Sub-adult	M	1.0708	2.5029	2.1968	13.934	8.00E+05	0.922	7.47E+05	8.68E+05
7/8/08	Raccoon	Adult	M	1.0269	2.5198	1.7748	41.977	6.50E+07	0.596	6.33E+07	1.09E+08
7/8/08	Raccoon	Adult	X	1.0356	2.3786	1.6871	40.987	1.40E+07	0.611	1.35E+07	2.29E+07
7/8/08	Raccoon	Sub-adult	M	1.285	2.4782	1.8677	32.687	3.90E+07	0.865	3.04E+07	4.51E+07
7/15/08	Raccoon	Adult	M	1.0588	2.4624	1.8023	36.625	8.80E+08	0.671	8.31E+08	1.31E+09
7/16/08	Raccoon	Adult	M	1.0445	2.5624	1.6391	56.33	4.70E+06	0.456	4.50E+06	1.03E+07
7/16/08	Raccoon	Adult	M	1.0231	2.5561	1.9373	31.941	5.00E+05	0.696	4.89E+05	7.18E+05
7/16/08	Raccoon	Adult	M	1.0181	2.4742	2.1473	15.224	1.46E+09	0.863	1.43E+09	1.69E+09
7/18/08	Raccoon	Adult	M	1.0215	2.3555	1.6502	42.74	1.03E+07	0.585	1.01E+07	1.76E+07
7/19/08	Raccoon	Adult	F	1.05	2.3881	2.1138	12.977	2.67E+08	0.914	2.54E+08	2.92E+08
7/19/08	Raccoon	Adult	F	1.05	2.4289	2.0647	17.639	2.78E+09	0.865	2.65E+09	3.21E+09
7/20/08	Raccoon	Adult	M	1.03	2.4751	1.8953	30.591	6.20E+08	0.715	6.02E+08	8.67E+08
7/20/08	Raccoon	Adult	F	1.03	2.4019	1.7211	39.556	5.20E+08	0.623	5.05E+08	8.35E+08
7/21/08	Raccoon	Adult	M	1.0805	2.5705	1.8221	41.073	3.80E+06	0.637	3.52E+06	5.97E+06
7/21/08	Raccoon	Adult	M	1.0916	2.4257	1.7883	35.643	1.96E+07	0.703	1.80E+07	2.79E+07
7/21/08	Raccoon	Adult	M	1.0603	2.3567	1.6713	41.01	4.50E+07	0.625	4.24E+07	7.19E+07

Date of Trapping	Species	Age	Sex	Wt. (g)	Moisture Content			Plate Count	Dry Weight	*E. coli*/ g of fecal material	
					Wet Wt. (g)	Dry Wt. (g)	%			Wet Basis	Dry Basis
7/21/08	Raccoon	Adult	F	1.0953	2.572	1.7617	45.995	5.30E+06	0.592	4.84E+06	8.96E+06
7/21/08	Raccoon	Adult	M	1.0013	2.6805	2.2343	19.97	3.40E+06	0.801	3.40E+06	4.24E+06
7/21/08	Raccoon	Sub-adult	M	1.0636	2.6619	1.9203	38.619	2.00E+05	0.653	1.88E+05	3.06E+05
7/21/08	Raccoon	Adult	F	1.0697	2.5766	2.211	16.536	1.40E+06	0.893	1.31E+06	1.57E+06
7/22/08	Raccoon	Adult	M	1.0927	2.4236	1.6508	46.814	6.20E+07	0.581	5.67E+07	1.07E+08
7/22/08	Raccoon	Adult	F	1.0802	2.4148	1.9115	26.33	3.20E+07	0.796	2.96E+07	4.02E+07
7/23/08	Raccoon	Adult	M	1.0657	2.543	1.6634	52.88	7.70E+06	0.502	7.23E+06	1.53E+07
7/23/08	Raccoon	Adult	F	1.0557	2.467	1.6173	52.538	9.60E+06	0.501	9.09E+06	1.92E+07
7/23/08	Raccoon	Adult	M	1.0432	2.4406	1.7725	37.693	2.70E+06	0.650	2.59E+06	4.15E+06
7/23/08	Raccoon	Adult	F	1.0421	2.5395	1.8697	35.824	1.00E+06	0.669	9.60E+05	1.50E+06
7/25/08	Raccoon	Adult	M	1.0289	2.4358	1.8184	33.953	5.20E+06	0.680	5.05E+06	7.65E+06
7/25/08	Raccoon	Adult	M	1.0136	2.4128	1.8259	32.143	6.60E+06	0.688	6.51E+06	9.60E+06
7/25/08	Raccoon	Adult	M	1.0388	2.4607	1.8147	35.598	5.00E+05	0.669	4.81E+05	7.47E+05
7/26/08	Raccoon	Adult	M	1.0567	2.5299	1.7805	42.089	3.34E+09	0.612	3.16E+09	5.46E+09
7/26/08	Raccoon	Adult	F	1.0795	2.5163	1.8585	35.394	3.10E+08	0.697	2.87E+08	4.44E+08
8/30/08	Raccoon	Adult	X	1.0438	2.5475	1.7659	44.261	6.10E+08	0.582	5.84E+08	1.05E+09
8/30/08	Raccoon	Adult	X	1.0474	2.5546	1.7258	48.024	4.10E+08	0.544	3.91E+08	7.53E+08
8/31/08	Raccoon	Adult		1.011	2.5098	1.6967	47.922	4.80E+07	0.527	4.75E+07	9.12E+07
9/2/08	Raccoon	Adult	M	1.0534	2.6713	1.6055	66.384	7.20E+07	0.354	6.84E+07	2.03E+08

APPENDIX B

Table B.1 – Data for three cattle samples subjected to four different temperature conditions

C1					C2				C3				
	Date			0°C		Date			0°C		Date			0°C
1 h	7/17/09			2.31E+06	1 h	7/24/09			8.00E+04	1 h	7/26/09			1.39E+05
24 h	7/18/09	1.50E+06	4.00E+05	1.40E+06	24 h	7/25/09	3.00E+04	4.00E+04	1.00E+04	24 h	7/27/09	1.53E+05	1.33E+05	1.34E+05
72 h	7/20/09	7.00E+05	7.00E+05	4.00E+05	72 h	7/27/09	3.62E+05	3.44E+05	3.48E+05	72 h	7/29/09	1.32E+05	1.06E+05	1.08E+05
120 h	7/22/09	3.70E+05	4.00E+05	3.90E+05	120 h	7/29/09	4.40E+04	4.00E+04	4.10E+04	120 h	7/31/09	2.73E+05	2.81E+05	2.44E+05
168 h	7/24/09	6.00E+05	5.30E+05	4.40E+05	168 h	7/31/09	6.10E+04	6.40E+04	6.20E+04	168 h	8/2/09	1.00E+04	2.10E+04	1.80E+04
	Date			10°C		Date			10°C		Date			10°C
1 h	7/17/09			3.44E+06	1 h	7/24/09			8.00E+04	1 h	7/26/09			1.20E+05
24 h	7/18/09	1.39E+07	1.15E+07	1.38E+07	24 h	7/25/09	1.10E+05	1.70E+05	7.00E+04	24 h	7/27/09	1.00E+05	2.00E+05	1.00E+05
72 h	7/20/09	5.90E+07	5.00E+07	5.40E+07	72 h	7/27/09	3.20E+05	1.80E+05	2.00E+05	72 h	7/29/09	2.20E+05	1.60E+05	1.90E+05
120 h	7/22/09	4.10E+07	3.20E+07	2.40E+07	120 h	7/29/09	2.00E+05	2.30E+05	2.10E+05	120 h	7/31/09	1.62E+06	1.68E+06	2.06E+06
168 h	7/24/09	1.75E+08	2.71E+08	2.03E+08	168 h	7/31/09	2.34E+05	1.89E+05	1.52E+05	168 h	8/2/09	8.60E+05	9.80E+05	1.47E+06
	Date			20°C		Date			20°C		Date			20°C
1 h	7/17/09			5.11E+06	1 h	7/24/09			9.00E+04	1 h	7/26/09			1.47E+05
24 h	7/18/09	4.90E+07	2.40E+07	7.70E+08	24 h	7/25/09	7.80E+07	5.60E+07	3.20E+08	24 h	7/27/09	2.72E+08	3.88E+08	4.28E+09
72 h	7/20/09	1.05E+10	1.65E+10	8.30E+09	72 h	7/27/09	3.50E+08	1.80E+08	2.40E+08	72 h	7/29/09	2.62E+09	2.56E+09	2.02E+09
120 h	7/22/09	5.00E+08	1.00E+08	2.00E+08	120 h	7/29/09	2.29E+09	2.18E+09	2.65E+09	120 h	7/31/09	1.40E+09	3.10E+09	1.20E+09
168 h	7/24/09	1.83E+09	2.57E+09	2.17E+09	168 h	7/31/09	5.00E+08	6.10E+09	5.00E+08	168 h	8/2/09	1.68E+09	8.70E+08	6.20E+08
	Date			50°C		Date			50°C		Date			50°C
1 h	7/17/09			2.73E+08	1 h	7/24/09			2.00E+04	1 h	7/26/09			1.29E+05
24 h	7/18/09	0.00E+00	0.00E+00	0.00E+00	24 h	7/25/09	0.00E+00	0.00E+00	0.00E+00	24 h	7/27/09	0.00E+00	0.00E+00	0.00E+00
72 h	7/20/09	0.00E+00	0.00E+00	0.00E+00	72 h	7/27/09	0.00E+00	0.00E+00	0.00E+00	72 h	7/29/09	0.00E+00	0.00E+00	0.00E+00

120 h	7/22/09	0.00E+00	0.00E+00	0.00E+00	120 h	7/29/09	0.00E+00	0.00E+00	0.00E+00	120 h	7/31/09	0.00E+00	0.00E+00	0.00E+00
168 h	7/24/09	0.00E+00	0.00E+00	0.00E+00	168 h	7/31/09	0.00E+00	0.00E+00	0.00E+00	168 h	8/2/09	0.00E+00	0.00E+00	0.00E+00

Table B.2 – Data for three Raccoon samples subjected to four different temperature conditions

R1					R2				R3				
	Date			0°C		Date			0°C		Date			0°C
1 h	7/26/09		9.20E+06		1 h	8/2/09		1.24E+03		1 h	8/2/09		9.14E+03	
24 h	7/27/09	1.00E+06	3.00E+05	2.40E+06	24 h	8/3/09	1.34E+02	4.00E+01	3.23E+02	24 h	8/3/09	9.90E+02	2.98E+02	2.38E+03
72 h	7/29/09	1.69E+07	1.78E+07	1.87E+07	72 h	8/5/09	2.27E+03	2.39E+03	2.52E+03	72 h	8/5/09	1.68E+04	1.77E+04	1.86E+04
120 h	7/31/09	2.00E+06	1.00E+06	0.00E+00	120 h	8/7/09	2.69E+02	1.34E+02	1.12E+02	120 h	8/7/09	1.99E+03	1.05E+03	9.10E+02
168 h	8/2/09	1.28E+07	1.00E+05	3.00E+05	168 h	8/9/09	1.72E+03	1.30E+01	4.00E+01	168 h	8/9/09	1.27E+04	9.94E+01	2.98E+02
				10°C		Date			10°C		Date			10°C
1 h	7/26/09		8.80E+06		1 h	8/2/09		1.18E+03		1 h	8/2/09		8.70E+03	
24 h	7/27/09	1.00E+05	2.00E+05	2.00E+05	24 h	8/3/09	1.40E+01	2.70E+01	2.80E+01	24 h	8/3/09	9.80E+01	1.99E+02	2.04E+02
72 h	7/29/09	1.72E+07	1.60E+07	1.48E+07	72 h	8/5/09	2.31E+03	2.15E+03	1.99E+03	72 h	8/5/09	1.71E+04	1.59E+04	1.47E+04
120 h	7/31/09	6.00E+06	6.00E+06	1.30E+07	120 h	8/7/09	8.00E+02	8.10E+02	1.75E+03	120 h	8/7/09	5.90E+03	6.10E+03	1.29E+04
168 h	8/2/09	1.68E+08	9.20E+07	1.05E+08	168 h	8/9/09	2.26E+04	1.24E+04	1.41E+04	168 h	8/9/09	1.67E+05	9.10E+04	1.04E+05
				20°C		Date			20°C		Date			20°C
1 h	7/26/09		1.71E+07		1 h	8/2/09		2.30E+03		1 h	8/2/09		1.70E+04	
24 h	7/27/09	2.00E+06	3.00E+06	1.00E+06	24 h	8/3/09	9.40E+04	9.80E+04	9.70E+04	24 h	8/3/09	1.74E+06	2.58E+06	3.24E+06
72 h	7/29/09	7.50E+07	7.00E+07	8.00E+07	72 h	8/5/09	2.11E+07	2.88E+07	3.26E+07	72 h	8/5/09	2.21E+08	2.30E+08	2.40E+08
120 h	7/31/09	4.50E+07	4.40E+07	3.70E+07	120 h	8/7/09	6.50E+07	5.90E+07	4.70E+07	120 h	8/7/09	5.32E+08	7.76E+08	5.44E+08
168 h	8/2/09	3.90E+06	2.00E+06	1.60E+06	168 h	8/9/09	1.42E+08	1.42E+08	1.42E+08	168 h	8/9/09	1.31E+10	1.19E+10	1.11E+10
				50°C		Date			50°C		Date			50°C
1 h	7/26/09		4.82E+06		1 h	8/2/09		6.40E+03		1 h	8/2/09		4.80E+03	
24 h	7/27/09	0.00E+00	0.00E+00	0.00E+00	24 h	8/3/09	0.00E+00	0.00E+00	0.00E+00	24 h	8/3/09	0.00E+00	0.00E+00	0.00E+00
72 h	7/29/09	0.00E+00	0.00E+00	0.00E+00	72 h	8/5/09	0.00E+00	0.00E+00	0.00E+00	72 h	8/5/09	0.00E+00	0.00E+00	0.00E+00

120 h	7/31/09	0.00E+00	0.00E+00	0.00E+00	120 h	8/7/09	0.00E+00	0.00E+00	0.00E+00	120 h	8/7/09	0.00E+00	0.00E+00	0.00E+00
168 h	8/2/09	0.00E+00	0.00E+00	0.00E+00	168 h	8/9/09	0.00E+00	0.00E+00	0.00E+00	168 h	8/9/09	0.00E+00	0.00E+00	0.00E+00

Table B.3 – Data for three Cattle samples subjected to four different moisture conditions

C1					C2				C3				
	Broth =	53 * 10^7 / 10 mL				Broth =	259 * 10^5 / 9 mL			Broth =	51 * 10^6 / 9 mL		
		Moisture Content				Moisture Content					Moisture Content		
	Date	1%			Date	1%				Date	1%		
1 h	7/9/09	1.12E+04			1 h	7/10/09	3.86E+05			1 h	7/18/09	1.29E+05	
24 h	7/10/09	3.00E+02	5.00E+02	2.00E+02	24 h	7/11/09	2.57E+04	2.89E+04	3.05E+04	24 h	7/19/09	4.80E+04	1.50E+04	2.00E+04
72 h	7/12/09	1.00E+02	1.10E+02	8.00E+01	72 h	7/13/09	9.40E+03	4.10E+03	1.22E+04	72 h	7/21/09	4.60E+03	7.00E+02	9.00E+02
120 h	7/14/09	1.00E+01	2.00E+01	0.00E+00	120 h	7/15/09	9.20E+03	7.80E+03	9.90E+03	120 h	7/22/09	8.00E+02	2.30E+03	2.90E+03
168 h	7/16/09	0.00E+00	0.00E+00	0.00E+00	168 h	7/17/09	9.30E+03	7.00E+03	2.40E+04	168 h	7/25/09	3.80E+03	1.70E+03	2.00E+02
	Date	25%				Date	25%				Date	25%		
1 h	7/9/09	4.22E+07			1 h	7/10/09	1.97E+06			1 h	7/18/09	2.18E+07		
24 h	7/10/09	1.92E+09	1.52E+09	2.29E+09	24 h	7/11/09	5.29E+08	4.86E+08	5.08E+08	24 h	7/19/09	5.70E+08	3.40E+08	6.00E+08
72 h	7/12/09	4.70E+09	5.50E+09	2.50E+09	72 h	7/13/09	2.73E+09	1.95E+09	4.34E+09	72 h	7/21/09	3.08E+09	2.78E+09	3.18E+09
120 h	7/14/09	6.40E+09	5.10E+09	4.60E+09	120 h	7/15/09	5.10E+09	6.30E+09	5.50E+09	120 h	7/22/09	2.30E+09	2.80E+09	7.60E+09
168 h	7/16/09	1.30E+09	3.10E+09	9.70E+09	168 h	7/17/09	6.90E+09	5.60E+09	4.80E+09	168 h	7/25/09	5.40E+09	8.60E+09	1.38E+10
	Date	56.5%				Date	56.5%				Date	56.5%		
1 h	7/9/09	5.00E+06			1 h	7/10/09	4.90E+06			1 h	7/18/09	5.80E+07		
24 h	7/10/09	1.49E+08	2.48E+08	2.77E+08	24 h	7/11/09	1.04E+08	8.50E+07	1.14E+08	24 h	7/19/09	3.80E+08	2.10E+08	3.40E+08
72 h	7/12/09	7.60E+08	5.60E+08	2.60E+08	72 h	7/13/09	1.02E+09	1.68E+09	1.69E+09	72 h	7/21/09	8.50E+08	1.78E+09	9.80E+08
120 h	7/14/09	1.96E+09	2.03E+09	2.09E+09	120 h	7/15/09	1.55E+09	1.72E+09	1.49E+09	120 h	7/22/09	1.38E+09	5.80E+08	6.20E+08
168 h	7/16/09	1.28E+09	1.04E+09	1.54E+09	168 h	7/17/09	2.68E+09	1.68E+09	1.59E+09	168 h	7/25/09	1.02E+09	7.20E+08	6.50E+08
	Date	83%				Date	83%				Date	83%		

1 h	5/21/09			3.56E+07	1 h	7/10/09			5.20E+05	1 h	7/18/09		4.30E+06
24 h	5/22/09	3.44E+08	3.92E+08	3.56E+08	24 h	7/11/09	1.75E+07	1.88E+07	3.92E+07	24 h	7/19/09	5.90E+07	1.57E+08	3.46E+08
72 h	5/24/09	1.20E+08	4.90E+08	3.30E+08	72 h	7/13/09	1.45E+08	3.04E+08	6.60E+07	72 h	7/21/09	4.20E+08	1.60E+08	3.60E+08
120 h	5/26/09	5.10E+08	5.90E+08	4.60E+08	120 h	7/15/09	2.30E+08	4.90E+08	4.60E+08	120 h	7/22/09	5.10E+08	7.10E+08	4.80E+08
168 h	5/28/09	1.90E+08	3.60E+08	3.70E+08	168 h	7/17/09	1.80E+08	1.60E+08	9.00E+07	168 h	7/25/09	2.80E+08	2.30E+08	2.00E+08

Table B.4 – Data for three Raccoon samples subjected to four different moisture conditions

R1					R2				R3				
			Broth =	3.20E+09			Broth =	1.80E+07			Broth =	1.13E+10
		Moisture Content				Moisture Content					Moisture Content		
	Date			1%		Date			1%		Date			1%
1 h	5/21/09			9.00E+06	1 h	5/22/09			3.60E+06	1 h	6/2/09			1.30E+05
24 h	5/22/09	1.66E+04	1.85E+04	1.08E+04	24 h	5/23/09	2.60E+03	4.00E+03	1.70E+03	24 h	6/3/09	7.00E+03	1.01E+04	8.20E+03
72 h	5/24/09	7.40E+03	6.50E+03	5.40E+03	72 h	5/25/09	4.00E+02	9.00E+02	8.00E+02	72 h	6/5/09	2.90E+03	3.20E+03	4.90E+03
120 h	5/26/09	5.10E+03	8.80E+03	6.70E+03	120 h	5/27/09	3.90E+02	5.40E+02	4.00E+02	120 h	6/7/09	3.90E+02	5.40E+02	4.00E+02
168 h	5/28/09	1.23E+04	1.07E+04	1.70E+04	168 h	5/29/09	3.30E+02	4.00E+02	3.40E+02	168 h	6/9/09	1.64E+03	1.66E+03	1.57E+03
	Date			25%		Date			25%		Date			25%
1 h	5/21/09			6.00E+06	1 h	5/22/09			3.22E+07	1 h	6/2/09			5.60E+07
24 h	5/22/09	1.90E+08	2.88E+08	3.26E+08	24 h	5/23/09	4.60E+07	6.10E+07	4.90E+08	24 h	6/3/09	3.92E+09	4.53E+09	4.18E+09
72 h	5/24/09	1.94E+09	1.33E+09	1.47E+09	72 h	5/25/09	9.90E+08	1.38E+09	1.33E+09	72 h	6/5/09	2.40E+09	2.70E+09	2.30E+09
120 h	5/26/09	8.50E+08	9.20E+08	8.30E+08	120 h	5/27/09	1.43E+09	2.12E+09	1.66E+09	120 h	6/7/09	3.90E+09	2.80E+09	4.80E+09
168 h	5/28/09	7.20E+08	9.20E+08	5.20E+08	168 h	5/29/09	2.57E+09	2.78E+09	2.85E+09	168 h	6/9/09	1.70E+09	2.10E+09	9.00E+08
	Date			56.5%		Date			56.5%		Date			56.5%
1 h	5/21/09			2.40E+07	1 h	5/22/09			2.38E+07	1 h	6/2/09			3.50E+08
24 h	5/22/09	2.73E+08	2.08E+08	4.99E+08	24 h	5/23/09	2.43E+08	2.29E+08	1.62E+08	24 h	6/3/09	1.34E+09	1.47E+09	9.50E+08
72 h	5/24/09	8.20E+08	4.50E+08	3.90E+08	72 h	5/25/09	6.30E+08	6.80E+08	6.00E+08	72 h	6/5/09	7.00E+08	1.60E+09	1.00E+09

120 h	5/26/09	1.80E+08	1.70E+08	2.50E+08	120 h	5/27/09	5.60E+08	5.10E+08	1.38E+09	120 h	6/7/09	1.72E+09	1.19E+09	2.54E+09
168 h	5/28/09	2.20E+08	2.30E+08	5.70E+08	168 h	5/29/09	5.80E+08	7.60E+08	8.20E+08	168 h	6/9/09	1.43E+09	2.21E+09	1.96E+09
	Date			83%		Date			83%		Date			83%
1 h	5/21/09			1.00E+06	1 h	5/22/09			2.76E+07	1 h	6/2/09			2.83E+07
24 h	5/22/09	3.90E+06	1.20E+06	6.30E+06	24 h	5/23/09	1.05E+08	7.40E+07	5.30E+07	24 h	6/3/09	4.12E+08	3.06E+08	4.26E+08
72 h	5/24/09	1.08E+08	1.34E+08	1.02E+08	72 h	5/25/09	3.00E+07	6.30E+07	1.38E+08	72 h	6/5/09	1.48E+09	1.22E+09	1.11E+09
120 h	5/26/09	1.32E+08	2.23E+08	1.51E+08	120 h	5/27/09	1.97E+08	1.85E+08	1.94E+08	120 h	6/7/09	1.27E+09	7.70E+08	1.21E+09
168 h	5/28/09	5.90E+08	7.10E+08	5.20E+08	168 h	5/29/09	3.10E+08	3.70E+08	3.20E+08	168 h	6/9/09	1.01E+09	1.06E+09	1.09E+09

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