Impact of Climate Change on Microbial Safety of Leafy Green Vegetables

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Cheng Liu

Thesis

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Chapter 1

General Introduction

1.1 Background

Food is a critical contributor to health, well-being and a major source of pleasure, worry and stress in the daily lives of many (Rozin et al. 1999, Wilcock et al. 2004). All expect that food is safe but, unfortunately, absolute safety does not exist. Most people, for example, will experience a foodborne disease at some point in their lives. More than 200 diseases are spread through food and this results in millions of people falling ill every year due to hazardous food (WHO 2011). Food contamination can occur at different stages in the food supply chain that ranges from on-farm production, harvest, processing, storage, transport, retail and preparation. Such food contamination not only influences human health and well-being, but also affects the economy and society as a whole. For example, the EHEC O104:H4 (Enterohemorrhagic *Escherichia coli* O104:H4) outbreak in 2011 had far-reaching effects starting in Germany. During this outbreak 18 people died (Muniesa et al. 2012) and lettuce, cucumbers and tomatoes that were falsely blamed to cause the outbreak, became difficult to sell. This outbreak led to huge economic losses especially for local producers. The food chain is becoming longer and more complicated through globalization of food production and trade. This could cause more foodborne disease outbreaks and challenges tracing their contamination source.

Food contamination and associated foodborne diseases are strongly related to local climatic conditions (D'Souza et al. 2004). Climate change may affect the prevalence of bacteria, growth of fungi or pests and therefore alter the risk of foodborne disease (Miraglia et al. 2009, Jacxsens et al. 2010, Tirado et al. 2010). Roughly one-third (population attributable fraction) of salmonellosis cases in England, Wales, Poland, the Netherlands, the Czech Republic and Switzerland can now be attributed to higher temperatures (Semenza and Menne 2009). In Australia, the rate of salmonellosis also increases with decreasing latitude and consequently with increasing average yearly temperatures (Hall et al. 2002). D’Souza et al. (2004) found a statistical correlation between these seasonal salmonellosis patterns and the mean monthly temperature of the previous month. The mechanisms underlying the observed seasonality in foodborne disease are, however, not fully understood, but they are likely a complex interplay of different factors. These factors include bacterial growth and survival, human behaviour, consumption patterns and agriculture management practices (Van Staveren et al. 1986, Ziegler et al. 1987, Franz et al. 2014).

Climate change and food safety thus both affect human health. Climate change not only has an impact on crop production or food security (Fischer et al. 2005, Gregory et al. 2005) but also on food safety and incidence and prevalence of foodborne diseases (Miraglia et al. 2009, Tirado et al. 2010, Bezirtzoglou et al. 2011, Lal et al. 2012). Before I further specify the research gap and the objectives of this thesis, the two research fields 'climate change' and 'food safety' will be introduced and defined.

Food Safety

Food safety is an umbrella term for a system of measures aimed at minimizing the risk of foodborne disease from the farm to fork food chain including food handing, preparation and storage. Food safety research studies microbiological hazards and natural and man-made chemical hazards. Microbiological hazards contain enteric bacteria, viruses and parasites. Natural chemical hazards contain mycotoxins that are produced by moulds and may occur in juices, acid sauces (e.g. ketchup) or dried products (e.g. apricots or peanut butter). Man-made chemical hazards contain pesticide and heavy metals residues. m

A high level of food safety requires an adequate food quality management system from farm to fork to prevent foodborne disease. In industrial food production and retail this is usually covered by Hazard Analysis and Critical Control Points (HACCP) (Pierson and Corlett 1992, Mortimore and Wallace 2013). It is a management system in which food safety is addressed through the analysis and control of biological, chemical and physical hazards from harvest to consumption. In agriculture this is represented by hygiene guidelines like Good Agricultural Practices (GAPs). GAPs implementation includes proper GAPs education and training, worker health and hygiene, irrigation water quality, manure use and land selection, on-farm sanitation and record keeping (Bihn et al. 2006). For example, the use of animal manure as fertilizer increased the risk of E. coli contamination in both organic and conventional farms (Mukherjee et al. 2007). The use of contaminated surface water increases foodborne pathogens contamination on fruits and vegetables (Islam et al. 2004a, Islam et al. 2004b, Steele and Odumeru 2004). Infected persons who work with fresh fruits and vegetables, also increase the risk of transmitting foodborne diseases (Definitions and Water 1998). Farmers should be trained to understand and follow basic hygienic principles to lower the possibilities of contaminating food, water supplies and other workers. Throughout my thesis research, agriculture management practices turned out to be very important for food safety. Although agriculture management practices were not the main focus in my thesis, they are still an important part of the study. Therefore they are introduced here.

In this thesis, I especially focus on the microbial safety of fresh produce measured by the contamination rate with *Escherichia coli* (*E. coli*). Since the presence of *E. coli* indicates faecal contamination, it is valid to state that the presence of *E. coli* implies an increased risk of pathogen presence (Edberg et al. 2000, Tallon et al. 2005). The chance of having pathogens in samples is relatively low due to the high food quality standard, especially in the developing countries, and the challenge of representative sampling. Instead, *E. coli* is extensively studied, which gives more data to model and the chance to compare different studies. Although often challenged, the common assumption is that its presence indicates an increased probability of pathogen presence (Holvoet et al. 2014). Hygienic status is therefore used in my thesis to represent the microbial safety of LGVs.

Climate Change

Climate is commonly defined as the weather averaged over a long time period. The standard averaging period is thirty years. Climate change refers to a statistically significant variation in either the mean state of the climate or in its variability, persisting for an extended period of decades or longer (IPCC 2013a). Climate change is a current global concern and, despite some continuing controversy about the magnitude of its effects, has affected the food production systems and supply chain (IPCC 2014a, b).

Natural and anthropogenic greenhouse gases that change the Earth's energy budget are drivers of climate change. Radiative forcing quantifies the change in the energy budget caused by these changes (IPCC 2013b). Positive radiative forcing leads to surface warming, negative radiative forcing leads to surface cooling. The current total radiative forcing is positive. The largest contribution to total radiative forcing is caused by the increase in atmospheric CO_2 concentrations due to anthropogenic greenhouse gases emissions since 1750 (IPCC 2013b).

Many changes in the atmosphere have been observed based on local measurements and remote sensing from satellites. These changes include changes in greenhouse gas concentrations, temperature increase, precipitation pattern changes, changes in extreme events and changed radiation budgets. The global surface temperature increase for the end of the 21st century is likely to exceed 1.5°C to 4.5°C, extremely unlikely to be less than 1°C and very unlikely to be greater than 6°C (IPCC 2013b). Global warming increases the evaporation of water from land and ocean and allows the atmosphere to hold more moisture. This change leads to more extreme precipitation. Annual mean precipitation is likely to increase by the end of this century in the high latitudes and tropical regions. In mid-latitude areas, arid regions will likely become drier and wet regions will likely become wetter (IPCC 2013b). Extreme events (heat waves, droughts and extreme precipitation) will very likely become more frequent, more intense and of longer duration and occasional cold winter extreme will continue to occur (IPCC 2013b). The downward thermal and net radiation has been increasing since the early 1990s.

Climate Change and Food Safety

The gap between climate change and food safety studies indicates lacking understanding and methods or tools to study and quantify future climate change impacts on food safety. Climate change and food safety usually were considered separated scientific disciplines before 2010. In 2010 a special issue entitled “Climate Change and Food Science” was published in Food Research International. This special issue defined the agenda for this emerging interdisciplinary research field. It has now positioned itself and is gradually gaining more attention. Several review articles describe the climate-change impacts on food safety qualitatively. Two major recent reviews (Miraglia et al. 2009) from this special issue were based on a Food and Agricultural Organisation report (FAO 2008) and the EU FP6 project SAFE FOODS respectively. Overall, these reviews conclude that climate change could negatively affect food safety and that more research helps to improve understanding of the consequent problems and develop adaptation strategies.

Veg-i-Trade project

The European funded large collaborative FP7 Veg-i-Trade project was launched in 2010 for a 4-year period to fill this research gap. The project was introduced in the 2010 Food Research International Special Issue on Climate Change (Jacxsens et al. 2010). It was set up to study the impact of international trade and climate change on fresh produce safety. Fresh produce includes fresh fruits and vegetables. This commodity includes a variety of crops and cultivars with a high diversity in production practices. Moreover, fresh produce is a commodity often grown in open fields with intensive use of water and is thus vulnerable to local weather conditions. Veg-i-Trade combined field studies, statistical analyses, scenario analyses and risk assessments. The project investigated to which extent climate change increases the prevalence or levels of enteric pathogens, pesticide use, mould growth and associated mycotoxin production. Furthermore, the project studied possible increased use of chemical crop protection products, because of increasing pest and disease pressures due to more favourable future conditions compared to the current West European temperate climate. Climate and climate change will in particular likely affect the introduction of biological or chemical contaminants at the pre-harvest stage of fresh produce production. Other phases of the food chain will be less affected, because processing and transport are generally done in controlled inside environments.

Leafy green vegetables (LGVs) are an important part of a healthy diet. They provide fibre, vitamins, minerals and phytochemicals for our daily need. Fresh, fresh-cut or ready-to-eat leafy vegetables (e.g. lettuce, spinach, cabbages, chicory and endive) are frequently consumed. And this consumption is increasing because it is promoted as part of a healthy diet. LGVs are identified as the fresh produce commodity group of highest concern from a microbiological safety perspective (FAO/WHO 2008), because they are often grown in the open field and vulnerable to contamination from manure used as fertilizer, soil, water used for irrigation, and contact with (faeces of) wildlife (FAO/WHO 2008). Moreover, they are grown and consumed raw and in large volumes. Bacteria, such as *Salmonella* spp. and pathogenic *Escherichia coli* strains are the main pathogens causing foodborne disease through LGVs (Takkinen et al. 2005, Friesema et al. 2008, Söderström et al. 2008, Gajraj et al. 2012).

A Horticultural Assessment Scheme (HAS) has been developed in the Veg-i-Trade project to assess the level of microbiological quality of leafy vegetables. HAS is a systematic approach to sample, analyse and standardise the sampling scheme in various regions within Veg-i-Trade. HAS defined the identification of critical sampling locations, the selection of microbiological parameters, the assessment of sampling frequency, the selection of sampling method and method of analysis, and finally data processing and interpretation (Holvoet et al. 2011). All Veg-i-Trade sampling data used in this thesis were collected and analysed under HAS.

My thesis research was part of the Veg-i-Trade project. Within the framework of Veg-i-Trade, my role was to bridge climate change and food safety by introducing the research methods, i.e. climate scenario analysis, from climate change studies to food safety research. I have prepared climate scenario data for other impact studies in Veg-i-Trade on pesticide and mycotoxin. Besides that, my research focused on microbial safety of LGVs, i.e. lettuce and spinach.

1.2 Objective, Research Questions and Scope

In line with the above-mentioned research gaps, this thesis aims **to quantify the impacts of climate change on the microbial safety of pre-harvested leafy green vegetables.** The hygienic status of LGVs as measured by contamination with generic *E. coli* was taken as a proxy for the microbial safety. To achieve this objective, my research requires literature review, statistical model development, climate data downscaling and multi-criteria scenario analysis (Figure 1.1). This interdisciplinary research will bring new methods/ tools and mind sets to food safety research. This connected food safety and climate change studies will enable food safety scientists to assess food microbial safety risks using a systems analysis approach.

The main question of this thesis is **"What are the climate-change impacts on the microbial safety of leafy green vegetables?"** This main question is addressed through the following research questions:

1. What are the impacts of climate change on contamination sources and pathways of foodborne pathogens?
2. How do climatic conditions quantitatively affect the *E. coli* contamination of pre-harvested leafy greens?
3. How to downscale climate and climate-change data for local food safety analysis?
4. How does the safety of LGVs evolve under future climate scenarios?

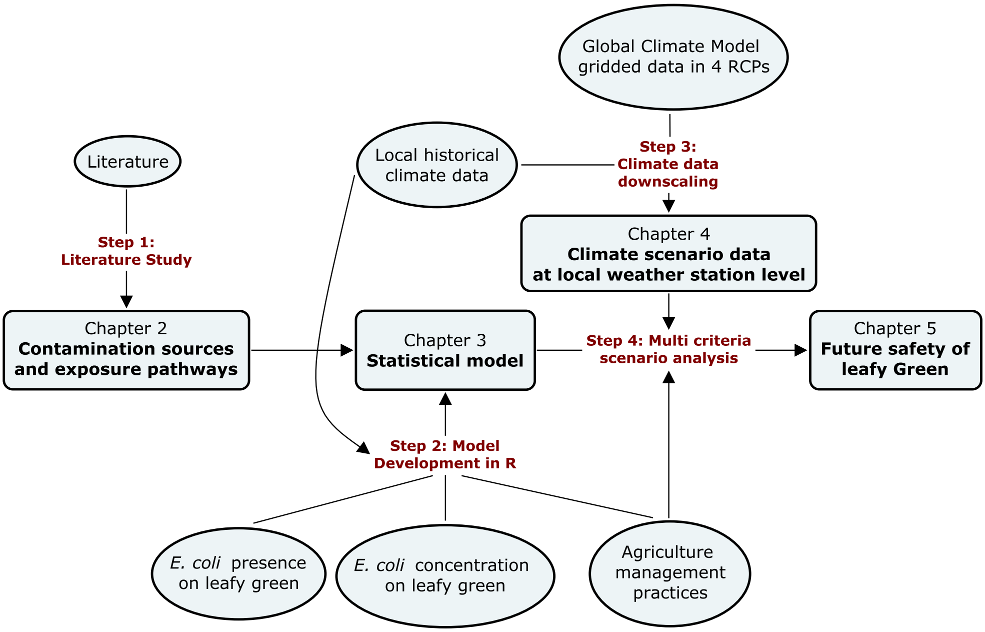

Figure 1.1 Process and methods of this thesis. Round boxes are data and knowledge inputs and square boxes are major results in this thesis (Chapter 2 to 5). Arrows are data and knowledge flow.

To answer these research questions, the process and methods of this thesis are indicated in Figure 1.1. Each step answers a specific research question. Firstly, I reviewed the literature for and synthesised major impacts of climate change (temperature increases and precipitation pattern changes) on contamination sources (manure, soil, surface water, sewage and wildlife) and pathways of foodborne pathogens (focussing on *Escherichia coli* O157 and *Salmonella* spp.) on pre-harvested leafy greens. Secondly, with these qualitative impacts as knowledge background, I developed a statistical model in R (R Core Team 2013) using sampling data, climate data and agriculture management information as inputs. This model identified the climate and management variables that are associated with the probability of LGVs contamination with generic *E. coli* using regression analysis. This model also explored how climatic conditions directly and indirectly affect the *E. coli* concentration on LGVs. Thirdly, to apply suitable climate data in the statistical model to study future impacts, I have prepared a tool in MATLAB and Excel to downscale coarse climate data for local food safety scenario analysis. I also illustrated how this tool can be used with impact models. Finally, I applied the downscaled data to the statistical model and used multi-criteria scenario analysis to explore future food safety.

1.3 Outline of the Thesis

This introduction (Chapter 1) and the combined discussion and conclusion (Chapter 6) of this thesis are written to connect the four independent scientific papers that are presented in Chapters 2 to 5.

Chapter 2 is the knowledge background of this thesis and answers Research Question 1. This chapter synthesises major impacts of climate change on contamination sources and pathways of foodborne pathogens (focussing on *Escherichia coli* O157 and *Salmonella* spp.) on pre-harvested LGVs. These are formulated as a conceptual framework. Major positive and negative impacts of temperature increases and precipitation pattern changes on pathogen prevalence in each contamination source and pathways have been reviewed. I started my research on foodborne pathogens. However due to the, fortunately, limited positive samples of pathogens on leafy greens, to develop a robust model was very challenging. After this review, I chose generic *E. coli* as an indicator to do the rest of the analysis.

Chapter 3 is the core data analysis of this thesis and answers Research Question 2. This chapter identifies the climate and management variables that are associated with the probability of LGVs contamination with generic *E.coli* and to explore how weather conditions directly and indirectly affect the *E. coli* presence and concentration on LGVs. I have used *E. coli* data of 562 LGV samples between 2011 and 2013 taken from 23 open-field farms from Belgium, Brazil, Egypt, Norway and Spain. This is the first large-scale meta-analysis on *E. coli* presence and concentration on LGVs. Meta-analysis allows a generic model to identify the statistically significant variables for *E. coli* contamination throughout the regions. I used logistic mixed effect regression and linear regression models to study the statistical relationship between these variables. In general, climate and good management practices should be studied together for *E. coli* presence and concentration on LGVs.

Chapter 4 is the data preparation connecting Chapter 3 and Chapter 5 of this thesis, and answers Research Question 3. I present a tool in this chapter to prepare climate and climate change data for local food safety scenario analysis. This chapter also illustrates how this tool can be used with bacterial growth model. As an example, coarse gridded data from two general circulation models, HadGEM2-ES and CCSM4, are selected and downscaled using the 'Delta method' with quantile-quantile correction for the official weather station Ukkel in Belgium. Observational daily temperature and precipitation data from 1981 to 2000 are used as a reference for this downscaling. Data are provided for four future representative concentration pathways (RCPs) for the periods 2031–2050 and 2081–2100. The climate projections for these RCPs show that both temperature and precipitation will increase towards the end of the century in Ukkel. The climate change data are then used with Ratkowsky's bacterial growth model to illustrate how projected climate data can be used for projecting bacterial growth in the future. This approach helps food safety researchers to perform their own climate change scenario analysis.

Chapter 5 is the future projection and answers Research Question 4. This chapter explores the development and application of a multi-criteria scenario analysis tool to study future food safety. I apply climate scenario analysis and multi-criteria scenario analysis on the statistical model presented in Chapter 3 using pre-harvest spinach in Spain as an example. I demonstrate the tool step by step with a sensitivity analysis to show the possibility of including different perspectives of interests in food safety studies. Moreover I calculate the future *E. coli* concentration changes on spinach in RCP8.5 and RCP 2.6 at the end of the century in Spain. This multi-criteria tool provides a platform to study changes in weather or climate, and management impacts on future food safety together with different perspectives or interests of stakeholders. The tool provides the opportunity to involve different stakeholders in the analysis and support their decision making process. In this way a multi-criteria analysis delivers a new mind set and method to study food safety in a systematic way and enhances the quality of agricultural management decisions for leafy green vegetables.

Lastly, Chapter 6 synthesised the main findings and methodological lessons learnt and concludes the thesis.

Chapter 2

Impacts of Climate Change on the Microbial Safety of Pre-harvest Leafy Green Vegetables

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Nynke Hofstra

Eelco Franz

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Abstract

The likelihood of leafy green vegetables (LGVs) contamination and the associated pathogen growth and survival are strongly related to climatic conditions. Particularly temperature increase and precipitation pattern changes have a close relationship not only with the fate and transport of enteric bacteria, but also with their growth and survival. Using all relevant literature, this study reviews and synthesises major impacts of climate change (temperature increases and precipitation pattern changes) on contamination sources (manure, soil, surface water, sewage and wildlife) and pathways of foodborne pathogens (focussing on *E. coli* O157 and *Salmonella* spp.) on pre-harvested LGVs. Whether climate change increases their prevalence depends not only on the resulting local balance of the positive and negative impacts but also on the selected regional climate change scenarios. However, the contamination risks are likely to increase. This review shows the need for quantitative modelling approaches with scenario analyses and additional laboratory experiments. This study gives an extensive overview of the impacts of climate change on the contamination of pre-harvested LGVs and shows that climate change should not be ignored in food safety management and research.

2.1 Introduction

Fresh fruit and vegetables are increasingly recognized as an important source of foodborne disease outbreaks in many parts of the world (Cummings et al. 2001, Beatty et al. 2004, Sivapalasingam et al. 2004, FAO/WHO 2008, Hanning et al. 2009, Wendel et al. 2009, Moretti et al. 2010, Gajraj et al. 2012). The risks of foodborne disease caused by fresh produce are illustrated by multiple outbreaks with high numbers of illnesses in several regions of the world, such as in Europe (Horby et al. 2003, Söderström et al. 2005, Takkinen et al. 2005, Friesema et al. 2008, Nygård et al. 2008, Söderström et al. 2008), the United States (Ackers et al. 1998, Wendel et al. 2009, Mody et al. 2011), Japan (Michino et al. 1999) and Australia (FAO/WHO 2008). Every year approximately 76 million people in the US become ill from foodborne disease and over 12% of these disease cases are linked to fresh produce (Klonsky 2006). The European percentage is similar (Miraglia et al. 2009). One of the causes of foodborne disease is contamination of fresh produce by foodborne pathogens originating from manure, soil, sewage, surface water or wildlife.

Leafy green vegetables (LGVs) are identified as the fresh produce commodity group of highest concern from a microbiological safety perspective (FAO/WHO 2008), because they are often grown in the open field and vulnerable to contamination from contaminated manure used as fertilizer, soil, water used for irrigation, and contact with (faeces of) wildlife (FAO/WHO 2008). Moreover, they are grown and consumed raw and in large volumes. Bacteria, such as *Salmonella* spp. and pathogenic *Escherichia coli* strains, are the main pathogens causing foodborne disease through LGVs (Takkinen et al. 2005, Friesema et al. 2008, Söderström et al. 2008, Gajraj et al. 2012).

The incidence in foodborne disease is generally correlated with climate conditions (Miraglia et al. 2009, Jacxsens et al. 2010, Tirado et al. 2010). Roughly one-third (population attributable fraction) of salmonellosis cases in England, Wales, Poland, the Netherlands, the Czech Republic, and Switzerland can be linked to higher temperatures (Semenza and Menne 2009). In Australia, the rate of salmonellosis also increases with decreasing latitude and consequently with increasing average yearly temperatures (Hall et al. 2002). These seasonal salmonellosis patterns were statistically correlated with the mean monthly temperature of the previous month (D'Souza et al. 2004). Similarly, in the Australian subtropical and tropical regions, temperature and rainfall were positively associated with the number of salmonellosis cases (Zhang et al. 2010). The mechanisms underlying the observed seasonality in foodborne disease are not fully understood, but they are likely a complex interplay of different factors. These include human behaviour and consumption patterns (Van Staveren et al. 1986, Ziegler et al. 1987), pathogen prevalence in the animal reservoir and pathogen environmental survival patterns. The risk of foodborne disease is directly related to the prevalence of bacteria on LGVs. The likelihood of LGV contamination and the associated pathogen concentrations are strongly related to environmental conditions. Though uncertain, the observed seasonality and climate relationships should thus not be ignored; they may result in higher risks.

Changes in temperature, distribution of precipitation (including more extreme events, such as floods and droughts), UV and moisture content are already observed worldwide (Meehl et al. 2007). Temperature has increased since the start of observations in 1654 (Camuffo and Bertolin 2012). Droughts have already become more common, especially in the tropical and subtropical regions since the 1970s (Meehl et al. 2007). Consistent with precipitation changes, runoff is notably reduced in southern Europe and increased in Southeast Asia and at high latitudes. The larger simulated runoff changes reach a 20% increase compared to 1980 to 1999 mean values. These changes will likely become more apparent in the future (Meehl et al. 2007). Climate changes will mainly impact the contamination sources and pathways of bacteria onto LGVs during the pre-harvest phase. Other phases of the food chain will be less affected, because generally processing and transport are done in controlled environments.

Several studies focus on climate change and foodborne diseases (Rose et al. 2001, FAO 2005, Lafferty 2009, Semenza and Menne 2009). There is also considerable understanding of how climatic variables affect pathogen survival in different environments. However, only few studies (e.g. Miraglia et al. 2009, Moretti et al. 2010, Tirado et al. 2010) have addressed the relationship between climate change impacts and the microbial safety of LGVs. Moretti et al. (2010), for example, qualified the impacts of temperature on post-harvest fresh produce quality from a biochemical perspective and summarised that the crop will mature sooner with higher temperature during the growing season. A systematic overview encompassing the impacts of temperature and precipitation changes on the contamination sources and pathways of bacteria on LGVs is, however, still missing. Such an overview is essential to quantitatively assess the impact of climate change on LGVs safety.

This paper therefore aims to review and synthesise major impacts of climate change (temperature increases and precipitation pattern changes) on contamination sources and pathways of foodborne pathogens (focussing on *E. coli* O157 and *Salmonella* spp.) on pre-harvested LGVs. Relevant literature, including peer review scientific papers and grey literature, on LGVs but limited to *E. coli* O157 and *Salmonella* spp., has been studied for each contamination source and pathway, and for their relationship to different climate variables. Each contamination source has been searched in combination with each of these two pathogens, and with temperature and precipitation. In this study, firstly, contamination sources and pathways of *E. coli* O157 and *Salmonella* spp. onto LGVs are identified. These are formulated as a conceptual framework (Section 2.2). Then we summarised major positive and negative impacts of temperature increases and precipitation pattern changes on pathogen prevalence in each contamination source and pathway (Section 2.3). Although the reviewed literature and data was relatively limited to Europe and North America, the study aims to provide a generic worldwide overview.

2.2 Contamination sources and pathways

Two foodborne pathogens will be discussed in this review: *Escherichia coli* O157 and *Salmonella* spp. in general. Different *Salmonella* serotypes will be discussed depending on the literature. This choice was made because these pathogens are the leading cause of bacterial foodborne illness on LGVs and are well documented in many studies (Beuchat 1996, Bach et al. 2002, Sivapalasingam et al. 2003, Sivapalasingam et al. 2004, Maurer and Lee 2005, Hanning et al. 2009). These two pathogens are also representative for other foodborne bacterial pathogens and their abundant literature will likely result in a deeper analysis.

The contamination sources and pathways associated with LGVs contamination with *E. coli* O157 and *Salmonella* spp. form the basis for the conceptual framework (Figure 2.1). Beuchat (2006) reviewed the literature for generic contamination sources and pathways for fresh produce. We build on this by summarising these findings and adding more recent literature. The principal reservoir for *E. coli* O157 is cattle and other small ruminants such as sheep and deer (Hancock et al. 2001). The main reservoirs for *Salmonella* spp. are pigs (Fedorka-Cray et al. 2000) and poultry (Aserkoff et al. 1970, Vandeplas et al. 2010). The pathogens shed in the faeces of these animals can subsequently contaminate LGVs directly or indirectly by contamination of soil and water. Additionally, the pathogens can enter the environment via shedding from incidental hosts (e.g. humans and insects) or wildlife. We therefore consider manure, soil, surface water, sewage and wildlife to be the most likely contamination sources.

Manure

Contaminated manure from livestock and faeces from wildlife form the primary source of environmental contamination with zoonotic pathogens such as *E. coli* O157 and *Salmonella* spp. Livestock and wildlife may defecate on land (Figure 2.1, arrow a) or directly into surface water (Figure 2.1 arrow b). The pathogens in livestock manure are killed by different treatments, such as long-term storage and/or composting. The use of improperly treated manure is an important risk factor for the microbial safety of LGVs (Franz and Bruggen 2008, Jiang and Shepherd 2009). Such livestock manure may contaminate LGVs when applied during plant growth (Figure 2.1 arrow c) and by contaminating water supplies (for example surface water) via surface and subsurface runoff (Figure 2.1 arrow d) (Jackson et al. 1998). Contamination of LGVs that grow on the manure-amended soils might occur by splash dispersal (Figure 2.1 arrow e) during rain events (Madden 1997, Pielaat and van den Bosch 1998, Franz et al. 2008b, Monaghan and Hutchison 2012).

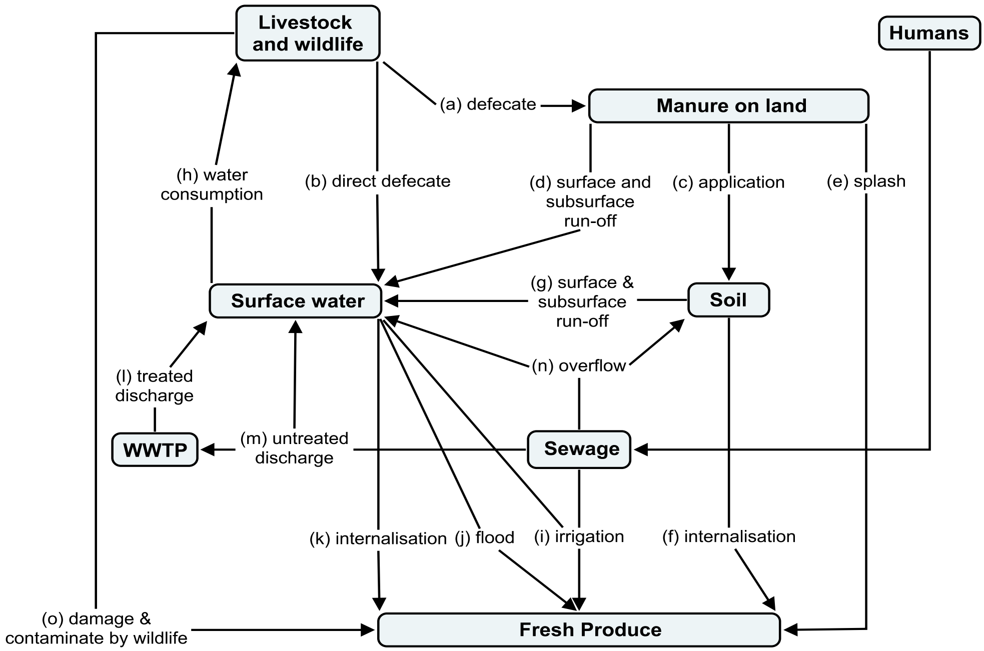

Figure 2.1 Bodies and pathways of pathogenic bacteria on leafy green vegetables. Boxes show bodies of pathogenic bacteria, arrows and words in the middle of arrows indicate pathogen flow. WWTP stands for waste water treatment plant. The letters a-o are referred to in the text.

Soils

Soil amended with contaminated manure or faeces can be a source of *E. coli* O157 and *Salmonella* spp. which can persist in the soil up to several months (Jamieson et al. 2002, Unc and Goss 2006, Barak and Liang 2008, Franz et al. 2008a, Semenov et al. 2009, Van der Zaag et al. 2010, Franz et al. 2011). When plants are grown in contaminated soils internalization (Figure 2.1 arrow f) of pathogenic *E. coli* and *Salmonella* spp. via root uptake has been described in laboratory settings (Solomon et al. 2002, Franz et al. 2007, Deering et al. 2012). This process however is thought to be rare in field conditions and, additionally, *E. coli* O157 does not persist in the leaves more than seven days (Erickson et al. 2010a). The evidence for internalisation of *Salmonella* spp. via soil has not been found. *E. coli* O157 (Donnison and Ross 2009), *Salmonella* spp. *and Salmonella* infantis (Miner et al. 1967, Jacobsen and Bech 2012) can be transferred by runoff from soils via the surface and subsurface to the surface water (Figure 2.1 arrow g).

Surface water

Cooley et al (2007) reported that surface water is a possible vehicle of transmission of *E. coli* O157 for pre-harvest LGVs contamination. Livestock and wildlife may get (re)infected by consumption of contaminated water (Figure 2.1 arrow h). Surface water may not only contaminate fruits and vegetables by irrigation (Rose et al. 2001, Bach et al. 2002, Okafo et al. 2003, Sivapalasingam et al. 2003, Islam et al. 2004a, Islam et al. 2004b, Steele and Odumeru 2004, Erickson et al. 2010b) (Figure 2.1 arrow i) but also as result of flooding (Figure 2.1 arrow j) of production fields after (extreme) rain events (Cooley et al. 2007, Orozco et al. 2008).

Like with root uptake from the soil, application of contaminated irrigation water can lead to internalization of both *E. coli* O157 and *Salmonella* spp. into the edible part of LGVs through open stomata (Kroupitski et al. 2009) (Figure 2.1 arrow k). Extreme weather conditions (i.e. drought and heavy rains) have been shown to increase the levels of internalized *Salmonella* Typhimurium into lettuce leaves (Ge et al. 2011).

Sewage

In developing countries and arid regions, sewage is often used for irrigation (Nichols et al. 1971, Amoah et al. 2005, WHO/UNICEF JMP 2010) (Figure 2.1 arrow i). It is cheap and efficient, as sewage also contains a high concentration of bioavailable nitrogen and phosphorus from domestic waste. Normally sewage flows back to surface water after being treated in waste water treatment plant (Figure 2.1 arrow l). Untreated sewage (Figure 2.1 arrow m) or improperly treated effluents from wastewater treatment plants used for irrigation may contain high levels of pathogens (Nichols et al. 1971, Gale 2005, Gerba and Smith 2005). Sewer overflows (Figure 2.1 arrow n) may cause many *Salmonella* serotypes (Claudon et al. 1971) and commensal *E. coli* (McLellan et al. 2007) to enter the surface water and/or soil directly.

Wildlife

Wildlife (e.g. insects, birds and mammals) may carry pathogenic bacteria in their digestive and respiratory systems, skin, hooves and hair or feathers (Ray and Bhunia 2008, WHO 2011). These wildlife share similar exposure pathways with livestock (c.f. Figure 2.1). Moreover, they might damage the leaves (Figure 2.1 arrow o), which provide vulnerable entry points for foodborne pathogens into the plant and leaching of nutrients that will facilitate pathogen persistence (Orozco et al. 2008). The wildlife driven contamination is further excluded from this review, as contamination by wildlife is random and currently unpredictable. This makes quantification very difficult. Moreover, climate change impacts on wildlife are species specific (McCarthy et al. 2001, Root and Schneider 2002, Petzoldt and Seaman 2005, Mawdsley et al. 2009). We expect that in the area where LGVs are grown wildlife will remain present under current climate change scenarios even though specific species may vary. Additionally, producers will often attempt to keep wild life out of the fields by fencing or removing vegetation around the field.

Summary

Contamination sources and pathways vary depending on the practical farming management in different parts of the world. In general, manure amended soil and irrigation water are better studied sources.

2.3 Influence of climate variables

Climate is commonly defined as the weather averaged over a long time. The standard averaging period is thirty years. As mentioned in the introduction, the incidence of foodborne disease is related to climatic conditions. Temperature and precipitation patterns and other climate factors are expected to change due to an increase in the radiation balance of the earth caused by greenhouse gas emissions. Standard practice in climate research is the application of different scenarios. These scenarios comprise plausible changes in factors driving climate change, such as population growth and land use changes. Climate models are run for these different scenarios to determine projected changes in climate variables worldwide. These changes differ by scenario and region, but generic changes are as follows:

Modelling studies project that temperature will continue to increase gradually over time, resulting in a 2°C to 5°C increase of 1-in-20 year extreme daily maximum temperature by the late 21st century (IPCC 2012). Highest temperature increases will be over land and at high northern latitudes (Figure 2, IPCC 2007).

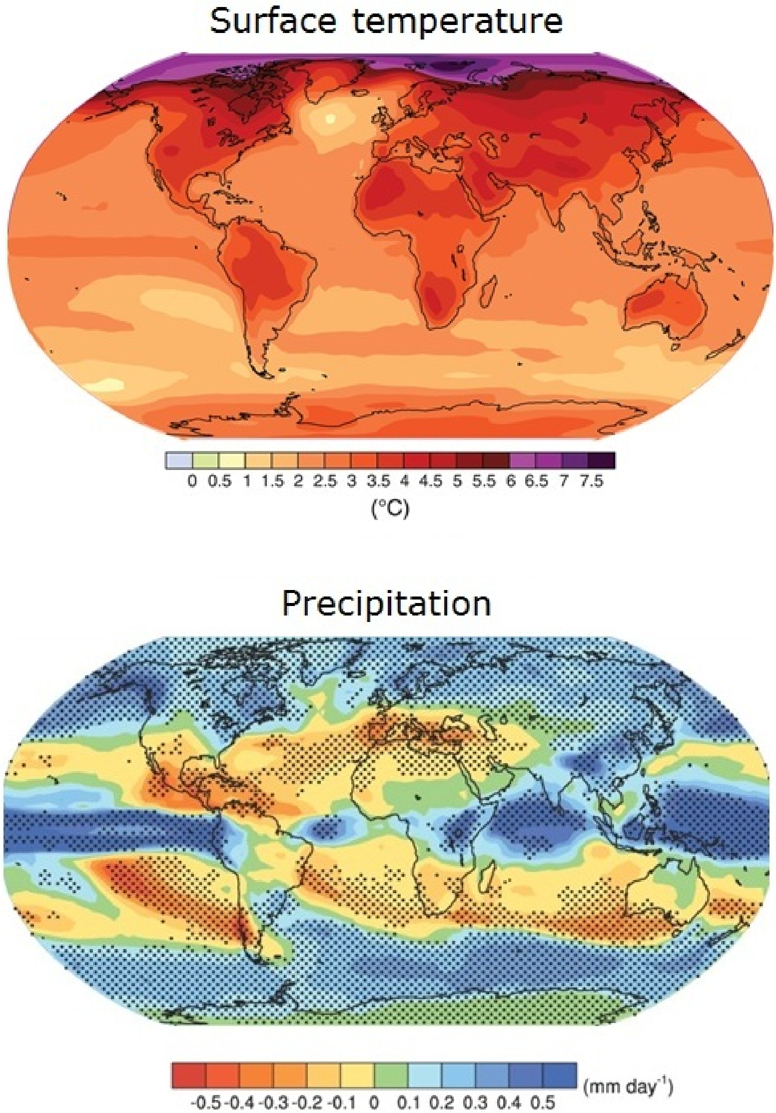

Figure 2.2 Multi-model mean projections of changes in surface air temperature (°C) (top) and precipitation (mm day$^{=1}$) (bottom). Changes are annual means for the SRES A1B scenario for the period 2090 to 2099 relative to 1980 to 1999. General Circulation Models, representing physical processes in the atmosphere, ocean, cryosphere and land surface, are the most advanced tools currently available for simulating the response of the global climate system to increasing greenhouse gas concentrations. Key assumptions of SRES A1B scenarios: a future world with very rapid economic growth, low population growth, rapid introduction of new and more efficient technology and balanced energy sources. Credit: Climate Change 2007: The Physical Science Basis. Working Group I Contribution to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, Figure spm6 and Figure 10.12. Cambridge University Press.

The amount of precipitation is expected to increase in some areas (e.g. high latitude and tropical regions, and in winter in the northern mid-latitudes) and decrease in others (e.g. southern Europe and the Mediterranean region, central Europe, central North America, Central America and Mexico, northeast Brazil, and southern Africa) (IPCC 2012) (Figure 3, Meehl et al. 2007). Moreover, the distribution of precipitation is expected to change, resulting in an increase in the number of extreme precipitation events even in areas with decreasing precipitation. (Meehl et al. 2007, IPCC 2012). The characteristics of what is called extreme weather may vary from place to place. But an extreme weather event would normally be as rare as or rarer than the 10^{th} or 90^{th} percentile (IPCC 2007). These events might intensify floods or droughts in some catchments, areas and seasons (IPCC 2012). This has already been observed in several regions (Meehl et al. 2007).

Due to temperature and precipitation changes, evapotranspiration changes affect atmosphere and soil moisture. Increased land precipitation intensity together with increased temperature lead to a higher moisture content of the atmosphere at a rate of about 7% for every 1°C rise (Trenberth et al. 2007). The annual mean soil moisture decreases in the subtropics and the Mediterranean region and increases in east Africa, central Asia, and some other regions with increased precipitation (Meehl et al. 2007). We discuss soil humidity changes together with precipitation changes.

Ultraviolet (UV) radiation is another variable that impacts bacterial contamination of LGVs and that is influenced by climate change. UV radiation changes have been simulated by radiative transfer models for all IPCC scenarios. The amount of UV at the surface result from ozone concentrations in the upper troposphere and lower stratosphere, cloud cover and the aerosol type, content and distribution (Penner et al. 1999). Under clear skies, UV light can effectively kill microbes (Yaun et al. 2003). Cloud cover, however, is very difficult to predict due to geometrical complexity and temporal variability of clouds (Penner et al. 1999, Sausen et al. 2005). Future UV changes on foodborne pathogens will therefore not be further discussed in this paper.

Climate change may impact on the contamination sources and pathways of *E. coli* O157 and *Salmonella* spp. These impacts may increase the likelihood of LGVs contamination and human disease associated with consumption of contaminated LGVs. Climate change may, therefore, increase the risk of disease due to foodborne contamination. Seasonality of the human prevalence of infections (mostly acquired through direct contact with animals or manure, and increasingly through consumption of raw vegetables) indicates climate change impacts (Section 2.1). The *E. coli* O157 incidence of human disease is generally higher in summer months and early fall (Douglas and Kurien 1997, Van Duynhoven et al.

2004, Rangel et al. 2005) and the daily *Salmonella* incidence closely follows ambient temperature with the a lag of 2 to 14 days (Naumova et al. 2007). However, the causal mechanisms behind the seasonal patterns remain elusive and may vary among different pathogens and geographic regions due to several confounding factors (e.g. animal housing, diet). Furthermore, there may be heterogeneity between strains of a particular pathogen. For instance, some strains may be better adapted to higher temperatures or tolerate drier conditions, which may enhance their environmental survival capacities and ultimately the likelihood of LGVs contamination (Franz et al. 2011).

From this literature review the relationship between climate change and contamination sources and pathways of *E. coli O157* and *Salmonella* spp. is evident. The next section discusses for each source of both pathogens first possible impacts of temperature and then possible impacts of precipitation. Manure and soil are combined as the climate change impacts on these sources are similar. Also the important seasonal relationships are discussed. Table 2.1 summarises all impacts.

2.3.1 Manure and Soil

Temperature
Seasonality of E. coli O157 in livestock

Many studies are available on the seasonal patterns of *E. coli* O157 prevalence among livestock, from which general relations between temperature and pathogen prevalence and/or shedding rates can be deduced. Ultimately these relations can be used, to a certain extent, to predict the likelihood of having contaminated manure. Since the causal relations remain unclear, these have not been explicitly added to Figure 2.1. Higher prevalence and/or increased shedding rates have been observed in cattle during summer months (Heuvelink et al. 1998, Hussein and Sakuma 2005, Schouten et al. 2005, Ogden et al. 2006, Berends et al. 2008). The role of temperature in the seasonality of *E. coli* O157, however, has been questioned: The North-American latitudinal gradient of *E. coli* O157 prevalence, for example, showed an opposite relation to the temperature gradient (Meyer-Broseta et al. 2001). Seasonality has also been observed in regions with little seasonal temperature fluctuations (Miller et al. 2004). Interestingly, a strong correlation between increased day length and *E. coli* O157 prevalence in cattle was observed in North America (Edrington et al. 2006). This, is unlikely to be affected by climate change. This indicates that indeed also other factors may be involved.

Seasonality of Salmonella in livestock

In the US, the occurrence of Salmonella on dairy farms increases with increasing seasonal temperature (Pangloli et al. 2008). In Denmark, the seasonal variation of the prevalence in pork and the human incidence is similar (Hald and Andersen 2001). However, several factors, such as management practices, concurrent diseases and elevated temperatures that lead to stress and higher multiplication rates of Salmonella, could well cause seasonal trends (Hald and Andersen 2001). Salmonella prevalence in Danish finisher pig herds was also higher in summer and fall as compared to spring and winter (Baptista et al. 2009).

Direct effects of climate change on environmental fate of E. coli O157 and Salmonella

Weather conditions influence transport and dissemination of pathogens from their reservoirs into the environment, food crops like LGVs and other hosts. Higher soil temperatures may lead to an increased use of potentially contaminated animal manure due to a faster depletion of soil nutrients as a result of increased biological soil activity (Franz et al. 2008a) (Table 2.1, a).

Temperature increase has a close relationship with foodborne bacteria growth and survival (Ratkowsky et al. 1982, Himathongkham et al. 1999, Beuchat 2002, Jiang et al. 2002, D'Souza et al. 2004, Mukherjee et al. 2006, Beuchat and Mann 2008, FAO 2008, Franz and Bruggen 2008, Lake et al. 2009, Nelson 2009, Pan and Schaffner 2010). Although commensal E. coli has been found to establish a stable population in the soil environment (Byappanahalli and Fujioka 2004, Ishii et al. 2006), the conditions for survival of foodborne pathogens are considered to be unfavourable once excreted from the animal gut. However, pathogens like E. coli O157 and Salmonella spp. are able to survive for extended periods (up to months) in manure and soil (Franz and Bruggen 2008). The survival of E. coli O157 and many Salmonella serotypes in soil and manure decreases with increasing temperature (Wang et al. 1996, Kudva et al. 1998, Himathongkham et al. 1999, Mukherjee et al. 2006, Semenov et al. 2007, Danyluk et al. 2008) (Table 2.1, b). The main reason for this inverse relation between temperature and persistence in soils is the increased levels of microbial competition due to increased (metabolic) activity of the native microflora (Semenov et al. 2007).

Indirect effects of climate change on the ecology of E. coli O157 and Salmonella

Several indirect effects of climate change can be defined. Higher temperatures might lead to increased susceptibility of livestock to animal disease. This, might make them more vulnerable to (asymptomatic) colonization by human enteric pathogens. Higher temperatures might also affect feeding strategies which can have a profound effect on the prevalence and shedding rate of human pathogens by altered ecological conditions in the animal gut (Jacob et al. 2009). Direct or indirect effects of climate might also affect the super-shedding phenomenon (i.e. some cattle may harbour and shed bacteria at higher levels that others), which strongly influence dissemination pathogens into the environment and ultimately to humans (Matthews et al. 2006). In addition, with higher temperatures cattle may graze more outside where they are more exposed to pathogens. They then feed on grass, which affects survival and shedding rates (Jacob et al. 2009). These indirect effects are not considered and thus not summarized in Table 2.1.

Precipitation for *E. coli* O157 and *Salmonella*

The impact of precipitation on bacteria contamination of manure and soil is relatively limited. Increased land precipitation intensity together with increased temperature lead to a higher moisture content of the atmosphere at a rate of about 7% for every 1°C rise (Trenberth et al. 2007) and a higher soil moisture content. Such higher air and soil humidity could enhance survival of pathogens in moisturised soil and manure (Warriner 2005, Warriner et al. 2009) (Table 2.1, e).

Higher intensity of rain events also enhance the chance of splashing manure and soil particles to fresh produce (Madden et al. 1996, Franz et al. 2008b, Cevallos-Cevallos et al. 2012) (Table 2.1, f).

2.3.2 Surface water

Temperature for *E. coli* O157 and *Salmonella*

Generally pathogen cell numbers decline over time when added to surface water (Vital et al. 2008). *E. coli* O157, however, has been observed to grow in surface water at 30 °C with low carbon concentration (Vital et al. 2008). The survival of both *E. coli* O157 and *Salmonella* spp. in surface water decreases with increasing temperatures (Rhodes and Kator 1988): the survival of *E. coli* O157 in surface water is up to 13 weeks at 8 °C (Wang 1998) and it strongly decreases with increasing temperatures. But it can still survive up to 8 weeks at 25°C (Wang 1998) (Table 2.1, c). An unusually prolonged outbreak in the summer of 1991 of bloody diarrhoea and hemolytic-uremic syndrome caused by *E. coli* O157 was traced to shallow swimming water (Keene et al. 1994). This outbreak suggests that these foodborne pathogens survive in lake water. Survival of *Salmonella* spp. is greater than that of *E. coli* (the faecal indicator) in surface water. In low water temperatures (less than 10°C), more than 83% of salmonellae survived after 1 week compared to less than 6% of *E. coli* during the same period (Rhodes and Kator 1988) (Table 2.1, c).

Precipitation for *E. coli* O157 and *Salmonella*

Increased temperatures and decreased precipitation enhance evapotranspiration (Meehl et al. 2007). This results in an increased need for irrigation of crops (Table 2.1, g). On the other hand, water scarcity in dry regions may result in future technological and management changes, such as subsurface drip irrigation instead of overhead sprinkler (Fonseca et al. 2011) or new water treatment methods which have lower contamination risk. Such technological changes may lower the risk of contamination via irrigation water.

Intensive precipitation may increase surface and subsurface runoff, which might be an intermediate contamination pathway of pathogens from manure at livestock farms and from grazing pastures (Table 2.1, h). When crops are irrigated with this water, contamination might be increasing.

Flooding as a result of extreme precipitation events can bring pathogens from surface water to fresh produce and might contaminate whole fields (Orozco et al. 2008, Donnison and Ross 2009) (Table 2.1, i).

2.3.3 Sewage

The impact of temperature on pathogen survival in sewage water is very limited. Decreased cell numbers of the *Enterobacteriaceae* family and *Salmonella* genus have been observed with temperature increase (Wolna-Maruwka et al. 2009) (Table 2.1, d). The die-off of commensal *E. coli* and *Salmonella* spp. in wastewater is related to desiccation of the sewage and was faster in warmer and drier conditions (Horswell et al. 2007).

Heavy rainfall in a relatively short time could cause sewer overflows to surface water and/or soil (Tierney et al. 1977, Watkins and Sleath 1981) (Table 2.1, j). This increases the risk of contaminated irrigation water. In drought-prone regions, the dilution of sewage in streams is reduced when surface water discharge decreases. So the concentration of pathogens in the surface water increases. This, increases the concentration of pathogens in the surface water (Senhorst and Zwolsman 2005, Hofstra 2011) (Table 2.1, k). In addition, a shortage of irrigation water and the high costs of artificial fertilizer may increase the use of sewage as a source of water and nutrients (Table 2.1, l).

2.4 Concluding remarks

The objective of this paper was to review and synthesise major impacts of climate change (temperature increases and precipitation pattern changes) on contamination sources and pathways of foodborne pathogens (focussing on *E. coli* O157 and *Salmonella* spp.) on pre-harvested LGVs.

Contamination sources (e.g. soil, manure, water, etc.) and pathways (irrigation, splash, contact with faeces, etc.) of *E. coli* O157 and *Salmonella* spp. onto LGVs were identified. Then the positive and negative impacts of temperature increases and precipitation pattern changes on pathogen prevalence in each contamination source and pathway have been elaborated.

Temperature likely increases everywhere, but precipitation patterns differ largely by region. Already arid regions are expected to become drier, while wet regions are expected to become wetter and extreme precipitation events are expected to occur more often worldwide. These changes have both positive and negative impacts on contamination sources and pathways that influence *E. coli* O157 and *Salmonella* spp. survival in manure, soil and water.

Whether climate change increases the prevalence of *E. coli* O157 and *Salmonella* spp. on pre-harvest LGVs depends on the balance of the positive and negative impacts and on the applied climate change scenarios for specific areas. There are, however, to date no quantitative studies assessing this balance and talking into account all positive and negative impacts. This review shows the need for quantitative modelling approaches with scenario analyses to understand the net impact of climate change on the contamination of pre-harvested LGVs. Also additional laboratory experiments, such as splash tests for both pathogens and LGVs and contamination of LGVs after irrigation with contaminated surface water – issues that appear to be missing from the literature-, would aid our understanding.

This study gives an innovative and extensive overview of the impacts of climate change on the contamination of pre-harvested LGVs. Although the balance of positive and negative impacts requires further study, this review clearly shows that climate change should not be ignored in food safety management and research.

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Table 2.1 The influence of climatic changes on contamination pathways and pathogens survival. *+/- explains the positive/negative relation between the columns "Climate variables" and "Changes". For example, e) precipitation is positively correlated with survival of pathogens in manure and moist soil. So, survival of pathogens in manure and moist soil will increase with increased precipitation and decrease with decreased precipitation. ** the relationship between survival of *E. coli* O157:H7 in sewage and increased temperature is unclear.

Climate variables	Contamination sources	Relationship*	Changes	Pathogens	Reference
Temperature	manure & soil	+	a) use of manure		Franz et al., 2008a
		-	b) survival of pathogens in manure and soil	*E.coli* O157:H7	Himathongkham et al., 1999, Kudva et al., 1998, Mukherjee et al., 2006, Semenov et al., 2007, Wang et al., 1996,
				Salmonella	Danyluk et al., 2008, Himathongkham et al., 1999, Semenov et al., 2007
	surface water	-	c) survival of pathogens	*E.coli* O157:H7	Wang, 1998
				Salmonella	Rhodes and Kator, 1988
	sewage	-	d) survival of pathogens	*E.coli* O157:H7**	
				Salmonella	Wolna-Maruwka et al., 2009
Precipitation	manure & soil	+	e) survival of pathogens in manure and moist soil	*E.coli* O157:H7	Warriner, 2005, Warriner et al., 2009
				Salmonella	Warriner, 2005, Warriner et al., 2009
		+	f) chance of splash		Cevallos-Cevallos et al., 2012, Franz et al., 2008b, Madden et al., 1996

	surface water	-	g) amount of irrigation water	
		+	h) surface and subsurface run-off	
		+	i) chance of flood	Donnison and Ross, 2009, Orozco et al., 2008
	sewage	+	j) chance of sewage overflow	Tierney et al., 1977, Watkins and Sleath, 1981
		-	k) concentration of waste water in surface water stream	Hofstra, 2011, Senhorst and Zwolsman, 2005
		-	l) use of sewage as a source of water and nutrients	

Chapter 3

Modelling Leafy Green Contamination by *Escherichia coli* at Pre-harvest Stage

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Abstract

The observed foodborne disease seasonality suggests that climatic conditions play a role and that changes in the climate may affect pathogens presence. However, whether this effect is direct or indirect through other factors, e.g. farm management remains elusive. This study aimed to identify the climate and management variables that are associated with the contamination (presence and concentration) of leafy green vegetables (LGVs) contamination with generic *E. coli*. This study used *E. coli* data of 562 LGVs (lettuce and spinach) samples taken between 2011 and 2013 from 23 open-field farms from Belgium, Brazil, Egypt, Norway and Spain. Mixed effect logistic regression and linear regression models were used to study the statistical relationship among the dependent and independent variables. Climate and agriculture management practices together both had influence on *E.coli* presence and concentrations. Temperature had a stronger influence (had a significant parameter estimate and highest R-squared) than management practices for *E. coli* presence and concentrations on LGVs. Minimum temperature of the sampling day (odds ratio [OR] 1.47), region and application of inorganic fertilizer were important for *E. coli* presence. Maximum temperature three days before and region were important for concentrations (R^2 = 0.75). *Region* was a variable masking many management variables including rain water, surface water, manure, inorganic fertilizer and spray irrigation. Climate variables had a positive relationship with *E. coli* presence and concentrations. Temperature, irrigation water type, fertilizer type and irrigation method should be considered systematically in fresh produce safety studies in the future.

3.1 Introduction

Leafy green vegetables (LGVs) have been identified as the fresh produce commodity group of the highest concern from a microbiological safety perspective (FAO/WHO 2008), because they are often grown in the open field and vulnerable to contamination from manure used as fertilizer, soil, water used for irrigation, and contact with (faeces of) wildlife (FAO/WHO 2008). Moreover, LGVs are grown and consumed raw and in large volumes. Pathogenic *Escherichia coli* strains are one of the main concerns with respect to foodborne disease associated with LGVs (Takkinen et al. 2005, Friesema et al. 2008, Söderström et al. 2008, Gajraj et al. 2012).

The incidence of foodborne disease is generally correlated with climate conditions (Miraglia et al. 2009, Jacxsens et al. 2010, Tirado et al. 2010). Roughly one-third (population attributable fraction) of salmonellosis cases in England, Wales, Poland, the Netherlands, the Czech Republic, and Switzerland can be linked to higher temperatures (Semenza and Menne 2009). In Australia, the rate of salmonellosis also increases with decreasing latitude and consequently with increasing average annual temperatures (Hall et al. 2002). The correlation between foodborne disease and climatic conditions is (partly) reflected in a strong seasonality of many foodborne diseases. The seasonal salmonellosis patterns have been statistically correlated with the mean monthly temperature of the previous month (D'Souza et al. 2004). Similarly, in the Australian subtropical and tropical regions, temperature and precipitation have been positively associated with the number of salmonellosis cases (Zhang et al. 2010).

The mechanisms underlying the observed seasonality in foodborne disease are not fully understood, but they are likely a complex interplay of different factors. Besides climatic conditions, these factors include human behavior and consumption patterns (Van Staveren et al. 1986, Ziegler et al. 1987), farm management practices, pathogen prevalence in the animal reservoir and pathogen environmental survival patterns. The risk of foodborne disease associated with LGVs is directly related to the likelihood of occurrence and the subsequent level of contamination. The observed seasonality suggests that climatic conditions influence pathogens presence and/or level (Liu et al. 2013). Improved understanding of this is important for better control and surveillance of LGV contamination particularly in the face of ongoing climate change. Whether the effect of climate on LGV contamination is direct or indirect through other factors, e.g. farm management remains elusive. These farm management practices are affected by climate and could be influenced even more or in different ways by climate change. Though uncertain, the effect of seasonality and climate on produce food safety should thus not be ignored as it may result in higher risks (Liu et al. 2013).

Changes in temperature, distribution of precipitation (including more extreme events, such as floods and droughts), ultraviolet radiation (UV) and moisture content are already observed worldwide (Meehl et al. 2007). Temperature has increased since the start of observations in 1654 (Camuffo and Bertolin 2012). Consistent with precipitation changes, runoff is notably reduced in southern Europe and increased in Southeast Asia and at high latitudes. The larger simulated runoff changes reach a 20% increase compared to 1980 to 1999 mean values. Droughts have already become more common, especially in the tropical and subtropical regions since the 1970s (Stocker et al. 2013c). These changes will likely become more apparent in the future (Stocker et al. 2013c). Climate changes will mainly impact the contamination sources and pathways of bacteria onto LGVs during the pre-harvest phase. Other phases of the food chain will be less affected, because generally processing and transport are done in controlled environments.

Good agricultural management practices are essential for food safety control and they are often applied in response to particular climatic conditions (Manning and Baines 2004). Response strategies have been developed to adapt to the pressures on fresh produce safety due to climate change (Kirezieva et al. 2015). The use of animal manure for fertilization of production fields increased the risk of *E. coli* contamination in both organic and conventional farms significantly (Mukherjee et al. 2007, Park et al. 2013, Park et al. 2014). Farmers should follow good agricultural practices for handling animal manure in order to minimize the risk of introducing pathogens. Such practices include manure composting and minimizing direct or indirect contact between manure and produce (Definitions and Water 1998). The use of contaminated surface water may also lead to contamination (Islam et al. 2004a, Islam et al. 2004b, Steele and Odumeru 2004). Groundwater is generally less likely to be contaminated with pathogens than surface water. Shallow wells and improperly constructed or old wells may be more likely to be susceptible to contamination (Definitions and Water 1998, Ceuppens et al. 2014). Infected persons who work with fresh produce also increase the risk of transmitting foodborne illnesses (Definitions and Water 1998). Farmers should understand and follow basic hygienic principles to lower the possibilities of contaminating fresh produce, water supplies and other workers.

A review of the impacts of climate change on micro-organisms (Liu et al. 2013) improved the qualitative understanding that will now be used to study these impacts quantitatively. Farmers are likely to change management practices to adapt to climate change (Kirezieva et al. 2015). It is important to have a better understanding of the quantitative changes in the face of management schemes and climate change. Such quantitative analyses are sparse due to little data availability, so aggregation of the available information in a meta-analysis may be useful to achieve a higher statistical power and generalizability.

This study aimed to explore how climate and agriculture management factors contribute to *E. coli* contamination on LGVs across different regions. The focus was on identifying a combination of statistically significant variables that best explained observed variation in *E.coli* presence and contamination level throughout regions. We addressed these objectives by applying statistical modeling (Section 3.2) on *E. coli* presence and concentration data from production fields in different regions. The meta-analysis in this study combined findings from independent studies from different regions within the Veg-i-Trade project. Subsequently, the results of logistic and linear regressions with climate and management variables were presented and summarized (Section 3.3). Finally data complexity and limitations were discussed and concluded with lessons learnt in this meta-analysis (Section 3.4).

3.2 Methods

3.2.1 Data

The data used in this study were collected within the Veg-i-Trade project which aimed to study the impact of climate change and globalisation on safety of fresh produce. A Horticultural Assessment Scheme (HAS) has been developed in the Veg-i-Trade project to assess the level of microbiological quality of leafy green vegetables. HAS was a systematic approach to sample, analyse and standardise the sampling scheme in various regions within Veg-i-Trade. HAS defined the identification of critical sampling locations, the selection of microbiological parameters, the assessment of sampling frequency, the selection of sampling method and method of analysis, and finally data processing and interpretation (Holvoet et al. 2011). All Veg-i-Trade sampling data used in this study were collected and analysed under HAS.

This meta-analysis included raw sampling data from Holvoet et al. (2013), Ceuppens et al. (2014), Uyttendaele et al. (2014) and Castro-Ibáñez et al. (2014). Our study used *E. coli* data of 562 LGVs samples taken from 23 open-field farms from six regions (Figure 3.1): Belgium (n = 160), Brazil (n = 69), Egypt (n = 18), Norway (n = 99) and Spain (n = 216). All farms grew lettuce, except for the farms in Spain that grew spinach. The data were collected from 2011 to 2013 by different laboratories of the local universities or research institutes within the Veg-i-Trade project. Each laboratory had its own detection limits

(Table 3.1). All samples were taken at the moment of harvest or, one, two or three weeks before harvest. Climate variables included daily average (*Tavg*, *Tavg3*, *Tavg7*), minimum (*Tmin*, *Tmin3*, *Tmin7*) and maximum (*Tmax*, *Tmax3*, *Tmax7*) temperature of the sampling day, three days and seven days before, daily precipitation (*P*), three days (*P3*) and seven days total precipitation (*P7*). Management variables included categorical variables (*Region* and toilet distance (*ToiletD*)) and binary variables (drinking water (*DrinkingW*), rain water (*RainW*), groundwater (*GroundW*), surface water (*SurfaceW*), drip irrigation (*Drip*), spray irrigation (*Spray*), flood irrigation (*Flood*), composted manure (-derived) (*Manure*) including composted manure and mixture with composted manure, inorganic fertilizer (*Inorganic*), non-animal organic fertilizer (*NonAOrganic*), farm animal presence (*FarmA*)). Missing values were omitted when regression models can only run with complete observations. Temperature and precipitation data were collated from the most nearby weather station for each farm. The data sources are summarized in Table 3.1. Management information was collected by means of a farmer questionnaire.

Figure 3.1 Sampling farms distribution. Black dots are sampling farms cooperated within Veg-i-Trade project.

Table 3.1 Summary of leafy green vegetables samples collected in this meta-regression study. Composted manure (-derived) category includes composted manure and mixture with composted manure. Non-animal organic category includes compost and mixture of mineral and organic fertilizer.

	Belgium	Brazil	Egypt	Norway	Spain	Total
Total # of samples	160	69	18	99	216	562
***E. coli* Detected**	20	14	10	3	12	59
Percentage (%)	12.5	20.3	55.6	3	5.6	10.5
Detection limit (log10 CFU/g)	0.7	1	1	1	2	
***Tavg* Min-Max (mean) (°C)**	12.4-19.6 (17.4)	15.5-29.8 (24.1)	15.4-25.9 (19.8)	7.7-16.2 (12.5)	8.4-17.3 (12.8)	
***Tavg3* Min-Max (mean) (°C)**	10.8-25.0 (16.9)	16.3-27.6 (22.9)	16.1-27.3 (19.9)	5.2-21.6 (12.5)	8.5-19.8 (13.2)	
***Tavg7* Min-Max (mean) (°C)**	13.8-21.5 (17.6)	15.6-30.5 (23.3)	13.8-29.6 (21.0)	6.4-16.4 (13.1)	7.1-16.9 (12.5)	
***Tmax* Min-Max (mean) (°C)**	15.5-28.1 (22.0)	16.0-36.0 (29.8)	22.3-31.2 (25.8)	3.0-21.7 (14.9)	12.32-24.7 (18.2)	
***Tmax3* Min-Max (mean) (°C)**	13.2-33.7 (22.4)	22.0-35.0 (28.9)	20.9-34.8 (26.6)	0.4-22.9 (15.0)	13.2-24.0 (19.14)	
***Tmax7* Min-Max (mean) (°C)**	8.6-15.7 (12.2)	16.0-36.0 (28.9)	20.4-38.3 (28.0)	8.6-21.7 (15.2)	10.8-24.7 (17.7)	
***Tmin* Min-Max (mean) (°C)**	6.9-16.6 (13.2)	11.0-22.0 (17.9)	10.0-21.6 (14.3)	1.7-21.8 (10.0)	0.8-12.8 (7.6)	
***Tmin3* Min-Max (mean) (°C)**	4.8-18.5 (12.0)	10.0-23.0 (17.0)	8.2-19.8 (13.7)	-1-28.00 (10.42)	2.2-15.2 (8.1)	
***Tmin7* Min-Max (mean) (°C)**	8.6-15.7 (12.2)	11.0-22.0 (16.7)	7.4-23.0 (14.4)	1.7-21.6 (10.9)	1.0-12.9 (7.7)	
Maximum precipitation amount: *P*, *P3*, *P7*(mm)	17.5, 38.3, 67.0	42.1, 110, 214.6	0.0, 0.0, 0.0	21.4, 40.2, 58.0	16.1, 20.2, 36.8	
Weather data source	The Royal	Instituto	www.tutiempo.net	Norwegian	Sistema de	

	Meteorological Institute of Belgium (RMI)	Naciional de Meteorologia (INMET)	www.worldweatheronline.com	Meteorological Institute web portal eKlima, LandbruksMeteorologisk Tjeneste (lmt.bioforsk.no)	Información Agrario de Murcia (SIAM) (Siam.imida.es)
Rain water (# of samples)	160	57	0	0	0
Surface water (# of samples)	0	0	12	48	216
Drinking water ((# of samples)	0	0	0	27	0
Groundwater (# of samples)	0	12	6	0	0
Spray irrigation (# of samples)	160	57	0	99	216
Drip irrigation (# of samples)	0	12	12	0	0
Flood irrigation (# of samples)	0	0	6	0	0
Composted manure (derived) (# of samples)	24	57	18	51	216
Inorganic fertilizer (# of samples)	88	0	0	48	0
Non animal organic (# of samples)	48	12	0	0	0
Farm Animal Presence (# of samples)	113	24	No information	0	0
Toilet Distance	0-100m	0-100m 100-200m 200-500m	No information	No information	0-100m 100-200m 200-500m

3.2.2 Statistical model

The data were analysed using the statistical software package R version 3.0.2. All statistical tests were assessed for significance at the 95% confidence level ($p < 0.05$) except for univariate analysis ($p < 0.25$).

The data were checked for collinearity by the Variance Inflation Factor (VIF) between categorical and binary variables and the phi coefficient between binary variables. In case of collinearity (VIF > 2 or phi coefficient > 0.6) the variable with the least biological relevance was omitted from further analysis.

We focussed on assessing the relationship of *E. coli* with climate variables first and then assessed management variables. In this way, impacts of climate or management variables can be analysed separately in order to further understand how climate or management individually influence the LGVs safety. After that all variables were combined in the final model to study the overall effects of climate and management influence on LGVs safety. Due to the hierarchical structure in the data (with repeated sampling at the same farm) a mixed effects model with Farm as random effect was applied. All variables from Table 3.1 are included in the models.

The stepwise selection method developed by Hosmer and Lemeshow (2004) was used to select variables for the logistic regression model. Spearman's rank correlation was used to assess correlations between numeric and ordinal variables. Univariate analysis was applied by fitting a univariable regression model to obtain the estimated coefficient, the estimated standard error, the likelihood ratio test for the significance of the coefficient and the univariable Wald statistic. Any variable with a likelihood ratio test that has a *p*-value < 0.25 is a candidate for the multivariable model. Using a more tolerant significance level ($p < 0.25$ instead of $p < 0.05$) allowed for inclusion of variables that are of potential importance at the model building stage (Bendel and Afifi 1977, Mickey and Greenland 1989). With these variables, we followed the backward selection method to choose the variables for the multivariable model. The overall importance of each categorical variable included in the multivariable model was verified by an examination of the Wald statistic. Variables which were not selected for the multivariable model were added back into the model. By doing this we could identify the variables that by themselves are not significantly related to the *E. coli* present but make an important contribution in the presence of other variables (Hosmer Jr and Lemeshow 2004). Interactions and quadratic terms were checked for the variables in the model.

Finally the mixed-effect model was implemented through the "lme4" package (Bates et al. 2014) and "lmeTest" package (Kuznetsova et al. 2014) in R software with the random effect Farm. In this study, all samples were combined and treated as one data set. *Region* was taken as fixed variable to exacerbate the differences in the sampling effort and detection limits among regions.

Logistic regression

To investigate the *E. coli* presence/absence on LGVs in the open field farms data (n = 562) were fitted to a logistic regression model, combining the different variables and locations together. This model aimed to separately assess the contributions of the climate and management variables to the observed variation in *E. coli* presence.

In logistic regression model, Akaike Information Criterion (AIC) was used to compare and select the best model. AIC measures the relative quality of a model for model selection (Akaike, 1974). After the final model was chosen, the odds ratio (OR) was calculated from the parameter coefficients. The parameter coefficients give the change in the log odds of the *E. coli* presence for a one unit increase in the predictor variable.

To assess the robustness of a model's predictive ability, a 10-fold cross validation was conducted. The data were randomly divided into 10 subsets of equal size and 9 subsets were used for training the model while the 10^{th} subset was used to test the model's predictive ability. This process was repeated 10 times, every time with a different test subset. So all observations were used for both training and testing, and each observation was used for validation exactly once. The mean area under the curves (AUC) was calculated. An area of 100% represents a perfect test and an area of 50% represents a worthless test.

Multiple linear regression

In the next step, we studied the observed variation in log10 transformed *E. coli* concentration levels to approximate data normality. The *E. coli* positive data (n = 59) was fitted to a linear mixed effect model, combining the different variables as fixed effects variables and Farm as a random effect. Visual inspection of residual plots (standardized residuals with fitted value) for homoscedasticity test and a q-q plot for normality test were performed to check the assumptions for linear regression. Such linear regression model assessed the relative contributions of the climate and management variables to the observed variation in *E. coli* concentrations. We again used the Hosmer and Lemeshow (2004) method and the backward approach to select independent variables. The F-test was used to select the best model fit. Root Mean Square Error (RMSE) and Mean Absolute Error (MAE) were calculated to evaluate models. Lower values are better.

3.3 Results

3.3.1 Data

In total 59 *E. coli* samples were positive. Very few samples (in total 18) were tested for *E. coli* on LGVs on farms in Egypt but more than half of them were positive (Table 3.1). Among 99 samples in Norway, only 3 of them were *E. coli* positive (Table 3.1). The presence ranged between 3% in Norway to 20.3% in Brazil. But these differences should be taken with awareness of large differences in the lower detection limit among studies, ranging from 0.7 log10 CFU/g in Belgium to 2 log10 CFU/g in Spain (Table 3.1).

The mean (median) concentration of the positive samples was 1.91 (2.00) log10 CFU/g with standard deviation of 0.81. In general, the highest *E. coli* concentrations were found in samples from Brazil (Figure 3.2). The observed *E. coli* concentrations on LGVs range from the detection limits to 3.9 log10 CFU/g in Brazil (Figure 3.2). *E. coli* concentrations below the detection limits are indicated as 0 log10 CFU/g in Figure 3.2.

The *Tavg* in Brazil was very high (up to 30°C, Table 3.1). Brazil also had highest *P* among all regions (Table 3.1). In contrast, Egypt had no rain on the sampling days. In general, the days which *E. coli* positive samples were found were dry in all regions, except for Brazil (Table 3.1). The variation in climate was not only due to the different geographic locations, but also to different growing seasons. For example, farmers in Spain grow spinach in their winter time from September to March to avoid the high temperature in their summer time, while farmers in the rest of the regions grow lettuce during their summer time. Consequently, the temperature during the sampling period in Spain had a similar range as the temperature during the sampling period in other regions, such as Norway (Table 3.1). In total about 60% of the sampling days had precipitation amount less than 0.1 mm.

In this dataset management variables were region specific. Some of the regions happened to have only one type of irrigation water or irrigation method. For instance, flood irrigation was applied only in Egypt. All samples from Belgium used rain water and all samples from Egypt and Spain used composted manure or a mixture with composted manure. The details for each management variable are summarised in Table 3.1.

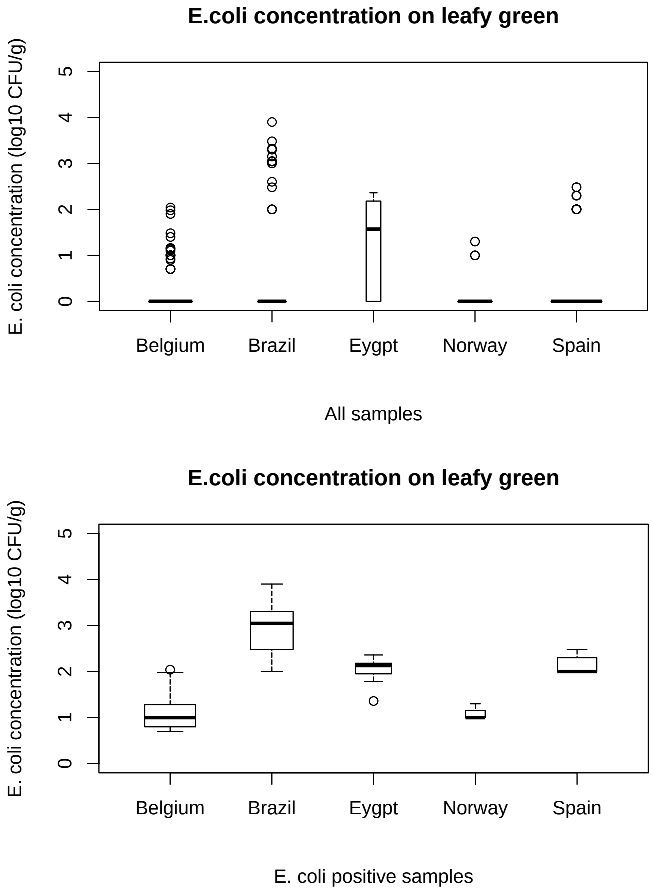

Figure 3.2 E. coli concentration (top: all samples, bottom: positive samples only) on lettuce sampled at farms in Belgium, Brazil, Egypt, and Norway and on spinach at farms in Spain. The box width is proportional to the square-roots of the number of observations in the regions.

3.3.2 *E. coli* presence on leafy green vegetables

Logistic regression was applied separately for both climate variables and management variables to assess relations with *E. coli* presence on LGVs using the method described in Section 3.2.2. Results are presented accordingly in this section.

Climate variables and *E. coli* presence

For climate variables, univariate analysis results showed that all variables in this analysis had a *p*-value <0.25 except for P. Since temperature or precipitation variables were not independent, only one variable should be selected for temperature and one for precipitation. The univariate Wald Test suggested that *P3* was the only significant precipitation variable (p < 0.05). And *Tmin* had the lowest AIC value. So *Tmin*, *P3* and *Region* were chosen after the univariate analysis.

Upon completion of the univariate analyses, firstly the variables *Tmin*, *P3* and *Region* were selected for the multivariable analysis. With backward selection, the Wald test for importance of the categorical variable and interaction check, the best model was the following:

$$ln \frac{p}{(1-p)} = \beta_0 + \beta_1 Tmin + \beta_2 P3 + \beta_{3,Region} + \varepsilon_{Farm} \qquad \text{Eq.1}$$

Where p = the probability of having an *E. coli* positive sample, *βi* are constants, *Tmin* = minimum temperature of the sampling day in °C, *P3* = total precipitation amount of three days before sampling day, $\beta_{3,Region}$ = dummy variable for region, ε_{Farm} is the random effect for farm. The β estimates are available in Table 3.2. However some samples had no *E.coli* presence at high *Tmin* and some samples had *E. coli* presence with low P3.

The estimated coefficients showed the odds of having *E. coli* positive samples on LGVs increased when temperature and cumulative precipitation amount increased with one measurement unit (°C or mm). For 1°C increase in daily minimum temperature, the odds of having *E. coli* positive samples on LGVs increased by a factor of 1.48 (95%CI 1.27-1.73) (Table 3.2). For 1mm increase in three days cumulative precipitation, the odds of having *E. coli* positive samples on LGVs increased by a factor of 1.02 (95%CI 1.01-1.03) (Table 3.2). *Tmin* and *P3* had a statistically significant relation with *E. coli* presence.

Table 3.2 Final mixed effect logistic regression models to estimate generic Escherichia coli absence/presence on lettuce and spinach.

	β_i Estimate	Odds Ratio(95% CI)	p-value
Climate model (n = 559):			
Variance (standard deviation) was 3.06 (1.75) for random effect Farm			
β 0	-8.339	0.00(0.00-0.01)	<0.000
β 1 (Tmin)	0.391	1.48(1.27-1.73)	<0.000
β 2 (P7)	0.019	1.02(1.01-1.03)	0.003
β 3,Belgium	Reference	Reference	Reference
β 3,Brazil	-1.203	0.30(0.02-3.70)	0.348
β 3,Egypt	3.092	2.20 (1.64-295.47)	0.020
β 3,Norway	-2.088	0.12(0.00-3.61)	0.225
β 3,Spain	0.327	1.39(0.06-29.69)	0.834

Management model (n = 520): Variance (standard deviation) was 1.33 (1.15) for random effect Farm			
β 0	-1.184	0.31(0.02-4.28)	0.379
β 1,RainW	2.497	12.14(1.80-81.99)	0.010
β 2,Spray	-3.315	0.04(0.00-0.86)	0.040
Joint model (n = 520): Variance (standard deviation) was 1.81 (1.35) for random effect Farm			
β 0	-9.016	0.00(0.00-0.00)	<0.000
β 1 (Tmin)	0.401	1.51 (1.29-1.76)	<0.000
β 2,SurfaceW	0.844	2.33 (0.23-23.78)	0.476
β 3,Inorganic	1.334	3.80 (0.50-29.01)	0.198
β 0	-9.433	0.00(0.00-0.00)	<0.000
β 1 (Tmin)	0.388	1.47(1.26-1.72)	<0.000
β 2,Belgium	Reference	Reference	Reference
β 3,Brazil	0.664	1.94(0.14-27.11)	0.621
β 4,Norway	-17.589	0.00(0.00-0.00)	0.997
β 5,Spain	2.273	9.71(0.00-168.09)	0.118
β 6,Inorganic	2.714	1.51(0.92-247.23)	0.057

Management variables and *E. coli* presence

For management variables both univariate analysis results and Wald test results showed that all variables in this analysis had a p-value < 0.25 except for NonAOrganic. Drinking water was not used for any samples after the missing values were removed. DrinkingW was therefore dropped from multivariate analysis. All other management variables were selected as candidates of the multivariate model (n = 520). The VIF results showed that *Region* had collinearity with *RainW*, surfaceW and Spray. This meant the region effect in *E. coli* presence model may be a proxy for these management practices. Although we preferred to keep *Region* in the model to exacerbate the differences in the sampling effort and detection limits among regions, it would be a huge sacrifice to avoid so many variables which have more relevance factors. Therefore *Region* was not included in the selection.

Upon completion of the univariate analyses, all variables were selected for the multivariable analysis. With backward selection and the Wald test for importance, the best model was following with Farm as a random effect term:

$$ln\frac{p}{(1-p)} = \beta_0 + \beta_{1,RainW} + \beta_{2,Spray} + \varepsilon_{Farm} \qquad \text{Eq.2}$$

Where p = the probability of having an *E. coli* positive samples, β_i are constants, $\beta_{1,\ RainW}$ = dummy variable for rain water, $\beta_{2,\ Spray}$ = dummy variable for spray irrigation, ε_{Farm} = random effect for farm. Spray irrigation showed a protective effect (Table 3.2). Using rain water for irrigation versus other irrigation water types increased the odds of having *E. coli* contamination on LGVs by a factor of 12.14 (Table 3.2).

All variables and *E. coli* presence

The backward selection was performed once more combining significant (p-value < 0.25) climate and management variables to predict *E. coli* presence on LGVs. Region was again not included in the backward selection. The combined model has the following form:

$$ln\frac{p}{(1-p)} = \beta_0 + \beta_1 Tmin + \beta_{2,SurfaceW} + \beta_{3,Inorganic} + \varepsilon_{Farm} \qquad \text{Eq.3}$$

Where p = the probability of having an *E. coli* positive samples, *Tmin* = minimum temperature in °C, β_i are constants, $\beta_{2,SurfaceW}$ = dummy variable for surface water, $\beta_{3,Inorganic}$ = dummy variable for inorganic fertilizer, and ε_{Farm} = random effect for farm. Because *Region* had collinearity with SurfaceW, the joint model was run again with the variable *Region* instead of SurfaceW. Comparing to Eq.3 a significantly lower AIC was found in that model with the following form:

$$ln\frac{p}{(1-p)} = \beta_0 + \beta_1 Tmin + \beta_{2,Region} + \beta_{3,Inorganic} + \varepsilon_{Farm} \qquad \text{Eq.4}$$

Where p = the probability of having an *E. coli* positive samples, *Tmin* = minimum temperature in °C, β_i are constants, $\beta_{2,Region}$ = dummy variable for regions, $\beta_{3,Inorganic}$ = dummy variable for inorganic fertilizer, and ε_{Farm} = random effect for farm. The odds ratio (Table 3.2) shows that for a 1°C increase in minimum temperature, the odds of having *E. coli* contamination on LGVs increase by a factor of 1.47, assuming management remains the same. In both Eq.3 and Eq.4, *Tmin* had significant influence on estimating *E. coli* contamination. Although other variables did not have a significant p-value (< 0.05), they improved the model fit significantly according to the AIC test. Therefore management variables were also included in the final mixed model. Although Eq.4 was the best model fit for this meta-analysis, we also presented Eq. 3 because it provided more understanding of the variable *Region* and it would be more useful than Eq.4 for future studies on prediction model in specific region. Cross validation analysis of Eq.4 showed that the mean AUC was 88% (range 79% to 95%) (Figure 3.3). So the model has a good predictive value.

Since *Tmin* was the only significant variable in the joint model, we concluded that although climate and management variables together influence *E. coli* presence on LGVs, *Tmin* had stronger influence on *E. coli* presence than management variables.

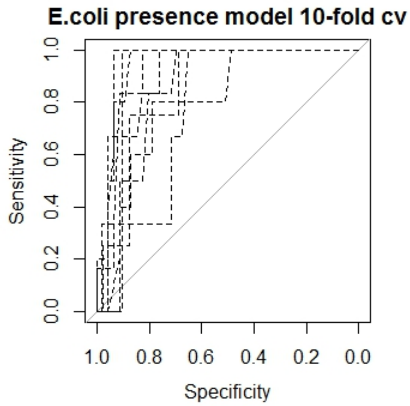

Figure 3.3 Receiver operating characteristic (ROC) curves for each of the 10-fold cross validation.

3.3.3 *E. coli* concentrations on leafy green vegetables

Visual inspection of normality plots of standardized residuals and the homoscedasticity test (residual plot of standardized residuals with fitted values) did not reveal any obvious deviation from normality. The random effect Farm was dropped because its effect was negligible to the smaller dataset (59 samples from 16 farms). To assess the effect of the climate variables and management variables on the *E. coli* concentrations, *E. coli* positive samples were fitted to the linear regression model with the method explained in Section 3.2.2. Results of climate and management variables are presented in this section.

Climate variables and *E. coli* concentration

Based on univariate analysis, variables *Tavg, Tavg3, Tavg7*, *Tmax*, *Tmax3* (highest R-squared: 0.38), *Tmax7*, *Tmin* and *P7*were significant (p < 0.25). Since the temperature variables were not independent, only one variable should be selected. So *Tmax3*, *P7*and *Region* were chosen after the univariate analysis. With backward selection, the Wald test for importance of the categorical variable, the F-test to select the best model fit and the interaction check, the final model had the following form:

$$Y = \beta_0 + \beta_1 Tmax3 + \beta_{2,Region} \qquad \text{Eq.5}$$

Where *Y* = *E. coli* concentration in log10 CFU/g, β_i are constants, *Tmax3* = maximum temperature of three days before sampling day in °C and $\beta_{2,Region}$ = dummy variable for region. Maximum temperature was significantly and positively correlated with *E. coli* concentrations (Table 3.3). This model gave RMSE of 0.38 and an MAE of 0.30 indicating a high accuracy. The adjusted R-squared was 0.75 with an associated *p*-value less than 0.00. Figure 3.4 graphically shows the regression of *Tmax3* and *E. coli* concentration for each region. This regression gave an adjusted R-squared of 0.38 and an associated *p*-value of 0.00 (Figure 3.4) indicating *E. coli* concentrations had a significant positive correlation with *Tmax3*.

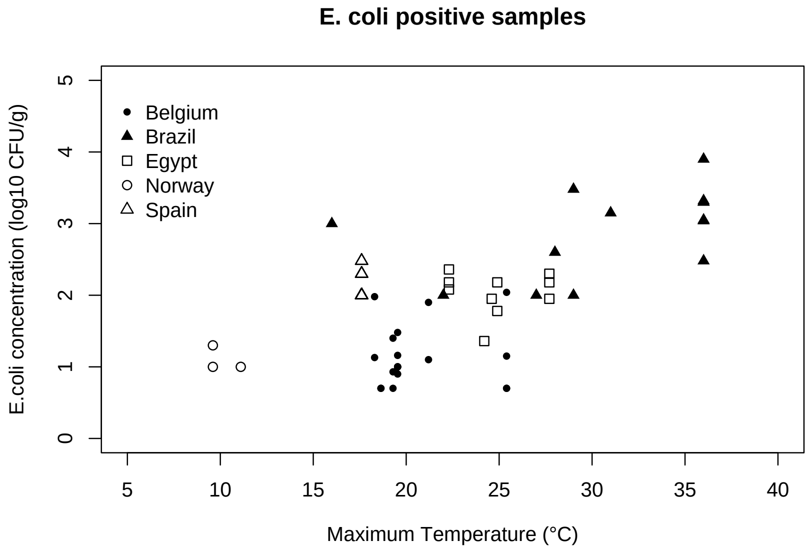

Figure 3.4 Correlation of E. coli concentration with daily average temperature for each region.

Table 3.3 Parameter estimates, standard error and p-value in the final linear regression models to estimate generic *Escherichia coli* (log10 CFU/g) on lettuce and spinach.

	β_i Estimate	Std. Error	p-value
Climate model (n = 59, R^2 = 0.75)			
β 0 (Intercept)	0.258	0.432	0.553
β 1 (Tmax3)	0.041	0.020	0.043
β 2,Belgium	Reference	Reference	Reference
β 2,Brazil	1.432	0.215	<0.000
β 2,Egypt	0.765	0.169	<0.000
β 2,Norway	0.287	0.294	0.333
β 2,Spain	1.090	0.151	<0.000

Management model (n = 56, R^2 = 0.67)			
β 0 (Intercept)	1.561	0.236	<0.000
β 1,RainW	0.471	0.185	0.014
β 2,Spray	0.594	0.195	0.004
β 3,GroundW	1.663	0.357	<0.000
β 4,Inorganic	-1.544	0.169	<0.000
Joint model (n = 56, R^2 = 0.69)			
β 0	-0.502	0.589	0.399
β 1 (Tmax3)	0.083	0.016	<0.000
β 2,Manure	0.613	0.249	0.017
β 3,Inorganic	-0.557	0.266	0.041
β 4,Spray	0.391	0.168	0.024

Management variables and *E. coli* concentration

In the management model, variables *FarmA* and *ToiletD* were excluded in the univariate analysis, because the missing values in these two variables were 17% and 25% of the total samples. Too many samples would have to be excluded for the regression analysis if these two variables were included in the dataset. Three samples from Norway were excluded from the dataset due to the missing values in irrigation water type. Upon completion of the univariate analysis, all variables had a *p*-value < 0.25 except for *SurfaceW* and *Flood*. The VIF results showed that *Region* had multi-collinearity with *RainW*, *Manure*, *Inorganic* and *Spray*. This meant that the region effect may be a proxy for these management practices. Therefore *Region* was not included in the selection. With backward selection, F-test and interaction check, the final model (n = 56) had the following form:

$$Y = \beta_0 + \beta_{1,RainW} + \beta_{2,Spray} + \beta_{3,GroundW} + \beta_{4,Inorganic} \quad \text{Eq.6}$$

Where Y = *E. coli* concentration in log10 CFU/g, β_i are constants, $\beta_{1,RainW}$ = dummy variable for rain water, $\beta_{2,Spray}$ = dummy variable for spray irrigation, $\beta_{3,GroundW}$ = dummy variable for groundwater and $\beta_{4,Inorganic}$ = dummy variable for inorganic fertilizer. The influence of rain water, spray irrigation, groundwater and inorganic fertilizer were significantly different from all other irrigation water types and fertilizer types (Eq.6). This model gave an RMSE of 0.44 and a MAE of 0.32 indicating a high accuracy. Adjusted R-squared is 0.67 with a *p*-value less than 0.00. The model parameter coefficients are given in Table 3.3. Inorganic fertilizer gave a protective effect compared with other fertilizer types (Table 3.3). *E.coli* concentrations were positively related with *RainW*, *Spray* and *GroundW*.

All variables and *E. coli* concentration

Climate variables and management variables were then combined to predict *E. coli* concentrations. All variables selected for the multivariable analysis in the previous two models were combined for the stepwise selection. According to this joint model, the *E.coli* concentrations on LGVs were estimated with the following equation:

$$Y = \beta_0 + \beta_1 Tmax3 + \beta_{2,Manure} + \beta_{3,Inorganic} + \beta_{4,Spray} \qquad \text{Eq.7}$$

Where *Y* = *E. coli* concentration in log10 CFU/g, β_i are constants, $\beta_{2,Manure}$ = dummy variable for composted manure (-derived), $\beta_{3,Inorganic}$ = dummy variable for inorganic fertilizer, $\beta_{4,Spray}$ = dummy variable for spray irrigation. In the joint model *Tmax3*, *Manure*, *Inorganic* and *Spray* were selected to estimate *E.coli* concentrations. This model gave RMSE of 0.42 and a MAE of 0.31 indicating a high accuracy. Adjusted R-squared was 0.69 with a *p*-value less than 0.00. Inorganic fertilizer again had a protective effect compared to other fertilizer types (Table 3.3). *E.coli* concentrations were positively related to higher maximum temperature and using composted manure (-derived) and spray irrigation.

This model had a lower adjusted R-squared than Eq.5. Multi-collinearity was present among *Region*, *Manure*, *Inorganic* and *Spray*. Eq.5 was the best model fit for estimating *E .coli* concentration on LGVs based on this meta-regression analysis and the variable *Region* was masking three other management variables. Eq.7 was, though, useful for future prediction modelling in a specific region.

We concluded that both climate and management variables influence the *E.coli* concentration significantly. *Tmax* had the strongest influence (adjusted R-squared of 0.38) among all variables.

3.4 Discussion

Our study identified a combination of statistically significant variables that best explained observed variation in *E. coli* presence and concentrations. A two-step approach was taken to first study the relation between climate and management variables and *E. coli* presence and secondly to study the relationship between climate and management variables and *E. coli* concentrations. For climate variables, *Tmin* and *P7* were important for the presence. *Tmax3* was important for the concentration. For management variables, *RainW* and *Spray* were important for both presence and concentrations. In addition to these two variables, *GroundW* and *Inorganic* were also important to estimate *E. coli* concentrations. When climate and management variables were combined, both temperature and management practices influenced the *E. coli* presence and concentrations together. Temperature had a stronger influence (shown by significant parameter estimate and highest R-squared) than management practices for *E. coli* presence and concentration on LGVs.

Inorganic fertilizer had a positive parameter estimate in the *E. coli* presence model. This differed from our expectation since inorganic fertilizer should be sterile. However, the parameter estimate was not significant, meaning that, although inorganic fertilizer significantly explained data variation, the parameter estimate was not representative for indicating directional changes in *E. coli* contamination. The contra-intuitive risk factor of inorganic fertilizer most likely is due to the nature of the dataset and the fact that regression analysis does not identify true causal relations. Many of the positive samples arose from farms using inorganic fertilizer but the true contamination source may be other factors not considered in this study. The farmers applying inorganic fertilizer happened to have a higher percentage of positive samples than the ones using composted manure. Almost all of these positive samples from farmers applying inorganic fertilizer were from the same farm in Belgium. The contamination cannot be from the inorganic fertilizer, but is most likely due to the hygiene conditions on the farm or other factors that cannot be explained by our dataset. Non-significant variables should not be used in predicting future *E. coli* presence, while we think the *E .coli* concentration model with an adjusted R-squared of 0.75 is applicable for scenario analysis.

Our results for *E.coli* presence show similar results compared to other studies. Results from Park et al. (2015) indicated that the *E.coli* presence was determined by the environment (state). Strawn et al (2013) also summarised that manure application, irrigation water, temperature and precipitation increase the risk of pathogen contamination. Pagadala et al (In press) stated with their univariate analysis that irrigation water source was a significant variable for all indicator bacteria on tomatoes. And *Region* was a significant variable for total coliforms levels.

This study on *E. coli* concentrations has slightly different results compared to the study of Park et al. (2014, 2015) which are, to our knowledge, the first studies on *E. coli* presence and concentration combined with climate and management variables. They concluded that *E.coli* presence was determined by farm management (manure application), environment (state) and climate variables (29 days average precipitation). Once a contamination event had occurred, the count of generic *E.coli* on spinach was determined by weather only (mean precipitation of the past 29 days and mean maximum temperature over the past 9 days) (Park et al. 2015). The results from our study showed that if *E. coli* is present on LGVs, the concentration was determined by *Tmax3* and *Region* which was a variable with high collinearity with management variables. We found that the influence on *E. coli* presence was a combination between climate and management. This is probably because the different model objectives and experimental set up. The study of Park et al. (2015) tested a cumulative weather effect which considered the survival of *E. coli* between contamination and sampling. We did not take the dynamics in the period between contamination and sampling into consideration due to lack of information on UV which is the most important factor for bacteria survival.

Our results showed that the *E. coli* presence and concentrations had positive relationships with temperature. Castro-Ibáñez et al (2014) also found that coliform counts were positively related to temperature. An increasing temperature due to climate change may increase the *E. coli* concentrations in the future although the actual *E. coli* concentrations increase with 1°C is low. A direct positive effect of temperature on *E. coli* presence and contaminations is, however, not expected given the general observed negative relation between temperature and environmental persistence (Franz et al. 2014). From a microbiology perspective, *E. coli* is expected to have reduced survival with increasing temperature in soil, manure or water (Wang and Doyle 1998, Mukherjee et al. 2006, Liu et al. 2013, Franz et al. 2014). Temperature may affect environmental factors like wildlife intrusion, insect activity, and irrigation frequency, which in turn directly affect *E. coli* presence and concentrations. These environmental factors should be included in the future sampling and analysis to cover more potential contamination pathways.

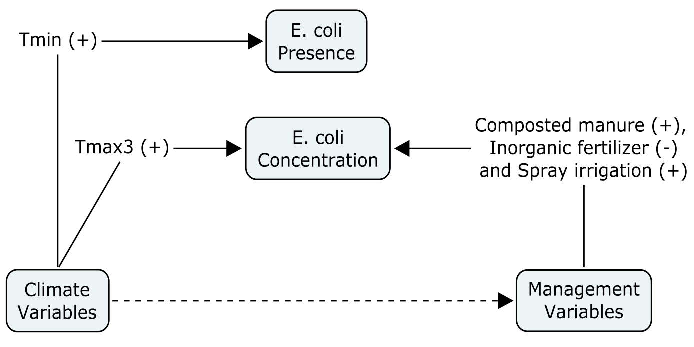

Figure 3.5 A diagram shows statistically significant effect of climate and management variables on *E. coli* presence and concentration. Solid arrows indicate quantified significant effects from this study. Dash arrows indicate unquantified potential association. Plus (minus) signs indicate positive (negative) relationship between variables and *E. coli* presence/concentration.

The relationships found in this study for *E. coli* concentrations, climate and management variables are illustrated in Figure 3.5. Both climate and management variables had a direct and positive relationship with *E. coli* presence and concentrations. In addition, climate may indirectly relate to management variables and then influence the *E. coli* concentrations. Although these management practices vary in different temperature zones, this difference is often due to different social-economic conditions in these zones. Fertilizer type and irrigation water type may also be influenced by Gross Domestic Product (GDP) and GDP happens to be lower in higher temperature countries in this study. We do not have sufficient data to conclude on this. Further research is needed to prove climate variables influence *E. coli* concentrations strongly via management practices.

The current study has some minor limitations. First, LGV samples used in the modelling may not represent the situation in large farms due to high uncertainty of the measurements. Secondly, the length of measurement period was short to observe the changing management behaviour due to climate change. However, a broad temperature range in the data was able to cover that. Third, the precipitation data used may not fully represent the precipitation over the farms. Although the most nearby weather station next to the LGVs farm were taken, the distance still ranged up to 50 kilometres away from the farm. Fourth, only little precipitation was observed on the sampling days and we have thus far not looked at rain amounts over more than 7 days. Medina-Martínez et al (2015) found that lettuce was contaminated after a flood in Spain and bacteria disappeared after 7 days due to UV radiation. Fifth, overall warmer regions happened to have comparatively many positive samples. We did not study whether this is also because of the other social-economic factors, e.g. facility availability and knowledge of the farmers. Sixth, besides climate and management variables, many other environmental variables influence *E. coli* contamination on LGVs, e.g. wildlife fencing around the farms, wildlife appearance frequency. These environmental factors were not included in the regression analysis.

Meta-analysis provides opportunities to perform statistical analysis with limited positive samples from each region. Including the regions with different climate conditions and *E. coli* concentrations enlarges the range of temperature and *E. coli* concentrations. This way, our analysis is founded on a larger range of data. Although *Region* appears to be an important variable for *E. coli* contaminations in this study, the meta-analysis allows a generic model to identify the statistically significant variables for *E. coli* contamination throughout the regions. From this study we have learnt several lessons for future meta-analysis: a) Experimental design in meta-analysis has to be standardized as much as possible. In our study all samples were taken according to the HAS which is developed in the Veg-i-Trade project. Each partner used the same sampling method and questionnaire. However, the sampling and analysis in different regions were not designed for a meta-analysis from the start. Even though the sampling was performed according to the same scheme, we had to work with two produce types (lettuce and spinach) and, more importantly, different detection limits. b) Meta-analysis has more strict sampling requirements than individual studies. The combined datasets need to have a representation that is as balanced as possible of all levels of management variables. If a study is set up for a meta-analysis, then every management variable would ideally have the same amount of samples. It is better to have all management variables covered in each region. When the data from other studies outside the Veg-i-Trade are included, some management variables only occurred in one region. Therefore it was impossible to include other studies outside the Veg-i-Trade project in this meta-analysis. This highlights the need for a coordinated future international sampling collection effort and for development of study design and reporting standards to assure that the data collected and results reported in different regions are comparable and could be used in subsequent meta-analyses. Including studies with very different sampling efforts may give different results than the studies with similar sampling efforts. c) Some of the regional differences are not defined in meta-analysis and they should not be ignored. The differences in joint models with and without *Region* show that the variable *Region* explained the regional variations in many management practices, but also additional regional differences. I recommend to enlarge the model boundary in future studies by including these additional differences (e.g. variation in detection limits, experimental material and equipment, local hygiene, social economic development levels, presence of wildlife intrusion, insects activity, irrigation frequency, soil type and slope/topography) to complete the system analysis of LGVs safety.

This is the first large scale meta-analysis on *E. coli* presence and concentrations on LGVs. This study combined climate and management variables from 23 farms and included more than 562 samples. The current study sets the baseline for future monitoring of climate and contamination relationships. The significant climate and management variables (temperature, fertilizer and irrigation water types and irrigation methods) determined in this study should be considered systematically in fresh produce safety studies in the future.

Acknowledgement

The authors thank Dr. Evert-Jan Bakker for his statistical advice and help in this study. This research is funded by the EU FP7 Veg-i-Trade project (Grant agreement no 244994).

Chapter 4

Preparing Suitable Climate Scenario Data to Assess Impacts on Local Food Safety

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Abstract

Quantification of climate change impacts on food safety requires food safety assessment with different past and future climate scenario data to compare the current and future conditions. This study presents a tool to prepare climate and climate change data for local food safety scenario analysis and illustrates how this tool can be used with impact models. As an example, coarse gridded data from two General Circulation Models (GCMs), HadGEM2-ES and CCSM4, are selected and downscaled using the 'Delta method' with quantile-quantile correction for Ukkel, Belgium. Data are provided for four future Representative Concentration Pathways (RCPs) for the periods 2031-2050 and 2081-2100. The climate projections for these RCPs show that both temperature and precipitation will increase towards the end of the century in Ukkel. The climate change data are then used with Ratkowsky's bacterial growth model to illustrate how projected climate data can be used for projecting bacterial growth in the future. This example shows that this downscaling method can be applied to assess future food safety. Our approach helps food safety researchers to perform their own climate-change scenario analysis. The actual algorithm of the downscaling method and its detailed manual is available in the supplementary material of the original publication.

4.1 Introduction

The likelihood of food contamination is strongly related to prevailing weather and climate (FAO 2008, Nelson 2009, Lake et al. 2010, Liu et al. 2013). Temperature and precipitation patterns are, for example, closely related with not only the fate and transport of enteric bacteria but also with their growth and survival. A temperature increase and shifts in precipitation intensity and patterns change contamination processes (Liu et al. 2013). Additionally, climatic change, affects toxigenic fungi colonization and diffusion, and enhances the production of mycotoxins (Miraglia et al. 2008). Moreover, increased temperature and changing precipitation more rapidly degrade pesticides and thus can increase the use and costs of pesticides on certain crops (Chen and McCarl 2001). Pests from the southern areas may occur in the North due to temperature increase, although pesticide reformulation can be expected with new technology (Delcour et al. this issue). Liu et al (2013) clearly showed that considering climate change will be important in food safety research and management.

Identification and quantification of climate change impacts on food safety requires impact modelling with different climate scenarios (Jacxsens et al. 2010, Liu et al. 2013). Such a modelling exercise requires the best possible climate and climate change data to specify both current and future conditions. These data are provided by the Intergovernmental Panel on Climate Change (IPCC) for specific future scenarios, which are commonly used by ecologists, hydrologists and agronomists to assess impacts on ecosystems, floods and droughts and food security respectively (Stocker et al. 2013b). Scenarios are plausible descriptions on how the future may unfold base on if-then propositions (Tirpak 1990). Changes in temperature, precipitation and other climate variables are calculated with General Circulation Models (GCMs). GCMs simulate the horizontal and vertical flow of matter (e.g. water, clouds, aerosols and air) and energy in the atmosphere and the oceans. The whole system is driven by the sun’s radiative energy and involves many complex interactions between, for example, ice, land, topography and greenhouse gases. The basic physics of this complex system are well understood (Sillmann et al. 2013, Stocker et al. 2013b). The main uncertainties in understanding the climate system stem from subtle feedbacks and other interactions, and stochastic or tele-connected processes, such as the proverbial flap of the Amazonian butterfly wing causing a later storm in the North Atlantic (Brayshaw et al. 2009). Many different GCMs are developed to understand past, present and future climate change. All these different GCMs have slightly different objectives and focus, and together form a model ensemble, which captures some of the uncertainties (Kharin and Zwiers 2002, Tebaldi and Knutti 2007). Results from individual GCM and averages/ranges from ensembles describe future climate conditions that could be used in impact studies (Christensen and Lettenmaier 2007).

Until the most recent IPCC assessment came out in 2013, the radiative forcing levels resulted from socio-economic scenarios (e.g. SRES, the Special Report on Emission Scenarios by Nakicenovic *et al.* (2000)). Recently a new scenarios development procedure (Moss et al. 2010b) was generated by the climate change research community. The procedure starts from radiative forcing levels. For this procedure, representative concentration pathways (RCPs) have been distilled from the scenario literature to cover the best possible range of future atmospheric greenhouse gas concentrations. Four typical pathways were selected. These lead to radiative forcing levels of 8.5 W/m^2 (business as usual), 6.0 W/m^2 (slowdown in emissions), 4.5 W/m^2 (mitigation) and 2.6 W/m^2 (strong mitigation) by the end of this century (van Vuuren et al. 2011). The 'strong mitigation' RCP likely keeps climate change within the desired 2°C target of the politically agreed Copenhagen Accords. Using RCPs as input data, GCMs calculate climate, atmospheric and carbon cycle projections to study the impacts (van Vuuren et al. 2011).

While climate change projections are calculated, various socio-economic scenarios can be developed that are consistent with the specified RCPs. This procedure is substantially faster than the earlier procedure, but the RCPs only provide a future climate that results from the specific change in radiative forcing. Their outputs have become a look-up table and are no longer based on consistent social-economic assumptions, like in the SRES emission scenarios. Making consistent assumptions for additional policy scenarios or for local and regional scenario interpretations is straightforward for SRES (e.g. Metzger et al. 2008), but extremely difficult for the RCPs (van Vuuren et al. 2011). To conform to the latest trends in climate research, we do use the GCM results for the new RCPs for this paper.

Direct GCM outputs are inadequate for assessing local and regional food safety (Ramirez-Villegas and Challinor 2012). The spatial GCM resolution (typically 200×200 km) is much coarser than the detailed resolution of food safety impact models. The GCM outputs are averages of large grid cells (40000 km^2). This implies that these data are 'smooth' compared to local data, probably underestimating temperature and precipitation extremes of actual field situation (Hofstra et al. 2010). Additionally, the available temporal resolution of GCMs (typically daily averages) is also too crude for many food safety models (especially those that model pesticide use (Karpati et al. 2004)). These two issues result in a spatial and temporal resolution mismatch between the GCM output and the input required by food safety models. The data thus need to be processed before they can be beneficially applied. This is generally done by combining climate data from local observations and GCM outputs.

This study describes an appropriate methodology for combining climate and climate change data for food safety assessments. A methodology to downscale the GCM data to a locality (e.g. a field) for food safety modelling is developed (Section 4.2). Subsequently, the downscaled data are presented and summarised (Section 4.3) and an example in which these data are used to estimate future bacterial growth illustrates how the data can be used (Section 4.4). Finally, data uncertainties and limitations are discussed to show the robustness and applicability of our approach (Section 4.5).

4.2 Methodology

This section discusses the selected data sources and models, and presents how spatial and temporal scales and resolutions of the data are selected and prepared for food safety modelling.

4.2.1 Observational data

We take Ukkel, Belgium as an example location, since many food safety studies are performed on fields near Ukkle (Wesemael and Moens 2008). We could, however, select any other example site. Daily minimum and maximum temperature and precipitation data have been obtained from the Belgium Royal Meteorological Institute.

4.2.2 The CMIP5 data and model choice

GCM outputs from the fifth phase of the Coupled Model Intercomparison Project (CMIP5; (Taylor et al. 2012)) were used in this study. CMIP5 is a standard experimental climate change protocol for GCMs. All CMIPs' data can be downloaded from the Earth System Grid Federation Portal (http://pcmdi9.llnl.gov/esgf-web-fe/). CIMP5 includes the most recent global GCM outputs available. These are also used in the most recent assessment report (AR5) of IPCC (Stocker et al. 2013b).

To represent the full range of outputs, the full multi-model ensemble (including 61different GCMs) for climate impact studies should ideally be used (Houtekamer and Derome 1995, Tebaldi and Knutti 2007). However, since we merely develop an approach to assess climate-change impacts on food safety (and running impact models 61 times is time-consuming), we feel that using the full models ensemble does not add information in this paper. On the other hand, using a single GCM projection as a representative of the possible change can lead to anecdotal future conditions and thus to misleading conclusions. When an uneven number of models are used, choosing the middle one as the 'most likely' one is tempting. For these reasons, data from two renowned GCMs are used: The Hadley Centre Global Environmental Model 2- Earth System (HadGEM2-ES) (Collins et al. 2008, Collins et al. 2011, Jones et al. 2011) and the Community Climate System Model version 4 (CCSM4) (Gent et al. 2011). The reasons for using HadGEM2-ES and CCSM4 GCM output are that they model temperature and most precipitation indices, including extreme precipitation, most robustly (Flato et al. 2013, Sillmann et al. 2013). These indices are important climate variables for food safety modelling (Liu et al. 2013). The HadGEM2-ES model is used for the core climate simulations carried out by the Met Office Hadley Centre for the CMIP5 project and the HadGEM2 series is one of the most important and commonly used GCMs for future climate projections. The open access CCSM4 model is developed and used by a community of scientists and students from universities, national laboratories and other institutions. This model is available from CCSM's website (http:www.cesm.ucar.edu/models/ccsm4.0/).

4.2.3 Spatial resolution and scale

Gridded temperature and precipitation data from GCMs are used in this study. These gridded data should be interpreted as average values of an infinite number of points in the grid (Harvey et al. 1997). To get a feel for what the gridded data look like, maximum temperature from both GCMs for the grid on top of Ukkel, Belgium is presented in Figure 4.1. The modelled current gridded data (grey lines) from the CCSM4 model are on average 1°C higher than from the HadGEM2-ES model. The size of the grid indicates the model's spatial resolution (HadGEM2-ES: 1.25°×1.88°, CCSM4: 1.25°×0.9°). The difference in modelled current maximum temperature is determined by these different resolutions. The HadGEM2-ES grid-cell covers a part of the cooler North Sea, while the CCSM4 grid-cell only covers land. This shows that the use of more than a single GCM is important for determining the proper climate context.

Gridded data have less variability and less local climate characteristics, especially for precipitation, than an agricultural field (Hofstra et al. 2010). Keeping local data variability, however, is important for future field-level food safety studies. This requires adequate downscaling procedures that produce point data from gridded data. Such an approach is applied in this study by using the data from actual local weather stations near the fields as a reference for the climate variability.

Grid cell selection

Selection of a grid cell for downscaling is very much an expert judgement because weather stations are rarely located in the centre of a grid. Therefore, to estimate the best possible climate value, a locality can be assumed similar to its grid or interpolated from its surrounding grids (Leemans and Cramer 1991, New et al. 1999) Another approach is to calculate the average of the surrounding grids (Wilby and Wigley 1997, Crawford et al. 2007). The difference between simply using the single grid in which the station is located and interpolation, however, is small at local scale (Prentice et al. 1992). Calculating an average introduces arbitrariness and reduces the local patterns (Prentice et al. 1992). Therefore, we use the single grid cell on top of the local weather station in this study.

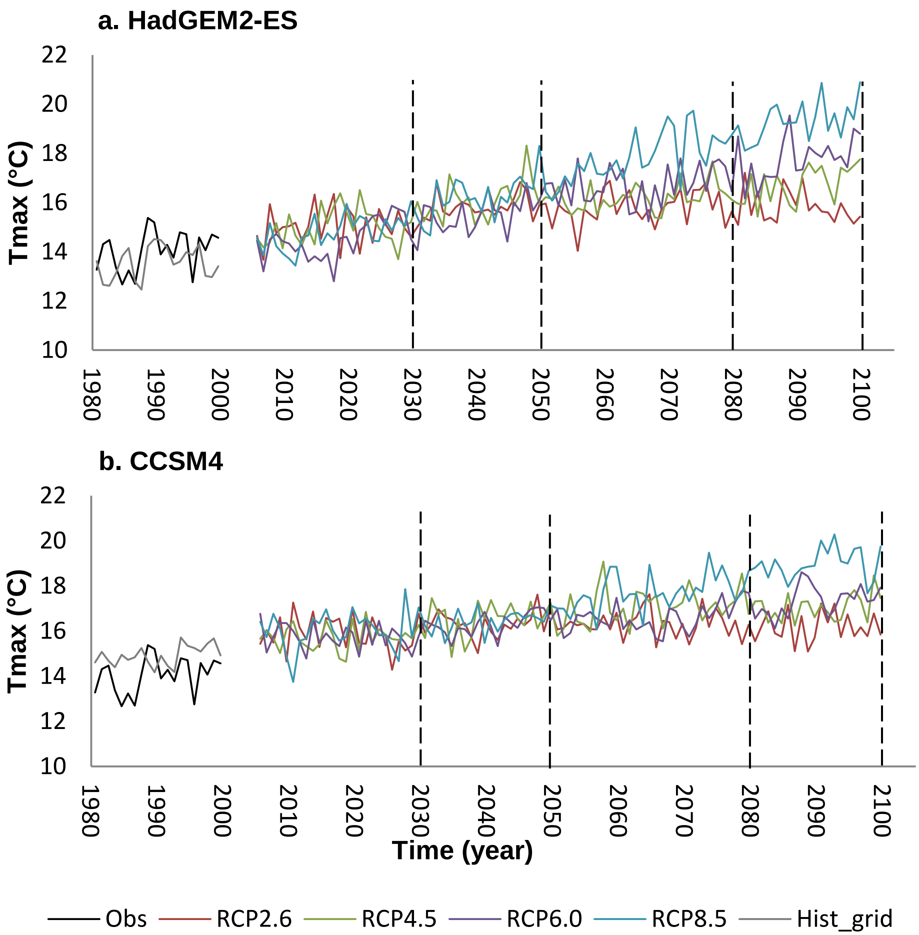

Figure 4.1 Annual average maximum temperature at 2m height of the grid on top of Ukkel, Belgium: comparing observation data (1981-2000) with GCMs (a. HadGEM2-ES and b. CCSM4) gridded outputs (2006-2100), i.e. modelled current data and modelled scenario data. This study focuses on two future periods (2031-2050 and 2081-2100) which are indicated with dashed lines.

Delta method

Many downscaling methods are used to produce weather data for impact models (Hawkins et al. 2013). The 'delta' or 'change factor' method is used in this study by overlaying the GCM's projected climate with observed climate (Wilby and Wigley 2000, Arnell et al. 2003, Diaz-Nieto and Wilby 2005) (Eq.8). This ensures that the current climate is the actually observed climate and not the simulated GCM-based current climate with its obvious errors. Temperature change can simply be added to the observed temperature. Such addition, however, can result in negative precipitation values. Here a multiplicative approach (i.e. relative change) is usually used (Hawkins et al. 2013) (Eq.9). The method adds variability to the gridded data.

Future station data = observation current data + (modelled scenario data – modelled current data) Eq.8

Future station data = observation current data × (modelled scenario data / modelled current data) Eq.9

Quantile-quantile correction

We apply quantile-quantile correction to ensure that the future station data and the observed data are distributed similarly. This correction is analogous to the quantile-perturbation approach (Ntegeka and Willems 2008, Willems and Vrac 2011). The observed data are perturbed with the modelled data, considering the projected changes in percentiles. The method has been developed in Excel and contains the following steps (repeat for all months in the twenty year period (i.e. all 240 months) and scenarios):

1. Modelled current and scenario data are ranked and percentiles are calculated.

2. An intermediate summary or lookup table is produced. The columns of this table contain the percentiles, the modelled current value for each percentile and the modelled scenario value for each percentile.

3. Anomalies are calculated for each percentile by subtracting the modelled current value from the modelled scenario value for temperature or dividing the modelled scenario data by the modelled current data for precipitation. The resulting anomalies represent the future change in temperature or precipitation.

4. Observed data are ranked and percentiles are calculated for each day.

5. Then the percentile of each observed day is retrieved from the table and the anomaly value for that percentile is then added to the observed value for temperature (Eq.8) or multiplied with the observed value for precipitation (Eq.9).

These five steps produce the final output: daily future data for the location of the observational station.

A routine with this quantile-quantile correction and its detailed manual is available in the supplementary material of the original publication. To apply the routine, daily station data for a twenty-year period, modelled data for the grid on top of the station for the same twenty-year period and corresponding modelled twenty-year scenario data are required. This procedure can be used for any meteorological station.

4.2.4 Temporal resolution and scale

The projected climate data from GCMs have various temporal resolutions: monthly, daily, 6 hourly and 3 hourly. Most of the impact models are driven by daily weather inputs and observed weather data are generally available with a daily time step. Some food safety studies, for instance on pesticide residues (Chen and McCarl 2001, Van Boxstael et al. 2013) and mycotoxins (Van de Perre et al. 2014a) require, however, hourly data to more accurately assess impacts. This can be achieved by coupling large-scale datasets with weather generators (Ramirez-Villegas and Challinor 2012).

Hourly weather generators are models calibrated on observed hourly weather series over an appropriate period for a site or a grid. Hourly weather is stochastically generated with precipitation considered to be the primary variable and other variables (e.g. maximum and minimum temperature) determined by a regressed relationship with precipitation (Ivanov et al. 2007, Fatichi et al. 2013). Hourly weather generators can be used to gain an even finer temporal resolution for food safety modelling. A suitable example is the weather generator specially developed for agricultural applications by Ivanov *et al.* (2007).

Our tool (see Section 4.2.3), does not provide hourly data. If needed, Ivanov *et al.*'s (2007) weather generator can be used to produce hourly input. This generator, though, requires much additional input data, such as cloudiness, shortwave radiation, wind speed and humidity. Alternatively, a simpler but less realistic approach can be used to estimate hourly temperature from daily maximum and minimum temperatures. Schaub (1991), for example, fits a hyperbolic tangent function through minimum and maximum temperature. Two straightforward methods from Waichler and Wigmosta (2003) predict hourly precipitation. Their first method uniformly distributes the daily precipitation over all 24 hours. This will strongly underestimate extreme precipitation events. The second method uses a relative month-hour fraction for each month. Also this downscaling method poorly fits observations (Waichler and Wigmosta 2003). Using daily or observed hourly precipitation instead would be much better. Although these simple temporal downscaling methods may not strongly influence the results of impact models, they strongly simplify reality. Testing the influence of temporal downscaling on impact model outputs could easily be done by running the impact model with observed hourly data and with temporally downscaled data and compare both results.

The database provided in this study for food safety modelling includes daily values from 2006 to 2100. For the presentation, twenty years of these data are used to account for inter-annual variability. The twenty-year reference and two scenario periods are specified as 1981-2000, 2031-2050 for the near future and 2080-2100 for the far future.

4.3 Results

Daily data of temperature and precipitation are presented for Ukkel, Belgium, to illustrate the changes in climate over the period 1981-2100. Daily data are provided for the four RCPs and the two GCMs, HadGEM2-ES and CCSM4. The resulting data are summarised in Figures 4.2, 4.3 and 4.4.

The annual average maximum temperature (Figure 4.2) and annual total precipitation (Figure 4.3) are calculated for current and future scenarios for both GCMs. Maximum temperature and precipitation increase towards the end of the century in all four RCPs (Figure 4.2 and 4.3). In the near future (i.e. 2031-2050; Figure 4.2a and 4.2c) the differences in temperature increase are small because the radiative forcing is still very similar as this is still strongly dominated by historic emissions. However, there are slight differences in the details. For example, RCP6.0 starts with a lower radiative forcing increase than RCP4.5. For the far future (i.e. 2081 – 2100; Figure 4.2b and 4.2d), the temperature increase diverge and range from 2^{o}C to 7^{o}C. RCP8.5 projects the highest (i.e. 5-7°C) increase by the end of this century (Figure 4.2b and 4.2d). Modelled scenario data values from the HadGEM2-ES GCM are approximately 1°C higher than for the CCSM4 GCM (Figure 4.2). This could be an artefact of the CCSM4 model as it simulates current climate for the Ukkel grid approximately 1°C warmer than HadGEM2-ES. The simulated future climates are similar but this leads to a larger change (Eq. 8).

Data users can choose other GCM data projections for their impact studies. Figure 4.4, for example, gives daily maximum temperature averaged over twenty years. Daily maximum temperature is projected under RCP8.5 to increase approximately 2°C (CCSM4) and 4.5 °C (HadGEM2-ES) in January and 5°C (CCSM4) and 6°C (HadGEM2-ES) in August (Figure 4.4).

4.4 Data application example

Climate data are used with Ratkowsky's (1983) bacterial growth model to illustrate how projected climate data can be used for bacterial growth modelling. Our results do not necessarily represent reality, because we did not thoroughly collect all necessary data for the model parameters and did no proper model validation. This example is included for illustration purposes only. The Ratkowsky model describes the bacterial growth rate throughout its entire temperature range and is widely used:

$$r = [b(T - T_{min})]^2 \times \{1 - \exp[c(T - T_{max})]\} \qquad \text{Eq.10}$$

Where r is the growth rate (h^{-1}), b (°$C^{-1}h^{-0.5}$) and c (°C^{-1}) are the Ratkowsky parameters, T_{min} and T_{max} are the minimum and maximum temperature at which growth is observed (°C). Zwietering et al (1991) estimated b, c, T_{min} and T_{max} values and we use their approximations for *Lactobacillus plantarum* (Table 4.1). T is the hourly temperature downscaled from projected daily temperature from each scenario (RCPs, HadGEM2-ES and CCSM4) for Ukkel, Belgium. In this example, a hyperbolic tangent function (Schaub Jr 1991) through daily minimum and maximum temperature is used to downscale daily data to hourly data. The Ratkowsky model is then run at an hourly time-step for the current and two future periods (i.e. 1981-2000, 2031-2050 and 2081-2100).

Table 4.1 Ratkowsky parameters used in this example, as estimated for *Lactobacillus plantarum* by Zwietering et al (1991).

Ratkowsky parameters	Estimates for *Lactobacillus plantarum* by Zwietering et al. 1991
b	0.041 °$C^{-1}h^{-0.5}$
c	0.161°C^{-1}
T_{min}	3.99 °C
T_{max}	43.7 °C

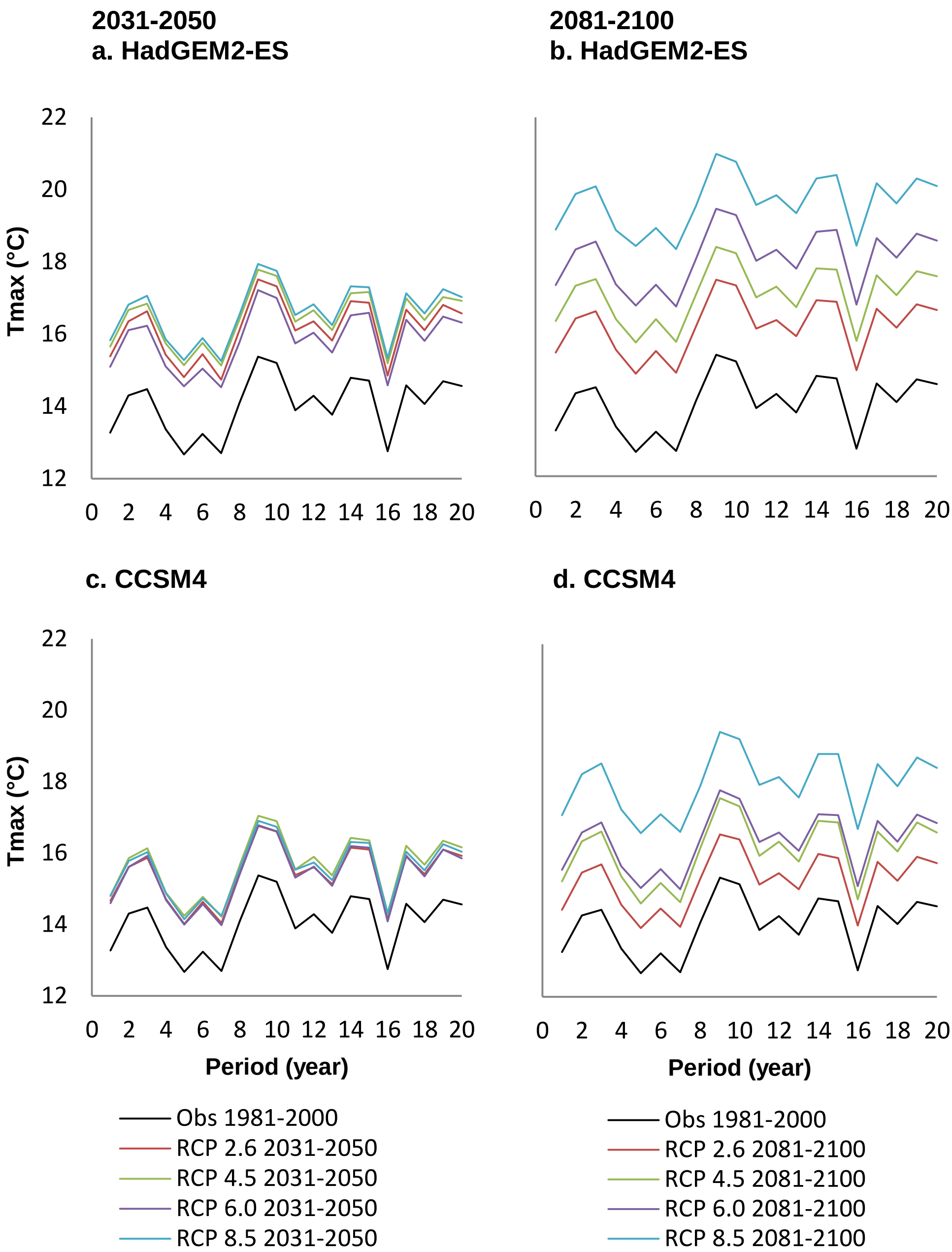

Figure 4.2 Annual average maximum temperature (measured at 2m) at weather station Ukkel, Belgium: comparing observed data (1981-2000) and future scenario data from GCMs HadGEM2-ES (a and b) and CCSM4 (c and d) in the near (2031-2050, a and c) and far future (2081-2100, b and d).

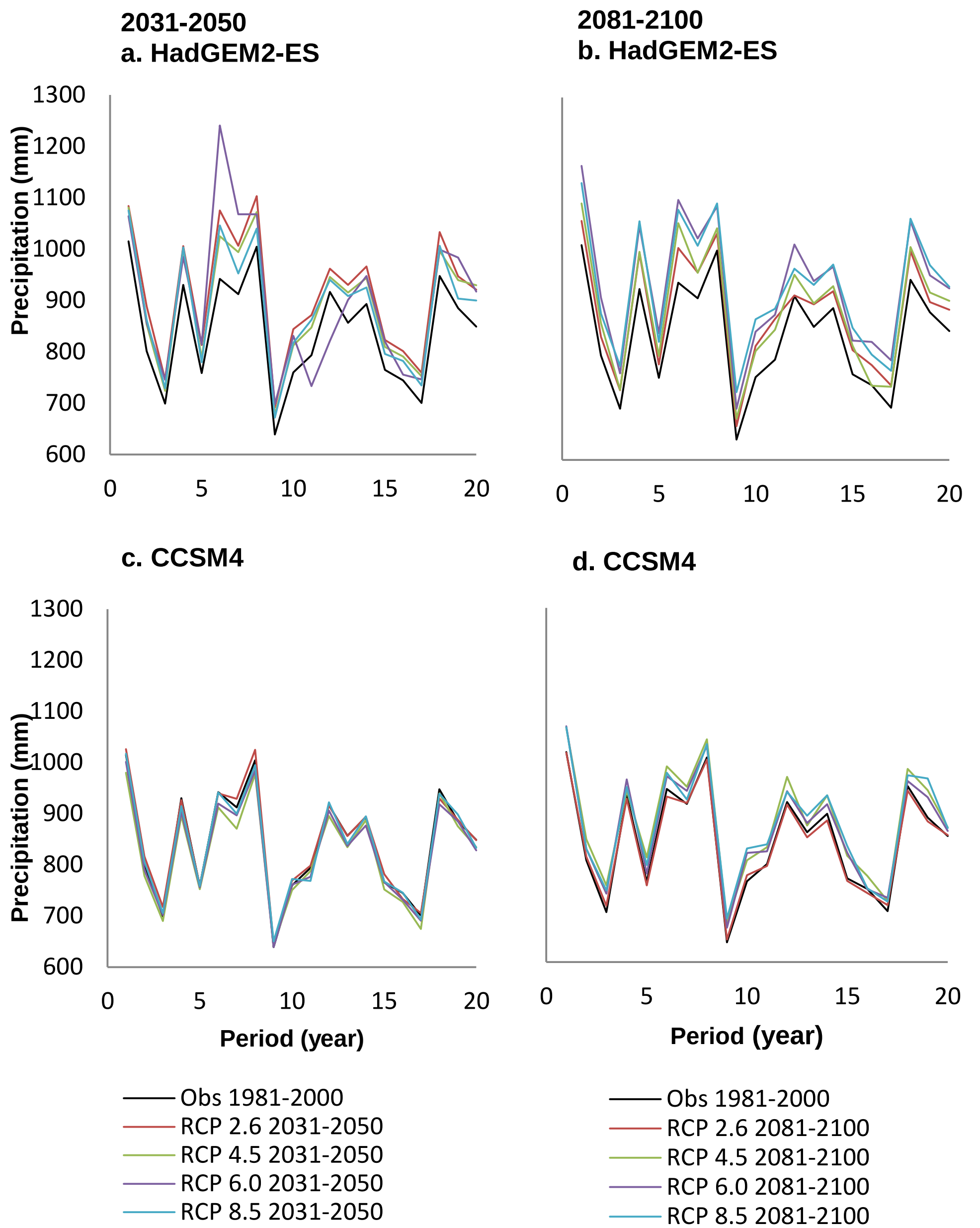

Figure 4.3 Annual total precipitation at weather station Ukkel, Belgium: comparing observation data (1981-2000) and downscaled scenario data from model HadGEM2-ES (a and b) and CCSM4 (c and d) in the near (2031-2050, a and c) and far future (2081-2100, b and d).

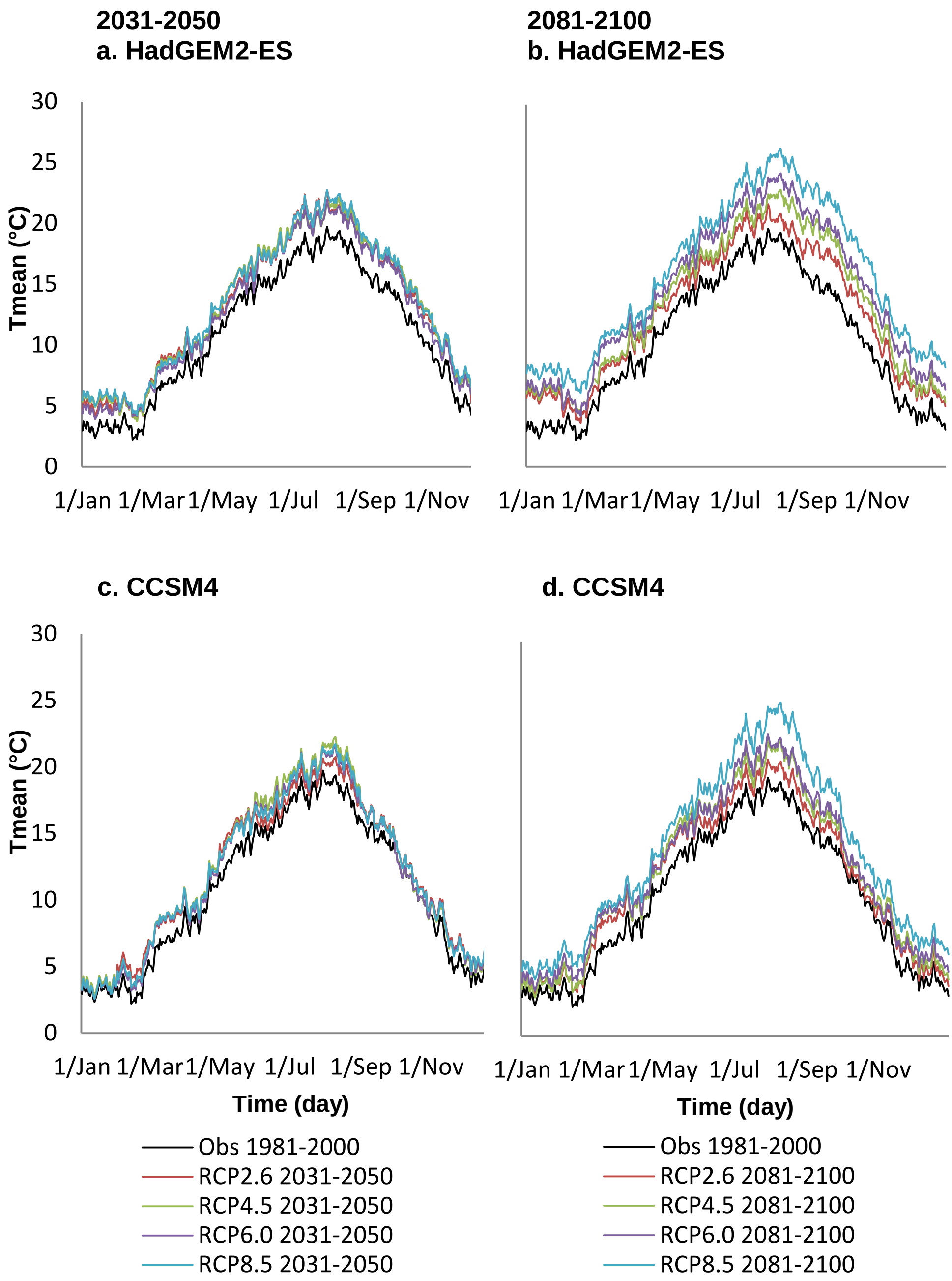

Figure 4.4 Daily mean temperature averaged over 20 years at weather station Ukkel, Belgium: comparing observation data (1981-2000) and downscaled scenario data from model HadGEM2-ES (a and b) and CCSM4 (c and d) in the near (2031-2050, a and c) and far future (2081-2100, b and d).

We determine how bacterial growth changes if temperature rises as projected with the HadGEM2-ES and CCSM4 GCMs for the four RCPs. Hourly *Lactobacillus plantarum* growth rates averaged over 20 years are presented in Figure 4.5. The upper and bottom lines show the maximum and minimum *Lactobacillus plantarum* growth rate averaged over 1981-2000. The variability of the growth rate during the growing season is largest in August (Figure 4.5). Maximum and minimum *Lactobacillus plantarum* growth rate per day during the growing season averaged over 20 years are calculated for each scenario in the far future (2081-2100) and compared with the current growth rate (1981-2000) (Figure 4.6). For both GCMs and all RCPs, the only day on which daily maximum temperature (43.8°C) is higher than T_{max} is calculated by HadGEM2-ES for RCP8.5 in August 2090. The growth rate is projected to increase in the future and the highest growth rate for Ukkel will be in August.

We can also determine the number of days that temperature is suitable for *Lactobacillus plantarum* to grow. This number of days is calculated for each scenario and compared with the numbers between 1981 and 2000 (Figure 4.7). In both models and all scenarios, the number of days that the bacteria can grow increases due to the substantial increase in number of days that daily average temperature is higher than T_{min}.

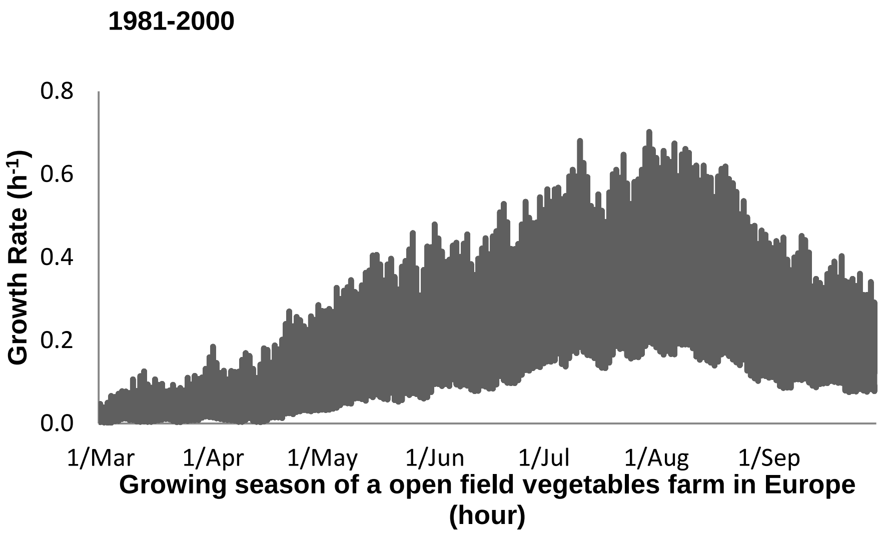

Figure 4.5 Hourly *Lactobacillus plantarum* growth rate averaged over 20 years at Ukkel, Belgium: calculated from temporally downscaled observation data (1981-2000).

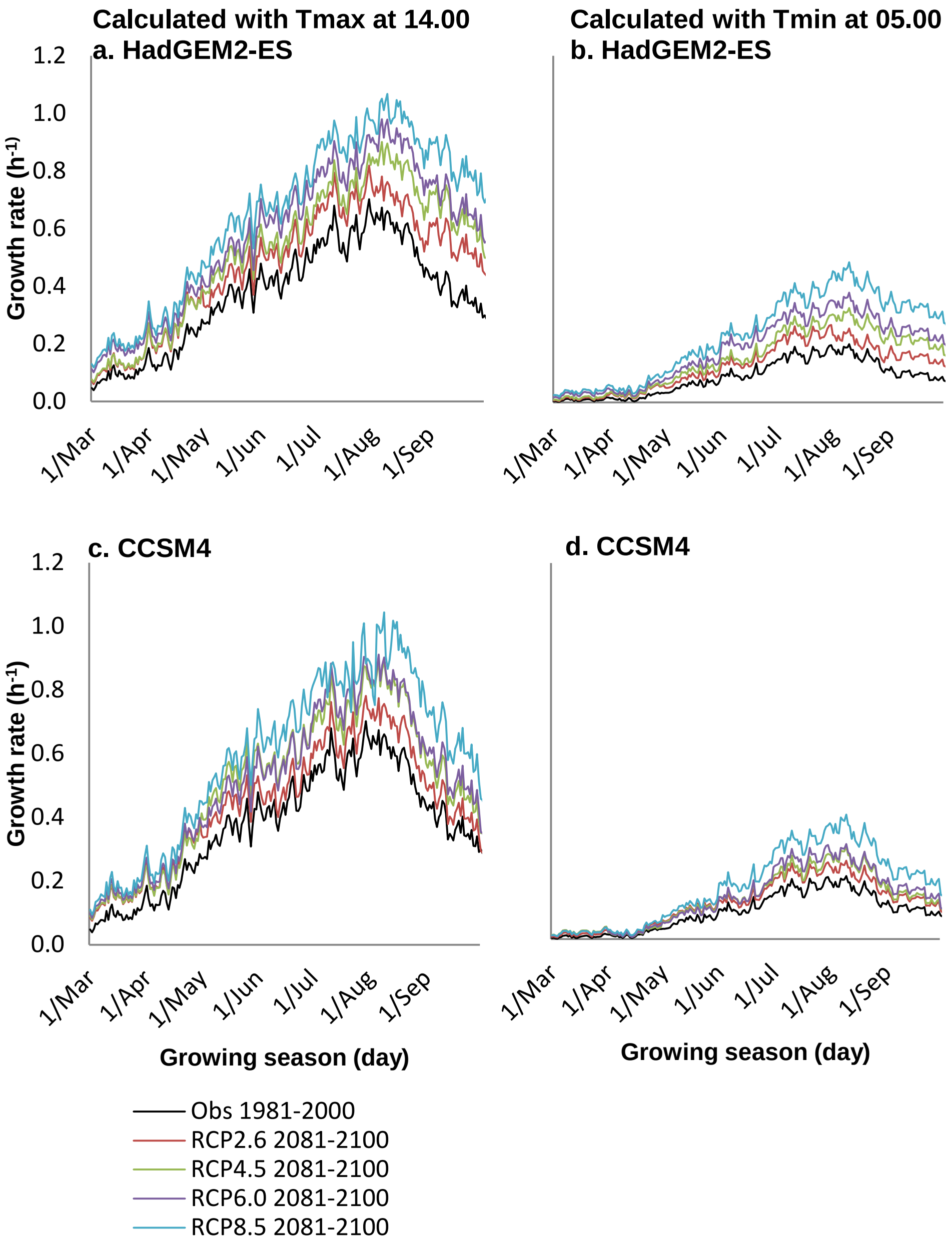

Figure 4.6 Maximum (a and c) and minimum (b and d) Lactobacillus plantarum growth rate averaged over 20 years at Ukkel, Belgium: calculated from observation data (1981-2000) and downscaled scenario data from model HadGEM2-ES (a and b) and CCSM4 (c and d) in the far future 2081-2100.

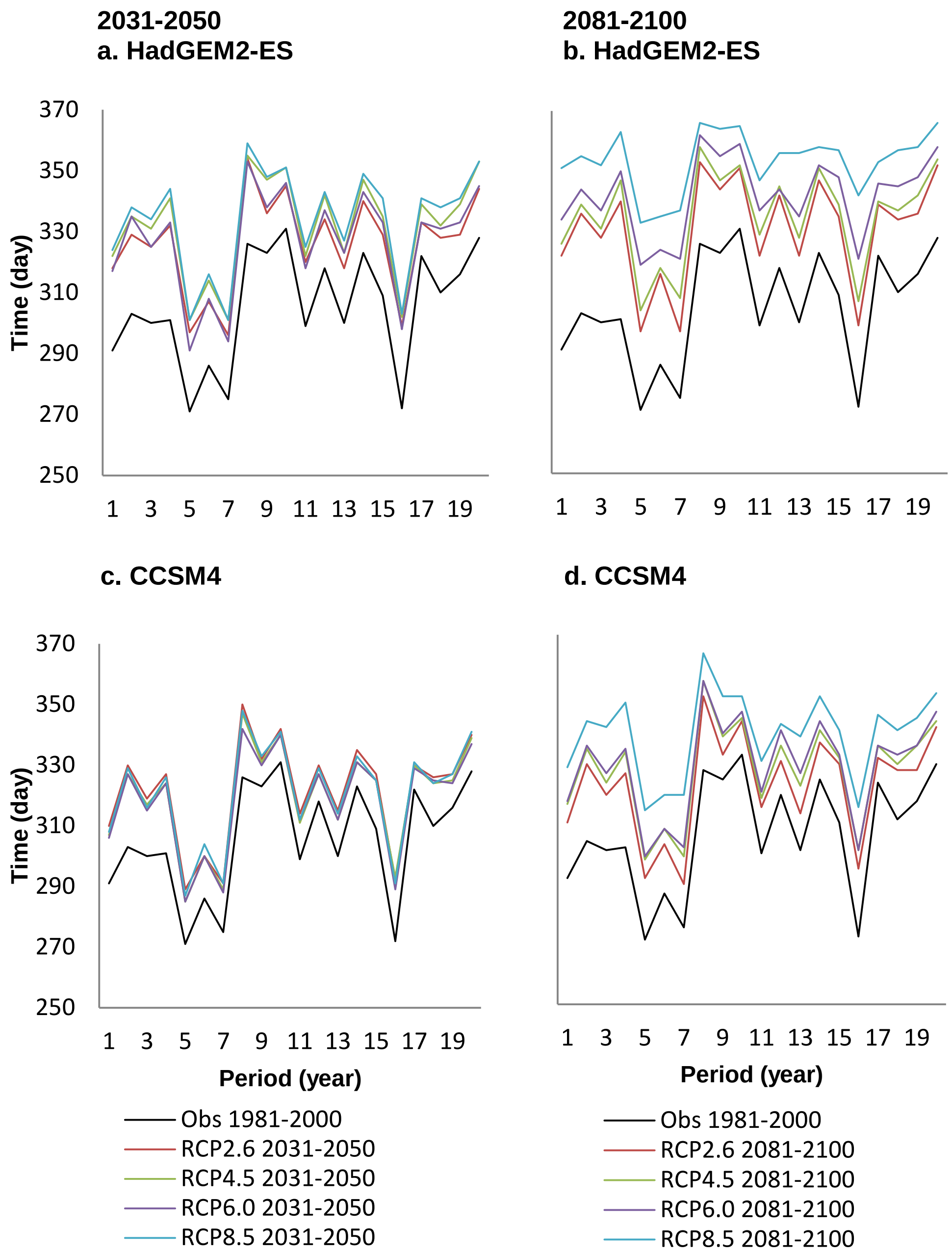

Figure 4.7 Over 20 year averaged number of days that Lactobacillus plantarum may grow at Ukkel, Belgium: calculated from observation data (1981-2000) and downscaled scenario data from model HadGEM2-ES (a and b) and CCSM4 (c and d) in the near (2031-2050, a and c) far future (2081-2100, b and d).

4.5 Discussion and Conclusion

In this study we described a tool to prepare climate data for use in local food safety scenario analysis. We also illustrated how the data can be used with impact models. Unfortunately, how the future will unfold is unknown. Therefore, the results of scenario analysis must always be interpreted carefully. These results are not precise predictions but just plausible futures (Jones 2000). Scenario users should be aware that the processes to prepare and downscale climate data introduce uncertainties, because these uncertainties will propagate through the impact assessment (Jones 2000). The main uncertainties are:

- Forcing, model and natural variability uncertainties (Hawkins and Sutton 2009, Deser et al. 2012, Taylor et al. 2012): Forcing uncertainties are due to limited knowledge of factors and their interactions influencing the climate system. Model uncertainties arise due to the differences in, for example, physical and numerical formulations. Natural variability uncertainties relate to the obvious variability of the climate system. Many studies (i. e. Meehl et al. 2007, Moss et al. 2010b) review these uncertainties of climate change projections, and show that they are real but not questioning the general trends of climate change.
- The uncertainty of precipitation extremes projections. These are so high that their practical utility can be questioned (Fatichi et al. 2013).
- The uncertainties related to the grid cell selection and downscaling method in producing climate data for the impact models (Hawkins et al. 2013). Ho *et al.* (2012) compare a so-called 'bias correction' method with the 'delta method' and conclude that both methods give different future climates. The uncertainties stemming from these differences could be equal to those from the different RCPs.
- The technical uncertainties in the temporal downscaling from daily to hourly data. These uncertainties relate to our ability to implement mathematical formulations in statistical analysis (Waichler and Wigmosta 2003). Additionally, weather generators are constructed for specific locations, creating uncertainties when the generator is applied elsewhere (Semenov and Barrow 1997, Semenov et al. 1998, Wilks and Wilby 1999). Which weather generator should be used when requires a careful decision, since hourly data for calibrating each climate variable are lacking (Semenov and Barrow 1997, Semenov and Brooks 1999, Semenov 2007). Moreover, weather generators are not weather forecasting tools. Weather generators just statistically describe weather data, but any particularly generated weather sequence cannot replace weather observations at a given time in either the past or future (Wilks and Wilby 1999).

The original ‘delta method’ uses a mean monthly anomaly, since the anomaly value varies little over the observation period. The daily values, however, vary much more. Therefore, we used the quantile-quantile correction. This enables adding different anomaly values based on the ranked daily values and thus strengthens the data variability of the observational station. The quantile-quantile correction straightforwardly uses the distribution of observational data, which describes the local variability, but requires the availability of a representative period of observations. Using the distribution from the observation data for all future scenarios can be disadvantageous because it standardizes the variability among scenarios. This neglects possible future shifts in extremes.

In this study we described a method for preparing climate and climate change data for food safety assessment. Our method was exemplified by determining how the bacteria growth rate may change in Ukkel if temperature changes according to the four RCP scenarios. We conclude that the downscaling method is satisfactorily applicable for food safety assessment. Impact modellers in the field of food safety are recommended to work directly with climate modellers to better understand the limitations and uncertainties in scenario analysis. Our results show that the outputs from each GCM differ. Researchers who will apply this method in food safety scenario analysis, should therefore use as many GCM outputs and scenarios as possible to obtain a plausible range of outcomes. This will increase the confidence in their impact assessment. Our tool will stimulate food safety researchers who are interested to study the impacts of climate change in their own scenario analysis.

Acknowledgment

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Chapter 5

Exploring a Multi-criteria Scenario Analysis Tool to Study Future Food Safety

--- Assessment of microbial safety for pre-harvest leafy greens in Spain

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Abstract

Food safety is a complex interplay of different climate and management variables and the future food safety is likely affected by climate change. This study explores the development and application of a multi-criteria scenario analysis tool to statistically model future food safety using a pre-harvest vegetable (spinach) in Spain as an example. Subsequently, the tool was demonstrated step by step with a sensitivity analysis to show the possibility of, for example, stakeholders' interests in food safety studies. This study calculated the future *E.coli* concentration changes on spinach in the scenarios 'RCP 8.5' and 'RCP 2.6' at the end of the century in Spain. The results indicate that *E.coli* concentrations are projected to increase by 0.2 log10 CFU/g to 0.3 log10 CFU/g (depending on the climate scenarios and management options applied) due to higher temperature at the end of the century compared with the *E. coli* concentrations at the end of the last century. This comparison assumed no changes in agriculture management practices. The results show this tool is appropriate to select the best management practices considering climate change and other. This multi-criteria tool provides a platform to study changes in weather or climate, and management impacts on future food safety together with different stakeholders' perspectives or interests. Such a multi-criteria analysis likely delivers a new mind set and method to determine study food safety and it enhances the quality of agricultural management decisions for leafy green vegetables.

5.1 Introduction

The incidence in foodborne disease is generally correlated with local weather and climate conditions (Miraglia et al. 2009, Jacxsens et al. 2010, Tirado et al. 2010). The observed foodborne disease seasonality suggests that climatic conditions play a role and that climate changes may affect pathogens presence and concentration. A recent review of the impacts of climate change on micro-organisms (Liu et al. 2013) improved the qualitative understanding and this will now be used to study these impacts quantitatively. The mechanisms underlying the observed seasonality in foodborne disease are not yet fully understood, but they are likely a complex interplay of different factors. Besides climatic conditions, these factors include human behavior and consumption patterns (Van Staveren et al. 1986, Ziegler et al. 1987), farm management practices (Kirezieva et al. 2015), pathogen prevalence in the animal reservoir and pathogen environmental survival patterns (Liu et al. 2013). Farmers are likely to change management practices to adapt to climate change (Kirezieva et al. 2015). For example, farmers in some areas may need to set up an alternative water sources for irrigation (e.g. valley dams, rain harvesting systems and ponds) to adapt to the drought in the future. Diverting from surface water to rain water irrigation likely increases contamination probabilities, because rain water is collected and stored in reservoirs which are often open to birds and insect (and their droppings).

A previous study (Liu et al. Accepted) combined climate and management variables and included more than 560 samples from 23 farms in Belgium, Brazil, Egypt, Norway and Spain. They presented a regression model and concluded that temperature, manure, inorganic fertilizer and spray irrigation are statistically significant for *E. coli* concentration on leafy green vegetables. In this study we use a pre-harvest leafy green vegetable (i.e. spinach) samples in Spain and the regression model from the previous study as an example to study future food safety with a multi-criteria scenario analysis tool. The concepts of scenario, climate scenario and multi-criteria scenario analysis which all will be used in this study are introduced as follows.

Scenario: Scenario in this study is defined as a plausible combination of current and possible future agricultural management practices. Such scenario starts from the current state with a specific set of current practices and develop towards a future state with specific set of current and future practices.

Climate scenario: A climate scenario is defined as plausible and often simplified representations of future climate, based on an internally consistent set of climatological relationships that has been constructed for explicitly investigating the potential consequences of anthropogenic climate change, often serving as input to impact models (IPCC 2013a). The climate change research community uses scenarios to improve understanding on how the future may unfold base on '*what if*' propositions. These scenarios include the complex interactions of the climate system, ecosystems, and human activities and conditions. The outputs of scenario analyses provide plausible descriptions of what may happen. They are not predictions but merely not-implausible projections. Scenarios help to evaluate uncertainties in the full climate system (the human contributions, responses of the Earth system and impacts of climate change) and the implications of implementation of different mitigation (i.e. measures to reduce net emissions) and adaptation (i.e. actions that facilitate coping responses to the new climate conditions) measures (Moss et al. 2010a).

A new scenarios development procedure (Moss et al. 2010b) was generated by the IPCC research community and used for IPCC's latest assessment report (Stocker et al. 2013a). Representative concentration pathways (RCPs) have been distilled from the scenario literature to cover the best possible range of future atmospheric greenhouse gas concentrations. Four typical pathways were selected. These lead to radiative forcing levels of 8.5 W/m^2 (i.e. business as usual), 6.0 W/m^2 (i.e. slowdown in emissions), 4.5 W/m^2 (i.e. mitigation) and 2.6 W/m^2 (i.e. strong mitigation) by the end of this century (van Vuuren et al. 2011). The 'strong mitigation' RCP likely keeps climate change within the desired 2°C target of the politically agreed Copenhagen Accords (van Vuuren et al. 2011). The RCP scenarios provide outputs that answer the question: "What is the future climate (e.g. temperature) if radiative forcing changes?"

Multi criteria scenario analysis: A multi criteria analysis is a decision-support tool, developed for complex problems that include multiple quantitative and/or qualitative problematic aspects and interactions (Macoun and Prabhu 1999). Multi-criteria scenario analysis is used to select the best scenarios based on several criteria, including climate change and various management options. To our knowledge, only one study from Mazzocchi et al. (2013) used multi criteria analysis on regulating mycotoxin contents in cereals.

This chapter explores the development and application of a multi-criteria scenario analysis tool to study future food safety. This objective is addressed by applying and combining climate-scenario analysis and multi-criteria analysis on a statistical model using pre-harvest spinach in Spain as an example (Section 5.2). Subsequently, the tool is demonstrated step by step by determining future microbial safety on spinach in Spain as an example. A sensitivity analysis was performed to show the possibility of including different stakeholders' perspectives or interests in food safety studies (Section 5.3). After that, strengths and weakness of this tool are discussed and concluded (Section 5.4).

5.2 Material and Method

5.2.1 Data

General Circulation Models (GCMs) outputs from the fifth phase of the Coupled Model Intercomparison Project (CMIP5: Taylor et al. 2012) were used in this study. CMIP5 is a standard experimental climate change protocol for GCMs. All CMIPs' data can be downloaded from the Earth System Grid Federation Portal (http://pcmdi9.llnl.gov/esgf-web-fe/). CIMP5 includes the most recent global GCM outputs available. These are also used in the most recent assessment report (AR5) of IPCC (Stocker et al. 2013d). Many different GCMs are developed to understand past, present and future climate change. All these different GCMs have slightly different objectives, focus and parameterizations, and together their output form a model ensemble, which captures the major range of uncertainties (Kharin and Zwiers 2002, Tebaldi and Knutti 2007). The future temperature data were downscaled using the 'Delta method' with quantile-quantile correction which is explained in the precious work (Liu et al. 2014). The coarse gridded data were from the GCM Community Climate System Model version 4 (CCSM4). Outputs from one model are used in this study to illustrate our approach. It thus only provides one realisation from the possible ensemble range. In future more comprehensive applications, we recommend to use the full ensemble to capture the climate model uncertainties (Liu et al. 2014). Observational daily temperature data from 1981 to 2000 were used as a reference for this downscaling. Downscaled data were provided for four future RCPs for the periods 2031-2050 and 2081-2100. In this study RCP2.6 and RCP 8.5 are used to illustrate the range of the climate change projection from the CCSM4 model.

5.2.2 Statistical Model

The *E. coli* concentration model (Eq.11: Liu et al., submitted) had an adjusted R-square of 0.69. The model gave Root Mean Square Error (RMSE) of 0.422 and a Mean Absolute Error (MAE) of 0.308. Together, this indicates a high accuracy. Therefore, we used this model to estimate *E.coli* concentration in the future. This estimation was used to demonstrate the tool's appropriateness to study future food safety. The model had the following form:

$$Y = -0.502 + 0.083 \times X_1 + 0.613 \times X_2 - 0.557 \times X_3 + 0.391 \times X_4 \qquad \text{Eq.11}$$

Where Y = *E. coli* concentration in log10 CFU/g, X_1 = maximum temperature three days before the sampling day in °C, X_2 = the application of manure (yes = 1, no = 0), X_3 = the application of inorganic fertilizer (yes = 1, no = 0), X_4 = the application of spray irrigation (yes = 1, no = 0). In this model, all dependent variables had significant influence on estimating *E. coli* concentration.

5.2.3. Multi criteria scenario analysis

This tool evaluates the relative importance of all criteria involved and reflect their importance in the final decision making process (Dodgson et al. 2009). The procedure of our Multi criteria analysis follows the approach by Dodgson et al. (2009) and is summarised in Box 5.1.

5.3 Results

Step 1 Establish the decision context.

The overall objective of the multi-criteria scenario analysis was to explore the future food safety of pre-harvest leafy greens and select the most suitable management scenario. And this objective was converted to four criteria: *E. coli* concentration, cost, yield and nutrient loss from soil.

Step 2 Identify management scenarios to be appraised.

The results from Liu et al. (in preparation), showed that using manure, inorganic fertilizer and spray irrigation had a statistically significant influence on *E. coli* concentration. The combinations of using manure, inorganic fertilizer, spray irrigation, other irrigation ways and other fertilizer type were used in this study as management scenarios. These six scenarios are presented in Table 5.1.

Step 3 Identify criteria.

The first criterion was the *E. coli* concentration on the spinach calculated with Eq.11. Cost and Yield were criteria identified by farmers and local policy makers and relate to their perspective and interest. Nutrient loss from soil/soil health from the environment impact perspective was the criteria important to environmental scientists and local policy makers. These criteria were chose as examples to represent the interests of each expert group. *E. coli* is the hygiene indicator for food safety (Holvoet et al. 2014). Cost and yield are two of the main factors that farmers and policy makers considered in their management strategies. Nitrogen and/or phosphorus loss from the fertilizer applied in the soil may flash into the water and cause eutrophication in the river systems (Mayorga et al. 2010).

Box 5.1. Procedure for applying multi-criteria analysis

Step 1. Establish the decision context.

a. Identify the overall objective
b. Convert objective to measurable criteria

Step 2. Identify the management scenarios to be appraised.

Step 3. Identify criteria. Identify criteria for assessing the consequences of each scenario.

Management criteria were identified based on farmers, local policy makers and environmental scientists' perspectives.

Step 4. 'Scoring'. Assess the expected performance of each scenario against the criteria.

The *E. coli* concentrations were calculated with daily maximum temperatures from 1981 to 2000 as a reference period for climate change. A distribution was fitted with these twenty years concentration data in @RISK version 5.7. The concentrations were calculated again with future (from 2081 to 2100) daily minimum temperatures which are projected under RCP 2.6 and RCP 8.5. These two climate scenarios' outputs from CCSM4 model are used in this tool to illustrate one possibility of highest and lowest RCP projections in the climate change ensemble.

Step 5. 'Weighting'. The weighting of the criteria takes place in two steps:

a. Ranking the importance of criteria.
b. Assigning the weights of the different criteria on a 0-1 scale to achieve a total weight of 1.

Step 6. Combine the weights and scores for each scenario to derive an overall value. The overall preference score (S_i) of option i was the sum of all weighted average scores on each criterion.

$$\boldsymbol{S_i = w_1 S_{i1} + w_2 S_{i2} + \cdots + w_n S_{in} = \sum_{j=1}^{n} w_j S_{ij}} \quad \text{(Eq. 2)}$$

Where $\boldsymbol{w_j}$ = weight for criterion j, $\boldsymbol{S_{ij}}$ = score for option i on criterion j, n = amount of criteria taken into account.

Step 7. Examine the results.

Step 8. Sensitivity analysis.

a. Different weights were given to each criterion to illustrate various desirable futures in the perspective of different stakeholders.
b. Different scores are given to each scenario to test the sensitivity of this tool.

Step 4 'Scoring'.

The procedure of giving scores was explained by means of an example in which four scenarios of agriculture practices are compared (Table 5.1). *E. coli* concentration, costs, yield and nutrient loss from the soil were the criteria defined in the previous step.

For the first criterion, the mean *E. coli* concentrations in 20 years for each scenario in the reference period and in the future under RCP2.6 and RCP8.5 are presented in Table 5.1. Projected daily maximum temperature was downscaled by the tool published in Liu et al. (2014). The mean *E.coli* concentration slightly increased in the future (2081-2100) compared with the reference period between 1981 and 2000. The concentration differences between scenarios were small (0.2 log10 CFU/g). We used values of RCP 8.5 for further analysis to represent the highest change. The combination of using manure and spray irrigation gave the highest (2.7 log10 CFU/g) mean *E. coli* concentration in the far future.

The scores for other criteria are examples on how this tool can be used. In other applications, expert team should give scores for each criterion based on the local fertilizer price and soil types to calculate nutrient loss from the soil. Costs are normalized figures in which the value for the most expensive scenario (combination of other irrigation ways and inorganic fertilizer) is set at 1 and the costs of other scenarios are calculated in proportion to the most expensive scenario. Yield and nutrient loss are scored on the categories low and high. In this example, application of inorganic fertilizer and manure got a low score for yield because the yield is lower than the production when organic material are used on the produce (Sanwal et al. 2006). Inorganic fertilizer provided similar yield with composted manure (Warman and Havard 1996), in general we gave it a low score. Inorganic fertilizer also got a low score in this example for nutrient loss from soil. When inorganic fertilizer are used, 30% of total phosphorus is lost from the soil, while manure causes 60% loss of total phosphorus from the soil (Tabbara 2003). This is, however, strongly determined by soil type. Therefore in other applications, specific scores should be assigned based on the actual local soil type.

In order to make the performance of these different criteria comparable to each other, they were scaled in a way that the lowest score/category was given a value 0.0 and the highest score/category 100. The lowest scores are 1.15 for *E. coli* concentration, 0.2 for costs, low for yield and nutrient loss. The highest scores are 2.72 for *E. coli* concentration, 1.0 for costs, high for yield and nutrient loss. Performance scores between these values are calculated by linear interpolation, which transforms Table 5.1 to Table 5.2.

Step 5 'Weighting'.

In this example equal importance of criteria were assumed. Each criterion got a weight of 0.25, which cumulates to a total weight of 1.00.

Step 6 & 7 Combine the weights and scores for each scenario to derive an overall value and examine the final results.

The weighting and the calculated overall performance scores on the basis of the performance given in Table 5.2 are shown in Table 5.3. Consequently the combination of using spray irrigation and other fertilizer type and the combination of other irrigation ways and other fertilizer type had the highest overall performance value (76 and 72) in this example. Since two scenarios got similar high scores, a further analysis by, for example, applying a sensitivity analysis, was needed to test the performance and uncertainties of this tool and the significance of the outcome.

Step 8 Sensitivity analysis.

A sensitivity analysis was performed by running the procedure again with different values of criterion weight representing the different perspective in the decision process (Table 5.4). When the *E. coli* concentration gets a weight of 0.7 representing a strong focus on food safety, the scenario of using inorganic fertilizer and other irrigation ways got the highest performance value (80). If decision making is inclined to farmer's perspective with low cost (weight 0.4) and high yield (weight 0.4), then using spray irrigation and other fertilizer type got the highest performance value of 79. If the assessors tend to avoid eutrophication in the water system, then environmental impacts related criteria get high weight of 0.7. This gave the highest performance value of 90 on the combination of using spray irrigation and other fertilizer type and 89 on the combination of using other irrigation ways and fertilizer type. These results showed that the sensitivity analysis was especially useful when the assessors have different opinions about the weight or scores. Such sensitivity analysis shows that in this example scenario spray irrigation and other fertilizer types almost always had highest score.

5.4 Discussion and Conclusions

This study calculated the future *E.coli* concentration changes on pre-harvest leafy green vegetables in RCP 8.5 and RCP 2.6 at the end of the century in Spain. The results indicate the *E.coli* concentration are projected to increase 0.2 log10 CFU/g to 0.3 log10 CFU/g (depending on the climate scenarios and management options applied) due to higher temperature at the end of the century, assumed no changes in agriculture management practices, compared to the concentrations at the end of the last century. This positive relationship, however, is opposite to the expectation that, at least in temperate climate zones, *E. coli* concentrations would decline faster with higher temperature (Franz et al. 2014). Temperature influences many additional factors, such as wildlife appearance, insects' activities and irrigation frequency, which might be important. Therefore, we speculate that the increase in *E. coli* contamination is probably due to an indirect effect of increasing temperature, and not directly related to warmer days (Liu et al., submitted). The study also demonstrated step by step a multi-criteria scenario analysis tool to study future safety of pre-harvest spinach in Spain as an example. The results show this is a very useful, flexible and easy to apply tool to select the best management practices considering climate change and indicators for other factors, such as management practices.

Multi-criteria analysis is a method commonly used in environmental science, to include several stakeholders and to a better inform decision-making process. Although, several strengths and weaknesses of stakeholders' participation in decision-support processes exists, involving them actively in research is supported by Bulkeley and Mol's (2003) review. In this study we adapted this commonly used method to study future food safety and developed the multi-criteria scenario tool. Our multi-criteria scenario analysis tool has several strengths, which correspond to the evaluation from Bulkeley and Mol's (2003) review: 1) Since food safety is influenced by both climate and agriculture management practices (Liu et al. submitted), the tool provides a possibility to simultaneously study climate and management impacts on future food safety changes; 2) The tool includes different stakeholders' perspective or interests and prioritises these interests; 3) It gives a more complete picture of the impacts of each individual scenario; and 4) It shows the decision makers or farmers the best way forward to effectively adapt to climate change.

To demonstrate the tool we kept the actual scoring and weighting relatively simple. Sensitivity analysis can test not only the changes in weight but also in scores. In this example only the sensitivity of weight was tested. In other applications multi-criteria scenario analysis may have the following challenges: 1) involving all relevant stakeholders and obtaining their preferences (i.e. weights) can be difficult; 2) A small and active group of stakeholders may dominate the process; and 3) A mutual understanding among stakeholders about the problem and the goals is rarely obtained.

Since this is an exploratory study, the changes of other criteria besides climate-change induced temperature increase were not included. For example, a higher soil temperature leads to an increased use of potentially contaminated animal manure due to a faster depletion of soil nutrients resulting from increased biological soil activity (Franz et al. 2008a). These, most likely major socio-economic changes, should not be ignored in more realistic applications. Apply, for example, the new scenario framework 'Shared Socioeconomic Pathways' for climate change research, which has been developed to study the climate change on socioeconomic aspects (O'Neill et al. 2014), could well complement the different climate-change scenarios and ensembles.

This multi-criteria tool provides a platform to study changes in weather or climate, and management impacts on future food safety together with different stakeholders' perspectives or interests. The tool allows to better involve different stakeholders in the analysis and likely supports their decision making process. In this way, such a multi-criteria scenario analysis delivers a new mind set and method to study food safety and enhances the quality of agricultural management decisions for leafy green vegetables.

Table 5.1 Performance matrix of six agriculture management practices combinations. Other fertilizer type is non animal organic fertilizer. Other irrigation ways include flood irrigation and drip irrigation.

Scenarios	**Mean of *E. coli* concentration distribution in 20 years (min, mode, max of Triangular distribution)**			**Costs (-)**	**Yield (*)**	**Nutrient loss from soil/soil health (*)**
Manure + Other irrigation ways	2.04 (1.32, 1.50, 3.32)	2.14 (1.39, 1.58, 3.44)	2.33 (1.54, 1.72, 3.72)	0.4	Low	High
Inorganic fertilizer + Other irrigation way	0.87 (0.21, 0.26, 2.15)	0.97 (0.28, 0.34, 2.28)	1.15 (0.44, 0.44, 2.56)	1	Low	Low
Spray + Other fertilizer type	1.82 (1.11, 1.27, 3.10)	1.92 (1.18, 1.35, 3.22)	2.10 (1.33, 1.48, 3.50)	0.5	High	Low
Manure + Spray	2.44 (1.69, 1.91, 3.71)	2.53 (1.76, 1.99, 3.83)	2.72 (1.91, 2.13, 4.11)	0.2	Low	High
Inorganic fertilizer + Spray	1.27 (0.57, 0.71, 2.54)	1.36 (0.65, 0.78, 2.66)	1.54 (0.80, 0.88, 2.95)	0.7	Low	Low
Other irrigation ways + Other fertilizer type	1.44 (0.73, 0.88, 2.70)	1.53 (0.81, 0.95, 2.83)	1.71 (0.95, 1.07, 3.11)	0.8	High	Low

Table 5.2 Performance of the scenarios on a 0 (lowest) – 100 (highest) scale. Other fertilizer type is non animal organic fertilizer. Other irrigation ways include flood irrigation and drip irrigation.

Scenarios	**Mean *E.coli* concentration 2081-2100 RCP 8.5**	**Costs**	**Yield**	**Nutrient loss from soil/soil health (*)**
Manure + Other irrigation ways	25	75	0	0
Inorganic fertilizer + Other irrigation way	100	0	0	100
Spray + Other fertilizer type	40	63	100	100
Manure + Spray	0	100	0	0
Inorganic fertilizer + Spray	75	38	0	100
Other irrigation ways + Other fertilizer type	64	25	100	100

Table 5.3 Calculation of weighted and overall performance values. Other fertilizer type is non animal organic fertilizer. Other irrigation ways include flood irrigation and drip irrigation.

Scenarios	**Mean *E.coli* concentration 2081-2100 RCP 8.5**	**Costs**	**Yield**	**Nutrient loss from soil/soil health (*)**	**Overall performance value**
Manure + Other irrigation ways	6	19	0	0	25
Inorganic fertilizer + Other irrigation way	25	0	0	25	50
Spray + Other fertilizer type	10	16	25	25	**76**
Manure + Spray	0	25	0	0	25
Inorganic fertilizer + Spray	19	10	0	25	44
Other irrigation ways + Other fertilizer type	16	6	25	25	**72**
Weight	0.25	0.25	0.25	0.25	1

Table 5.4 Sensitivity analysis. Other fertilizer types include composted manure and non animal organic fertilizer. Other fertilizer type is non animal organic fertilizer. Other irrigation ways include flood irrigation and drip irrigation.

Scenarios	**Mean *E.coli* concentration 2081-2100 RCP 8.5**	**Costs**	**Yield**	**Nutrient loss from soil/soil health (*)**	**Overall performance value**
Manure + Other irrigation ways	18	8	0	0	26
Inorganic fertilizer + Other irrigation way	70	0	0	10	**80**
Spray + Other fertilizer type	28	6	10	10	54
Manure + Spray	0	10	0	0	10
Inorganic fertilizer + Spray	53	4	0	10	67
Other irrigation ways + Other fertilizer type	45	3	10	10	68
Weight	**0.7**	0.1	0.1	0.1	1
Manure + Other irrigation ways	3	30	0	0	33
Inorganic fertilizer + Other irrigation way	10	0	0	10	20
Spray + Other fertilizer type	4	25	40	10	**79**
Manure + Spray	0	40	0	0	40
Inorganic fertilizer + Spray	8	15	0	10	33
Other irrigation ways + Other fertilizer type	6	10	40	10	66
Weight	0.1	**0.4**	**0.4**	0.1	1
Manure + Other irrigation ways	3	8	0	0	11
Inorganic fertilizer + Other irrigation way	10	0	0	70	80
Spray + Other fertilizer type	4	6	10	70	**90**
Manure + Spray	0	10	0	0	10
Inorganic fertilizer + Spray	8	4	0	70	82
Other irrigation ways + Other fertilizer type	6	3	10	70	**89**
Weight	0.1	0.1	0.1	**0.7**	1

Chapter 6

Synthesis and Conclusion

This thesis aims to quantify the impacts of climate change on the microbial safety of pre-harvested leafy green vegetables (LGVs). The hygienic status of LGVs as measured by contamination with generic *E. coli* was taken as a proxy for the microbial safety. This research was part of the EU-funded interdisciplinary Veg-i-Trade project. Four research questions (RQs) guided my analysis:

1) What are the impacts of climate change on contamination sources and pathways of foodborne pathogens?
2) How do climatic conditions quantitatively affect the *E. coli* contamination of pre-harvested leafy greens?
3) How to downscale climate and climate-change data for local food safety analysis?
4) How does the safety of LGVs evolve under future climate scenarios?

I structured these questions in a logic way to answer the main question of my thesis: **What are the climate-change impacts on microbial safety of leafy green vegetables?** My research process and methods are described in Figure 1.1. These research questions are answered and discussed in section 6.1 to 6.4. Methodological lessons learnt are discussed (Section 6.5) before I conclude with the main findings (Section 6.6).

6.1 Impacts of climate change on contamination sources and pathways (RQ1)

RQ1 was answered in two steps. Firstly, contamination sources and pathways were defined. These sources included manure, soil, surface water, sewage and wildlife. Contamination pathways included irrigation, splash, contact with faeces, surface run-off and overflow. Secondly, I elaborated the positive and negative impacts of temperature increases and changes in precipitation pattern on pathogen prevalence for each contamination source and pathway. The net climate-change impacts depended on the balance of the positive and negative impacts, and on the applied climate change scenarios for specific areas.

This interdisciplinary study started with a mutual understanding of the terminology in the two research fields, 'climatology' for climate change and 'food microbiology' for food safety. I started this research with little knowledge about food microbiology research and its research methods. A systematic review was more than necessary for me to learn the fields' state-of-the-art and to combine the knowledge comprehensively into a conceptual diagram (Figure 2.1) and a table (Table 2.1), which qualitatively indicate the climate impacts on foodborne pathogens. I eventually limited this review to two foodborne pathogens to achieve a more in-depth review. The pathogen *Listeria* was also reviewed but was dropped during the revision to give a better focus on the other two pathogens. The risk factors for *Listeria* contamination in fruits and vegetables are summarised by Park et al (2012). They also concluded that irrigation water and soil are two main contamination sources. In this thesis, I initially focused on pathogenic contamination of LGVs in Chapter 2. Then I switched to the hygiene indicator generic *E. coli* in the rest of the chapters. *E. coli* was better covered by data compared to pathogens. This review ended with an identified research gap: lack of quantitative studies with scenario analyses to understand the net impact of climate change on the contamination of pre-harvest LGVs. I fill this research gap by addressing the remaining RQs and the further research in this thesis.

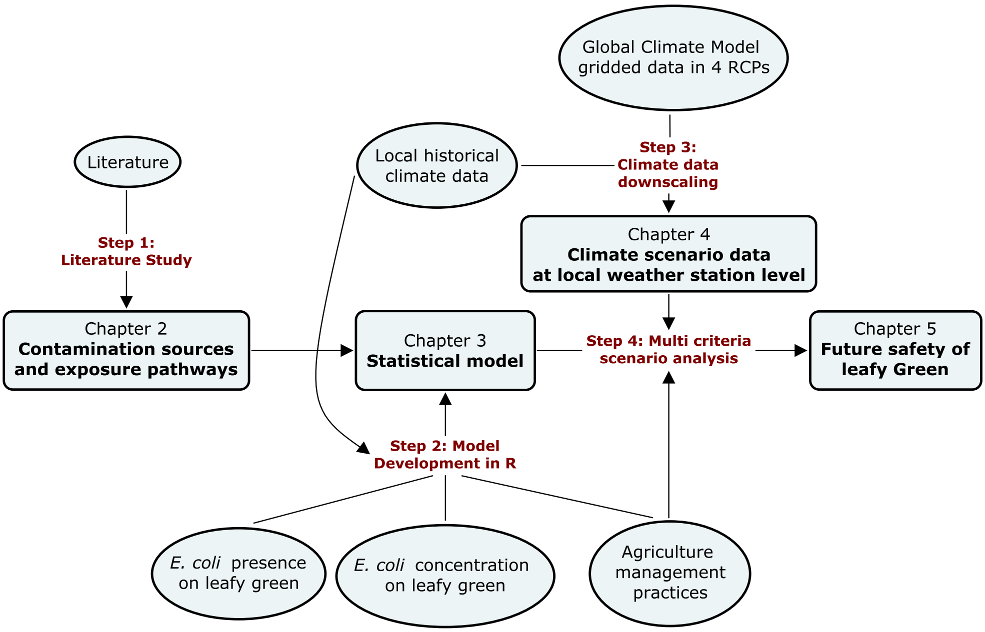


Figure 1.1 Process and methods of this thesis. Round boxes are data and knowledge inputs and square boxes are major results in this thesis (Chapter 2 to 5). Arrows are data and knowledge flow.

6.2 Statistical modelling Meta-regression analysis (RQ2)

RQ2 was answered by mixed effect logistic regression and linear regression models. Climate and agricultural management practices both had influence on *E. coli* presence and concentration. Temperature had a stronger influence than management practices for *E. coli* presence and concentration on LGVs. Minimum temperature on the sampling day, region and application of inorganic fertilizer are important for *E. coli* presence (odds ratio

[OR] 1.47). Maximum temperature on the three days before the sampling day and region are important for *E. coli* concentration (R^2 = 0.75). The variable *Region* masks many management variables including irrigation water use and fertilizer use. Temperature has a positive relationship with *E. coli* presence and concentration. Climate, irrigation water type, fertilizer type and irrigation method should be considered systematically in fresh produce safety studies in the future.

After defining the conceptual framework in Chapter 2, I started this study with the goal of developing a simulation model based on Franz et al. (2008b). Such a model has the advantage of identifying explicit mechanism and causal relationships (Adams et al. 2013), predicting under uncertainty and applying what-if scenario analysis. However, including several contamination pathways (e.g. splash, irrigation and manure application) and climate influences in this simulation model was too ambitious. The contamination pathways were too complicated to be included in one model and, more importantly, the quantitative influences by climate were unknown. Instead, a statistical model was developed to study the quantitative impact of climate and climate change. During the process of model development, I have learned a lot about statistical modelling as summarised in Section 6. 5.

Meta-analysis was used in Chapter 3 to answer the second research question. I did not limit myself only to the data collected in the Veg-i-Trade project, but also requested data from the literature studying climate and management impacts on *E. coli* contamination on leafy green vegetables. These studies included Loncarevic et al. (2005), Mukherjee et al. (2007), Ailes et al. (2008), Strawn et al. (2013) and Park et al. (2014). As a result, Park et al. (2014) shared their sampling data with me, the others sadly did not respond or responded positively but never followed up with actual data. Combining different studies with big differences in the sampling set up and data structure, however, gave a high degree of pseudoreplication. For this reason, I decided to use only Veg-i-Trade data in Chapter 3 and summarised the lessons learnt during this excise for future meta-analysis in Section 6. 5.

Chapter 3 aimed to identify a combination of statistically significant variables that best explained observed variation in *E.coli* presence and contamination level. However, several of these best explaining variables have counter-intuitive relations with *E. coli* contamination. Inorganic fertilizer had a positive parameter estimate in the *E. coli* presence model. This differed from my expectation since inorganic fertilizer should be sterile. However, the parameter estimate was not significant meaning that, although inorganic fertilizer significantly explained the data variation, the parameter estimate was not representative for indicating directional changes in *E. coli* contamination. The counter-intuitive risk factor of inorganic fertilizer most likely is due to the dataset's nature and the fact that regression analysis does not identify true causal relations. Many of the positive samples arise from farms using inorganic fertilizer but the true cause of contamination may be other factors not considered in this study. Similarly, a direct positive effect of temperature on *E. coli* presence and contamination is not expected given the general observed negative relation between temperature and environmental persistence (Franz et al. 2014). Temperature may affect environmental factors like wildlife intrusion, insect activity, and irrigation frequency, which in turn directly affect *E. coli* presence and concentrations. These environmental factors should be included in the future sampling and analysis to cover more potential contamination pathways.

6.3 Climate and climate change data downscaling (RQ3)

RQ3 was answered with a new method described in Chapter 4 for preparing climate and climate change data for food safety assessment. I selected HadGEM2-ES and CCSM4 models and downscaled data using the 'Delta method' with quantile-quantile correction. I illustrated the method by determining how the bacteria growth rate may change if temperature changes according to the four representative concentration pathways (RCP) scenarios. The downscaling method was satisfactorily applicable for food safety assessment.

The quantile-quantile correction straightforwardly uses the distribution of observational data, which describes the local variability, but requires the availability of a representative period of observations. Using the distribution from the observation data for all future scenarios can be disadvantageous because it standardizes the variability among scenarios. This unrealistic future having the same pattern as the present observational station data neglects possible future shifts in extremes.

This study took place at the end of 2013 when some of the GCM teams were computing their final RCP runs. Shortly after the data analysis, the fifth assessment report of the Intergovernmental Panel on Climate Change (IPCC) was published documenting the various RCPs. I recommend checking the errata information for CCSM4 (used in Chapter 4) and CESM1 models (available on www.cesm.ucar.edu/CMIP5/errata) before choosing the model runs because these new scenarios are definitely work-in-progress. For example, CCSM4 RCP2.6 r6i1p1 run was withdrawn from the CMIP5 archive due to an error found in the data. This simulation will not be re-run since RCP2.6 is not often used. Despite, this limitation, all food safety impact studies are recommended to rely on the latest climate-change scenarios.

My downscaling tool provided a user friendly platform (in MATLAB and Excel) and was developed especially for food safety researchers to explore the impact of climate change on their impact models, such as the climate change impact study on mycotoxins (van de Perre et al. 2014b). More downscaling tools are, however, available to downscale the RCP data. A commonly used method in meteorology is the Climate Data Operator (Schulzweida et al. 2006) available from https://www.mpimet.mpg.de/cdo. This operator requires more experience in programming and data management in Linux systems. A package (code also written in MATLAB) developed by Oregon State University's College of Engineering was released shortly after my manuscript was submitted. The package is available at http://globalclimatedata.org/. This tool also produces a delta downscaled data with a 30 arc-second monthly precipitation surface. However, its temporal scale is still too coarse for food safety research. For this research my tool is still the most appropriate.

6.4 Future projection (RQ4)

RQ4 was answered in Chapter 5 by applying climate change data to the *E. coli* concentration model presented in Chapter 3 using pre-harvest spinach in Spain as an example. I explored the development and application of a multi-criteria scenario analysis tool in Chapter 5. The step by step demonstration and the sensitivity analysis showed this is an applicable tool to select the best management practices, considering climate change and other indicators.

Although the main focus of Chapter 5 was to explore the tool, I also calculated the future *E.coli* concentration changes on Spanish spinach in climate-change scenario RCP 8.5 and RCP 2.6 at the end of this century. This way, all research questions become connected and RQ4 was addressed. The results indicated that the mean *E.coli* concentration over 20 years were projected to increase between 0.2 log10 CFU/g and 0.3 log10 CFU/g (depending on the climate scenarios and management options applied) due to higher temperature at the end of the century, compared with the concentrations at the end of the last century. This analysis assumed no changes in agriculture management practices.

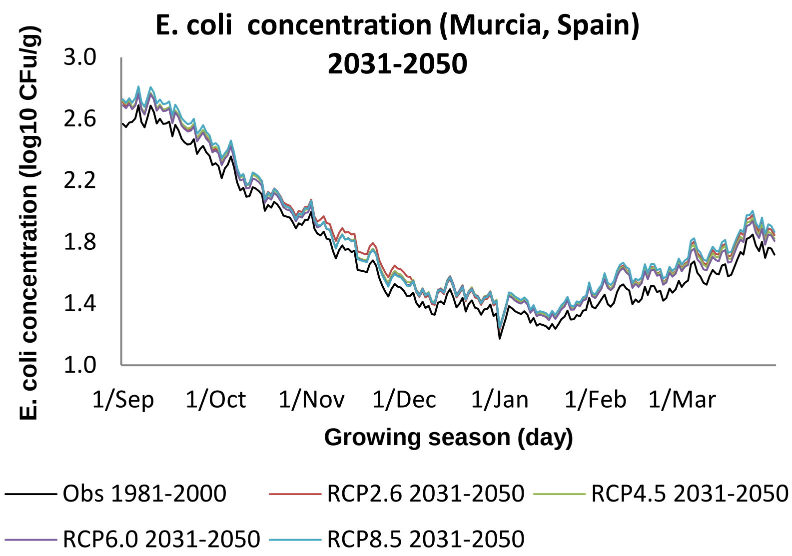
To further detail the calculation for *E. coli* concentrations, historical concentrations are compared with near and far future concentrations during the growing season for four RCPs (Figure 6.2). These are computed with historical and projected daily maximum temperature from the CCSM4 (Gent et al. 2011). Daily concentrations are averaged over 20 years. *E. coli* concentrations on spinach in Murcia, Spain increase in all four RCPs (Figure 6.2). In the near future (i.e. 2031-2050), the differences in *E. coli* concentration increase are small because the radiative forcing for this period is still similar to that of today. Temperature change in this period is still strongly dominated by historic emissions.

For the far future (i.e. 2081-2100), the *E. coli* concentration increases diverge between the different RCPs and range from 0.05 log10 CFU/g to 0.40 log10 CFU/g. RCP8.5 projects the highest (i.e. between 0.22 log10 CFU/g - 0.40 log10 CFU/g) average increase for this period.

Climate change data from only one GCM were used in this analysis. *E. coli* concentrations calculated with climate-change data from other GCMs are likely different but the calculated direction of the trends will probably be very similar. For example, projected temperature data from the HadGEM2-ES GCM are approximately 1 °C cooler than for the CCSM4 GCM (Chapter 4). To capture the full range of climate change uncertainties, I recommend to use either the ensemble mean (the most cost-effective approach) or the full ensemble range (the most informative approach) as supported by Christensen et al. (2007), Kharin et al. (2002) and Tebaldi et al. (2007). How these different ensembles were generated is explained in Chapter 4.

Since this was an exploratory study, the changes in management practices due to climate change (e.g. adaptation efforts) were excluded. For example, a higher soil temperature may lead to an increased use of potentially contaminated animal manure due to a faster depletion of soil nutrients as a result of increased biological soil activity (Franz et al. 2008a). Such processes and their interactions should not be ignored in other, more realistic applications. Applying, for example, the new scenario framework 'Shared Socio-economic Pathways', which has been developed to consistently combine socio-economic aspects with the RCP scenarios (O'Neill et al. 2014), could well complement the different climate-change scenarios and ensembles.

At the start of this PhD, I planned to study the *E. coli* concentration changes in the future due to future climate change induced temperature and precipitation changes. Chapter 3, however, showed management also had significant influences on the fate of *E. coli.* The methodology of multi-criteria analysis was then adapted to be used as a tool to study the future food safety including both climate and management influences together. I kept the actual criteria selection, scoring and weighting overly simplistic for the purpose to demonstrate the application of the tool without forming an expert team or diving into the literature. In other applications, more appropriate criteria, scores and weights should be discussed among expert teams and assigned to each scenario based on local situations.



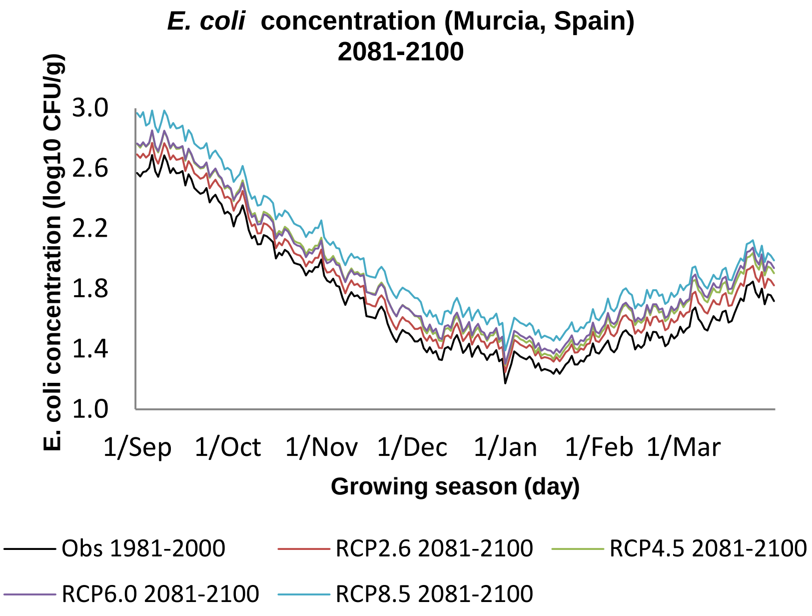


Figure 6.2 Comparison of historical (1981-2000), near future 2031-2050 (top) and far future 2081-2100 (bottom) *E. coli* concentration on spinach in Murcia (Spain) during growing season. The future *E. coli* concentration were calculated with future daily maximum temperature in four Representative Concentration Pathways (RCPs) projected by global circulation model CCSM4.

6.5 Methodological lessons learnt

This section summaries some of the most important methodological lessons that I have learnt throughout my thesis.

Meta-analysis provides opportunities to perform statistical analysis with limited positive samples from each region. From this study, I have learnt several lessons for future meta-analyses:

a) Experimental design in meta-analysis has to be standardized as much as possible. In my study all samples were taken according to the Horticultural Assessment Scheme (HAS) which is developed in Veg-i-Trade project. This sampling protocol enables the same sampling method and information input. However, the sampling and analysis in different regions initially were not fully designed to conduct a meta-analysis. Even though the samples were taken using the same scheme, I had to work with two types of produce and, more importantly, different detection limits.
b) Meta-analysis has more strict sampling requirements compared to individual studies. The resulting combined datasets need to have the best-possible balanced representation of management variables and their various levels. If a study is aimed to do a meta-analysis, then its sampling would ideally have the same amount of samples for each management variable. Ideally all management variables should also be covered in each region. For this reason it was impossible to include other studies outside the Veg-i-Trade project in my meta-analysis. This highlights the need for a better coordinated future international sampling collection effort and for development of study design and reporting standards. Because a better standardisation will assure that the data collected and results reported in different regions are comparable and compatible, and can be used in subsequent meta-analyses. Including studies with very different sampling efforts may give totally different results.

Some of the regional differences are not well defined in the meta-analysis, but they should not be ignored. The difference in my joint regression models with and without the variable *Region* show that this variable not only explains the regional variations in many management practices, but also additional regional differences. I recommend to enlarge the model boundary in future studies by including these additional differences (e.g. variation in detection limits, experimental material and equipment, local hygiene, social economic development levels, presence of wildlife intrusion, insects activity, irrigation frequency, soil type and slope/topography) to complete the system analysis of LGVs safety.

The climate data downscaling tool presented in Chapter 4 can be used for data outputs from any GCM. However, each GCM has different parameterizations and settings for the model. For example, HadGEM2-ES has 360 days in a year and its base date is December 1, 1859 versus 365 days (no leap year) and January 1, 1850 in the CCSM4 model. These differences have to be verified and adjusted in the downscaling process. Because of such inconsistencies, I recommend to use programming software, such as MATLAB or R, rather than ArcGIS. ArcGIS is easier to use for visualisation and for selecting a specific location, but does not quickly allow addressing and adjusting the inherent inconsistencies of the required GCM results.

E. coli is used as a hygiene indicator in this thesis to study microbial safety of LGVs. Since the presence of *E. coli* indicates faecal contamination, it is valid to state that the presence of *E. coli* implies an increased risk of pathogen presence (Edberg et al. 2000, Tallon et al. 2005). However, the behaviour (growth and survival) of generic *E. coli* and enteric pathogens may significantly differ, even for pathogenic *E. coli* (Franz et al. 2014). To further study the risks of foodborne diseases in the future, the prevalence and the link between hygienic indicator and foodborne pathogens should be included.

6.6 Main findings

This thesis assessed and quantified the impacts of climate change on microbial safety of pre-harvested leafy green vegetables contaminated with generic *E. coli.* With one degree increase in minimum temperature of the sampling day, the odds of having *E. coli* presence on leafy green vegetable increase by a factor of 1.5. The mean *E. coli* concentrations over twenty years are expected to increase between 0.2 log10 CFU/g and 0.3 log10 CFU/g (depending on the climate scenarios and management options applied) due to higher temperature towards the end of this century, compared to the concentrations at the end of the last century. For this conclusion, no changes in current agriculture management practices were assumed. I obtained these quantitative climate-change impacts on future food microbial safety in a series of sequential steps which construct my thesis (Figure 1.1).

The pioneering research presented in my thesis not only quantified climate change impacts on LGVs contamination by *E. coli* for the first time, but also brought new methods and tools to food safety research. The climate change data downscaling tool presented in Chapter 4 provides detailed temporal and spatial climate data for climate scenario analysis in food safety assessment studies. The multi-criteria scenario analysis tool presented in Chapter 5 provides a platform to study the consequences of changes in weather or climate, and management impacts on future food safety together with different perspectives or interests of stakeholders. This tool especially provides an opportunity to involve different stakeholders in the analysis and support their decision making process. A more traditional way of studying the fate of bacteria is to follow the bacteria from the contamination reservoirs (e.g. manure or soil) to the produce. Chapters 3 and 5 deliver a new mind set to systematically study food safety by simultaneously combining climate and management variables. The results in these two chapters show that climate and agricultural management practices both had influence on *E. coli* presence and concentration.

The climate data downscaling tool demonstrated in Chapter 4 has already been used in a climate change impacts on a mycotoxin study (van de Perre et al. 2014b) in Poland and Spain and a pesticide study in Belgium (Veg-i-Trade Consortium 2014). This example can stimulate food safety researchers, who are interested to study the impacts of climate change, by providing a comprehensive scenario analysis for any location in the world. The multi-criteria scenario analysis tool and the quantified climate change impacts on LGVs can be used by multiple stakeholders, including farmers and policy makers, during their decision making process to achieve mutual understanding of their challenges and the best strategies to adapt to climate change.

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Summary

Climate change is generally recognized as a major threat to humans and the environment. With respect to food production, climate change does not only affect crop production or food security, but possibly also effects on food safety by affecting the prevalence and levels of bacteria, fungi or other pests and pesticides. Fresh-cut or ready-to-eat leafy vegetables (e.g. lettuce and spinach) are increasingly consumed because they are promoted as part of a healthy diet. Such leafy green vegetables (LGVs) are identified as the fresh produce commodity group of highest concern from a microbiological safety perspective, because they are often grown in the open field and therefore vulnerable to contamination and contact with (faeces of) wildlife. Moreover, they are grown and consumed in large volumes and often consumed raw. Bacteria, such as *Salmonella* spp. and pathogenic *Escherichia coli* strains are the main pathogens causing foodborne disease through LGVs. A major knowledge gap is understanding how climate change may directly or indirectly affect the contamination of LGVs. This primarily relates to the current lack of methods and tools to link climate data and climate change scenarios to food safety.

My thesis aims to quantify the impacts of climate change on microbial safety of pre-harvested LGVs. To achieve this, I reviewed the literature and synthesised major impacts of climate change on contamination sources and pathways of foodborne pathogens (focussing on *Escherichia coli* O157 and *Salmonella* spp.) on pre-harvested LGVs (Chapter 2). Subsequently, I developed a statistical model that identifies the weather and management variables that are associated with the LGVs contamination with generic *E. coli* using regression analysis (Chapter 3). To apply suitable climate data to this statistical model to assess future impacts, I have prepared a tool to downscale coarse climate and climate change data for local food safety scenario analysis (Chapter 4). Finally, I applied the downscaled data to the statistical model and used multi-criteria scenario analysis to explore future food safety (Chapter 5). *E.coli* is used as a hygienic indicator in this thesis to study microbial safety of LGVs. Its presence is indicative for an increased pathogen presence probability. *E. coli* and many foodborne bacteria share the same contamination pathways and climate change is expected to similarly impact on both bacteria. Hygienic status is therefore used in my thesis as a proxy for the microbial safety of LGVs.

The major result of the literature review in Chapter 2 is that the impact of climate change on LGV contamination depends on the resulting local balance of the positive and negative impacts. The review shows that the interactions between climate change and

contamination are real but poorly understood. Therefore, integrative quantitative modelling approaches with scenario analyses and additional laboratory experiments are needed.

With this knowledge background, mixed effect logistic regression and linear regression models were developed to identify the climate and management variables that are associated with the presence and concentration of *E. coli* on LGVs (Chapter 3). These models used *E. coli* data of 562 lettuce and spinach samples taken between 2011 and 2013 from 23 open-field farms from Belgium, Brazil, Egypt, Norway and Spain. Weather and agriculture management practices together had a systematic influence on *E.coli* presence and concentration. Temperature explained most of the observed variation on *E. coli* prevalence and concentration on LGVs. Minimum temperature of the sampling day (odds ratio [OR] 1.47), region and application of inorganic fertilizer explained a significant amount of variation in *E. coli* prevalence. Maximum temperature on three days before sampling and region best explained the variation in *E. coli* concentration (R^2= 0.75). *Region* is a variable masking many management variables including use of rain water, surface water, manure, inorganic fertilizer and spray irrigation. Climate variables and *E. coli* presence and concentration are positively related. The results indicate that climate change will have an impact on microbiological safety of LGVs. These impacts can be directly through an increasing temperature, but also indirectly through changes in irrigation water type, fertilizer type and irrigation method. Therefore, climate change and farm management should be considered more systematically in an integrated way in future studies on fresh produce safety.

To prepare climate data for local food safety scenario analysis, a climate data downscaling tool was presented and demonstrated (Chapter 4). Coarse gridded data from two general circulation models, HadGEM2-ES and CCSM4, were selected and downscaled using the 'Delta method' with quantile-quantile correction for the Belgium meteorological station in Ukkel. Observational daily temperature and precipitation data from 1981 to 2000 were used as a reference period for this downscaling. Data were provided for four future representative concentration pathways (RCPs) for the periods 2031–2050 and 2081–2100. These RCPs are radiative forcing scenarios for which future climate conditions are projected. The climate projections for these RCPs show that both temperature and precipitation will increase towards the end of the century in Ukkel. The climate change data were subsequently used with Ratkowsky's bacterial growth model to illustrate how projected climate data can be used for projecting bacterial growth in the future. In this example, the future growth rate of Lactobacillus plantarum and the number of days that the bacteria are able to grow are both projected to increase in Ukkel. This example illustrates that this downscaling method can be applied to assess future food safety. This downscaling tool is relatively straightforward compared to other more complex downscaling tools, so the food safety researchers can easily understand and apply it to their impact studies.

With the statistical model (Chapter 3) and downscaled climate data (Chapter 4), a multi-criteria scenario analysis tool was developed to explore future food safety using pre-harvest spinach in Spain as an example (Chapter 5). The future *E. coli* concentrations on spinach were projected to change in RCP 8.5 and RCP 2.6 by the end of the century in Spain. The *E. coli* concentration was projected to increase between 0.2 log10 CFU/g and 0.3 log10 CFU/g (depending on the climate scenarios and management options applied) due to higher temperature by the end of the century compared to the concentrations by the end of the last century. This comparison assumed no changes in agricultural management practices. This tool can be used to help selecting the best management practices considering climate change and other indicators.

The pioneering research presented in my thesis brought new methods and tools, and another mind set to food safety research. The climate-change data downscaling tool provides detailed temporal and spatial climate data for climate scenario analysis in food safety assessment studies. The multi-criteria scenario analysis tool provides a platform to study changes in weather or climate, and management impacts on future food safety. This tool also allows for inclusion of different stakeholders' perspectives or interests and supports their decision making processes. Moreover, the thesis presents a statistical model that can be used to study the relationship between climate and *E. coli* contamination.

My thesis quantified the impacts of climate change on microbial safety of pre-harvested LGVs contaminated with generic *E. coli* for the first time. With one degree increase in minimum temperature of the sampling day, the odds of having *E. coli* presence on LGVs increase by a factor of 1.5. The mean *E. coli* concentrations are also expected to increase. Climate change should not be ignored in food safety management and research.

Samenvatting

Klimaatverandering wordt algemeen beschouwd als een groot risico voor mens en milieu. Als het gaat om voedselproductie beïnvloedt klimaatverandering niet alleen de wereldwijde gewasopbrengst en de voedselbeschikbaarheid, maar mogelijk ook de voedselveiligheid door verandering van de verspreiding van bacteriën, schimmels en andere contaminanten. De productgroep met het hoogste risico op microbiologische verontreiniging zijn verse bladgroenten, zoals spinazie en sla. Deze groenten worden aanbevolen als onderdeel van een gezond dieet. Verse bladgroenten worden veelal rauw geconsumeerd en vaak in grote volumes in het open veld geteeld. Hierdoor kan het gewas in contact komen met wilde dieren of uitwerpselen daarvan. De verontreinigingen van verse bladgroenten omvatten bacteriën, zoals *Salmonella* spp en pathogene stammen van *Escherichia coli*. Een belangrijke vraag is hoe klimaatverandering de voedselveiligheid van verse bladgroenten zal beïnvloeden. Hierover is nog weinig bekend. Er is nog geen algemeen erkende methode om klimaatgegevens en klimaatscenario's te koppelen aan voedselveiligheid.

In mijn onderzoek kwantificeer ik het effect van klimaatverandering op de microbiologische veiligheid van verse bladgroenten in het veld. Om dit te bereiken heb ik de belangrijkste literatuur samengevat om besmettings-routes van ziekteverwekkers op verse bladgroenten en de effecten van klimaatverandering erop in beeld te brengen (Hoofdstuk 2). Hierbij heb ik mij voornamelijk gericht op *Salmonella* spp. en *Escherichia coli* O157. *E. Coli* wordt in dit onderzoek gebruikt als indicatororganisme voor de microbiologische verontreiniging van verse bladgroenten. De aanwezigheid van deze bacterie duidt op verhoogde kans op de aanwezigheid van andere pathogenen. *E. coli* en veel andere door voedsel overgedragen bacteriën hebben immers dezelfde besmettingsroutes. Klimaatverandering zal naar verwachting een vergelijkbare invloed hebben op deze bacteriën. Daarom wordt de microbiologische veiligheid van verse bladgroenten in dit onderzoek weergegeven middels de concentratie van *E. Coli*.

Vervolgens heb ik een statistische regressieanalyse toegepast waarmee de weer- en teeltvariabelen zijn geïdentificeerd, die worden verbonden aan de verontreiniging van verse bladgroenten met goedaardige *E. coli* (Hoofdstuk 3). Weervariabelen uit klimaatscenario's kunnen in deze regressieanalyse worden toegepast om toekomstige effecten op de voedselveiligheid te voorspellen. Hiervoor heb ik een instrument ontwikkeld waarmee klimaatgegevens en klimaatanomalieën uit mondiale

klimaatmodellen kunnen worden gedetailleerd voor een scenarioanalyse van voedselveiligheid in een specifiek teeltgebied (Hoofdstuk 4). Vervolgens heb ik de deze gedetailleerde gegevens toegepast in het regressiemodel uit Hoofdstuk 3. Met de resultaten van dit regressiemodel en een multicriteria scenarioanalyse schets ik een beeld van veranderingen in de voedselveiligheid als gevolg van klimaatverandering (Hoofdstuk 5).

Uit mijn literatuuronderzoek van Hoofdstuk 2 blijkt dat het netto effect van klimaatverandering op het risico op microbiologische verontreiniging van verse bladgroenten afhangt van de lokale balans tussen positieve en negatieve invloeden. Mijn literatuuronderzoek laat zien dat er een reëel verband is tussen klimaatverandering en verontreiniging. Dit is verband is echter onvoldoende onderzocht. Integrale, kwantitatieve modellen in combinatie met scenarioanalyse en aanvullende laboratoriumonderzoeken kunnen worden gebruikt om deze kennisleemte op te vullen. Met deze motivatie zijn gemengde logistische en lineaire regressiemodellen ontwikkeld waarmee de klimaat- en teeltvariabelen zijn geïdentificeerd die kunnen worden gekoppeld aan de aanwezigheid en concentratie van *E. coli* op verse bladgroenten (Hoofdstuk 3). Deze modellen gebruiken de gemeten *E. coli* concentraties van 562 monsters van spinazie en sla, genomen tussen 2011 en 2013 op 23 tuinbouwbedrijven (teelt op open grond) in België, Brazilië, Egypte, Noorwegen en Spanje. De combinatie van het weer en de teeltpraktijk heeft een systematische invloed op zowel het voorkomen als de concentratie van *E. coli* op verse bladgroenten. De meeste variatie hierin kan worden verklaard door de temperatuur. De minimum dagtemperatuur tijdens de monstername (Odds Ratio [OR] 1.47), de regio en de toepassing van kunstmest verklaren samen een significant deel van de variatie in het voorkomen van *E. coli*. De maximum temperatuur van de 3 dagen voor de monstername en de regio hebben de meeste verklarende waarde voor de concentratie van *E. coli* (R^2=0.75). De geografische regio is een verklarende variabele die verschillende lokale teeltvariabelen maskeert, zoals het gebruik van regenwater of oppervlaktewater voor irrigatie, het gebruik van dierlijke mest of kunstmest en het gebruik van sproei-irrigatie. Klimaatvariabelen en de aanwezigheid en concentratie van *E. coli* zijn positief gerelateerd. De resultaten laten dan ook zien dat klimaatverandering invloed zal hebben op de microbiologische veiligheid van verse bladgroenten. Dit kan een een directe invloed zijn door hogere temperaturen tijdens het groeiseizoen, maar ook een indirecte invloed door veranderingen in het gebruik en de herkomst van irrigatiewater, het gebruik en de herkomst van dierlijke mest, en het gebruik van kunstmest. Deze teeltvariabelen en hun gevoeligheid voor klimaatvariabelen zullen verder moeten worden gekwantificeerd om de toekomstige voedselveiligheid van verse producten te bepalen.

Een instrument voor het detailleren van klimaatgegevens voor een scenarioanalyse van de lokale voedselveiligheid wordt gepresenteerd in Hoofdstuk 3. Grove roosterdata van twee algemene circulatiemodellen (HadGEM2-ES en CCSM4) zijn geselecteerd. Deze data zijn gedetailleerd voor het Belgische weerstation Ukkel (nabij Brussel) met behulp van de zogenoemde “Delta Methode” met een kwantiel-kwantiel correctie. De gedetailleerde klimaatdata zijn vervolgens toegepast in het Ratkowsky model voor bacteriegroei. Op deze manier kunnen klimaatscenario’s worden gebruikt om de theoretische groeisnelheid van bacteriën in een veranderd klimaat te voorspellen. Op basis van de gedetailleerde klimaatdata van Ukkel wordt voorspeld dat de theoretische groeisnelheid van *Lactobacillus plantarum* en het aantal dagen met gunstige groeicondities voor deze bacterie zullen toenemen in de toekomst. Deze illustratieve toepassing laat zien dat de gebruikte methode voor het neerschalen van klimaatdata geschikt is voor het beoordelen van de toekomstige voedselveiligheid van gewassen in het open veld. Bovendien is de gebruikte methode relatief eenvoudig toe te passen en daarom geschikt voor studies naar de klimaateffecten in de voedingswetenschappen.

Met het regressiemodel (Hoofdstuk 3) en de gedetailleerde klimaatdata (Hoofdstuk 4) is een multicriteria scenarioanalyse ontwikkeld. Dit instrument is toegepast op spinazie in het open veld in Spanje (Hoofdstuk 5). Een verandering van de concentraties van *E. coli* op het gewas wordt voorspeld voor de klimaatscenario’s RCP 8.5 en RCP 2.6 aan het einde van de 21^{e} eeuw. De E-coli concentratie neemt toe met 0.2 log10 CFU/g tot 0.3 log10 CFU/g (afhankelijk van het betreffende klimaatscenario en de toegepaste teeltvariabelen) als gevolg van temperatuurstijging en in vergelijking met de gemodelleerde concentraties aan het einde van de 20^{e} eeuw. Voor deze vergelijking is aangenomen dat er geen veranderingen zijn geweest in de teeltpraktijk. De multicriteria scenarioanalyse kan worden gebruikt om de beste teeltpraktijk af te stemmen op de verwachte klimaatverandering.

Naast nieuwe methoden en instrumenten, heeft dit vernieuwend onderzoek een nieuwe invalshoek in het onderzoek naar voedselveiligheid opgeleverd. Het instrument voor het detailleren van klimaatdata produceert de ruimtelijke en temporele noodzakelijke klimaatvariabelen voor scenario-analyses van voedselveiligheid bij klimaatverandering. De multicriteria scenarioanalyse maakt het mogelijk om veranderingen van het klimaat in combinatie met veranderingen in de teeltpraktijk te beschouwen. Hierbij kunnen verschillende belangen en methoden in de agrarische praktijk worden meegenomen. Op die manier kan het instrument het opstellen van milieu- en landbouwkundig beleid onder klimaatverandering ondersteunen. Het in dit promotieonderzoek ontwikkelde statistische model beschrijft de relatie tussen besmetting met *E. coli* en het klimaat.

Nog niet eerder is het effect van klimaatverandering op de microbiologische veiligheid van verse bladgroenten gekwantificeerd. Als de minimum temperatuur tijdens monstername stijgt met één graad Celsius neemt de kans op aanwezigheid van *E. coli* op verse bladgroenten toe met een factor van 1.5. Er wordt dan ook verwacht dat toekomstige *E. coli* concentraties zullen toenemen. Daarom mag klimaatverandering niet worden genegeerd in het onderzoek naar voedselveiligheid.

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About the author

Cheng Liu was born on September 14th 1985 in Qingdao, China. She has studied land and water management in China Agricultural University, Beijing and later received a diploma of River Delta Management (2008) from Van Hall Larenstein in Velp, the Netherlands. During her Bachler thesis internship at DHV Beijing in 2008, she contributed to the preparation phase of China-Europe River Basin Management Project. Her research there focused on improving efficiency of wastewater treatment plants designed by DHV in China. After that she specialised herself in Environmental Systems Analysis in Wageningen University. She studied past and future trends in grey water footprints of anthropogenic nitrogen and phosphorus inputs to major world rivers as her master thesis. This study calculated grey water footprint for more than 1000 rivers for the first time and projected the future pollution trends. During her internship at Netherlands environmental Assessment Agency (PBL) in 2010, she contributed to development of the global model which estimate the nutrient flow emitted by shellfish farming. In September 2010 she started her PhD at the chair group Environmental Systems Analysis of Wageningen University and worked for EU FP7 funded Veg-i-Trade project. Cheng is experienced in data processing, statistical modelling, climate scenario analysis and impact modelling on global and regional scales. Within the Veg-i-Trade project she put her efforts on bridging climate science and food safety studies. She applied the climate data downscaling method on food safety assessment. Her research focused on climate change impacts on microbial safety of pre-harvest leafy green vegetables. The dissertation is finished on May 2015. Since June 2015 she has been working as a postdoctoral researcher at RIKILT (Institute of food safety) in the Netherlands developing mycotoxins prediction models for cereals.

List of selected publications

Liu, C., Hofstra, N., & Franz, E. (2013). Impacts of climate change on the microbial safety of pre-harvest leafy green vegetables as indicated by *Escherichia coli* O157 and *Salmonella* spp. *International journal of food microbiology*, *163*(2), 119-128.

Liu, Cheng, Hofstra, Nynke, & Leemans, Rik. (2014). Preparing suitable climate scenario data to assess impacts on local food safety. *Food Research International*.

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Van de Perre, E., Jacxsens, L., Liu, C., Devlieghere, F., & De Meulenaer, B. (2014). Climate impact on Alternaria moulds and their mycotoxins in fresh produce: The case of the tomato chain. *Food Research International*.

Liu, Cheng, Hofstra, Nynke, & Franz, Eelco. Accepted with minor revision. Regression analysis of climate variables and management variables' impacts on leafy green contamination by *Escherichia Coli* at pre-harvest stage.

Liu, Cheng, Hofstra, Nynke, & Leemans, Rik. In preparation. Sensitivity/scenario analysis of management variables' impacts on leafy green contamination by *Escherichia Coli* at pre-harvest stage.

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Other PhD Courses

- Introduction to R for Statistical Analysis, Wageningen University (2011)
- Techniques for Writing and Presenting a Scientific paper, Wageningen University (2012)
- Project and Time Management, Wageningen University (2012)
- IMPACT2C Summer School, Lüneburg, Germany (2013)

Management and Didactic Skills Training

- Supervising essay groups and assisting seminars for the course 'Introduction to Global Change' and Seminar 'Interdisciplinarity in Scientific Research and Education', Wageningen University (2014)

Oral Presentations

- *Impact of climate change on fresh produce contamination by pathogenic bacteria.* Veg-i-Trade 2nd Consortium meeting, 24-28 January 2011, Ghent, Belgium
- *WP9: Impacts of climate change on food safety*. Veg-i-Trade 3rd Consortium Meeting, 6-8 July 2011, Oslo, Norway
- *Preparing climate data for fresh produce safety modelling*. Veg-i-Trade 5th Consortium Meeting, 18-22 March 2013, Coimbatore, India
- *Impact of climate change on microbial safety of pre-harvest leafy green vegetables*. IAFP European Symposium on Food Safety, 15-17 May 2013, Marseille, France
- *Microorganisms under climate change*. Veg-i-Trade 6th consortium meeting, 17-23 March 2014, Pretoria, South Africa
- *Climate change impacts on fresh produce safety.* FACCE-JPI SAFE - Food Safety Implications of Climate Change and Climate Variability Workshop, 24-25 September 2014, Bucharest, Romania

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