Assessment of irrigation water quality for the Quebec horticulture industry

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Dedication

This thesis is dedicated to my late father, Harsh Kumar Gupta. I thank Him for showering all His love, care and blessings on me.

Abstract

Ready-to-eat vegetables when irrigated with untreated surface water cause risk of gastrointestinal infection to humans. Greenhouse and field studies were conducted to quantify *Escherichia coli* in the irrigation water and vegetables irrigated with untreated water. The Quantitative Microbial Risk Assessment (QMRA) model used data from the greenhouse and field studies to estimate the health risk to humans on the consumption of irrigated fresh fruits and vegetables.

The field study analyzed pathogenic *E. coli* in the irrigation water during the May-October growing seasons in 2013 and 2014 from two field sites, St-Remi and Rougemont in Quebec. In Rougemont, the maximum concentration of *E. coli* was found during the May-June period for both years. Whereas in St-Remi, the maximum *E. coli* concentration was found during the May-June and the September-October. The greenhouse study was conducted in controlled environmental conditions at the Macdonald campus to confirm the level of contamination that was transferred to fruits and soil over a 30 days' time period. The application of *E. coli* contaminated irrigation water resulted in the contamination of vegetables and of soil using four different treatments. The highest risk for lettuce was observed in the Sprinkler+Organic treatment, followed by the Sprinkler+Mineral and the Drip+Organic treatments, but risk with the Drip+Mineral treatment was observed only on the 20^{th} day. There was a risk observed in tomatoes only on the 10^{th} day in the Drip+Organic treatment.

The QMRA model used data from field experiments and the combined annual disease burden for all the pathogens was found in the range of 10^{-3} to 10^{-2} DALYs (Disability Adjusted Life Years) for lettuce and tomatoes. Whereas, the combined gastrointestinal (GI) risk was in the range of 10^{-2} to 10^{-1} and 10^{-3} to 10^{-1} for lettuce and tomatoes respectively. Comparison of the models used for

vegetables, showed that lettuce consumption would result in higher risk because ranges are greater compared to tomato consumption.

Another QMRA study was conducted using data from St-Esprit, Quebec, to confirm the quantified gastrointestinal risks when crops were irrigated with untreated surface water. Drip irrigation showed less risk (1.8×10^{-8} to 3.9×10^{-4} DALYs) than sprinkler irrigation (9.8×10^{-8} to 2.1×10^{-3} DALYs) across all scenarios. Washing fresh vegetables for 2 min showed the least risk (10^{-8}-10^{-5} DALYs) as compared to washing vegetables for 3-4 sec (10^{-8}-10^{-4} DALYs) and to no washing (10^{-7}-10^{-3} DALYs). Among five vegetables, lettuce showed the highest risk compared to tomatoes, squash (zucchini), cauliflower and broccoli.

Therefore, it is recommended to wash fresh vegetables for 2 min prior to consumption; and use drip irrigation and mineral soil to grow the vegetables to be eaten raw.

Résumé

Les légumes prêts à consommer, lorsqu'ils sont irrigués avec de l'eau de surface non traitée, causent un risque gastro-intestinal pour les humains. Des études en serre et sur le terrain ont été menées pour quantifier *Escherichia coli* dans l'eau d'irrigation et les légumes irrigués avec de l'eau non traitée. Le modèle quantitatif d'évaluation des risques microbiens (QMRA) a utilisé des données des études de serre et de terrain pour estimer le risques pour la santé des humains sur la consommation de fruits et légumes frais irrigués.

L'étude de terrain a analysé *E. coli* pathogène dans l'eau d'irrigation au cours des saisons de croissance de mai à octobre en 2013 et 2014 sur deux sites de terrain, l'un à St-Rémi et l'autre à Rougemont, au Québec. À Rougemont, la concentration maximale d'*E. coli* a été observée pendant la période de mai-juin pour les deux années. Alors qu'à St-Remi, la concentration maximale d'*E. coli* a été observée pendant les mois de mai-juin et septembre-octobre. L'étude en serre a été menée dans des conditions environnementales contrôlées au campus Macdonald afin de confirmer le niveau de contamination transféré aux fruits ainsi qu'au sol sur une période de 30 jours. À l'aide de quatre différents traitements, l'application d'eau d'irrigation contaminée à l'*E. coli* a causé la contamination des récoltes et du sol. Le risque le plus élevé pour la laitue a été observé avec le traitement « gicleur+terre organique » suivi du « gicleur+ terre minérale » puis du « goutte à goutte+ terre organique », mais le risque associé au traitement « goutte à goutte+ terre minérale » a été observé seulement à partir du 20ème jour. Pour la tomate, un risque a seulement été observé avec le traitement « goutte à goutte+terre organique » à partir du 10ème jour.

Le modèle QMRA a utilisé des données provenant d'expériences sur le terrain et la charge annuelle de morbidité combinée pour tous les pathogènes a été estimée entre 10^{-3} et 10^{-2} AVAI

(années de vie ajustées sur l'incapacité) pour la laitue et la tomate. Tandis que le risque combiné de maladies gastro-intestinales se situait dans les fourchettes de 10^{-2} à 10^{-1} pour la laitue et 10^{-3} à 10^{-1} pour la tomate. La comparaison entre la modélisation de la laitue et de la tomate a démontré que la consommation de laitue entraînerait un risque plus élevé puisque la fourchette est plus étendue que celle liée à la consommation de la tomate.

Une autre étude de QMRA a été menée à l'aide des données de St-Esprit, au Québec, afin de valider les risques de maladies gastro-intestinaux quantifiés lorsque les cultures étaient irriguées avec de l'eau de surface non traitée. Pour tous les scénarios, l'irrigation goutte à goutte a montré moins de risques (1.8 $X10^{-8}$ to 3.9 $X10^{-4}$ AVAI) que l'irrigation à l'aide de gicleurs (9.8 $X10^{-8}$ to 2.1 $X10^{-3}$ AVAI). Laver les légumes frais pendant 2 minutes minimise le plus les risques (10^{-8}-10^{-5} AVAI) lorsque comparé à un lavage de 3-4 secondes (10^{-8}-10^{-4} AVAI) ou ne pas les laver du tout (10^{-7}-10^{-3} AVAI). Parmi les cinq légumes suivant: la tomate, la courge (courgette), le chou-fleur, le brocoli et la laitue, cette dernière présente le plus haut risque de transmission de maladie.

Par conséquent, il est recommandé de laver les légumes frais pendant 2 min avant la consommation; et d'utiliser l'irrigation goutte à goutte et un sol minéral pour faire pousser les légumes à manger crus.

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Contribution of authors

All manuscripts presented in this dissertation were authored by Divya Gupta (first author) and Dr. Chandra A. Madramootoo (second author, supervisor). Divya Gupta designed all of the experiments, conducted the field and greenhouse work, laboratory analysis of field and greenhouse samples, preparation of *E. coli* inoculated water for greenhouse study, testing for *E. coli* in water, soil, lettuce leaves and tomato fruits, development of MATLAB code for risk modelling. She also performed all the statistical analyses, risk modelling and wrote all the manuscripts. Dr. Chandra A. Madramootoo, the second author, supervised the research, provided financial support for this project, and guidance on various aspects of experimental design and reviewed all the manuscripts.

Table of Contents

List of Tables

List of Figures

Acronyms

ATCC American Tissue Culture Collection

BD Becton, Dickinson and Company

CCME Canadian Council of Ministers of the Environment

CDC Centers for Disease Control and Prevention

DALYs Disability Adjusted Life Years

DOC Dissolved Organic Carbon

E. coli Escherichia coli

eFOSS Electronic Foodborne and Non-Foodborne Gastrointestinal Outbreak Surveillance System

EMB Eosin Methylene Blue

ETo Reference Evapotranspiration

FAO Food and Agricultural Organization

FBDO Foodborne Disease Outbreak

FDA Food and Drug Administration

GI Gastrointestinal

HT Health Target

HUS Haemolytic Uraemic Syndrome

LB Luria Bertani

MAPAQ Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec

MPN Most Probable Number

NACMCF National Advisory Committee on Microbiological Criteria for Foods

NESP National Enteric Surveillance Program

NPK Nitrogen Phosphorus Potassium

ONPG o-nitrophenyl- b-d-galactopyranoside

PHAC Public Health Agency of Canada

PHE Public Health England

pppy per person per year

QMRA Quantitative Microbial Risk Assessment

RTE Ready to Eat

STEC Shigatoxigenic Escherichia coli

Stx Shiga toxin

TSB Tryptic Soy broth

TSS Tryptone Saline Solution

USEPA United States Environmental Protection Agency

VTEC Verotoxigenic Escherichia coli

WHO World Health Organization

NaCl Sodium Chloride

$MgCl_2$ Magnesium Chloride

KH_2PO_4 Monopotassium Phosphate

CFU Coliform Forming Unit

CFU/ml Coliform Forming Unit per milliliters

log CFU/ml logarithm value of (Coliform Forming Unit per milliliters)

log CFU/g logarithm value of (Coliform Forming Unit per gram)

Chapter 1: General Introduction

1.1 Background

Over the last few decades, food and water safety have emerged as major global concerns. The consumption of fresh fruits and vegetables has been reported to cause various gastrointestinal illnesses and other diseases (Beuchat 2002). Excluding chemical agents and metal toxicity, the possible reason for the gastrointestinal illnesses and other diseases could be the presence of pathogenic microorganisms in fresh fruits and vegetables (Beuchat 2002). Fresh fruits and vegetables could get contaminated with pathogenic microorganisms while growing in the fields or during distribution and preparation, or cooking at home (CDC, 2015). According to the World Health Organization (2003), point and nonpoint sources of pollution such as municipal sewage waste, runoff from agricultural fields, and fecal materials, not only impair the quality of water for drinking, recreation, aquaculture and irrigation purposes, but also pose significant health risks to people, and affect environmental health by contributing to pathogenic contamination.

Public Health Agency of Canada (2011) reported that, every year, 11 million people in Canada suffer from food-related illnesses. These illnesses can show minor symptoms such as nausea, vomiting and diarrhea. However, some of these cases are parts of outbreaks or sporadic cases of foodborne illnesses. In recent years, an average of about 440 cases of *E. coli* infection have been reported annually in Canada.

Contaminated irrigation water, contaminated manure (fertilizer) applied to fruits and vegetables are the potential carriers of the pathogens, which then affect consumers. Islam et al. (2004) reported that contaminated manure can contact the produce directly or indirectly. Many countries are predominantly using untreated surface water, wastewater and groundwater for irrigation,

without the prior monitoring of pathogens or fecal coliforms. Contaminated soil used to grow fruits and vegetables can also be the carrier of pathogenic organisms. These pathogens in soil can move into the roots and through the vascular system, and may transfer to the fruit. This is known as internalization of the pathogen (Hirneisen et al., 2012). Buck et al. (2003) reported that runoff from cattle feedlots can also be a potential source of contamination. Various environmental factors such as light intensity, moisture, irradiation and high temperature significantly influence the growth of pathogens (Oliveira et al., 2011).

1.2 Problem definition

Irrigation water supplies may become contaminated through runoff from cattle feedlots, sewage wastes, agricultural runoff, sewer overflows, leakages or sewer drainage, and from fecal materials from domesticated animals and wildlife. Irrigation water is less intensively monitored than drinking or recreation water. Therefore, there is a need to understand the quality of irrigation water supplies for fecal indicators and *Escherichia coli (E. coli)*. The *E. coli* organism is an indicator of fecal pollution and is usually harmless, but some strains of *E. coli* can result in gastrointestinal problems and serious complications which may lead to kidney failure or death.

When untreated surface irrigation water is applied to RTE vegetables, consumers are at risk of infection. The risk to consumers has to be quantified in order to mitigate the causes of infection and manage the risks due to RTE vegetables. The fate and transport of pathogens in RTE vegetables using different irrigation methods and crops grown in different soil types has not been studied in Quebec. Climatic conditions, water quality, irrigation method, soil type and soil microflora in Quebec vary from those of studies conducted elsewhere on the risk quantification

for the consumption of RTE vegetables. Therefore, the overall picture of risk quantification is important for people consuming RTE vegetables in Quebec. RTE vegetables (such as lettuce, spinach, leafy greens) are mostly eaten raw or in salads in many parts of the world. When these vegetables are irrigated with untreated water the consumption can lead to foodborne illnesses and outbreaks, due to carrying contaminants or pathogens to consumers. The main problem is that farmers are using untreated wastewater for irrigation without prior monitoring of pathogens, which can lead to contaminant transfer from water to plants and from plants to consumers i.e. through farm to fork continuum. In order to quantify the risk to consumers, the pathogen concentrations in water and on edible parts of the plants must be known, and this necessitates risk assessment modelling in water and crops. Models such as Quantitative Microbial Risk Assessment for a particular crop give an idea of the probable risk of illness or foodborne infection. The QMRA model on irrigation water and RTE vegetables has not been run in Quebec to the best of our knowledge. However, the QMRA model has been applied at 17 Canadian water treatment facilities (Tfaily et al., 2015). There have been a few studies conducted in Quebec to assess the public health risks of microbial contamination in recreational water, using satellite imagery (PHAC, 2015).

1.3 Research Objectives

The overarching goal of this research project was to quantify the risk to humans of the consumption of fresh vegetables which could be contaminated when irrigated by untreated water. This study utilizes the QMRA modelling approach in order to assess the risk of pathogens from the consumption of fresh fruits and vegetables. This goal was attained through the following specific objectives:

I. To study the quality of untreated irrigation water for two years (2013-2014) at two field sites, St-Remi and Rougemont, producing lettuce and tomatoes, respectively.

II. To analyze the fate and transport over a time period of 30 days, of the bacterial contaminant (*E. coli*) on lettuce and tomatoes, when irrigated by known amounts of *E. coli* in irrigation water. These crops were grown under four different treatments comprised of two soil types (organic and mineral) and two irrigation methods (drip and sprinkler) in the greenhouse of Macdonald Campus, McGill University.

III. To develop the QMRA model based on the field and greenhouse studies in order to estimate the potential risk of three pathogens (pathogenic *E. coli*, *Campylobacter* and *Rotavirus*) in humans due to the consumption of lettuce and tomatoes irrigated with untreated water at two field sites.

IV. To estimate the annual disease burden in humans consuming contaminated fruits and vegetables grown under four different treatments (comprised of drip or sprinkler irrigation and mineral or organic soil) in the greenhouse and harvested after the 10^{th}, 20^{th} or 30^{th} day of the inoculation.

V. To estimate the pathogenic risk through consumption of fresh lettuce, tomato, broccoli, cauliflower and squash (zucchini), sprinkler or drip irrigated with untreated surface water at Saint-Esprit, Quebec. The risk was quantified based on three different scenarios: washing vegetables for 3-4 sec prior to consumption, for 2 min prior to consumption, or not washing at all.

1.4 Thesis Organization

This dissertation comprises nine chapters arranged as follows:

Chapter 1 General introduction: provides a general background on the foodborne illnesses and their causes, defines the research problem and objectives of this project, and outlines the organization of the thesis.

Chapter 2 Literature review: provides a synopsis of literature on foodborne illnesses and diseases' occurrence in Canada and globally, past knowledge of the contaminated irrigation water, causative pathogens, and risk assessment studies using the QMRA modelling approach.

Chapter 3 *Escherichia coli* contamination on Ready-To-Eat (RTE) vegetable, lettuce with different soil types and irrigation methods: This chapter describes the fate and transport of *E. coli* on lettuce leaves and soil over a time period of 30 days. A known amount of bacterial inoculum in irrigation water was given to lettuce crops, grown under four different treatments comprised of organic and mineral soil types; and drip and sprinkler irrigation methods. This chapter addresses the second objective of the research project.

Chapter 4 Fate and transport of *Escherichia coli* in Tomato production: The fate and transport of *E. coli* was analyzed on the tomato fruits and in the soil over a period of 30 days in the greenhouse. These tomato plants grown under four different treatments were irrigated with laboratory prepared contaminated water. This chapter addresses the second objective of the project.

Chapter 5 QMRA model associated with the consumption of contaminated lettuce and tomatoes grown in the greenhouse and at two field sites: This chapter addressed the first, third and fourth objectives of this research project i.e. potential risk to humans can result from the consumption of contaminated fruits and vegetables grown under four different treatments and harvested after the 10^{th}, 20^{th} or 30^{th} day of inoculation, and irrigated with untreated surface water at the field

sites (Rougemont and St-Remi) for over two years 2013-2014. The QMRA model was used to estimate the annual disease burden due to pathogens such as *Escherichia. coli*, *Campylobacter* spp. and *Rotavirus*.

Chapter 6 Scenario analysis study using QMRA for the consumption of ready-to-eat (RTE) vegetables: This chapter addresses the fifth and last objective of this research project i.e. to estimate the pathogenic risk through consumption of fresh lettuce, tomato, broccoli, cauliflower and squash (zucchini), sprinkler or drip irrigated with untreated surface water at Saint-Esprit, Quebec. Also, the risk was quantified for humans based on scenarios including washing of vegetables for 3-4 sec or 2 min prior consumption or no washing.

Chapter 7 General summary and conclusions: The general summary and conclusions resulting from this research are presented in this chapter, as are the contributions towards the improvement of scientific knowledge as well as suggestions for future research.

Chapter 8 Bibliography: Presents all of the literature cited in this dissertation.

Appendices: This chapter contain eight appendices.

Chapter 2: Literature Review

2.1 Source of contamination

There are a number of factors responsible for epidemiological foodborne outbreaks or illnesses associated with fresh fruits and vegetables. Due to the presence of pathogenic microorganisms, consumption of leafy vegetables, fresh fruits and vegetables leads to gastrointestinal problems and various other illnesses (Buck et al., 2003; FDA 2007; Pachepsky et al., 2011). The CDC (2000) described foodborne outbreaks as incidents when two or more persons experience a similar illness or symptoms after ingestion of a common food. The contamination can take place during any step of the farm-to-fork continuum. One of the possible reasons for the contamination could be contaminated irrigation water applied to the vegetables and fruits, or contaminated soil in the field. Leifert et al. (2008) reported that the irrigation water sources can be generally ranked by the microbial contamination hazards in order of increasing risk from potable or rain water, groundwater from deep or shallow wells, surface water and finally to fresh or inadequately treated wastewater. In many countries, surface water is the predominant source for irrigation and is mostly utilized untreated (Pachepsky et al., 2011).

Another potential source of contamination is manure (fertilizer), for example, chicken manure, which is always infected with pathogens (Pachepsky et al., 2011) or becomes contaminated with irrigation water at a later stage. Contaminated manure can, directly or indirectly, contaminate the crops through its use as a soil fertilizer. The crops can become colonized through infiltration of contaminated irrigation water or while washing the fruits and vegetables (Doyle, 1990). Runoff from cattle feedlots and application of contaminated irrigation water to soil also represents the

possible sources of contamination (Buck et al., 2003). Oliveira et al. (2011) observed that there was more contamination on the outer lettuce leaves than on the inner leaves. To support this finding, Oliveira et al. (2011) also reported that the outer leaves are more exposed to environmental conditions, therefore, more vulnerable to contamination as they are in direct contact with the soil. Some environmental factors such as light intensity, moisture, irradiation and high temperature influence the growth of pathogens, resulting in more contamination (Oliveira et al., 2011). There has been increased consumption of fresh fruits and vegetables due to convenience, and to nutritive and health benefits. These fresh vegetables and fruits, when imported from countries having lower sanitation standards, could result in heightened concern for food safety (NACMCF, 1999).

2.2 Pathogens responsible for contamination

The most common pathogens that are responsible for contamination of RTE vegetables include bacteria, viruses and protozoans like *Campylobacter* spp., enterohemorrhagic *Escherichia coli* (e.g., *E. coli* O157:H7), enterotoxigenic *Staphylococcus aureus*, *Clostridium* spp., enterotoxigenic *Bacillus cereus*, *Listeria monocytogenes*, *Salmonella* spp., *Shigella* spp., *Vibrio cholerae*, *Yersinia* spp., *Cryptosporidium* spp., *Cyclospora cayetanensis*, *Giardia* spp., *Entamoeba histolytica*, and adenoviruses, enteroviruses, noroviruses, and rotaviruses (Pachepsky et al., 2011). Pachepsky et al. (2011) also reported that *E. coli O157:H7* has been isolated from many animals such as sheep, pigs, deer and goats (Ferens and Hovde, 2011) but found that cattle are the primary reservoir of this pathogen, with a prevalence of up to 36.8% (Chapman et al., 1997) shown in Appendix 1.

2.3 *E. coli*, the microbe of interest

Escherichia coli, usually called *E. coli*, refers to a group of bacteria commonly found in the intestines of warm-blooded animals. It has been used as an indicator organism of fecal pollution in aquatic environments (Clesceri et al., 1998). *E. coli* strains are usually harmless but some of them can result in many gastrointestinal problems and serious complications that can lead to kidney failure. *E. coli* infections can be caused by contaminated food, untreated drinking water, unpasteurized milk and milk products, including raw milk cheese, and direct contact with animals at farms or zoos. Similarly, fresh fruits and vegetables can become contaminated with *E. coli* while in the field by improperly composted manure, contaminated water, wildlife or poor hygiene by farm workers.

The *E. coli* incubation period can vary between 1-10 days but symptoms such as stomach cramps, diarrhea, vomiting and fever start within 3-4 days after exposure. It can lead to hemolytic uremic syndrome (HUS) in elderly people and young children, which can be fatal. In other cases, some people have seizures, strokes, or need blood transfusions and kidney dialysis, or suffer from permanent kidney damage. It was observed that pregnant women or people with weakened immune system would be at higher risk to develop serious complications. In adults, infection generally lasts for a week or less; however, infection can last for three weeks in children. It has been observed that the *E. coli* outbreak can occur in a single or in multiple provinces. The Canadian Notifiable Disease Surveillance System tracks the total number of *E. coli* infection cases each year, which gives a better understanding of fluctuations in the number of cases over time. In the 2000-2004 period, 129 *E. coli* outbreaks and illnesses in Canada were

reported to PHAC (2013). Table 2.5 shows the incidence rates of *E. coli* from 2002 to 2011 as reported through the Agency's National Enteric Surveillance Program (NESP). Table 2.6 lists the annual national totals and rates for *E. coli* compared with other major organism groups as reported through the National Enteric Surveillance Program (NESP) from 2006 to 2011.

Kaper et al. (2004) reported that there are several highly adapted *E. coli* clones that have acquired specific virulence attributes and cause a broad spectrum of diseases. These virulence attributes are frequently encoded on genetic elements that can be mobilized into different strains, or on genetic elements that might once have been mobile, but have now evolved to become 'locked' into the genome (Kaper et al., 2004). These successful combinations of virulence factors which have persisted are called 'pathotypes' of *E. coli.* Symptoms include enteric/diarrhoeal disease, urinary tract infections (UTIs) and sepsis/meningitis. There are six well described categories among the intestinal pathogens (Kaper et al., 2004) such as:

- enteropathogenic *E. coli* (EPEC),
- enterohaemorrhagic *E. coli* (EHEC),
- enterotoxigenic *E. coli* (ETEC),
- enteroaggregative *E. coli* (EAEC),
- enteroinvasive *E. coli* (EIEC) and
- diffusely adherent *E. coli* (DAEC).

And some of the extraintestinal pathogenic *E. coli* (ExPEC) are Uropathogenic *E. coli* (UPEC), which cause urinary tract infections and Meningitis-associated *E. coli* (MNEC) causes meningitis and sepsis (Kaper et al., 2004). All the pathotypes mentioned above can cause disease in humans and animals due to virulence factors present in them (See Appendix 2).

Enteropathogenic *E. coli* (EPEC): EPEC was the first pathotype of *E. coli* to be recognized, due to large outbreaks of infant diarrhea in 1945. Attaching and effacing (A/E) is a characteristic intestinal histopathology associated with EPEC. This pathotype after attaching to intestinal epithelial cells, cause cytoskeletal changes (including the accumulation of polymerized actin directly beneath the adherent bacteria) and microvilli in the intestine are effaced and pedestal-like structures on which the bacteria perch frequently rises from the epithelial cell (Kaper et al., 2004). This ability is induced by gene on pathogenicity island (PAI) called the locus of enterocyte effacement (LEE), which encodes for the protein called intimin and this helps the bacteria in attachment to epithelial cells.

Enterohaemorrhagic *E. coli* (EHEC): EHEC was first recognized in 1982 as a cause of human disease and results in bloody diarrhea (haemorrhagic colitis), non-bloody diarrhea and haemolytic uremic syndrome (HUS). The principal reservoir of EHEC is bovine intestinal tract and resulted in outbreaks associated with the consumption of undercooked hamburgers, sausages, unpasteurized milk, lettuce, radish sprouts, etc. The key virulence factor is Stx (Shigella-like toxin or verocytotoxin, VT), produced in colon and travels by the bloodstream to the kidney and damages the renal endothelial cells and occludes the microvasculature through a combination of direct toxicity and induction of local cytokine and chemokine production, resulting in renal inflammation (Kaper et al., 2004). However, EHEC can lead to haemolytic anaemia, thrombocytopenia and potentially fatal acute renal failure, which results in haemolytic uremic syndrome (HUS).

Enterotoxigenic *E. coli* (ETEC): ETEC is an important cause of childhood diarrhea in developing countries, causing watery diarrhea ranging from mild, self-limiting disease to severe purging disease. This pathotype produces enterotoxins (heat-labile enterotoxins (LTs) and heat-stable enterotoxins (STs)). ETEC colonizes the surface of the small bowel mucosa and elaborate enterotoxins, which give rise to intestinal secretion; colonization is mediated by one or more proteinaceous fimbrial or fibrillar colonization factors (CFs) (Kapers et al., 2004).

Enteroaggregative *E. coli* (EAEC): EAEC are increasingly recognized as a cause of diarrhea among children and adults in developing and developed countries. This pathotype does not secrete LT or ST but adhere to HEp-2 cells in an auto-aggregative pattern, where bacterial cells adhere to each other in a 'stacked-brick' configuration by virtue of fimbrial structures known as aggregative adherence fimbriae (AAFs) (Kaper et al., 2004). Therefore, these cells colonize the intestinal mucosa (of colon) and secrete enterotoxins and cytotoxins.

Enteroinvasive *E. coli* (EIEC): EIEC are biochemically, genetically and pathogenically closely related to *Shigella* spp. This pathotype causes watery diarrhea that is indistinguishable from that due to infection by other *E. coli* pathogens, but resemble *Shigella* as both pathogens share essential virulence factors. EIEC/*Shigella* pathogenesis comprises epithelial cell penetration, followed by endocytic vacuole lysis, intracellular multiplication, directional movement through the cytoplasm and extension into adjacent epithelial cells (Kaper et al., 2004). These virulence genes are present on large virulence plasmid composed of insertion sequence (IS) elements.

Diffusely Adherent *E. coli* (DAEC): DAEC causes diarrhea in children more than 12 months of age, this pathotype is defined by the presence of a characteristic diffuse pattern of adherence to HEp-2 cell monolayers. These strains produce fimbrial adhesion called F1845 (belong to Dr family of adhesins), and induce a cytopathic effect by the development of long cellular extensions and wrap around the adherent bacteria (Kaper et al., 2004). DAEC infection could induce inflammatory bowel diseases.

2.4 *Salmonella*

Salmonella sps. can cause infections known as salmonellosis, caused by eating contaminated food or water. Other than food and water, *Salmonella* bacteria can be carried through pets such as dogs, cats, amphibians, reptiles and their food. The symptoms caused by salmonellosis are fever, headache, stomach cramps, diarrhea and vomiting. Salmonellosis generally has incubation period for 4-7 days. *Salmonella* sps. were found to cause contamination on consuming fresh RTE vegetables. Islam et al. (2004) reported that *Salmonella enterica* serovar *Typhimurium* was detected on roots and leaves of lettuce and parsley contaminated with irrigation water and manure compost. Guo et al. (2001) supported by observing the migration of *Salmonella* from soil directly into the stem scar tissue of green tomatoes. Table 2.4 reported information on the *Salmonella braenderup* infections. Other *Salmonella* infections are reported in Table 2.6. Nebraska Public Health Laboratory reported that in 1996, the most common *Salmonella* serotypes reported from the West North Central Region of the U.S. in descending order were *Salmonella* serotype *Enteritidis* (Group D1), *Salmonella* serotype *Typhimurium* (Group B), *Salmonella* serotype *Heidelberg* (Group B), *Salmonella* serotype *Newport* (Group C2), and

Salmonella serotype *Braenderup* (Group C1). The potential sources for *Salmonella braenderup* are cattle, chicken and turtles; this serovar can also penetrate the eggs of turtles. Serovar *braenderup* resulted in multiple outbreaks with total 775 cases during 1993-2012, mostly occurred in United States-multistate, England and Japan and associated sources of contamination were mangoes, tomatoes, lettuce, chicken and lunch boxes (https://confluence.cornell.edu/display/FOODSAFETY/Salmonella+Braenderup).

2.5 Campylobacter

Campylobacter jejuni and *C. coli* are the most common causes for foodborne gastroenteritis worldwide (Karenlampi and Hanninen, 2004). Consumption of uncooked food, unpasteurized milk and contaminated drinking water could result in campylobacteriosis and attacks the digestive system causing diarrhea, abdominal pain, fever, nausea and vomiting. This bacterium is gram negative, non-spore forming, microaerophilic and thermotrophic motile spiral rod and moves by corkscrew-like motion. The main reservoirs for *Campylobacter* are poultry, birds, swine, cattle; they are unable to multiply in foods under normal storage conditions (such as temperatures below 30 ºC). Park and Sanders (1992) reported that *Campylobacter* was isolated at rate of 3.3% in spinach, 3.1% in lettuce, 2.7% in radishes, 2.5% in green onions, 2.4% in parsley and 1.6% in potatoes, sampled from farmers' outdoor markets (533 samples), whereas 1031 samples from supermarkets were all negative. Another study reported that *C. jejuni* was found in 2 (1 spinach and 1 fenugreek) out of 56 samples (Kumar et al., 2001).

2.6 Rotavirus

Rotavirus is the most common cause of diarrheal disease among infants and young children. It belongs to double stranded RNA viruses. This virus is transmitted by the fecal-oral route and on infection damages the cells in the small intestine and causes gastroenteritis. Infected children may have symptoms such as severe watery diarrhea, vomiting, fever and abdominal pain (available at https://www.cdc.gov/rotavirus/about/symptoms.html). Virus can spread by contaminated food and water. Children are most likely to get *rotavirus* in the winter and spring i.e December through June (available at https://www.cdc.gov/rotavirus/about/transmission.html). Rotavirus vaccine is available and is the best way to control the rotavirus illnesses in infants. CDC (2016) reported that 9 out of 10 children will be protected from severe rotavirus illness after routine vaccination of infants with either of the two available oral vaccines: RotaTeq® (RV5) or Rotarix® (RV1). Contaminated market lettuce were found to be positive for *Rotavirus* in three sample pools in Costa Rica (Hernandez et al., 1997). Badawy et al. (1985) reported that *Rotavirus* survived on lettuce longer than on radishes and carrots, may be due to the large surface area and rough surfaces provided by lettuce.

2.7 Water quality standards and guidelines

According to Pachepsky et al. (2011) when irrigation water is monitored, indicator organisms are measured rather than actual pathogens in the vast majority of cases. WHO has recommended that fresh fruits and vegetables be irrigated with treated irrigation water or water that has undergone disinfection to achieve a coliform level of not more than 100 coliforms per 100 ml in 80% of

samples. For unrestricted irrigation, WHO has recommended a limit of 1000 fecal coliforms per 100 ml of water (Shuval 2007). The Canadian water quality guidelines (CCME 2008) recommends more stringent guidelines and suggests a maximum allowable count of 100 fecal coliforms per 100 ml and 1000 total coliforms per 100 ml of irrigation water.

Indicator organisms have been selected mainly to indicate fecal contamination, if any, rather than detecting the presence of any specific pathogen in the irrigation water. Ashbolt et al. (2001) reported that the major indicator organisms are *E. coli*, fecal streptococci, enterococci and organisms such as *Bacteroides*, *E. coli* specific phages, but none of them have been widely adopted. There has been no regular reporting on the biological properties of irrigation water and this is due to the extensive sampling required, and its associated costs. In addition, farmers who have the biological or microbe data of their fields, might not share their data (Suslow, 2010). Recently, *E. coli* and in some cases fecal streptococci have been commonly used as indicator organisms. Blumenthal et al. (2000) reported the adoption of additional standards which include nematode and helminth egg counts. The high concentrations of indicators are tolerated in surface irrigation until and unless irrigation water does not come in contact with the edible parts of plants. Some of the pathogens in the irrigation water used for agriculture have been reported in Table 2.1.

The microbiological water quality standards should distinguish between irrigation water sources, method of irrigation, crop type, and land use (See Appendix 3). In Appendix 3, water quality standards for four Canadian provinces (Alberta, British Columbia, Manitoba, and Saskatchewan) are listed and it was found that the water quality standards at the regional level can be very different from each other. For example, Alberta's water quality standards for surface irrigation water are 1000 cfu/100ml for total coliforms and 100 cfu/100ml for fecal coliforms, whereas for

British Columbia, the standards are 1000 cfu/100ml for total coliforms and 200 cfu/100ml for fecal coliforms (In Appendix 3).

Restricted irrigation is the irrigation used for crops likely to be eaten uncooked, and unrestricted irrigation is used for crops that will be cooked. Blumenthal et al. (2000) and Marr (2001) reported that restricted and unrestricted irrigation were distinguished on the basis of wastewater usage for irrigation. It has been observed that some states do not allow wastewater effluent of any type to be used for crop irrigation. For example, Florida allows drip and sprinkler irrigation, rather than spray irrigation with effluent water, of edible crops (USEPA, 2004). Also, there is growing concern about using wastewater for irrigation, as wastewater could contain organic contaminants such as antibiotics, endocrine disrupting compounds, and pesticide residues (Pachepsky et al., 2011). These contaminants are an emerging concern for public and environmental health.

Therefore, as discussed in section 2.1, irrigation water is considered as the potential source of contamination for fresh fruits and vegetables. Irrigation water can be wastewater, groundwater or surface water, depending on the availability or accessibility of the water resources. Hess (1986) reported that more than 2.7×10^9 m^3 of water was used annually for irrigation on agricultural lands in Canada. Roughly 3.3% of this total water was withdrawn from groundwater sources. In provinces such as Ontario and British Columbia, groundwater plays an important role in satisfying the irrigation water demand, with over 10% of the total water used for irrigation (http://ceqg-rcqe.ccme.ca/download/en/131). To date, no database on the microbial quality of irrigation water has been compiled. Also, irrigation water quality guidelines are expressed only

on the basis of chemical pollutants like pesticides, metals etc. without incorporating the microbial pollutants (CCME 1999).

Agricultural practices in Quebec: Statistics Canada (2010) reported that percentage of total Quebec's irrigated area was only 3%. In Quebec, there were 670 irrigated farms whereas 105 farms were non-irrigated. Irrigation in Quebec (Statistics Canada 2010) was conducted on field crops (a total irrigated area of 2810 hectares), fruits (2820 hectares) and vegetables (8710 hectares) during May-October, 2010. In Quebec, the number of farms using sprinkler, micro and surface irrigation method were 440, 265 and 80 respectively. Irrigation water sources in Quebec are on-farm surface water (used by 500 farms) and on-farm underground or well water (used by 255 farms) (Statistics Canada, 2010).

2.8 Mechanism of contamination of fruits and vegetables

There are a number of modes through which pathogens can enter the plant and contaminate it. Entry of pathogens may be through stoma, scar tissue, or wounds as a consequence of irrigation water contacting leaf surfaces or from raindrop splashes from the soil surface (Kroupitski et al., 2009; Materon et al., 2007; Mitra et al., 2009). Guo et al. (2001) observed the migration of *Salmonella* from soil directly into the stem scar tissue of green tomatoes. Another possible reason could be spray or sprinkler irrigation, which produces bioaerosol which can contaminate crops that are likely to be eaten uncooked. Pathogens can enter plants via the root system (Bernstein et al., 2007a, 2007b; Solomon et al., 2002a, 2002b), and the in-field splash can transport microorganisms from the soil surface into the crop or to other areas of the plant (Boyer, 2008).

Incidents of foodborne pathogens on fruits and vegetables vary from region to region. Developing countries usually report higher levels of pathogen in irrigation water (Pachepsky et al., 2011) as compared to the developed countries. The reason could be that developed countries have comparably better sanitation and hygienic conditions. WHO (2008) concluded that the role of contaminated water used in the production of vegetable crops as the vector for the transmission of these pathogens to humans, was yet to be proved.

The intrinsic (the nature of epithelium, protective cuticle, tissue pH, presence of antimicrobials) and extrinsic factors (environment in which plants are grown) associated with fresh fruits and vegetables, determine the growth of pathogens (Beuchat, 2002). Bruised and cut surface tissues exude fluids containing nutrients and numerous phytoalexins and other antimicrobials that may enhance or retard the growth of naturally occurring microflora and pathogens. Beuchat (2002) reported that the presence of soil or fecal material on the surface of vegetables and fruits can permeate bruised tissue, altering the ecological environment and the behavior of pathogens and other microorganisms. The growth of molds in these environments may result in increased pH, thus enhancing the probability of pathogenic bacteria growing (Beuchat, 2002). Understanding the growth of pathogenic bacteria remains a major challenge as its source is not readily identifiable.

Lettuce is the fresh leafy vegetable most frequently involved in foodborne disease outbreaks. Human bacterial pathogens may be experimentally internalized into lettuce plants, but the occurrence of natural microflora inside lettuce leaves has not been elucidated. According to Hou et al. (2013), the bacterial genes involved in attachment and biofilm formation are likely important for the contamination of lettuce plants with Shiga toxin-producing *E. coli* strains. Hou et al (2013) reported that spore forming bacteria and traditional epiphytic bacterial genera were

frequently detected in surface-sterilized commercial lettuce leaves. Hou et al. (2013) reported that despite the common occurrence of internalized bacteria, only *Enterobacter* was related to *Escherichia coli* O157:H7 and *Salmonella*. Table 2.2 reports the pathogens that were isolated from the fresh vegetables.

2.9 Case studies of foodborne outbreaks

There were several foodborne illnesses and outbreaks which have been observed and reported. According to WHO (2015), thirty-one foodborne hazards causing 32 diseases are reported, being 11 diarrheal disease agents (1 virus, 7 bacteria and 3 protozoa), 7 invasive infectious disease agents (1 virus, 5 bacteria and 1 protozoan), 10 helminths and 3 chemicals. Globally, these 31 hazards caused 600 million foodborne illnesses and 420,000 deaths in 2010 (WHO, 2015). Among diarrheal disease agents (most frequent causes of foodborne illness), were *Norovirus* and *Campylobacter* spp. Foodborne diarrheal disease agents caused 230,000 deaths, particularly from non-typhoidal *Salmonella enterica* (NTS). *Salmonella typhi*, *Taenia solium*, hepatitis A virus, and aflatoxin were other major causes of foodborne deaths (available at http://apps.who.int/iris/bitstream/10665/200046/1/WHO_FOS_15.02_eng.pdf?ua=1). In 2010, the global burden of foodborne disease (from 31 hazards) accounted for 33 million DALYs and 40% of burden was among children under five years of age. WHO (2015) also reported that foodborne diarrheal disease agents such as NTS and EPEC lead to 18 million DALYs worldwide in 2010, other foodborne hazards included were *Salmonella typhi* and *Taenia solium*. Foodborne burden estimates were even reported for 3 other bacterial and 1 chemical hazards for some sub-regions, the global estimate for these hazards was not feasible (WHO, 2015).

Some of the epidemiological foodborne outbreaks are reported in Table 2.3, and others are listed below:

2.9.1 Outbreak in United States, 2007

As shown in Figure 2.1, the Centers for Disease Control and Prevention (CDC) reported that an outbreak in the U.S. in 2007 resulted in a total of 21,244 foodborne illnesses and 18 deaths. 34% of these reported illnesses were due to bacteria like *Salmonella*, *Listeria monocytogenes*, *E. coli* O157:H7, and *Clostridium botulinum* and resulted in eleven deaths out of eighteen. Whereas, 38% of the illnesses were linked to viruses, most specifically *Norovirus*. Less than 1% were attributed to parasites. Chemicals contributed to just one percent of the illnesses and multiple causes were cited in three percent. The most interesting fact of this study (CDC (2010); https://www.cdc.gov/mmwr/preview/mmwrhtml/mm5931a1.htm) was that in 24% of the foodborne outbreak associated illnesses, the cause was unknown.

2.9.2 Outbreaks in Canada, 2008-2014

There were 115 foodborne outbreaks reported in Canada during the 2008-2014 period (PHAC, 2015). Of these outbreaks, 106 reported cases were caused by an etiologic agent. The most commonly reported agents were *Salmonella* (40.9%), *Escherichia* coli O157 VTEC (14.8%), *Campylobacter* (1.7%) and norovirus (12.2%).

2.9.3 Outbreak reported in England and Wales, 2010

As shown in Figure 2.2, in 2010, 61 general outbreaks of foodborne infectious diseases in England and Wales were reported to the Public Health England's (PHE's) electronic Foodborne and Non-Foodborne Gastrointestinal Outbreak Surveillance System (known as eFOSS). 1,396 people were affected by these 61 general foodborne outbreaks, of whom 616 were laboratory confirmed cases, 82 required hospitalizations, and 5 resulted in death. Most of these outbreaks were reported in South East England, with the fewest in West Midlands. The cases reported in 2010 were fewer than in 2009 (there were 91 general outbreaks in year 2009), according to the PHE agency's latest data analysis. The number of outbreaks dropped in 2010 due to a decline in the *Salmonella* outbreaks. There were only eight *Salmonella* outbreaks in 2010. However, the lowest number of *Salmonella* outbreaks, since the surveillance system was established, was reported in 1992. *Campylobacter* was the causative agent in 80% of outbreaks, whereas *Salmonella* accounted for 13% (8/61). It was observed that in 2010, *Campylobacter* displaced *Salmonella* as the most frequently implicated causative agent in reported foodborne outbreaks (19/61 outbreaks, 31%), followed by *Norovirus* (10/61, 16%) and those of unknown aetiology (suspected to be *Norovirus*) (10/61, 16%).

2.9.4 *E. coli* O104:H4 outbreak in Germany and France, 2011

Soon et al., (2013) reported on the *Escherichia coli* O104: H4 outbreak from sprouted seeds that occurred in France and Germany during May-July 2011. This was one of the largest outbreaks of haemolytic uraemic syndrome (HUS) and of bloody diarrhoea caused by the Shiga toxin-producing *Escherichia coli* (STEC) O104:H4. The hypothetical origin of the outbreak strain was a combination of enteroaggregative *E. coli* and an enterohaemorrhagic *E. coli* which had the

ability to resist multi-antibiotics and produce Shiga-toxin 2. The combination of aggregative ability, antibiotic resistance and the production of Shiga-toxin 2 significantly affected the severity of the symptoms present.

2.9.5 *E. coli* O157:H7 illnesses in Maritimes and Ontario, Canada in 2012-2013

Due to *E. coli* O157:H7 outbreak, 30 cases of illness were reported in Nova Scotia, New Brunswick and Ontario. The last case of this outbreak was reported on January 9, 2013. Since then, no cases of this outbreak were reported, some cases may remain unreported. According to an investigation (PHAC, 2013) http://www.phac-aspc.gc.ca/fs-sa/phn-asp/2013/ecoli-0113-eng.php), the most probable bacterium responsible for this outbreak was *E. coli* O157:H7. This bacterium spread from shredded lettuce distributed by FreshPoint Inc. primarily to some KFC and KFC-Taco Bell restaurants.

2.9.6 *Salmonella* illnesses related to mangoes, 2012

Mangoes originating from Mexico were the source of this outbreak. They were sold between July 12 and August 28, 2012, contaminated with *Salmonella braenderup*. In total, 21 cases were reported as shown in Table 2.4.

Eating contaminated food or drinking contaminated water generally causes *Salmonella* infections known as Salmonellosis. Pets, such as dogs, cats, amphibians and reptiles can be carriers of the bacteria. The symptoms are fever, headache, stomach cramps, diarrhea and vomiting. Salmonellosis generally runs its course in four to seven days.

2.10 Methodology for assessing and analysing human health risks:

There are a number of quantitative methods being used to assess the quality of water and to quantify human health risks caused by pathogens. Some of the Quantitative approaches are as follows:

2.10.1 Multiple-tube fermentation technique or the Most Probable Number (MPN) Technique

To assess the human health risks of contaminated surface water or of irrigation water with microbial pathogens, there is a need for quantitative data on the concentration of pathogenic microorganisms (Ferguson et al. 2003; Jenkins et al., 2008). The MPN method with the confirmatory tests can be used to quantify pathogens such as *E. coli* O157:H7, *E. coli* non-O157:H7, *Campylobacter* and *Salmonella*. The MPN method was widely used by studies to determine and quantify the concentration of *E. coli* O157:H7 in food (Chapman et al., 2001), feces (Fegan et al., 2003; Stephens et al., 2007) and surface waters of watershed with animal agriculture or wildlife (Jenkins et al., 2008). Thus, the 95% probable count for the pathogenic microorganisms was determined. Jenkins et al. (2008) developed a culture-based five tube-four dilution MPN method for enumerating *Salmonella* in environmental waters in order to understand and manage the fate and transport of *Salmonella* in agricultural watersheds. Jenkins et al. (2008) also reported that *Salmonella* densities in pond inflow samples were associated with *Escherichia coli* and fecal *enterococci* densities, indicating fecal contamination of streams. In this manner, the MPN method would improve understanding of the fate and transport of pathogens in agricultural watersheds if used for irrigation water. The MPN method can be the basis for collections of data on environmental pathogens.

2.10.2 Colilert-18/ Quanti-Tray system

The Quanti-Tray system identifies total coliforms through β-galactosidase activity, which is determined from the hydrolysis of o-nitrophenyl- b-d-galactopyranoside (ONPG), which causes the release of a diffusible yellow pigment. (Fremaux et al., 2009). However, its accuracy is questionable. Pisciotta et al. 2002; Chao et al. 2004 observed that the accuracy varies extensively according to the types and locations of water being tested.

2.10.3 Quantitative Microbial Risk Assessment (QMRA)

To quantify the human risk due to consumption of fresh fruits and vegetables, the model known as the Quantitative Microbial Risk Assessment (QMRA) has been widely used. Pathogen concentration is used as a dose and the response is estimated based on mathematical models, and thus the probability of illness or risk can be estimated. In this manner, the consumers would be at less risk of foodborne illnesses and outbreaks.

The QMRA model establishes a link or relationship between the pathogen concentration in the irrigation water, produce and the probability or risk of illness. This model is comprised of two statistical models: the exposure model and the infectivity model. No single mathematical equation or formulation exists for the irrigation QMRA (Pachepsky et al., 2011). The exposure model computes the daily dose as the product of the concentration of the pathogen in irrigation water, the volume of irrigation water retained on the unit mass of produce, the fraction of pathogen in produce that remained infective at harvest time, the fraction of pathogen that remained infective between harvest and consumption, and the mass of produce consumed daily. The infectivity model uses the dose and the number of consumption days as inputs and provides the probability of illness as the output (Pachepsky et al., 2011). The QMRA model is evolving

rapidly and can be developed for specific pathogens and its related illness and risks, for managing agricultural fields and produce, source of irrigation water, and the environmental conditions.

Based on the National Academy of Sciences framework for Quantitative Risk Analysis, the QMRA model has been divided into four stages when used to estimate the risk due to pathogens: hazard identification, dose-response, exposure assessment and risk characterization. These four stages are explained briefly below:

1. **Hazard Identification**: This stage describes a pathogen and the disease it causes, including symptoms, severity, and death rates from the microbe. Then the identification of sensitive populations that are particularly prone to that particular infection is carried out.
2. **Dose-Response**: In this stage, the relationship between the dose, or the number of microbes received or captured on the food, and the probable health effects or risks on humans or animals is computed. The data sets from human and animal studies allow the construction of mathematical models that predict the dose-response of the particular pathogen. With the available information, the dose-response relation is computed.
3. **Exposure Assessment**: The pathways which allow a microbe to reach humans and cause infection (through air, drinking water, irrigation water, food etc.) would be described at first. Secondly, the size and duration of exposure by each pathway is described. Lastly, the number of people exposed and the categories or age groups of people affected is estimated.

4. **Risk Characterization**: This last step of the QMRA model integrates all the information from steps 1, 2 and 3. It develops a single mathematical model to estimate the risk or probability of infection, illness or death.

   Steps 1, 2 and 3 would give a range of values for exposure, dose and hazard, using these values, risks would be calculated. This calculation of risk is called the **Monte-Carlo Analysis** when 10,000 or 1,000,000 trial simulations would run to give the full range of possible risks including the average and worst-case scenarios and these estimated risks would be looked at by the decision and policy makers, and government authorities.

The QMRA model was first developed for wastewater irrigation and was adopted by WHO when developing guidelines for water related diseases (Fewtrell and Bartram, 2001). The QMRA model has also been used for assessing viruses on lettuce (Petterson et al., 2001); enteric virus colonization on cucumber, broccoli, cabbage or lettuce (Hamilton et al. 2006); *Cryptosporidium* and *Giardia* on irrigated bell peppers, cucumbers, and lettuce (Mota et al., 2009); and norovirus and *Ascaris* infection (Mara, 2010). Earlier versions of the QMRA model supported the use of indicator organisms and employed the conversion of indicator organism concentrations to concentrations of the pathogen of interest (Blumenthal et al., 2000). Also, Stine et al. (2005) used the reverse procedure to determine the concentration of Hepatitis A virus and *Salmonella* in irrigation water.

2.11 Conclusion of literature review

According to the literature, there have been numerous foodborne illnesses and outbreaks reported worldwide. There is limited database for the microbial quality of irrigation water for a particular region. In addition to chemical pollutants in the irrigation water, microbiological contaminants also play an important role in causing contamination to transfer to the crops. To therefore fill in the gaps in our knowledge, this research project was carried out to evaluate the importance that irrigation water is a potential source of infection and a carrier of pathogen causing foodborne illness or disease. There are studies which examine the comparative analysis of different treatments on the fate and transport of *E. coli* on crops such as lettuce and tomatoes, but not utilizing four treatments comprising of two irrigation methods and two soil types.

The following studies have shown comparison of irrigation methods in the fate and transport of pathogens. Makkaew et al. (2016) reported that no *E. coli* was detected in lettuce from drip irrigated beds using treated domestic wastewater; whereas, all lettuce samples were positive for *E. coli* when spray irrigated. Whereas, Moyne et al. (2011) found that there was no influence of drip and overhead sprinkler irrigation on the persistence of attenuated *E. coli* O157:H7 in the lettuce phyllosphere. Moyne et al. (2013) also reported that shortly (2 hours) after inoculation of *E. coli* O157:H7 (using drip and spray irrigation) onto lettuce plants, most cells either died or were no longer in a culturable state. Similarly, Zhu et al. (2011) reported that there was no significant effect of *E. coli* survival on romaine and iceberg lettuces harvested from fields irrigated using drip, furrow and sprinkler irrigation systems and stored under three different conditions of temperature and relative humidity. The contamination could transfer during harvesting as well, suggested by McEvoy et al. (2009), preventing contamination of coring knife and cored lettuce, as well as prompt chilling of fresh cored lettuce heads are necessary steps to ensure the safety of field-cored iceberg lettuce.

The QMRA model is used in assessing the pathogenic risks in the irrigation and in drinking water. Most of the studies have used the QMRA model to assess or estimate the risk in wastewater, drinking or recreation water. Whereas in this research, the QMRA model was used to assess the risk due to untreated surface water used for irrigation. Also, the QMRA model has been widely used for lettuce and other leafy vegetables, but in this research project it is the first use of the model to estimate the risk due to the consumption of tomatoes, irrigated with untreated surface water. The QMRA model was also used for the first time to estimate the risk on lettuce and tomatoes grown in a greenhouse under four different treatments plans and on produce harvested on three different days (10, 20 and 30) after having been irrigated with contaminated water. The QMRA model was further used to estimate the pathogenic risk on the consumption of fresh broccoli, cauliflower, squash (zucchini), lettuce and tomato under different scenarios of washing and irrigation methods.

Table 2.1: Pathogens in Irrigation water used for food crops

Pathogen and its forms	**Prevalence (%)**	**Country**	**Reference**
Microsporidia	28	USA and several Central American countries	Thurston- Enriquez et al. (2002)
Giardia cysts	60		
Cryptosporidium oocysts	36		
Salmonella spp.	9	USA	Duffy et al. (2005)
Cryptosporidium, *Giardia*, or both	11	USA	Moulton-Hancock et al. (2000)
E. coli	75	New Zealand	Close et al. (2008)
Campylobacter spp.	12	New Zealand	Close et al. (2008)
E. coli O157:H7	2	Nigeria	Chigor et al. (2010)
E. coli O157:H7	1, 2	Canada	Johnson et al. (2003), Gannon et al. (2004)
Salmonella spp.	6		
Fecal coliforms (>100/100ml)	8	Canada	Cross (1997)
Salmonella spp.	6	Greece	Arvanitidou et al. (1997)
E. coli O157:H7	3	Netherlands	Schets et al. (2005)

Source: Pachepsky et al. 2011

Table 2.2: Examples of pathogenic bacteria isolated from fresh vegetables

Vegetables	**Prevalence (%)**	**Pathogen**	**Country**
Broccoli	31.3	*Aeromonas*	USA
Cabbage	2.2	*L. monocytogenes*	Canada
	1.1	*L. monocytogenes*	USA
Egg Plant	2.2	*L. monocytogenes*	USA
Bean sprouts	85	*L. monocytogenes*	Malaysia
Cucumber	80	*L. monocytogenes*	Malaysia
		B. cereus	USA
Lettuce	3.1	*Campylobacter*	Canada
	50	*L. monocytogenes*	Sri lanka
		Aeromonas	USA
Potatoes	27.1	*L. monocytogenes*	USA
Prepacked salads	2.7	*Camplylobacter*	Canada
Radish	36.8	*L. monocytogenes*	USA
Seed sprouts	24	*Staphylococcus*	Canada
Spinach	3.3	*Camplylobacter*	Canada
Sprouting seeds	57	*B.cereus*	USA
Tomato	13.3	*L.monocytogenes*	Pakistan

Source: Beuchat, 2002

Table 2.3: Impact of the contamination: Epidemiological foodborne outbreaks

Vegetable	***Pathogen***	**Country**	**Year**
Seed Sprouts	*Bacillus cereus*	USA	1973
Cabbage	*Clostridium botulinum*	USA	1987
	Listeria monocytogenes	Canada	1981
Lettuce	*E. coli O157:H7*	USA	1995
	Shigella sonnei	USA	1986
	Hepatitis A virus	USA	1988
	Cyclospora cayetanensis	USA	1997
Lettuce, Tomato	*Listeria monocytogenes*	USA	1979
Bagged fresh spinach and lettuce (Fremaux et al. 2009)	*E. coli O157:H7*	USA	2006
Carrots	*E. coli (enterotoxigenic)*	USA	1993
Tomatoes	*Salmonella javiana*	USA	1990
	Salmonella Montevideo	USA	1993
	Hepatitis A virus	USA	1994

Source: Beuchat, 2002

Table 2.4: Location and number of *Salmonella braenderup* infections as of August 29, 2012

Location	**Confirmed cases**
British Columbia	16
Alberta	5
TOTAL	21

Table 2.5: Incidence rates of *E. coli* reported to PHAC through the NESP, 2002 to 2011

Year	***E. coli* O157** **Incident rate/100 000**	***E. coli* non-O157** **Incident rate/100 000**
2002	3.80	0.29
2003	3.00	0.35
2004	3.31	0.32
2005	2.27	0.22
2006	2.99	0.41
2007	2.83	0.25
2008	1.98	0.12
2009	1.56	0.25
2010	1.18	0.22
2011	1.39	0.28

Table 2.6: Annual national totals and rates (per 100,000) for major organism groups reported to NESP, 2006 to 2011†

Group	**2006**		**2007**		**2008**		**2009**		**2010**		**2011**	
	Total	**Rate**	**Total**	**Rate**	**Total**	**Rate**	**Total**	**Rate**	**Total**	**Rate**	**Total**	**Rate**
*Campylobacter**	1958	5.99	1959	5.93	1614	4.83	1751	5.17	1837	5.36	1938	5.60
E. coli O157‡	978	2.99	934	2.83	661	1.98	529	1.56	404	1.18	482	1.39
Listeria											132	0.38
Parasites*	1705	5.22	1678	5.08	1783	5.33	1570	4.64	1585	4.63	1190	3.44
Salmonella	5724	17.51	6419	19.42	6351	18.99	6084	17.97	7251	21.17	6809	19.68
Shigella	526	1.61	636	1.92	680	2.03	631	1.86	739	2.16	860	2.49
Vibrio	43	0.13	37	0.11	39	0.12	47	0.14	51	0.15	47	0.14
Virus*	4057	12.41	4657	14.09	3248	9.71	3184	9.40	4662	13.61	4441	12.83
Yersinia	578	1.77	488	1.48	414	1.24	382	1.13	341	1.00	381	1.10
Total	15569		16808		14790		14178		16870		16280	

† Rates calculated using the population estimates for Canada as of July 1 for years 2006 to 2011 as reported by Statistics Canada. * *Campylobacter*, Parasitic (*Giardia, Cryptosporidium, Entamoeba histolytica/dispar* and *Cyclospora*) and viral infections (*Norovirus, Rotavirus* and *Adenovirus*) are not routinely reported to the provincial or central reference laboratories and are greatly underrepresented in NESP. ‡ Only cases of *E. coli* O157 are included in this table, as *E. coli* non-O157 is not consistently reported by provinces.

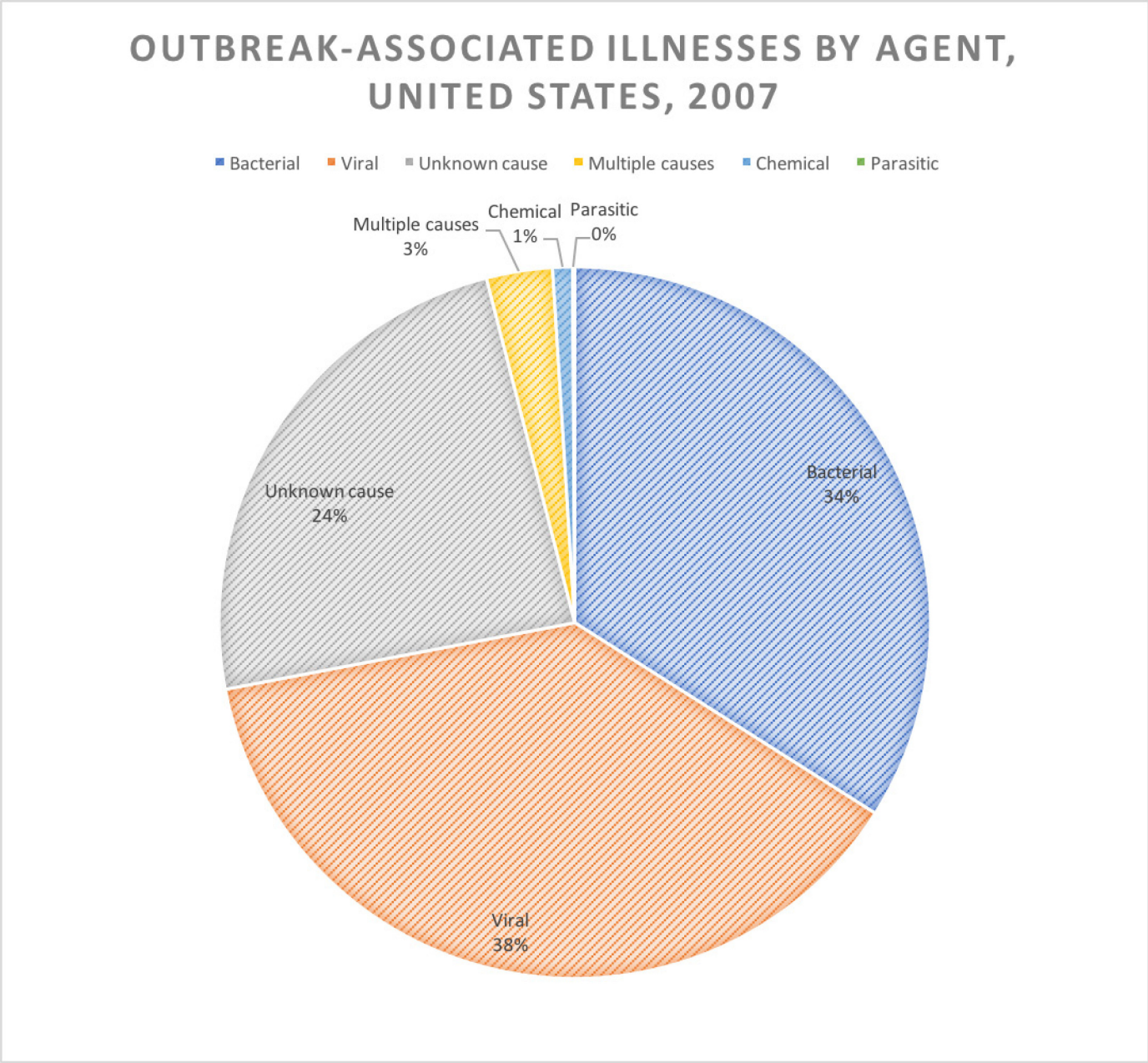

Figure 2.1: Foodborne outbreak in United States, 2007

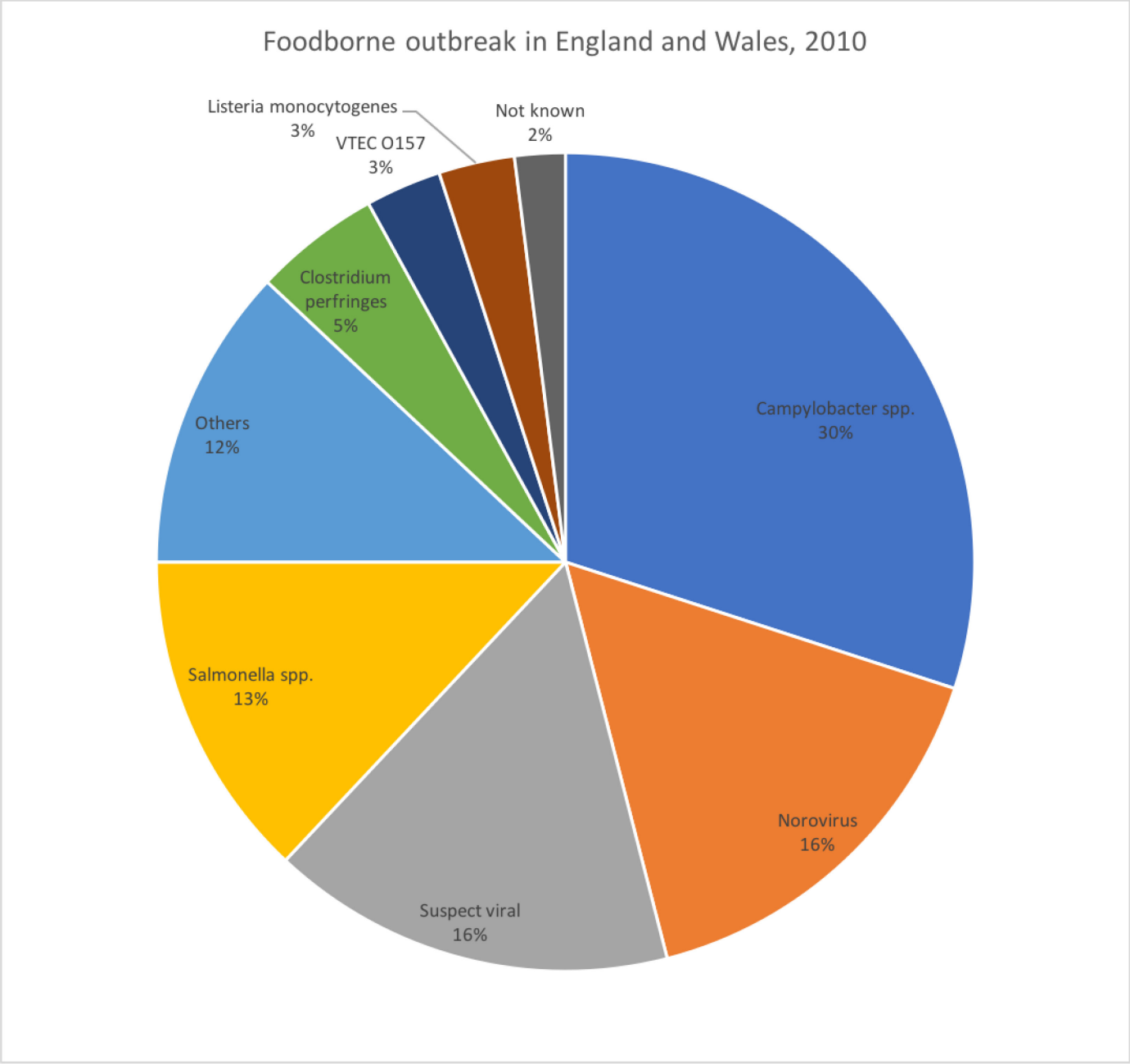

Figure 2.2: Foodborne outbreak in England and Wales, 2010

Connecting text to Chapter 3

Chapter two of this dissertation reviewed the available literature on foodborne illnesses and outbreaks with case studies, the sources, pathogens, mechanisms, responsible for causing the contamination and disease, as well as methodology to quantify the pathogens and estimating the risk due to consumption of contaminated fresh fruits and vegetables. The irrigation water before application to crops, are rarely quantified for the indicator microorganisms or *E. coli*. The usage of untreated surface water, wastewater and groundwater for irrigation purposes can lead to these foodborne illnesses. *E. coli*'s fate and transport in lettuce was studied using four treatments (comprised of two soil types and two irrigation methods) in the greenhouse of the Macdonald Campus of McGill University, St-Anne-de-Bellevue, Quebec. This study was addressed in Chapter three, with the use of different treatments. Lettuce was grown in four different treatments comprised of two soil types namely, organic and mineral soil, and two irrigation methods namely, sprinkler and drip irrigation method. The contamination retained on the lettuce leaves and soil was studied over a time period of 30 days after inoculation. This chapter fills in the knowledge gap by explaining the fate and transport of bacteria *E. coli* on lettuce under four different treatments. The manuscript from this study, which addressed the second objective of this dissertation, was published.

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Chapter 3: *Escherichia coli* contamination on Ready-To-Eat (RTE), Lettuce

Divya Gupta and Chandra A. Madramootoo

Abstract

Escherichia coli outbreaks associated with Ready-to-Eat vegetables have brought attention to irrigation water contaminated with pathogens. A greenhouse study was conducted to determine the potential transfer and survival of *E. coli* from contaminated irrigation water to lettuce leaves and in soil. Contamination of lettuce was studied using two irrigation methods (drip and sprinkler irrigation) and two soil types (organic and mineral soil) during June–August 2014. There were pots inoculated with bacteria *E. coli* ATCC8739, and un-inoculated pots without bacteria. The four treatments were replicated five times each for the inoculated and un-inoculated pots. One-time application of *E. coli*-contaminated bacterial water (7.23 log cfu/ml) was used to irrigate lettuce, after two weeks of transplanting the seedlings into pots. The transfer and survival of bacteria were observed in the soil and lettuce leaves over a time period of 30 days after inoculation. It was observed that there was highest transfer and survival of bacteria on organic soil with drip irrigation, i.e., 7.03 log cfu/g (63.1% bacterial retention) and dropped to 4.71 log cfu/g (0.3% retention) on the 30th day. For lettuce leaves, maximum contamination was observed with plants growing in organic soil with sprinkler irrigation and it decreased from 5.32 log cfu/g (1.23% retention) to 2.88 log cfu/g (0.004% retention) by the 30th day. Soil type significantly influenced survival of the bacteria, as organic soil showed more contamination (i.e., 63.1 and 0.57% retention) than mineral soil (i.e., 0.47 and 0.04% retention) on the 2nd day. Also, an early bacterial decay or movement to lower depths was observed in mineral soil compared to

the organic soil. Irrigation method has a significant influence in contaminating the lettuce leaves, as sprinkler irrigation resulted in higher transfer and retention of bacteria on the edible portion of the plant. Also, in the case of sprinkler irrigation, fate and transport of bacteria were independent of the soil type. Drain water from the pots was also collected to study bacteria transport, but none were positive for the bacterial presence.

Keywords: Ready-to-Eat (RTE) vegetables, irrigation, *E. coli,* contamination, lettuce, greenhouse, soil.

3.1 Introduction

Foodborne illnesses and outbreaks have become a major concern in the last few years. Foodborne disease outbreak is an incident in which two or more persons experience a similar illness resulting from the ingestion of a common food (CDC 2000). These outbreaks can lead to several deaths and illnesses. Tauxe et al. (1997) reported outbreaks of foodborne illness associated with fresh fruits and vegetables in the USA have nearly tripled since 1973. CDC (2012) reported that there was a multistate outbreak of *Escherichia coli* O157:H7 due to the consumption of romaine lettuce and 58 persons were infected from nine states. Contaminated Ready-to-Eat (RTE) vegetables can be one of the sources of foodborne outbreaks. Oliveira et al. (2011) reported that these vegetables are of major concern and are gaining popularity with consumers because of nutrition, health concerns, and convenience. RTE vegetables such as lettuce, spinach, celery, other leafy vegetables, tomatoes, and carrots are minimally processed, consumed mostly raw in salads, and have a short shelf life of 7–14 days (Garcia-Gimeno and Zurera-Cosano 1997). Beuchat (1996) reported that RTE vegetables are often colonized by

microorganisms, which can be pathogenic. RTE vegetables are generally colonized by different microorganisms such as bacteria, viruses, yeast, and fungi, which are responsible for spoilage (Abadias et al. 2008) and infections.

Pathogenic bacteria such as *Salmonella, Shigella, Escherichia coli O157:H7, Clostridium botulinum,* and *Listeria monocytogenes* can be present in RTE vegetables. Oliveira et al. (2010) found that contamination of lettuce occurs predominantly by gram-negative bacteria, which belong to the family of Pseudomonadaceae and Enterobacteriaceae. *Escherichia coli (E. coli)* is widely used as an indicator organism of fecal contamination and water pollution (Clesceri et al. 1998), as well as a tool for bacterial source tracking (USEPA 2000; Hamilton et al. 2006). *E. coli* refers to a group of gram-negative rod-shaped facultative anaerobic bacteria commonly found in the intestines of humans and warm-blooded animals. *E. coli* gets excreted to the external environment through the primary host (animal or human) and can be transported to different niches, thereby making the external environment a secondary (open or non-host-associated) habitat (Savageau 1983).

There are several foodborne outbreaks reported due to the presence of *E. coli* O157:H7 on RTE vegetables. In July 1995, *E. coli* O157:H7 contaminated lettuce and infected the residents of Montana (Ackers et al. 1998). Between 1995 and 1998, different states (Missouri, Michigan, California, Washington, Arizona, and Nevada) suffered from nine foodborne outbreaks due to consumption of contaminated fresh vegetable sprouts by *Salmonella* or *E. coli* O157:H7 (Buck et al. 2003). In 2006, *E. coli* O157:H7 contaminated baby spinach and infected people of 26 states in the USA, which resulted in a major outbreak (FDA 2007). The Public Health Agency of Canada reported that in January 2013, shredded lettuce was contaminated by *E. coli* O157:H7 and led to an outbreak and illnesses in the Maritimes and Ontario, Canada. In December 2013,

the Centers for Disease Control and Prevention (CDC) reported that a multistate outbreak of *E. coli* O157:H7 occurred in Arizona, Texas, California, and Washington due to contaminated RTE salads. Moreover, in August 2014, CDC reported that six states of the USA were infected with *E. coli* O121 strain through raw clover sprouts.

Other bacteria such as *Listeria monocytogenes* and *Shigella* also resulted in foodborne illnesses and outbreaks. In 1981, *Listeria monocytogenes* outbreak was first reported in the Maritime provinces of Canada through *Listeria*-infected cabbage (Schlech et al. 1983). This outbreak resulted in seven adults and 34 perinatal infections. In 1986, *Shigella* infected shredded lettuce and tomatoes, which resulted in two Shigellosis outbreaks in Texas (Doyle 1990).

These outbreaks depict that fresh produce acts as a vehicle for foodborne illnesses and outbreaks. RTE vegetables may become contaminated during planting, growing, harvesting, postharvest handling, storage, distribution, and even during preparation. Pathogens can contaminate RTE vegetables through different sources such as municipal sewage, runoff, contaminated surface and groundwater, and fecal waste materials from domesticated and wild animals (WHO 2003). In many countries, surface water is a predominant source of irrigation, and is monitored much less intensively than drinking or recreational water. Therefore, irrigation water can be a possible source of contamination. Buck et al. (2003) reported that runoff water from cattle feedlots and application of contaminated irrigation water to the soil also represents possible sources of contamination.

In Quebec, it was revealed in 2007 that 70% of fruit and vegetable producers irrigated their fields, with nearly 75% of them pumping water from surface sources (such as a pond or a river), known to be prone to microbial contamination (Groupe AGECO 2007). Despite serious concerns

regarding the issues of food safety and traceability, only 10% of the producers tested the quality of their irrigation water regularly (i.e., more than twice a year) and 50% of them admitted to never testing (Groupe AGECO 2007). Hogg (2010) reported that irrigation water quality monitored at 14 locations in Saskatchewan's main irrigation areas between 2007 and 2009, showed that 11 of 180 samples exceeded irrigation guidelines for fecal coliforms. An assessment of pond irrigation water used for fruit and vegetable crops on farms with little or no livestock production in Ontario revealed that between 2 and 22% of water samples were unacceptable for levels of *E. coli* and also that the concentrations of fecal indicators varied widely over the growing season (Steele and Odumeru 2004). Another potential reason for contamination could be contaminated manure, which is in direct contact with the produce (Islam et al. 2004). Semenov et al. (2009) also found that *E. coli* O157:H7 survived significantly longer in soil with slurry than with manure.

There are different modes of entry for pathogens into the plants. Entry of pathogens may be through stomata, hydathodes, scar tissue, or wounds (as a consequence of irrigation water contacting leaf surfaces) or from raindrop splashes from the soil surface (Gu et al. 2011, 2013a; Kroupitski et al. 2009; Mitra et al. 2009). Guo et al. (2001) observed that there is a migration of *Salmonella* from soil, directly into the stem scar tissue of green tomatoes. Other possible reasons could be spray or sprinkler irrigation; bioaerosols/aerosols produced during sprinkler irrigation (Cevallos–Cevallos et al. 2012b; Pianietti et al. 2004). Also, Cevallos–Cevallos et al. (2012a) demonstrated the dispersal of *Salmonella* by splashing water (artificial rain or irrigation). Oliveira et al. (2011) observed more contamination on the outer leaves of the lettuce than on the inner leaves. The outer leaves are more exposed to environmental conditions and are more favorable to contamination by direct contact with the soil. Some environmental factors like light

intensity, moisture, irradiation, and high temperature can significantly influence the growth of pathogens. The pathogen internalization through roots to plant vascular tissues, xylem and phloem (Hirneisen et al. 2012) and its transportation to the edible portion of the crop has received much debate over the last few years (Solomon and Sharma 2009). Internalization of pathogens is defined as the process of pathogen uptake through the roots into the intercellular spaces between plant cells and plant vascular tissues (Hirneisen et al. 2012). The pathogens can enter plants via root systems (Solomon et al. 2002a, b; Bernstein et al. 2007a, b) and the in-field splash can transport microorganisms from the soil surface quite far (Boyer 2008). Gu et al. (2011) studied microscopically an enteric pathogen, which traveled through the phloem. On the other hand, some researchers (Mitra et al. 2009; Zhang et al. 2009; Erickson et al. 2010) reported that there was no internalization of bacteria in the plant treated with inoculated irrigation water, manure, and soil. Internalization of pathogens into the edible part of the crop through contaminated soil and irrigation water is still unclear and contradictory.

Different soil types (Franz et al. 2008), management practices (Franz et al. 2005), microbial diversity (Gu et al. 2013c) have an effect on the growth of pathogens and showed differences in the decline rate of pathogen in soil with time. Some studies (Franz et al. 2008; Semenov et al. 2010) showed the comparison of two soil types. Franz et al. (2008) observed that *E. coli* O157:H7 survived better in clayey soil than sandy soil because there is higher availability of protective pore spaces in clayey soil than sandy soil, against feeding by soil fauna. Semenov et al. (2010) reported that high dissolved organic carbon (DOC) in soil supported better survival of pathogens.

In our study, the fate and transport of *E. coli* were carried out in a greenhouse. The bacterial contaminated soil and lettuce leaves were analyzed over a time period of 30 days. A known

amount of bacterial inoculum was given to lettuce crops through contaminated irrigation water. These crops were grown under four different treatments. The contamination retained on the crops and soil indicates the actual condition in the field when irrigation water becomes contaminated. This would prove our hypothesis of irrigation water as a potential source of foodborne illnesses and outbreaks. Also, there could be a movement of bacteria through roots to the edible tissues and thereby contaminating lettuce leaves. This research would even show that the *E. coli* fate and transport could be affected using four different treatments that comprised two soil types (organic and mineral) and two irrigation methods (drip and sprinkler). The present study would help farmers in reducing the bacterial contaminants on lettuce crop by selecting appropriate treatment and harvesting time. If farmers chose the treatment with high risk as shown in results, then irrigation water quality need to be checked, before using for irrigation.

3.2 Materials and Methods

3.2.1 Experimental Design

Greenhouse experiments were conducted on the Macdonald Campus of McGill University, St-Anne-de-Bellevue, Quebec during June–August 2014. The study area was between latitude 45.24 N and longitude 73.56 W with an elevation of 32 m. There were four treatments with different soil types and irrigation methods. Each treatment had five replicates and pots were randomly placed in the greenhouse as shown in Fig. 3.1. The greenhouse room was divided into two parts; one had un-inoculated pots with no bacterial water application and the other had inoculated pots with bacterial water application. The four treatments were each replicated five times for the inoculated and un-inoculated pots. To avoid any cross-contamination among the pots and

treatments, the pots were separated by a distance of almost 1 m in both the x and y directions. For drip irrigation, a drip line was connected to the pots using rubber tubes. To regulate the flow of water in the drip line, a flow meter and a pressure gauge were installed. A check valve was installed to prevent the backflow of bacterial inoculum contaminated water to the pots with no bacteria application. For sprinkler irrigation, a hand sprayer was used and water was given regularly to plants. For the experiments, a photoperiod of 12 h, constant temperature of 27 C, and humidity of 40–45% were maintained in the greenhouse (Gupta and Madramootoo 2016a).

3.2.2 Lettuce Production

Seedlings of Romaine lettuce (Lactuca sativa var. Green Towers) with few (4–5) true leaves were transplanted on 2nd July, 2014 into the plastic pots of height, upper and lower diameter of 25, 32.5, and 28, respectively. A mineral soil was collected from the Macdonald Campus Farm. A commercial organic potting soil was purchased from a local supplier (Laniel Prodamex). The mineral soil was sieved to remove all gravel and stones before filling the pots. Both the soil types were tested for *E. coli* but none of the soils showed any *E. coli*. Organic and mineral soil pots weighed 9.7 and 26.7 kg, respectively. Soil properties of mineral and organic soil are shown in Table 3.1. In comparison to an organic soil, the mineral soil has higher bulk density, percentage of sand, silt, and clay, low gravimetric moisture content, and a very low amount of organic matter. Non-inoculated irrigation water was applied to all the pots starting from 1st July until 21st August 2014 as shown in Table 3.2. Reference Evapotranspiration (ETo) was estimated using FAO-56 Penman–Monteith Equation (Allen et al. 1998) as shown in Eq. 1.

$$ET_o = [\{0.408\Delta(R_n-G)+\gamma(900/(T+273))u_2(e_s-e_a)\}/\{\Delta+\gamma(1+0.34u_2)\}] \quad (1)$$

Where, ET_o is the reference evapotranspiration (mm/d), R_n is the net radiation at the crop surface ($MJ/m^2/d$), G is the soil heat flux ($MJ/m^2/d$), T is the mean daily air temperature at 2 m height (°C), u_2 is the wind speed at 2 m height (m/s) which was assumed as 1.5 m/s for greenhouse conditions, e_s is the saturation vapor pressure (kPa), e_a is the actual vapor pressure (kPa), e_s-e_a is the saturation vapor pressure deficit (kPa), Δ is the slope vapor pressure curve (kPa/°C) and γ is the psychrometric constant (kPa/°C).

For estimating the crop evapotranspiration (ET_c), ET_o is multiplied with the crop coefficients (k_c) as shown in Table 3.2. Irrigation frequency or interval was calculated using Equation 2.

$$IR = D / ET_c \qquad (2)$$

Where, IR is the irrigation frequency or interval in days, D is the depth of water applied (mm).

During the same time period, the plants were fertilized thrice with 2.9 g of 20-20-20 NPK water-soluble fertilizer. Each pot was punctured at the bottom. The pots were placed on plastic trays to collect the drain water and to avoid spills and contamination of the greenhouse surrounding. Drain water collected after harvesting the lettuce, was tested for the presence of microorganisms. Harvesting of lettuce was done on 23rd August 2014. The limitations for greenhouse experiments are mentioned in Appendix 4.

3.2.3 Preparation of Inoculum, Inoculation of Irrigation Water and its Application to Lettuce

In this study, *E. coli* ATCC8739 was used and it is reported as a surrogate organism for *E. coli* O157:H7 in some studies (Evrendilek et al., 1999; Li and Zhang, 2004; Orlowska et al., 2015). It is non-pathogenic bacterium and does not carry vero-toxin genes (stx1 and stx2). This bacterium belongs to Biosafety level 1 and is isolated from feces. *E. coli* ATCC8739 pellets (American Type Culture Collection, USA) were transferred aseptically in 5 ml of Luria Bertani (LB) miller broth (Sigma-Aldrich, USA) and mixed well using a vortex mixer. A bacterial culture of 0.1 ml from the LB tube was spread plated on BBL™ Levine Eosin Methylene Blue (EMB) agar (BD, Becton, Dickinson and Company, USA). EMB agar is a selective differential medium, selective for gram-negative bacteria against gram-positive bacteria and differential for lactose fermenters and non-lactose fermenters. *E. coli*, being gram-negative and vigorous lactose fermenter, produces green metallic sheen colonies with dark centers. Green metallic sheen on EMB agar confirms the presence of *E. coli* ATCC8739. Spread plating was replicated on five different EMB agar plates. LB tube and five EMB agar plates were incubated at 37°C for 24 h. Colonies of *E. coli* ATCC8739 on EMB plates were transferred in 20 ml of sterile Bacto™ Tryptic Soy broth (TSB) (BD, USA) and then incubated at 37°C for 24 h with agitation. After bacterial growth, 20 ml of TSB bacterial culture was transferred to twelve 1.5 ml Eppendorf tubes and centrifuged at 7.7×1000 RPM (4000×g) for 20 min, to harvest the bacterial cells. These Eppendorf tubes were then washed with 0.1% Tryptone Saline Solution (TSS) (1 g/L tryptone (Sigma-Aldrich, USA) and 8.5 g/L NaCl (Fischer Scientific, USA)) in 1 L of 0.1% TSS. The optical density of TSS with *E. coli* ATCC8739 was estimated using a spectrophotometer set at a wavelength of 600 nm according to previously determined standard curves. The serial

suspension dilutions of TSS were plated on the EMB agar plates. Plates were then incubated at 37°C for 24 h, to count the colony forming units per ml (cfu/ml) of TSS.

Similarly, four liters of contaminated irrigation water with inoculum, *E. coli* ATCC8739 at 7.23 log cfu/ml was prepared under sterile laboratory conditions. There was only one-time application of contaminated irrigation water to lettuce. Bacterial water was given (on 20th July, 2014) after two weeks, since transplanting the seedlings into the pots. Each inoculated pot was provided with 150 ml of bacterial water using the two different irrigation methods. For drip irrigation, a container was filled with 2 L of contaminated water and an equal amount of 150 ml of water was applied to all pots in 15 min. In the case of sprinkler irrigation, the pots were manually irrigated with 150 ml of contaminated irrigation water using a sterile hand sprayer. A hand sprayer applied water uniformly over the plant from the distance of 25 cm. Water application was done carefully to prevent any splashes or spills of the inoculum. At the same time when inoculated pots were irrigated with bacterial inoculum, each of un-inoculated pots was irrigated with 150 ml of non-contaminated water using both irrigation methods.

3.2.4 Soil and Lettuce Leaves Sample Collection

All lettuce leaves and soil samples were collected from the greenhouse using sterilized gloves, scissors, and spatulas. To avoid contamination of un-inoculated samples, sampling was carried out firstly from the un-inoculated pots and then from the inoculated pots. In order to confirm sterile conditions, samples were collected from all the pots prior to application of bacterial water. From all the pots, samples were collected on 2nd, 5th, 10th, 15th, 20th, 25th and 30th day from the day of bacterial inoculation i.e. from 22nd July to 20th August 2014. At each sampling time, approximately 20 g of soil at 5-10 cm depth was obtained aseptically from each pot and placed in

sterile plastic sampling bags. Similarly, approximately 5 g of inner and outer lettuce leaves were obtained randomly and aseptically from each pot and were placed in sterile sampling bags. The samples were then carried immediately to the laboratory and stored in a refrigerator at 4°C until the analyses were done, within 48 h. The leaf area index (LAI) and dry biomass of lettuce were measured and reported in Appendix 5 (Table 1).

3.2.5 Microbiological Analysis

All the lettuce leaves and soil samples were evaluated in the laboratory for *E. coli* ATCC8739. For the analyses, 10 g of soil was taken from 20 g sample and added to 90 ml of 0.1% TSS in a 250 ml Erlenmeyer flask and homogenized well by shaking at 150 RPM for 20 min. Simultaneously, 5 g of lettuce leaves was mixed with 45 ml of 0.1% TSS in sterile plastic bags. TSS with leaf sample was rinsed well by rubbing and vigorously agitating by hand for two minutes. After homogenization, serial dilutions (1:10) of soil and lettuce samples were prepared in sterile test tubes using 0.1% TSS. An amount of 0.1 ml of each dilution was spread onto sterile EMB agar plates. The plates were then incubated at 37°C for 24 h. *E. coli* colonies were counted on plates spread with serial dilution. For inoculated and non-inoculated treatments, the above-mentioned method was used for counting the *E. coli* and non-*E. coli* colonies.

Drain water analysis was carried out to check for the presence of bacteria. After harvesting the crop, the pots were saturated with water and the drain water was collected (almost 500 ml) in sterile bottles. For analysis, 100 ml of water was taken from each bottle. The membrane filtration technique was carried out to confirm the presence of *E. coli* in the drain water. In this technique, 100 ml sample was used to prepare serial dilutions (1:10) using phosphate buffer (1.25 ml/L of 0.25M KH_2PO_4 at pH 7.2 and 5 ml/L 0.4M $MgCl_2$). These were filtered through 47 mm diameter, 0.45µm pore size mixed cellulose esters sterile membrane filter paper (Fischer

Scientific, USA) under vacuum suction. The filter with the residue was then placed on mcoliBlue24® broth (HACH Company, USA) absorbed on pads in sterile 47 mm diameter petri plate (Fischer Scientific, USA). Plates were then incubated at 37°C for 24 h. mcoliBlue24® broth is a selective medium for *E. coli* and shows blue colonies for *E. coli* presence and pink colonies for other coliforms (USEPA, 2003).

3.2.6 Statistical Analysis

Analysis of variance (ANOVA) was carried out using Student's t test on JMP software version 11.2.0 at 95% confidence interval. Before running ANOVA, assumptions of normality and homogeneity of variances were checked. One-way ANOVA and two-way ANOVA tests were then performed for all the sampling days. One-way ANOVA was performed for the irrigation methods, soil types and individual treatments. Two-way ANOVA was performed to check the statistical significance of the interaction effect between irrigation method and soil type (see Appendix 6).

3.3 Results

3.3.1 Transfer and Survival of *E. coli* in Soil

There was no contamination of the soil observed prior to the bacterial inoculation. After a bacterial inoculation, bacteria were observed in all the treatments on the 2^{nd}, 5^{th}, 10^{th}, 15^{th}, 20^{th}, 25^{th} and 30^{th} day. There was a decreasing trend in the concentration of bacteria in all the treatments. This may be due to movement of bacteria to deeper soil depths. As seen in Figure 3.2, among all treatments, D+O shows the highest bacterial transfer of 7.03 log cfu/g on 2^{nd} day.

In contrast, D+M shows the lowest bacterial transfer of 3.85 log cfu/g. This shows that soil type significantly influences survival of the bacteria in soil. On the 5th day, there was a decrease in concentration of *E. coli* by 1 log cfu/g for the treatment D+M. However, a similar trend was noticed between the 15th and 20th day for the treatment D+O. An early bacterial decay or movement to lower depths was observed in mineral soil compared to the organic soil. Also, it can be due to little or no organic matter available for bacteria in the mineral soil as compared to the organic soil.

From Figure 3.2, it was noticed that in case of the sprinkler irrigation, fate and transport of bacteria were independent of the soil type. As both treatments with sprinkler irrigation had almost the same concentration of bacteria about 4.9-4.95 log cfu/g on the 2nd day after inoculation. For the first 25 days there was an identical bacterial concentration for these two treatments i.e. S+O and S+M. Afterwards, bacterial concentration started to diverge, as there was a greater decline in bacterial concentration in the mineral soil, probably because of less nutrient availability.

For D+O treatment, the 30th day bacterial concentration was highest (4.71 log cfu/g), followed by S+O treatment (3.59 log cfu/g). Therefore, it shows that the organic soil retained a greater concentration of bacteria irrespective of the irrigation type. In the case of the mineral soil, lowest bacteria survival (0.79 log cfu/g) was found with drip irrigation compared to sprinkler irrigation (about 3.38 log cfu/g).

There was no contamination of *E. coli,* but non–*E. coli* colonies were observed on un-inoculated pots. These colorless or pink colonies on EMB agar could be aerobic mesophilic, *Enterobacteriaceae* or *Pseudomonas* spp. populations (Oliveira et al., 2012).

Table 3.3 also shows the statistically significant differences between the treatments. D+O treatment was significantly different from D+M to S+M treatments for all sampling days. It was noticed that the D+O treatment was not significantly different from S+O treatment after 3 weeks (20th day) of bacterial inoculation. The S+O treatment was found to be significantly different from the D+M treatment for all sampling days. However, it was not significantly different from S+M treatment for most of the days except on the 30th day. The S+M treatment was significantly different from D+M treatment for all sampling days except for the 25th day. Two-way ANOVA found that the interaction was always statistically significant on all sampling days. This means that the interaction between the two factors (soil type and irrigation method) influence the bacteria transport and survival.

3.3.2 Transfer and Survival of *E. coli* on Lettuce Leaves

There was no contamination on the leaves (edible part of the plant) prior to inoculation. On the days of sampling, the bacteria were found on leaves in mostly all the inoculated treatments except in the D+M treatment. All the other treatments showed that the bacterial concentration was declining with time, as bacteria from the edible part would be washed off or diluted with clean irrigation water and would move to lower soil depths.

As seen in Fig. 3.3, the S+O treatment shows the highest bacterial transfer and survival of 5.32 log cfu/g on the 2nd day, followed by S+M treatment (4 log cfu/g) and D+O treatment (3.27 log cfu/g). Unlike drip irrigation, sprinkler irrigation showed a higher bacterial contamination due to direct contact with contaminated water onto the leaves. In the case of drip irrigation, bacteria from the soil were transported to the leaves through the xylem, phloem, and root system (Hirneisen et al. 2012). Treatment D+M showed nil or lowest bacterial survival of 0 log cfu/g on

the 2nd day. This shows that the irrigation method has a significant influence in contaminating the lettuce leaves, as sprinkler irrigation resulted in higher transfer and retention of bacteria on the edible portion of the plant.

Figure 3.3 shows that survival of bacteria in the case of S+M and D+O treatments reaches almost zero with time (on 30th day), whereas S+O treatment on the 30th day shows a considerably high amount of survived bacteria i.e., 2.88 log cfu/g. In the S+O treatment, there was long lasting contamination due to the presence of organic matter, which provides food and nutrients for bacterial growth and through direct association of bacterial contamination on leaves. In the case of D+M treatment, some bacterial survival was noticed on the 20th day, and then it again dropped to zero, possibly due to experimental error or the fact that bacteria could not sustain the conditions for survival and multiplication.

Sprinkler irrigation resulted in the direct contact of bacteria with the leaves whereas no direct contact with the leaves was observed in the case of drip irrigation. In Table 3.4 it was noticed that the S+O treatment was always significantly different from other treatments on all sampling days because it had maximum contamination. D+O treatment was significantly different from D+M and S+M treatments for most of the time (almost 2 weeks). Similarly, it was noticed for the D+M treatment and S+M treatment that they were initially significantly different from each other, but after 20 days there was no significant difference between them as bacterial concentration reduced to zero. Two-way ANOVA showed that the interaction effect and fixed effects were always significantly different from each other. Therefore, interaction effect between the two factors (soil type and irrigation method) was found to be significant in terms of bacterial survival on lettuce leaves.

3.3.3 Survival of *E. coli* in Drain Water and *E. coli* Count Balance Analysis

Drain water (500 ml) was collected from all the pots after harvesting the crop and was tested in the laboratory for *E. coli*. It was observed that drain water samples from all bacterial treated pots were devoid of bacteria. None of the inoculated pots showed positive results for bacteria draining from the soil and root zone of plants as shown in Table 3.5. It seems that the soil acted as a filter and has the ability to bind with bacteria. The amount of bacteria given was 7.23 log cfu/ml of water. For certain treatments, bacteria were not transferred and survived on lettuce leaves and soil. The bacterial loss could be due to bacterial death, decay, lack of nutrients to grow or survive, or retention in the lower layers of soil. If the drain water was contaminated under field conditions then it could have resulted in contamination of groundwater and this groundwater need to be tested and/or treated before use in order to avoid the spread of contamination.

3.4 Discussion and conclusion

Organic soil treatments (S+O and D+O) confirmed higher bacterial contamination than mineral soil treatments (S+M and D+M), due to high organic matter and carbon content. Similarly, Semenov et al. (2010) noticed that high dissolved organic carbon (DOC) favors or supports the growth of pathogens. There was a sudden decline of bacteria of 1 log CFU/g by day 21st in D+O treatment which may be due to the physiological stress placed on *E. coli* cells in soil, as also observed by Sharma et al. (2009). D+O treatment showed maximum contamination as the organic soil is favorable for microbial growth due to the availability of organic matter (Semenov et al. 2010). Also in the D+O treatment, drip irrigation played a vital role in contamination as the inoculated water was in direct contact with the soil (Forslund et al. 2012). High *E. coli* concentration in organic potting soil could be due to the reason that the organic potting soil

is very different from organically managed soil (Franz et al. 2005). Franz et al. (2005; 2008) reported in organically managed soil, *E. coli* and *Salmonella* are generally suppressed due to microbial competition (Gu et al. 2013b).

The D+O treatment also confirmed that bacteria could have been transported or entered into the leaves through the root system as there was contamination observed on the lettuce leaves. Hirneisen et al. (2012) reported that there was internalization of the pathogens through the plant roots grown in inoculated soil. However, Erickson et al. (2014) reported that there was little or no internalization of bacteria. Also, Solomon and Matthews (2006) observed lettuce leaves positive for *E. coli* O157:H7 when grown in manure-amended soil inoculated with *E. coli* O157:H7. The potential internalization of bacteria can be due to damaged roots of plants and wounding of the root tips (Bernstein et al. 2007a).

Brandl and Amundson (2008) reported that young lettuce leaves were more susceptible to bacterial contamination than old leaves. Our results also support that young leaves were more susceptible to contamination. Young leaves that received the inoculation at 7.23 log CFU/ml level showed more contamination during the initial days and then contamination began to decline with time. Sprinkler irrigation (S+M and S+O treatments) showed more contamination of lettuce leaves than drip irrigation (D+M and D+O treatments) as the leaves were in direct contact of inoculum and bacteria could enter the leaves through stoma or through aerosols (Cevallos–Cevallos et al. 2012b). Also, Solomon et al. (2002b) reported that sprinkler irrigation poses high risk of contamination than drip irrigation on spinach and rocket.

Dong et al. (2003) expected that *E. coli* O157:H7 has the highest probability of internalization and contamination compared to other pathogens, and higher inoculum levels would result in more contamination and internalization into the plants. However, our experimental results do not

support this hypothesis because there was little or no contamination and internalization of bacteria to leaves in the case of the D+M treatment, compared to other treatments. Miles et al. (2009) support our results to some extent as they observed that with the inoculation of 7 log CFU/ml every 14th day for 70 days resulted in no internalization of bacteria to stem, fruit, or leaves of the tomato plant. Although, in our study we observed that there was difference in bacterial retention and contamination on the soil and edible leaves, for all the four (D+O, S+O, S+M, and D+M) treatments that comprised different soil types and irrigation methods. The possible reason of bacterial loss could be bacterial death and decay. Other reasons could be that the bacteria did not find favorable amount of nutrients to grow or survive in soil and leaves. Bacteria may remain retained or adsorbed with the soil particles between the depths of 10–25 cm (Gupta and Madramootoo 2016a).

Application of *E. coli* ATCC8739 contaminated irrigation water resulted in contamination of lettuce leaves and soil using different irrigation methods and soil types. Unlike mineral soil, organic soil showed more contamination in soil and leaves, as organic soil is rich in organic matter and has the ability to bind with bacteria. It was found that there could be bacterial movement to the edible tissues in the case of D+O treatment. Movement of bacteria to leaves might have occurred via vascular tissues, xylem and phloem, entering through the root system or via aerosols produced during sprinkler irrigation. Sprinkler irrigation was found to be effective in the contamination of edible part as contaminants were in direct contact with the plant.

All the four treatments with different soil types and irrigation methods behaved differently and showed difference in bacterial retention and contamination. Drain water was negative for the bacterial contamination in all the treatments. This could be due to the fact that the soil acted as a filter and bacteria were adsorbed onto the soil particles in lower soil depths. Bacterial

contamination of RTE vegetables can cause detrimental effect and can pose significant risk to human and environmental health. Also, the contaminated vegetables can cross-contaminate the food during preparation of food. Irrigation water must be tested and treated well prior to application on crops as it can pose risks to consumers. The *E. coli* level used in our study was far greater than usually found in the field under natural conditions. However, a low level of *E. coli* can result in health hazards and detrimental effects. Therefore, the source and distribution of irrigation water, manure use, and history of land should be well known, to limit the pathogen introduction to crops in the fields.

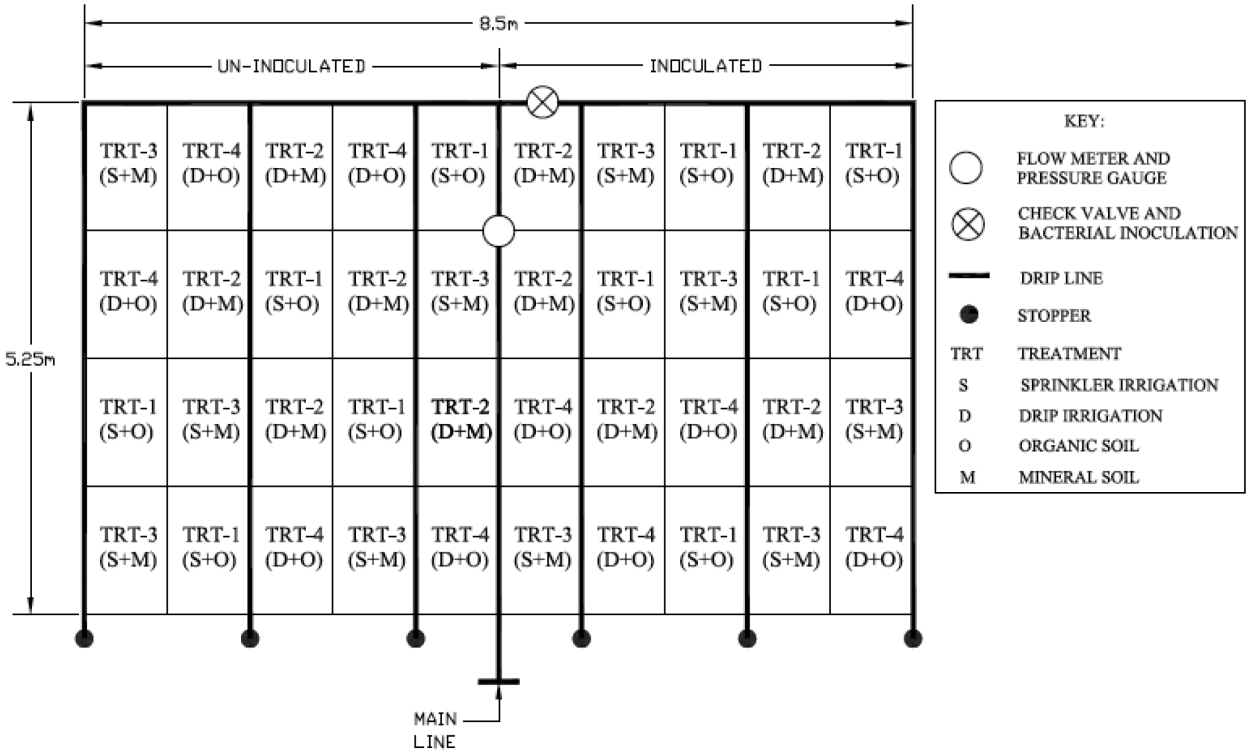
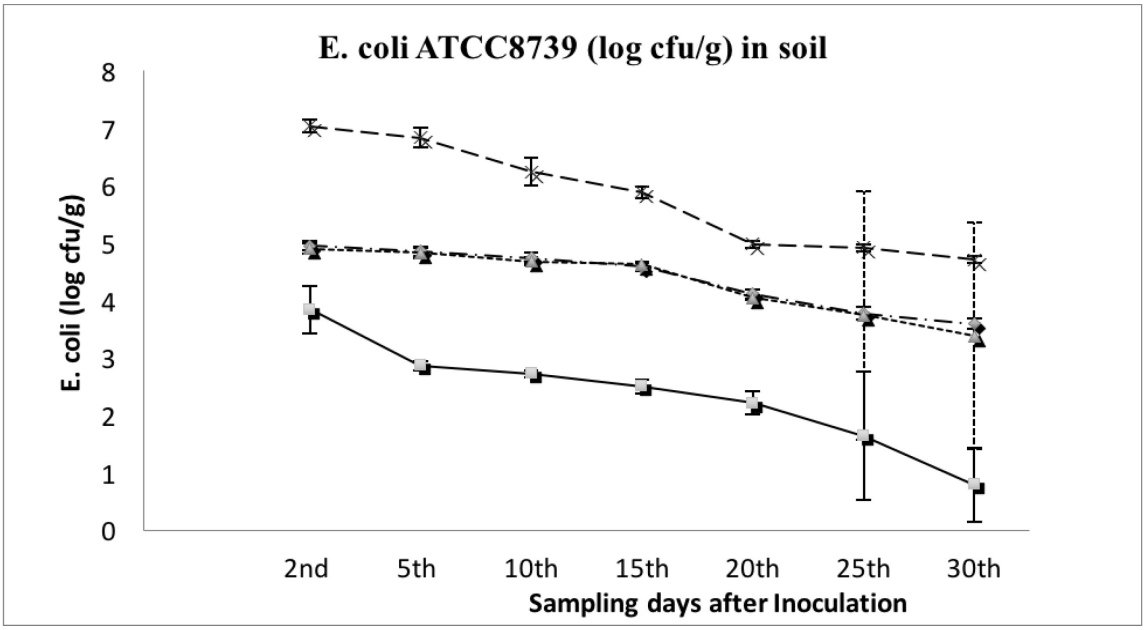


Figure 3.1: Layout of Experimental design with different treatments (TRT-1, TRT-2, TRT-3 and TRT-4) in Macdonald Campus greenhouse, where TRT-1: with sprinkler irrigation and organic soil (S+O), TRT-2: with drip irrigation and mineral soil (D+M), TRT-3: with sprinkler irrigation and mineral soil (S+M) and TRT-4: with drip irrigation and organic soil (D+O).



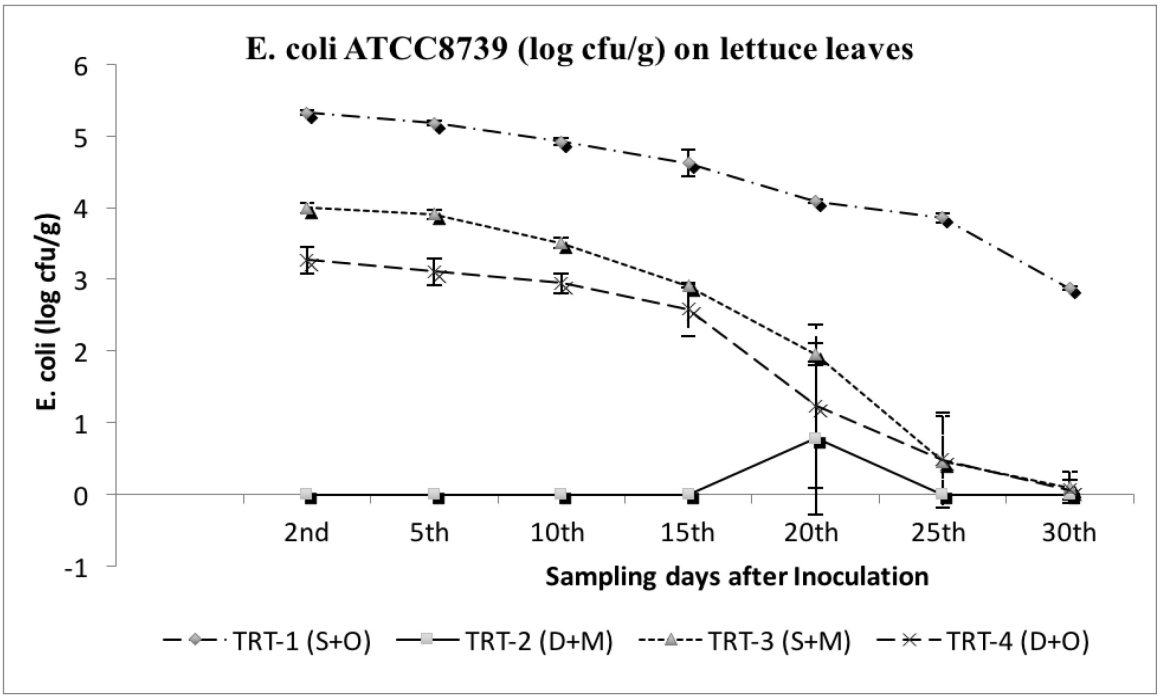


Figure 3.2 and 3.3: *E. coli* ATCC8739 (log cfu/g) in soil and on lettuce leaves with respect to time in different treatments. Represented with mean (point with marker) and standard deviation (error bars).

Table 3.1: Soil properties for soil used in different treatments

Soil type	Sand (%)	Silt (%)	Clay (%)	Organic matter (%)	Bulk density (g/cm3)	Gravimetric moisture content (%) at pot filling
Mineral Soil	50.9	27.2	21.9	0.1	1.5	15.37
Organic Soil	12	11	6	71	0.35	65.19

Table 3.2: Irrigation water application and frequency based on growth stages and crop evapotranspiration

Growth stages	Length of growth stages (days)	K_c	ET_o (mm/d)	ET_c ((mm/d)	Lettuce growth stages in 2014	D (mm)	IR (days)
Initial	20	0.7	2.59	1.81	17th June-6th July	5-10	2-days Interval
Developmental*	30	0.7-1.15	2.43	1.81-2.45	7th July-5th August	10-15	3-days interval
Mid	15	1.15	2.13	2.45	6th-20th August	11-15	3-days interval
Late	10	1.15-0.8	1.89	2.45-1.51	21st-30th August	10	One time on 21st August 2014

*lettuce was inoculated with bacteria during developmental stage i.e. 20th July, 2014.

Where, length of growth stages (days) was generated from Allen et al. (1998), K_c is crop coefficient for all the specific stages and was calculated based on crop coefficient curve (Allen et al. 1998), ET_o is the reference evapotranspiration (mm/d), ET_c is the crop evapotranspiration (mm/d), D is the depth of water applied (mm) and IR is the irrigation frequency or interval in days.

Table 3.3: Significant difference among the treatments for *E. coli* ATCC8739 concentration (log cfu/g) in lettuce soil

Treatments	2nd Day	5th Day	10th Day	15th Day	20th Day	25th Day	30th Day
			Contrast differences Probabilities (Prob>F)				
D+O vs D+M	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*
D+O vs S+M	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	0.0047*	0.0014*
D+O vs S+O	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	0.1538	0.1085
S+O vs D+M	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	0.0016*	0.0002*
S+M vs D+M	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	0.0612	0.0214*
S+O vs S+M	0.788	0.5937	0.6239	0.3256	0.5868	0.0939	0.0467*
			Two way ANOVA				
Irrigation method (Drip and Sprinkler)	0.0003*	0.9201	0.0007*	<.0001*	<.0001*	0.7138	0.5508
Soil type (Organic and mineral)	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	0.0001*	<.0001*
Irrigation method*Soil type (Interaction effect)	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	0.0246*	0.0082*

*** means they are significantly different from each other (p<0.05)**

Table 3.4: Significant difference among the treatments for *E. coli* ATCC8739 concentration (log cfu/g) on lettuce leaves

Treatments	2nd Day	5th Day	10th Day	15th Day	20th Day	25th Day	30th Day
Contrast differences Probabilities (Prob>F)							
D+O vs D+M	<.0001*	<.0001*	<.0001*	<.0001*	0.3433	0.1198	0.4774
D+O vs S+M	<.0001*	<.0001*	<.0001*	0.0314*	0.1623	0.9463	0.6342
D+O vs S+O	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*
S+O vs D+M	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*
S+M vs D+M	<.0001*	<.0001*	<.0001*	<.0001*	0.0266*	0.1348	0.2429
S+O vs S+M	<.0001*	<.0001*	<.0001*	<.0001*	0.0005*	<.0001*	<.0001*
Two way ANOVA							
Irrigation method (Drip and Sprinkler)	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*
Soil type (Organic and mineral)	<.0001*	<.0001*	<.0001*	<.0001*	0.0019*	<.0001*	<.0001*
Irrigation method*Soil type (Interaction effect)	<.0001*	<.0001*	<.0001*	0.0003*	0.0294*	<.0001*	<.0001*

*** means they are significantly different from each other (p<0.05)**

Table 3.5: Bacterial count balance for inoculated lettuce pots after 30th day of inoculation

Bacterial count balance after 30th day of inoculation					
Treatments	**Total amount of *E. coli* given in irrigation water (log cfu/ml)**	***E. coli* in soil on 30th day (log cfu/g)**	***E. coli* on lettuce leaves on 30th day (log cfu/g)**	***E. coli* drained (log cfu/g)**	***E. coli* stored in soil at depth of 10-25 cm or death and decay of *E. coli* (log cfu/g)**
TRT-1 (S+O)	7.23	3.59	2.88	0	0.76
TRT-2 (D+M)	7.23	0.79	0.00	0	6.44
TRT-3 (S+M)	7.23	3.38	0.10	0	3.75
TRT-4 (D+O)	7.23	4.71	0.06	0	3.98

Connecting text to Chapter 4

Chapter three of this dissertation showed that contaminated irrigation water is one possible source or carrier of pathogen to the lettuce leaves. *E. coli*'s fate and transport was analyzed on lettuce crop, grown under four different treatments comprised of two soil types namely, organic and mineral soil, and two irrigation methods namely, sprinkler and drip irrigation method. The pathogen fate and transport was found to be different for each treatment in the case of lettuce leaves and soil. The treatment with drip irrigation and mineral soil showed the least pathogen transfer and survival over the time period of 30 days. Chapter four fills in the knowledge gap with respect to tomato, fresh fruit which can be contaminated and cause foodborne illnesses. Tomato was grown similar to lettuce with four different treatments. Manually contaminated irrigation water was applied to analyze the fate and transport of *E. coli* in all different treatments over the time period of 30 days after inoculation. The manuscript from this study, which addressed objective second of this dissertation was accepted and published in *Exposure and Health* Journal.

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Chapter 4: Fate and transport of *Escherichia coli* in Tomato production

Divya Gupta and Chandra A. Madramootoo

Abstract

E. coli can result in foodborne illnesses and outbreaks to consumers through consumption of contaminated fresh fruits and vegetables. A greenhouse study was conducted to understand the fate and transport of bacteria in soil and tomato fruits. *E. coli* contaminated irrigation water was applied to tomato plants grown in the greenhouse. Two soil types, namely organic and mineral soil and two irrigation methods, namely drip and sprinkler irrigation were used to generate four different treatments. Tomato fruits in two treatments (drip irrigation and organic soil (D+O), and sprinkler irrigation and mineral soil (S+M)) showed bacterial contamination. However, the D+O treatment might have internalization of bacteria in the tomato fruit. Bacterial contamination in the soil (at the depth of 5-10 cm) was decreasing with time for all the treatments. Organic soil showed more bacteria retention than mineral soil because organic soil is rich in organic matter and organic matter can carry bacteria with it. Sprinkler irrigation posed a higher risk of contamination on the fruits on the 2nd day after inoculation than drip irrigation because the edible part of the crop is in direct contact with the contaminated water. A bacterial count balance study showed that the bacteria were retained mostly in the soil at lower depths of 10-25 cm, in addition to bacterial death in various habitats. The treatment with drip irrigation and mineral soil (D+M) was noticed as the best treatment because it did not show any sign of bacterial contamination in the tomato fruits.

Keywords: *E. coli*, irrigation, treatments, tomato, internalization, fate.

4.1 Introduction

Foodborne disease outbreak (FBDO) is an incident in which two or more persons experience a similar illness resulting from the ingestion of a common food (CDC 2000). There are several foodborne outbreaks reported in the past few years (Sivapalasingam et al., 2004), which are due to the consumption of tomatoes or its products. It has been reported that foodborne outbreaks associated with fresh fruits and vegetables in the USA have nearly tripled since 1973 (Tauxe et al. 1997). Valadez et al. (2012) reported that between 1979-2009 there were about 21 outbreak cases notified in North America and Europe, associated with contaminated tomatoes or foods involving tomatoes.

Pathogenic microorganisms may colonize the fruits and vegetables (Beuchat 1996). Some of the bacteria responsible for contamination and foodborne outbreaks are enlisted as *Salmonella*, *Shigella*, *Escherichia coli O157:H7*, *Clostridium botulinum* and *Listeria monocytogenes*. *Escherichia coli* is the bacterium that is commonly used as an indicator organism for fecal contamination and water pollution (Clesceri et al. 1998). *Escherichia coli O157:H7* or Enterohemorrhagic *E. coli* (EHEC) is a pathogenic bacterium and belongs to the family of *Enterobacteriaceae.* This bacterium is associated with shiga toxin or verotoxin and results in hemolytic uremic syndrome (HUS) (Mathusa et al. 2010). Nguyen and Carlin (1994) reported that *Escherichia coli* has been found in fresh fruit and vegetable salads and can lead to various gastrointestinal problems. Therefore, *E. coli* contaminated irrigation water delivered to crops can be a potential source of contamination to fresh fruits and vegetables (Cevallos-Cevallos et al. 2012a; Gu et al. 2013a).

The mechanism of entry of pathogen into plants is unclear and contradictory (Gu et al. 2013a). One of the hypothesis is that entry of pathogens could be through contaminated irrigation water contacting the leaf surface's stoma, stem's scar tissue, or through wounds or from raindrop splashes from the soil surface (Kroupitski et al. 2009; Mitra et al. 2009; Gu et al. 2011; Cevallos-Cevallos et al. 2012b; Gu et al. 2013b). It was observed that there was a migration of *Salmonella* from the soil to stem scar tissue of the green tomatoes (Guo et al. 2001). Another possible reason of contamination could be bioaerosols (Pianietti et al. 2004), which are produced by sprinkler irrigation. These bioaerosols with microorganisms could stay on the plant or the fruit's surface to cause contamination. Cevallos-Cevallos et al. (2012b) observed that *Salmonella* can reach tomato fruits, when fruits are exposed to aerosols formed by rain. Solomon et al. (2003) reported that spray irrigation is linked to the contamination of crops and suggested that repeated exposure of contaminated water to the plants increases the level of *E. coli* O157:H7. Islam et al. (2004) found that *E. coli* O157:H7 persisted in soil for more than 5 months after application of contaminated compost or irrigation water, irrespective of source or crop type.

Pathogen contamination and internalization through roots to plant vascular tissues (xylem and phloem) and its transportation to the edible portion of the crop has received much debate over the last few years (Solomon and Sharma et al. 2009; Hirneisen et al. 2012). Hirneisen et al. (2012) defined internalization of pathogens as the process of pathogen uptake through the roots into the intercellular spaces between plants cells and plant vascular tissues. Gu et al. (2011) were the first to show microscopically that an enteric pathogen travels through the phloem. Some other studies also proved that the bacteria can enter the plants and its edible part via root systems (Solomon et al. 2002a; 2002b; Bernstein et al. 2007a; 2007b) but others disagreed with this hypothesis and reported that there is no internalization of bacteria into the plants and its parts with the inoculated

irrigation water, manure or soil (Mitra et al. 2009; Zhang et al. 2009; Erickson et al. 2010). Therefore, further research is required on the internalization of pathogens into the plants and its edible parts through contaminated soil and irrigation water.

Oliveira et al. (2011) reported that there are environmental factors like light intensity, moisture, irradiation and high temperature that can significantly influence the growth of pathogens. Also, there are effects of aerobic and anaerobic conditions (Semenov et al. 2011), temperature (Semenov et al. 2007), soil conditions and in particular microbial diversity (Semenov et al. 2008; Gu et al. 2013c) on the growth of pathogens. Different soil types and soil management practices exhibit differences in decline rate of human pathogens in the soil with time. Franz et al. (2008) reported that soil texture plays an important role to prolong the survival of *E. coli* O157:H7 in the soil. Franz et al. (2008) observed that there was prolonged survival of *E. coli* O157:H7 associated with clayey (fine-textured) soil compared to sandy (coarse-textured) soil. Franz et al. (2005) showed that *E. coli* O157:H7 declined faster in organic management practices than in conventional management practices. However, Semenov et al. (2008) reported that decline of *E. coli* O157:H7 was more irregular in conventional management practices and loamy soil than in organic management practices and sandy soils. Semenov et al. (2010) reported that pathogens survive or grow better at high dissolved organic carbon (DOC) concentrations, and maintain high population densities, which are sufficient to cause disease in humans.

Movement of *E. coli* O157:H7 and other pathogens in the soil profile are affected by the water flow, soil texture, pH, temperature and structure of root system in the soil (Kemp et al. 1992; Semenov et al. 2009). However, even small amounts of rainfall or irrigation can carry significant numbers of pathogens through soil profile to groundwater (Artz et al. 2005; Lang and Smith 2007; Semenov et al. 2009). Semenov et al. (2009) showed that slurry had two times higher

concentration of dissolved organic carbon and nitrogen than manure. Slurry rich in organic matter moved faster resulting in higher risk of pathogen movement to the bottom of the soil columns than manure (Semenov et al. 2009).

Tomatoes are a popular commodity around the world and eaten fresh or in processed form. Tomatoes have both nutritional and health benefits; therefore, consumption of tomatoes is steadily increasing. Tomatoes have a short shelf life of 7 to 14 days (Garcia-Gimeno and Zurera-Cosano 1997). Fresh tomatoes can be contaminated with pathogens and can result in foodborne illnesses and outbreaks. Guo et al. (2001) reported there were 176 cases of foodborne outbreaks in Illinois, Michigan, Minnesota, and Wisconsin in 1990, associated with consumption of fresh tomatoes. CDC (1993) identified that tomatoes were the vehicle of *Salmonella enterica* infection and resulted in multistate outbreak. *Salmonella* also resulted in an outbreak in geographically separated regions of United States with the consumption of diced tomatoes (Guo et al., 2001). There were 22 cases of foodborne illnesses due to consumption of tomatoes during time period 2004-2010 (http://www.foodpoisonjournal.com). The tomatoes are mostly consumed fresh and there is no "kill-step" in the processing for the elimination of pathogens (Maitland et al. 2011; Valadez et al. 2012). Thus, contamination of tomatoes by foodborne pathogens creates challenges to the fresh produce industry, public health organizations and officials (Valadez et al. 2012). Lynch et al. (2009) reported that it is often difficult to isolate the causative organism from the fresh, processed or consumed product.

In this study, the fate and transport of *E. coli* was analyzed in the tomato fruits and in the soil over the period of 30 days in the greenhouse. Tomato plants grown under four different treatments were inoculated with contaminated irrigation water. The contamination retained in tomato fruits and soil and probable bacterial internalization through the root system to the

vascular tissues were studied. The contaminated irrigation water would indicate the actual condition of the field when bacterial contaminated irrigation water is applied to the crops, which could result in foodborne illnesses or outbreaks to consumers. This would prove our hypotheses of irrigation water as a potential source of contamination and there could be internalization of bacteria through the root system to the edible part of the tomato plant.

4.2 Materials and Methods

4.2.1 Experimental design

A greenhouse study was conducted on the Macdonald Campus of McGill University, St-Anne-de-Bellevue, Quebec during June-November 2014. The greenhouse area is located at latitude 45.24° N and longitude 73.56°W with an elevation of 32 m. Four treatments (five replicates each) with two different soil types and irrigation methods were used as shown in Table 4.1. Tomato pots were categorized as inoculated and un-inoculated and these were randomly placed in the greenhouse as shown in Figure 4.1. Inoculated pots had a one-time bacterial water application; whereas, un-inoculated pots had no bacterial water application. Both inoculated and un-inoculated pots had four treatments with five replicates each. Tomato pots were separated by a distance of 1 m in x-y directions, to avoid any cross-contamination among inoculated and un-inoculated pots and different treatments. Irrigation methods, namely drip and sprinkler irrigation and soil types, namely organic and mineral soil were used. For installation of drip irrigation, a drip line was connected to the pots using spaghetti rubber tubes. A flow meter with pressure gauge was installed to regulate the flow of water. To prevent backflow of bacterial contaminated water to un-inoculated pots, a check-valve was installed. Sprinkler irrigation was carried out

using a hand sprayer and the water was given regularly to all the plants. For the greenhouse experiments, a constant temperature of 27°C, photoperiod of 12 h and humidity of 40-45% was maintained.

4.2.2 Tomato production

Tomato (*Solanum lycopersicum* var. Ramapo) seeds were germinated in small seed-starting containers or "cell flats" in the greenhouse. Two weeks after germination i.e. on 2nd July, 2014, seedlings were transplanted to plastic pots. The plastic pots were of height 25 cm with an upper and lower diameter of 32.5 cm and 28 cm. Two types of soil, a commercial organic potting soil (71% organic matter) was obtained from a local supplier (Laniel Prodamex) and a mineral soil was obtained from the Macdonald Campus Farm. Gravel and stones were removed from the mineral soil through sieving. Soil properties as shown in Table 4.2, were estimated for the two soil types before filling the pots. In comparison to the organic soil, the mineral soil had a higher bulk density, percentage of sand, silt and clay, low gravimetric moisture content and low amount of organic matter. Before filling the pots, 10 samples each from both the soil types were tested for *E. coli* and there was no *E. coli* found in any of the samples. Twenty pots for each soil type were filled and weighed to 9.7 kg for organic soil and 26.7 kg for mineral soil. Irrigation water without any inoculum was applied regularly to all the pots starting from 1st July until 8th November 2014 as shown in Table 4.3. Crop evapotranspiration and irrigation frequency were calculated from equations 1 and 2. Reference Evapotranspiration (ET_o) was estimated using the FAO-56 Penman-Monteith equation (Allen et al. 1998):

$$ET_o = \frac{0.408 * \Delta * (R_n - G) + \gamma * \frac{900}{T+273} * u_2 * (e_s - e_a)}{\Delta + \gamma * (1 + (0.34 * u_2))} \quad (1)$$

where, ET_o is the reference evapotranspiration (mm/d), R_n is the net radiation at the crop surface ($MJ/m^2/d$), G is the soil heat flux ($MJ/m^2/d$), T is the mean daily air temperature at 2 m height (°C), u_2 is the wind speed at 2 m height (m/s) which was assumed as 1.5 m/s for greenhouse conditions, e_s is the saturation vapor pressure (kPa), e_a is the actual vapor pressure (kPa), e_s-e_a is the saturation vapor pressure deficit (kPa), Δ is the slope vapor pressure curve (kPa/°C) and γ is the psychrometric constant (kPa/°C).

For estimating crop evapotranspiration (ET_c), ET_o is multiplied by the crop coefficients (k_c) as shown in Table 4.3. Irrigation frequency or interval was calculated as follows:

$$IR = \frac{D}{ET_C} \quad (2)$$

where, IR is the irrigation frequency or interval in days and D is the depth of water applied (mm).

During the same time period from 1st July to 8th November 2014, the plants were fertilized twice per month with 5.8 g of 20-20-20 NPK water-soluble fertilizer. All the pots were punctured at the bottom and placed on the plastic trays to collect the drain water to avoid spills and contamination. Drain water was collected and tested for the presence of bacteria, after crop harvest on 10th November 2014. The limitations for greenhouse experiments are mentioned in Appendix 4.

4.2.3 Bacterial inoculum preparation, inoculation of irrigation water and its application to tomato crops

E. coli ATCC8739 used in this study, is a facultative anaerobic chemo-organotroph, non-pathogenic, gram negative bacterium and was obtained from the American Type Culture Collection (ATCC), USA. *E. coli* ATCC8739 was isolated from feces and belongs to Biosafety level 1 because it does not carry any vero-toxin genes (stx1 and stx2). Some studies (Evrendilek et al. 1999; Li and Zhang 2004) reported that *E. coli* ATCC8739 is a potential surrogate organism of *E. coli* O157:H7. *E. coli* pellets supplied by ATCC were transferred to 5 ml of Luria Bertani (LB) miller broth (Sigma-Aldrich, USA) and followed by mixing the culture using a vortex mixer. An amount of 0.1 ml of bacterial culture from the LB prepared tube was spread plated on BBL™ Levine Eosin Methylene Blue (EMB) agar (BD, Becton, Dickinson and Company, USA). EMB agar is selective for gram-negative bacteria against gram-positive bacteria and differential for lactose fermenters and non-lactose fermenters. *E. coli*, being gram-negative and vigorous lactose fermenter, produces green metallic sheen colonies with dark centers. Green metallic sheen on EMB agar confirms the presence of *E. coli*. Spread plating was replicated on five different EMB agar plates. LB tube and five EMB agar plates were incubated at 37°C for 24 h. Colonies of *E. coli* on EMB plates were transferred in 20 ml of sterile Bacto™ Tryptic Soy broth (TSB) (BD, USA) and then incubated at 37°C for 24 h with agitation. After bacterial growth, 20 ml of TSB bacterial culture was transferred to twelve 1.5 ml Eppendorf tubes and centrifuged at 7.7×1000 RPM (4000×g) for 20 minutes, to harvest the bacterial cells. These Eppendorf tubes were then washed with 0.1% Tryptone Saline Solution (TSS) (1 g/L tryptone (Sigma-Aldrich, USA) and 8.5 g/L NaCl (Fischer Scientific, USA)) in 1 L of 0.1% TSS. The optical density of TSS with *E. coli* was estimated using a spectrophotometer set at a

wavelength of 600 nm according to previously determined standard curves. The serial suspension dilutions of TSS were plated on EMB agar plates. Plates were then incubated at 37°C for 24 h, to count the colony forming units per ml (cfu/ml) of TSS.

Four liters of contaminated irrigation water with inoculum *E. coli* ATCC8739 at 7.23 log cfu/ml were prepared under sterile laboratory conditions, similar to the methodology for preparation of the inoculum. Contaminated irrigation water was only applied once on 30th September 2014 when all the plants were fruit bearing. Each of the inoculated pots was provided with 150 ml of bacterial inoculated water using two different irrigation methods. For drip irrigation, the container was filled with 2 L of contaminated water to provide an equal amount of water to all pots. A 150 ml of inoculated water was provided to each pot through drip irrigation in 15 min. In the case of sprinkler irrigation, the pots were manually irrigated with 150 ml of contaminated irrigation water using a sterile hand sprayer over the entire plant. The sprayer was kept at a distance of 25 cm from the watered pot, and water was uniformly applied to the plant. Sprinkler irrigation was done carefully to prevent any splashes or spills of the inoculum and, thereby avoiding cross-contamination or aerosol formation (Cevallos-Cevallos et al. 2012b). On the same day, each of un-inoculated pots was irrigated with 150 ml of non-contaminated water using the two irrigation methods.

4.2.4 Soil and tomato fruit samples collection

Tomato fruits and soil samples were collected from un-inoculated and inoculated pots under aseptic conditions. Samples were collected from all the pots on the 2nd, 5th, 10th, 15th, 20th, 25th and 30th days from the day of bacterial inoculation i.e. from 2nd to 30th October 2014. On every sampling day, approximately 20 g of soil were collected in sterile plastic bags from 5-10 cm soil

depth, using a sterile spatula. The soil sampling was carried out at different regions on the surface of the soil from each pot. For tomato fruit samples, one fruit was collected on every sampling day and placed in sterile plastic bags for analysis. These soil and fruit samples were immediately carried to the laboratory and stored in a refrigerator at 4°C until the analyses were done. Tomato fruit weights and shoot lengths were measured and reported in Appendix 5 (Table 2).

4.2.5 Microbiological analysis

The fruit and soil samples from inoculated and un-inoculated pots were evaluated in the laboratory for *E. coli*. For soil analysis, 10 g of soil was taken from 20 g sample and added to 90 ml of 0.1% TSS in a 250 ml Erlenmeyer flask. This flask was homogenized well by shaking at 150 RPM for 20 min on the shaker. Serial dilutions (1:10) of soil solution were prepared in sterile test tubes using 0.1% TSS. An amount of 0.1 ml of each soil sample dilution was spread onto sterile EMB agar plates, which were then incubated at 37°C for 24 h. Simultaneously, each fruit of about 50 g was mixed with 50 ml of 0.1% TSS in sterile plastic bags. Each sample was rinsed well by rubbing, crushing and vigorously agitating by hand for two minutes. Serial dilutions (1:10) of fruits were spread plated on EMB agar plates, and then incubated at 37°C for 24 h. *E. coli* colonies were counted after 24 h from all the plates.

Drain water was collected in 500 ml sterile bottles for analysis after harvesting the crop from all the pots. For laboratory analysis, 100 ml of water were taken from each bottle and the membrane filtration technique was conducted. Serial dilutions (1:10) of water samples were prepared using a phosphate buffer (1.25 ml/L of 0.25M KH_2PO_4 at pH 7.2 and 5 ml/L 0.4M $MgCl_2$). The dilutions were filtered through 47 mm diameter, 0.45µm pore size mixed cellulose esters sterile

membrane filter paper (Fischer Scientific, USA) under vacuum. The filter with the residue was then placed on mcoliBlue24® broth (HACH Company, USA) absorbed on pads in sterile 47 mm diameter petri plates (Fischer Scientific, USA). The plates were then incubated at 37°C for 24 h to confirm the presence of *E. coli*. mcoliBlue24® broth is a selective medium for *E. coli* and shows blue colonies for *E. coli* presence and pink colonies for other coliforms (USEPA 2003). *E. coli* and non-*E. coli* colonies were counted the next day from the plates.

4.2.6 Statistical analysis

Analysis of variance (ANOVA) was carried out on JMP software version 11.2.0, for fate and transport of bacteria in soil and fruits. Before running the ANOVA, assumptions of normality and homogeneity of variances were checked and the data of soil contamination were found to be normally distributed. Therefore, the Student's t Test (with 95% confidence interval) was performed to find the significant differences between the irrigation methods, soil types and among the treatments. Whereas data for tomato fruits contamination were not normally distributed therefore, the student's t test is not applicable to this data. Therefore, non-parametric Wilcoxon or Mann-Whitney or Kruskal-Wallis test (with 95% confidence interval) was carried out to obtain significant differences between the irrigation methods, soil types and among the treatments. One-way ANOVA and two-way ANOVA tests were then performed for all the sampling days to the study fate and transport of bacteria in soil and fruits. A one-way ANOVA was performed for the irrigation methods, soil types and the individual treatments. A two-way ANOVA was performed to check the statistical significance of the interaction effect between irrigation method and soil type (see Appendix 6).

4.3 Results

4.3.1 Fate and transport of *E. coli* in soil over the test period

There was no bacterial contamination of the soil before inoculation. On initial days of sampling from the day of inoculation, it was observed that all the treatments were positive for *E. coli.* However, all the treatments showed a decreasing trend in concentration of *E. coli* with respect to time as shown in Figure 4.2. The decreasing trend could be due to the movement of bacteria to lower soil depths or death and decay of bacteria. Among all the treatments, D+O shows the highest bacterial contamination of 4.9 log cfu/g on the 2nd day, followed by S+O (4.7 log cfu/g) on the same day. The S+M and D+M treatments were similar and showed 4.6 and 4.62 log cfu/g bacterial transport on the 2nd day respectively. On the 2nd day after inoculation, soil type significantly influences the fate and transport of bacteria as organic soil had more concentration of bacteria compared to mineral soil with the two irrigation systems. On the 5th day, D+M treatment showed a decrease in concentration of *E. coli* by about 1 log cfu/g. This immediate decline of 1 log could be due to bacterial death or movement along with the water to lower soil depths. This early bacterial decay or movement to lower depths was observed in mineral soil compared to the organic soil. However, it was noticed that there was a sudden decline in the concentration of *E. coli* in D+M treatment within 10 days i.e. from the 10th day to 20th day. Whereas, S+M treatment with the same soil type did not show any such immediate decline in concentration of *E. coli*. Therefore, irrigation method could also significantly affect the fate and transport of bacteria to lower depths, or eventual death and decay.

The D+O and S+O treatments showed similar and overlapping results for most of the time as shown in Figure 4.2. The concentration of *E. coli* was almost the same for both treatments except on the 15th and 30th days. The bacteria concentration in D+O treatment was higher than the S+O

treatment, but on the 30th day it was noticed that the D+O treatment showed a decline in concentration from 3.65 log cfu/g to 3.25 log cfu/g. Whereas, the S+O treatment maintained the concentration of *E. coli* from 3.67 log cfu/g to 3.64 log cfu/g on the 30th day. The reason for this decline in D+O treatment could be that drip irrigation was in direct contact with the soil and may have resulted in bacterial dilution by the clean water applied on subsequent periods. There could also be a movement of bacteria to lower soil depths. However, this is not the case observed with sprinkler irrigation (S+O treatment). Therefore, sprinkler irrigation (S+O and S+M) resulted in more soil contamination compared to drip irrigation (D+O and D+M) over the period of 30 days. Also, organic soil (D+O and S+O) resulted in more contamination than mineral soil (D+M and S+M) over the same time period.

The un-inoculated pots showed contamination of non–*E. coli* colonies, which were pink or colorless on EMB agar. These colonies could be aerobic mesophilic, *Enterobacteriaceae* or *Pseudomonas* spp. populations (Oliveira et al. 2012).

One-way ANOVA and two-way ANOVA using student's t test were performed for all the sampling days. Table 4.4 represents the significant effects of treatment and interactions. Irrigation methods were not significantly different from each other for most days, but on the 25th and 30th day irrigation methods were significantly different as noticed in the case of D+O and S+O treatments. However, mineral and organic soil were found to be significantly different for most days except for the 2nd day. Table 4.4 also shows the significant differences among treatments. The D+O treatment showed significant differences from the D+M treatment for all sampling days except for the 2nd day as the bacterial concentration was not significantly different between the two treatments on the 2nd day. The D+O treatment showed no significant difference from the S+O treatment, as the bacterial concentration for both treatments was almost the same

for all the sampling days. Also, the D+O and S+O treatments were significantly different from the S+M treatment for only 5th and 10th day. The D+M treatment was significantly different from all the other three treatments for most of the time except for the 2nd day.

A two-way ANOVA found that the interaction was not significant for initial days but from 20th day it was significant until the 30th day. This means that the interaction between the two factors (soil type and irrigation method) does not influence the bacteria fate and transport for the first few days, but on and after the 20th day of inoculation the interaction was influencing the bacterial concentration.

4.3.2 Fate and transport of *E. coli* in tomato fruits over the test period

The tomato fruits showed no contamination before 30th September 2014 i.e. the day of bacterial inoculation. As shown in Figure 4.3, the D+O and S+M treatments showed a declining trend in the concentration of bacteria on tomato fruits unlike S+O and D+M treatments. The S+O and D+M treatments showed no *E. coli* contamination for most days, but it was noticed that there was *E. coli* concentration peak in both cases on the 15th day and 25th days, respectively. This peak could be due to cross-contamination or experimental error or aerosol formation (Cevallos-Cevallos et al. 2012b) as shown by the error bar in Figure 4.3. Whereas on the 2nd day, it was observed that there was contamination of bacteria on tomato fruits in the S+M and D+O treatments with *E. coli* concentrations of 3.68 log cfu/g and 3.2 log cfu/g, respectively. The S+M treatment showed the highest *E. coli* concentration on the 2nd day compared to the D+O treatment, because the sprinkler irrigation initiates direct contact of bacteria on the tomato fruits. However, *E. coli* was not observed on and after the 5th day in the S+M treatment. The possible

reason for no bacteria in the S+M treatment could be that bacteria got washed off from the tomato fruits as plants were irrigated by clean irrigation water.

The D+O treatment showed *E. coli* contamination in the fruits on 2nd, 5th and 10th day as 3.20, 2.72 and 2.32 log cfu/g respectively. On and after the 15th day, there was no bacterial contamination observed in the fruits for D+O treatment. Drip irrigation provided bacteria directly to the soil during inoculation and there was no direct contact of bacteria with the fruits. This shows that organic soil supported the growth of bacteria given by drip irrigation and bacteria may have gotten transported to the tomato fruits through the xylem, phloem and root system (Hirneisen et al. 2012). This means that there could be internalization of bacteria through the vascular tissues to the fruits. The bacterial contamination lasted for the first 10 days and then there was no contaminant transport. During later days, the bacteria must have been diluted with the clean irrigation water and moved to lower soil depths below the root system or there could be death or decay of bacteria with time.

One-way ANOVA and two-way ANOVA using Kruskal-Wallis test were performed for all the sampling days. Table 4.5 represents the significant effect of treatments and interactions. It was found that irrigation methods were not significantly different from each other except on the 5th and 10th day. The D+O treatment showed bacterial contamination on the 5th and 10th day, but sprinkler irrigation (S+O and S+M) showed no bacteria on the same days. This was due to the fact that bacteria on fruits in the S+O and S+M treatment would be washed out with clean irrigation water applied (Forslund et al. 2012) as clean water will be in direct contact with the fruits.

The soil types were not significantly different from each other for all the sampling days. The D+O and D+M treatments were significantly different from the S+O and S+M treatments,

respectively for all the sampling days. This showed that irrigation method significantly influenced the fate and transport of bacteria. Sprinkler irrigation resulted in direct contact of bacteria on the tomato fruits (Solomon et al. 2002b; Forslund et al. 2012), whereas no direct contact was observed in the case of drip irrigation. Also, we observed that all the treatments were significantly different with one other from the 20th day to 30th day because there was no contamination observed on the fruits during that time. A two-way ANOVA was performed after ranking the data, as data were not normally distributed. This showed that the interaction effect between the two factors (soil type and irrigation method) was significant on all the sampling days except on the 5th and 10th day.

4.3.3 Fate and transport of *E. coli* through drain water and a bacterial count balance for *E. coli* in tomato crops

Drain water (500 ml) was collected from all the pots and was studied in the laboratory for the presence of *E. coli* after harvesting the tomato crop. It was observed that the drain water from the inoculated pots for all treatments didn't show any *E. coli*. The bacteria did not drain out of the soil and root zone of the plants. This shows that the soil acts as a filter and has the ability to bind the bacteria within. Bacterial count balance was computed for all the inoculated tomato pots after the 30th day of inoculation. Bacteria concentration in soil lower depths (10-25 cm) was calculated by subtracting bacteria retained in drain water, tomato fruits and soil (at the depth of 5-10 cm) from total bacteria inoculated. The treatment D+M showed that there was no bacterial retention on tomato fruits and soil (at the depth of 5-10 cm) over the 30 days period. In the D+M treatment, probably all the bacteria died or moved to lower soil depths.

4.4 Discussion and Conclusion

Our results confirmed that organic soil (D+O and S+O) resulted in more *E. coli* contamination than mineral soil (D+M and S+M) over the 30 days' time period due to high organic matter and carbon content. Franz et al. (2008) reported that the soil texture plays an important role in prolongation of bacterial survival. Semenov et al. (2010) also found that pathogens survive better in high dissolved organic carbon concentrations. Similarly, another study showed that the total carbon levels in the soil influence the survival of *E. coli* and affect their ability to internalize into the root tissues (Vidovic et al. 2007). The decline in the concentration of bacteria as clearly noticed in D+M treatment could be due to physiological stress placed on *E. coli* cells in the soil. Sharma et al. (2009) reported that stress placed on *E. coli* cells and their limited mobility in soil, could prevent the ability of these cells to internalize into spinach root tissues. Also, sprinkler irrigation (S+O and S+M) resulted in more soil contamination compared to drip irrigation (D+O and D+M) over the 30 days' period. This could be due to more aerosol production in the case of sprinkler irrigation. Similarly, Cevallos-Cevallos et al. (2012a; 2012b) observed that splashes and aerosols produced by rain, cause contamination of tomato plants. Initially, the D+O treatment showed more contamination of soil than S+O treatment because drip irrigation is in direct contact with the soil surface (Forslund et al. 2012) but on the 30^{th} day, maximum bacterial concentration was observed in S+O treatment. This was due to the clean water application on subsequent days, which diluted or moved bacteria to lower soil depths as soil in the D+O treatment was in direct contact with the clean water (Solomon et al. 2002b; Forslund et al. 2012). However, it has been reported by Semenov et al. (2009) that slurry rich in organic matter moves faster to lower depths of soil.

Our results suggested that bacteria could have been internalized through the root system to vascular tissues and finally to tomato fruits, as observed in the case of D+O treatment. However, this internalization of bacteria in tomato plants did not last for a long time as fruits were devoid of bacteria from the 15th day. Similarly, Erickson et al. (2010) reported that internalization of *E. coli* O157:H7 occurs rarely and if it occurs then does not persist after 7 days. Few other studies reported that there was internalization of the pathogens through the plant's root system, which are grown in inoculated soil (Solomon et al. 2006; Hirneisen et al. 2012). Whereas, Erickson et al. (2014) reported that there was little or no internalization of bacteria noticed with the plants grown in bacterial inoculated soil. Solomon et al. (2002b) reported that *E. coli* O157:H7 may internalize via vascular tissue from the roots to the edible portion of the plant. Fonseca et al. (2011) reported that when contaminated water is sprayed onto the lower side of leaves then the survival and internalization of bacteria is increased, compared to water sprayed on the upper side (Erickson et al. 2010). Zhang et al. (2009) reported that there is potential for internalization regardless of the field conditions such as heat and moisture.

Studies also reported that plant age and exposure time would affect the bacterial internalization. Young leaves were more susceptible to bacterial contamination than old leaves (Brandl and Amundson 2008). We observed in the D+O and S+M treatments that there was contamination of tomato fruits during initial days of sampling, probably because the tomato fruits were young and more susceptible to bacteria.

Solomon et al. (2002b) reported that there is a high risk of contamination on spinach and rocket due to sprinkler than drip irrigation. Sprinkler irrigation causes a high risk of contamination, because contaminated water has direct contact with the crop surface; whereas in surface drip irrigation there is direct contact at the soil surface and not with the crop surface (Forslund et al.

2012). High risk of contamination by sprinkler irrigation was found in our results as the S+M treatment had 51% bacteria retention, i.e. more than the D+O treatment (44.3%). However, the S+M treatment showed bacterial contamination only on the 2nd day as the inoculum was in direct contact with the outer covering of the tomato fruit. Eventually, the bacteria on the tomato could have washed out with the clean irrigation water or died.

Higher inoculum levels result in more contamination and internalization of bacteria into the plants (Hirneisen et al. 2012). Also, *E. coli* O157:H7 has the highest probability of internalization and contamination compared to *E. coli* K12 and other *Salmonella* serovars (Dong et al. 2003). But according to our experimental results, S+O and D+M treatments did not show contamination and internalization to the tomato fruits even with higher inoculum level. Therefore, our study disproves that a higher inoculum level results in higher contamination and internalization to plants. Miles et al. (2009) also observed that with the inoculation of 7 log CFU/ml every 14th day for 70 days resulted in no internalization of bacteria to stem, fruit or leaves of the tomato plant but root samples were positive for bacteria. This shows that it is not the inoculum level and frequency of inoculation which causes the contamination of fruits. However, the causative factors for contamination and internalization of fruits and vegetables could be the type of pathogen present in irrigation water, age of plant, exposure time to plant, crop type and its root system, growth stage of plant, soil type and irrigation method used to irrigate the plants.

We can say that the D+M treatment was more suitable for tomato crop as it did not retain any bacteria over the test period. This treatment is therefore the most favorable for the tomato production.

According to our findings, *E. coli* ATCC8739 contaminated the soil and fruits and could have been internalized into the tomato crop through the root system. However, this contamination and internalization in the fruits was only significant for a few days from the day of inoculation. We could say that the D+O treatment might have had internalization of bacteria through the root system. Internalization of pathogens might have occurred; we cannot confirm that internalization happened in our study as there was no microscopy performed for the vascular tissues. Although, some studies have reported that there might be internalization due to contaminated irrigation water or manure. Organic soil showed a considerably higher amount of contamination in soil and fruits than mineral soil, as organic soil is rich in organic matter and carbon content which is favorable for the bacterial movement. Sprinkler irrigation was found to cause a high risk of contamination to the crops as bacterial water was in direct contact with the fruit surface. Also, there is possibility of aerosol formation during sprinkler irrigation and resulting in contamination of crops. However, in sprinkler irrigation, clean irrigation water used after inoculation can wash off or dilute the bacterial concentration from the plants. However, the repeated use of contaminated irrigation water on crops can lead to higher contamination of fruits.

Drain water from the bottom of the pots did not show any bacteria for all the treatments. The bacterial count balance showed that all the retained bacteria in the soil were either at a depth of 10-25 cm, or bacterial death and decay occurred. We found that the D+M treatment did not retain bacteria in the tomato fruits over the 30 days' time period and can be considered as the best treatment for tomato crop production. Therefore, there are very few studies which analyzed the fate and transport of *E. coli* contamination under different irrigation systems and soil types. This present study could help farmers in selecting appropriate treatment and harvesting time, for ready to eat vegetables and fruits in order to reduce the bacterial contamination occurring in the tomato

crops. If farmers are using treatment with high fate and transport of bacteria as shown, then they need to make sure the quality of irrigation water meets the standards to avoid any risk to consumers. However, bacterial contamination on the fresh fruits and vegetables can cause detrimental effects on human and environmental health. These contaminated fruits and vegetables can even cross-contaminate other foods. Therefore, irrigation water prior application must be treated and tested for pathogens, as this would reduce the risk of contamination to consumers. Irrigation water quality and manure applications require special attention. Wildlife can also be a potential source of fecal contamination to surface irrigation water sources.

Through our study, we found that non-contaminated irrigation water can reduce health risks. However, the bacteria inoculum level used for our experiment was relatively higher than usually found in the field under natural conditions, but pathogen even in low quantities can cause food contamination. Such a level of contamination might not be realistic in terms of level of contamination occurring in the environment. There are some studies which have used higher level of contamination. Guo et al (2001) applied 7.5 log cfu of S*almonella* cells to the stems of tomatoes. Similarly, Fonseca et al (2011) applied 10^8-10^9 cells ml^{-1} of *E. coli* to lettuce through different irrigation methods. These high level of contamination might occur in wastewater irrigation and also if there is manure application (Islam et al., 2004). Therefore, knowledge of the irrigated field and source of irrigation water are necessary to limit pathogen introduction and foodborne illnesses and outbreaks through consumption of fresh fruits and vegetables.

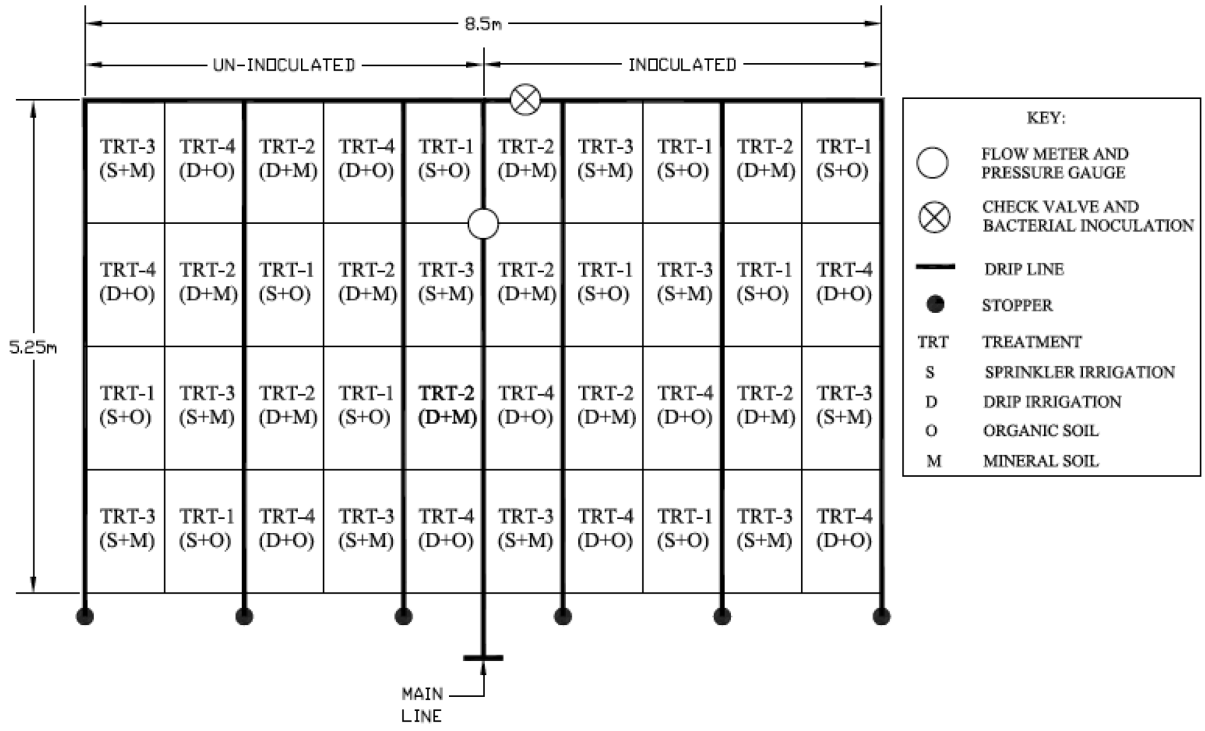

Figure 4.1: Layout of Experimental design with different treatments (TRT-1, TRT-2, TRT-3 and TRT-4) in Macdonald Campus greenhouse for Tomato

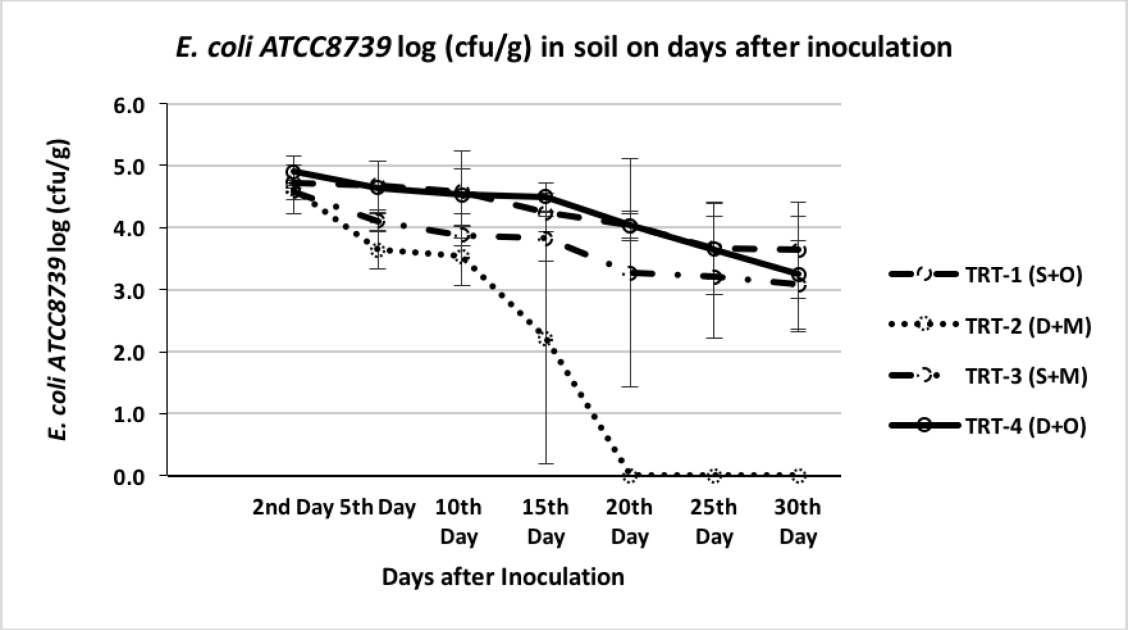

Figure 4.2: *E. coli ATCC8739* log (cfu/g) in soil for tomato with respect to time in different treatments

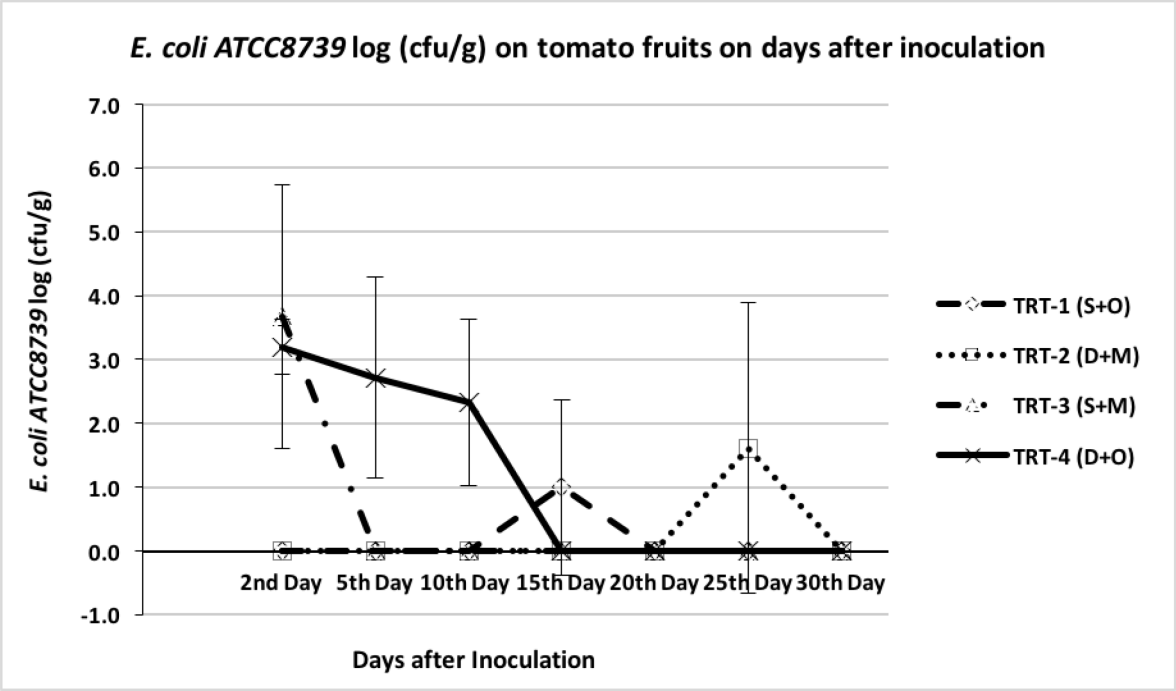

Figure 4.3: *E. coli ATCC8739* log (cfu/g) on tomato fruits with respect to time in different treatments

Table 4.1: Treatments used

Treatments	**Irrigation method**	**Soil type**	**Abbreviation for treatments**
TRT-1	Sprinkler	Organic	S+O
TRT-2	Drip	Mineral	D+M
TRT-3	Sprinkler	Mineral	S+M
TRT-4	Drip	Organic	D+O

Table 4.2: Soil properties for soil used in different treatments

Soil type	Sand (%)	Silt (%)	Clay (%)	Organic matter (%)	Bulk density (g/cm3)	Gravimetric moisture content (%) at pot filling
Mineral Soil	50.89	27.20	21.87	0.11	1.51	15.37
Organic Soil	12	11	6	71	0.35	65.19

Table 4.3: Irrigation water application and frequency based on growth stages and crop evapotranspiration

Growth stages	Length of growth stages (days)	K_c	ET_o (mm/d)	ET_c (mm/d)	Tomato growth stages in 2014	D (mm)	IR (days)
Initial	30	0.60	2.59	1.55	17th June to 16th July	Between 5 and 10	2-days Interval
Developmental	40	Between 0.60 and 1.15	2.43	Between 1.55 and 2.45	17th July to 25th August	Between 10 and 15	3-days interval
Mid	45	1.15	2.13	2.45	26th August to 9th October	Between 11 and 15	3-days interval
Late	30	Between 1.15 and 0.80	1.89	Between 2.45 and 1.51	10th October to 8th November	Between 10 and 15	3-days interval

Table 4.4: Analysis of Variance (ANOVA) for treatments with respect to soil

One way ANOVA: Contrast differences Probabilities (Prob>|t|) using Student's t Test							
Comparisons	**2nd Day**	**5th Day**	**10th Day**	**15th Day**	**20th Day**	**25th Day**	**30th Day**
Drip vs Sprinkler	0.3635	0.3066	0.5029	0.2643	0.0523	0.0296*	0.0124*
Organic vs Mineral	0.0645	0.0001*	0.0006*	0.0181*	0.0022*	0.0036*	0.005*
D+O vs D+M	0.0892	0.0002*	0.0039*	0.0033*	<.0001*	<.0001*	<.0001*
D+O vs S+M	0.0555	0.0207*	0.0409*	0.3256	0.2168	0.3459	0.2168
D+O vs S+O	0.2715	0.8535	0.8699	0.7038	0.9999	0.9653	0.9999
S+O vs D+M	0.5121	0.0001*	0.0028*	0.0076*	<.0001*	<.0001*	<.0001*
S+M vs D+M	0.8016	0.0429*	0.2696	0.0273*	<.0001*	<.0001*	<.0001*
S+O vs S+M	0.3682	0.0141*	0.0295*	0.5395	0.2167	0.3251	0.2167
Two way ANOVA							
Irrigation method (Drip and Sprinkler)	0.3388	0.1109	0.368	0.1683	0.0012*	0.0001*	<.0001*
Soil type (Organic and mineral)	0.071	<.0001*	0.0009*	0.0109*	<.0001*	<.0001*	<.0001*
Irrigation method*Soil type (Interaction effect)	0.5409	0.1742	0.4994	0.0639	0.0012*	0.0001*	0.0006*
*** means they are significantly different from each other (p<0.05)**							

Table 4.5: Analysis of Variance (ANOVA) for treatments with respect to tomato fruits

One way ANOVA: Nonparametric comparison probabilities using Wilcoxon or Mann-Whitney or Kruskal Wallis Test							
Comparisons	**2nd Day**	**5th Day**	**10th Day**	**15th Day**	**20th Day**	**25th Day**	**30th Day**
Drip vs Sprinkler	0.4647	0.0007*	0.0007*	0.4647	1	1	1
Organic vs Mineral	0.268	0.268	0.268	0.268	0.0562	0.0562	0.0562
D+O vs D+M	0.077	0.7104	0.7104	<.0001*	<.0001*	<.0001*	<.0001*
D+O vs S+M	0.7104	0.0007*	0.0007*	0.006*	0.0001*	0.0001*	0.0001*
D+O vs S+O	0.0016*	0.0375*	0.0375*	<.0001*	<.0001*	<.0001*	<.0001*
S+O vs D+M	0.077	0.077	0.077	0.536	0.0001*	0.0001*	0.0001*
S+M vs D+M	0.0375*	0.0016*	0.0016*	0.0001*	<.0001*	<.0001*	<.0001*
S+O vs S+M	0.0007*	0.077	0.077	0.0004*	0.0001*	0.0001*	0.0001*
Two way ANOVA (after ranking the data)							
Irrigation method (Drip and Sprinkler)	0.3009	0.0006*	0.0006*	0.0926	1	1	1
Soil type (Organic and mineral)	0.1284	0.1284	0.1284	0.0163*	<.0001*	<.0001*	<.0001*
Irrigation method*Soil type (Interaction effect)	0.0006*	0.3009	0.3009	<.0001*	<.0001*	<.0001*	<.0001*
*** means they are significantly different from each other (p<0.05)**							

Connecting text to Chapter 5

Chapter 4 explains that the four different treatments (comprised of organic and mineral soil, and drip and sprinkler irrigation) have different fates and transport *E. coli* bacteria differently. This gives us the knowledge of which treatment combination of soil type and irrigation method can result in a minimum or a maximum contamination of the fresh fruits and vegetables. It gives farmers information on which treatment is the safest to use to grow fresh vegetables and fruits, and to test for pathogens in the irrigation water before application. Chapter 5 focusses on the first, third and fourth objectives of this dissertation. Chapter 5 develops the QMRA model and gives in-depth information on the potential risks to humans that is associated with the consumption of contaminated fruits and vegetables. For the greenhouse study, the scenarios were based on manually contaminated irrigation water, and on crops grown under four different treatments and harvested on the 10^{th}, 20^{th} and 30^{th} days after irrigating with contaminated water. At field scale, the QMRA model was run on untreated surface water, which was used to irrigate lettuce and tomatoes at two field sites in Quebec. Using the QMRA model, pathogens such as *Escherichia. coli*, *Campylobacter* spp. and *Rotavirus* were used as the target in estimating the annual disease burden. To the best of our knowledge, this is the first study in Quebec to estimate risk using the QMRA model on crops grown under different treatments, on crops harvested 10, 20 and 30 days after irrigation, and for those irrigating with untreated surface water. The following manuscript, co-authored by Dr. C.A. Madramootoo, resulted from this study and will soon be submitted.

Gupta, D. and Madramootoo, C.A. (2016) Quantitative Microbial Risk Assessment (QMRA) model associated with the consumption of contaminated lettuce and tomatoes grown in the greenhouse and at two field sites.

Chapter 5: Quantitative Microbial Risk Assessment (QMRA) model associated with the consumption of contaminated lettuce and tomatoes grown in the greenhouse and at two field sites

Divya Gupta and Chandra A. Madramootoo

Abstract

Consumption of contaminated, fresh or uncooked vegetables may result in human gastrointestinal or diarrheal illnesses. These illnesses can be due to pathogenic microorganisms contaminating the vegetables, and these health risks from pathogens can result from the use of untreated or contaminated irrigation water. This study estimated the annual disease burden from pathogenic *E. coli* in greenhouse; and three pathogens namely, pathogenic *E. coli*, *Campylobacter* and *Rotavirus* in untreated irrigation water at two sites (Rougemont and St-Remi) in Quebec. The Quantitative Microbial Risk Assessment (QMRA) model was used to estimate pathogenic risks. The sprinkler irrigation method used in greenhouse (6.2×10^{-2} DALYs (disability adjusted life years) pppy) resulted in higher risk than drip irrigation (5.5×10^{-2} DALYs pppy) for both crops. There was significant high risk in lettuce with all treatments and for all harvested days (10^{th}, 20^{th} and 30^{th} day) except in the Drip+Mineral treatment, where risk was found only on the 20^{th} day (1.7×10^{-3} DALYs). The highest risk on lettuce was observed in the Sprinkler+Organic treatment (1.3×10^{-2} to 3.8×10^{-2} DALYs); followed by the Sprinkler+Mineral (7.4×10^{-5} to 2.1×10^{-2} DALYs) and the Drip+Organic (6.3×10^{-5} to 1.4×10^{-2} DALYs) treatments. The risk decreased from the 10^{th} day to 30^{th} day for all the treatments, due to dilution with clean irrigation water. For tomatoes, risk was observed on the 10^{th} day in the Drip+Organic treatment (1×10^{-2} DALYs) only. At two field sites, the QMRA for untreated surface water is believed to be

the first attempt to estimate the annual disease burden from these three pathogens in lettuce and tomato. However, the risk always exceeded the WHO health target of 10^{-6} DALYs across all the scenarios. The combined annual disease burden is the summation of annual disease burden associated with all the pathogens considered (DALYs pppy). The combined annual disease burden was found in the range of 10^{-3} to 10^{-2} DALYs for lettuce and tomato. The combined risk is the probability of at-least one illness from any of the three pathogens. The combined risk was in the range of 10^{-2} to 10^{-1} and 10^{-3} to 10^{-1} for lettuce and tomato, respectively. The QMRA model showed significant risk associated with tomato and lettuce consumption. Therefore, it is recommended to use drip irrigation when using treated irrigation water to significantly reduce the number of pathogens at these sites, for the consumption of fresh fruits and vegetables.

Keywords: Annual disease burden, greenhouse, treatments, tomato, lettuce

5.1 Introduction

5.1.1 Ready-to-eat vegetables and contamination

Ready-To-Eat (RTE) vegetables and fruits such as lettuce, spinach, tomatoes and other leafy greens are gaining popularity with consumers due to their high nutritional content, health benefits, and convenience (Oliveira et al., 2011). These fruits and vegetables are minimally processed, mostly consumed as fresh and have a short shelf life (Garcia-Gimeno and Zurera-Cosano 1997). They are often colonized by microorganisms such as bacteria, viruses, yeast, and fungi, which can be pathogenic (Beuchat 1996; Abadias et al., 2008). Contamination of lettuce predominantly occurs by gram-negative bacteria which belongs to the family *Pseudomonadaceae* and *Enterobacteriaceae* (Oliveira et al., 2010). *Escherichia coli* O157:H7 is

an example of a pathogenic bacterium commonly found in RTE vegetables (Ackers et al., 1998; Buck et al., 2003; FDA, 2007).

There is evidence that increasing consumption of fresh fruits and vegetables is a major contributor of human gastrointestinal or diarrheal illness (Buck et al., 2003; FDA 2007; Scharff, 2009; Oliveira et al., 2011; Pachepsky et al., 2011). The contaminated vegetables and fruits, when eaten fresh or uncooked can result in these illnesses (Beuchat 1996; Fan et al., 2009; Sapers et al., 2009; Warriner et al., 2009; Pachepsky et al., 2011) due to the presence of pathogenic microorganisms (*E. coli, Campylobacter, Listeria, Rotavirus, Norovirus* and *Giardia*). The produce could be contaminated through different sources such as municipal sewage, contaminated surface water and groundwater, and fecal waste materials from domesticated and wild animals (WHO, 2003). Irrigation accounts for over 70% of freshwater withdrawals from the available water resources (FAO, 2012) and the sources of irrigation water have been placed in the order of increasing health contamination risks (Leifert et al., 2008; Pachepsky et al., 2011) from potable or rain water to fresh or inadequately treated wastewater.

5.1.2 Irrigation practices and water quality in Quebec

In Quebec, irrigation is practiced during the summer growing season months i.e. May-October. The precipitation ranges from 700-750 mm during the May-October period (Environment Canada, 2014), and 82% of the total irrigation volume is mostly applied during June-August (Statistics Canada, 2013). In Quebec, the irrigation water sources are on-farm surface water (73%) and on-farm underground water (17%) (Statistics Canada, 2013). In 2012, 380 farms in Quebec used on-farm surface water, and 235 farms used on-farm underground water for irrigation (Statistics Canada, 2013). Most farmers utilize water sources located on or near their farms such as rivers, ponds or dugouts, as on-farm surface water, and utilize wells as an

underground water source for irrigation. Sprinkler and micro-irrigation are the major irrigation methods practiced in Quebec. In 2012, sprinkler and micro-irrigation were practiced on 285 and 300 farms, respectively; followed by surface irrigation on 65 farms (Statistics Canada, 2013).

Surface water bodies are most vulnerable to pollution because of their easy accessibility for the disposal of wastewaters and non-point pollutants. Natural and anthropogenic sources of pollution influence surface water quality (Carpenter et al., 1998; Jarvie et al., 1998; Singh et al., 2004). Municipal and industrial water discharges constitute major pollution sources for water bodies. Buck et al. (2003) reported that runoff from cattle feedlots and agricultural fields can contaminate surface irrigation water. In Quebec, Ministère du Développement durable, de l'Environnement et de la Lutte contre les changements climatiques (http://www.mddelcc.gouv.qc.ca/eau/potable) reported that groundwater is generally of better quality than surface water, owing to the soil's natural filtering capacity, but it may be vulnerable to contamination, and precautions must be taken to ensure a supply of good quality water to crops at all times.

In Saskatchewan, irrigation water quality was monitored at 14 locations from 2007 to 2009 and 11 of 180 samples exceeded the irrigation guidelines for fecal coliforms (Hogg, 2010). In Ontario, pond irrigation water quality assessment found that 2-22% of water samples were unacceptable for the levels of *E. coli*. The concentration of fecal indicators varied over the growing season (Steele and Odumeru, 2004). The irrigation of vegetables and fruits is practiced worldwide with polluted irrigation water or wastewater. Petterson et al. (2001) reported an annual risk ranging from 0.55 to 31 per 10000 exposed, if untreated wastewater was used for irrigation. It was reported that there is a clear link between consumption of crops irrigated by wastewater and community illness (Blumenthal and Peasey 2002; Barker et al., 2014).

5.1.3 Quantitative Microbial Risk Assessment (QMRA) model

The Quantitative Microbial Risk Assessment (QMRA) is a modelling technique used to estimate the health risks due to a pathogens' exposure. WHO (2004) reported that the QMRA is a valuable and informative tool to validate water safety plans and to assess if the irrigation scheme meets the health standard of 10^{-6} DALYs pppy. The Disability-Adjusted Life Years (DALYs) method has been recommended (Havelaar and Melse 2003; WHO 2004) to assess risk and to understand the outcomes of exposure. The DALY is an overall disease burden measure, expressed as the number of years lost to disability, illness or premature death (Barker et al., 2013). The QMRA model has been widely used in recent years to estimate the disease burden caused by individual pathogens (Haas et al., 1999; Howard et al., 2006). The QMRA has been used for estimating the disease burden associated with drinking water (Machdar, 2013; George et al., 2015), urban water systems (Labite et al., 2010; Lulani et al., 2008; Barker et al., 2014), consumption of wastewater irrigated vegetables (Ackerson and Awuah, 2012; Drechsel and Seidu, 2011; Seidu et al., 2008), consumption of street food salads (Barker et al., 2014), and home-produced lettuce (Barker et al., 2013). The QMRA model has been found to be a successful and powerful approach in estimating the risks associated with different water systems and crops after harvest (Ackerson and Awuah, 2012; Barker et al., 2013; Barker et al., 2014; Drechsel and Seidu, 2011; George et al., 2015; Hamilton et al., 2006; Labite et al., 2010; Lulani et al., 2008; Machdar 2013; Seidu et al., 2008). Also, McKellar et al. (2014) provided information on the probabilistic models to predict the fate of *E. coli* O157:H7 on field-grown leafy green vegetables. However, there is not enough knowledge on the risk associated with the consumption of tomatoes and on use of the untreated surface irrigated water.

Hazard assessment and exposure

In Canadian cities, 86% of acute gastrointestinal illnesses result in diarrhea (Canadian Digestive Health Foundation). FoodNet Canada (2013; 2014) reported that in 2013 and 2014, *Campylobacter* and *Salmonella* were the major causes of human enteric illness across Canada. *Campylobacter* and verotoxin producing *E. coli* (VTEC) were found in untreated surface water, irrigation canals, and ditches in two watersheds of British Columbia (FoodNet Canada, 2013). FoodNet Canada (2014) found VTEC in 25% of the irrigation water samples in British Columbia and Alberta in 2014. Canada Communicable Disease Report (2015) reported that during 2008-2014, 73% of foodborne outbreak cases were due to bacteria (comprising 14.8% and 1.7% cases from VTEC and *Campylobacter*, respectively), and 14.8% of cases were due to viruses. WHO (2014) reported that in the Americas, diarrheal diseases represent 20% of all infectious and parasitic diseases with a disease burden of 2.8×10^{-3} DALYs per person per year (pppy), which was similar to HIV/AIDS (3.6×10^{-3} DALYs pppy) in 2012. WHO (2014) also reported that in 2012, the male and female populations aged between 1-59 months and those older than 70 years, were the largest segments infected from diarrheal diseases. Kirk et al (2010) reported that people older than 65 years of age and children below two years of age (FDA, 2015) are more susceptible to gastrointestinal problems (Smith, 1998).

When pathogens are ingested, the host creates a defensive mechanism to remove or inactivate the pathogen. It is usually assumed that microorganisms follow the Poisson distribution in the inoculum (Haas et al., 1999; Teunis & Havelaar, 2000; Teunis et al., 2004). There is a simple exponential dose-response relation for a Poisson distributed inoculum and a constant probability of infection for an ingested pathogen. This implies that the probability of infection is equal for any single pathogen in any host (Teunis et al., 2004). A more realistic model, however,

incorporates heterogeneity between the pathogen-host interactions by implementing probability distribution to describe variations in the probability of infection of individual pathogens. This model is called the Beta Poisson (hypergeometric) dose-response model, commonly used in QMRA (Teunis et al., 2004) and has two parameters. In this model, the probability of infection cannot exceed the probability of exposure (Teunis & Havelaar, 2000; Teunis et al., 2004).

The QMRA has been mostly applied to wastewater or greywater irrigation (Surinkul and Koottatep, 2009; Barker et al., 2013), and has ignored risks in the use of surface irrigation water. Studies (Ottoson and Stenstrom, 2003; Barker-Reid et al., 2010; Barker et al., 2013; Barker et al., 2014) have used *Rotavirus* as a model viral pathogen to assess the health risk due to greywater irrigation. In addition, the QMRA model has been rarely used for assessing risk due to *Campylobacter* spp. George et al. (2015) assessed *Campylobacter* spp. risk in Mysore drinking water. Most of the studies (Hamilton et al., 2006; Barker et al., 2013; Mok et al., 2014) have used lettuce and other leafy vegetables to assess the quality of greywater irrigation. However, to the best of our knowledge, no research has been done using the QMRA model for assessing risks from the consumption of irrigated fresh tomatoes.

When untreated wastewater, surface water or reclaimed water is used for irrigating the RTE vegetables and fruits, it carries the potential pathogens causing gastrointestinal illnesses or other diseases to consumers. Implementation of high technology treatments can be a solution to reduce these potential pathogens in the water but such systems are very expensive (Brennan et al., 2003; Robinson 2003; Hamilton et al., 2006) and only 10% of wastewater undergoes treatment (Homsi 2000). Hamilton et al. (2006) reported that there could be two steps to consider when addressing the safety of horticulture; the first is to determine the risks associated with the consumption of fresh fruits and vegetables and the second is to understand the risks in managing low technology

schemes. To determine risks associated with a particular pathogen, or when considering worst case scenarios, a QMRA model would need to be developed. In this study, the results of the greenhouse and field studies would be analyzed using this novel way of considering the Quantitative Microbial Risk Assessment (QMRA) model. Using this novel approach, this study would quantify the gastrointestinal risks to humans due to the consumption of irrigated fresh vegetables which could be contaminated when irrigated by untreated water.

In this greenhouse study, the QMRA model was used to analyse the risks from water and crops. The water model refers to the irrigation water used to irrigate crops such as lettuce and tomato. The crops model refers to lettuce and tomatoes grown under four different treatments. The four different treatments consist of two irrigation methods (drip and sprinkler irrigation) and two soil types (mineral and organic soil). This is the first study that estimated the contamination risk from the consumption of lettuce and tomatoes grown under the Drip+Mineral, Drip+Organic, Sprinkler+Mineral and Sprinkler+Organic treatments. The QMRA model was used to estimate the risk of pathogenic *E. coli* in water and on the crops (lettuce and tomato) harvested after 10, 20 and 30 days of irrigation. To the best of our knowledge, this is the first study that estimated the risks for crops harvested after 10, 20 and 30 days of irrigation.

This study also focused on the potential risks to humans due to the consumption of lettuce and tomatoes irrigated with untreated surface water in Quebec. The study was conducted using data from two field sites namely, Rougemont and St-Remi over a two-year period (2013 and 2014). The QMRA model was used to estimate the annual disease burden in DALYs per person per year (pppy) due to the following pathogens: *Escherichia. coli*, *Campylobacter* spp. and *Rotavirus*. All three pathogens are transmitted fecal-orally as they could survive in water and fresh vegetables.

5.2 Methodology

5.2.1 Greenhouse study area and experimental design

Greenhouse experiments were conducted on the Macdonald Campus of McGill University, St-Anne-de-Bellevue, Quebec during June-November 2014. Four treatments with two soil types (organic and mineral) and two irrigation methods (drip and sprinkler) were used for lettuce and tomatoes. These four treatments were represented as Sprinkler+Organic, Sprinkler+Mineral, Drip+Mineral, and Drip+Organic. Each of the four treatments had five replicates for each crop and the pots of lettuce and tomatoes were placed randomly in the greenhouse. Drip irrigation was installed using the drip line, flow meter, check valve and pressure gauge. For sprinkler irrigation, a hand sprayer was used to irrigate the crops in order to avoid over watering and to control the flow. Mineral soil (with 50.9% sand, 27.2% silt and 21.9% clay) was collected from the Macdonald Campus farm, and organic soil (with 71% organic matter) was obtained from a local supplier, Laniel Prodamex. Lettuce and tomato seedlings were transplanted into plastic pots containing soil. *E. coli* contaminated water (7.23 log cfu ml^{-1}) was prepared in the laboratory at Macdonald Campus and was applied once on July 20 to the lettuce, and once on Sept 30 to the tomato plants. Each lettuce and tomato pot was also sprinkler or drip irrigated with *E. coli* contaminated water. This high concentration of 7.23 log cfu per ml was used because in some countries wastewater with such levels of contamination is used for irrigation. There are some studies which used higher contamination such as 7.5 log cfu of S*almonella* cells to the stems of tomatoes (Guo et al., 2001); 10^8-10^9 cells ml^{-1} of *E. coli* to lettuce through different irrigation methods (Fonseca et al., 2011).

5.2.2 Field location and study area

Two field sites located in Rougemont and Saint-Rémi, Quebec were selected. Both sites are located in the Montérégie administrative region on the south-shore of the St. Lawrence River.

Saint-Rémi is part of Les Jardins-de-Napierville Regional County Municipality having a latitude: 45°14' N, longitude: 73°40' W and elevation of 50 m. At the site, lettuce, onions and celery were grown. The Rougemont site has a latitude: 45°27' N, longitude: 73°00' W and elevation of 38 m and is located within the Rouville Regional County Municipality. This site grows tomatoes, apples, strawberries, squash and corn. During the study period, lettuce and tomatoes were grown at the Saint-Rémi and Rougemont sites, respectively.

At both the sites, untreated surface water stored in ponds was used for irrigation. During precipitation, water accumulates in the pond. When there is insufficient water in the pond for irrigation, water from wells is pumped into the pond. The method of irrigation varied at both the fields; the lettuce field was sprinkler irrigated, whereas the tomato field was drip irrigated. Saint-Rémi's soil is organic whereas Rougemont's is mineral. Organic matter content in the organic soil was about 80%. The mineral soil was Saint-Hyacinthe silty clay loam with 15-25% sand, 45-55% silt, 25-35% clay and 0.1% organic matter (http://sis.agr.gc.ca/cansis/publications/surveys/pq/index.html).

5.2.3 Sampling and analytical methods

a) For greenhouse experiments

Lettuce and tomato samples were collected from all four treatments in the greenhouse on the 10th, 20th and 30th day after a one-time irrigation water application as previously mentioned. On sampling days, lettuce leaves and tomato fruits were collected in sterile plastic bags and were analyzed in the laboratory for *E. coli*. Each sample of lettuce and tomato was tested for *E. coli* using serial dilutions and spread plating on BBL™ Levine Eosin Methylene Blue (EMB) agar (BD, Becton, Dickinson and Company, USA) plates. *E. coli* colonies were counted from the plates after incubation at 37°C for 24 h. For data analysis, the *E. coli* concentrations

(mean±standard deviation; # log cfu g^{-1}) on lettuce leaves and tomato fruits on the 10^{th}, 20^{th} and 30^{th} day for all the treatments were recorded as shown in Table 5.1.

b) For field experiments

Water samples for the two study years were collected from the two fields during the May-October growing season. Irrigation water samples were collected biweekly, in autoclaved glass bottles, with five replicates at a depth of 0.3 m, from two locations in the same pond. The samples were immediately stored in a cooler and then refrigerated until analyzed in laboratories on the Macdonald Campus of McGill University. Quantification of *E. coli* was carried out by the membrane filtration technique. In this technique, 100 ml of sample or its' dilutions were filtered onto 0.45 μm pore-sized (47-mm diameter) glass fiber filters (Hotto et al., 2005), and these filters were then kept on m-coli blue media plates followed by incubation at 37°C for 24 h. Blue colonies on the media plates confirmed the presence of *E. coli*. For *E. coli,* data analysis (mean±standard deviation; # log cfu 100 mL^{-1}) was divided into three time periods namely, May-June, July-August and September-October as represented in Table 5.2.

The percentage of pathogenic *E. coli* (C_e; # log cfu 100 mL^{-1}), *Campylobacter* (C_c; # log cfu 100 mL^{-1}) and *Rotavirus* (C_r; # log cfu 100 mL^{-1}) were obtained from the literature as 8%, 6.6% and 0.001% respectively, of the total measured *E. coli* concentration (C_E; # log cfu 100 mL^{-1}) (Haas et al., 1999; George et al., 2015) and described as:

$$C_e = 0.08 C_E \quad (1)$$

$$C_c = 0.066 C_E \quad (2)$$

$$C_r = 10^{-5} C_E \quad (3)$$

5.2.4 Dose-response models' structure, implementation and risk characterization

a) For greenhouse

For the greenhouse study, two different exposure models were developed to estimate the annual disease burden on humans. The first model was of the contaminated irrigation water used to irrigate lettuce and tomato. The second model was the crops model, developed for lettuce and tomatoes grown under the following four different treatments: Sprinkler+Organic, Sprinkler+Mineral, Drip+Mineral, and Drip+Organic. This QMRA model was used to estimate the risk of pathogenic *E. coli* in water and on the crops (lettuce and tomato) harvested after 10, 20 and 30 days of irrigation. The water model estimated the pathogen concentration in the fresh lettuce and tomatoes, based on the volume of water retained on lettuce and on tomatoes through drip or sprinkler irrigation. In the crops model, the actual *E. coli* concentration found on lettuce and tomatoes for all four treatments on 10^{th}, 20^{th} and 30^{th} day, as shown in Table 5.1, was used to estimate the risk.

b) For fields (Rougemont and St-Remi)

For both of the fields, two different exposure models were developed to estimate the annual disease burden due to pathogens (pathogenic *E. coli*, *Campylobacter* and *Rotavirus*) from the consumption of tomatoes and lettuce irrigated with untreated surface water. The first model used irrigation water for growing tomatoes at the Rougemont site. The model estimated the pathogen concentration of the fresh tomatoes, based on the volume of water retained on the tomatoes through drip irrigation. The second model estimated the pathogen concentration on lettuce leaves based on the volume of water retained on the lettuce through sprinkler irrigation at St-Remi.

In these models, only the risk associated with eating fresh fruits and vegetables (without washing) was considered. Raschid-Sally and Jayakody (2008) and Barker et al. (2014) reported

that there could be other risks such as exposure risks to the farmers and their families. However, those risks were not considered in this study.

The summary of the model input parameters for calculating annual disease burden is represented in Table 5.3. To develop the dose-response models for lettuce and tomatoes, the mean per capita consumption of lettuce or tomatoes (C; kg yr^{-1}) in 2013 and 2014 was obtained from Agriculture and Agri-Food Canada Statistics (http://www.agr.gc.ca). The consumption data were then converted to mean per capita consumption of lettuce or tomatoes in g per day (C_d; g day^{-1}) and is defined as:

$$C_d = 1000\ C / 365 \quad (4).$$

The volume of water retained on the surface of lettuce and tomatoes was based on sprinkler (0.11 ml g^{-1}) and drip irrigation (0.02 ml g^{-1}) (Shuval et al., 1997; van Ginneken and Oron, 2000; Keuckelaere et al., 2015) as shown in Table 5.3. It was found that sprinkler irrigation, because of the direct contact of the water with the plants, resulted in more water being retained on fruits and leaves than was the case with drip irrigation. For the lettuce and tomato models, the dose of pathogenic *E. coli* per consumption (D_e; # day^{-1}) was defined as:

$$D_e = C_d\ V\ C_e \quad (5)$$

where C_d is the mean per capita consumption of lettuce or tomatoes (g day^{-1}), V is the volume of water retained on the surface of lettuce or tomatoes, following the sprinkler or drip irrigation respectively (mL g^{-1}) and C_e is the concentration of pathogenic *E. coli* in irrigation water (# log cfu 100 mL^{-1}).

For the models, the dose of *Campylobacter* per consumption (D_c; # day^{-1}) was defined as:

$$D_c = C_d\ V\ C_c \quad (6)$$

where C_d is the mean per capita consumption of lettuce or tomatoes (g day^{-1}), V is the volume of water retained on the surface of lettuce or tomatoes following sprinkler or drip irrigation respectively (mL g^{-1}) and C_c is the concentration of *Campylobacter* in irrigation water (# log cfu 100 mL^{-1}).

Similarly, for the models, the dose of *Rotavirus* per consumption (D_r; # day^{-1}) was defined as:

$$D_r = C_d V C_r \quad (7)$$

where C_d is the mean per capita consumption of lettuce or tomatoes (g day^{-1}), V is the volume of water retained on the surface of lettuce or tomatoes following sprinkler or drip irrigation respectively (mL g^{-1}) and C_r is the concentration of *Rotavirus* in irrigation water (# log cfu 100 mL^{-1}).

For the crops model, the dose of pathogenic *E. coli* per consumption of lettuce or tomatoes harvested on the 10^{th}, 20^{th} or 30^{th} day after irrigation (D_{ep}; # day^{-1}) was represented as:

$$D_{ep} = C_d C_{ep} \quad (8)$$

where C_{ep} is the concentration of pathogenic *E. coli* on lettuce or tomatoes (# gm^{-1}) for all the treatments on the 10^{th}, 20^{th} and 30^{th} days after irrigation as shown in Table 5.1.

Dose-response relation

Dose response models have been developed for all three pathogens' using the Beta Poisson (hypergeometric) dose-response model. The β-Poisson dose-response was used to calculate the daily probability of pathogenic *E. coli* (*E. coli* O157:H7) infection (Teunis et al., 2004) ($P_{infe,d}$; P_{inf} day^{-1}) and defined as:

$$P_{infe,d} = 1-[1+(D_e/\beta)]^{-\alpha}, \text{ (for water)} \quad (9)$$

$$P_{infe,d} = 1-[1+(D_{ep}/\beta)]^{-\alpha}, \text{ (for crops)} \quad (10)$$

where D_e and D_{ep} are the dose of pathogenic *E. coli* per consumption or exposure event (# day^{-1}) through water and crops respectively, and α and β are the fit parameters as shown in Table 5.3.

Medema et al. (1996) developed the dose-response model for the probability of *Campylobacter* infection and the full β-Poisson model was used to calculate the daily probability of infection ($P_{infc,d}$; P_{inf} day^{-1}) and defined as:

$$P_{infc,d} = 1-[1+(D_c/\beta)]^{-\alpha} \quad (11)$$

where D_c is the dose of *Campylobacter* per consumption or exposure event (# day^{-1}), and α and β are the distribution parameters given in Table 5.3.

Teunis and Havelaar (2000) developed the dose-response model for the probability of *Rotavirus* infection and the full β-Poisson model was used to calculate the daily probability of infection ($P_{infr,d}$; P_{inf} day^{-1}) and defined as:

$$P_{infr,d} = 1-[1+(D_r/\beta)]^{-\alpha} \quad (12)$$

where D_r is the dose of *Rotavirus* per consumption or exposure event (# day^{-1}), and α and β are the parameters as shown in Table 5.3.

Annual risk:

The annual probability of infection explained in equations 13, 14 and 15 (Barker et al., 2014), due to pathogenic *E. coli*, *Campylobacter* and *Rotavirus* ($P_{infe,y}$, $P_{infc,y}$, $P_{infr,y}$) respectively, was estimated as:

$$P_{\inf e,y} = 1 - \prod_{k=1}^{d} \left(1 - P_{\inf e,d}\right) \quad (13)$$

$$P_{\inf c,y} = 1 - \prod_{k=1}^{d} \left(1 - P_{\inf c,d}\right) \quad (14)$$

$$P_{\inf r,y} = 1 - \prod_{k=1}^{d} \left(1 - P_{\inf r,d}\right) \quad (15)$$

where $P_{infe,d}$ is the daily probability of pathogenic *E. coli* infection, $P_{infc,d}$ is the daily probability of *Campylobacter* infection, $P_{infr,d}$ is the daily probability of *Rotavirus* infection and d is the number of exposure events per year (i.e. 365 days) from k = 1 to 365.

Probability of annual gastrointestinal illness due to pathogenic *E. coli*, *Campylobacter* and *Rotavirus* (P_{ille}, P_{illc}, P_{illr}) was estimated as:

$$P_{ille} = P_{infe,y} P_{ill/infe} \quad (16)$$

$$P_{illc} = P_{infc,y} P_{ill/infc} \quad (17)$$

$$P_{illr} = P_{infr,y} P_{ill/infr} \quad (18)$$

where $P_{infe,y}$, $P_{infc,y}$ and $P_{infr,y}$ are the annual probability of pathogenic *E. coli*, *Campylobacter* and *Rotavirus* infection respectively, and $P_{ill/infe}$, $P_{ill/infc}$ and $P_{ill/infr}$ are the probability of gastrointestinal illness per pathogenic *E. coli*, *Campylobacter* or *Rotavirus* infection as shown in Table 5.3.

Annual disease burden in the fields:

The annual disease burden from these three pathogens (D_e, D_c, D_r) was represented using DALYs as shown in Table 5.3 and annual disease burdens (DALYs pppy) were estimated as:

$$D_e = B_e P_{ille} \quad (19)$$

$$D_c = B_c P_{illc} \quad (20)$$

$$D_r = B_r P_{illr} \quad (21)$$

where P_{ille}, P_{illc} and P_{illr} are the probability or risk of annual diarrheal disease illness due to pathogenic *E. coli*, *Campylobacter* and *Rotavirus* respectively, and B_e, B_c, B_r are the disease burdens (DALYs per case of illness) given in Table 5.3.

Disease burdens (B_e, B_c, B_r) were calculated in Table 5.4 as DALYs =Number of symptomatic cases*severity*duration in years (George et al., 2015), where case fatality ratio for pathogenic *E.*

coli and *Rotavirus* were based on Howard et al., 2006 and *Campylobacter* from Haas et al., 1999. And the severity and duration of illness of pathogenic *E. coli* and *Rotavirus* were based on Havelaar and Melse, 2003 and *Campylobacter* were from Kemmeren et al., 2006.

Combined GI risk in the fields:

Finally, the combined risk of illness from the three pathogens was computed for the studied field sites. The combined risk (P_{ill_C}) is a total estimated probability of gastrointestinal illness (Schoen and Ashbolt, 2010; USEPA, 2010; Barker et al., 2014) and determined as,

$$P_{ill\_C} = 1 - \prod_{k=1}^{d} \left(1 - P_{ill\_k}\right) \quad (22)$$

where k is an individual pathogen (pathogenic *E. coli*, *Campylobacter* and *Rotavirus*) and P_{ill_k} is the daily probability of illness for each pathogen. It is assumed that the risk of illness from each pathogen is statistically independent. Therefore, this is the probability of at-least one illness from any of the three pathogens. The combined annual disease burden was determined by the summation of the annual disease burden of all three pathogens (Barker et al., 2014).

To estimate the risk per exposure event, Monte-Carlo simulations with 1,000,000 trial runs were conducted. All the modelling and analysis were performed using MATLAB version R2015a (8.5.0 from Mathworks®) and Microsoft Excel 2016. For all the model outputs, the risks were computed with their mean values. A health target of 10^{-6} DALY pppy (WHO, 2004; Barker et al., 2013; George et al., 2015) was considered for the annual disease burden.

5.3 Results and discussion

5.3.1 Risk assessment and annual disease burden for greenhouse study

The annual probability of gastrointestinal illness ($year^{-1}$) and the mean annual disease burden (DALYs pppy) in the water and crops models, are represented in Table 5.5. In the water model,

the risk assessment for lettuce and tomato with sprinkler and drip irrigation was compared. The known amounts of *E. coli* concentration were used to irrigate the plants. Based on this, the annual disease burden on humans is shown. The annual disease burden exceeded the health target of 10^{-6} DALYs pppy for both irrigation methods and for both of the crops in the water model. Sprinkler irrigation showed a higher risk of infection to humans than drip irrigation. Spray irrigation can lead to a higher risk of infection than drip irrigation and other irrigation practices (Hamilton et al., 2006). The mean annual disease burden was found to be 6.2×10^{-2} DALYs pppy, during sprinkler irrigation for both the crops. Similarly, the mean annual disease burden was found to be 5.5×10^{-2} DALYs pppy, during drip irrigation. There was no variation in the risks between the crops because the volume of water retained on the crops during sprinkler and drip irrigation were the same i.e. 0.11 and 0.02 ml, (Keuckelaere et al., 2015) and the consumption of lettuce and tomatoes during 2014 was not that different from each other (Agriculture and Agri-Food Canada Statistics at http://www.agr.gc.ca).

In the crops model, it was found that consumption of lettuce would lead to higher risks of gastrointestinal illnesses compared to tomatoes as shown in Table 5.5. Lettuce showed risk in all the four treatments and for all the days (10, 20 and 30) except Drip+Mineral which showed risk only on the 20th day. Whereas, tomatoes showed risk only in Drip+Organic treatment on the 10th day and after the 10th day there was no risk estimated when consuming tomatoes. This is because lettuce is a leafy vegetable and it has a larger surface area for exposure and contamination than does the tomato. Robertson (2013) supported our results that people consuming lettuce are more prone to infections. The risks of infection or illness due to lettuce consumption for all the treatments and harvested days are explained below:

In the treatment with sprinkler irrigation and organic soil (Sprinkler+Organic), it was observed that the annual disease burden was found to exceed the health target of 10^{-6} DALYs pppy for all the harvesting days (10, 20 and 30). The overall range of mean annual disease burden for this treatment was found to be 1.3×10^{-2} to 3.8×10^{-2} DALYs pppy. The mean annual disease burden was found to be maximum i.e. 3.8×10^{-2} DALYs pppy on the 10^{th} day and then it reduced to 2.8×10^{-2} DALYs pppy on the 20^{th} day, and further reduced to 1.3×10^{-2} DALYs pppy on the 30^{th} day. The results showed that for the present case, even after the 30^{th} day, the lettuce was not safe to be consumed if irrigated with untreated wastewater and grown with sprinkler irrigation in organic soil.

In the Sprinkler+Mineral treatment, it was observed that the overall range of the mean annual disease burden for the treatment was broad, 7.4×10^{-5} to 2.1×10^{-2} DALYs pppy. However, on all of the harvesting days, the annual disease burden exceeded the health target. The mean annual disease burden was highest on the 10^{th} day after irrigation i.e. 2.1×10^{-2} DALYs pppy. The annual disease burden for humans was 3.7×10^{-3} DALYs pppy on the 20^{th} day and 7.4×10^{-5} DALYs pppy on the 30^{th} day for this treatment.

In the drip irrigation and organic soil (Drip+Organic) treatment, the risk was found on all of the days, and the mean annual disease burden exceeded the health target of 10^{-6} for all three days. The annual disease burden for all the cases can be observed in Table 5.5 and the range of risks was found to be broad. The highest mean annual disease burden for this treatment was found to be 1.4×10^{-2} DALYs pppy on the 10^{th} day, 3.4×10^{-3} DALYs pppy on the 20^{th} day, and least was 6.3×10^{-5} DALYs pppy on the 30^{th} day.

In the treatment with drip irrigation and mineral soil i.e. Drip+Mineral, the risk due to consumption of lettuce was found only on the 20^{th} day, this is shown in Table 5.5. The observed

risks exceeded the annual disease burden of 10^{-6} DALYs pppy i.e. the health target. After the 20th day of irrigation, there was no risk observed in this treatment. In comparison to other treatments for lettuce, Drip+Mineral treatment showed the least risk to humans in terms of magnitude and duration.

For the crops model on lettuce, the comparison of all the treatments showed that the Sprinkler+Organic treatment resulted in the maximum risk in terms of magnitude. Also in this treatment, the overall range of the annual disease burden was found to be narrower and farther from the health target of 10^{-6} DALYs pppy than in the other treatments. After the Sprinkler+Organic treatment, the annual disease burden was found to be the highest in the Sprinkler+Mineral treatment, less in the Drip+Organic treatment and the least in the Drip+Mineral treatment. The annual disease burden showed that lettuce from the Drip+Mineral treatment, is safe to be used after the 20th day of irrigation. Lettuce was found to be very sensitive to these pathogenic *E. coli* infections as it has a large surface area that is exposed to contamination (Robertson 2013). Similarly, Drechsel and Seidu (2011) reported that the annual disease burden due to lettuce consumption was found to be 1.7×10^{-2} DALYs pppy, which supports our results. Hamilton et al. (2006) found that the annual risk of infection ranged from 10^{-3} to 10^{-1} when irrigated water ceased to be used 1 day before harvest, 10^{-6} to 10^{-2} for one week before harvest and 10^{-9} to 10^{-3} for two weeks before harvest. The annual risk of infection was found to be higher than the benchmark of 10^{-4} (Macler et al., 1993; USEPA 1989) for all vegetables which have been studied, except in the case of broccoli and cucumber (Hamilton et al., 2006).

When the crops model was applied to tomatoes, only the Drip+Organic treatment showed risk. This was the case only when consuming tomatoes that were harvested on the 10th day from the

contaminated irrigation water application. On the 10^{th} day of the Drip+Organic treatment, the mean annual disease burden was found to be 1×10^{-2} DALYs pppy, which could be an experimental or an unknown error. Though after 10^{th} day in the Drip+Organic treatment, tomatoes were found to be safe to be eaten fresh. Drip+Organic treatment could have internalization of bacteria in the tomato fruit (Gupta and Madramootoo, 2016a) through contaminated organic soil. Also, organic soil being rich in organic matter and carbon content is favorable for the bacterial contamination (Gupta and Madramootoo, 2016b). All other treatments (Sprinkler+Organic, Sprinkler+Mineral and Drip+Mineral) showed no risk which proved that tomatoes are safe to be used when grown with all the other treatments.

The health target of 10^{-6} DALYs pppy (WHO 2004) was never attained in our results, and all the mean annual disease burden values and ranges exceeded the WHO target. Similarly, George et al. (2015) reported that pathogenic *E. coli* exceeded the health target, with an annual disease burden of 3.6×10^{-6} DALYs pppy for the drinking water in India. Machdar (2010) reported significantly higher risk of pathogenic *E. coli* in drinking water in Ghana, and George et al. (2015) observed risk in drinking water in India due to the pathogenic *E. coli*. Pathogenic *E. coli* risk was found to be significantly higher in irrigation water applied and lettuce consumed, irrespective of different treatments. The four treatments due to variation in their properties, showed differences in bacterial transport to fruits and vegetables (Gupta and Madramootoo, 2016a; 2016b).

Comparison of the water and crop model: the crop model is better than the water model because when harvested produce is consumed, the risk to consumers is better identified using the crop model as the pathogen tested was in direct contact with the fruits and vegetables. The water model identifies the risk in irrigation water, but the possibility exists that the pathogens may not

come in contact with the crop. Therefore, the crop model is more significant in terms of identifying the risks to consumers.

5.3.2 Microbial risk assessment and disease burden at two field sites in Quebec

The measured *E. coli* concentration as shown in Table 5.2 was used to estimate the concentration of pathogens on the lettuce and tomatoes. The pathogenic risk assessment in Rougemont (tomato) and St-Remi (lettuce) was shown in Tables 5.6 and 5.7, respectively. The results showed the mean of annual probability of diarrheal illness ($year^{-1}$), combined GI risk due to all three pathogens, annual disease burden (DALYs pppy), combined annual disease burden for all three pathogens. Annual disease burden estimates were highly variable across the two models and three pathogens.

In Table 5.6, it is found that for Rougemont (tomato), the annual disease burden ranges from 1.4×10^{-3} to 6.4×10^{-2}; 7.3×10^{-5} to 3.5×10^{-3}; 5.8×10^{-6} to 2.8×10^{-4} (DALYs pppy) for pathogenic *E. coli*, *Campylobacter*, *Rotavirus*, respectively, in 2013-2014. The annual disease burden for Rougemont (tomato) always exceeded the 10^{-6} DALYs pppy for all three pathogens. The annual disease burden estimates increased from *Rotavirus* to *Campylobacter* and were highest with pathogenic *E. coli*. Therefore, the pathogenic *E. coli* causes the most diarrheal illnesses followed by *Campylobacter* and *Rotavirus* infections.

When considering St-Remi (lettuce) for the years 2013-2014 it was seen that the annual disease burden ranged from 6.9×10^{-3} to 5×10^{-2} (DALYs pppy) for pathogenic *E. coli*; 3.7×10^{-4} to 2.7×10^{-3} (DALYs pppy) for *Campylobacter*; and 3×10^{-5} to 2.2×10^{-4} (DALYs pppy) for *Rotavirus.* The annual disease burden for St-Remi (lettuce) always exceeded the 10^{-6} DALYs pppy for all the three pathogens. Similar to unwashed tomato consumption, unwashed lettuce consumption can

cause gastrointestinal illnesses mostly from pathogenic *E. coli* and *Campylobacter*, followed by *Rotavirus* as seen from the annual disease burden estimates.

For the drinking water in India, George et al. (2015) found that the annual disease burden range from 4.56×10^{-8} to 2.43×10^{-7} DALYs pppy for *Rotavirus* which did not exceed the WHO health target, but *Campylobacter* and pathogenic *E. coli* exceeded the WHO target with 2.6×10^{-6} and 3.6×10^{-6} DALYs, respectively. In comparison, the DALYs for pathogenic *E. coli* and *Campylobacter* were higher in our results. Comparatively, Barker et al. (2014) found 2×10^{-3} DALYs pppy, and Machdar (2013) found 2.6×10^{-2} DALYs pppy, for *Rotavirus*, which is higher than our results.

Combined DALYs (DALY pppy) were found by adding the DALYs for all the pathogens for each year and for all models. The combined DALYs are represented in Tables 5.6 and 5.7 for all the time periods over the two years. For the tomato model as shown in Table 5.6, the combined annual disease burden was found to be highest (0.068 DALYs pppy) for May-June, 2013 and lowest (0.001 DALYs pppy) for September-October, 2014. For the lettuce model as shown in Table 5.7, the combined annual disease burden among all the time periods was found to be highest (0.053 DALYs pppy) for May-June, 2014 and lowest (0.007 DALYs pppy) for July-August, 2014. The combined DALYs were found in the range of 10^{-3} to 10^{-2} for lettuce and tomatoes, similar to the combined DALYs (for *Rotavirus*, *Norovirus* and *Ascaris*) reported by Barker et al. (2014) i.e. of the order of 10^{-3}.

Combined gastrointestinal (GI) risk was computed from the daily probability of illness from all the pathogens, using equation 22 and represented in Tables 5.6 and 5.7. In Table 5.6 for the tomato model, the combined GI risk was highest (0.149) for May-June, 2013 and lowest (0.003) for September-October, 2014. Whereas in Table 5.7, for the lettuce model, the combined GI risk

was seen highest (0.118) for May-June, 2014 and lowest (0.017) for July-August, 2014. This shows that combined annual disease burden (sum DALYs) and combined GI risk are highly correlated as the time periods for the highest and lowest combined DALYs and combined risk are the same in each model. However, all the values and ranges exceeded the health target of 10^{-6} (WHO, 2004), although our results for combined GI risk were lower than those reported by Barker et al. (2014). Therefore, the implementation of treatment of the surface irrigation water and vaccination needs to be considered in order to prevent the spread of these illnesses. It is important for children to take *Rotavirus* vaccination as recommended (Health Canada, 2007) in order to avoid *Rotavirus* infections, but susceptibility to pathogenic *E. coli* and *Campylobacter* infections remains high. Pathogenic *E. coli* and *Campylobacter* presence in the irrigated water is largely responsible for the gastrointestinal illnesses according to our results. The results of our study correlate with the trends found by Machdar (2010) in the drinking water in Ghana and George et al. (2015) in the drinking water in India i.e. the risk of *Rotavirus* infection was lower compared to those of pathogenic *E. coli* and *Campylobacter*.

Out of the two models (tomato and lettuce), it was found that lettuce consumption resulted in a greater risk of infection (combined GI risk range: 10^{-2} to 10^{-1}) by the three pathogens compared to the tomato consumption (combined GI risk range: 10^{-3} to 10^{-1}). People are more prone to infections through lettuce consumption as it is a leafy vegetable and has larger surface area for exposure and contamination (Robertson, 2013). Tomatoes can also lead to a significant number of infections if they are eaten unwashed, fresh or uncooked. Also, the range for the combined annual disease burden (7×10^{-3} to 5.3×10^{-2} DALYs pppy) for the lettuce model was more stringent when compared to the range (1×10^{-3} to 6.8×10^{-2} DALYs pppy) for the tomato model. Drechsel and Seidu (2011) reported that annual disease burden due to lettuce consumption was

1.7×10^{-2} DALYs pppy, which is similar to our results. See Appendix 7 for risk distribution diagrams.

The quality of the irrigation water and the crop type were given importance in our study. The crop model assumed that the fruits and vegetables were eaten fresh and unwashed, and the pathogenic contamination was from the water only. Other possible sources of contamination could be soil splashes from rain water or irrigation water. In the same context, Amoah et al. (2007) reported that while irrigation water might be uncontaminated, the crop could be contaminated by the surface soil and/or manure application. Other parameters such as pH, presence of macro- and micro-elements could influence the growth of bacteria in soil resulting in contamination (Appendix 8).

Limitations of the QMRA model used:

1. The consumption of fruit or vegetable was based on data from Agriculture and Agri-Food Canada which is country-specific or region-specific. From our results, it can been seen that the consumption of lettuce was higher than tomato consumption which resulted in higher risk to consumers in consuming lettuce than tomatoes. Therefore, the model should be modified based on the consumption data for different regions or countries.
2. Bacterial (pathogenic *E. coli* and *Campylobacter*) and viral (*Rotavirus*) estimates used in the model (8%, 6.6% and 0.001% respectively) provide approximate risk of gastrointestinal diseases to consumers because in our studies *E. coli* was used an indicator organism. If we know the actual number of pathogens in water or on crops then the QMRA model would give information on the actual gastrointestinal risk or annual disease burden to consumers.

5.4 Conclusion

Pathogens not always exceeded the WHO health target of 10^{-6} DALYs pppy for the fields studied. In the water model, it was observed that sprinkler irrigation (6.2×10^{-2} DALYs pppy) resulted in a higher risk than drip irrigation (5.5×10^{-2} DALYs pppy) for both the lettuce and tomato crops. Irrigation types (sprinkler and drip irrigation) and soil types (organic and mineral soil) impacted the degree of exposure of pathogenic *E. coli* on the crops. The crops model resulted in significantly high risk in lettuce for all the treatments and for all the harvested days except in the Drip+Mineral treatment, where the risk was observed on lettuce only on the 20th day after irrigation. The highest risk on lettuce was observed in the Sprinkler+Organic treatment followed by the Sprinkler+Mineral and the Drip+Organic treatments. The risk decreased from the 10th day to 30th day for all the treatments, due to dilution with clean irrigation water. The crops model showed that there was a risk observed in tomato, only on the 10th day of the Drip+Organic treatment. Drip irrigation with mineral soil was the best treatment for minimizing the gastrointestinal risks due to lettuce consumption. However, for tomatoes, all the treatments proved to be good other than drip irrigation with organic soil, probably due to soil type, as organic soil is the source of carbon for the pathogenic *E. coli*.

The QMRA for tomatoes is believed to be the first attempt to estimate the annual disease burden from the pathogens. The untreated surface water had a significant concentration of pathogens that cause a high annual disease burden in humans. In Rougemont, the maximum risk from all three pathogens was found during the May-June period for both years. Whereas in St-Remi, the maximum risk from all three pathogens was found during May-June and September-October periods for both years. The combined annual disease burden for all the pathogens (DALYs pppy)

was found in the range of 10^{-3} to 10^{-2} for lettuce and tomatoes. Similarly, combined GI risk was in the range of 10^{-2} to 10^{-1} and 10^{-3} to 10^{-1} for lettuce and tomatoes respectively.

Comparison of St-Remi and Rougemont, shows that lettuce consumption would result in a higher risk and ranges are narrower compared to tomato consumption. Even though lettuce consumption shows a higher risk, the models demonstrate that the risks are still quite significant with either lettuce or tomato consumption. In order to reduce the risks, irrigation water should be applied using drip rather than the sprinkler irrigation method. Washing of vegetables and fruits was not considered in this study. The present model can be applied as a systemic approach by farmers, government and regulatory agencies in order to control the gastrointestinal illness risks of consumption of fresh fruits and vegetables. The study also raises the importance of monitoring the quality of the irrigation water in order to reduce the risk of contamination.

Figure 5.1: Flowchart for QMRA model to estimate risk and annual disease burden

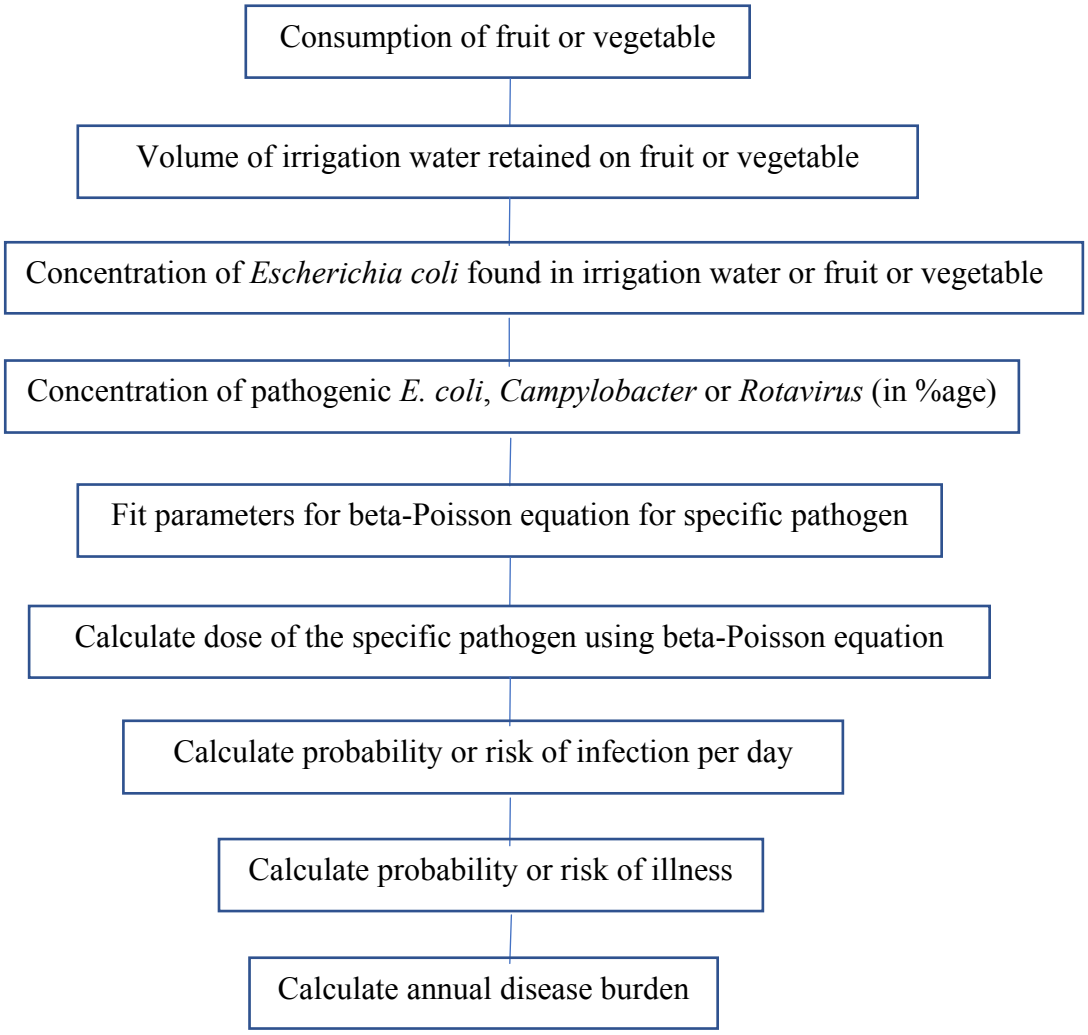

Table 5.1: *E. coli* (log cfu/g) concentration on lettuce leaves and tomatoes after inoculation day (2 log cfu/100ml allowed for irrigation (CCME (2008))

	E. coli (log cfu/g) on lettuce leaves			*E. coli* (log cfu/g) on tomato fruits		
Treatments	10th Day	20th Day	30th Day	10th Day	20th Day	30th Day
Sprinkler+Organic	4.92a±0.04	4.09a±0.03	2.88a±0.02	0±0	0±0	0±0
Drip+Mineral	0d±0	0.78c±1.07	0b±0	0±0	0±0	0±0
Sprinkler+Mineral	3.51b±0.08	1.95b±0.15	0.1b±0.21	0±0	0±0	0±0
Drip+Organic	2.95c±0.14	1.24bc±1.14	0.06b±0.13	2.32±1.3	0±0	0±0

Table 5.2: *E. coli* (log cfu/100ml) concentration in the irrigation water at two sites in Quebec (Rougemont and St-Remi) for 2013 and 2014 (2 log cfu/100ml allowed for irrigation (CCME (2008))

Year	2013				2014			
Site	Rougemont		St-Remi		Rougemont		St-Remi	
Time period	Mean	St. deviation	Mean	St. deviation	Mean	St. deviation	Mean	St. deviation
May-June	2.11	0.02	0.99	0.01	1.43	0.35	1.05	0.42
July-Aug	0.68	0.12	0.54	0.05	0.86	0.26	0.33	0.22
Sept-Oct	1.16	0.05	1.07	0.07	0.42	0.13	1.01	0.09
Total (May-Oct)	1.08	0.08	0.74	0.05	0.89	0.25	0.71	0.24

Table 5.3: Summary of model input parameters and equations (from 1 to 22) for calculating annual disease burden

Variable	Description	Units	Equations and/or values	Reference
C	Consumption of lettuce and tomato	kg person^{-1} year^{-1}	Lettuce: 9.38 (2013), 9.14 (2014); Tomato: 8.92 (2013), 8.68 (2014)	Agriculture and Agri-food Canada Statistics
V	Volume of irrigation retained on fruits and vegetables (tomatoes) for on-surface drip irrigation	ml g^{-1}	0.02	(Van Ginneken and Oron 2000)
V	Volume of water retained on lettuce for sprinkler irrigation	ml g^{-1}	0.11	(Shuval 1997)
Ce	Concentration of pathogenic *E. coli*		8%	(Haas et al., 1999; Howard et al., 2006; George et al., 2015)
Cc	Concentration of *Campylobacter*		6.60%	(Smeets 2008; Machdar et al., 2013; George et al., 2015)
Cr	Concentration of *Rotavirus*		0.001%	(Mara et al., 2007; Machdar et al., 2013; George et al., 2015)
α and β for pathogenic *E. coli*	Fit parameters for beta-poisson equation		α = 0.05 and β = 1.001	(Teunis et al., 2004; Haas et al., 1999; George et al., 2015)
α and β for *Campylobacter*	Fit parameters for beta-poisson equation		α = 0.145 and β = 7.59	(Medema et al., 1996)
α and β for *Rotavirus*	Fit parameters for beta-poisson equation		α = 0.167 and β = 0.191	(Teunis and Havelaar, 2000; Barker et al., 2014)
Pill/infe	Probability of gastrointestinal illness given pathogenic *E. coli* infection	Probability of illness per infection	0.25	(George et al., 2015)
Pill/infc	Probability of gastrointestinal illness given *Campylobacter* infection	Probability of illness per infection	0.3	(George et al., 2015)
Pill/infr	Probability of gastrointestinal illness given *Rotavirus* infection	Probability of illness per infection	0.5	(George et al., 2015)
Be	Disease burden for pathogenic *E. coli*	DALY per case	0.56	Calculated in Table 5.4
Bc	Disease burden for *Campylobacter*	DALY per case	0.08	Calculated in Table 5.4
Br	Disease burden for *Rotavirus*	DALY per case	0.56	Calculated in Table 5.4
Run 1,000,000 Monte-Carlo simulations				
Assumption of concentration of *E. coli*: normal distributed				
Assumption: no prior washing of vegetables and fruits before consumption				

Table 5.4: Disease burden for pathogens (in this study) causing gastroenteritis

Pathogen	**Outcome**	**Severity**	**Duration (in years)**	**Disease burden per case (in DALYs)**
Pathogenic *E. coli*	Water diarrhea (53%)	0.07	0.01	0
	Bloody diarrhea (47%)	0.39	0.02	0
	Death from diarrhea (0.7%)	1	80.1	0.56
	Total diarrhea			0.56
Campylobacter	Gastroenteritis population (94%)	0.07	0.01	0
	Gastroenteritis-general practitioners (6%)	0.39	0.03	0
	Death from gastroenteritis	1	80.1	0.08
	Total gastroenteritis			0.08
Rotavirus	Mild diarrhea (85%)	0.1	0.02	0
	Severe diarrhea (14.4%)	0.23	0.02	0
	Death from diarrhea	1	80.1	0.56
	Total diarrhea			0.56

Table 5.5: Risk assessment for water and crops (lettuce and tomato) in greenhouse

Models		**Risk of illness (year^{-1})**	**persons in 100000 (USEPA target: 10 persons per 10000)**	**Annual disease burden (DALY pppy)**	**Number of hours lost (WHO health target 10-6 DALYs = 0.0087 hrs)**
Water	Lettuce (sprinkler)	1.1×10^{-1}	11000	6.2×10^{-2}	543.12
	Lettuce (drip)	9.8×10^{-2}	9800	5.5×10^{-2}	481.80
	Tomato (sprinkler)	1.1×10^{-1}	11000	6.2×10^{-2}	543.12
	Tomato (drip)	9.8×10^{-2}	9800	5.5×10^{-2}	481.80
Crops: Lettuce					
Sprinkler+Organic	10th day	6.8×10^{-2}	6800	3.8×10^{-2}	332.88
	20th day	5.0×10^{-2}	5000	2.8×10^{-2}	245.28
	30th day	2.2×10^{-2}	2200	1.3×10^{-2}	113.88
Drip+Mineral	20th day	3×10^{-3}	300	1.7×10^{-3}	14.89
Sprinkler+Mineral	10th day	3.7×10^{-2}	3700	2.1×10^{-2}	183.96
	20th day	6.5×10^{-3}	650	3.7×10^{-3}	32.41
	30th day	1.3×10^{-4}	13	7.4×10^{-5}	0.65
Drip+Organic	10th day	2.4×10^{-2}	2400	1.4×10^{-2}	122.64
	20th day	5.9×10^{-3}	590	3.4×10^{-3}	29.78
	30th day	1.1×10^{-4}	11	6.3×10^{-5}	0.55
Crops: Tomato					
Drip+Organic	10th day	1.8×10^{-2}	1800	1×10^{-2}	87.60

Table 5.6: Risk assessment for pathogenic *E. coli*, *Campylobacter* and *Rotavirus* in 2013 and 2014 at Rougemont

Time Period	Pathogens	Annual probability of illness ($year^{-1}$)	persons in 100000 (USEPA target: 10 persons per 10000)	Combined GI risk	combined GI risk (persons in 100000)	Annual disease burden (DALY pppy)	Number of hours lost (health target 10-6 DALYs = 0.0087 hrs)	Combined DALY (pppy) (SUM)	Combined DALYs (Number of hours lost)
May-June, 2013	Pathogenic *E. coli*	1.1×10^{-1}	11000	0.149	14900	6.4×10^{-2}	560.64	0.068	595.68
	Campylobacter	4.3×10^{-2}	4300			3.5×10^{-3}	30.66		
	Rotavirus	5×10^{-4}	50			2.8×10^{-4}	2.45		
July-August, 2013	Pathogenic *E. coli*	4.4×10^{-3}	440	0.006	600	2.5×10^{-3}	21.90	0.003	26.28
	Campylobacter	1.7×10^{-3}	170			1.3×10^{-4}	1.14		
	Rotavirus	1.9×10^{-5}	1.9			1.1×10^{-5}	0.10		
September-October, 2013	Pathogenic *E. coli*	1.3×10^{-2}	1300	0.018	1800	7.3×10^{-3}	63.95	0.008	70.08
	Campylobacter	4.9×10^{-3}	490			3.9×10^{-4}	3.42		
	Rotavirus	5.7×10^{-5}	5.7			3.2×10^{-5}	0.28		
Total (May-October), 2013	Pathogenic *E. coli*	1.1×10^{-2}	1100	0.015	1500	6.1×10^{-3}	53.44	0.006	52.56
	Campylobacter	4.1×10^{-3}	410			3.3×10^{-4}	2.89		
	Rotavirus	4.8×10^{-5}	4.8			2.7×10^{-5}	0.24		
May-June, 2014	Pathogenic *E. coli*	3.3×10^{-2}	3300	0.045	4500	1.8×10^{-2}	157.68	0.019	166.44
	Campylobacter	1.2×10^{-2}	1200			9.8×10^{-4}	8.58		
	Rotavirus	1.4×10^{-4}	14			7.9×10^{-5}	0.69		
July-August, 2014	Pathogenic *E. coli*	7.7×10^{-3}	770	0.011	1100	4.4×10^{-3}	38.54	0.005	43.80
	Campylobacter	2.8×10^{-3}	280			2.3×10^{-4}	2.01		
	Rotavirus	3.3×10^{-5}	3.3			1.8×10^{-5}	0.16		
September-October, 2014	Pathogenic *E. coli*	2.4×10^{-3}	240	0.003	300	1.4×10^{-3}	12.26	0.001	8.76
	Campylobacter	9×10^{-4}	90			7.3×10^{-5}	0.64		
	Rotavirus	1×10^{-5}	1			5.8×10^{-6}	0.05		
Total (May-October), 2014	Pathogenic *E. coli*	8.1×10^{-3}	810	0.011	1100	4.6×10^{-3}	40.30	0.005	43.80
	Campylobacter	3×10^{-3}	300			2.4×10^{-4}	2.10		
	Rotavirus	3.5×10^{-5}	3.5			1.9×10^{-5}	0.17		

Table 5.7: Risk assessment for pathogenic *E. coli*, *Campylobacter* and *Rotavirus* in 2013 and 2014 at St-Remi

Time Period	Pathogens	Annual probability of illness ($year^{-1}$)	persons in 100000 (USEPA target: 10 persons per 10000)	Combined GI risk	combined GI risk (persons in 100000)	Annual disease burden (DALY pppy)	Number of hours lost (health target 10-6 DALYs = 0.0087 hrs)	Combined DALY (pppy) (SUM)	Combined DALYs (Number of hours lost)
May-June, 2013	Pathogenic *E. coli*	5×10^{-2}	5000	0.068	6800	2.8×10^{-2}	245.28	0.03	262.8
	Campylobacter	1.9×10^{-2}	1900			1.5×10^{-3}	13.14		
	Rotavirus	2.2×10^{-4}	22			1.2×10^{-4}	1.05		
July-August, 2013	Pathogenic *E. coli*	1.8×10^{-2}	1800	0.025	2500	1×10^{-2}	87.60	0.011	96.36
	Campylobacter	6.8×10^{-3}	680			5.5×10^{-4}	4.82		
	Rotavirus	7.9×10^{-5}	7.9			4.4×10^{-5}	0.39		
September-October, 2013	Pathogenic *E. coli*	6×10^{-2}	6000	0.082	8200	3.4×10^{-2}	297.84	0.036	315.36
	Campylobacter	2.3×10^{-2}	2300			1.9×10^{-3}	16.64		
	Rotavirus	2.7×10^{-4}	27			1.5×10^{-4}	1.31		
Total (May-October), 2013	Pathogenic *E. coli*	2.8×10^{-2}	2800	0.039	3900	1.6×10^{-2}	140.16	0.017	148.92
	Campylobacter	1.1×10^{-2}	1100			8.7×10^{-4}	7.62		
	Rotavirus	1.2×10^{-4}	12			7×10^{-5}	0.61		
May-June, 2014	Pathogenic *E. coli*	8.7×10^{-2}	8700	0.118	11800	5×10^{-2}	438.00	0.053	464.28
	Campylobacter	3.4×10^{-2}	3400			2.7×10^{-3}	23.65		
	Rotavirus	3.9×10^{-4}	39			2.2×10^{-4}	1.93		
July-August, 2014	Pathogenic *E. coli*	1.2×10^{-2}	1200	0.017	1700	6.9×10^{-3}	60.44	0.007	61.32
	Campylobacter	4.6×10^{-3}	460			3.7×10^{-4}	3.24		
	Rotavirus	5.3×10^{-5}	5.3			3×10^{-5}	0.26		
September-October, 2014	Pathogenic *E. coli*	5.2×10^{-2}	5200	0.071	7100	2.9×10^{-2}	254.04	0.031	271.56
	Campylobacter	2×10^{-2}	2000			1.6×10^{-3}	14.02		
	Rotavirus	2.3×10^{-4}	23			1.3×10^{-4}	1.14		
Total (May-October), 2014	Pathogenic *E. coli*	3×10^{-2}	3000	0.041	4100	1.7×10^{-2}	148.92	0.018	157.68
	Campylobacter	1.1×10^{-2}	1100			9.2×10^{-4}	8.06		
	Rotavirus	1.3×10^{-4}	13			7.4×10^{-5}	0.65		

Connecting text to Chapter 6

Chapter 5 gives information on the potential health risks to humans, when applying the QMRA model to the water and crop studies at the greenhouse scale, and for the two field sites in Quebec. For the greenhouse study, the model was based on manually contaminated irrigation water, and on crops grown under four different treatments and harvested on the 10^{th}, 20^{th} and 30^{th} days after irrigation with contaminated water. At field scale, the QMRA model was used for the untreated surface water, which was used to irrigate lettuce and tomatoes at two field sites in Quebec. Chapter 6 develops thirty different scenarios based on the QMRA model including the washing conditions (no washing, washing for 3-4 sec or washing for 2 min prior consumption), irrigation methods (drip and sprinkler) and vegetables type (broccoli, cauliflower, zucchini, lettuce and tomatoes). This study provided information and knowledge on the pathogenic risk caused by pathogenic *E. coli*, *Campylobacter* and *Rotavirus* after the consumption of these fresh vegetables grown in Saint-Esprit, Quebec after they were irrigated with untreated surface water. This study gave detailed information on which irrigation method, in combination with the washing conditions, best reduces the health risks of consumption of fresh vegetables. To the best of our knowledge, this is the first study that uses the QMRA model to estimate the risks on vegetables when considering growth scenarios based on different irrigation methods and three different washing conditions (no washing, washing for 3-4 sec or washing for 2 min). The following manuscript prepared from this study and co-authored by Dr. C.A. Madramootoo will be submitted soon.

Gupta, D. and Madramootoo, C.A. (2016) Scenario analysis study using Quantitative Microbial Risk Assessment (QMRA) for the consumption of ready-to-eat (RTE) vegetables.

Chapter 6: Scenario analysis study using Quantitative Microbial Risk Assessment (QMRA) for the consumption of ready-to-eat (RTE) vegetables

Divya Gupta and Chandra A. Madramootoo

Abstract

The QMRA model examining different scenarios was developed for Saint-Esprit, Quebec. Fecal coliform concentration in the St-Esprit stream (untreated surface water) was obtained for the May-October growing season for the 1997-2008 time period. Based on the fecal coliform concentration, thirty different scenarios using the Beta Poisson dose-response model were constructed including five vegetables namely, broccoli, cauliflower, squash (zucchini), lettuce, and tomatoes. Two irrigation methods namely, drip and sprinkler and three washing conditions i.e. no washing, washing for 3-4 sec and washing for 2 min were considered. All the scenarios estimated the annual disease burden and combined gastrointestinal (GI) risk from three pathogens (pathogenic *E. coli*, *Campylobacter* and *Rotavirus*). The Disability-Adjusted Life Year (DALY) is an overall disease burden measure, expressed as the number of years lost to disability, illness or premature death. Drip irrigation showed less risk (1.8×10^{-8} to 3.9×10^{-4} DALYs) than sprinkler irrigation (9.8×10^{-8} to 2.1×10^{-3} DALYs) across all scenarios. Washing fresh vegetables for 2 min prior to consumption showed the least risk (mostly in the range of 10^{-8}-10^{-5} DALYs) compared to washing vegetables for 3-4 sec (range of 10^{-8}-10^{-4} DALYs) and no washing (range of 10^{-7}-10^{-3} DALYs). Therefore, drip irrigation and washing vegetables for 2 min is highly recommended as the best way to reduce health risks. *Rotavirus* showed the least or no risk as compared to *Campylobacter* and pathogenic *E. coli* during both irrigation practices. Lettuce showed the highest risk among all the vegetables studied. The combined GI risk showed

a similar trend as the annual disease burden, across all the scenarios. The QMRA model will help irrigators and vegetable producers in deciding the appropriate practice to lower risk by analyzing the health risks caused by different scenarios.

Keywords:

DALYs, risk, pathogens, vegetables, irrigation, washing

6.1 Introduction:

The agricultural sector consumes 70% of the world's accessible freshwater (FAO, 2012). The irrigation water requirement for agriculture creates large demands on water resources. However, (FAO, 2012) reported in 2010, that only 0.16% of freshwater demand in Canada was for irrigation Scott et al. (2004) reported that even after the high percentage of freshwater withdrawal, in many parts of the world, agricultural irrigation is done with wastewater and untreated water. In Quebec, the irrigation water sources are on-farm surface water (73%) and on-farm underground water (17%) (Statistics Canada, 2013). Irrigation water in Quebec is generally untreated. Vegetable growers rely on water sources located on or near their farms such as rivers and ponds. If the water source is not located close by, the precipitated water gets stored in ponds or dugouts, which is used as on-farm surface water. Underground water sources, like wells, are the other sources of irrigation water in food production.

In 2006, Quebec accounts for about 21.6% of all the vegetables growing farms reported in Canada (Statistics Canada, 2006). In Quebec, 82% of the total irrigation volume is mostly applied during June-August (Statistics Canada, 2013). Major irrigation methods practiced in

Quebec are sprinkler and micro irrigation. Irrigation sources like rivers, ponds and dugouts can be polluted by agricultural field runoff or non-point sources in the watershed. Buck et al. (2003) reported that runoff from cattle feedlots and agricultural fields can be the source of pollution of surface irrigation water. However**,** Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec (MAPAQ) reported that groundwater is generally of better quality than surface water, owing to the soil's natural filtering capacity, but it may be vulnerable to contamination. Precautions must be taken to ensure a supply of good quality water to crops at all times. Therefore, it is important to study and analyze the quality of irrigation water.

To develop programs and regulations on agricultural pollution, MAPAQ (2015) monitored the fecal coliform concentrations from surface water streams for 51 stations in the agricultural areas of Quebec. MAPAQ found that, during the growing period (May-October) when crop irrigation is primarily carried out, fecal coliform concentrations exceeded the water quality criteria for agriculture and recreational uses. During the November-April period, water was found to be less contaminated as there was less agricultural activity at this time of year.

The fecal coliform concentrations in the irrigation water are the indicators of water pollution and can be used to quantify the health risks associated with the consumption of fresh vegetables irrigated with infected water. Hamilton et al. (2006) developed a QMRA model for enteric virus infection associated with the consumption of fresh broccoli, cabbage, cucumber and lettuce irrigated with infected secondary effluent. The mean annual risk of infection was less for cucumber than for other vegetables such as broccoli, cabbage and lettuce. Hamilton et al. (2006) advised incorporating a burden of disease end point, such as disability adjusted life year (DALY) in the model. Hamilton et al. (2006) and Nauta (2000) reported that, the QMRA model is the

recommended approach in assessing the viral and bacterial risks from the consumption of vegetables and milk. Also, Hamilton et al. (2006) and Nauta (2000) estimated the risks in food through Monte-Carlo simulations.

The QMRA model has been used for risk assessment of wastewater (Barker et al., 2013), non-disinfected secondary effluent (Hamilton et al., 2006) and drinking water (George et al., 2015). Limited information is available on the application of the QMRA model for foodborne illness risk assessment associated with ready to eat vegetables irrigated with surface water. Therefore, there is a need to construct a QMRA model which considers foodborne illness risk when surface water is used for irrigating the vegetables which can be eaten fresh such as broccoli, cauliflower, squash (zucchini), lettuce and tomatoes. In this study, QMRA model based scenarios for pathogenic *E. coli*, *Campylobacter* and *Rotavirus* infection associated with the consumption of fresh vegetables irrigated with untreated surface water stream, are presented. All of the different scenarios were constructed using two irrigation methods and three washing conditions. These scenarios will help irrigators and vegetable producers in deciding the appropriate practice to minimize the associated health risks.

6.2 Methodology

6.2.1 Study area

The area considered for this study was the Saint-Esprit watershed of 26.1 sq. km. The watershed is located approximately 50 km north of the city of Montreal in the St. Esprit river basin which forms part of the larger L'Assomption river basin. The St. Esprit river basin is located in a region with the longest growing season in Quebec. The climate of the

watershed is temperate. The St-Esprit region was selected due to following reasons: a) there are 179 ha of vegetables grown (excluding the greenhouses) in the 13 reported farms (Statistics Canada 2006); b) most of the vegetables grown are ready-to-eat vegetables such as broccoli, cauliflower and zucchini; c) Lapp et al. (1998) reported that 19 farms on the watershed are involved in livestock production; d) the water used for irrigation is untreated and could be infected by livestock feces. The density of livestock on the watershed is 0.81 animal units per hectare (Lapp et al., 1998; MAPAQ 2015). The upper region of the watershed has loamy and sandy soils whereas the lower region has clay and clay loams (Lapp et al., 1998). With its tributaries, the length of the water channel on the basin is approximately 9 km. One of the tributaries was examined for fecal coliforms on the watershed (MAPAQ 2015). The time period considered for analyzing the water quality in St. Esprit was 1997-2008. The washing of fruits and vegetables on the farm was considered for three scenarios: no washing, washing for 3-4 sec and washing for 2 min.

6.2.2 Dose-response models' structure, implementation and risk characterization

The fecal coliform concentrations during 1997-2008 were reported as 275 cfu 100 mL^{-1} for the May-October growing season and 111 cfu 100 mL^{-1} for the non-growing season (November-April) (MAPAQ 2015). *Escherichia coli* (*E. coli*) concentration was assumed as 95% of the total fecal coliform concentration and was converted to # log cfu 100 mL^{-1}. The percentage of pathogenic *E. coli* (C_e; # log cfu 100 mL^{-1}), *Campylobacter* (C_c; # log cfu 100 mL^{-1}) and *Rotavirus* (C_r; # log cfu 100 mL^{-1}) were obtained from the literature as 8%, 6.6% and 0.001% respectively, of the total measured *E. coli* concentration (C_E; # log cfu 100 mL^{-1}) (Haas et al., 1999; Howard et

al., 2006; Mara et al., 2007; Smeets 2008; Machdar et al., 2013; George et al., 2015) and described as:

$$C_e = 0.08C_E \quad (1)$$

$$C_c = 0.066C_E \quad (2)$$

$$C_r = 10^{-5}C_E \quad (3)$$

The exposure model was developed as a means of estimating the annual disease burden due to pathogens (pathogenic *E. coli*, *Campylobacter* and *Rotavirus*) from the consumption of untreated surface water irrigated broccoli, cauliflower, zucchini, lettuce and tomatoes. There were thirty (5 vegetables*3 washing conditions*2 irrigation methods) scenarios considered for all of the vegetables (broccoli, cauliflower, zucchini, lettuce and tomatoes) with respect to three washing conditions namely, no washing, washing for 3-4 seconds and washing for 2 minutes and two irrigation methods i.e. sprinkler and drip irrigation. Fifteen scenarios with drip irrigation (5 vegetables*3 washing conditions) were used to estimate the health risks based on the volume of water retained on the fresh vegetables through drip irrigation, irrespective of washing or not. Similarly, another fifteen scenarios with sprinkler irrigation measured the health risks based on the volume retained on vegetables in this manner. In these scenarios, health risks associated with eating these fresh vegetables (washing for 3-4 sec, 2 min or without washing) were considered.

The summary of the model input parameters for calculating the annual disease burden is represented in Table 6.1. To develop the dose-response model for broccoli,

cauliflower, zucchini, lettuce and tomatoes, the mean per capita consumption for the respective vegetables (C; kg yr^{-1}) was obtained from Agriculture and Agri-Food Canada Statistics (http://www.agr.gc.ca) as reported in Table 6.1. It was then converted into g per day (C_d; g day^{-1}) and is defined as:

$$C_d = 1000\ C / 365 \quad (4).$$

The volume of water retained on the surface of vegetables was based on the type of irrigation method used, as reported in Table 6.1.

For all of the scenarios with no washing of vegetables prior to consumption, the doses of pathogenic *E. coli* per consumption (D_e; # day^{-1}), *Campylobacter* per consumption (D_c; # day^{-1}) and *Rotavirus* per consumption (D_r; # day^{-1}) were defined as:

$$D_e = C_d\ V\ C_e \quad (5)$$

$$D_c = C_d\ V\ C_c \quad (6)$$

$$D_r = C_d\ V\ C_r \quad (7)$$

where C_d is the mean per capita consumption of broccoli, cauliflower, squash (zucchini), lettuce or tomatoes (g day^{-1}), V is the volume of water retained on the surface of vegetables, following the sprinkler or drip irrigation respectively (mL g^{-1}) and C_e is the concentration of pathogenic *E. coli* in irrigation water (# log cfu 100 mL^{-1}), C_c is the concentration of *Campylobacter* in irrigation water (# log cfu 100 mL^{-1}), and C_r is the concentration of *Rotavirus* in irrigation water (# log cfu 100 mL^{-1}).

For all of the scenarios considered with the washing of vegetables for 3-4 seconds or 2 minutes' prior to consumption, the doses of pathogenic *E. coli* per consumption

(Dw_e; # day^{-1}), *Campylobacter* per consumption (Dw_c; # day^{-1}) and *Rotavirus* per consumption (Dw_r; # day^{-1}) were defined as:

$$Dw_e = C_d\, V\, C_e\, 10^{-w} \quad (8)$$

$$Dw_c = C_d\, V\, C_c\, 10^{-w} \quad (9)$$

$$Dw_r = C_d\, V\, C_r\, 10^{-w} \quad (10),$$

where C_d is the mean per capita consumption of broccoli, cauliflower, squash (zucchini), lettuce or tomatoes (g day^{-1}), V is the volume of water retained on the surface of vegetables, following the sprinkler or drip irrigation respectively (mL g^{-1}), C_e is the concentration of pathogenic *E. coli* in irrigation water (# log cfu 100 mL^{-1}), C_c is the concentration of *Campylobacter* in irrigation water (# log cfu 100 mL^{-1}), C_r is the concentration of *Rotavirus* in irrigation water (# log cfu 100 mL^{-1}) and w is the $\log_{10}$ reduction in pathogen concentration from washing crops prior to consumption i.e. 1 $\log_{10}$ reduction for 3-4 seconds washing and 1.4 $\log_{10}$ reduction for 2 minutes washing, by dipping the crops in cold water (Amoah et al., 2007).

The model that is commonly used in the QMRA is called the Beta Poisson (hypergeometric) dose-response model (Teunis et al., 2004) and has two parameters, α and β. This model was chosen as it is a more realistic model and incorporates heterogeneity between the pathogen-host interactions by implementing probability distribution to describe variations in the probability of infection of individual pathogens (Haas et al., 1999; Teunis & Havelaar, 2000; Teunis et al., 2004). Also, in this model, the probability of infection cannot exceed the probability of exposure (Teunis & Havelaar, 2000; Teunis et al., 2004).

Beta Poisson (hypergeometric) dose-response model has been developed for all the three pathogens. Teunis et al. (2004) developed the dose-response model for the probability of pathogenic *E. coli* (*E. coli* O157:H7) infection. The β-Poisson model was used to calculate the daily probability of pathogenic *E. coli* infection ($P_{infe,d}$; P_{inf} day^{-1}) and defined as:

$$P_{infe,d} = 1-[1+(D_e/\beta)]^{-\alpha}, \text{ (no washing)} \quad (11)$$

$$P_{infe,d} = 1-[1+(Dw_e/\beta)]^{-\alpha}, \text{ (after washing)} \quad (12)$$

where D_e or Dw_e is the dose of pathogenic *E. coli* per consumption or exposure event (# day^{-1}) with or without washing, and α and β are the fit parameters as shown in Table 6.1.

Similarly, Medema et al. (1996) and Teunis and Havelaar (2000) developed the dose-response model for the probability of *Campylobacter* and *Rotavirus* infection, respectively. The full β-Poisson model was used to calculate the daily probability of *Campylobacter* infection ($P_{infc,d}$; P_{inf} day^{-1}) and *Rotavirus* infection ($P_{infr,d}$; P_{inf} day^{-1}) and was defined as:

$$P_{infc,d} = 1-[1+(D_c/\beta)]^{-\alpha}, \text{ (no washing)} \quad (13)$$

$$P_{infc,d} = 1-[1+(Dw_c/\beta)]^{-\alpha}, \text{ (after washing)} \quad (14)$$

$$P_{infr,d} = 1-[1+(D_r/\beta)]^{-\alpha}, \text{ (no washing)} \quad (15)$$

$$P_{infr,d} = 1-[1+(Dw_r/\beta)]^{-\alpha}, \text{ (after washing)} \quad (16)$$

where D_c or Dw_c is the dose of *Campylobacter* per consumption or exposure event (# day^{-1}) with or without washing, D_r or Dw_r is the dose of *Rotavirus* per consumption or exposure event (# day^{-1}) with or without washing and α and β are the distribution parameters given in Table 6.1.

Annual risk:

The annual probability of pathogenic *E. coli*, *Campylobacter* and *Rotavirus* ($P_{infe,y}$, $P_{infc,y}$, $P_{infr,y}$) infection, was estimated as:

$$P_{\inf e,y} = 1 - \prod_{k=1}^{d} \left(1 - P_{\inf e,d}\right) \quad (17)$$

$$P_{\inf c,y} = 1 - \prod_{k=1}^{d} \left(1 - P_{\inf c,d}\right) \quad (18)$$

$$P_{\inf r,y} = 1 - \prod_{k=1}^{d} \left(1 - P_{\inf r,d}\right) \quad (19)$$

where $P_{infe,d}$ is the daily probability of pathogenic *E. coli* infection (with or without washing), $P_{infc,d}$ is the daily probability of *Campylobacter* infection (with or without washing), $P_{infr,d}$ is the daily probability of *Rotavirus* infection (with or without washing) and d is the number of exposure events per year (i.e. 365 days) from k = 1 to 365.

Probability or Risk of annual diarrheal disease illness due to pathogenic *E. coli*, *Campylobacter* and *Rotavirus* (P_{ille}, P_{illc}, P_{illr}) was estimated as:

$$P_{ille} = P_{infe,y} P_{ill/infe} \quad (20)$$

$$P_{illc} = P_{infc,y} P_{ill/infc} \quad (21)$$

$$P_{illr} = P_{infr,y} P_{ill/infr} \quad (22)$$

where $P_{infe,y}$, $P_{infc,y}$ and $P_{infr,y}$ are the annual probability of pathogenic *E. coli*, *Campylobacter* and *Rotavirus* infection, respectively (with or without washing vegetables prior consumption), and $P_{ill/infe}$, $P_{ill/infc}$ and $P_{ill/infr}$ are the probability of diarrheal disease illness per pathogenic *E. coli*, *Campylobacter* or *Rotavirus* infection as shown in Table 6.1.

Annual disease burden:

The annual disease burden from these three pathogens (D_e, D_c, D_r) was represented using DALYs as shown in Table 6.1 and the annual disease burdens (DALYs pppy) were estimated as:

$$D_e = B_e P_{ille} \quad (23)$$

$$D_c = B_c P_{illc} \quad (24)$$

$$D_r = B_r P_{illr} \quad (25)$$

where P_{ille}, P_{illc} and P_{illr} are the probability or risk of annual diarrheal disease illness due to pathogenic *E. coli*, *Campylobacter* and *Rotavirus* respectively, and B_e, B_c, B_r are the disease burdens (DALYs per case of illness) in Table 6.1.

The health risks were categorised as medium, low risk, etc based on the annual disease burdens. If the risks were of order 10^{-3}, 10^{-4} and 10^{-5} DALYs then they were categorised as high, medium and low risks, respectively. And if they were of the order of 10^{-6} DALYs then the risks were considered almost equal to the health target of 10^{-6} DALYs pppy. Risks in the order of 10^{-7} DALYs and less, were categorised as below the health target (HT).

Combined risk:

The combined risk (P_{ill_C}) is a total estimated probability of gastrointestinal illness (Schoen and Ashbolt, 2010; USEPA, 2010; Barker et al., 2014). It is the probability of obtaining at least one illness from any of the three pathogens. The combined risk of illness from all three pathogens was determined as,

$$P_{ill\_C} = 1 - \prod_{k=1}^{d} \left(1 - P_{ill\_k}\right) \quad (26)$$

where k is an individual pathogen (pathogenic *E. coli*, *Campylobacter* and *Rotavirus*) and P_{ill_k} is the probability of illness for each pathogen.

Thirty different scenarios to estimate the risk per exposure event were constructed. Monte-Carlo simulations with 1,000,000 trial runs were conducted to estimate the health risk and annual disease burden in the form of DALYs. All the modelling and analysis were performed in MATLAB version R2015a (8.5.0 from Mathworks®) and Microsoft Excel 2016. For all of the model outputs, the risks and annual disease burdens were computed with their mean values. A health target of 10^{-6} DALY pppy (WHO, 2004; Barker et al., 2013; George et al., 2015) was considered for the annual disease burden.

6.3 Results and Discussion

The pathogenic risk assessment was estimated using the QMRA approach in terms of the annual disease burden (DALYs) and probabilities of illness (also, combined GI risk). The human health risks were presented in Tables 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 6.10, 6.11 and 6.12. The tables show the mean of the risk of annual diarrheal illness ($year^{-1}$), combined GI risk due to all three pathogens, and annual disease burden (DALYs pppy). Annual disease burden estimates were highly variable across all the scenarios for the three pathogens considered.

Effect of sprinkler and drip irrigation on the consumption of five types of fresh vegetables

In Tables 6.3, 6.4, 6.5, 6.6 and 6.7, the risk assessment for broccoli, cauliflower, zucchini, lettuce and tomatoes irrigated with the sprinkler method (with or without washing) are presented. Similarly, in Tables 6.8, 6.9, 6.10, 6.11 and 6.12, the risk assessment for vegetables irrigated using the drip irrigation method (with or without washing) are presented.

6.3.1 Effect with or without washing

In the Tables 6.3, 6.4, 6.5, 6.6 and 6.7, the estimates for the annual disease burden were found to be in the range of 10^{-6}-10^{-3} DALYs for fresh vegetables not washed prior to consumption, followed by vegetables washed for 3-4 sec (range of 10^{-7}-10^{-4} DALYs) and finally for vegetables washed for 2 min (range of 10^{-8}-10^{-5} DALYs).

Similarly, in the Tables 6.8, 6.9, 6.10, 6.11 and 6.12, the estimates for the annual disease burden were found in the range of 10^{-7}-10^{-4} DALYs for fresh vegetables not washed prior to consumption, then vegetables washed for 3-4 sec (range of 10^{-8}-10^{-5} DALYs) and lastly for vegetables washed for 2 min (range of 10^{-8}-10^{-5} DALYs). In summary, to reduce the disease burden to an acceptable health target, it is recommended to wash the vegetables for 2 min rather than 3-4 sec because it results in a 1.4 log reduction in fecal coliform concentration (Amoah et al., 2007). Vegetables, when drip irrigated are safe to be used after washing for 2 min prior to consumption as there was no risk observed after washing the drip irrigated vegetables such as broccoli, cauliflower and zucchini.

6.3.2 Effect of pathogens studied

In the Tables 6.3, 6.4, 6.5, 6.6 and 6.7, it was observed that among all the scenarios, irrespective of washing and the type of vegetables irrigated, *Rotavirus* exhibited the least risk (i.e. in the range of 10^{-8}-10^{-6} DALYs) followed by *Campylobacter* (10^{-6}-10^{-4} DALYs) and then by pathogenic *E. coli* (10^{-5}-10^{-3} DALYs). Similar findings were observed by George et al., (2015) for drinking water in India. As well, Machdar (2010) reported a higher risk of pathogenic *E. coli* in drinking water in Ghana.

Supportively, pathogenic *E. coli* also showed a higher risk in the scenarios with drip irrigation. Among all of the five scenarios in the Tables 6.8, 6.9, 6.10, 6.11 and 6.12, *Rotavirus* (range of 10^{-8}-10^{-6} DALYs) and *Campylobacter* (range of 10^{-7}-10^{-5} DALYs) had a lower range to the pathogenic *E. coli* (range of 10^{-6}-10^{-4} DALYs). Therefore, the risks of *Rotavirus* and *Campylobacter* were approximately equal to the health target (HT) or below HT in the case of drip irrigated vegetables. There could be a risk of pathogenic *E. coli* resulting from the consumption of non-washed and washed vegetables. Vegetables that have been washed for 2 min are safe to be consumed as most of the pathogenic risks were found to be below or approximately equal to the HT.

6.3.3 Effect through consumption of different vegetables

In the Tables 6.3, 6.4, 6.5, 6.6 and 6.7, the maximum risk was observed when consuming lettuce (3.6 $X10^{-7}$ to 2.1 $X10^{-3}$ DALYs), followed by tomatoes (3.2 $X10^{-7}$ to 1.9 $X10^{-3}$ DALYs), squash (zucchini) (1.3 $X10^{-7}$ to 7.6 $X10^{-4}$ DALYs), cauliflower (1 $X10^{-7}$ to 6.1 $X10^{-4}$ DALYs) and lastly broccoli (9.8 $X10^{-8}$ to 5.6 $X10^{-4}$ DALYs). However, the risks to fresh broccoli and cauliflower were roughly similar across all scenarios. Lettuce,

however, had the highest risk compared to all the vegetables studied and across all scenarios. Similarly, Hamilton et al. (2006) found that the annual risk of infection was higher than the benchmark of 10^{-4} (Macler et al., 1993; USEPA 1989) for all the vegetables studied (cabbage, lettuce), except in the case of broccoli and cucumber for all water qualities given a 14-day with-holding period.

A similar trend was noticed in Tables 6.8, 6.9, 6.10, 6.11 and 6.12. The maximum risk was observed with the consumption of lettuce (6.5 $X10^{-8}$ to 3.9 $X10^{-4}$ DALYs), followed by tomatoes (5.9 $X10^{-8}$ to 3.5 $X10^{-4}$ DALYs), squash (zucchini) which was in the range of 2.3 $X10^{-8}$ to 1.4 $X10^{-4}$ DALYs, followed by cauliflower (1.8 $X10^{-8}$ to 1.1 $X10^{-4}$ DALYs) and broccoli (1.8 $X10^{-8}$ to 1.1 $X10^{-4}$ DALYs). Risks associated with the consumption of lettuce and tomatoes was higher than the consumption of squash, cauliflower and broccoli (Agriculture and Agri-food Canada Statistics (http://www.agr.gc.ca)). The risks posed by the consumption of these vegetables can vary in different regions of the world.

6.3.4 Effect of combined GI risk due to washing scenarios

Of all the five scenarios in Tables 6.3, 6.4, 6.5, 6.6 and 6.7, the maximum combined GI risk was found when vegetables were not washed (1.3 $X10^{-3}$ to 5.1 $X10^{-3}$), followed by vegetables washed for 3-4 sec (1.3 $X10^{-4}$ to 5.1 $X10^{-4}$) and the least risk was found after washing vegetables for 2 min prior to consumption (5.5 $X10^{-5}$ to 1.9 $X10^{-4}$). When sprinkler irrigated, vegetables should be washed for at least 2 min prior to consumption to reduce the risk.

A similar trend was observed in all five scenarios (no washing, washing for 3-4 sec and washing for 2 min) in Tables 6.8, 6.9, 6.10, 6.11 and 6.12. The maximum combined GI risk was observed when vegetables were not washed (2.6 $X10^{-4}$ to 9.5 $X10^{-4}$), followed by vegetables washed for 3-4 sec (2.6 $X10^{-5}$ to 9.5 $X10^{-5}$) and the least combined GI risk results after washing for 2 min prior to consumption (1 $X10^{-5}$ to 3.6 $X10^{-5}$).

6.3.4 Comparison of drip and sprinkler irrigation

Drip irrigation showed less annual disease burden estimates (1.8 $X10^{-8}$ to 3.9 $X10^{-4}$ DALYs) compared to sprinkler irrigation (9.8 $X10^{-8}$ to 2.1 $X10^{-3}$ DALYs). Similarly, drip irrigation showed less combined GI risk (1 $X10^{-5}$ to 9.5 $X10^{-4}$) than sprinkler irrigation (5.5 $X10^{-5}$ to 5.1 $X10^{-3}$), for all the vegetables and for all the washing scenarios. Therefore, drip irrigation is recommended over sprinkler irrigation as a better means of reducing the health risks (Hamilton et al., 2006). Also, washing of the vegetables for 2 min prior to consumption is highly recommended in order to reduce the bacteria and viruses from fresh vegetables.

To the best of our knowledge, this is the first study using the QMRA model to determine the estimated risk on vegetables when considering different scenarios based on irrigation methods and three different washing conditions (no washing, washing for 3-4 sec or washing for 2 min).

6.4 Conclusion

The QMRA model allowed for the testing of different scenarios to estimate pathogenic risks. Drip irrigation showed less risk (1.8 $X10^{-8}$ to 3.9 $X10^{-4}$ DALYs) than sprinkler

irrigation (9.8 X10^{-8} to 2.1 X10^{-3} DALYs) across all scenarios. Drip irrigation is safer to use than sprinkler irrigation as the volume of water retained on the vegetables is less in the case of drip than in sprinkler irrigation. Washing fresh vegetables for 2 min prior to consumption showed the least risk (mostly in the range of 10^{-8}-10^{-5} DALYs, compared to washing vegetables for 3-4 sec (range of 10^{-8}-10^{-4} DALYs) and no washing (10^{-7}-10^{-3} DALYs). Therefore, drip irrigation and washing vegetables for 2 min is highly recommended as the way to reduce health risks. *Rotavirus* showed the least or no risk (range of 10^{-8}-10^{-6} DALYs) compared to *Campylobacter* (10^{-6}-10^{-4} DALYs) and pathogenic *E. coli* (10^{-5}-10^{-3} DALYs) during sprinkler irrigation. During drip irrigation, pathogens such as *Rotavirus* (10^{-8}-10^{-6} DALYs) and *Campylobacter* (10^{-7}-10^{-5} DALYs) showed very low risk, compared to pathogenic *E. coli* (10^{-6}-10^{-4} DALYs). Among the five vegetables, lettuce showed the highest risk. The combined GI risk showed a similar trend as the annual disease burden, across all the scenarios.

Therefore, there could be high risks in developing countries where wastewater is used for irrigating vegetables that are eaten fresh. Gastrointestinal risks can be reduced if the quality of the water is analyzed, and only pathogen free water is used for irrigating the crops.

Table 6.1: Summary of model input parameters and equations for calculating annual disease burden

Variable	Description	Units	Equations and/or values	Reference
C	Consumption of broccoli, cauliflower and squash (zucchini), lettuce and tomatoes	kg person^{-1} year^{-1}	Broccoli (2.58), Cauliflower (2.67), squash (3.35), lettuce (9.46), tomatoes (8.55)	Agriculture and Agri-food Canada Statistics
V	Volume of water retained on fruits and vegetables through on-surface drip irrigation	ml g^{-1}	0.02	(Van Ginneken and Oron 2000)
V	Volume of water retained on vegetables through sprinkler irrigation	ml g^{-1}	0.11	(Shuval 1997)
Ce	Concentration of pathogenic *E. coli*		8%	(Haas et al., 1999; Howard et al., 2006; George et al., 2015)
Cc	Concentration of *Campylobacter*		6.60%	(Smeets 2008; Machdar et al., 2013; George et al., 2015)
Cr	Concentration of *Rotavirus*		0.001%	(Mara et al., 2007; Machdar et al., 2013; George et al., 2015)
α and β for pathogenic *E. coli*	Fit parameters for beta-Poisson equation		α = 0.05 and β = 1.001	(Teunis et al., 2004; Haas et al., 1999; George et al., 2015)
α and β for *Campylobacter*	Fit parameters for beta-Poisson equation		α = 0.145 and β = 7.59	(Medema et al., 1996)
α and β for *Rotavirus*	Fit parameters for beta-Poisson equation		α = 0.167 and β = 0.191	(Teunis and Havelaar, 2000; Barker et al., 2014)
Pill/infe	Risk of diarrheal illness given pathogenic *E. coli* infection	Probability of illness per infection	0.25	(George et al., 2015)
Pill/infc	Risk of diarrheal illness given *Campylobacter* infection	Probability of illness per infection	0.3	(George et al., 2015)
Pill/infr	Risk of diarrheal illness given *Rotavirus* infection	Probability of illness per infection	0.5	(George et al., 2015)
Bbe	Disease burden for pathogenic *E. coli*	DALY per case	0.56	Calculated in Table 6.2
Bbc	Disease burden for *Campylobacter*	DALY per case	0.08	Calculated in Table 6.2
Bbr	Disease burden for *Rotavirus*	DALY per case	0.56	Calculated in Table 6.2
Run 1,000,000 Monte-Carlo simulations				
Assumption of concentration of *E. coli*: normal distributed				

Table 6.2: Disease burden for pathogens (in this study) causing gastroenteritis

Pathogen	**Outcome**	**Severity**	**Duration (in years)**	**Disease burden per case (in DALYs)***
Pathogenic *E. coli*	Water diarrhea (53%)	0.07	0.01	0
	Bloody diarrhea (47%)	0.39	0.02	0
	Death from diarrhea (0.7%)	1	80.1	0.56
	Total diarrhea			0.56
Campylobacter	Gastroenteritis population (94%)	0.07	0.01	0
	Gastroenteritis-general practitioners (6%)	0.39	0.03	0
	Death from gastroenteritis	1	80.1	0.08
	Total gastroenteritis			0.08
Rotavirus	Mild diarrhea (85%)	0.1	0.02	0
	Severe diarrhea (14.4%)	0.23	0.02	0
	Death from diarrhea	1	80.1	0.56
	Total diarrhea			0.56
*DALYs =Number of symptomatic cases*severity*duration in years (George et al., 2015)				

Table 6.3: Risk assessment from consumption of Broccoli, irrigated with sprinkler

Time period	**Pathogens**	**Risk of illness (year^{-1})**	**persons in 100000**	**Combined GI risk**	**combined GI risk (persons in 100000)**	**Annual disease burden (DALYs pppy)**	**Number of hours lost (health target 10^{-6} DALYs = 0.0087 h)**	**Risk**
Sprinkler irrigation, no washing of vegetables								
Growing season (May-October)	Pathogenic *E. coli*	9.9×10^{-4}	99	1.3×10^{-3}	130	5.6×10^{-4}	4.906	Medium risk
	Campylobacter	3.9×10^{-4}	39			3.2×10^{-5}	0.280	Low risk
	Rotavirus	4.6×10^{-6}	0.46			2.6×10^{-6}	0.023	≈ health target (HT) of 10^{-6}
Sprinkler irrigation, washing of vegetables with cold water for 3-4 sec dipping i.e. 1 log reduction (Amoah et al., 2007)								
May-October	Pathogenic *E. coli*	9.9×10^{-5}	9.9	1.3×10^{-4}	13	5.6×10^{-5}	0.491	Low risk
	Campylobacter	3.9×10^{-5}	3.9			3.2×10^{-6}	0.028	≈ health target (HT) of 10^{-6}
	Rotavirus	4.6×10^{-7}	0.046			2.6×10^{-7}	0.002	Below HT
Sprinkler irrigation, washing of vegetables with cold water for 2 min dipping i.e. 1.4 log reduction (Amoah et al., 2007)								
May-October	Pathogenic *E. coli*	4×10^{-5}	4	5.5×10^{-5}	5.5	2.2×10^{-5}	0.193	Low risk
	Campylobacter	1.5×10^{-5}	1.5			1.2×10^{-6}	0.011	≈ health target (HT) of 10^{-6}
	Rotavirus	1.8×10^{-7}	0.018			9.8×10^{-8}	0.001	Below HT

Table 6.4: Risk assessment from consumption of Cauliflower, irrigated with sprinkler

Time period	**Pathogens**	**Risk of illness ($year^{-1}$)**	**persons in 100000**	**Combined GI risk**	**combined GI risk (persons in 100000)**	**Annual disease burden (DALYs pppy)**	**Number of hours lost (health target 10^{-6} DALYs = 0.0087 h)**	**Risk**
Sprinkler irrigation, no washing of vegetables								
Growing Season (May-October)	Pathogenic *E. coli*	1.1×10^{-3}	110	1.5×10^{-3}	150	6.1×10^{-4}	5.344	Medium risk
	Campylobacter	4.1×10^{-4}	41			3.3×10^{-5}	0.289	Low risk
	Rotavirus	4.8×10^{-6}	0.48			2.7×10^{-6}	0.024	≈ health target (HT) of 10^{-6}
Sprinkler irrigation, washing of vegetables with cold water for 3-4 sec dipping i.e. 1 log reduction (Amoah et al., 2007)								
May-October	Pathogenic *E. coli*	1.1×10^{-4}	11	1.5×10^{-4}	15	6.1×10^{-5}	0.534	Low risk
	Campylobacter	4.1×10^{-5}	4.1			3.3×10^{-6}	0.029	≈ health target (HT) of 10^{-6}
	Rotavirus	4.8×10^{-7}	0.048			2.7×10^{-7}	0.002	Below HT
Sprinkler irrigation, washing of vegetables with cold water for 2 min dipping i.e. 1.4 log reduction (Amoah et al., 2007)								
May-October	Pathogenic *E. coli*	4.1×10^{-5}	4.1	5.7×10^{-5}	5.7	2.3×10^{-5}	0.201	Low risk
	Campylobacter	1.6×10^{-5}	1.6			1.3×10^{-6}	0.011	≈ health target (HT) of 10^{-6}
	Rotavirus	1.8×10^{-7}	0.018			1×10^{-7}	0.001	Below HT

Table 6.5: Risk assessment from consumption of Squash (Zucchini), irrigated with sprinkler

Time period	**Pathogens**	**Risk of illness (year^{-1})**	**persons in 100000**	**Combined GI risk**	**combined GI risk (persons in 100000)**	**Annual disease burden (DALYs pppy)**	**Number of hours lost (health target 10^{-6} DALYs = 0.0087 h)**	**Risk**
Sprinkler irrigation, no washing of vegetables								
Growing season (May-October)	Pathogenic *E. coli*	1.3×10^{-3}	130	1.8×10^{-3}	180	7.6×10^{-4}	6.658	Medium risk
	Campylobacter	5.1×10^{-4}	51			4.1×10^{-5}	0.359	Low risk
	Rotavirus	5.9×10^{-6}	0.59			3.3×10^{-6}	0.029	≈ health target (HT) of 10^{-6}
Sprinkler irrigation, washing of vegetables with cold water for 3-4 sec dipping i.e. 1 log reduction (Amoah et al., 2007)								
May-October	Pathogenic *E. coli*	1.3×10^{-4}	13	1.8×10^{-4}	18	7.6×10^{-5}	0.666	Low risk
	Campylobacter	5.1×10^{-5}	5.1			4.1×10^{-6}	0.036	≈ health target (HT) of 10^{-6}
	Rotavirus	5.9×10^{-7}	0.059			3.3×10^{-7}	0.003	Below HT
Sprinkler irrigation, washing of vegetables with cold water for 2 min dipping i.e. 1.4 log reduction (Amoah et al., 2007)								
May-October	Pathogenic *E. coli*	5.1×10^{-5}	5.1	7×10^{-5}	7	2.9×10^{-5}	0.254	Low risk
	Campylobacter	1.9×10^{-5}	1.9			1.6×10^{-6}	0.014	≈ health target (HT) of 10^{-6}
	Rotavirus	2.2×10^{-7}	0.022			1.3×10^{-7}	0.001	Below HT

Table 6.6: Risk assessment from consumption of lettuce, irrigated with sprinkler

Time period	**Pathogens**	**Risk of illness ($year^{-1}$)**	**persons in 100000**	**Combined GI risk**	**combined GI risk (persons in 100000)**	**Annual disease burden (DALYs pppy)**	**Number of hours lost (health target 10^{-6} DALYs = 0.0087 h)**	**Risk**
Sprinkler irrigation, no washing of vegetables								
Growing season (May-October)	Pathogenic *E. coli*	3.7×10^{-3}	370	5.1×10^{-3}	510	2.1×10^{-3}	18.396	High risk
	Campylobacter	1.4×10^{-3}	140			1.1×10^{-4}	0.964	Medium risk
	Rotavirus	1.7×10^{-5}	1.7			9.5×10^{-6}	0.083	≈ health target (HT) of 10^{-6}
Sprinkler irrigation, washing of vegetables with cold water for 3-4 sec dipping i.e. 1 log reduction (Amoah et al., 2007)								
May-October	Pathogenic *E. coli*	3.7×10^{-4}	37	5.1×10^{-4}	51	2.1×10^{-4}	1.840	Medium risk
	Campylobacter	1.4×10^{-4}	14			1.1×10^{-5}	0.096	Low risk
	Rotavirus	1.7×10^{-6}	0.17			9.5×10^{-7}	0.008	Below HT
Sprinkler irrigation, washing of vegetables with cold water for 2 min dipping i.e. 1.4 log reduction (Amoah et al., 2007)								
May-October	Pathogenic *E. coli*	1.4×10^{-4}	14	1.9×10^{-4}	19	8.1×10^{-5}	0.710	Low risk
	Campylobacter	5.5×10^{-5}	5.5			4.4×10^{-6}	0.039	≈ health target (HT) of 10^{-6}
	Rotavirus	6.4×10^{-7}	0.064			3.6×10^{-7}	0.003	Below HT

Table 6.7: Risk assessment from consumption of tomatoes, irrigated with sprinkler

Time period	**Pathogens**	**Risk of illness (year^{-1})**	**persons in 100000**	**Combined GI risk**	**combined GI risk (persons in 100000)**	**Annual disease burden (DALYs pppy)**	**Number of hours lost (health target 10^{-6} DALYs = 0.0087 h)**	**Risk**
Sprinkler irrigation, no washing of vegetables								
Growing season (May-October)	Pathogenic *E. coli*	3.4×10^{-3}	340	4.7×10^{-3}	470	1.9×10^{-3}	16.644	High risk
	Campylobacter	1.3×10^{-3}	130			1×10^{-4}	0.876	Medium risk
	Rotavirus	1.5×10^{-5}	1.5			8.5×10^{-6}	0.074	≈ health target (HT) of 10^{-6}
Sprinkler irrigation, washing of vegetables with cold water for 3-4 sec dipping i.e. 1 log reduction (Amoah et al., 2007)								
May-October	Pathogenic *E. coli*	3.4×10^{-4}	34	4.7×10^{-4}	47	1.9×10^{-4}	1.664	Medium risk
	Campylobacter	1.3×10^{-4}	13			1×10^{-5}	0.088	Low risk
	Rotavirus	1.5×10^{-6}	0.15			8.5×10^{-7}	0.007	Below HT
Sprinkler irrigation, washing of vegetables with cold water for 2 min dipping i.e. 1.4 log reduction (Amoah et al., 2007)								
May-October	Pathogenic *E. coli*	1.3×10^{-4}	13	1.8×10^{-4}	18	7.3×10^{-5}	0.639	Low risk
	Campylobacter	5×10^{-5}	5			4×10^{-6}	0.035	≈ health target (HT) of 10^{-6}
	Rotavirus	5.7×10^{-7}	0.057			3.2×10^{-7}	0.003	Below HT

Table 6.8: Risk assessment from consumption of Broccoli, irrigated through drip

Time period	**Pathogens**	**Risk of illness ($year^{-1}$)**	**persons in 100000**	**Combined GI risk**	**combined GI risk (persons in 100000)**	**Annual disease burden (DALYs pppy)**	**Number of hours lost (health target 10^{-6} DALYs = 0.0087 h)**	**Risk**
Drip irrigation, no washing of vegetables								
Growing season (May-October)	Pathogenic *E. coli*	1.9×10^{-4}	19	2.6×10^{-4}	26	1.1×10^{-4}	0.964	Medium risk
	Campylobacter	7.2×10^{-5}	7.2			5.9×10^{-6}	0.052	≈ health target (HT) of 10^{-6}
	Rotavirus	8.4×10^{-7}	0.084			4.7×10^{-7}	0.004	Below HT
Drip irrigation, washing of vegetables with cold water for 3-4 sec dipping i.e. 1 log reduction (Amoah et al., 2007)								
May-October	Pathogenic *E. coli*	1.9×10^{-5}	1.9	2.6×10^{-5}	2.6	1.1×10^{-5}	0.096	Low risk
	Campylobacter	7.2×10^{-6}	0.72			5.9×10^{-7}	0.005	Below HT
	Rotavirus	8.4×10^{-8}	0.0084			4.7×10^{-8}	0.0004	Below HT
Drip irrigation, washing of vegetables with cold water for 2 min dipping i.e. 1.4 log reduction (Amoah et al., 2007)								
May-October	Pathogenic *E. coli*	7.3×10^{-6}	0.73	1×10^{-5}	1	4.1×10^{-6}	0.036	≈ health target (HT) of 10^{-6}
	Campylobacter	2.7×10^{-6}	0.27			2.2×10^{-7}	0.002	Below HT
	Rotavirus	3.2×10^{-8}	0.0032			1.8×10^{-8}	0.0002	Below HT

Table 6.9: Risk assessment from consumption of Cauliflower, irrigated through drip

Time period	**Pathogens**	**Risk of illness ($year^{-1}$)**	**persons in 100000**	**Combined GI risk**	**combined GI risk (persons in 100000)**	**Annual disease burden (DALYs pppy)**	**Number of hours lost (health target 10^{-6} DALYs = 0.0087 h)**	**Risk**
Drip irrigation, no washing of vegetables								
Growing season (May-October)	Pathogenic *E. coli*	1.9×10^{-4}	19	2.6×10^{-4}	26	1.1×10^{-4}	0.964	Medium risk
	Campylobacter	7.5×10^{-5}	7.5			6.1×10^{-6}	0.053	≈ health target (HT) of 10^{-6}
	Rotavirus	8.6×10^{-7}	0.086			4.8×10^{-7}	0.004	Below HT
Drip irrigation, washing of vegetables with cold water for 3-4 sec dipping i.e. 1 log reduction (Amoah et al., 2007)								
May-October	Pathogenic *E. coli*	1.9×10^{-5}	1.9	2.6×10^{-5}	2.6	1.1×10^{-5}	0.096	Low risk
	Campylobacter	7.5×10^{-6}	0.75			6.1×10^{-7}	0.005	Below HT
	Rotavirus	8.6×10^{-8}	0.0086			4.8×10^{-8}	0.0004	Below HT
Drip irrigation, washing of vegetables with cold water for 2 min dipping i.e. 1.4 log reduction (Amoah et al., 2007)								
May-October	Pathogenic *E. coli*	7.5×10^{-6}	0.75	1×10^{-5}	1	4.2×10^{-6}	0.037	≈ health target (HT) of 10^{-6}
	Campylobacter	2.8×10^{-6}	0.28			2.3×10^{-7}	0.002	Below HT
	Rotavirus	3.3×10^{-8}	0.0033			1.8×10^{-8}	0.0002	Below HT

Table 6.10: Risk assessment from consumption of Squash (Zucchini), irrigated through drip

Time period	**Pathogens**	**Risk of illness (year^{-1})**	**persons in 100000**	**Combined GI risk**	**combined GI risk (persons in 100000)**	**Annual disease burden (DALYs pppy)**	**Number of hours lost (health target 10^{-6} DALYs = 0.0087 h)**	**Risk**
Drip irrigation, no washing of vegetables								
Growing season (May-October)	Pathogenic *E. coli*	2.5×10^{-4}	25	3.4×10^{-4}	34	1.4×10^{-4}	1.226	Medium risk
	Campylobacter	9.3×10^{-5}	9.3			7.6×10^{-6}	0.067	≈ health target (HT) of 10^{-6}
	Rotavirus	1.1×10^{-6}	0.11			6.1×10^{-7}	0.005	Below HT
Drip irrigation, washing of vegetables with cold water for 3-4 sec dipping i.e. 1 log reduction (Amoah et al., 2007)								
May-October	Pathogenic *E. coli*	2.5×10^{-5}	2.5	3.4×10^{-5}	3.4	1.4×10^{-5}	0.123	Low risk
	Campylobacter	9.3×10^{-6}	0.93			7.6×10^{-7}	0.007	Below HT
	Rotavirus	1.1×10^{-7}	0.011			6.1×10^{-8}	0.001	Below HT
Drip irrigation, washing of vegetables with cold water for 2 min dipping i.e. 1.4 log reduction (Amoah et al., 2007)								
May-October	Pathogenic *E. coli*	9.4×10^{-6}	0.94	1.3×10^{-5}	1.3	5.3×10^{-6}	0.046	≈ health target (HT) of 10^{-6}
	Campylobacter	3.5×10^{-6}	0.35			2.9×10^{-7}	0.003	Below HT
	Rotavirus	4.1×10^{-8}	0.0041			2.3×10^{-8}	0.0002	Below HT

Table 6.11: Risk assessment from consumption of lettuce, irrigated through drip

Time period	**Pathogens**	**Risk of illness (year^{-1})**	**persons in 100000**	**Combined GI risk**	**combined GI risk (persons in 100000)**	**Annual disease burden (DALYs pppy)**	**Number of hours lost (health target 10^{-6} DALYs = 0.0087 h)**	**Risk**
Drip irrigation, no washing of vegetables								
Growing season (May-October)	Pathogenic *E. coli*	6.9×10^{-4}	69	9.5×10^{-4}	95	3.9×10^{-4}	3.416	Medium risk
	Campylobacter	2.6×10^{-4}	26			2.1×10^{-5}	0.184	Low risk
	Rotavirus	3.1×10^{-6}	0.31			1.7×10^{-6}	0.015	≈ health target (HT) of 10^{-6}
Drip irrigation, washing of vegetables with cold water for 3-4 sec dipping i.e. 1 log reduction (Amoah et al., 2007)								
May-October	Pathogenic *E. coli*	6.9×10^{-5}	6.9	9.5×10^{-5}	9.5	3.9×10^{-5}	0.342	Low risk
	Campylobacter	2.6×10^{-5}	2.6			2.1×10^{-6}	0.018	≈ health target (HT) of 10^{-6}
	Rotavirus	3.1×10^{-7}	0.031			1.7×10^{-7}	0.001	Below HT
Drip irrigation, washing of vegetables with cold water for 2 min dipping i.e. 1.4 log reduction (Amoah et al., 2007)								
May-October	Pathogenic *E. coli*	2.6×10^{-5}	2.6	3.6×10^{-5}	3.6	1.5×10^{-5}	0.131	Low risk
	Campylobacter	1×10^{-5}	1			8.1×10^{-7}	0.007	Below HT
	Rotavirus	1.1×10^{-7}	0.011			6.5×10^{-8}	0.001	Below HT

Table 6.12: Risk assessment from consumption of tomatoes, irrigated through drip

Time period	**Pathogens**	**Risk of illness (year^{-1})**	**persons in 100000**	**Combined GI risk**	**combined GI risk (persons in 100000)**	**Annual disease burden (DALYs pppy)**	**Number of hours lost (health target 10^{-6} DALYs = 0.0087 h)**	**Risk**
Drip irrigation, no washing of vegetables								
Growing season (May-October)	Pathogenic *E. coli*	6.3×10^{-4}	63	8.7×10^{-4}	87	3.5×10^{-4}	3.066	Medium risk
	Campylobacter	2.4×10^{-4}	24			1.9×10^{-5}	0.166	Low risk
	Rotavirus	2.7×10^{-6}	0.27			1.5×10^{-6}	0.013	≈ health target (HT) of 10^{-6}
Drip irrigation, washing of vegetables with cold water for 3-4 sec dipping i.e. 1 log reduction (Amoah et al., 2007)								
May-October	Pathogenic *E. coli*	6.3×10^{-5}	6.3	8.7×10^{-5}	8.7	3.5×10^{-5}	0.307	Low risk
	Campylobacter	2.4×10^{-5}	2.4			1.9×10^{-6}	0.017	≈ health target (HT) of 10^{-6}
	Rotavirus	2.7×10^{-7}	0.027			1.5×10^{-7}	0.001	Below HT
Drip irrigation, washing of vegetables with cold water for 2 min dipping i.e. 1.4 log reduction (Amoah et al., 2007)								
May-October	Pathogenic *E. coli*	2.4×10^{-5}	2.4	3.3×10^{-5}	3.3	1.3×10^{-5}	0.114	Low risk
	Campylobacter	9×10^{-6}	0.9			7.3×10^{-7}	0.006	Below HT
	Rotavirus	1×10^{-7}	0.01			5.9×10^{-8}	0.001	Below HT

Chapter 7: General Summary and Conclusions

7.1 General Summary

Foodborne illnesses and outbreaks due to the consumption of fresh fruits and vegetables, irrigated with untreated water, was studied in this project. The study found that irrigation water is a source and carrier of pathogens to vegetables and fruits that are eaten fresh. In most countries, the water used for irrigation is untreated and not tested prior to its use in the field. Therefore, irrigation water quality and its' microbiological analysis are important considerations when attempting to reduce and to mitigate water-related foodborne illnesses. Contaminated soil and field irrigation practices are other major factors to consider as sources of contamination of fresh fruits and vegetables. Greenhouse and field studies were conducted, and the Quantitative Microbial Risk Assessment (QMRA) model used data from these studies to quantify the gastrointestinal risks to humans due to the consumption of fresh vegetables irrigated with untreated water.

The water samples were collected for two years, 2013 and 2014, at St-Remi and Rougemont to quantify the fecal coliforms, predominantly *E. coli*. A greenhouse study was conducted at the Macdonald campus of McGill University to analyze the concentration of *E. coli*. *E. coli* contaminated water was applied using sprinkler and drip irrigation to the vegetables grown on organic and mineral soils. Only one type of organic and mineral soil was considered for the greenhouse experiment because the organic soil replicated the soil type of St-Remi, and the mineral soil replicated the soil type of Rougemont. These two soils used, replicated the soil properties in Quebec context for the greenhouse study. A QMRA study was carried out using data from the field and greenhouse experiments. To validate the results, the QMRA model used

data from the commercial field site, Saint-Esprit. St-Esprit was chosen due to the following reasons: it is a major fresh vegetable producing site; has vegetables such as zucchini, broccoli, cauliflower, and others with large commercial production; and it is in a region with the longest growing season in Quebec.

The summary of results is as follows:

A) Irrigation water samples were tested for the two field sites namely Rougemont and St-Remi in Quebec for two years (2013 and 2014). The quantification of *E. coli* showed that there was exceedance in *E. coli* concentration (i.e. over 2 log cfu/100ml) only during May-June time period for site Rougemont in year 2013.

B) From the greenhouse studies, it was found that irrigation practices and soil type significantly influence the fate and transport of the pathogens in the plants. Lettuce and tomato crops showed the least contamination when treated with drip irrigation and mineral soil (D+M). D+M treatment was the safest treatment to use in order to reduce the effect of foodborne illnesses on crops. Other treatments such as S+O, S+M, D+O showed considerable amounts of contamination on the lettuce leaves, tomato fruits and soil over the 30 day time period. It can be concluded that the plants irrigated using the sprinkler irrigation system have a greater risk of contamination compared to the drip irrigation system. As well, plants grown in organic soil also have a greater chance of contamination compared to mineral soil.

C) The two years of field data were used to run a QMRA model and the potential risk to humans was estimated in the annual disease burden (DALYs pppy) for three pathogens. The annual disease burden was compared to the WHO health target of 10^{-6} DALYs pppy for both the crops (lettuce and tomatoes). In the case of tomatoes, the maximum risk from

all three pathogens was found during May-June for both years. For lettuce, the maximum risk was found during May-June and September-October in years 2013 and 2014. The combined annual disease burden and combined risk for all the pathogens (DALYs pppy) were found to exceed the health target. Therefore, it can be concluded that there was potential risk on the RTE vegetables and fruits if the surface water used for irrigation was untreated.

D) At the greenhouse level, the potential risk to humans was estimated using the QMRA model for the different treatments. In this study, the pathogen of interest was only *E. coli* because it was used to manually contaminate the irrigation water. The manually prepared irrigation water was then used to irrigate lettuce and tomato grown under four different treatments. The study looked at the potential risk to consumers when vegetables are grown under four different treatments, and these are harvested after the 10^{th}, 20^{th} or 30^{th} day of the contaminated water application. In the QMRA model considering irrigation method, sprinkler irrigation resulted in a higher risk than drip irrigation for both crops. The four treatments impacted the exposure of pathogenic *E. coli* on vegetable crops to different degrees. Lettuce was found to be more contaminated with *E. coli*, compared to tomatoes. Among all treatments, the highest risk was found in the Sprinkler+Organic treatment, followed by the Sprinkler+Mineral, Drip+Organic, and Drip+Mineral treatments. Though the risk of contamination was present in the order of 10^{-5}-10^{-2} DALYs in all of the treatments, it never attained the WHO health target of 10^{-6} DALYs.

E) When considering the washing scenarios, the study used data from St Esprit. It was found that drip irrigation showed less risk (1.8×10^{-8} to 3.9×10^{-4} DALYs) than sprinkler irrigation (9.8×10^{-8} to 2.1×10^{-3} DALYs) across all scenarios. Also, washing fresh

vegetables for 2 min prior to consumption showed the least risk (mostly in the range of 10^{-8}-10^{-5} DALYs) compared to washing vegetables for 3-4 sec (range of 10^{-8}-10^{-4} DALYs) and no washing (10^{-7}-10^{-3} DALYs). Therefore, drip irrigation and washing of vegetables for 2 min is highly recommended for reducing health risks. Among all of the vegetables, lettuce showed the highest risk. The combined GI risk showed a similar trend as the annual disease burden, across all of the scenarios.

7.2 Conclusions

The following conclusions were drawn from this research:

Objective I: To study the quality of untreated irrigation water for two years 2013-2014 at two field sites, St-Remi and Rougemont, growing lettuce and tomatoes respectively.

The quality of irrigation water at the two field sites was analysed for *Escherichia coli* (*E. coli*). In the case of the Rougemont (growing tomato), the maximum concentration of *E. coli* was found during the May-June period for both years. There was exceedance in *E. coli* concentration over 2 log cfu/100ml, only during May-June time period for site Rougemont in year 2013. In the case of the St-Remi (growing lettuce), the maximum *E. coli* concentration was found during the May-June and the September-October periods for both years.

Objective II: To analyze the fate and transport over a time period of 30 days, of the bacterial contaminant (*E. coli*) on lettuce and tomatoes, when irrigated by known amounts of *E. coli* contaminated irrigation water. These crops were grown under four different

treatments comprised of two soil types (organic and mineral) and two irrigation methods (drip and sprinkler) in the greenhouse of Macdonald Campus, McGill University.

The application of *E. coli* ATCC8739 contaminated irrigation water resulted in the contamination of lettuce leaves, tomato fruits and soil using different irrigation methods and soil types. Unlike mineral soil, lettuce leaves and tomatoes grown in organic soil, showed more contamination since the organic matter in this soil has the ability to bind with bacteria. In the case of the D+O treatment, it was found that there could be bacterial movement to the edible tissues. Movement of bacteria to leaves might have occurred via vascular tissues, xylem and phloem entering through the root system or via aerosols produced during sprinkler irrigation. Sprinkler irrigation brought contaminants in direct contact with the plants, resulting in high levels of contamination. All four treatments with the different soil types and irrigation methods showed different reactions, with different levels of bacterial retention and contamination. It was found that the D+M treatment did not retain bacteria and can thereby be considered as the best treatment for tomato and lettuce crop production. The drain water did not show any bacterial contamination in all of the treatments. This could be due to the fact that the soil acted as a filter and the bacteria were adsorbed onto the soil particles at deeper soil depths. Despite this, the repeated use of contaminated irrigation water on crops can lead to higher contamination of fruits and vegetables. Therefore, irrigation water, before being applied to crops, must be treated and tested for pathogens. These results can be used by vegetable growers to select the appropriate irrigation method in order to avoid the risk of *E. coli* contamination.

Objective III: To develop the quantitative microbial risk assessment (QMRA) model based on the field and greenhouse studies in order to estimate the potential risk of three

pathogens (pathogenic *E. coli*, *Campylobacter* and *Rotavirus*) in humans due to the consumption of lettuce and tomatoes irrigated with untreated surface water at two field sites.

The untreated surface water had a significant amount of pathogens which caused an annual disease burden in people. The presence of pathogenic *E. coli*, *Campylobacter* and *Rotavirus* exceeded the health target of 10^{-6} DALYs pppy across all the scenarios. The risk of *Rotavirus* can be prevented by the use of an available vaccination. In the case of the Rougemont (Tomato) model, pathogenic *E. coli* showed high risk (in the order of 10^{-3} DALYs), *Campylobacter* showed medium risk (in the order of 10^{-4} DALYs) and *Rotavirus* showed low risk (in the order of 10^{-5} DALYs) for all the time periods. Whereas in the case of the St-Remi (lettuce) model, pathogenic *E. coli* showed higher risk (in the order of 10^{-2} DALYs) for May-June and September-October time periods. The combined annual disease burden for all the pathogens (DALYs pppy) was in the range of 10^{-3} to 10^{-2} for lettuce and tomatoes. Similarly, the combined GI risk was in the range of 10^{-2} to 10^{-1} and 10^{-3} to 10^{-1} for lettuce and tomatoes respectively. Comparison of the two crops showed that lettuce consumption would result in higher risk because ranges are greater compared to tomato consumption. The developed QMRA model can be applied as a systemic approach by the regulatory agencies and government authorities in order to quantify and to avoid the risk of pathogens.

Objective IV: To estimate the annual disease burden in humans consuming contaminated fruits and vegetables grown under four different treatments (comprised of drip or sprinkler irrigation and mineral or organic soil) in greenhouse and harvested after the 10^{th}, 20^{th} or 30^{th} day ofthe inoculation.

The QMRA model for water and crops was used to estimate the pathogenic *E. coli* risk. Pathogenic *E. coli* always exceeded the WHO health target of 10^{-6} DALYs pppy across all of the scenarios. In the water model for the lettuce and tomato crops, it was observed that sprinkler irrigation (6.2×10^{-2} DALYs pppy) resulted in a higher risk than drip irrigation (5.5×10^{-2} DALYs pppy). The type of irrigation (sprinkler and drip irrigation) and the soil type (organic and mineral soil) impacted the level of exposure of pathogenic *E. coli* on crops. The crops model showed a significantly high risk in lettuce for all the treatments and for all of the harvested days except when the Drip+Mineral treatment was used. With the Drip+Mineral treatment, the risk to lettuce was observed only on the 20th day after irrigation. The highest risk on lettuce was observed in the Sprinkler+Organic treatment followed by the Sprinkler+Mineral and the Drip+Organic treatments. The risk decreased from the 10th day to 30th day for all the treatments, due to dilution of the irrigation water with clean water. However, the risk never decreased to the WHO health target level in any of the treatments. The crops model observed a risk in tomatoes only on the 10th day when using the Drip+Organic treatment. The results showed that consumption of fresh lettuce grown under all the treatments, can lead to more infections compared to tomatoes. The irrigation water should be applied using drip rather than the sprinkler irrigation method in order to reduce the risk of contamination. For lettuce, drip irrigation with mineral soil was the best treatment to minimize the gastrointestinal risks. This would provide information to farmers to understand the best possible harvesting time in order to avoid bacterial contamination in the edible part.

Objective V: To estimate the pathogenic risk through consumption of fresh lettuce, tomato, broccoli, cauliflower and squash (zucchini), sprinkler or drip irrigated with untreated

surface water at Saint-Esprit, Quebec. The risk was quantified based on three different scenarios: washing vegetables for 3-4 sec prior to consumption, for 2 min prior to consumption, or not washing at all.

The QMRA model was used to estimate the pathogenic risks of the different scenarios. Drip irrigation showed less risk (1.8×10^{-8} to 3.9×10^{-4} DALYs) than sprinkler irrigation (9.8×10^{-8} to 2.1×10^{-3} DALYs) across all scenarios. Drip irrigation is safer than sprinkler irrigation as the volume of water retained on the vegetables is less in drip than sprinkler irrigation. The washing of fresh vegetables for 2 min prior to consumption showed the least risk (mostly in the range of 10^{-8}-10^{-5} DALYs, compared to washing vegetables for 3-4 sec (range of 10^{-8}-10^{-4} DALYs) and no washing (10^{-7}-10^{-3} DALYs). Therefore, drip irrigation and washing vegetables for 2 min are highly recommended as ways to reduce health risks. *Rotavirus* showed the least or no risk (range of 10^{-8}-10^{-6} DALYs) compared to *Campylobacter* (10^{-6}-10^{-4} DALYs) and pathogenic *E. coli* (10^{-5}-10^{-3} DALYs) during sprinkler irrigation. During drip irrigation, pathogens such as *Rotavirus* (10^{-8}-10^{-6} DALYs) and *Campylobacter* (10^{-7}-10^{-5} DALYs) showed very low risk, compared to pathogenic *E. coli* (10^{-6}-10^{-4} DALYs). Among the vegetables, lettuce showed the highest risk. The combined GI risk showed a trend similar to the annual disease burden, across all the scenarios.

7.3 Contributions to Knowledge

The work presented in this thesis generates important information on the potential risks that are associated with the consumption of vegetables irrigated with untreated water and grown under

two soil types and two irrigation methods. This research has made the following contributions to knowledge:

1. This research, for the first time, performed a comparative analysis of the fate and transport of *E. coli* bacteria using four different treatments i.e. comprised of two irrigation methods and two soil types, for lettuce and tomato production. Foodborne illnesses are best controlled with drip irrigation on a mineral soil.

2. This research quantifies the pathogenic risk in water and on vegetables harvested at different times. This research for the first time, has shown that harvesting of tomatoes after the 10^{th} day had no *E. coli* contamination and thereby tomatoes are safe to be consumed.

3. This study for the first time, has come up with the knowledge that washing of vegetables for 2 min prior to consumption, would be safest way to eliminate the *Campylobacter* and *Rotavirus* infections.

7.4 Recommendation for future research

1. **To improve the Quantitative Microbial Risk Assessment (QMRA) model by including variables and parameters on age and gender**

Future studies could improve by including certain parameters and steps such as gender and different age group populations in the QMRA model to estimate the risks, and then establish protocols to substantially reduce or eliminate these risks.

2. **To confirm the internalization of the bacteria through the vascular system to the fruits or leaves of the plants**

   There could be probable contamination of leaves and fruits through internalization or movement of bacteria through the vascular system. The present research conducted in greenhouse left some questions unanswered on 'the internalization of bacteria'. To answer these questions, I recommend that further research can focus on different soil types, crop types and their root depth, and its effect on the internalization of bacteria through the vascular system to the fruits or leaves of plants.

3. **Aerosols produced during sprinkler irrigation**

   Other than the internalization of bacteria, aerosols could also be a probable source of contamination or cross-contamination. Some studies have proved that these aerosols can cause contamination but further research may be able to point to the extent and to the manner in which aerosols may work in combination with the internalization of bacteria.

4. **Monitoring and development of treatment technologies**

   There needs to be more monitoring of pathogenic contaminants, and treatment technologies and strategies to control the pathogenic contaminants in the irrigation water need to be developed.

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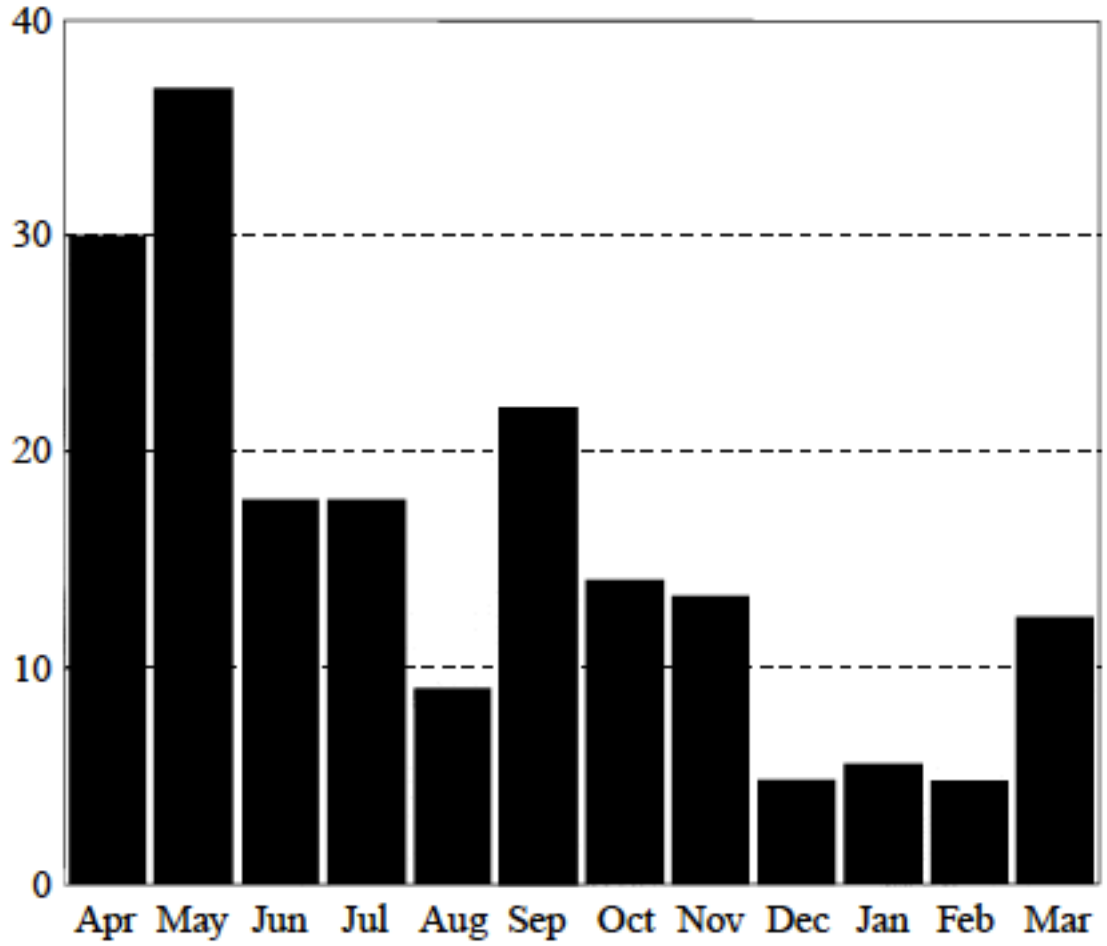
Appendices

Appendix 1: Seasonal distribution of *E. coli* O157 isolates from cattle during 1995-1996 (in % isolation) (Chapman et al., 1997)



Appendix 2: *E. coli* virulence factors (Kaper et al., 2004)

Table 1 | ***E. coli* virulence factors: colonization and fitness factors**

Factor	Pathotype	Activity/effect
IcsA (VirG)	EIEC	Nucleation of actin filaments
Intimin	EPEC, EHEC	Adhesin, induces T_H1 response; 10 variants described
Dr adhesins	DAEC, UPEC	Adhesin, binds to decay-accelerating factor (DAF), activates PI-3-kinase, induces MICA; >10 Dr adhesins described
P (Pap) fimbriae	UPEC	Adhesin; induces cytokine expression
CFAs	ETEC	Adhesin, >20 different factors designated CFA, CS or PCF
Type-1 fimbriae	All	UPEC adhesin; binds to uroplakin
F1C fimbriae	UPEC	Adhesin
S fimbriae	UPEC, MNEC	Adhesin
Bundle-forming pilus (BFP)	EPEC	Type IV pilus
Aggregative adherence fimbriae	EAEC	Adhesin; >4 subtypes
Paa	EPEC, EHEC	Adhesin
ToxB	EHEC	Adhesin
Efa-1/LifA	EHEC	Adhesin
Long polar fimbriae (LPF)	EHEC, EPEC	Adhesin
Saa	EHEC	Adhesin
OmpA	MNEC, EHEC	Adhesin
Curli	Various	Adhesin; binds to fibronectin
IbeA, B, C	MNEC	Promotes invasion
AslA	MNEC	Promotes invasion
Dispersin	EAEC	Promotes colonization; aids mucous penetration
K antigen capsules	MNEC	Antiphagocytic; >80 K types
Aerobactin	EIEC	Iron acquisition, siderophore
Yersiniabactin	Various	Iron acquisition, siderophore
IreA	UPEC	Iron acquisition, siderophore receptor
IroN	UPEC	Iron acquisition, siderophore receptor
Chu (Shu)	EIEC, UPEC, MNEC	Iron acquisition, haem transport
Flagellin	All	Motility; induces cytokine expression through TLR5; >50 flagella (H) serotypes
Lipopolysaccharide	All	Induces cytokine expression through TLR4; >180 O types

CFA, colonization factor antigen; CS, coli surface antigen; MICA, MHC class I chain-related gene A; PCF, putative colonization factor; PI-3-kinase, phosphatidylinositol 3-kinase; TLR, Toll-like receptor.

Appendix 3: Microbiological water quality standards (Pachepsky et al., 2011)

(NS: not specified)

					Concentration limits			
Source	**Type of water**	**Irrigation method**	**Land Use**	**Type of crop**	**TC (total coliforms, cell/100 ml)**	**FC (fecal coliforms, cell/100 ml)**	**EC (E. coli, cell/100ml)**	**FS (enterococci, cell/100ml)**
USEPA (1973)	Surface	NS	NS	NS	NS	1000	NS	NS
Canadian Council.. (1999)	NS	NS	NS	NS	1000	100	NS	NS
Alberta Environment (1999)	Surface	NS	NS	NS	1000-2400	100-200	NS	NS
British Columbia: Warrington (1988)	NS	NS	NS	eaten raw	1000-2400	200	77	20
British Columbia: Warrington (1988)	NS	NS	Open to public and grazing	other than eaten raw	1000-2400	NS	385	100
Manitoba: Williamson (2002)	NS	NS	NS	NS	NS	200	200	NS
Saskatchewan: Anonymous (2006)	Surface	NS	NS	eaten raw	1000	100	NS	NS

Blumenthal et al (2000)	wastewater	NS	NS	eaten raw	NS	1000	NS	NS
Blumenthal et al (2000)	wastewater	NS	NS	eaten processed	NS	100000	NS	NS
CSFSGLLGSC (2009)	NS	overhead	NS	eaten raw	NS	NS	126-235	NS
CSFSGLLGSC (2009)	NS	drip/ furrow	NS	eaten raw	NS	NS	126-576	NS
Vermont Water Agency (2009)	NS	NS	NS	NS	NS	200	77	NS
Johnson (2009)	NS	Overhead	NS	NS	NS	200	126	NS
Johnson (2009)	NS	Drip	NS	NS	NS	576	NS	NS
Bahri and Brissaud (2004)	Wastewater	Overhead, surface	NS	vegetables	1000	1000	NS	NS

Appendix 4: Limitations of greenhouse experiments

Plant replicates can be increased from 5 to 7 to reduce variability. Also, the experiment could be repeated 2-3 times to get enough replicates in total to reduce any experimental variability (Increasing experimental repeats would be advantageous).
Destructive sampling can be achieved by increasing the number of plants per treatment so that at every sampling time we can take away 5-7 plants for analysis.
Number of samples can be improved by pooling at least 3 samples per sampling time.
To collect samples for a longer duration instead of 30 days, we could include collecting fruits and leaves at different maturity levels for tomato and lettuce respectively.
Swab sampling from the surface of food is another option (not in our experiments)

Appendix 5: Tables for greenhouse experiments

Table 1:

Lettuce: Leaf Area Index (LAI) and Dry Biomass (in g) (Un-Inoculated and Inoculated)

Treatments	LAI (Un-Inoculated)	LAI (Inoculated)
S+O	1.99±0.37	2.19±0.5
D+M	1.89±0.07	2.14±0.06
S+M	1.65±0.25	1.09±0.17
D+O	1.46±0.21	1.44±0.19
Treatments	Dry biomass (Un-Inoculated)	Dry biomass (Inoculated)
S+O	48.73±8.2	49.26±5.93
D+M	24.2±4.39	30.95±4.25
S+M	24.64±6.53	23.72±5.97
D+O	48.54±5.59	36.87±2.35

Table 2:

Tomato: Total fruit weight (in g) and Shoot Length (in m) (Un-Inoculated and Inoculated)

Treatments	Un-inoculated (Total fruit weight)	Inoculated (Total fruit weight)
S+O	105±110	89±8
D+M	278±139	226±39
S+M	345±178	141±25
D+O	75±59	43±21
Treatments	Un-inoculated (Shoot length)	Inoculated (Shoot length)
S+O	1.7±0.3	1.6±0.2
D+M	1.7±0.1	1.5±0.1
S+M	1.4±0.1	1.2±0.1
D+O	1.3±0.2	1.1±0.1

Appendix 6: Two-way ANOVA for lettuce and tomato

Combinations	**Lettuce (soil)**	**Lettuce (leaves)**	**Tomato (soil)**	**Tomato (fruits)**
Irrigation method	0.5508	<.0001*	<.0001*	1
Soil type	<.0001*	<.0001*	<.0001*	<.0001*
Irrigation method*soil type	0.0082*	<.0001*	0.0006*	<.0001*

*** means they are significantly different from each other ($p<0.05$)**

Appendix 7: Risk distribution diagrams for field sites

Figure 1: The plotted graph shows the annual disease burden (DALYs pppy) against cumulative probability due to all three pathogens (*Rotavirus, Campylobacter* and pathogenic *E. coli*) to humans at age 1 year and 65 years at St-Remi (Lettuce) in year 2013

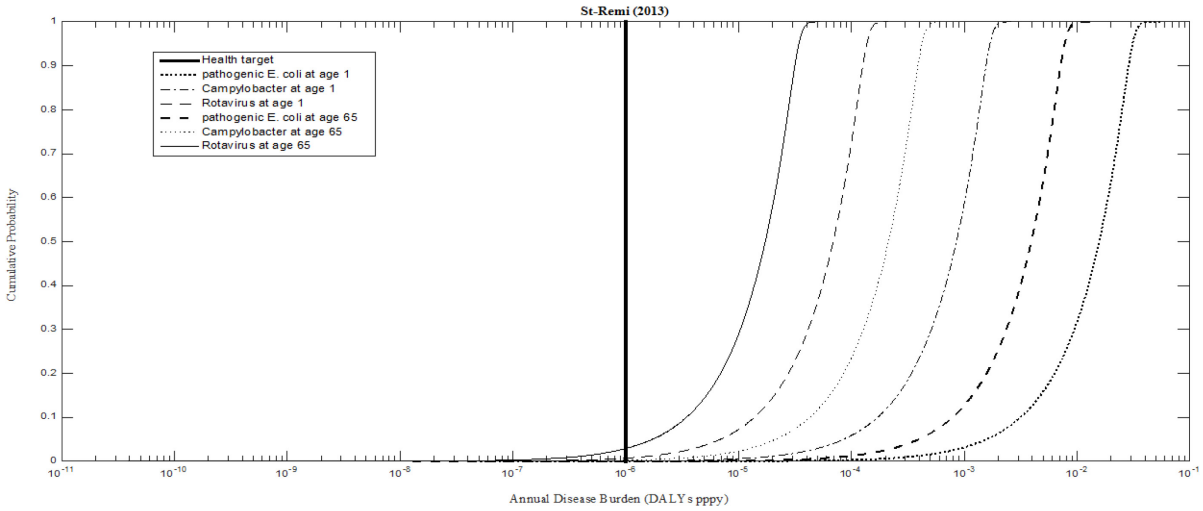


Figure 2: The plotted graph shows the annual disease burden (DALYs pppy) against cumulative probability due to all three pathogens (*Rotavirus, Campylobacter* and pathogenic *E. coli*) to humans at age 1 year and 65 years at St-Remi (Lettuce) in year 2014

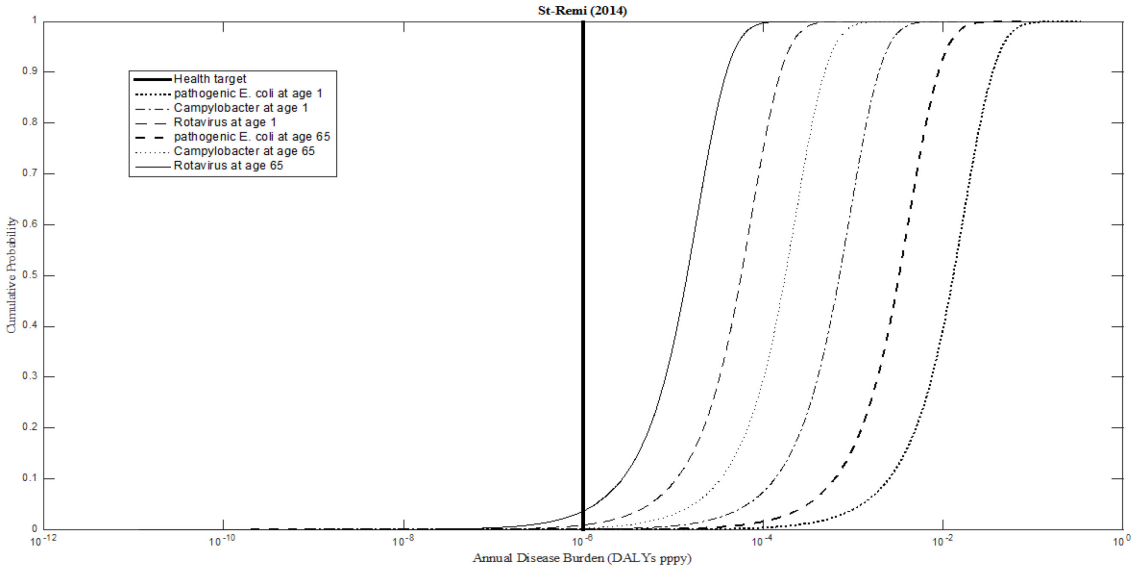


Figure 3: The plotted graph shows the annual disease burden (DALYs pppy) against cumulative probability due to all three pathogens (*Rotavirus, Campylobacter* and pathogenic *E. coli*) to humans at age 1 year and 65 years at Rougemont (Tomato) in year 2013

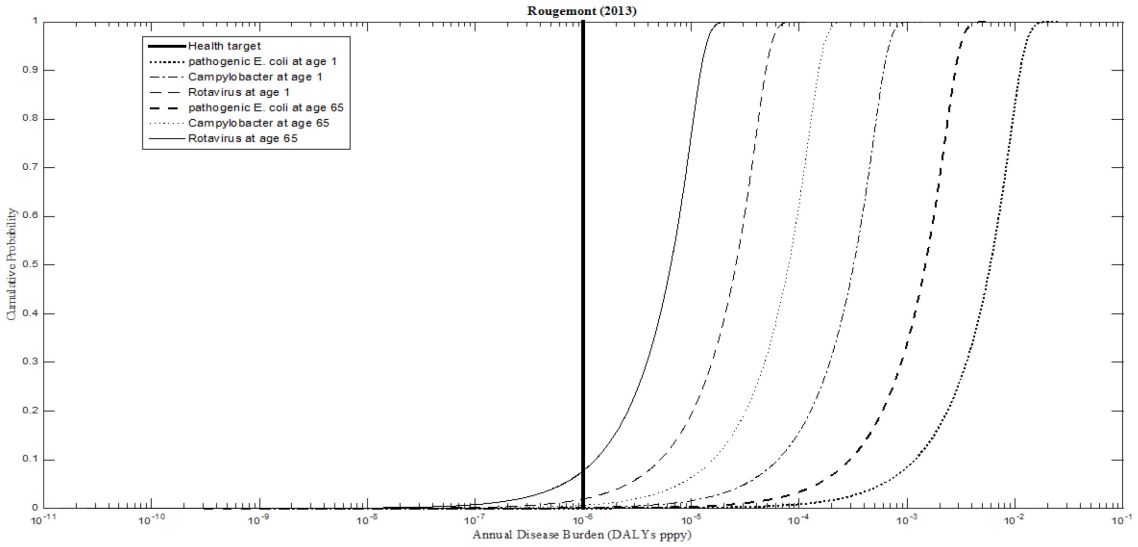
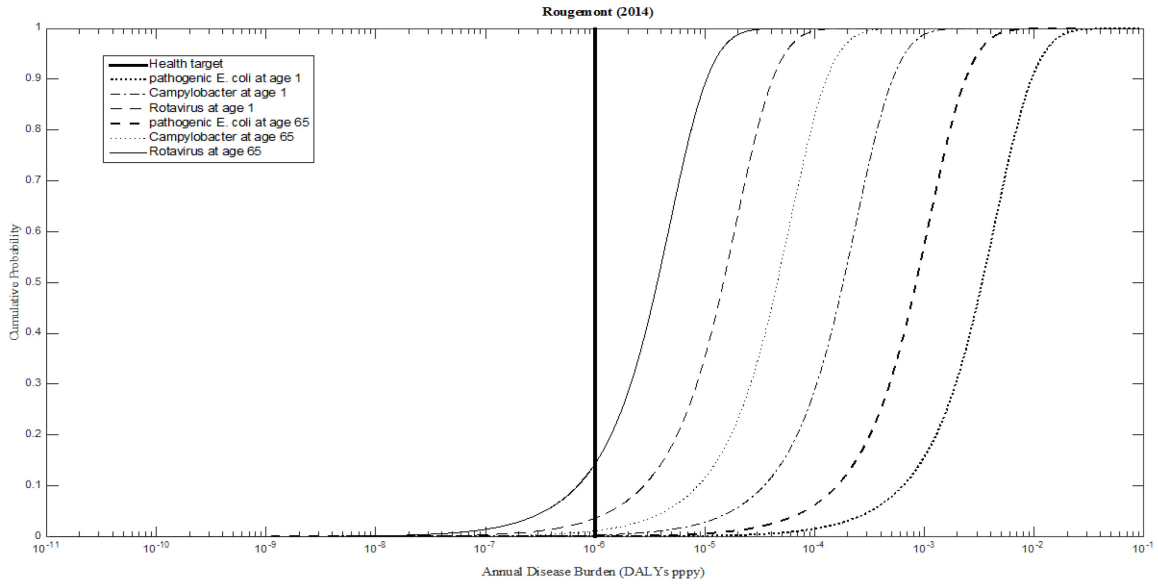


Figure 4: The plotted graph shows the annual disease burden (DALYs pppy) against cumulative probability due to all three pathogens (*Rotavirus, Campylobacter* and pathogenic *E. coli*) to humans at age 1 year and 65 years at Rougemont (Tomato) in year 2014



Appendix 8: Soil quality parameters at two sites in Quebec during 2013-2014

Parameters	Rougemont		St-Remi	
	Mean	St.Dev	Mean	St.Dev
Bulk Density (g/cm3)	1.66	0.06	0.26	0.07
Particle density (g/cm3)	2.58	0.20	0.62	0.17
% Porosity	35.63	2.23	57.39	11.59
% Organic matter	1.73	0.99	79.26	3.71
pH	6.71	0.32	5.98	0.15
P mg/kg	39.94	24.57	220.56	93.33
K mg/kg	155.67	49.96	588.82	323.34
N-NO3 mg/kg	2.40	0.92	132.03	128.90
N-NH4 mg/kg	2.09	0.51	5.07	1.92
Fe mg/kg	252.58	86.92	371.69	70.69
Ca mg/kg	2074.50	388.61	12755.84	2502.27
Al mg/kg	882.88	81.07	121.37	56.51
Mg mg/kg	462.82	89.24	1330.46	200.47