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Effect Of Soil And Irrigation Water On Microbial Quality Of Fresh Produce Grown In North Carolina

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Effect of Soil and Irrigation Water on Microbial Quality of Fresh Produce Grown in North Carolina

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Biographical Sketch

Tracie Davis was born on July 3, 1985, in Wilmington, North Carolina to Johnsie and Raymond Davis. She is the youngest with two older siblings, Danita Davis-Manly and Tamika Beirliene. Tracie graduated from New Hanover High school in Wilmington, North Carolina. She received the Bachelor of Science in Nutrition and Wellness, minor in Public Health from the University of North Carolina in Greensboro. She enrolled at North Carolina A&T State University Graduate Food and Nutritional Sciences program to further her education. While pursuing her Masters, she focused her research on food safety while under the advisement of Dr. Ipek Goktepe and Dr. Ibrahim Salam A. Ibrahim. Mrs. Davis has also served as Research Assistant in the Family and Consumer Sciences Department. She was inducted into the Gamma Sigma Delta honor society of Agriculture in 2011. After graduation, Ms. Davis plans to pursue a doctoral degree in food science.

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ANOVA Analysis of Variance

BSA Bismuth Sulfite Agar

CDC Center for Disease Control and Prevention

CFU Colony Forming Unit

EC *E. coli* species

GAP Good Agricultural Practices

HACCP Hazard Analysis and Critical Control Point

HUS Hemolytic Uremic Syndrome

MA Modified Atmosphere

MAC MacConkey Agar

MAP Modified Atmosphere Packaging, Passive MAP

NaOC NaOC

NC North Carolina

PACA Perishable Agricultural Commodities Act

PCR Polymerase Chain Reaction

ppm Parts per Million

SS *Salmonella spp.*

STEC Shiga Toxin Producing *E. coli*

Stx Shiga Toxin

TC Total Count

TE Tris-EDTA (Ethylenediamine Tetraacetic Acid; buffered solution)

TSA Tryptic Soy Agar

TSB Tryptic Soy Broth

USDA United States Department of Agriculture

USFDA United States Food and Drug Administration

UV Ultra Violet

VT Verocytotoxin

XLT4 Xylose-Lysine-Tergitol 4 agar

Abstract

Foodborne outbreaks involving *Listeria monocytogenes*, *Salmonella enteritidis*, and *E. coli* O157:H7 from contaminated fresh produce have been increasingly recognized all over the world. The purpose of this research was to determine the effect of irrigation water and soil on microbial quality of leafy greens and tomatoes grown in different parts of North Carolina (NC). Soil and water samples were collected from 4 small farms located in NC and inoculated onto selective media (TSA for total aerobic count, XLT4 for *Salmonella spp. (SS)*, and MacConkey for total *E. coli* species, *EC*). All plates were incubated for 48 hours at 37°C. Following incubation, colonies were counted and the numbers were expressed as Log CFU/ml. The identification of microorganisms was carried out by multiplex PCR analysis.

The results indicated that soil samples collected from the farms located in the eastern part of NC had the highest microbial load (7.46 Log CFU/ml on TSA, 6.68 Log CFU/ml on MAC, 5.61 Log CFU/ml on XLT4) in the summer. Both water and soil samples collected from farms located in the western part of the state had the lowest microbial counts, indicating that humidity and temperature directly affect the microbial content of soil and irrigation water. The PCR analysis confirmed the presence of *SS* only in soil samples collected from a farm located in the eastern part of the state. These findings indicate that improvements are needed to avoid pathogenic bacterial contamination in fresh produce farming operations in NC and this should be carried out by training farmers on produce safety.

CHAPTER 1

Introduction

Nutritional guidelines geared towards improving dietary choices have increased consumer preference for fresh produce within the decade. Dietary assessment of vegetable consumption in the U.S shows 17% increase over the past four decades. According to the USDA Economic Research Service, the average American consumes 1.7 cups of vegetables per day. This average is 11% above the 1970s average of 1.5 cups per day (USDA Economic Research Service; Thornsbury, Jerardo, & Hodan, 2012). In 2010, the total vegetable amount available for consumption in the U.S has increased by 17 % since 1970 (Thornsbury, Jerardo, & Hodan, 2012). Between 1970 and 2010, fresh vegetables availability showed 21% growth in availability in the U.S (Thornsbury, Jerardo, & Hodan, 2012). Specifically, romaine lettuce production has increase three fold between 1985 and 2010.

Research surveys have indicated that the consumption of cucumbers in the U.S increased by 15% in 2011 compared to 2004 (Absar Alum, 2011). The consumption of vegetable and melon crops, which ranked at a top value of 38%, is estimated to increase 1.7% annually (North Carolina Department of Agriculture and Consumer Services and The Agriculture Statistic Division, 2011). The production volume of vegetable and melons are also projected to increase at 0.8 % year, reaching 330 billion kilograms by 2020 (North Carolina Department of Agriculture and Consumer Services and The Agriculture Statistic Division, 2011) **.** Statistics on crop production value in North Carolina indicate an 82% increase from 2006 to 2010 (North Carolina Department of Agriculture and Consumer Services and The Agriculture Statistic Division, 2011). While consumer intakes of leafy greens increased 9% from 1996 to 2005,

foodborne outbreaks associated with leafy green produce increased 39% (Daniel, Carycoppoc, & Manasharma, 2011). Figure 1 indicates that consumption of fresh fruits and vegetables has strongly correlated with the higher rate of domestic foodborne illnesses in recent years.

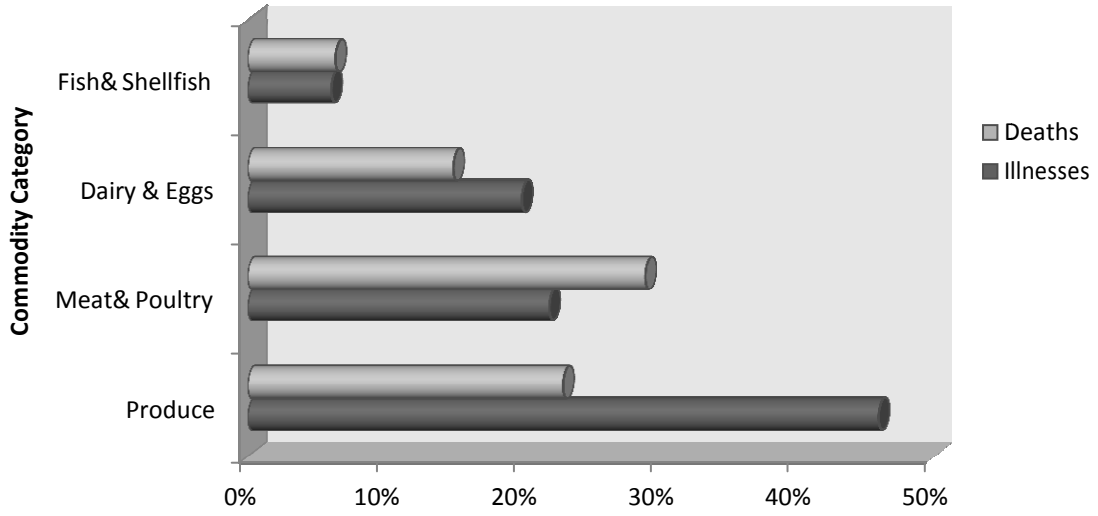

Figure 1.1 U.S Food Sources Associated with Estimated Illnesses & Death for 1998-2008.

Over the past several years, *E. coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes* have been the major concerns in the U.S fresh produce industry. Between January and August of 2011 twenty-five states reported an outbreak of *Salmonella* Agona strain among one-hundred and six individuals (CDC, 2011). Investigations traced the outbreak to the consumption of fresh papayas imported from Mexico (CDC, 2011a). On July the 5th 2011, twenty-five individuals, between the age of 12 and 77, in five states reported having *Salmonella* Enteritis (CDC, 2011b). Investigation by CDC, FDA, and state and local regulatory agencies linked the outbreak to Evergreen Fresh Produce Sprouts, LLC alfalfa sprout and spicy sprouts grown in Moyie Spring, Idaho (CDC, 2011c). Another case reported on June 20 of 2011

involving twenty individuals located in ten states were infected with *Salmonella enterica* Panama strain, which was traced back to cantaloupe harvested from a Guatemalan farm (CDC, 2011d).

In another case just recently reported in ten states within the U.S, sixty individuals were infected with *E. coli* O157:H7 in late 2011(CDC, 2011e). *Salmonella* Typhimurium and *Salmonella* Newport were recently associated with a 261-person outbreak associated with cantaloupe from Owensville, Indiana (CDC, 2011d). Figure 1.2 shows individuals infected with *Salmonella* Typhimurium and *Salmonella* Newport linked to the cantaloupe in United States from the time of illness onset. These recent associations of *E.coli* O157:H7 and *Salmonella spp.* outbreaks with consumed fresh produce has lead to investigation within fresh produce farming practices. The objective of this research is to assess the association of season soil differences and farming practices among fresh produce susceptible to *E.coli* O157:H7, *Salmonella spp.*, and *Listeria monocytogenes*.

Within North Carolina *E.coli* O157:H7 outbreaks associated with animal contact at the 2011 State Fair have notably documented. However, fresh produce grown in North Carolina has not been fully researched in possible risks as a source of foodborne outbreaks in recent years. Within North Carolina alone, previous soil and water microbial testing lack extensive research. The various climatic regions for various fresh produce farms can have significant influences on the risks for pathogenic contamination. Climatic conditions involving seasonal temperatures, sun exposure, moisture content, humidity ranges effect the quality of produce irrigation systems, and nutrient content within available soil. The adoption of the most suitable practices of agricultural management is essential in order to improve produce safety. Thus, the purpose of this concurrent prospective study was to assess the risk of microbial quality related to soil and water in small-scale farms located throughout North Carolina in summer and fall. The

information obtained will provide further data for potential risk factors in fresh produce contamination.

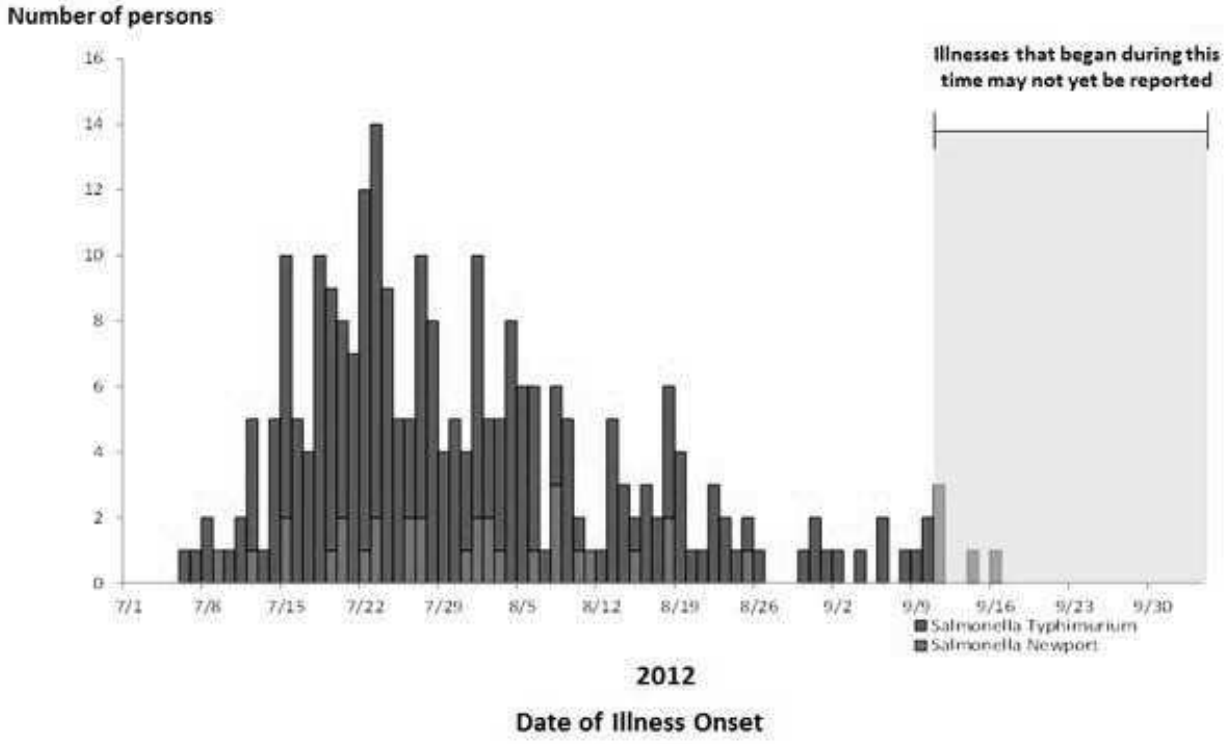

Figure 1.2 Total population (n=261) for whom information was reported as of October 4, 2012. Persons infected with the outbreak strain of *Salmonella* Typhimurium and *Salmonella* Newport linked to Cantaloupe in U.S, by date of illness onset.

Source: CDC 2012

Table 1

Pathogens contributing significantly to foodborne illnesses and hospitalization within the U.S. [1]

Pathogen	**Estimated number of hospitalizations**	**90% Credible Interval**	**%**
***Salmonella*, nontyphoidal**	19,336	8,545–37,490	35
Norovirus	14,663	8,097–23,323	26
Campylobacter spp.	8,463	4,300–15,227	15
Toxoplasma gondii	4,428	3,060–7,146	8
***E.coli* (STEC) O157**	2,138	549–4,614	4

[1.] Pathogens not exclusive to fresh produce contamination.

Source: CDC 2013

CHAPTER 2

Literature Review

2.1. Common Foodborne Pathogens

Though great strides are being taken to prevent food contamination, it is evident that not enough is being done to determine the source of foodborne diseases. There are thirty one known pathogenic agents transmitted through food within the United States. Foodborne diseases account for an estimated 48 million illnesses, 128,000 hospitalizations, and 3000 deaths each year in the United States (Wendy Marcason, 2011). Fresh Produce related foodborne illnesses contributed to 131 outbreaks, over 14,000 illnesses and 34 deaths between 1996 and 2010 (FDA, 2013). Common pathogens contributing to the acquirement of foodborne illnesses are: *E.coli* O157:H7, mainly entero- hemorrhagic related pathogen in the United States, spread predominantly from human fecal carriage to person-to-person contact: *Salmonella* spp., a facultative anaerobic gram-negative rod shaped bacteria that can cause clinical conditions of enteric fever, and non-typhoidal systemic infections; and *Listeria monocytogenes*, a unique, facultative anaerobe foodborne pathogen that enters the human host cell, proliferates, and directly transmits to neighboring cells. Additional virulent foodborne pathogens include; *Clostridium botulinum*, a gram positive, obligate anaerobic pathogen that produces botulinum toxin causing botulism; *Campylobacter*, a genus type under the *Camplybacter-aceae* family, are S-shaped gram negative bacterium frequently associated with diarrheal illness.

2.2. *Escherichia coli* O157:H7

Escherichia coli is typically a harmless predominant facultative anaerobe of the mammalian colonic flora. This microorganism generally colonizes the gastro-intestinal tract of infants usually remains confined to the intestinal lumen. However *E. coli* strains still have the ability to cause infections and are considered a public health concern. *E.coli* is considered a species under the genus *Escherichia* within in the *Enterobacteriaceae* family. Specific combinations of somatic (O) and flagellar (H) antigens serotype *E.coli*. These serotypes are identification markers that strongly correlate with virulence characteristics of *E. coli* strains. The process of infection associated *E. coli* is believed to involve colonization of a mucosal site, evasion of host defenses, replication, and host cell damage. All *E.coli* strains have fimbriae attachments that are suggested to enhance attachment and colonization of hosting site.

A recently published study evaluated *E. coli* (STEC) O157:H7 adherence to spinach leaves with the aid of curli fimbriae and cellulose (Macarisin, Patel, Bauchan, & Vijay, 2012). Both components are characteristics of *E.coli* extracellular structure and enhance microbial attachments to animal cells and intestinal mucosal surfaces. The cellulose component consists of polysaccharide embedded within the membrane of *E.coli*. In addition, curli combined with cellulose have demonstrated the ability to contribute cell-to-cell aggregation, increasing pathogenic resistance (Macarisin, Patel, Bauchan, & Vijay, 2012). Within the comparative study, researchers' utilized Shiga toxin-producing *E.coli* O157:H7 wild type strains and their isogenic mutant deficient counterparts. Mutant *E.coli* strains lacked either the curli fimbriae or cellulose components or both within the study. Spinach leaves were inoculated with 100 *ul* of

either the mutant or non-mutant bacterial strains for a 7 log colony forming unit (CFU) (Macarisin, Patel, Bauchan, & Vijay, 2012). Un-inoculated spinach leaves were also used as a control group. Samples were incubated at 22° C for 0, 24, 48 hours and observed for adherence strength (Macarisin, Patel, Bauchan, & Vijay, 2012). A statistical analysis using randomized complete block design per treatment was conducted to analyze data. Treatments were conducted three times for accuracy and analyzed by a two-way analysis of variance (ANOVA) to determine the effect of bacterial strain and sampling period. Results indicated that attached curli-deficient mutant strains were significantly lower than curli expressing bacterial strains. The study further confirmed that curli fimbria is essential for strong attachment to spinach leaves thus increasing the pathogens resistance ability (Macarisin, Patel, Bauchan, & Vijay, 2012).

Large outbreaks involving *E.coli* O157:H7 have occurred within multiple developed countries including the United States. The *E.coli* serotype O157:H7 is the main cause of enter hemorrhagic illnesses and accounts for 75,000 cases every year in the U.S (Ashley D. Duffitt, 2011). Approximately 54% of *E.coli* O157:H7 illnesses in the United States were associated with contaminated produce (Duffit, 2011). Contaminated fecal matter among soil and water within contact of food products are always the source of *E. coli* O157:H7 illnesses. *Escherichia coli* O157:H7 are unusually tolerant to a broad range of environmental conditions as well as demonstrate long-term survival in manure. This single organism may exist in short chains or in pairs with one or more flagella.

Associated infection characteristic includes hemorrhagic colitis (bloody diarrhea), non-bloody diarrhea, and kidney disease, such as hemolytic-uremic syndrome (HUS). HUS causes kidney damage and may progress to organ failure and death. Infected individuals may also exhibit no signs or symptoms referred to asymptomatic infection (Thomas J. Montville,

Enterohemorrhagic *Escherichia coli*, 2005). The shiga-like verotoxin can spread throughout the body by attaching exteriorly to neutrophils leading to extensive host cell and tissue death. An infectious dose of *E.coli* O157:H7 can be as few as 10 cells. Fecal shedding of the pathogenic strain can last for more than three weeks. Susceptible populations, such as children and elderly, have a higher risk of infection from *E.coli* O157:H7 due to their low dose threshold. Infectious outbreaks of the pathogen are highest during warmer seasons of the year.

There is no defined information concerning the survival or growth rate of *E.coli* O157:H7 within soil and water contents, but research indicates that some strains of *E. coli* have the ability to produce colicin, an antibacterial protein that eliminates competing microbial strains (Ashley D. Duffitt, 2011). Research indicates that surface application of *E. coli* O157:H7 on greenhouse lettuces are traceable for up to 20 days (Moyne, et al., 2011) Common route of introduction for *E.coli* O157:H7 onto agricultural crops includes contaminated water irrigation systems and soil. In such cases, nearby manure and soil amendments potentially contaminate water runoff used in food crop irrigations.

Researchers have found that the survival rate of *E.coli* O157:H7 among agricultural soil is determined by soil types (Thomas J. Montville, Enterohemorrhagic *Escherichia coli*, 2005). The difference in nutrient availability among various soil types, indicate a correlation with pathogenic persistence (Asbar, Gerba, & Enriquez, 2011). Among farming sites, cattle infected with *E.coli* O157:H7 can transfer viable pathogenic cells to their feces which can further cross-contaminate nearby irrigation water sources. Survival rate of *E.coli* O157:H7 may span from weeks to months among water and manure amended soil sources (Thomas J. Montville, Enterohemorrhagic *Escherichia coli*, 2005). Variations in soil types also raise concerns as a source of *Salmonella* spp. outbreak related to fresh produce.

2.3. *Salmonella* spp.

National outbreaks of *Salmonella* spp. are increasingly associated with fresh fruits and vegetables consumption. From 2002-2003, a reported 31 *Salmonella* spp. were linked to contaminated produce. According to FoodNet 2010 *Salmonella* spp. is the most common infection, attributing for 1.2 million U.S illnesses annually. *Salmonella* infections have actually increased since 2006-2008 (Figure 2.1) and almost three times the 2010 national health objective target (CDC, 2011b).

Source: CDC 2011

Figure 2.1. Reported rates, by year, of laboratory-confirmed infections with *Campylobacter*, *E.coli* O157:H7, *Listeria, Salmonella*, and *Vibrio*, compared with 1996-1998 rates.

Salmonella spp. is an aerobic organism that has the ability to metabolize nutrients through respiratory and fermentative routes. The rod-shaped gram-negative bacteria belong to the *Enterobacteriaceae* family. There are six subspecies and an estimated 2500 serovars for *Salmonella enterica*. The analysis of somatic (O) and flagellar (H) antigens are used to

distinguish bacteria into a specific serovars. The growth of *Salmonella* spp. is most favorable at 37° C, producing acid and gas by catabolizing carbohydrates such as D-glucose. However *Salmonella* spp. is able to survive and grow in temperature ranges of 7-48° C (Schneider & Fatica, 2011). *Salmonella* spp can survive within a pH range from 4.05 to 9.5, with optimum pH of 6.5-7.5 (Schneider & Fatica, 2011).

A study investigating the interactions of *Salmonella enterica* with lettuce leaves found that various time and temperature exposures can significantly impact Salmonella growth. Researchers visualized Salmonella on lettuce leaves by marking Salmonella Typhimurium SL1344 with cyan fluorescent protein (CFP). The tagged fluorescent pathogens were then visualized under a confocal microscope followed by a 3D analysis (Kroupitski, Pinto, Brandi, & Sela, 2009). Study found that cut regions of romaine lettuce had a higher attachment level of Salmonella after 2 hours at 5° C versus 18 hours at 4° C. Intact lettuce pieces contaminated with Salmonella for 9 days at 4° C only show a minimal population change (Kroupitski, Pinto, Brandi, & Sela, 2009).

In addition, *Salmonella* attached to lettuce leaves shown an increase tolerance for acidic conditions during storage. Some *Salmonella* strains can proliferate at extreme conditions by adapting to the surrounding environment. The ability of *Salmonella* spp. to survive extensive periods in foods held at freezing or room temperature is a serious safety concern in the food processing industry. Clinical conditions for *Salmonella* infections are dependent upon the serotype. Human *Salmonella* infections are predominately associated with *Salmonella enterica* serovars Typhimurium, and Enteritidis. Serious conditions associated with the typhoid strain include enteric fever, a human disease with an incubation period between 7 to 28 days. Symptoms normally associated with enteric fever include diarrhea, persistent fever, headaches,

fatigue, and abdominal cramping. Like typhoid *Salmonella* strain, non-typhoidal strains typically involve diarrhea, and abdominal pain. However, non-typhoidal *Salmonella* exhibit symptoms 8 to 72 hours after consumption of contaminated food (Montville & Matthews, 2008). One of the most common causes of foodborne diseases among Americans is *Salmonella enterica*. In 2011, it was estimated that 19,336 people were hospitalized from acquiring non-typhoidal *Salmonella*. It was estimated that 378 deaths were associated with *Salmonella* non-typhoidal strain in 2011, accounting for 28% known foodborne illnesses to cause death (CDC, 2011g). Over the last 15 years, *Salmonella* infections have not only been ongoing but have progressed between 2006 and 2008 (CDC, 2012).

Susceptibility to *Salmonella* illness is higher among infants, elderly, and individuals who are immune-deficient. Incidence reports indicated that 1,409 individuals aging 60 or older along with 2,217 children under 5 were infected with *Salmonella* in 2010 (CDC, 2011h). The typical infectious dose of *Salmonella* spp. may range from 10^6 to 10^8 CFU; however, infectious dosages less than 10^6 CFU can infect some human individuals within the population (Schneider & Fatica, 2011). Human foodborne salmonelloses have been associated with various produce across the United States.

Once *Salmonella* has reached agricultural crops, risk of contamination is mainly dependent on the pathogens survival in soil, and their pathway into fresh produce irrigation systems. Close range infected cattle and wild life are possible carriers of *Salmonella* spp. and may contribute to crop contamination. The persistence of *Salmonella* spp. among animals is dependent of species type, health, herd population, and their residing environment (Bech & Carsten, 2012).

Various factors are associated with *Salmonella* spp. survival within soil including temperature, moisture content, soil type, UV exposure, and the initial organisms present. Studies suggest that *Salmonella* spp. has the ability to persist up to 332 days within amended soil (Jacobsena & Bech, 2011). *S.* Typhimurium compared to *E. coli* O157:H7 has a higher resistance to environmental stressors (Bech & Carsten, 2012).

Wastewater used to treat agricultural land can contain animal waste which frequently carries pathogenic bacteria such as *Salmonella* spp., *Escherichia coli*, and *Campylobacter* species. According to the Environmental Protection Agency (EPA) sewage sludge must meet the criteria under the 40 CFR Part 503 sewage sludge standards before being applied. Under this standard sewage sludge must meet specific alternatives for either Class A or Class B pathogen reduction levels. The EPA states *Salmonella* spp. bacterial load must be under 3 most probable numbers (MPN) per 4 grams in treated sludge before use in agricultural spreads. However research has shown increase *Salmonella* spp. populations by 10^6 CFU/ g in Class A sludge stored under anaerobic conditions (Bech & Carsten, 2012). Class B bio solids stored in anaerobic conditions prior to crop application showed a regrowth up to 10^5 CFU/g (Bech & Carsten, 2012). Primary port of entry of *Salmonella* spp. to fresh produce is via seeds planted in manure-based soil (Bech & Carsten, 2012). Cattle or livestock urine in sandy soil have proven to significantly increase S. Typhimurium survival rate (Bech & Carsten, 2012). Microbial population in soil can also influence the survival of *Salmonella* spp once introduced within soil. High populations of microbial competition can decrease *S. enterica* survival. *S. enterica* has shown to be able to colonize tomato plants from both contaminated irrigation systems and soil amendments, but the highest microbial load of the pathogen was found in irrigation water (Bech & Carsten, 2012).

2.4. *Listeria monocytogenes*

2.4.1. Characteristics of *L. monocytogenes*. *Listeria monocytogenes*, a genus of *Listeria*, is primarily a human pathogen that causes listeriosis. Characteristically, gram-positive bacterium is uniformly covered across the body surface in flagella for motility and consists of thirteen serotypes which opportunistically take advantage of immune-compromised individuals. Serotypes 1/2a, 1/2b/ and 4b are commonly isolated in clinical cultures with 1/2a serotype having the highest prevalence in food (McLaughlin, Casey, Cotter, & Gahan, 2011). In addition, this bacterium has the distinctive ability to cause hemolysis of red blood cells.

2.4.2 Pathogenes of *L. monocytogenes*: Outbreaks and symptoms. *L. monocytogenes* is a prevalent species of *Listeria* in foodborne outbreaks. Symptoms associated with *L. monocytogenes* infection include; meningitis, encephalitis, septicemia, low -fever, liver abscess, and miscarriage among pregnant women (E. Galdiero, 1997). Current estimates indicate that out of 1600 listeriosis cases, 260 lead to death each year (CDC, 2011i). Among the population in the US, pregnant mothers, infants, elderly, and persons with immune deficiencies have a higher possibility of falling ill to listeriosis. Conducted surveillances demonstrated that pregnant women are nearly thirteen times more at risk of infection than the overall population. Individuals with AIDS have roughly 300 times more of a chance to become infected by listeriosis than those with normal immune function (CDC, 2012). Similar to *E.coli* O157:H7 and *Salmonella* spp., *L. monocytogenes* has the potential to grow in favorable environmental conditions among soil.

2.4.3. Sources of *L. monocytogenes* . The sources of *L. monocytogenes* can be found in soil, water, fresh produce and the digestive system of mammals including humans. Infectious dosages are typically greater than 100 CFU/g. *L. monocytogenes* may enter the food-processing

system through several outlets, one including contaminated raw produce. Low temperatures and moisture within soil provide favorable conditions for *L. monoctyogenes* to thrive within fresh produce farm settings.

2.4.4. Impact of Environmental Factors on *L. Monocytogenes*. *L. monocytogenes* is able to sufficiently grow at temperatures of 0 to 45^0C and acidic pH values of 4.4 (Montville & Matthews, 2008). Average salt concentrations of 6.5% can induce elevated growth rates of *L. monocytogenes*. When the salt concentration is increased, this pathogen's survival rate can be extended to longer periods, however the bacterium cannot endure heat processing.

Researchers found that this bacterium has a greater capacity to survive within soil condition of 8° C as opposed to temperature ranges of 25° -30°C (McLaughlin & Casey, 2011). However further research has indicated *L. monocytogenes* can sustain survival in wounded apple tissue at a temperature range of 10º and 20 º C. A study conducted by Conway and colleagues with U.S Department of Agriculture, Beltsville, Maryland investigated the treatment of lytic bacteriophages and a bacteriocin on fresh-cut produce contaminated with *Listeria monocytogenes*. Within the study fresh cut apples and honey dew melons were inoculated with 24 *ul* of *L. monocytogenes* followed by phage and nisin treatments to the aliquots. To determine the influence of bacterial concentration on the efficacy of phage treatment, researchers inoculated freshly cut honey dew melon squares at 10^5 and 10^6 CFU/ml before applying phage treatment. Samples were placed in 10° C storage and quantified on the 1^{st}, 2^{nd}, 5^{th}, and 7^{th} day. The log CFU results for tested samples were compared to control inoculated samples without treatment. *L. monocytogenes* population continued to increase on produce decayed by *G. cingulata*, a common fungal culture among produce (Nastou, Rhoades, P., Kontominas, & Likotrafiti, 2012). Though *L. monocytogenes* is often found in cold, moist environments such as

refrigerators, Conway et. al concludes that the microbial population of this species can be minimized if produce is kept at a recommended 4° C refrigerated temperature. A recent article in International Journal of Food Microbiology supports this conclusion and further investigates the efficacy of household methods to reduce *L. monocytogenes* in fresh produce (Nastou, Rhoades, P., Kontominas, & Likotrafiti, 2012).

Several studies have researched the growth and survival of *L. monocytogenes* on both refrigerated and ambient temperature conditions. In 2005, Fless and colleagues studied the survival of *L. monocytogenes* on fresh and frozen strawberries (Fless & Harris, 2005). Within this study cut and whole strawberries were inoculated with a prepared nalidixic acid resistant *L. monocytogenes* cocktail that consisted of five pathogenic strains: V7, LCDC 81-861, Scott A, 101 M, and 108 M (Fless & Harris, 2005). Fifteen microliters of the *L. monocytogenes* cocktail were inoculated on the surface of sliced side of strawberries (Fless & Harris, 2005). After inoculation strawberries were air dried under a biological hood for one hour with a fan at 24° C. Strawberries were then stored at either 4° C for up to 7 days or 24° C for a period 48 hours (Fless & Harris, 2005). An additional bag of inoculated cut strawberries were stored at freezing temperatures of -20° C with and without sucrose, to model typical consumer and retail freezer conditions (Fless & Harris, 2005). Observed results for inoculated whole strawberries stored at 24° C for 48 hours showed a significant decline of 2.2 log CFU in pathogenic growth. However, inoculated cut strawberries under the same conditions showed no significant reduction in *L. monocytogenes* populations (Fless & Harris, 2005). Whole and cut strawberries placed in 4° C temperatures were stored for up 7 days. A total 3 log CFU reduction was observed for whole strawberries after 7 days of storage (Fless & Harris, 2005). Cut strawberries evaluated under the

same conditions showed less than 1 log decline in *L. monocytogenes* population (Fless & Harris, 2005). The survival rate of *L. monocytogenes* in cut strawberries, without sucrose and stored at – 20°C, decline by 1 log within the first 24 hours. After 28 days in storage 1.2 total log CFU was observed among inoculated strawberries without sucrose. In contrast, strawberries with added sucrose maintained a steady microbial population after 28 day in – 20 ° C storage (Fless & Harris, 2005). The study concluded that storage temperature, time and the acidity of produce are influential in the survival of L. *monocytogenes* (Fless & Harris, 2005).

In a similar study, researchers evaluated various strains of *L. monocytogenes* and *Listeria innocua* within soil particles in order to determine factors that can affect the survival of these pathogens (McLaughlin, Casey, Cotter, & Gahan, 2011). Three strains of *L. monocytogenes*, EGDe, CD83, and CD1038 and three strains of *L. innocua*, CLIP, FH2117, and FH2157, were inoculated into a gram of soil and incubated at either 8,25, or 30 °C in order to observe the influence of temperature. Growth rates for each sample were counted using direct enumeration of colony forming (CFU). Results of the study did not indicate any specific survival differences between *L. monocytogenes* and *L. innocua*. However the survival rate of *L. monocytogenes* CD83 remained significantly higher than all other tested strains at various time periods of monitoring. *L. monocytogenes* CD83 exhibited the highest survival rate at 25° C. In comparison to 25° C incubation temperatures, 30° C resulted in rapid cell decline among all six *Listeria spp.* evaluated (McLaughlin, Casey, Cotter, & Gahan, 2011). The study further investigated the effect water loss has on the contaminated soil samples. Each strain of *L. monocytogenes* and *L. innocua* of were independently inoculated into 1 gram of soil and kept in either sealed or unsealed tubes for a period of 2 weeks. Results indicated that unsealed tubes with soil samples

were affected by subsequent moisture loss after a week of exposure (McLaughlin, Casey, Cotter, & Gahan, 2011). During the study, researchers also determined a correlation between survival rates and motility among Listeria strains. An analysis determined that both pathogenic strains *L. monocytogenes* CD83 and *L. innocua* FH2152 had the highest rate of motility and microbial count among all six strains observed (McLaughlin, Casey, Cotter, & Gahan, 2011). To confirm these findings, Mclauglin compared the survival of *L. monocytogenes* CD83 and *L. monocytogenes* EGDe to non-motile mutant strains of *L. monocytogenes* within soil. The mutant non-motile pathogenic strains proved to decline in persistence within soil at a quicker rate compared to motile strains CD83 and EGDe. Overall, the study emphasizes the importance of understanding the factors that influence the survival *Listeri*a in order to comprehend this pathogens route from the environment into the food chain (McLaughlin, Casey, Cotter, & Gahan, 2011).

Several researchers have studied the correlation between the behavior of *L. monocytogenes* and environmental factors. In 2005, International Journal of Food Microbiology published a study that evaluated the growth rate of *L. monocytogenes* on fresh cut iceberg lettuce under various temperature ranges (Koseki & Isobe, 2005). Within this study each 100 g lettuce sample separated into plastic bags and inoculated with a six strain *L. monocytogenes* cocktail that had a 3-4 log CFU concentration. The six *L. monocytogenes* strain used included: ATCC 1911, ATCC19117, ATCC19118, ATCC 13932, ATCC15313, and ATCC35152. Samples were then stored at either 5,10,15,20 or 25° C and observed at specific intervals during incubation (Koseki & Isobe, 2005). The observations indicate a shorter lag time for *L. monocytogenes* incubated at 25° C. In contrast, incubation temperatures of 5° C exhibited a longer lag time in pathogenic growth in comparison with the four other temperature conditions observed. The results of the

study suggest fluctuating temperatures have an influential impact on the survival of *L. monocytogenes* on produce (Koseki & Isobe, 2005).

2.4.5 Paths of *L. monocytogenes* contamination. The facultative anaerobe is able to resist antibiotic contact and grow by entering host cells, proliferating and transferring to neighboring cells causing diseases. Some strains of *L. monocytogenes* are resistant to antibiotics including; tetracycline, gentamicin, penicillin, ampicillin, streptomycin, erythromycin, kanamycin, sulfonamide, trimethoprim, and rifampicin (Zhang, et al., 2007). Only *L. monocytogenes* and *L. inanovii* are virulent among the *Listeria* genus (Zhang, et al., 2007).

Both the Food Safety and Inspection Service (FSIS) and the Food and Drug Administration (FDA) have issued "zero tolerance" for *L. monocytogenes* on all ready-to-eat foods including produce. Foods found to contain this pathogenic organism are withheld from distribution or recalled.

2.5. Susceptibility of Fresh Produce

The increasing association of fresh produce with food borne outbreaks has lead to further research surrounding the correlation and concern. Leafy green vegetables are excellent sources of vitamins and phytonutrients that may provide beneficial anticancer and aging properties (Luo, et al., 2011). *The Dietary Guidelines for American 2010* encourages vegetable consumption for optimal vitamin and mineral intake. Current recommendations suggest a diet consistent of at least 2.5 cups of vegetables per day (United States Department of Agriculture, 2012).

2.5.1 Produce Contamination. The trend in the consumption of fresh cut, uncooked produce has highlighted the importance of food safety. Fresh produce has an increased risk of

exposure to human pathogens for more than a decade. HACCP (Hazard Analysis and Critical Control Points) based procedures along hygienic practices are important requirements among the application produce industry (Lehto, Risto, Maatta, Kymalainen, & Maki, 2011). Fresh-cut fruits and vegetables are exposed to rapid deterioration and can sustain large proliferation of microorganism, before and after the processing. Majority of processed foods undergo irradiation, which is used to kill microbes and sterilize product before consumption. However fresh produce, often eaten raw, does not undergo irradiation during processing, thus its main form of microbial containment is temperature control. Several, detailed steps must be considered during processing in order to maintain the safety and quality of produce; including abiding to good manufacturing practices and sanitation procedures (Zagory, 1999).

Operations such as cutting, slicing, chopping, and mixing are important processing steps for ready to eat fresh produce products. These procedures can result in an increase in microbial growth on fresh produce through the transfer of microorganisms from the equipment to the product (Montville & Matthews, 2008). During processing, conditions including low humidity, low oxygen, and high levels of carbon dioxide within packaging can influence microbial growth on produce (Zagory, 1999). Pathogenic adaptation and colonization are influenced not only by good manufacturing practices but also the genotypic differences among fresh produce. The root, stem, and surface fissures are known port of entries for pathogenic contamination among fresh produce. Research has suggested the type of produce grown may determine the prevalence of pathogen.

In a recent study, *Salmonell*a spp. contamination among radish, turnip, and broccoli is significantly higher than lettuce or tomatoes grown in contaminated soil (Critzer & Doyle, 2010). Research suggests that *Salmonella* spp. have reduced attachment to the phyllospheres of lettuce

and tomatoes causing lower occurrences in contamination (Critzer & Doyle, 2010). Strong biofilming producing strains are proposed to have a higher ability to attach to fruits and vegetables compared to weak biofilm producers. Flagella have proven to be a mechanism for pathogenic attachment among fresh produce.

Research using romaine lettuce has shown that *E. coli* has a stronger preference for the interior of the leaves during early development as oppose to those that have matured (Greb, 2008). The findings are believed to be a result of the younger plants higher exuded level of nitrogen and carbon. Food borne pathogens can use these compounds as nutrients to enhance growth and proliferation. Microbial contamination can be introduced at several sources among the environment. Contaminated water irrigation, improper composting of soil, insects, and short periods of field replanting, and plant injury are some of the possible causes for crop contamination (Greb, 2008). Lettuces injured or damaged during harvesting supported growth of *E. coli* O157:H7 as well as generic *E. coli*. (Seymour & Appleton, 2001). Fresh fruits and vegetables that had soft rot also aided in *Salmonella* and *E. coli* O157:H7 contamination. *Salmonella* contamination occurred twice as much in fresh produce that had rot-producing organism compared to healthy produce (Critzer & Doyle, 2010).

2.5.2 Produce Disinfectant Methods. Surface disinfectants are commonly used to kill these enteric pathogens among the surface of fresh produce before consumption. However pathogenic microbes are able to thrive internally within plants through natural openings such as the stomata or damaged areas of the plant's phyllosphere or rhizosphere (Critzer & Doyle, 2010). Phyllosphere encompass the plant surface embodies the leaf surface anchored below the soil (U.S Food and Drug Administration, 2009). *E.coli* O157:H7 have the ability to survive within

the stomata, surface of the trichome, and crevices of lettuce even after treatment with 200 ppm chlorine (Critzer & Doyle, 2010).

Although *E. coli* O157:H7 has a low infectious dose, the severity of illness intensifies with amount consumed (Luo & McEvoy, 2010). Storage temperatures are an important factor in affecting the quality in produce and microbial growth. Studies indicate that microbial growth among fresh tomatoes and melons are strongly associated with elevated storage temperatures. In order to prevent temperature abuse during processing fresh cut tomatoes and melons are required by the FDA to be maintained in a refrigerated environment of 5 º C or less (Luo & McEvoy, 2010). Storage temperature of 1° to 3º C is recommended to maintain quality and reduce pathogenic risk (Luo & McEvoy, 2010). Lettuce inoculated with *E. coli* O157:H7 show significant population increase when stored at 12° C whereas no significant growth is detected on lettuce at storage temperatures of 5° C (Luo & McEvoy, 2010). The rate of physiological deterioration and microbial growth generally decrease in low temperature environments. However, research indicates that lettuce inoculated with *E. coli* O157:H7 maintained high visual quality for the first 3 days of storage indicating that pathogenic growth can occur while package still appears acceptable for consumption.

Food and Drug Administration confirmed that from 1996 to 2008, eighty two foodborne illness outbreaks were associated with the fresh produce consumption (2009). Thirty four percent of these outbreaks were linked to leafy green produce that accounted for 949 illnesses and 5 deaths (U.S Food and Drug Administration, 2009). The foodborne pathogens predominately associated with these produce related outbreaks were *Escherichia coli* O157:H7 and *Salmonella* spp. . United States health officials have proposed that foodborne illnesses associated with fresh produce are largely due to animal origin pathogens (U.S Food and Drug Administration, 2009).

Contamination of produce can occur during harvesting, postharvest handling, processing, shipping or marketing. During pre-harvesting phase, *Listeria monocytogenes* is a prevalent pathogen within soil (Beuchat, 2006) . Common factors that link animal based pathogenic microbes to fresh produce contamination are type of tillage, crop variation, and improper use of manure (via treatment, storage, and processing methods; FDA, 2009). In addition, contaminated wash water in the processing facility, irrigation water contaminated with runoff from areas grazed by animals, and drip or splash from contaminated floors, drains, overhead pipes or cooling system are major challenges that contribute to fresh produce contamination (Zagory, 1999).

2.5.3. Good Agricultural Practices (GAP). Good Agricultural Practice systems (GAP) have been recommended to reduce fresh produce contamination during harvesting, cultivation, packaging, and storage. The U.S National Advisory Committee on Microbiology Criteria for Foods recommends establishing GAP guidelines for reduction in fresh produce pathogen. These guidelines are prerequisites for the Hazard Analysis and Critical Control Point (HACCP) plan on farm levels (Yoon, et al., 2010). Under the GAP system produce growers are advised on appropriate treatments to reduce pathogenic levels, application of manure, and animal feces. Treatments to reduce pathogen levels involve a variety of methods. Growers may use organic farm materials or supplies for passive or active treatments.

A passive treatment is dependent upon environmental factors, such as temperature, moisture, and ultraviolent irradiation in conjunction with time to minimize microbial hazards (FDA, 1998). Manure is fully aged and decomposed before applying to fields with the passive treatment method (FDA, 1998). The aging period for manure is dependent upon regional and seasonal climatic conditions and source of manure. Active treatment methods include

pasteurization, heat drying, anaerobic digestion, alkali stabilization, and aerobic digestion in combination or independently (FDA, 1998). Active treatment known as composting is generally used against microbial hazards in raw manure (FDA, 1998). Composting a controlled process by which organic substances are aerobically or anaerobically digested. The high temperatures produced by this method are capable of eliminating most pathogens in a few days. Growers can ensure adequate treatment by turning outside edges into the center of compost piles to prevent pockets that do not receive treatment and risk re-contaminating the entire batch. Growers purchasing treated manure are advised to obtain specification sheet for each shipment from the supplier. Specification sheets should contain information about the method of treatment for the manure purchased. Expert assistance for handling manure may be available through agricultural colleges or cooperative extension services.

In conjunction with methods of manure treatment, appropriate handling and applications may promote further decrease in microbial contamination of fresh produce. Growers are advised to review existing practices to identify potential contamination sources. Treatment and manure site should be located as practically far as possible from produce handling areas to prevent risk of microbial hazard (FDA, 1998). The necessary distance is determined by the farm layout, slope of land, runoff controls, rainfall amount, wind flow, the quantity, and containment of manure. Physical barriers are recommended for manure storage and treatments sites where runoff or wind spread may pose a concern (FDA, 1998). Covering manure piles under a roof or a form of covering prevents contact with rainfall resulting in possible microbial contaminated leachate (FDA, 1998).

Farming equipment can also be potential pathogenic hazard if in contact with untreated or partially untreated manure and used in produce fields. Equipment should be cleaned with high

pressure water or steam sprays prior to fresh produce contact (FDA, 1998). Raw untreated manure applied to produce fields holds a higher risk for contamination than treated manure, and should be incorporated into soil before planting. This process may reduce pathogens through competition with soil microorganisms (FDA, 1998). Health officials do not recommend untreated manure application to produce fields during the growing season prior to harvest (FDA, 1998). Researchers have indicated that hazardous microbes may survive in untreated manure for a year on longer depending on the environmental conditions (FDA, 1998). Thus growers are advised to prolong manure application to produce areas to the greatest extent possible (FDA, 1998). These recommendations are also given to treated manure might not kill pathogenic microbes. Fresh produce farmers may need to consider animal waste from adjacent fields, waste storage facilities, and wildlife, especially is produce is grown in a low lying field. Precautions can include physical barriers such as ditches, mounds, sod waterways, and vegetative buffer areas (FDA, 1998). In general, food safety officials encourage growers to follow these GAP guidelines to minimize direct and indirect manure contact with produce.

2.5.4. Industry Sanitizers. Sanitizers used to wash or assist in antimicrobial activity are regulated by the U.S Food and Drug Administration in accordance to the Federal Food, Drug and Cosmetic Act. Sanitizers have proven effective in reducing pathogenic populations; however sensory quality is most likely to be compromised during the process. Effective chemical sanitizing agents have the ability to kill microorganism within a specific time. The most common sanitizers used during fresh produce processing are chlorine, chlorine dioxide, organic acids, and surfactants.

Within the U.S, chlorine is the most widely used sanitizing compound in the fresh produce industry because it is inexpensive, provides rapid antimicrobial results, and easy to

apply. Within the fresh produce industry chlorine chemical agents are generally used in washed and sprays. Research indicates hypochlorous acid is most effective form of chlorine currently (Luo, et al., 2011). Within recent years researchers investigated the efficacy of sodium hypochlorite and peroxyacetic acid sanitizers to reduce *Listeria monocytogenes* and *Escherichia coli* O157:H7 on shredded iceberg lettuce and residual wash water (Baert, et al., 2009). Freshly cut iceberg lettuces were inoculated with 3 strains of *L. monocytogenes* and 3 strains of *E.coli* O157:H7 at two different concentration levels. The first experimental group inoculated cut lettuce at 7 logs CFU/ml of each strain. The second experimental group inoculated cut lettuce at a lower level of 3 log CFU/ml. Both experimental groups were treated with either NaOCl and PAA solutions or tap water. NaOCl concentration levels at 20 and 200 mg/ liter and PAA solution of 80 and 250 mg/ liter were used in experimental set. Tap water or treatment solutions at 500 ml were poured into a container containing 50 g of inoculated cut lettuce. After approximately 5 minutes of contact with treatment or tap water on a shaken platform, inoculated lettuce were spin dried for 1 minute. Ten grams of the inoculated lettuce were transferred for bacterial analysis.

For the first experimental group, effects of washing with tap water versus NaOCl indicated no significant difference in bacterial reduction. Researchers suggest the higher bacterial load in experimental group 1 decreased the efficacy of NaOCl. For the second experimental group, a 200 mg/ liter of NaOCl resulted in a 0.61 and 0.67 mean log CFU/g reduction for *L. monocytogenes* and *E.coli* O157:H7. No significant decline was seen for 20 mg/ liter for NaOCl treatment compared with tap water washing (Liming, Zhang, Meng, & Bhagwat, 2011) . However, peroxyacetic acid had shown no major influence by the higher microbial concentration in the first experimental study. For each experimental group microbial load

reductions were evident at 80 mg/ liter and more so in 250 mg/ liter of PAA compared to tap water.

The study extended the research into wash water quality and determined that 2 to 4 log CFU of bacterial pathogens per ml were detected in tap wash water. Wash water containing NaOCl or PAA , regardless of concentration, showed no residual pathogens and viruses upon analysis (Luo, et al., 2011). The study concluded that NaOCl or PAA are necessary sanitizers in the fresh produce industry to maintain recycled wash water microbiological quality. However microbial load among produce can decontamination ability of these sanitizers. Chlorine's antimicrobial ability is dependent on its availability in water to have contact with microbes. Chlorine concentrations of up to 50 ppm results in a significant reduction in microorganisms and fecal coliforms on leafy green salads, but increased concentrations of up to 200 ppm did not indicate considerable effects (Beuchat, 2006). In order to minimize corrosion to processing equipment, chlorine based sanitizers are typically applied at pH values of 6.0 and 7.5, yet the compound is proven most effective in acidic solutions (D., Martin-Diana, J., & Barry-Ryan, 2007). In a recent study, *E. coli* O157:H7 inoculated into to fresh cut romaine lettuce, were effectively inactivated after 30 second exposure to chlorine concentration solution of 0.5mg/liter.

Unlike chlorine-based disinfectants, chlorine dioxide is not weakened by changes in pH. However, chlorine dioxide compounds are unstable and can be explosive when with increase concentration (Seymour & Appleton, 2001). Though chlorine dioxide has an oxidizing capacity up to 5 time stronger than chlorine there is no indication of a difference in efficiency between the chemical agents (Seymour & Appleton, 2001). Organic acids such as acetic, citric, succinic, malic, tartaric, benzoic, propanoic and sorbic acids are effective agents against microbial growth among produce. However pathogens that effect the gastrointestinal tract can survive low pH

conditions, thus acid stable (Seymour & Appleton, 2001). Hydrogen peroxide is an applicable sanitizing agent against biofilms and equipment surfaces. Ozone, glutaraldehyde, and quaternary ammonium are additional sanitizing agents with antimicrobial ability. Though these chemical sanitizers can be effective in reducing microbial load they must be used properly (Montville & Matthews, 2008). Organic material such soil, food, bacteria, oils on equipment surfaces can react with sanitizers decreasing the chemical agent's effectiveness. Water impurities such as iron, manganese, nitrites, and sulfides can react with sanitizing agents and reduce its effectiveness (Montville & Matthews, 2008). Produce sanitizers lack access within crevices, creases, and plant openings furthermore reducing the effectiveness of eliminating residing pathogens.

2.5.5. Produce Traceability. Timely traceability in the recall of a fresh produce implicated in the transmission of infectious disease is currently being recognized as an important step in infectious control and food safety. In a recent current event, cantaloupes distributed from a Indiana farm were linked to a growing outbreak of Salmonella (CDC, 2011d). In the U.S. Traceability, requirements allowed us to trace the contaminated produce from farm to fork in order to contain further distribution of the implicated produce.

The objectives of traceability in fresh produce includes risks management and food safety, verification and control, supply chain efficiency, quality assurance of products, and information and communication to the consumer. In 1930 congress passed Perishable Agricultural Commodities Act (PACA). Part the PACA act required recordkeeping for produce transactions for shippers selling on the behalf of farmers. These regulation established the first fresh produce traceability system for shipment (U.E Service, 2012). Over the past several years, increase awareness of foodborne illness outbreaks has brought interest to food safety and

produce tracking. The FDA has addressed these concerns with the development of the GAPs guidelines in efforts to minimize the susceptibility of fresh produce microbial contamination. Part of the guidelines focus on improving traceability. Farmers can market produce through direct consumer contact at fresh produce stands or farmers markets. Additional marketing options include selling goods to processors and food industry companies. In 2002, 86 percent of vegetables and 69 percent of fruits within the U.S were wholesaled to processing industries (U.E Service, 2012). Retailers may require produce farmers to meet the standards under GAPs guidelines as well as present third party audits for compliance verification (U.E Service, 2012). While food service industries focus attention in linking contaminated produce to the exact shipper, farmers require a higher level of accuracy to detect the source of contamination.

Recently retail and food industry have begun tracking the source of the product and the area within field the product was grown (U.S Food and Drug Administration, 2009) . The cost for establishing and sustaining traceability program for fresh produce is generally less than other food goods. Normally containers for produce are only large enough hold goods from one grower. This type of segregation minimizes the risk for tracking errors in contamination detection.

However, fresh produce poses more difficulty in tracking than processed fruits and vegetables.

There are currently two systems in place for information pertaining to produce. The first system involves physical labels on boxes and pallets used to ship produce. The second system includes documentation through electronic data entry or manual recordkeeping allows traceability between various markets. Processed fruits and vegetables carry extensive tracking identification information on labels were as fresh produce are not expected to present this same information on its surface (U.S Food and Drug Administration, 2009) (U.E Service, 2012).

Pallet tags are often used to identify package produce placed on pallets. Typically pallet tags

may include packing date, packing shift, grower, lot number, grade, size, and type of produce. By law, pallet tags are not required but are effective in investigating the source of produce contamination. Though pallet tags provide a stronger link in traceability, most fresh produce have lost their forms of identification once they reach retail shelves. Unpackaged fresh fruits and vegetables displayed in retail stores are generally anonymous. Products contained in bags, plastic containers or marked with brand logo stickers do retain some of the identification needed for trace back. The increasing popularity of ready to eat fresh cut produce, and branded produce has pushed the continuing advancement of providing information to consumers.

Shippers in general sell produce to a wide range of purchasers, including retailers, food service establishments, and buyers. Traceability can be straightforward if shippers sell directly to retailers and food service buyers since PACA requires documentation to the first buyer. When commercial buyers receive produce shipments, information is entered into the buyer's data system that tracks the variety and arrival of the product. If a trace back is needed, commercial buyers must examine their records to indicate what was in stock during the time period in question, identify the purchase order linked to the produce, and contact the shipper (U.E Service, 2012).

The last step of produce traceability is the commercial buyer to the consumer. Consumers who observe poor quality among produce before its sell by date can return the product to retailers or identify the products origin if packaged. However most consumers who become ill and contact health authorities after the perishable produce or labeled package has been discarded. If the local health department can identify the nature of the contaminated produce along with the location and date of purchase, than the commercial buyers may possibly locate the shipper. Buyers can contact the shipper for additional information about the product and grower. Though

this process still brings uncertainty in the precise grower being implicated the U.S organization are exploring ways to encourage a standardize traceability system between each stage of produce processing (U.E Service, 2012).

Effective produce traceability programs begin with assessing the targeted farming community; in order appropriately promote the usage of traceability. Knowledge on Fresh Produce Safety and Traceability Survey forms were filled out by 22 farmers in North Carolina. This survey includes demographic information and questions testing the knowledge of farmers about fresh produce safety problems throughout the country. Highest percentages of farmers are 41-60 yrs old married Caucasian males with $ 25000-$ 50000 annual income, graduated from college. Most of them think there is a fresh produce contamination problem in USA but they are not familiar with GAP, GHP, SOP, SSOP and PTS procedures and they do not apply them in their farms. Nevertheless, they are all ready to get training and apply these procedures and Produce Tracking System in those farms.

2.6 Pathogenic Prevalence in Fresh Produce Industry

Researchers have examined ways to improve the quantification of microbial contamination among certain produce in relation to soil type and irrigation methods. Microbial population in soil is known to be diverse in microorganisms.

2.6.1 Soil Testing. Studies calculate that over 6000 various bacterial microbes can be detected per gram of soil (Nannipieri, et al., 2003). Recent focal laser scanning, traditional electron microscopy techniques have provided evidence of the location of microorganism populations within soil substance (Nannipieri, et al., 2003). Most research in relation to the analysis of soil activity primarily concentrates on the potential of microbial activity as oppose to

actual activity (Nannipieri, et al., 2003). These studies are conducted under synthetic environments that lack natural occurrence (Nannipieri, et al., 2003). Currently a testing technique known as BIOLOG has become a popular means for microbiological soil assessment. This technique has proven to be rapid, and simple in usage. Weaknesses of this form of technique are the microbial changes that can occur while using this method, posing a challenge replicating consistent results. Due to the uncertainty of microbiological methods with techniques such as BIOLOG, molecular methods are commonly used to support results. Molecular techniques in soil testing can allow determination of detected species being measured (Nannipieri, et al., 2003). Extracting Deoxyribonucleic acid (DNA) from soil involves several steps for purification in order to identify active bacterial microbes. Research regarding microbial assessment in soil has been based on synthetic inoculation of soil with microorganism, chemical approaches to reduce soil microbial load, or biological methods to reduce pathogens in soil (Nannipieri, et al., 2003). Links between microbial diversity and soil functioning among produce sites are poorly understood. Several factors including temperature, air composition, sun exposure, and available water can affect soil quality among produce sites.

2.6.2. Water Testing. Established irrigation systems on produce farms have indicate significant association with produce soil quality. *Listeria* and other potential pathogenic microorganisms are known to be associated with untreated irrigation water containing raw sewage or run off from sewage treatment facilities (Beuchat, 2006). Studies have examined sewage within 2 month intervals from 1991 to 1992 and discovered 84% to 100% of sludge contained *L. monocytogenes* or *L. innocua* (Beauchat & Ryu, 1997). Drip irrigation methods have proven to reduce produce contamination risks. Previous field studies indicate combination

of drip irrigation and plastic ground covers usage has minimize microbial contamination of cucumbers (Alum, Enriquez, & Gerba, 2011).

Contaminated surface drip irrigations have shown to affect the tomato and cucumber crops above and below soil ground. The roots of these studied tomatoes and cucumbers exhibited the highest degree of contamination, followed by the leaves and fruit (Alum, Enriquez, & Gerba, 2011). In comparison, contaminated subsurface drip irrigation system did not detect pathogenic populations in either of the above ground plant surfaces (Alum, Enriquez, & Gerba, 2011). However subsurface drip irrigation had consistently resulted in contamination of the plant roots compared to the stem. This research concluded that irrigation methods are the most significant factors in contamination trends of various parts of crop plants. Analysis of the major components of produce during the study, including roots , stem, and fruit, display different risk levels for microbial contamination through irrigation water (Alum, Enriquez, & Gerba, 2011).

CHAPTER 3

Materials and Methods

3.1 Sampling Methods

3.1.1. Collection of soil and water samples. Soil and water samples were randomly collected from different locations in the fields of four small-scale tomato and leafy green produce farms located in the mountain, piedmont and coastal regions of North Carolina for both summer and fall of 2011 and 2012. Summer core samples were taken from May 1 to September 28. Fall samples were collected from October 15 to December 17. Soil samples were collected using the systematic zigzag approach across each plot in order to receive varying samples (Appendix A.1) For each section of the field (beginning, middle, and end) two samples were collected, providing a total of 6 soil samples collected at each farm site. Two 20 mL water sample was collected at the water source of each site. A sterile spatula was inserted at a 45° angle at 7.5 to 8 cm beneath the surface of the soil. The sterile spatula obtains a 15 to 20 g core sample from each section. Samples were immediately put into 50 ml sterile plastic tubes transferred in an ice chest to Food Microbiology Laboratory at North Carolina A&T State University and stored at 5ºC overnight. The processing of samples was carried out the following day.

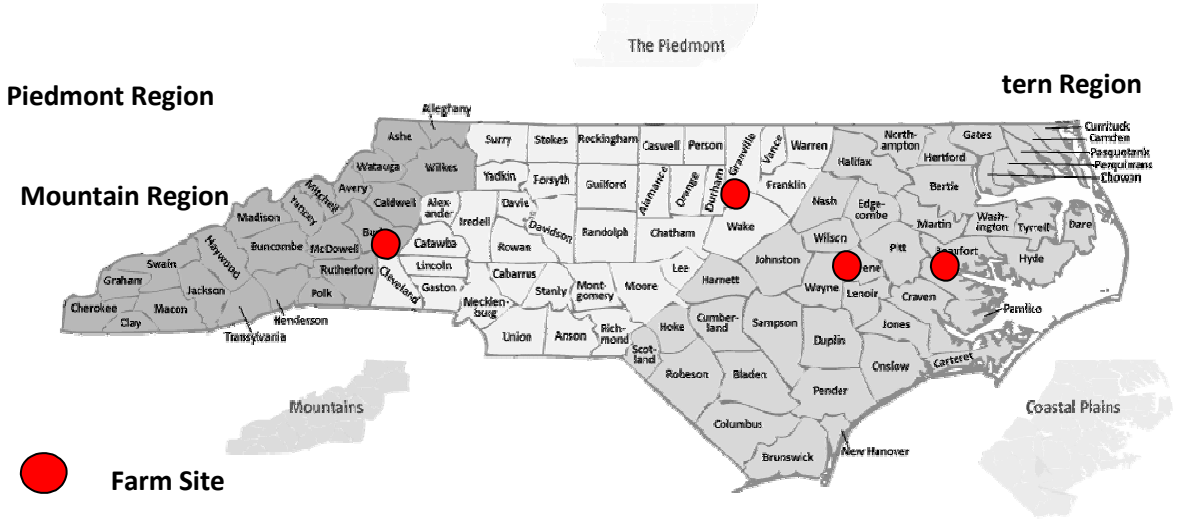

Figure 3.1. Location and region of participating fresh produce farming sites.

3.2. Verification of Pathogenic Strains with Microbiological Assay.

Quantitative colony forming unit (CFU) counts and microbial activity of collected soil and water samples were assessed using aseptic microbiological techniques. One gram of soil and 1 ml of water samples were mixed in 9 ml of sterile peptone water and appropriate dilutions were plated. Soil samples were further diluted through a series of 10 fold dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}) while water samples were diluted to 10^{-2}. Approximately 0.1 ml of each soil and water dilution were plated to medium agar; tryptic soy agar (TSA) for total aerobic count, xylose lysine Tergitol 4 (XLT4) as well as bismulth sulfite agar (BSA) for *Salmonella* spp., and MacConkey (MAC) agar for total *E. coli* species (Difco,USA). Tergitol in XLT4 inhibits non characteristic *Salmonella* spp . Salmonella fermentates xylose and lysine causing the pH to rise. When the environment within the media reaches alkaline conditions Salmonella will form red colonies due to Salmonella spp. production of hydrogen sulfide. Bacterium unable to decarboxylase lysine within the medium will undergo acidification from the sugar. This results in

a reduction in pH and lack of black pigmentation within XLT4 medium. In addition to XLT4, BSA provided further detection of *Salmonella* spp. by inhibiting the growth of gram-positive bacterial growth and provides ferrous sulfate in order for Salmonella spp to convert it to hydrogen sulfide. MacConkey agar inhibits gram-positive bacteria provides lactose which enteric bacteria can ferment. This fermentation within MacConkey media results in a pink pigmented colony formation, characteristic of pathogenic *E.coli* strains. All plates were incubated at 37ºC for 48 h. Microbial counts were expressed as Log CFU/ml. For the qualitative identification of *E. coli O157:H7* and *Salmonella enterica,* 1 gram of sample was inoculated into TSB for enrichment purposes and incubated at 37 ºC for 24h. Following the enrichment process, isolated samples underwent DNA extraction for further differentiation.

3.3. DNA Extraction from Soil and Water Samples for Molecular Assay.

To confirm microbial detections of potential *E. coli* O157:H7 or *S. enterica*, colonies were picked from TSB plates and diluted in 100 µl of sterile nanopure water for genomic DNA isolation preparation. Cultured colonies underwent particle washing with repeated centrifuging (5804R model Eppendorf Centrifuge) at 3500 rpm for 5 min. The genomic DNA was purified from homogenates by DNAzol and ethanol precipitation.

Once purification steps were completed DNA concentration was measured using a spectrophotometer (Genesys ThermoSpectronic 10uv) at 260 nanometer (nm) wavelengths. The machine is standardized prior to absorbance (A_{260}) reading by measuring blank 10mm×10mm cuvette with 1 ml TE buffer. In order to calculate the absorbance from DNA concentration, 10ul of the DNA solution from a series concentration was diluted by a factor of 0.5 in a resulting volume of 1000 ml. Cuvettes containing prepared diluted DNA samples are placed in

spectrophotometry sample holder for absorbance reading. A spectrophotometric A_{260} reading 1.0 is equivalent 50 µg/ml of pure double stranded DNA (Bunaciu, Hoang, & Aboul-Enein, 2013). Detection principles indicate that absorbance of the original DNA concentration is reciprocal to the diluted concentration and defined as:

$$\text{Original DNA concentration } \mu g/ml = 50\ \mu g/ml \times A_{260} \times \text{DNA dilution factor}$$

Each reading depicts the diluted DNA solutions and is multiplied by the 1/0.5 dilution factor ratio for the undetermined DNA concentration. A_{260} readings for each sample were determined and printed by the spectrophotometer.

3.4. Verification of Isolated Pathogenic Strains with Polymerase Chain Reaction.

The spectrophotometer calculations were used to prepare purified DNA samples for polymerase chain reaction (PCR) process. PCR amplification was carried out using *E. coli* O157:H7, *S. enterica*, and *L. monocytogene*s specific primers in a standard mix form. Table 2 shows the primer pairs selected for the multiplex PCR analysis.

Table 2

Multiplex PCR primer pairs

Microorganism	**Target gene**	**Primer Sequence**	**Size**	**Reference**
Salmonella enterica	*invA* gene (AY594274)	SAL-F: AAT TAT CGC CAC GTT CFF FCA A SAL-R: TCG CAC CGT CAA AGG AAC C	297	(Germini, Masola, Carnevali, & Marchelli, 2009)
L. monocytogenes	*prfA* gene (AY750900)	LIS-F: TCA TCG ACG GCA ACC TCG G LIS-R: TGA GCA ACG TAT CCT CCA GAG T	217	(Germini, Masola, Carnevali, & Marchelli, 2009)
E. coli O157:H7	*eaeA* gene (AF530554)	ESC-F: GGC GGA TAA GAC TTC GGC TA ESC-R: CGT TTT GGC ACT ATT TGC CC	397	(Germini, Masola, Carnevali, & Marchelli, 2009)

Samples then underwent PCR analysis using PCR kits obtained from Integrated DNA Technologies (IDT). A standard culture mixture containing *E.coli* O157:H7 strain RM 4407, *L. monocytogen*es strain 19115 and *Salmonella enterica* serovar Hadard Kentucky was used for every sample set tested during PCR analysis. There are three basic steps to PCR process that are based on amplification of specific fragments of cellular DNA. Initially double stranded DNA template is denatured to form to single-stranded pieces of DNA. Primers are utilized to amplify certain regions on the template DNA and are allowed to anneal to single stranded denatured DNA. Primers are short segments of DNA complementary to certain regions on the DNA template strand (Montville & Matthews, 2008). The final phase includes elongation and extension of the primer in order to make a complimentary copy of the DNA template (Montville & Matthews, 2008). These basic steps make up a single PCR cycle and are repeated a specified number of times.

After an initial 4 min at 94° C, 35 cycles were performed with the following steps: 1 min at 94°C, 1 min at 56°C, and 1 min at 72°C. Ten-minute extension process at 72°C followed by 4^0 C holding period concludes the final steps for optimal amplification (Germini, Masola, Carnevali, & Marchelli, 2009). The PCR products were then visualized as genetic bands on 1% ultrapure agarose gel (Invitrogen) stained with 10ul of ethidium bromide within the Fisher Scientific FB3000 gel box. Gel procedure included constant voltage at 200 volts for 23 minutes. Once time frame is completed the gel product is inserted into a UV tray on a PCR reader (BIO-RAD). Samples that displayed similar genetic bands as the referenced primer mix were then reanalyzed with PCR conditions using the specific primer that closely corresponded to the DNA bands discovered in the samples.

Prior to multiplex PCR analysis of DNA samples, appropriate testing for sensitivity was conducted for each target pathogen. Serial dilution using 24 h incubated (37° C) TSB cultures from each pathogen were prepared. Germini et al., (2009) performed serial dilutions, prior to

multiplex PCR testing, to determined detection limit among pathogen. Figure 3.2 provides the detection limit of *Listeria monocytogenes* inoculated into soil and identified through multiplex PCR application to after serial dilutions (10^9, 10^8, 10^7, 10^6, 10^5, 10^4, 10^3, 10^2, 10^1, 10, <10 cells/ ml).

3.4.1 Statistical Analysis. The data were analyzed using regression analysis (SAS, 2000). Significant differences among treatments were determined using t-test at $p< 0.05$.

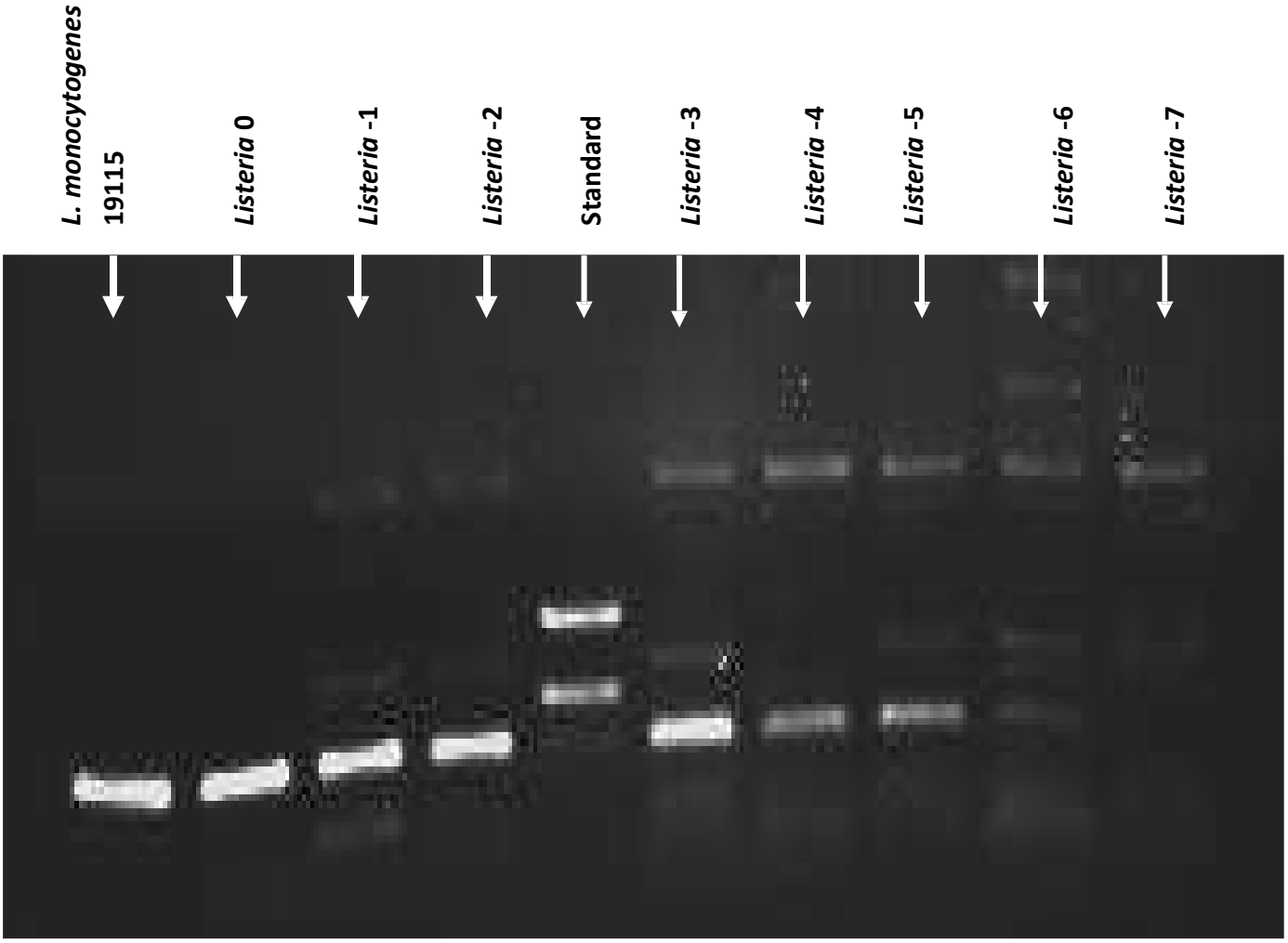

Figure 3.2. Multiplex PCR of *Listeria monocytogenes* detection limit. DNA marker *L. monocytogenes* strain 19115 is used a reference strain. (Germini et al., 2009)

CHAPTER 4

Results and Discussion

4.1. Verification of Pathogenic Strains Validity

The survival and proliferation of targeted microbial cells was assessed using total aerobic counts, *E.coli* counts, and *Salmonella* spp. counts. Samples collected for fall 2011 showed a significant differences among counts ($p<0.05$).Soil samples in the piedmont sites showed slightly higher total aerobic counts and *Salmonella* spp. counts on BSA media compared to the eastern farming locations. Coastal plain water samples also demonstrated a slight increase in all microbial counts in comparison to the mountainous region of N.C.

Collected soil samples within the summer harvesting seasons for both 2011 and 2012 showed similar averages in total aerobic counts for all regional growing areas. The piedmont farm showed slightly higher counts for yearly collected summer samples. Overall, the seasonally collected samples demonstrated marginally higher microbial growth among summer growing period.

Table 3

Total Aerobic E.coli and Salmonella Count for Log CFU/ml Fall (October-December) 2011 Samples after 48 h 37° C incubation.

Microbial Populations (Log CFU/Soil and Water Samples) on Differential Media				
Soil	**Farm 1**	**Farm 2**	**Farm 3**	**Farm 4**
Total aerobic count	6.41±0.02[a]	6.39±0.07[a]	6.83±0.39[a]	NR
E.coli count	4.88±0.04[a]	4.88±0.16[a]	5.63±0.44[b]	NR
Salmonella count on XLT4	3.60±0.18[a]	3.72±0.04[a]	5.08±0.90[b]	NR
Salmonella count on BSA	4.17±0.98[a]	4.00±0.15[a]	5.32±0.70[b]	NR
Water	**Farm 1**	**Farm 2**	**Farm3**	**Farm 4**
Total Aerobic Count	0.34±0.05[a]	2.39±0.19[b]	1.58±0.24[c]	NR
E.coli count	0.53±0.08[a]	1.90±0.09[b]	0.78±0.21[a]	NR
Salmonella count on XLT4	0.28±0.28[a]	1.85±0.13[b]	0.62±0.34[a]	NR
Salmonella count on BSA	0.28±0.28[a]	1.22±0.05[b]	0.18±0.15[a]	NR

Data with the same superscript in the raw are not significantly different ($p>0.05$).

Data with different superscript in the raw are significantly different($p<0.05$).

NR, not reported.

Farm	**Name**	**Water Type**
1	Eastern farm I	well
2	Eastern farm II	stream
3	Mountain	stream
4	Piedmont	pond

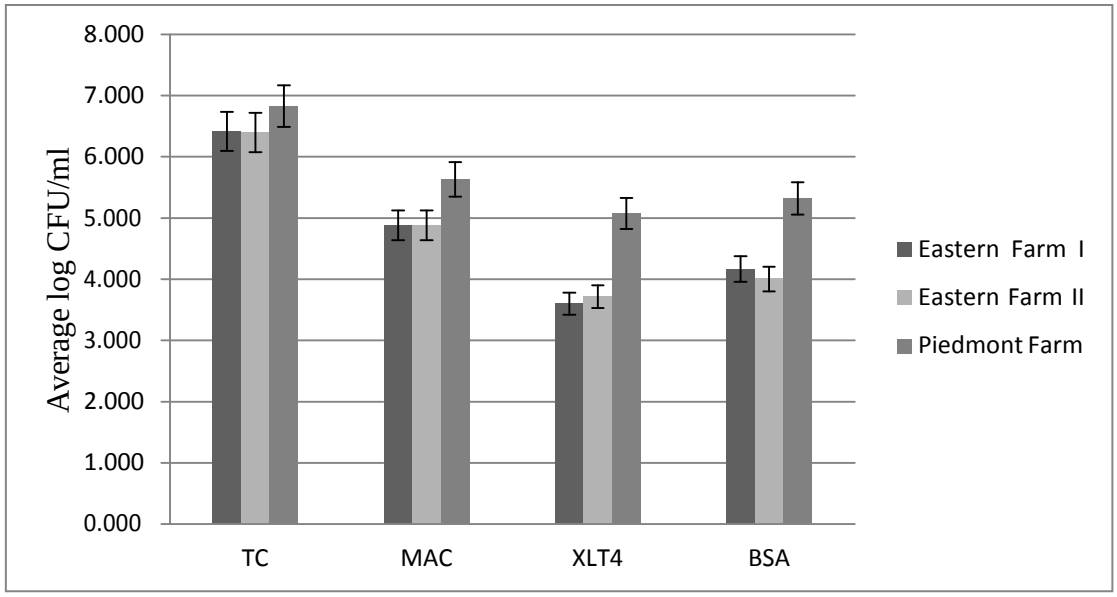

Figure 4.1. Fall 2011 total aerobic count, *E.coli* O157:H7, and *Salmonella* spp. detections of soil samples for Eastern Farm I, Eastern Farm II.

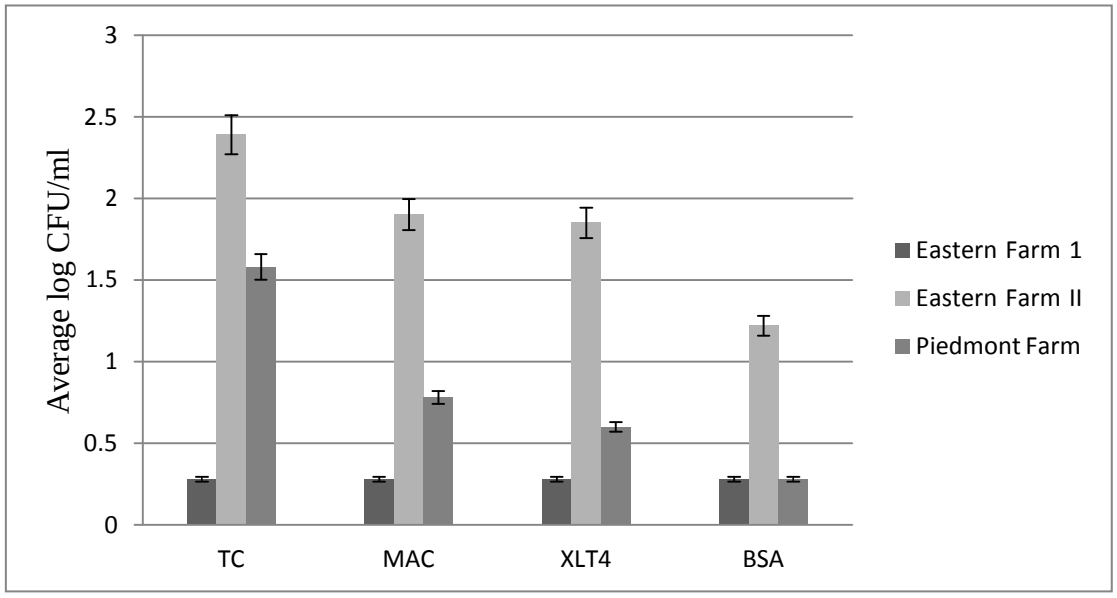

Figure 4.2. Fall 2011 total aerobic *E.coli* O157:H7, and *Salmonella* spp. detections of water samples for Eastern Farm I, Eastern Farm II .

Table 4

Total Aerobic E.coli and Salmonella Count for Log CFU/ml Fall (October-December) 2012 Samples after 48 h 37° C incubation.

Microbial Populations (Log CFU/Soil and Water Samples) on Differential Media				
Soil	**Farm 1**	**Farm 2**	**Farm 3**	**Farm 4**
Total aerobic count	6.96±0.25 [a]	4.42±0.01 [b]	6.04±0.37 [a]	6.78±0.14 [a]
E.coli count	6.26±0.09 [b]	4.36±0.06 [a]	3.52±0.11 [b]	4.73±0.58 [a]
Salmonella count on XLT4	4.10±0.36 [b]	ND	3.20±0.14 [a]	2.68±0.19 [a]
Salmonella count on BSA	2.07±0.06 [b]	3.58±0.06 [b]	3.16±0.19 [a]	3.12±0.10 [a]
Water	**Farm 1**	**Farm 2**	**Farm 3**	**Farm 4**
Total aerobic count	4.18± 0.33 [a]	1.15±0.09 [b]	0.98±0.02 [b]	4.11± 0.39 [a]
E.coli count	2.13±0.48 [b]	1.26±0.38 [b]	1.64±0.49 [b]	3.36± 0.20 [b]
Salmonella count on XLT4	1.38±0.90 [a]	0.28±0.01 [b]	0.94±0.36 [a]	2.93 ±0.02 [b]
Salmonella count on BSA	ND	0.28±0.01 [b]	0.60±0.02 [b]	2.06±0.92 [b]

[a] Data with the same superscript in the raw are not significantly different ($p>0.05$).

[b] Data with different superscript in the raw are significantly different($p<0.05$).

ND Not Detected.

Farm	Name	Water Type
1	Eastern farm I	well
2	Eastern farm II	stream
3	Mountain	stream
4	Piedmont	pond

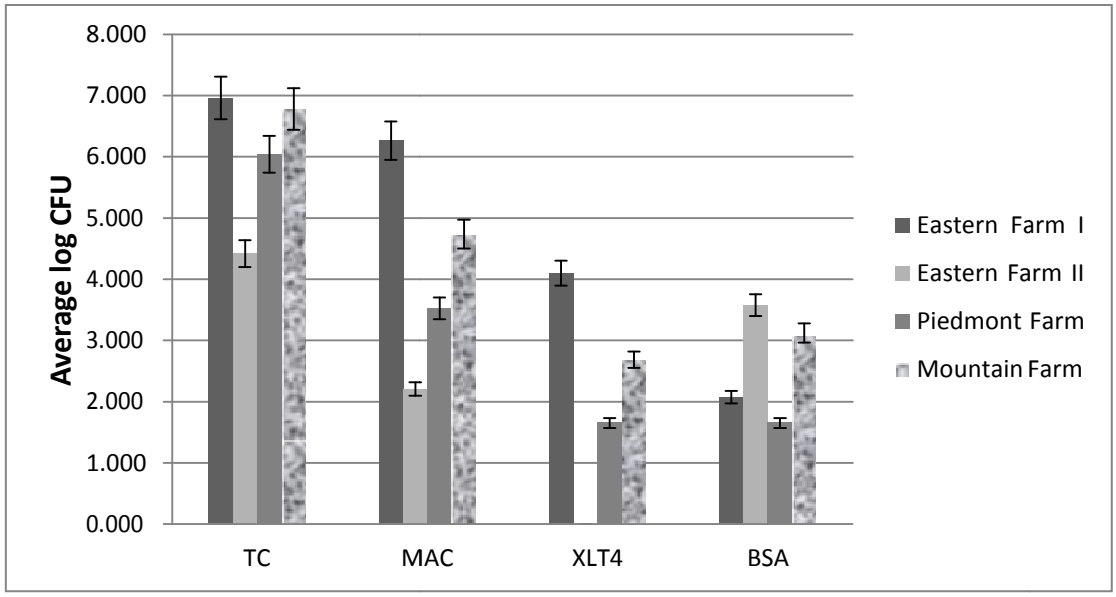

Figure 4.3. Fall 2012 total aerobic count *E.coli* O157:H7, and *Salmonella* spp. detections of soil samples for Eastern Farm I, Eastern Farm II.

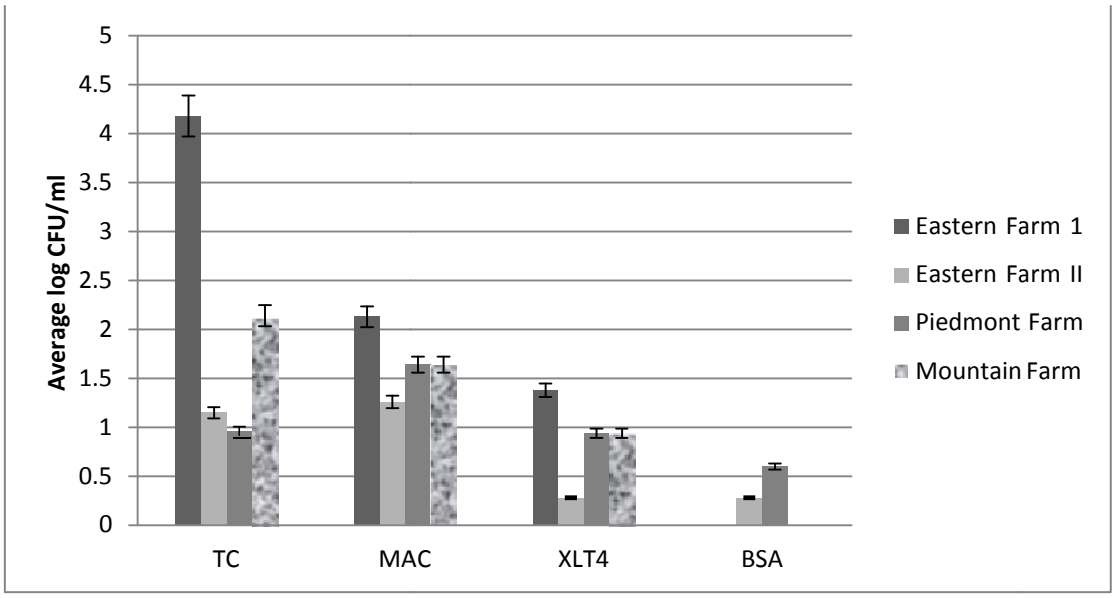

Figure 4.4. Fall 2012 total aerobic count *E.coli* O157:H7, and *Salmonella* spp. detections of water samples for Eastern Farm I, Eastern Farm II.

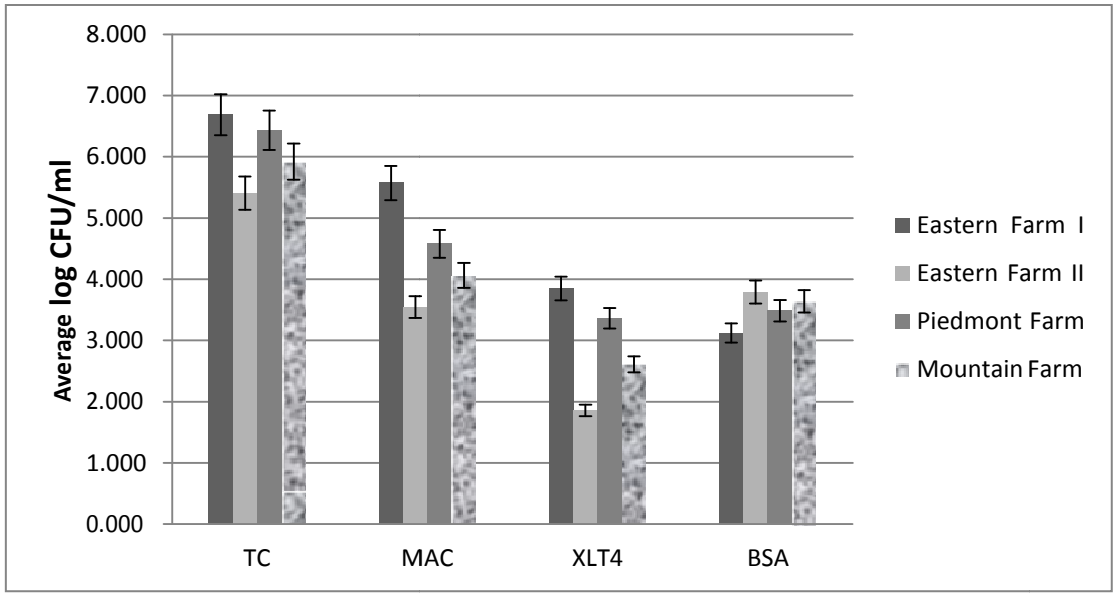

Figure 4.5. Average fall 2011/2012 total aerobic count *E.coli* O157:H7, and *Salmonella* spp. detections of water samples for Eastern Farm I, Eastern Farm II.

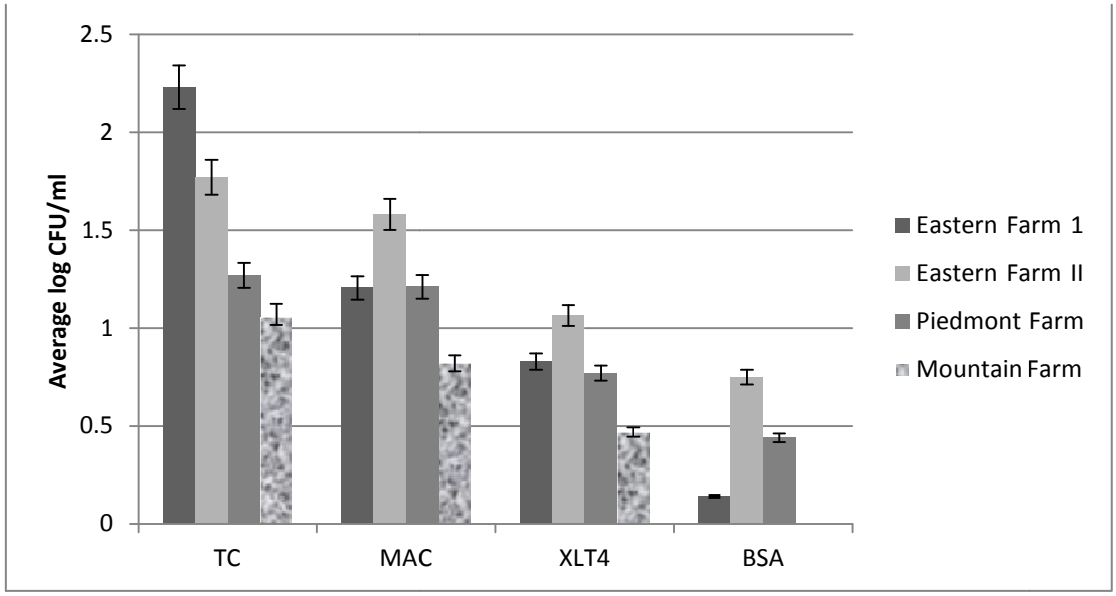

Figure 4.6. Average fall 2011/2012 total aerobic count *E.coli* O157:H7, and *Salmonella* spp. detections of water samples for Eastern Farm I, Eastern Farm II.

Table 5

Total aerobic, E.coli, and Salmonella count for Summer 2011(September) samples after 48 h 37° C incubation.

Microbial Populations (log CFU/Soil and Water samples) on Differential Media				
Soil	**Farm 1**	**Farm 2**	**Farm 3**	**Farm 4**
Total aerobic count	6.70±0.37 [a]	6.78±0.15 [a]	NR	NR
E.coli count	5.59±0.54 [a]	5.68±0.96 [a]	NR	NR
Salmonella count on XLT4	4.60±0.03 [a]	4.08±0.67 [a]	NR	NR
Salmonella count on BSA	5.15±0.14 [b]	3.91±0.19 [b]	NR	NR

[a] Raw data in the same superscript are not significantly different (p>0.05).

[b] Raw data in the same superscript are significantly different(p<0.05).

NR, not reported.

Farm	Name	Water Type
1	Eastern farm I	well
2	Eastern farm II	stream
3	Mountain	stream
4	Piedmont	pond

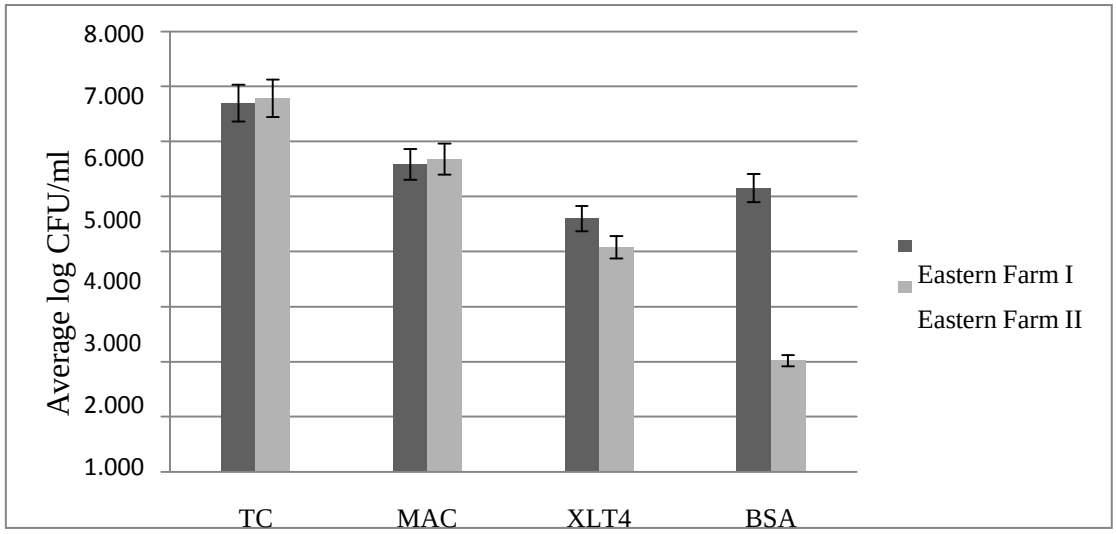

Figure 4.7. Summer 2011 total aerobic count *E.coli* O157:H7, and *Salmonella* spp. detections of soil samples for Eastern Farm I, Eastern Farm II.

Table 6

Total aerobic, E.coli, and Salmonella count for Summer 2012(May-September) samples after 48 h 37° C incubation.

Microbial Population (log CFU/Soil and Water Samples) on Differential Media				
Soil	**Farm 1**	**Farm 2**	**Farm 3**	**Farm 4**
Total aerobic count	6.71±0.01 [b]	6.07±0.14 [a]	5.75±0.78 [a]	6.00±0.34 [a]
E.coli count	5.33±0.05 [a]	4.25±0.21 [b]	4.50±0.43 [b]	5.26±0.44 [a]
Salmonella count on XLT4	4.00±0.11 [b]	3.61±0.29 [a]	3.79±0.05 [a]	ND
Salmonella count on BSA	5.05±0.07 [b]	ND	3.98±0.06 [b]	4.25±0.60 [a]
Water	**Farm 1**	**Farm 2**	**Farm 3**	**Farm 4**
Total aerobic count	0.60±0.02 [b]	2.26±0.32 [a]	2.36±0.10 [a]	2.40±0.53 [a]
E.coli count	0.28±0.01 [b]	1.69±0.26 [a]	1.67±0.28 [a]	2.35±0.62 [b]
Salmonella count on XLT4	0.28±0.02 [b]	1.68±0.02 [b]	1.00±0.05 [b]	2.63±0.59 [b]
Salmonella count on BSA	0.28±0.02 [b]	0.60±0.02 [b]	0.78±0.01 [a]	1.23±0.47 [a]

[a] Raw data in the same superscript are not significantly different ($p>0.05$).

[b] Raw data in the same superscript are significantly different($p<0.05$).

ND, not detected.

Farm	**Name**	**Water Type**
1	Eastern farm I	well
2	Eastern farm II	stream
3	Mountain	stream
4	Piedmont	pond

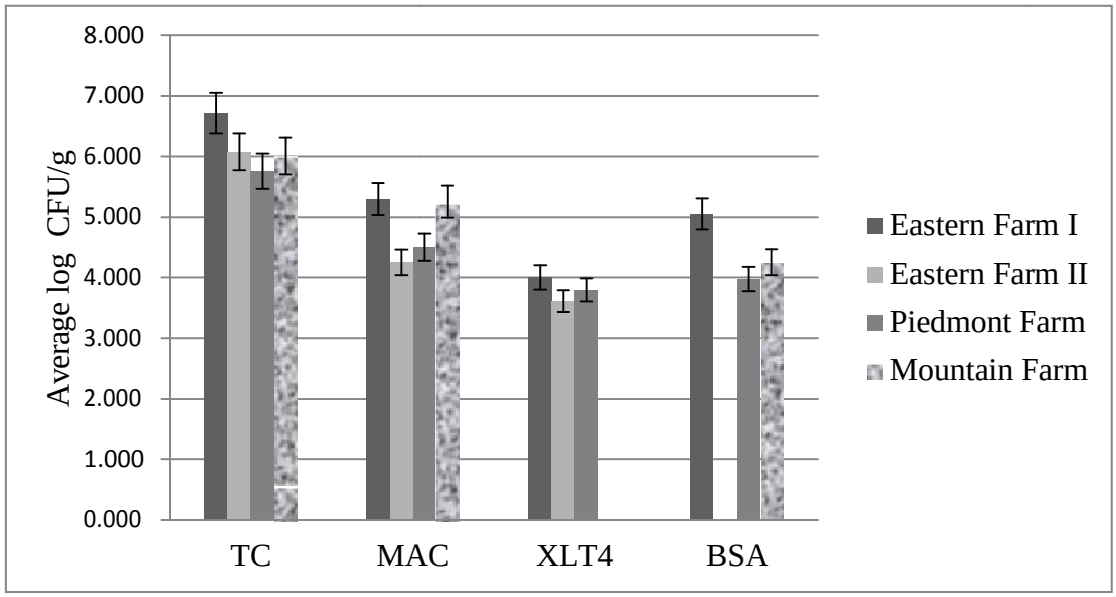

Figure 4.8 Summer 2012 total aerobic count *E.coli* O157:H7, and *Salmonella* spp. detections of soil samples for Eastern Farm I, Eastern Farm II.

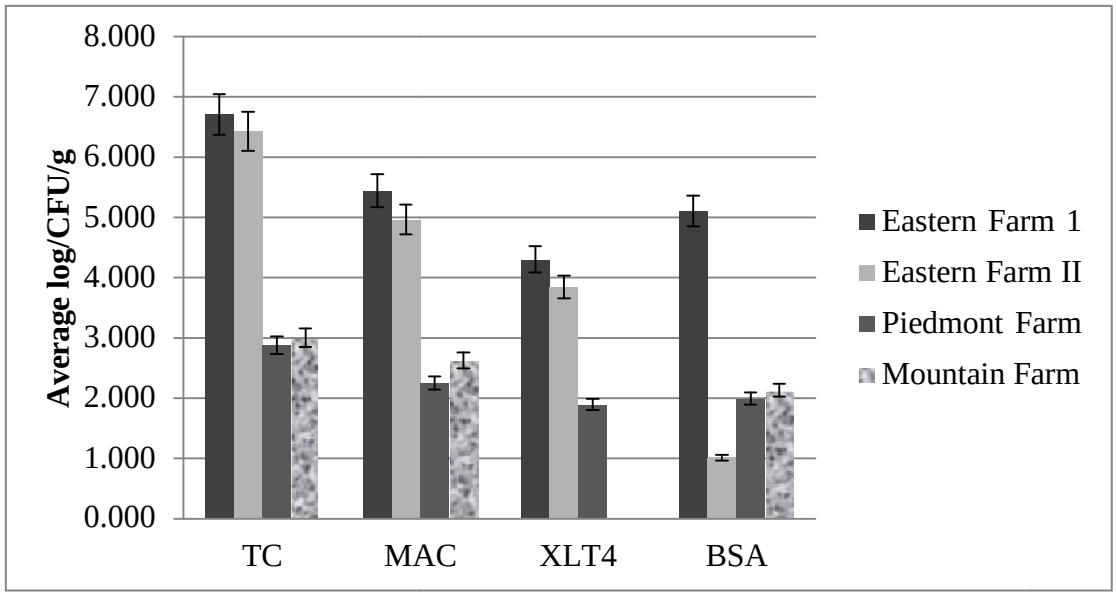

Figure 4.9. Average summer 2011/2012 total aerobic count *E.coli* O157:H7, and *Salmonella* spp. detections of soil samples for Eastern Farm I, Eastern Farm II.

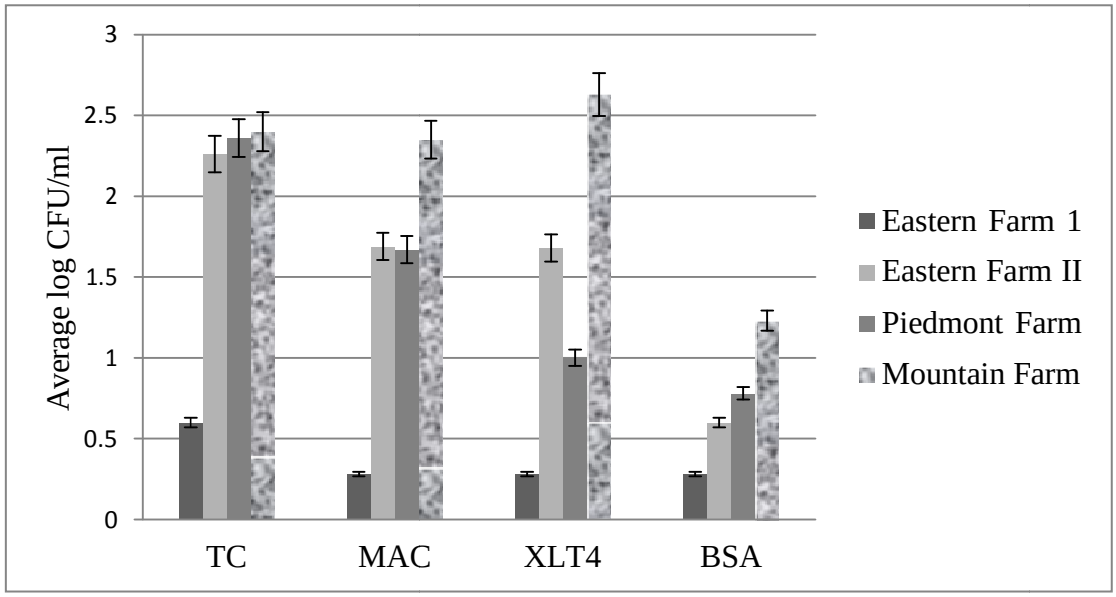

*Figure 4.10.*Summer 2012 total aerobic count *E.coli* O157:H7, and *Salmonella* spp. detections of water samples for Eastern Farm I, Eastern Farm II.

Microbial growth observed among selective media, XLT4, BSA, and MAC are assessed for targeted pathogenic characteristics. Suspected microbial pathogens among XLT4 media were found to have rigid un-uniform borders. BSA plated samples expressed black pigmentations with encaved appearance. Bacteria with rigid borders and diffusible pink pigment were presumed *Salmonella enterica* colonies.

Figure 4.11. Detection of *E. coli* O157:H7 and *Salmonella enterica* by Difco Agar MAC and BSA.

4.2. Efficacy of Polymerase Chain Reaction in verifying Pathogenic in Prepared Media

Suspected bacterial colonies were further analyzed for molecular verification. Multiplex polymerase chain reaction (PCR) was applied to verify the suspected presence of E.coli O157:H7, *Salmonella spp.*, and *L. monocytogenes* genes. Multiplex PCR base pair bands for samples were illustrated using gel electrophoresis. Visualized PCR products were compared with the standard band with multiple pathogenic base pairs for verification reference. The qualitative analysis did detect *Salmonella enterica* among two soil samples from Eastern farm I site during summer season 2011. For further positive verification both soil samples were analyzed by individual *Salmonella enterica*, *E.coli* O157:H7, and *L. monocytogenes* primers (Figure 4.12). Soil sample 2 from Eastern farm I indicated positive results with individual *Salmonella enterica* primers (Figure 4.12). Further detection of *E.coli, Salmonella spp.* and *L. monocytogenes* among all other farming sites have O157:H7 and *Salmonella enterica* in soil and water samples collected from different farms located in North Carolina during summer and fall seasons.

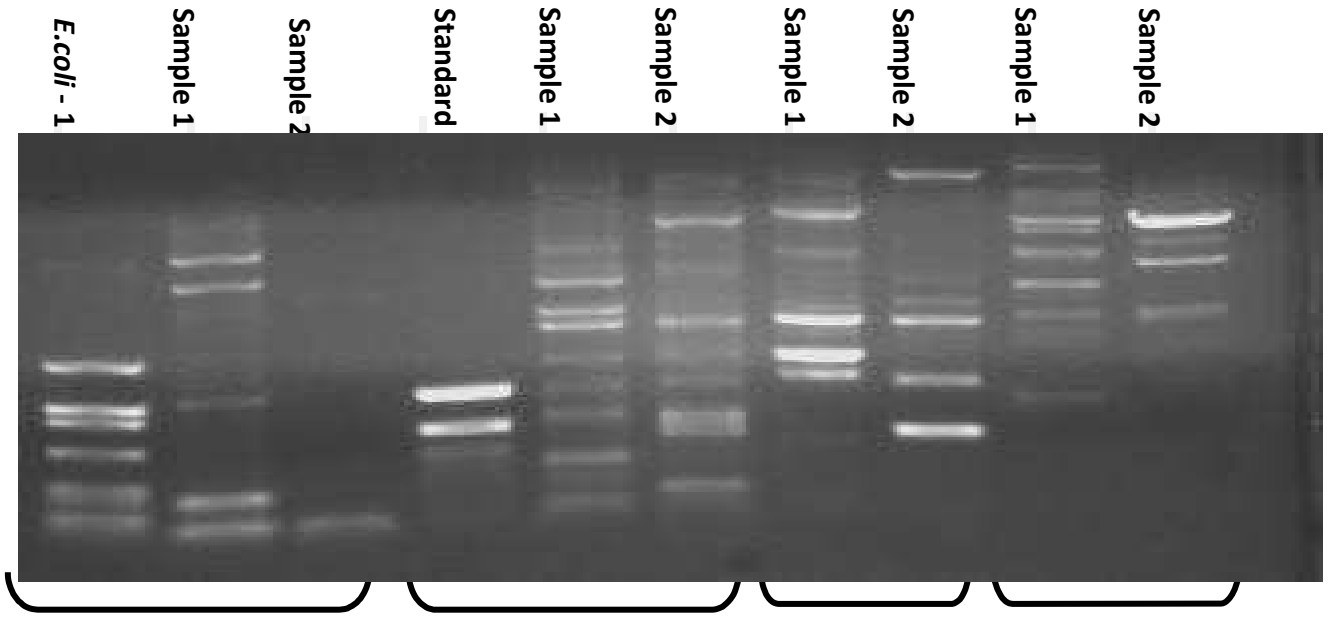

Figure 4.12. Multiplex PCR procedure for characteristic of genes in primer sets.

Targeted pathogens *Salmonella enterica, E. coli* O157: H7 and *L. monocytogenes*

primers were applied individually and simultaneously to DNA samples.

Table 7

Qualitative Identification of E. coli O157:H7 and Salmonella enterica in soil and water samples in various regional farming sites during the fall and summer months

Farms	*Salmonella enterica*		*E. coli* O157:H7	
	Summer	Fall	Summer	Fall
Soil samples				
Farm #1	(-)	(-)	(-)	(-)
Farm#2	(-)	(-)	(-)	(-)
Farm#3	(-)	(-)	(-)	(-)
Farm#4	(-)	(-)	(-)	(-)
Water samples				
Farm #1	(-)	(-)	(-)	(-)
Farm#2	(+)	(-)	(-)	(-)
Farm#3	(-)	(-)	(-)	(-)
Farm#4	(-)	(-)	(-)	(-)

The detection of *Salmonella enterica* and *E. coli* O157:H7 in water and soil samples taken among eastern farms may correlate with the intensity of microbial load within this region. Quantitative results of soil samples indicated that farms within the eastern part of N.C had the highest average microbial populations (6.70 log CFU/ml on TC, 5.44 log CFU/ml on MAC, and 4.30 log CFU/ml on XLT4) in the summer. Both water and soil samples collected from farms located in the piedmont part of the state, had the lowest microbial counts, indicating that humidity and temperature directly affect the microbial content of soil and irrigation water. Summer 2012 soil samples showed reduced total aerobic count (5.75 log CFU/ml) in comparison eastern regional farms (Eastern regional farm I 6.71 log CFU/ml). The statistical analysis showed that there is a significant difference between soil and water contamination

among various regional farming locations. Higher microbial log were observed in water samples taken from western farming region compared to additional produce site. Microbial populations reached 2.63 log CFU/ml on XLT4 medium and 2.35 log CFU/ml on MAC for western water samples taken during summer 2012.

Seasonal water and soil samples taken during the fall months indicate a higher microbial population for eastern regional farms in comparison to piedmont and western farming regions. In the fall of 2011 bacterial log CFU/ml populations were significantly higher among eastern farm II water samples (2.39 log CFU/ml in TSA, 1.9 log CFU/ml in MAC, 1.85 log CFU/ml in XLT4. Fall 2011 water samples soil samples did not show a correlation to soil samples taken during the same season. Higher microbial counts were detected in piedmont farming regions (6.82 log CFU/ml in TSA, 5.63 log CFU/ml in MAC, and 5.07 log CFU/ml in XLT4) compared to eastern farming regions. However fall 2012 samples indicated a correlation between soil and water samples with a higher *E.coli* count among eastern farm I(6.26 log CFU/ml). Soil data from the piedmont site showed one of the lowest microbial load for Salmonella spp. count and E.coli count (3.52 log CFU/ml in MAC and 1.651 log CFU/ml in XLT4). Result averages for summer 2011 and 2012 show eastern farm I with the higher soil microbial populations. Summer seasonal averages for soil show no significance in microbial load between piedmont and mountain farm regions.

Microbial populations intensity The PCR analysis confirmed the presence of *SS* and *E. coli* only in soil samples collected from a farm located in the eastern part of the state. These findings indicate that improvements are needed to avoid pathogenic bacterial contamination in fresh produce farming operations in NC and this should be carried out by training farmers on produce safety.

Initiatives towards fresh produce traceability are increasingly providing awareness to the

concern of safe food practices and sanitation. Knowledge on Fresh Produce Safety and Traceability Survey forms were filled out by 22 farmers in North Carolina. This survey includes demographic information and questions testing the knowledge of farmers about fresh produce safety problems throughout the country. Highest percentages of farmers are 41-60 yrs old married Caucasian males with $ 25000-$ 50000 annual income, graduated from college. Most of them think there is a fresh produce contamination problem in USA but they are not familiar with GAP, GHP, SOP, SSOP and PTS procedures and they don't apply them in their farms. However, they are all ready to get training and apply these procedures and Produce Tracking System in those farms.

CHAPTER 5

Conclusion

The microbial load of soil and water samples were overall higher in the Summer months than in the cooler months of the Fall, indicating that temperature is an important factor for microbial quality of fresh produce. Significant differences (p□0.05) in total aerobic, *Salmonella* spp., and total *E. coli* species were detected among soil samples between farms located in different regions in the summer. Both soil and water samples collected from the farms located in the eastern part of NC had the highest microbial load in the summer. The seasonal variations in viral contamination level could be due to the climatic changes in temperature and humidity. It was determined that water samples collected from a farm located in the eastern part of NC in summer 2011 tested positive for *E. coli* O157:H7, indicating that the adoption good agricultural practices is essential in order to improve environmental safety in this farm.

The microbial quality of water irrigation is critical to the safety of fresh fruits and vegetables. Irrigation methods among all participating farmers included drip irrigation system, which could be a factor in microbial growth and survival. Water quality or irrigation methods can be compromised due to surface water run-off from nearby animal herds or farms. Small scale farmers typically irrigate produce from nearby water sources such as ponds, streams, rivers, or wells. Eastern farming locations utilized stream or well as a water sources. Similarly, piedmont and mountain farming regions pumped well or pond water for irrigation sources.

Survey analysis of Fresh Produce Safety and Traceability questionnaire, completed by 22 North Carolina farmers, indicates that the majority of farmers know that produce contamination is a serious health concern. However, according to the survey answers, many of a farmers were unaware of hygienic and sanitation procedures such as; GAP, GHP, SSOP and PTS.

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Appendix A

Table A.1

Systematic zig-zag approach for soil sample collection.