SURVIVAL AND OVERLAND TRANSPORT OF FECAL COLIFORM UNDER CANADIAN PRAIRIE CONDITIONS

A Thesis

Submitted to the Faculty of Graduate Studies and Research

in Partial Fulfillment of the Requirements

for the Degree of

Doctor of Philosophy

in Environmental Systems Engineering

University of Regina

December 2016

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UNIVERSITY OF REGINA

FACULTY OF GRADUATE STUDIES AND RESEARCH

SUPERVISORY AND EXAMINING COMMITTEE

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Abstract

In-field winter feeding of cattle is becoming a common practice in the Canadian Prairie. Cattle feeding in-field during winter provides economic advantages to agricultural producers over feeding the cattle in confined corrals by eliminating the extra work and cost associated with feed transport and manure management. Despite the economic advantages; wintering grounds for livestock operations can have profound impacts on water quality. Animal waste accumulated during winter can be transported to surface water with spring snowmelt. The potential impact on snowmelt runoff quality of winter in-field bale-grazing under the cold climate of Saskatchewan is being evaluated under the Saskatchewan component of the Canada-wide Watershed Evaluation of Beneficial Management Practices (WEBs) program which is located in the Pipestone Creek watershed.

This study is intended to complement the Saskatchewan WEBs project and it aims to support the evaluation of in-field bale-grazing during winter. The design of the Saskatchewan WEBs project enabled the evaluation of in-field bale-grazing during winter (W) compared to the conventional manure management practice associated with wintering cattle in confined corrals, where the manure piled during winter is spread on field in the fall (F). The control fields did not receive any animal waste beyond the spring and summer grazing season.

The data collected over three years showed that adding extra animal waste to the field past the grazing season can significantly increase the number of bacteria in snowmelt runoff despite the age of the waste. The wintering fields (W) reported the highest flow-weighted count over the years (2674 CFU/100ml). However, the mean count of FC from W fields (497 CFU/100ml) was not significantly different ($p=0.74>0.05$) from the F fields (364 CFU/100 ml). The mean count of FC in snowmelt runoff from the control fields (110 CFU/100ml) can be well below the recreational water standards of 200 CFU/100 ml. As a result, feeding the animal in confinements during winter and spreading the collected manure on field the next fall may result in the same microbial pollution as that associated with the animal grazing in-field during winter. The study recommends including microbial pollution in the evaluation of beneficial management practices on pasture.

Acknowledgement

I am very fortunate to be part of the project team of the Saskatchewan Watershed Evaluation of Beneficial Management Practices (WEBs) project. The amount of learning and support that I received throughout my study cannot be described in words. I would like to thank the many people who helped me make this manuscript happen. I might not be able to mention them individually but I would like to extend special thank you to the following:

*Thank you Barbar*a. I would like to extend special thank you to Dr. Barbara Cade-Menun of Agriculture and Agri-foods Canada whose leadership and team spirit guided the work in this project and provided me and everyone in the team with all the support and guidance needed. Special thank you to Barbara for co-supervising my research.

*Thank you Den*a. I also would like to extend special thank you to my great and enthusiastic supervisor Dr. Dena McMartin who worked hard to solve every obstacle and created a great supervisor-student relation that made learning fun and inspiring.

Thank you Ulrike, Chris, Syed, and Kelvin. I would like also to send my thanks to every member of my committee for their valuable feedback and effort guiding me through every stages of my research.

Thank you Mahmoud, Zaid, Suhail and Arwa. Finally, I would like to send special thanks you for my husband Mahmoud and my sons Zaid and Suhail, and my daughter Arwa who believed in me and joined me in every adventure I took during the course of my research.

Thank you everyone. Thank you to everyone I worked with during this study who supported me in a direct and indirect way. Special thanks to Ymene and Katie from Agriculture and Agri-food Canada and to Tylor and Carmen form Dr. Chris Yost lab of the University of Regina.

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List of Abbreviations

AAFC	Agriculture and Agri-Food Canada
BMP	Beneficial (or Best) Management Practice
E.coli	Escherichia coli
FC	Fecal coliform
FS	Fecal Streptococci
MST	Microbial source tracking
PCR	Polymer Chain Reactor
PPR	Prairie Pothole Region
qPCR	Quantitative Polymer Chain Reactor
Prairie	Canadian Prairie with main focus on Saskatchewan in this study
TC	Total coliform
UR	University of Regina
WEBs	Watershed Evaluation of Beneficial Management Practices

Co-Authorship

This thesis is organized with three discrete papers intended for peer review publication. Due to this structure, there may be some redundancy in the chapters. Every effort has been made to minimize repetition without affecting the integrity of the independent chapters.

This thesis is based on three manuscripts:

Chapter 3: Baker-Ismail, S., Cade-Menun, B., McMartin, D.W. Loading of fecal coliform from grazing pasture under different grazing management practices during spring snowmelt in the Canadian Prairie.

Chapter 4: Baker-Ismail, S., Cade-Menun, B., McMartin, D.W. Bacteria and suspended solids loading pattern with snowmelt from agriculture watershed: is there a first flush?

Chapter 5: Baker-Ismail, S., Cade-Menun, B., McMartin, D.W. Survival of fecal coliform in cow dung in the Canadian Prairie

For each manuscript, the author of this dissertation, Samar Baker-Ismail, was responsible for the design and implementation of the study, data analysis and manuscript authoring. Barbara Cade-Menun and Dena McMartin provided ongoing feedback of study design and manuscript preparation.

1 INTRODUCTION

Across the Canadian Prairies, water scarcity creates challenges for appropriate allocation, preservation of quality, and threats to quantity and competition. In the semi-arid environment of the Prairie, these water-related challenges limit economic growth. The scarce water resources in southern Saskatchewan where agriculture, oil, gas, and potash mining industries dominate are exceptionally sensitive to changes in hydroclimatic conditions (Pomeroy *et al.* 2005, Sauchyn & Kulshreshtha 2008). Further, the growing intensity and density of industrial activities coupled with population growth in southern Saskatchewan have placed pressure on water quality, particularly in locations where non-point contributions to surface water courses are prevalent, and in locations immediately upstream of rural communities (PFSRB 2009).

Livestock operations such as cattle production are one of the major industries in Canada. There are more than 12.5 million cattle distributed across the country, with 9 million cattle distributed in the Canadian Prairie provinces alone (Statistics Canada 2011). Feeding cattle in winter can bring challenges to producers, especially due to the long and cold winters of the Prairie. In the conventional wintering scenario, hay is consolidated in the fall, then hauled to the corrals in piecemeal throughout winter. The dung deposited by the cattle is collected in piles inside the corrals and stored in the piles until the following fall. The manure is then hauled to the field and spread on the field. This extra work and cost can be eliminated with in-field bale-grazing during winter (SWA 2003, Haag 2007) where the cattle feed directly from bales placed in the field. The extra work associated with manure handling, storage, and hauling can also be eliminated as the cattle deposit their dung directly on the field. Therefore, in-field winter feeding of cattle is becoming a common practice in the Canadian Prairie where grazing in the field provides economic advantages to agricultural producers during Prairie winters (SWA 2003).

Despite the economic advantages, wintering grounds for livestock operations can have profound impacts on water quality. The livestock waste accumulated during the winter can be transported to surface water with spring runoff (Alberta Agriculture and Forestry 2012). Manure is composed of partially digested food materials of organic carbon nature (Dao *et al*. 2008). Cattle manure can be a great source of organic matter and nutrients for soil and plants (Ramos *et al.* 2006). However, pollution caused by nutrient loss (mainly nitrogen and phosphorus) from livestock fields can significantly impact the quality of surface and ground water (Hooda *et al.* 2000, Kay *et al*. 2007). The impact on receiving streams can affect aquatic life, as well as the reuse of the water downstream for agricultural, recreational or drinking purposes (Alberta Agriculture and Forestry 2012).

Microbial pollution is another serious pollution associated with livestock production. Microbial biomass accounts for almost 30% of the manure mass (Pachepsky *et al.* 2009). Manure contains bacteria, viruses, protozoa and parasites (Hooda *et al.* 2000, SWA 2003, Theron and Cloete 2002, Rosen *et al*. 2000). Many of the microorganisms shed in the feces of domestic and agricultural animals can pose a great health risk to humans (Field and Samadpour 2007). Examples of the pathogenic microorganisms in animal feces include *E. coli* O157:H7, Salmonella, *Giardia* spp, *Cryptosporidium* spp. *Campylobacter jejuni*, and hepatitis E virus. Soller *et al.* (2010) reported that the risk to human health after the exposure to recreational waters impacted by cattle feces contamination is not sustainably different from the exposure to waters contaminated with human sewage.

Microbial pollution has not received as much attention as nutrient pollution (Hooda *et al.* 2000, Kay *et al*. 2007, Kay *et al.* 2008). Several studies reported increased risk of microbial pollution from grazed fields compared to ungrazed fields. Doran and Linn (1979) reported a fecal coliform (FC) count that is 5 to 10 times higher in a grazed field compared to an ungrazed field. Jaswon *et al*. (1982) found that the effect of grazing on bacteriological quality of runoff persisted for more than one year after animals were removed from a pasture in the Pacific Northwest. Edwards *et al*. (2000) examined the effect of manure application strategy on the quality of runoff in a simulated runoff in a study field. The study found that the concentrations of FC in runoff from the manure-treated fields were significantly higher (two order of magnitude or higher) than the concentration in runoff from the *control* fields. However, the nutrient content in runoff from the manure-treated fields was not consistently different than that from the *control* field. Therefore, microbial pollution should be considered in the context of beneficial management practices for livestock production, along with nutrients and other suspended particles in the runoff.

Studies examining the impact of in-field grazing on the quality of runoff are mostly performed in rainfall runoff conditions. On the Canadian Prairie, cattle feeding in-field during winter will deposit dung on snow-covered and/or frozen ground. The water released from the snow pack during spring snowmelt may carry pollutants as it runs over land to surface water (NAO 2010). Snowmelt runoff is a major source of runoff from land to streams in the Canadian

Prairie where more than two-thirds of the annual peak flow takes place in March and April, due to snowmelt runoff (Shook and Pomeroy 2012).

The potential impact of winter in-field bale-grazing on snow runoff quality, in the cold climate of Saskatchewan, is being evaluated under the Saskatchewan component of the Canada-wide Watershed Evaluation of Beneficial Management Practices (WEBs) program. The program was available in nine locations across Canada and was designed to critically evaluate the field-scale impacts of agricultural management practices which have been deemed beneficial management practices (BMPs). The program evaluated the impact of the varied production models on the environment, economics, and the health of agricultural ecosystems.

The Saskatchewan Watershed Evaluation of Beneficial Management Practices project was located in the Pipestone Creek watershed. The project, extended over a period of four years, was led by Agriculture and Agri-Food Canada. The main objective of the project was to evaluate the effectiveness of agricultural beneficial management practices (BMPs) at the watershed scale. In the first year of the project and before the implementation of the BMPs, background data were collected on nutrients and suspended sediment in snowmelt runoff (Cade-Menun *et al.* 2013). Four BMPs were investigated by the Saskatchewan WEBs project including: (1) winter in-field bale- grazing, (2) nutrient management on annual cropland, (3) wetland restoration in pasture land, and (4) conversion of annual cropland to perennial cover (AAFC 2013). Within the Pipestone Creek Watershed, a study area consisting of 14 field-scale sites at three farms was established. The fields were operated by three separate producers who were also research partners. The producers are referred to as Producer M, Producer F and Producer B (Figure 1).

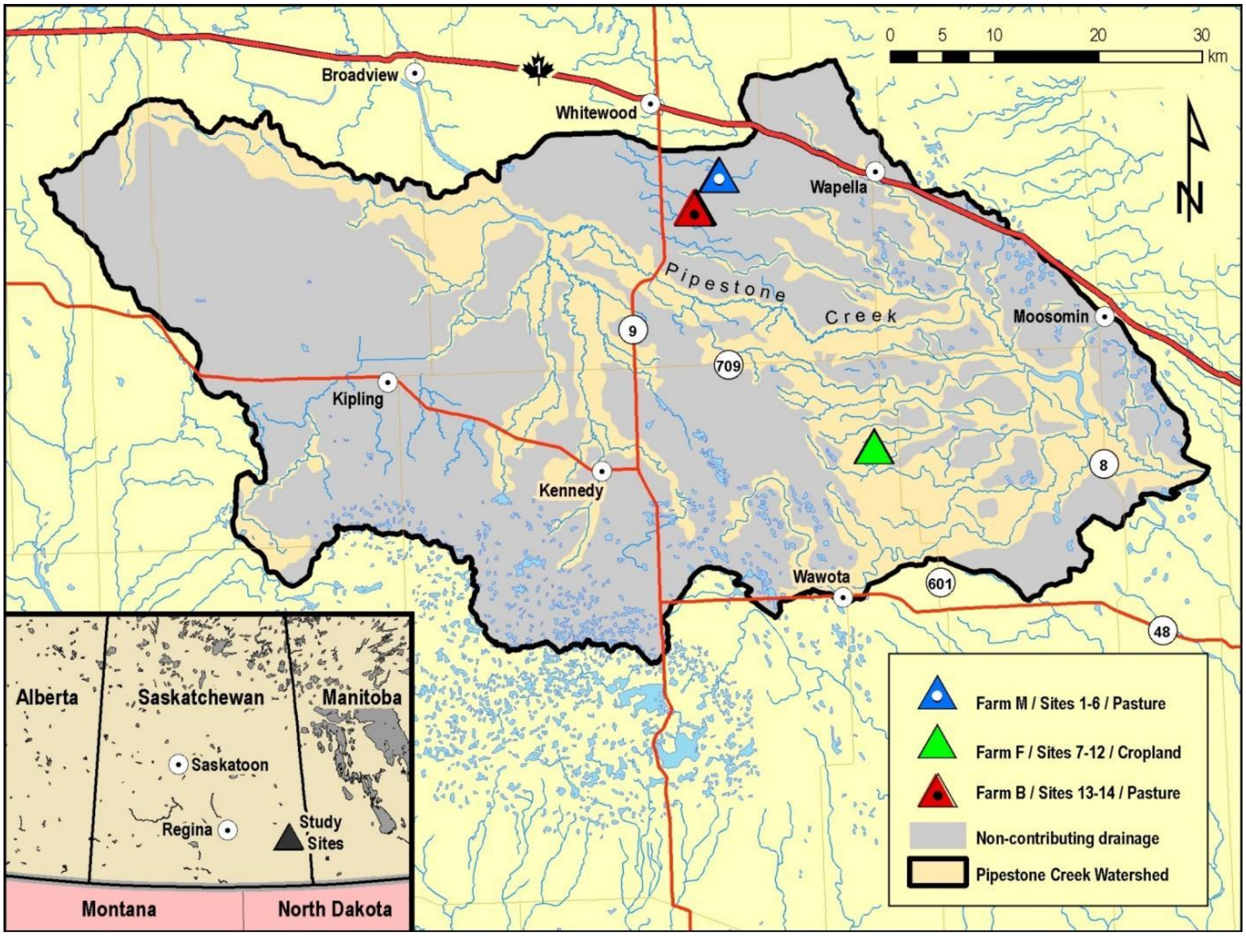

Figure 1. Saskatchewan WEBs project in the Pipestone watershed and the participating producers' sites

This study complemented the Saskatchewan WEBs project by providing data on the loading of fecal coliform with snowmelt runoff collected at edge-of-field from six sub-watersheds managed by producer M. The study is intended to support the evaluation of in-field bale-grazing during winter by providing the decision maker with information on FC removal and loading pattern with snowmelt runoff. The design of the Saskatchewan WEBs projects enabled the evaluation of in-field grazing during winter, compared to the conventional grazing practice where cattle graze in the field during the spring and summer season only, and the conventional manure handling practice where manure is piled in confined corrals during winter and is spread on the field in the fall.

1.1 Objectives

The main objective of this study is to complement the Saskatchewan WEBs project by providing information on fecal coliform (FC) removal and loading pattern at edge-of-field, with snowmelt runoff draining six sub-watersheds, and involving the three different grazing practices of in-field bale-grazing during winter, manure spreading in fall, and spring and summer grazing. Both *winter in-field bale-grazing* and *manure spreading in fall* were the considered treatments in this study and are each implemented in duplicate sub-watersheds. In the other two sub-watersheds, which were considered the *controls*, no extra animal waste was added to the field beyond the conventional grazing season in spring and summer. The study was also supplemented by two experiments conducted outdoors during the winter season and the spring/summer season, in order to examine the survival of FC during winter and the conventional grazing season in spring and summer.

The specific objectives of this study are as follows:

1. To compare the loading of FC from cattle wintering fields where the cattle feed in-field during winter to the loading of FC from a) fields grazed during spring and summer only and b) fields spread in manure in the fall; which represents the conventional manure management practice associated with wintering the cattle in confinement. The main hypotheses tested are:

Hypothesis (1): Fields receiving animal waste beyond the conventional grazing season (spring/summer), either by manure spreading in fall or by the grazing animals during winter, will contribute higher concentrations of fecal coliform in runoff compared to control fields where grazing takes place during spring and summer only.

Hypothesis (2): The fields receiving the fresh dung deposits during winter in-field bale-grazing are expected to contribute higher concentrations of FC in runoff compared to the fields receiving the old manure collected in the previous winter and spread on the field the following fall.

2. To examine whether the loading pattern of FC is similar to that of the loading pattern of other suspended particles in the runoff and to evaluate if the loading of FC exhibits a first-flush.

   Hypothesis (1): The loading pattern of FC is not identical to that of other suspended particles in the runoff.

   Hypothesis (2): Grazing practice and field conditions affect the temporal loading pattern and first flush might not be the only pattern.

3. To examine the survival of FC in dung under the cold climate of the northern hemisphere during the grazing season (spring and summer) and winter. The study also examined the

difference in temperature between ambient temperature and the temperature inside the snow-covered cowpats.

Hypothesis (1): Bacteria is expected to survive the cold winter and be available for transport with snowmelt runoff.

Hypothesis (2): the die-off of FC during spring/summer season is different than die-off during winter season. Initial growth is expected during the spring/summer season, while initial decline is expected during winter.

Hypothesis (3): The conditions inside the cowpats is expected to provide a shield for the bacteria from the expected diurnal fluctuations in temperature under the Canadian Prairie winter conditions.

1.2 Thesis Organization

The body of this thesis is presented in six chapters with three manuscripts inserted in Chapters 3, 4, and 5. The other three chapters include the Introduction in Chapter 1, the Literature Review in Chapter 2 and the Conclusion and Recommendations in Chapter 6. The material included in each chapter is as follows:

Chapter 2 provides a condensed literature review of relevant studies examining the survival of bacteria in dung and the release and transport of bacteria in the runoff. The study will also provide background on the currently acceptable methods for the assessment of microbial pollution in water and runoff samples, as well as the emerging methods and techniques under development. This chapter will also provide background information on the climate and hydrology of the Canadian prairie, its impact on field conditions, and the transport of bacteria during runoff.

Chapter 3 is the second manuscript which examines the loading of fecal coliform from six field scale sites within the Pipestone watershed. The fields are under three different treatments, applied in duplicates. The treatments include grazing during spring and summer only, manure spreading on the field in fall, and in-field winter bale grazing.

Chapter 4 extends the analysis of the data analyzed in Chapter 3 to include the analysis of first flush by examining the temporal loading of fecal coliform during snowmelt runoff compared to the loading of the other suspended particles in the runoff.

Chapter 5 represents the result of an experiment conducted under the cold winter conditions of the Canadian Prairie, as well as during the spring and summer seasons, which represents the conventional grazing season in the Canadian Prairie.

Chapter 6 provides an overview of the research program (conclusions) as well as recommendations for implementation activities and future work.

2 LITERATURE REVIEW

2.1 Introduction

The main water quality concerns related to livestock production are nutrients (nitrogen and phosphorus), organic materials (e.g. plant material mainly straw), and pathogens. Microbial biomass typically comprises around one-third of the fecal matter by weight which includes viruses, bacteria, and protozoa, some of which may be pathogenic. The health threat to humans may exist if the pathogenic fecal organisms find their way into the water and food supplies. Gastrointestinal illness is one of the water-borne diseases with symptoms that include diarrhea, nausea, abdominal pain, vomiting, and fever. Outbreaks of gastrointestinal illness are well known in the developing and developed countries (Pandey *et al.* 2014, Craun *et al.* 2006). Pathogens like Shigella spp., *Cryptosporidium parvum*, *Naegleria fowleri*, *E. coli* 0157:H7, Schistosoma spp., and Salmonella are a few of the commonly known agents of gastrointestinal illness that originate from fecal contamination.

The following paragraphs provide an overview of the available studies that examine the current methods for evaluating microbial pollution of runoff, the fate of bacteria in dung after deposition, and bacteria transport to surface water in runoff from fields receiving animal waste. The last section will provide a short background on the hydrology and climate of the Canadian Prairie and its expected effect on the survival of dung-based bacteria and its transport in overland flow.

2.2 Microbial Pollution, Current Indicators

Monitoring for all waterborne pathogens in water sources may be unrealistic and expensive with the current assaying techniques. There is great diversity in pathogens that make assaying for each pathogen separately very expensive, time-consuming and, possibly, technically complicated. Some of these pathogens are difficult to culture and may be rare, randomly spread, or only available in low concentrations in environmental waters. The infectious dose may also be different for each pathogen, and some of the pathogens may be highly infectious in low doses. Several approaches are constantly under development that aim to assay several pathogens simultaneously to reduce cost and time requirements. However, these assays are still under development and problems associated with sensitivity, specificity and quantification prevent these methods from being accepted for water quality monitoring (Field and Samadpour 2007).

The current alternative to the direct monitoring of the individual pathogens is to use indicator bacteria. Indicator bacteria are currently the accepted standards used to assess the microbiological quality of water (Theron and Cloete 2002, Field and Samadpour 2007). Indicator organisms are not necessarily pathogenic themselves, but they are used to indicate the potential presence of enteric pathogens (Theron and Cloete 2002). The main indicators used are total coliforms (TC), fecal coliform (FC), *E. coli,* and fecal *Streptococci* (FS). Total coliforms contain a broad group of aerobic and facultative anaerobic bacteria that can be from intestinal or environmental sources (Vaccari *et al.* 2006, Rosen *et al.* 2000). FC is a subgroup of the TC group incubated at a higher temperature in a selective media to eliminate the growth of environmental bacteria (Vaccari *et al*. 2006). *E. coli* is a member of the FC group, and it is found to correlate well with gastrointestinal illness outbreaks (Rosen *et al*. 2000). The fecal coliform test is an examination of *E. coli* (Thelin and Gifford 1983). FS is limited to the intestinal tract of warm-blooded animals. Doran and Linn (1979) compared the indicator bacteria count (TC, FC, and FS) from grazed and ungrazed fields. The study recommended that FC was the best indicator group for the impact of grazing.

A major limitation of the indicator bacteria is that it does not specify the source of contamination. When multiple sources of fecal pollution exist, the indicator bacteria cannot pinpoint the sources of pollution. The fecal indicator bacteria like *E.coli* and enterococci are shed in the feces of many different cold and warm blooded animals. In addition, some strains of the fecal indicator bacteria can adapt to environmental conditions and may persist and grow in many habitats, including aquatic sediments and terrestrial soils (Harwood *et al*. 2014).

Earlier efforts to identify the source of pollution were based on the fecal coliform/fecal *Streptococci* (FC/FS) ratios (Meays *et al.* 2004). The ratio of FC/FS provided a mean to evaluate the source of fecal pollution based on the fact that humans have higher FC concentrations relative to FS when compared with other animals. Geldreiche and Kenner (1969) studied the distribution of FC/FS ratios in warm-blooded animal feces and concluded that a ratio of FC/FS of more than 4 is found in human feces, whereas a ratio of less than 0.7 is found in all other warm-blooded animals. Doran and Linn (1979) proposed a ratio of below 0.05 as indicative of wildlife sources while a ratio above 0.1 is an indicator of cattle sources. These ratios were studied intensively afterward, and several studies questioned the validity of this ratio. Edwards *et al*. (1997) analyzed runoff from pastureland under different practices and found that the ratio of FC/FS varied widely from zero to more than 100 and concluded that it could not be a reliable indicator of fecal pollution source. Howell *et al.* (1996) studied the mortality rates of FC and FS at three different temperatures in a laboratory study and found that FC regrowth after deposition at warm temperatures can increase FC/FS ratios to levels that indicate contamination form human sources where no human presence existed. Another issue presents itself with the FC/FS ratio when multiple sources exist.

Microbial source tracking (MST) are emerging techniques that are constantly under development as an alternative to the fecal indicator bacteria (Harwood *et al*. 2014). The basic principal of MST is that certain strains of fecal microorganisms are strongly associated with the host animal. MST methods use this fact to associate the fecal microorganism with a particular host using signature molecules known as markers (Harwood *et al*. 2014, Layton *et al*. 2006). Research in the MST methods is currently moving towards quantitative methods that can simultaneously identify the host animal and quantify the target gene in the sample using the Real-Time Polymer Chain Reaction known as Real-Time PCR or Quantitative PCR or qPCR (Harwood *et al*. 2014). Real-time PCR is currently viewed as a promising tool to measure the bacteria in food, fecal and tissue samples, as well assess the quality of recreational waters, and assess in the establishment of total maximum daily load allocations.

Bacteroides-based MST methods are becoming the suggested alternative for the fecal indicator bacteria. Layton *et al.* (2006) presented the main arguments behind using *Bacteroides* as an alternative to the fecal indicator bacteria. *Bacteroides* are plentifully available in the fecal matter as they make up a total of 30 to 40% of the fecal bacteria which accounts for almost 10% of the fecal mass. Unlike the fecal indicator bacteria, *Bacteroides* are not likely to grow in the environment and exhibit a high degree of host specificity. *Bacteroides*-based methods target the whole *Bacteroides* population in the feces, which makes them suitable to detect contamination even in very small concentrations. *Bacteroides* based methods target the sequence within the *Bacteroides* 16S rRNA gene which is present in all mammalian feces.

The use of both conventional indicators and MST based methods is recommended for identification of fecal contamination (Savichtcheva and Okabe 2006). There are different *Bacteroides*-based markers available to differentiate the different sources of animal feces. Many of the qPCR markers developed target the human-associated *Bacteroides (*Harwood *et al.* (2014). Other markers are available like AllBac for all *Bacteroides* in all mammalian fecal samples (Layton *et al*. 2006), BoBac for bovine (Layton *et al*. 2006), CowM2 and CowM3 for bovine specific *Bacteroides* (Shanks *et al.* 2008), as well as several other markers that are targeting dog, pig or other animals specific *Bacteroides*. Harwood *et al*. (2014) provided a review of the currently available qPCR markers and the current studies presenting field testing of the markers compared to the levels of fecal indicator bacteria.

2.3 Fate and Survival

The overland transport of dung-based bacteria to surface water is contingent upon their survival in the dung and their release from the fecal source, either deposited cowpats or surface applied manure. The concentration of indicator bacteria in runoff is affected by the number of viable bacteria in the fecal source. Muirhead *et al*. (2005) exposed fresh cowpats to simulated rainfall in the lab and collected runoff and dung samples over 30 days. The study found that the number of indicator bacteria in runoff correlated with the numbers inside the cowpat.

The fresh cow deposit resembles a batch experiment where all of the nutrients are available once and are not resupplied (Vaccari *et al*. 2006). Bacteria go through exponential growth within the intestine of the host. Once deposited, the growth may continue outside the host while environmental conditions that promote growth last. The growth phase can last for a few days, after which the bacteria may enter a stationary phase where growth stalls but the bacteria may remain viable. Several studies investigated the survival of indicator bacteria in cow dung. Some of these studies were performed on naturally occurring cowpats (Buckhouse and Gifford 1976), manure samples (Wang *et al.* 2004), or on artificial cowpats made from fresh deposits (Meays *et al*. 2005, Muirhead *et al*. 2005, van Kessel *et al*. 2007, Sinton *et al*. 2007, Soupir 2008, Muirhead and Littlejohn 2009). The artificial cowpats were created from a mixture of feces of several animals to create replicated units of relatively uniform fecal deposits (Meays *et al.* 2005). Some of these studies were performed under controlled temperatures and conditions in the lab while others were performed under field conditions. In most of these studies, the change in moisture content was also measured.

Studies examining the survival of bacteria under warm temperatures reported growth after deposition (Muirhead *et al*. 2005, Soupir 2008). Van Kessel *et al.* (2007) suggested that temperatures between 20°C to 35°C would be the best for post-deposit growth. High temperature can inactivate or kill some of the indicator bacteria. Wang *et al.* (2004) examined the survival of FC, *E.coli* and FS in bovine feces under three constant temperatures (4°C, 27°C, 41°C). The study reported the initial growth of two orders of magnitude of *E. coli* and FC at 27°C with initial growth reported in one of two trials at 4°C. The high temperature (41°C) killed FC and *E. coli* in 35 days but did not affect FS. Similar results were reported under field conditions. Sinton *et al.* (2007) examined the survival of *E. coli*, FS, and enterococci under the field conditions of the southern hemisphere. The study reported an initial increase of up to 1.5 order of magnitude for the three indicators under the warm to hot seasons (summer, spring, and autumn) and a decline under winter conditions, except for enterococci.

Under cold temperatures, the bacteria may experience initial die-off but may survive longer compared to higher temperatures. Bach *et al*. (2005) examined the survival of *E. coli* O157:H7 under constant temperatures (-10, +4, +22) and reported a one-order of magnitude growth at 22°C during the first 2 weeks of incubation, but not at +4 or -10°C. Kudva *et al.* (1998) studied the short and long term survival of *E. coli* O157:H7 in inoculated manure samples incubated under different temperatures. The study reported an initial decrease of 2 logs in the first 24 hours after inculcation under cold temperatures (-20°C, and 4°C), whereas an initial increase of 1 log was reported in the first 24 hours at 23°C, and 37°C. The study reported longer survival of *E. coli* O157:H7 under freezing and moderate temperatures (-20°C, 4°C and 23 °C), compared to high temperatures (37°C, 45°C and 70 °C).

Temperature regime is another factor that affects the survival of indicator bacteria. Temperature is often stable in the host body but may be strongly fluctuating in a non-host environment (Van Elsas *et al.*, 2011). The effect of fluctuating temperature on the survival of *E.coli* in inoculated steer manure was examined by Semenov *et al.* (2007). The study examined the effect of different temperatures (7, 16, 23 and 33 °C) and three amplitudes (0, ±4, ±7 °C) representing daily oscillations. The study reported that the survival of indicator bacteria significantly declined with increasing mean temperatures and with increasing amplitude in daily temperature oscillations. The results indicated that the response of enteropathogens to fluctuating temperatures could not be constructed from temperature relationships determined under constant temperatures.

Several studies examined changes in moisture content and the effect on the survival of indicator bacteria. Thelin and Gifford (1983) found that manure deposits reach complete desiccation within two weeks under summer conditions. Sinton *et al*. (2007) studied changes in moisture content under field conditions of the four seasons of the southern hemisphere. The study reported the highest desiccation rate in the first 30 days during summer followed by spring, autumn, and then winter. Shading has an effect on the loss of moisture content. Van Kessel *et al*. (2007) reported that shading of cow pats could retard desiccation in the first month after deposition. Meays *et al.* (2005) studied the changes in moisture content in artificial cowpats under different levels of shading (0%, 40%, 80% and 100%). The study reported that moisture content declines slower under 100% shading compared to 0% shading, which enhanced the survival of *E. coli* in the shaded cowpats in the hot, dry summer weather. The study reported that while shading enhanced the survival of *E. coli* and retarded the loss of moisture content, the decline in moisture content was not associated with a reduction in *E. coli* concentrations. However, Van Kessel *et al.* (2007) reported an increase in *E. coli* and FC concentrations following rainfall events.

The combined effect of temperature and moisture content may have a different effect on the survival of indicator bacteria. Wang *et al*. (2004) reported that high temperatures and lower moisture content might decrease the survival of *E. coli* and FC. Under freezing temperatures, survival is promoted under low moisture content. Adhikari *et al*. (2007) studied the survival of TC in soil contaminated with human and animal feces in Alaska. The study reported 66% survival of TC over a period of 170 days at subfreezing temperatures from -15°C to -28°C with relatively dry soil (24% moisture content). High moisture content may lower the temperature inside the cowpat where no sun exposure is available. Van Kessel *et al.* (2007) reported that the temperature inside the shaded cowpats during the day was lower than air temperature when moisture content exceeded 50%.

The integrity of the cowpat can affect the survival of indictor bacteria. Intact cowpats form a crust that protects the bacteria (Thelin & Gifford, 1983) which may sustain its survival for years under field conditions (Buckhouse and Gifford 1976). Muirhead and Littlejohn (2009) examined the effect of smearing of the cowpat on the survival of *E. coli* under field conditions. The study reported that smearing can provide a greater surface area and increase the solar exposure and accelerates desiccation. However, disrupting the cowpat did not accelerate the die-off of *E. coli* when compared to intact cowpats.

All of the studies available investigated the aging of cowpats and the survival of indicator bacteria under warm to cold temperatures. There is a lack of studies on the aging of cowpats and indicator bacteria survival under the cold hemisphere climate, especially under freezing winter conditions. During the grazing season of spring and summer of the Canadian Prairie, the evaporation rate, and solar radiation can lead to quick desiccation of the deposited dung mainly during drought periods. Solar radiation can accelerate die-off of fecal bacteria, but dried dung can persist longer than wet dung (Vadas *et al*. 2011).

During the cold and long winter of the Canadian Prairie, diurnal fluctuations in temperature are expected. The microorganisms will be exposed to cold shock, freezing, and freeze-thaw cycles. All of these conditions will result in injury to the microorganisms and may even be lethal due to the membrane or cell damage (Walker *et al.* 2006). The effect of slow freezing followed by slow warming on *E. coli* was examined by Walker *et al*. (2006) over 48 freeze-thaw cycles. Sampling at 3, 12, 18, 24, and 48 cycles showed a dramatic decline for each sampling period. A decline of 3 orders of magnitude was reported after the first three cycles followed by a further decline with subsequent cycles. The colonies exposed to freeze-thaw treatment were smaller in diameter than unexposed colonies which were 2.7 times larger. Both the numbers and the complexity of the community decreased with repeated freeze-thaw cycles. However, bacteria that remained viable after the freeze-thaw treatment were >1,000-fold more tolerant to subsequent freeze-thaw cycles.

2.4 Release and Overland Transport

Bacteria transport to surface water is not well understood, but it is mostly related to overland flow (Ferguson *et al*. 2003, Jamieson *et al.* 2004). Several studies were designed to evaluate the microbial water quality of runoff from fields where animal waste have been applied. These studies were performed in the lab (Muirhead *et al*. 2005) or a research field or plot (Thelin and Gifford 1983, Kress and Gifford 1984, Edward *et al*. 2000, Mishra *et al*. 2008, Wanger *et al*. 2010). These studies examined several factors, including the age of the dung, grazing duration, and rainfall intensity and duration.

Earlier studies revealed that indicator bacteria concentrations in runoff decreased with manure age, presumably due to mortality. Thelin and Gifford (1983) reported a significant reduction of FC in runoff from cowpat aged 30 days compared to that from cowpats less than 5 days in age. Mishra *et al.* (2008) examined the concentrations of indicator bacteria in simulated rainfall runoff induced 1 and 2 days after manure application to treatment plots based on phosphorus agronomic rates. The study found that rainfall directly after the application of manure can lead to significant bacterial loading to streams even if phosphorus was applied based on agronomic rates.

The effect of rainfall intensity on the release of indicator bacteria is also dependent on the age of the cowpats. Thelin and Gifford (1983) reported that the release of FC from old cowpats was delayed by 5 minutes, which was interpreted as the time necessary for the dung to hydrate. Kress and Gifford (1984) examined the release of FC from cowpats aged 2, 10 and 20 days under different rainfall intensities; 23, 51, and 69 mm h^{-1}. The study found that rainfall intensity did not have a significant impact on FC release from the 2 or 10 day-old pats. The release of indicator bacteria from the 20 day-old cowpats was delayed under low-intensity rainfall. However, the highest peak count was reported under the lowest intensity. The FC peak count was also reduced with recurrent rainfall due to loss of bacteria from the fecal deposit.

The length of grazing duration and stocking rate of animals can add extra animal waste to the field and increase the availability of bacteria from the fresh manure. Edwards *et al.* (2000) examined the effect of grazing duration (4, 8 and 12 weeks) on the quality of runoff from fescue plots. The study analyzed runoff samples for nitrogen (N), phosphorus (P) and fecal coliform

(FC). The study found that the effect of grazing duration on P and FC was significant due to the increase in the amounts of manure on the plot. Wanger *et al.* (2010) evaluated the impact of different stocking rates on the concentration of bacteria in runoff from a watershed site in Texas, USA. The study found that converting from heavy to moderate stocking rate can reduce indictor bacteria levels in runoff by 67-85%. The study reported the highest concentrations of *E.coli* in runoff occurring within two weeks of stocking and reported a substantial decline after 30 days.

Manure constituents other than fecal coliforms as well as soil particles are transported with runoff and there is a growing interest in literature examining microbial transport in relation to the release and transport of soil particles and manure constituents. Waterborne pathogens are the size of clay and silt particles. However, they have a lower specific gravity, their supply is limited and they can be inactivated (Dorner *et al*. 2006). The density of microorganisms is very close to that of water. Therefore they are mostly suspended rather than dissolved (Pachepsky *et al*. 2006). Once suspended in surface flow, they can be transported as free cells, or attached to suspended manure or soil particles. Several studies reported that under rainfall runoff bacteria tend to transport as free cells. The released bacteria tend to transport as individual cells rather than in clumps where the impact of the rainfall drop may break the clump of bacteria (Murihead *et al.* 2005). Only a small percentage of indicator bacteria was reported to be transported in the attached form under rainfall runoff. Murihead *et al*. (2005) reported that only 8% of *E. coli* were transported in the attached form despite the cowpat age or moisture content. Soupir and Mostaghimi (2011) reported that 4.8% of *E.coli* and 13% of enterococci was transported in the attached form.

The attachment of bacteria to soil or organic particles in the runoff can be affected by the size distribution of the soil, its clay and organic content. Bacteria tend to attach to smaller particles and soil particles with higher clay content. Muirhead *et al*. (2006) suggested that bacteria cells that were not pre-attached to soil particles may attach to smaller particles of <2 μm during overland transport. Ling *et al*. (2002) evaluated strong and weak adsorption of *E. coli* onto two different soil types with 14% and 35% clay content. Results indicated that the percentage of adsorption of *E. coli* was significantly higher in soils containing higher clay content. Guber *et al*. (2007) examined the attachment of fecal coliforms (FC) released from bovine manure to the different soil fractions of clay, silt, and sand. The study reported that in the presence of manure, the attachment of FC to soil particles decreased. In the absence of manure colloids, FC attached mainly to silt and clay particles more than to sand particles without an organic coating. In the presence of manure collides, attachment to silt and clay decreased but did not decrease for sand. This indicates that in the presence of manure collides bacteria tend to be transported as free cell. This may be due to competition between bacteria and dissolved organic matter for attachment sites on soil or due to the modification of soil mineral surfaces, or bacterial surfaces by dissolved organic matter and manure constituents. However, Ramos *et al*. (2006) reported that the transport of FC was strongly related with organic matter in the sediment rather than total suspended solids in a lab scale experiment. Guber *et al.* (2006) reported a similar but not identical kinetic of FC release with colloidal manure components like organic carbon (OC), and water-soluble phosphorus (P) from manure slurry.

Ground conditions affect the flow regime of runoff and have been found to affect the loading pattern of bacteria in the runoff. Soupir *et al.* (2006) reported a delayed loading pattern in a lab setting associated with simulated runoff over saturated soil conditions for FC, *E. coli*, and Enterococcus where highest concentrations were recorded toward the end of runoff. However, in the same experiment with overland flow under dry soil condition, the concentrations of indicator bacteria was hydrograph associated where concentrations of indicator species increased as flow rate increased, peaking simultaneously.

Available studies are mostly performed under rainfall runoff and may not be comparable to the conditions of snowmelt runoff. The release and transport of bacteria under snowmelt conditions may be different from that under rainfall runoff conditions. The energy of the snowmelt runoff is governed by the field conditions. Snowmelt over frozen soil restricts soil particle detachment and favors the transport of smaller and less dense particles like clay and organic matter (Panuska and Karthikeyan 2010). Therefore, runoff over frozen soil from in-field wintering grazing sites is expected to have high organic content. The application of manure on top of snow can affect the infiltration rate by attenuating snowmelt peaks and retarding snowmelt (Kongoli and Bland, 2002). Williams *et al*. (2010), in a lab experiment, found that the application of manure on top of snow can increase infiltration and reduce runoff when compared to the application of manure before snowfall which may inhibit infiltration and increase runoff. During winter, the application of manure can create a mulching effect that increases infiltration of snowmelt. The timing of manure application, occurring either before soil freezing in the fall or on the frozen soil before the spring thaw, as well as the position within snowpack, can have an impact on soil temperature, infiltration, and erosion (Srinivasan *et al*. 2006).

All of the available studies performed at different scales confirmed that bacteria concentrations in runoff from fields where animal waste have been applied is usually higher than that from fields with no animal waste, whatever the nutrient management practice. The loading of bacteria from animal wintering fields during snowmelt runoff might be different than that under rainfall runoff conditions. Studies examining the loading of bacteria from animal wintering fields and their loading pattern during snowmelt can provide valuable information for decision makers to support their decision when evaluating agriculture BMPs.

2.5 Climate and Hydrology of the Canadian Prairie

Saskatchewan's main agricultural ecoregions are the Aspen Parkland and Mixed Grass ecoregions (Huel 2000). The area is part of the North America Prairie Pothole Region (PPR); Figure 2. The Canadian portion of the PPR occupies the southern part of the Canadian Prairie Provinces (Fang *et al*. 2007). The Saskatchewan portion of the PPR is characterized by lower precipitation and lower temperatures compared to the Alberta and Manitoba portions (Bailey *et al*. 2010). Alberta benefits from the Pacific maritime weather systems that arrive with westerly winds. The air mass cools as it rises over the Rocky Mountains and brings less precipitation to Saskatchewan. The southern weather systems that originate in the Gulf of Mexico bring moisture and warm air to Manitoba (Bailey *et al*. 2010).

Figure 2. Map of the Prairie Pothole Region (Ducks unlimited)

The continental and subhumid to semiarid climate of the Prairie is characterized by long cold winters, short hot summers, and low precipitation (Ecological Stratification Working Group 1995). The continental air masses bring clear skies to the Prairie and more than 2,400 hours of unobstructed sunshine annually. On average around 312 to 322 days a year have some hours of sunshine. The hours of annual sunshine in other regions of Canada ranges from 1,200-2,000 hours (McGinn 2010). The region is also characterized by high wind speed, mainly in spring and fall, which accelerates desiccation (McGinn 2010). The blowing wind during winter can transport and sublimate around 75% of snowfall in open fields. The redistribution of snow makes snow depth highly heterogeneous (Fang *et al*. 2007). The region is also characterized by high evaporation rates, especially during summer months, which consumes most of the rainfall (Fang *et al*. 2007).

On the Prairie, summer maximum temperatures are around 22°C higher than winter maximum and summer minimum temperatures are 28°C higher than winter minimum (McGinn 2010). Extreme low temperatures in winter and extremely high temperatures in summer are also common. Millett *et al.* (2009) reported that average daily minimum temperature across the PPR increased by 1°C from 1906 to 2000 with the largest increase of around 3.5°C reported in the Canadian Aspen Forests and Parkland ecoregion. The average winter minimum temperature increased more than the summer minimum. Sauchyn *et al*. (2009) described the increase in minimum temperature in Saskatchewan as, "Saskatchewan is not getting hotter, but rather less cold".

The flow of major rivers may not be affected by runoff from Prairie agricultural land as they originate from the Rocky Mountains and their foothills, whereas the flow of smaller streams is affected by local runoff (Shook and Pomeroy 2012). Snowmelt runoff is the dominant source of runoff from lands to streams in the prairie. Shook and Pomeroy (2012) analyzed data on annual peak flow in 89 prairie streams over the period of 1919-2009. The results indicated that more than two-third of the annual peak flow occurred during March and April due to snowmelt runoff.

Annual precipitation in the Canadian Prairie falls mainly as rainfall and only one-third of the precipitation is attributed to snowfall (Shook and Pomeroy 2012). Intense single-day summer storms are frequent during summer months. The less frequent frontal rainfall systems can persist for multiple days. During rainfall events, infiltration rates mostly exceed rainfall. Rainfall mostly run over unsaturated soil due to the high evapotranspiration rates which leave the soil unsaturated for most of the summer. Infiltration of excess runoff may develop under intense single-day summer thunderstorms, but it is mostly localized over small areas. Saturation excess runoff may be associated with prolonged frontal rainfall systems. Shook and Pomeroy (2012) analyzed monthly precipitation data obtained from the Historical Adjusted Climate Database for Canada (HACDC) over the periods 1901- 2000 and 1951-2000. The study reported that single-day summer rainfalls displayed a significant decrease over the study period, whereas summer multiple-day rains have shown a significant increase over the same period.

Unlike the localized summer rainfall events, snowmelt is more spatially extent (Shook and Pomeroy, 2012). Snow cover stores water for an extended period then releases it during the snowmelt event. Spring snowmelt is mostly controlled by the frozen soil conditions (Singh *et al*. 2009). The development of frozen soil is controlled by the presence or absence of a snowpack and the start of the snowfall in the season. Early snow cover can provide an insulation effect for the underlying soil and raise the minimum temperature in soil, which may leave the soil unfrozen through the rest of the winter (Edwards *et al.* 2007). In some cases, thick snow cover can insulate the ground and allow the frozen soil to thaw with the upward conductive heat from the bottom unfrozen layer (Iwata *et al*. 2008).

Hydraulic conductivity under frozen conditions is significantly smaller than that under unfrozen conditions (Iwata *et al*. 2008, Nishimura *et al*. 2011). Frozen soil inhibits infiltration and creates infiltration excess runoff conditions. Snowmelt may infiltrate into the partially frozen soil. In partially frozen soil, the frozen layer impedes infiltration and surface runoff begins once the unfrozen layer becomes saturated, which generates excess saturation conditions (Srinivasan *et al*. 2006, Nishimura *et al*. 2011). Snowmelt in excess of soil infiltration capacity is routed downslope in sheet or concentrated flow (Srinivasan *et al.* 2006, Wall *et al*. 2002).

Unsaturated hydraulic conductivity in frozen soil is a function of ice and liquid water content (Zhang *et al*. 2007). In a soil medium ice may not form at 0°C and some water may remain unfrozen (Zhang *et al*. 2007, Iwata *et al*. 2010). Unfrozen water may also exist within ice crystals. The forming of ice blocks the soil pores and affects the permeability of the soil (Flerchiner *et al.* 2005). Soil permeability will depend on the type of frost formed, which affects the ice content.

The type of frost formed is affected by the rate of freezing, pre-freezing soil moisture content, and soil physical properties (Flerchiner *et al.* 2005). As water freezes, an upward movement towards the freezing front is created. Rapid freezing of soil will freeze the water in place, and water movement will be minimized (Flerchiner *et al*. 2005, Zhang and Sun 2011). The presence of a frozen layer creates a freezing front and negative water potential, which creates an upward water movement and a drying effect (Christ *et al*. 2009, Flerchiner *et al*. 2005). Water movement will increase with the increase of pre-freeze moisture content in soil (Nishimura *et al*. 2011). The upward movement will decrease the liquid water content in the unfrozen layer. Liquid water content will also drop in the frozen layer as water freezes at the frost front (Nishimura *et al*. 2011). Significant upward movement of water towards the freezing front can create a concrete frost that reduces soil permeability and infiltration of snowmelt (USDA 2009, Flerchiner *et al*. 2005).

The fraction of precipitation falling as rain is increasing at many locations in the Prairie, mainly during fall and spring (Shook and Pomeroy 2012). The increase in rainfall fraction during fall can increase pre-freeze soil moisture and reduce infiltration during snowmelt. Spring rain and associated saturated conditions and above freezing temperatures can all accelerate snowmelt through heat exchange and direct contribution from rain (Shook and Pomeroy 2012, Srinivasan *et al*. 2006, Wall *et al.* 2003). Rain on partially frozen soil can form a surface seal that restricts infiltration; even in highly permeable soil, which increases surface runoff (Nishimura *et al*. 2011).

2.6 Summary

In the Canadian Prairie, only one-third of the annual precipitation falls as snowfall. However, snowmelt runoff is the primary source of runoff from lands to streams. The annual precipitation in Prairie is changing with increasing rainfall during the fall and the spring. The pre-freeze increase in moisture content during the fall can lead to the development of concrete frost which limits infiltration during snowmelt. Rain over snow in the spring can accelerate snowmelt. Therefore, snowmelt is expected to run over land to surface water during snowmelt where infiltration is minimal especially during the early stages of the runoff. Consequently, the quality of snowmelt runoff is expected to impact the quality of surface water. Pollutants loading from land to surface water during snowmelt runoff can be reduced or eliminated by implementing proper management practices designed specifically for the climate and hydrology of the Canadian Prairie.

3 LOADING OF FECAL COLIFORM FROM GRAZING PASTURE UNDER DIFFERENT GRAZING MANAGEMENT PRACTICES DURING SPRING SNOWMELT IN THE CANADIAN PRAIRIE

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3.1 Abstract

In-field wintering of cattle is becoming a common practice in the Canadian Prairies due to its economic advantages to agriculture producers compared to wintering animals in confined corrals. The potential impact on surface water of winter in-field bale-grazing, under the cold climate conditions of Saskatchewan, is being evaluated under the Saskatchewan component of the Canada-wide Watershed Evaluation of Beneficial Management Practices (SK-WEBs) program. This study complements the SK-WEBs project by providing information on fecal coliform (FC) removal with snowmelt runoff. The data were collected at edge-of-field over three years (2011-2013) from six sub-watersheds under three different grazing practices implemented in duplicate. The design of the SK-WEBs project enabled the evaluation of the microbial impact of in-field bale grazing during winter compared to the conventional manure handling practice associated with winter feeding in confined corrals; where the collected manure is spread on the field in the fall. The results of this study showed that grazing fields receiving animal waste past the spring and summer grazing season could contribute significantly higher loading of FC with

snowmelt runoff compared to *control* fields. Microbial loading with snowmelt runoff from the *control* fields receiving animal waste during the grazing season only (110 CFU/100 ml) can be well below the recreational water standards of 200 CFU/100ml. However, the loading of FC with snowmelt runoff from cattle wintering fields (497 CFU/100 ml) is not significantly higher compared to the fields spread with manure in the fall (364 CFU/100 ml). The study recommends including microbial pollution in the evaluation of beneficial management practices on pasture.

3.2 Introduction

Cattle production is a major industry in Canada. More than 70% of the cattle in Canada are distributed in the Canadian Prairie provinces alone (Statistics Canada 2011). Wintering sites where animals feed in-field provide economic advantages to agriculture producers over the conventional wintering scenario where animals feed in confined corrals. In the conventional wintering scenario, hay is consolidated in the fall and then hauled to the cows throughout winter. Animal deposits are stockpiled manually in the corrals and stored until the next fall. The manure collected in the winter is then hauled to the field and spread over the land. This extra work is mostly eliminated with in-field bale grazing, where the animals feed directly from bales distributed in the field. The grazing animals deposit their dung directly on the field, eliminating the extra work associated with manure management and storage. Therefore, wintering animals in the fields is becoming a common practice on the Prairies (SWA 2003, Haag 2007).

On the Canadian Prairies snowmelt runoff is the main source of runoff from land to streams, where more than two-thirds of stream peak flow occurs during March and April due to snowmelt runoff (Shook and Pomeroy 2012). In-field wintering animals will be depositing waste on frozen or snow-covered ground under long, cold winter conditions. The accumulated waste during winter will be transported to surface water with snowmelt carrying dung constituents. Dung is composed of partially digested food materials of an organic carbon nature, where microbial biomass accounts for almost 30% of the dung mass (Dao *et al*. 2008, Pachepsky *et al*. 2009). Many of these organisms shed in the feces can pose a great health risk to humans (Field and Samadpour 2007). Pathogens like Shigella spp., *Cryptosporidium parvum*, *Naegleria fowleri*, *E. coli* 0157:H7, Schistosoma spp., and Salmonella are a few of the commonly known agents of gastrointestinal illness that originate from fecal contamination. Earlier studies reported that the exposure to recreational waters contaminated by cattle feces can be as risky as the exposure to waters contaminated with human sewage (Soller *et al*. 2010). The risk of contamination may extend beyond the snowmelt season, as some of these pathogens may be deposited in sediments. Bacteria survive longer in sediment compared to the water column (Garzio-Hadzick *et al*. 2010, Kiefer *et al*. 2012). Manure colloids in the runoff can increase the organic content in the sediment, which may enhance the survival of bacteria and lower its sensitivity to changes in temperatures (Garzio-Hadzick *et al*. 2010). The sediment can become a reservoir for bacteria, which may be suspended in the water column during the warm season.

Indicator bacteria, total coliforms (TC), fecal coliform (FC), *E. coli,* and fecal *Streptococci* (FS), are the currently acceptable standard for assessing the microbiological quality of water (Theron and Cloete 2002, Field and Samadpour 2007). The total coliform group can be of environmental or intestinal origin. The FC group is a subgroup of TC and it is one of the indicators recommended for use in evaluating the microbial water quality of grazing fields (Doran and Linn, 1979). The FC group is incubated in a selective medium at a higher temperature than the TC to eliminate the growth of environmental bacteria (Vaccari *et al*. 2006). The FC group consists largely of *E. coli*, which is found to correlate well with gastrointestinal illness outbreaks (Rosen *et al*. 2000). The FS group is limited to the intestinal tract of warm-blooded animals. Indicator bacteria include a large group of aerobic and facultative anaerobic bacteria (Vaccari *et al*. 2006, Rosen *et al*. 2000) which may not be pathogenic themselves. The presence of indicator bacteria indicate the potential presence of enteric pathogens. (Theron and Cloete 2002). Therefore, indicator bacteria are considered the acceptable alternative to the direct monitoring of individual pathogens which can be expensive.

A major limitation of the indicator bacteria is that it does not specify the source of contamination as it is shed in the feces of many cold and warm blooded animals. In addition, some strains of the fecal indicator bacteria have the ability to adapt outside the host and can survive and multiply in many habitats, including aquatic sediments and terrestrial soils (Savichtcheva and Okabe, 2006, Harwood *et al*. 2014). Earlier efforts to identify the source of pollution were based on the fecal coliform/fecal *Streptococci* (FC/FS) ratios (Meays *et al.* 2004). Human feces tend to have higher concentrations of FC relative to FS when compared to other animals. Several ratios were proposed in the literature (Geldreiche and Kenner, 1969, Doran and Linn 1979). However, further examination of these ratios indicated that they cannot be reliable as indicators of the source of fecal pollution. Edwards *et al*. (1997) found that FC can regrow under warm temperatures and increase the FC/FS ratio to levels that indicate human contamination where no human presence existed.

The fecal anaerobic bacteria are becoming the new alternative for the fecal indicator bacteria. Unlike the fecal indicator bacteria, the fecal anaerobic are not likely to grow in an aerobic environment and exhibit a high degree of host specificity (Savichtcheva and Okabe, 2006). In addition, fecal anaerobic constitutes a significant portion of the fecal bacteria. *Bacteroides* spp. is one of the fecal anaerobic that constitutes 30 to 40% of the fecal bacteria which accounts for almost 10% of the fecal mass (Layton *et al.* 2006). The recent development of molecular methods and the development of the Quantitative PCR (qPCR) enabled the detection of a bacterial species that belongs to the order *Bacteroidales,* which includes the genus *Bacteroides*. Several genetic markers for human and different animals have been developed based on the *Bacteroidales* 16S rRNA which is present in all mammalian feces (Harwood *et al*. 2014, Layton *et al*. 2006, Shanks *et al*. 2008). *Bacteroidales*-based methods target the whole *Bacteroides* population in the feces, which makes them suitable for detecting contamination, even in very small concentrations. The use of both conventional indicators and *Bacteroidales*-based methods is recommended for identification of fecal contamination (Savichtcheva and Okabe 2006).

Earlier studies reported that the concentration of bacteria in runoff from fields where animal waste has been applied is mostly higher than bacteria concentrations from fields with no animal waste, regardless of the nutrient management practice (Edwards *et al*., 2000, Doran and Linn 1979). Several studies examined the loading of bacteria with runoff from land where animal waste has been applied at different scales (Muirhead *et al.* 2005, Thelin and Gifford 1983, Kress and Gifford 1984, Edward *et al*. 2000, Mishra *et al.* 2008, Wanger *et al*. 2010). These studies were mainly performed under the conditions of rainfall runoff, which may not be comparable to bacteria loading under conditions of snowmelt runoff. The release and transport of bacteria under snowmelt conditions may be different from that under rainfall runoff conditions. The field conditions govern the energy of the snowmelt runoff. The hydrological channel of the water released from the snow pack during snowmelt is affected by soil status at the time of melt (Srinivasan *et al.* 2006). Overland runoff is expected to dominate if the ground is frozen or saturated as soil permeability is reduced in frozen (Iwata *et al*. 2010, Bayard *et al.* 2005) and near-saturated soil (Granger *et al.* 1984). The application of manure on top of the snow can affect the infiltration rate by attenuating snowmelt peaks and retarding snowmelt (Kongoli and Bland, 2002). Williams *et al.* (2010) found in a lab experiment that the application of manure on top of snow can increase infiltration and reduce runoff when compared to the application of manure before snow fall, which may inhibit infiltration and increase runoff.

The Canada-wide Watershed Evaluation of Beneficial Management Practices (WEBs) program was designed to evaluate the impacts of agricultural management practices deemed beneficial management practices (BMPs) at the field-scale. The WEBs program was available in nine sites around Canada including Saskatchewan. The Saskatchewan WEBs project, located in the Pipestone Creek watershed, investigated the potential impact on surface water of winter in-field bale grazing under the cold climate conditions of Saskatchewan at the watershed scale. The Saskatchewan WEBs project, which extended over a period of four years, was led by Agriculture and Agri-Food Canada. The Saskatchewan WEBs project did also investigate three other BMPs, besides winter in-field bale grazing, including (1) nutrient management on annual cropland, (2) wetland restoration in pasture land, and (3) conversion of annual cropland to perennial cover (AAFC 2013).

This study was designed as part of the Saskatchewan WEBs project. The design of this study within the Saskatchewan WEBs projects enabled the evaluation of the microbial quality of snowmelt runoff from cattle wintering fields compared to that from fields spread with manure in the fall. The manure spreading in the fall practice is the conventional manure management practice associated with wintering the cattle in confinement. During winter, the manure is piled and stored till the following fall when it is hauled and spread on the field. Cattle wintering fields, and the fields spread with manure in the fall; both receive excess animal waste beyond the spring and summer grazing season. Both practices were considered treatments and compared to control fields that did not receive any animal waste beyond the spring and summer grazing season.

The study provides information on fecal coliform (FC) removal at edge-of-field to snowmelt runoff draining six sub-watersheds under the three practices delineated as; *winter in-field bale-grazing*, *manure spreading in fall*, and *control*. All practices were implemented in duplicate sub-watersheds. The study investigated whether the fields receiving animal waste past the spring and summer will have higher concentrations of fecal coliform in the snowmelt runoff compared to the fields where grazing takes place during spring and summer only. The study did also examine whether the *winter in-field bale grazing* practice will result in higher concentration of FC in the snowmelt runoff compared to the *manure spreading in the fall* practice. The study also examined if the fecal indicator bacteria identified in the collected samples comes mainly from bovine sources using the newly developed *Bacteroides* markers.

3.3 Materials and Methods

Study Sites

Data were collected over three years (2011-2013) from six sub-watersheds located in the Pipestone Creek Watershed in the south-eastern corner of Saskatchewan. The watershed is part of the Prairie Pothole Region which has a landscape defined by till plains, hummocky moraines, and glacial potholes. The Pipestone Creek flows into Manitoba, enters Oak Lake and becomes part of the Souris River Basin. The region is characterized by long winters of four to six months, short, hot summers, and low precipitation. The area is also characterized by clear skies where around 312 to 322 days a year have some hours of sunshine. July is the warmest month of the year with an average temperature of 18°C, while January is the coldest month with an average of -16°C. On average, summer maximum and minimum temperatures are roughly higher than winter maximum and minimum temperatures by 22°C and 28°C respectively (McGinn 2010). The region is also known for its high wind speed where blowing wind during winter can sublimate snow and results in highly heterogeneous snow width in open fields *(*Fang *et al.* 2007). Snowmelt runoff is the main source of runoff to streams on the Canadian Prairies. Annual precipitation falls mainly as rainfall, and only one-third falls as snowmelt. However, runoff from summer rainfall events is mostly localized, whereas spring snowmelt is more spatially extent. The average annual precipitation is 431 mm. Average snowmelt during winter is 13-20 cm per month between November and March. The highest precipitation falls as rainfall in June around 83 mm.

The study sites are located in a permanent alfalfa pasture operated by producer (M). The six sub-watersheds range in area from 0.5 to 2.3 ha and represent natural drainage with a slope of 5 to 6%. Three groups of treatments were implemented in duplicate. The different treatments represent a grazing practice or a grazing-related manure handling practice. Two of the sub-watersheds were left as controls, where no animal waste was added after the spring and summer grazing season. In the other four sub-watersheds, extra animal waste was added to the field after the spring and summer grazing season either by spreading manure in the fall or by the cattle grazing in-field during the winter. In this study the different treatments are delineated as *control* (C), *winter in-field bale-grazing* (W) and *manure spreading in fall* (F). The *manure spreading in fall* sites constitute another control for the winter in-field bale-grazing practice. The *manure spreading in fall* represents a conventional wintering scenario where manure is stockpiled in corrals and then spread on the fall. The amount of manure spread during the fall was phosphorus equivalent to the amount of dung estimated to be deposited by the wintering cattle. For each sub-watershed, a 2-inch 45-degree trapezoidal flume was installed, with wooden berms to direct runoff flow into the flumes.

Runoff Sample Collection

Runoff samples were collected at edge-of-field every second hour; where possible, throughout spring snowmelt. The samples were collected in plastic bottles using ISCO's continuous automated sampler (ISCO model 6712, ISCO Inc.) with flow rate data logger. This project was designed as part of the Saskatchewan WEBs project; therefore sample collection followed the protocol of the WEBs project that was designed primarily for evaluating nutrients. The samples analyzed by the WEBs project and this study provided a snapshot of the content of the snowmelt runoff samples including nutrients, suspended particles, and the indicator bacteria. No chemical disinfectant was used to sterilize the bottles. The bottles were thoroughly washed with ultra-pure water before sample collection. During sample collection, it was not possible to autoclave the bottles used in sample collection due to the large number of bottles used to collect samples from 14 sub-watershed. Analysis of 30 blank samples showed that the washing protocol did eliminate cross contamination during sample collection. The collected samples were placed on ice in a dark cooler after collection and were transported to the lab for analysis within 24 hours of collection. Fecal coliform was enumerated by membrane filtration using 0.45 µm filters plated on MFC ager then incubated for 24 ± 2 h at 44.5 ± 0.2°C. Preparation of different culture media, phosphate buffered dilution water, and enumeration procedures were performed according to the Standard Methods for Examination of Water and Wastewater method 9222 D. Fecal Coliform Membrane Filter Procedure (APHA, 1998). Each sample was filtered in triplicates and only plates recording colonies count less than 300 CFU were recorded. Fecal coliform counts were reported as CFU/100ml. Sub-samples of 100ml were frozen for molecular analysis throughout snowmelt runoff over the three years. Around forty-one samples of the frozen samples; representing the highest counts recorded for each treatment, were selected for molecular analysis with qPCR. Extraction of DNA and analysis using qPCR were performed in the Dr. Chris Yost lab at the University of Regina. The number of samples analyzed by molecular methods for each of the treatment groups (W, F, C) by year is presented in Table 1.

Table 1. The distribution of the runoff samples collected for molecular analysis with qPCR

	W	F	C	Total
2011	10	5	0	15

2012	7	3	3	13
2013	6	3	4	13
Total	23	11	7	41

DNA was extracted from environmental samples using MO BIO PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Inc.). All quantitative real-time PCR (qPCR) assays were performed using the StepOnePlus Real-Time PCR System (Applied Biosystems). A general *Bacteroidales* gene marker was detected using the AllBac and probe set (Tambalo *et al*., 2012, Table 2). A bovine-specific *Bacteroidales* gene marker was also detected using the BacBov-1 primers and probe set (Lee *et al*., 2010, Table 2). Mean log copy number/100mL was determined for all samples using the following equation:

$$\text{Log copy number/100mL} = \log_{10}(((((SQ/4)100)2)100)/V_{filtered}) \quad (1)$$

Where: SQ = Starting Quantity, V= Volume

Table 2. PCR primer and probe sequence used in this study

Name (Target)	Type	Primer/Probe Name	Sequences
AllBac	Forward primer	AllBac296f	GAGAGGAAGGTCCCCCAC
(General)	Reverse primer	AllBac412r	CGCTACTTGGCTGGTTCAG
	Probe	AllBac375Bhqr	FAMJCATTGACCAATATTCCTCACTGCTGCCT -BHQ1
BacBov-1	Forward primer	BacBov-F1	AAGGATGAAGGTTCTATGGATTGTAAA

(Bovine)	Reverse primer	BacBov-R1	GAGTTAGCCGATGCTTATTCATACG
	Probe	BacBov-TP1	FAM-ATACGGGAATAAAACC-NFQJMGB

Data Analysis

The conventional indicator:

The fecal coliform count in each sample; calculated as CFU/100ml, was compiled with flow data collected at 5-min intervals to calculate total fecal coliform count for each sampling interval. The sampling interval is calculated as the midpoint between two samples. For example, consider the following three samples; 1) sample A collected at 9:00 am, 2) sample B collected at 12:00 pm, and 3) sample C collected at 3:00 pm and runoff started at 8:00 am and ended at 5:00 pm. The sampling interval for sample A will be 8:00 am to 10:30 am (150 minutes). The sampling interval for sample B is 11:30 am to 1:30 pm (120 minutes), and the sampling interval for sample C is 1:30 pm to 5:00 pm (210 minutes). In general, the samples were collected every two hours where possible. Therefore, the sampling intervals for the different samples were almost identical. The total count at each sampling interval was accumulated for all samples collected in the day and divided by the cumulative flow for the day to calculate the daily flow-weighted count for each day. Events mean count was calculated as the average of the daily flow-weighted count calculated for each sub-watershed for each year. The normality of the data was tested using the Shapiro-Wilks w test. Statistical analysis was performed using R 3.3.2 with significance set at 0.05.

The *Bacteroidales* markers:

Detection frequency for each of the *Bacteroidales* markers was estimated by dividing the number of samples in which the marker was detected by the total number of samples collected. The relation between the FC count and *Bacteroidales* concentration in the samples is examined by calculating the correlation coefficient between $\log_{10}$ FC CFU/100 ml and the mean $\log_{10}$ copy/100 ml of AllBac marker and the Bovine marker in the detected samples.

3.4 Results

Results per year

The automated sampler was not installed for site C1 in the years 2011 and 2012 and was only added in the year 2013. Therefore only site C2 was available as a *control*. The snowmelt runoff started at different dates every year and lasted from a few days to over a week. **In the year 2011**, snowmelt runoff started later in the spring on April 8th and ended on April 21st. Runoff was interrupted with a few days of below freezing temperatures starting April 14th, and then resumed on April 19th. Sites W1 and F2 stopped flowing on April 13th. No flow data were available from the autosampler installed at site F1 on April 8th. Fecal coliform removal from site C2 and site F2 reported counts below the recommended recreational water standards of 200 CFU/100ml (Health Canada 2012). The counts for sites F1 and W2 exceeded the 200 CFU/100 ml on several occasions but remained low compared to site W1, which recorded a peak count of 1071 CFU/100ml (Table 3). All counts are reported as daily flow-weighted count. In general, all sites recorded peak counts at the beginning of the runoff events.

Table 3. Event flow-weighted mean count and peak count for each sub-watersheds in 2011

	Winter in-field bale-grazing	Control	Manure spreading in fall

	W1	W2	C2	F1	F2
Events mean count (CFU/100ml)	455	173	34	173	101
Peak count recorded	1071	501	90	343	151
Standard deviation	384	172	30	90	42
Total no. of samples collected	28	32	30	32	24

In the year 2012, snowmelt began late in winter on March 12th and ended on March 16th. Flow data from two sub-watersheds; W1 and F1, could not be collected due to errors with the two autosamplers caused by ice formation. Sub-watersheds W1 is one of the duplicate sites for the *winter in-field bale-grazing* treatment, and F1 is one of the duplicate sites for the *manure spreading in fall* treatment. Therefore, only one site was available for each treatment in the year 2012. Fecal coliform counts for site C2 were below the 200 CFU/100 ml recreational standard. However, for sites W2 and F2 fecal coliform counts were higher than 200 CFU/100ml, reaching a peak of 800 CFU/100ml for F2, and a peak of 2674 CFU/100 ml for W2 (Table 4). However, unlike the year 2011, the peak counts for W2 and F2 were recorded towards the end of the runoff event, whereas for C2 no peak was apparent and the count remained relatively constant throughout the snowmelt event.

Table 4. Event flow-weighted mean count and peak count for each sub-watersheds in 2012

	Winter-in-field bale-grazing		Control	Manure spreading in fall	
	W1	W2	C2	F1	F2
Events mean count (CFU/100ml)	NA	1245	113	NA	446
Peak count recorded	NA	2674	163	NA	800

Standard deviation	NA	1024	44	NA	324
Total no. of samples collected	29	19	21	23	16

In the year 2013, all sub-watersheds were equipped with an autosampler, and data were available for all sub-watersheds. Snowmelt runoff began on April 26th and ended on May 3rd. Sites F1 and F2 stopped flowing on April 28th. Only the two *control* sub-watersheds C1 and C2 were flowing until May 2nd. The sampler for C1 started collecting data on April 28th. Fecal coliform counts were above the 200 CFU/100 ml for all sites except site C1. Site C2 recorded the highest counts of all the years, with a peak of 1031 CFU/100ml. The peak daily flow-weighted mean count recorded in runoff from site C2 was as high as that recorded from the *winter in-field bale-grazing* and the *manure spreading in fall* sites and even higher than one of the *winter in-field bale-grazing* (site W2), as shown in Table 5**Error! Reference source not found.**. The peak count for all sites was mostly recorded at the beginning of the snowmelt runoff.

Table 5. Event flow-weighted mean count and peak count for each sub-watersheds in 2013

	Winter in-field bale-grazing		Control		Manure spreading in fall	
	W1	**W2**	**C1**	**C2**	**F1**	**F2**
Events mean count (CFU/100ml)	761	412	27	251	638	684
Peak count recorded	1122	632	34	1031	1058	999
Standard deviation	356	200	7	372	409	363
Total no. of samples collected	36	35	48	49	19	19

Results per treatment

The data collected over the three years (2011-2013) showed that the counts; reported as daily flow-flow weighted counts, ranged from 6 CFU/100ml to 2674 CFU/100ml. The cattle wintering sites under the *winter in-field bale grazing* treatment reported the highest count of 2674 CFU/100ml. The highest counts reported for the other two treatments were very close. The highest count reported for the *manure spreading in fall* sites was 1058 CFU/100 ml, while the highest count reported for the *control* sites was 1031 CFU/100ml. The mean count calculated for the different treatments showed that the fields under the *winter in-filed bale grazing* treatment reported the highest mean count of 497 CFU/100ml while the *control* sites reported the lowest mean count of 110 CFU/100ml. The mean count from the field under the *manure spreading in fall* treatment was 364 CFU/100ml (Figure 3).

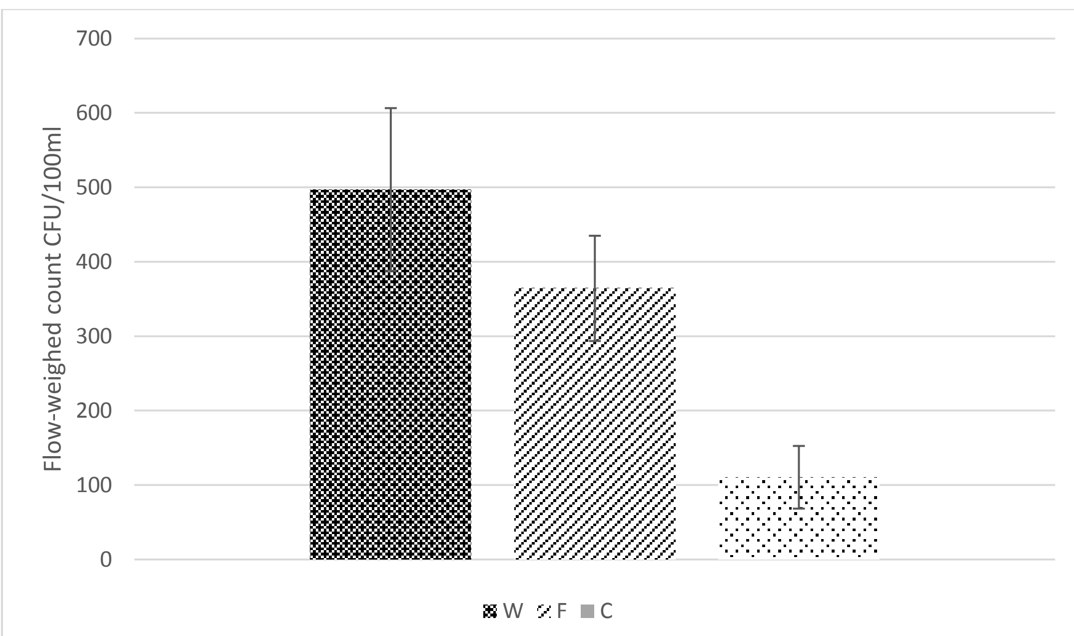

Figure 3. Flow-weighted count of fecal coliform (CFU/100 ml). Error bars are standard errors of the mean; n=28 for W, n=22 for F, and n=25 for C.

The results of the Shapiro-Wilks test showed that the daily flow-weighted counts for the different treatments did not follow a normal distribution. The nonparametric Kruskal-Wallis rank test was used to determine whether the difference between the means of the three treatments is statistically significant. The mean count for each treatment is calculated as the average of the daily flow-weighted counts for each treatment over the three years. The mean counts of the three treatment were found to be significantly different, and the null hypothesis stating that there is no difference between the means was rejected ($p=0.000088<0.05$).

The means were further compared using the nonparametric test Mann-Whitney-Wilcoxon Test to identify which treatments were statistically different. The Mann-Whitney-Wilcoxon Test was used to compare C with F, C with W, and F with W. The results showed that the mean count of FC in snowmelt runoff from the *control* sites was significantly different from the mean count from F fields ($p=0.0001 <0.05$) and the mean count from W fields ($p=0.0003<0.05$). However, there was no significant difference between the mean count from F and the W ($p=0.74>0.05$).

Bacteroidales qPCR markers

The percentage of samples detected by each marker per year is shown in Table 6. Overall AllBac was detected in 68% (28/41) of the analyzed samples. In 2011, AllBac was detected in 87% (13/15) of the samples but was only detected in 30% (4/13) of the samples in 2012. AllBac was detected in 85% (11/13) of the samples in 2013. The Bovine marker was detected in 39% (16/41) of the overall samples. In 2011, the Bovine marker was detected in 73% (11/15) of the samples but was only detected by 8% (1/13) of the samples in 2012 and by 30% (4/13) of the samples in

2013. The highest number of samples that were detected by both markers was recorded in 2011 with around 73% (11/13) of the samples detected by both markers. However, in 2013 only 30% (4/13) of the samples were detected by both markers. No sample was detected by both markers in 2012. The same year recorded the highest number of samples that did not detect any of the markers at 62% (8/13) compared to 13% (2/15) in 2011 and 15% (2/13) in 2013. The percentage of samples detected by AllBac only, and not bovine, was the highest in 2013 with 46% (6/13) followed by the years 2012 with 30% (4/13). The year 2013 had the lowest percentage (13%) of samples detected by AllBac and not detected by Bovine. In general, any sample that detected Bovine maker did also detect the AllBac, except in 2012 where one sample detected only Bovine and did not detect AllBac.

Table 6. Percentage of sample detection for each marker per year

	2011 (15 samples)		2012 (13 samples)		2013 (13 samples)	
	# of samples	%	# of samples	%	# of samples	%
Detected both	11	73	0	0	4	30
Detected AllBac	13	87	4	30	11	85
Detected Bovine	11	73	1	8	4	30
Detect AllBac only	2	13	4	30	6	46
Detect Bovine only	0	0	1	8	0	0
Did not detect	2	13	8	62	2	15

The detection of the markers varied with the different treatments (W, F, and C) as shown in Table 7. In general, the Bovine marker was only detected in W samples. The Bovine marker was only detected in 60% of the W samples over the years and was not detected in any of the samples in 2012. Only one C sample detected the Bovine marker in 2012. However, the same sample did not detect the AllBac marker which raises the question on the validity of the sample.

The AllBac marker was detected in samples collected from the different treatments at different percentages. Over the three years, the AllBac marker was detected in 90% of the W samples, in 45% of the F samples, and in 43% of the C samples. However, in the year 2012, the AllBac marker was only detected in W samples and was not detected in any of the C or F samples.

Table 7. Percentage of sample detection for each marker per year per treatment

		Winter in-field bale grazing	Manure spreading in fall	Control
2011	AllBac	10 (100%)	3 (60%)	NA
	Bovine	10 (100%)	0 (0%)	NA
2012	AllBac	4 (60%)	0 (0%)	0 (0%)
	Bovine	0 (0%)	0 (0%)	1 (30%)
2013	AllBac	6 (100%)	2 (70%)	3 (75%)
	Bovine	4 (70%)	0 (0%)	0 (0%)
Total	AllBac	20/23 (90%)	5/11 (45%)	3/7 (43%)
	Bovine	14/23 (60%)	0/11 (0%)	1/7 (14%)

Further analysis of the correlation between $\log_{10}$ FC CFU/100 ml and the mean $\log_{10}$ copy/100 ml of AllBac marker and the Bovine marker in the detected samples indicated that there is no relation between the FC count and the concentration of the AllBac in the detected samples (r^2=0.17). However, a weak relation (r^2=0.46) exists between the concertation of FC and the concentration of the Bovine marker in the detected samples.

3.5 Discussion

This study examined two main hypotheses. The first hypothesis states that fields receiving animal waste past the spring and summer grazing season, either by manure spreading in fall or by the grazing animals during winter, are expected to contribute higher loading of FC in snowmelt runoff compared to the *control* fields where grazing takes place during spring and summer only. The second hypothesis states that the fields receiving fresh animal deposits during winter are expected to contribute higher loading of FC in snowmelt runoff compared to the fields receiving the aged manure in the fall.

The data collected over the three years supported the claims stated in the two hypotheses. The results of this study showed that the fields receiving animal waste beyond the spring and summer grazing season did contribute significantly higher count of FC compared to the *control* fields that were only grazed during spring and summer. The *winter in-field bale grazing* practice, where the cattle grazed on bales during winter, did contribute a significantly higher count of FC compared to the *control* fields. The results showed that *winter in-field bale grazing* fields could contribute more than four times the FC load in snowmelt runoff compared to the *control* fields. The *manure spreading in the fall* practice did also contribute significantly higher counts of FC in snowmelt runoff compared to the *control* fields. The *manure spreading in fall* fields contributed more than three times the count of FC in snowmelt runoff compared to the *control* fields.

The results showed that the highest flow-weighted count of FC was reported in the snowmelt runoff samples from the *winter in-field bale grazing* fields with a mean of 497 CFU/100ml, whereas the lowest count was reported in the samples from the *control* fields (110 CFU/100ml). The mean flow-weighted count of FC in snowmelt runoff from the *manure spreading in fall* fields was 364 CFU/100ml. The results showed that the mean flow-weighted count of FC from the *control* fields could be well below the recreational water standards of 200 CFU/100ml. Though the *winter in-field bale grazing* fields reported the highest mean count of FC in snowmelt runoff samples, the results showed that the mean count of FC from the *winter in-field bale grazing fields* was not significantly higher than that from the *manure spreading in fall* fields.

The fresh manure deposited by the cattle during the winter can be a source of extra bacteria during snowmelt. The extra manure added to the field during the fall can also increase the bacteria concentration in the field compared to the *control* fields. The number of indicator bacteria in the runoff is related to the numbers of bacteria inside the cowpat (Muirhead *et al.*, 2005). Age of the dung is one of the factors that affect the microbial release from animal waste (Kress and Gifford 1984, Blaustein *et al*. 2015). The number of bacteria in the old manure is expected to be lower than the number of bacteria in the fresh dung. Earlier studies examining the release of bacteria from dung to rainfall runoff reported a decrease in bacteria concentration in runoff under rainfall runoff conditions as the dung ages (Kress and Gifford 1984, Hodgson *et al.*

2009). The old dung from the *control* fields is expected to have a lower count of bacteria compared to the freshly deposited dung in the *winter in-field bale-grazing* fields. The method of manure storage, as well as the length of storage, can all affect the number of bacteria in the old manure. Storage can also affect the distribution of bacteria in manure (Guber *et al.*, 2011). This may explain the difference observed between the years in the count of FC in runoff from fields with the *manure spreading in fall* treatment.

The results indicate that bacteria is expected to survive the cold winter and be available for transport to surface water with snowmelt runoff. The aging process might lower the number of bacteria in the dung, but may not completely kill the bacteria. The bacteria living in the surface layer of the dung may become inactivated due to desiccation and UV exposure or cold shock, which all can also be lethal to the bacteria (Hodgson *et al*. 2009). However, the formation of a crust can protect the bacteria from environmental stresses and the inner matrix of the dung may contain viable bacteria for an extended period of time (Murihead *et al*. 2005). This crust may protect the bacteria from UV exposure, and may shield the bacteria from the impact of diurnal variations in temperatures during winter and the stresses associated with freeze-thaw cycles.

The results of the study showed that the currently available *Bacteroidales* markers may not be suitable to evaluate the grazing practices examined in this study. The persistence of the markers may prohibit the detection of pollution from older dung, which can limit the validity of the markers to evaluate the pollution from the old dung versus that from the freshly deposited dung. The Bovine marker used in this study that was not able to detect bovine sources in any sample from the *manure spreading in the fall* fields and the *control* fields. The marker did only detect bovine sources in the samples collected from the *winter in-field bale-grazing* sites, which contained younger dung compared to the other fields. All fields have fecal matter of bovine source, either from the dung deposited by the cows during the grazing season or by the extra manure added in the fall. The persistence of the Bovine markers was also questioned by Tambalo *et al.* (2012) who conducted a study in Wascana Creek to examine the persistence of host-specific *Bacteroidales* qPCR markers *in situ* in two trials lasting over 14 to 16 days, and compared it to the persistence of *E. coli*. The study found that the persistence of *Bacteroidales* markers for human, ruminant, and bovine are significantly shorter than the conventional *E. coli*, and were not detectable beyond 12 days. The general *bacteroidales* marker AllBac was detected in all sites in varying percentages over the years for all treatments, which indicated that the pollution is from fecal sources. Tambalo *et al.* (2012) reported a longer persistence for the AllBac marker which was detected until the last day of the study and was comparable to the persistence of *E.coli*.

The persistence of the *bacteroidales* markers may also be affected by other environmental factors like temperature, pH and light (Tambalo *et al.*, 2012). This may explain the fact that the AllBac marker was not detected in any of the samples in 2012. The year 2012 recorded below average precipitation and early snowmelt compared to the years 2011 and 2013, which, on the contrary, recorded higher than normal precipitation and later snowmelt. Environmental factors may have affected the persistence of the AllBac markers differently between the years.

3.6 Conclusion and Recommendations

This study recommends that microbial pollution should be included in the evaluation of BMPs on pasture. The data collected over three years (2011-2013) showed that feeding the cattle in confinements during winter and spreading the collected manure on the field the next fall may result in the same microbial pollution as that associated with the animal grazing in-field during winter. These results can be included in future cost and benefit analysis of grazing practices and manure handling practices under the cold climate of the Canadian Prairie. Including the continuously evolving microbial source tracking methods in field-scale studies is vital for the development of reliable alternative methods for microbial source tracking. However, these methods are not yet suitable to be used alone for evaluating BMPs, especially over different years and varying environmental conditions.

4 BACTERIA AND SUSPENDED SOLIDS LOADING PATTERN WITH SNOWMELT FROM AGRICULTURE WATERSHED: IS THERE A FIRST FLUSH?

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4.1 Abstract

The overland transport of manure-borne bacteria during snowmelt runoff is not well understood. The comparison of bacteria transport patterns to that of other suspended particles in the runoff; such as soil particles, organic materials, and nutrients, can improve our understanding of bacteria transport in overland flow. This study examined the loading pattern of fecal coliform (FC) from grazing pastures, under different management practices, during snowmelt runoff compared to that of the other suspended particles (TSS) in the runoff. The study evaluated if there is a first-flush associated with the loading of FC and TSS during snowmelt runoff. The study found that pollutant loading from fields receiving animal waste beyond the grazing season, especially animal wintering fields, is dependent on precipitation levels and field conditions during snowmelt runoff. Animal wintering fields transport most of their pollution load, of both microbial and suspended solids, at the early stages of the snowmelt during high precipitation years. Loading of pollutants from fields where grazing occurs only during the grazing season are less dependent on the precipitation level and field conditions and exhibit first-flush in microbial loading. In general, the loading pattern of FC was not similar to that of TSS at all times.

Management practices for first-flush are recommended to retain the runoff water in the early stages of snowmelt (e.g. wetlands), mainly in high precipitation years to reduce the concentration of pollutants reaching surface water during snowmelt runoff.

4.2 Introduction

Manure-borne pathogens such as bacteria, protozoa and viruses are one of the main water quality concerns related to livestock production. Other water quality effects associated with livestock production include nutrients (nitrogen and phosphorus), and organic materials contained in manure; e.g. straw. The transport of manure-borne bacteria from land to water sources is not well understood. However, it is primarily related to overland flow (Jamieson *et al*. 2004). Bacteria are believed to transport as free cells under rainfall runoff (Murihead *et al*. 2005, Soupir and Mostaghimi 2011). The microorganisms tend to be mostly suspended in the water column. Bacteria may also attach to sediments or other particles in the runoff (Pachepsky *et al.* 2006). The attachment of bacteria to soil or organic particles in the runoff can be affected by the particle size distribution of the suspended material, clay content (surface charge interactions), and organic content. Bacteria tend to attach to smaller particles; less than 2µm, and to soil particles with a higher clay content (Murihead *et al*. 2006, Ling *et al.* 2002, Guber *et al.* 2007). In the presence of manure colloids, bacteria may favor free cell transport over attachment to small soil particles (Guber *et al*. 2007). Ground conditions may also affect the attachment of bacteria in overland flow. Murihead *et al.* (2006) found that during runoff under excess saturation conditions, where interaction with soil matrix is limited, bacteria tend to transport unattached. Whether attached or free, the transport pattern of bacteria may be different from that of other suspended particles in the runoff. Unlike other organic and inorganic suspended particles in the runoff, bacteria are susceptible to inactivation (Dorner *et al.* 2006). The transport of bacteria with runoff is dependent upon its release from the dung matrix. Therefore, the concentration of bacteria in the runoff is affected by the concentration of the bacteria in the manure source and flow rate (Pachepsky *et al.* 2009, Muirhead *et al*. 2005).

The release of bacteria to runoff and the subsequent overland transport during rainfall events is expected to be different than that during snowmelt. Limited data is available on the transport of bacteria from land to surface water during snowmelt runoff. Snowmelt over frozen soil restricts soil particle detachment and favors the transport of smaller and less dense particles like clay and organic matter (Panuska and Karthikeyan 2010), which may favor the transport of bacteria. Studies examining the loading of bacteria from animal wintering fields, and their loading pattern during snowmelt, can provide valuable information to decision makers supporting the evaluation of agriculture best management practices (BMPs). The cold conditions and fluctuating temperatures during winter may kill or injure the bacteria. Injured bacteria may recover as temperatures warm up and may be available for transport later during snowmelt. The application of manure on top of snow may create a mulching effect that increases infiltration of snowmelt and reduces runoff (Kongoli and Bland, 2002, Williams *et al.* 2010). The timing of manure application, either before soil freezing in the fall, or on the frozen soil before the spring thaw, as well as the position within snowpack, can have an impact on soil temperature, infiltration, and erosion (Srinivasan *et al*. 2006).

Wintering cattle in-field during the cold winters of the Canadian Prairies is becoming a common practice; known as *winter in-field bale grazing*. During winter, the grazing cattle deposits their dung on snow-covered or frozen ground. The impact on surface water receiving snowmelt runoff from cattle wintering fields is expected to be higher that the impact from grazing fields where animal graze during the grazing season; spring and summer, only. The cattle can also be wintered in confinements during the Canadian Prairie winter. However, the extra cost associated with feed preparation and transport during winter, as well as manure handling and storage, make this option less attractive to farmers compared to wintering cattle in-field. In addition, the manure collected during winter is stored till the following fall when it is hauled to field and spread; a practice known as *manure spreading in fall*. The extra manure added to the field in the fall beyond the grazing season might increase the impact on surface water with snowmelt runoff. In the Canadian Prairie, snowmelt runoff is the primary source of runoff from land to surface water (Shook and Pomeroy 2012).

The impact of the winter in-field bale-grazing practice on snowmelt runoff quality was examined as part of the Saskatchewan component of the Canada-wide Watershed Evaluation of Beneficial Management Practices (WEBs). The WEBs program aimed at evaluating agricultural management practices considered beneficial management practices (BMPs) and was available in nine locations across Canada. The program evaluated the field-scale impact on the environment, economics, and the health of agricultural ecosystems and production models. The Saskatchewan WEBs project, located in the Pipestone Creek watershed, evaluated four BMPs: (1) winter in-field bale-grazing, (2) nutrient management on annual cropland, (3) wetland restoration in pastureland, and (4) conversion of annual cropland to perennial cover (AAFC 2013).

This study is intended to support the Saskatchewan WEBs project by supporting the evaluation of the impact of *winter in-field bale grazing* on snowmelt runoff quality. This study investigated the loading pattern of bacteria; represented by fecal coliform (FC), and the other suspended particles (TSS), during snowmelt runoff. The study examined if the extra dung deposited by the grazing cattle on top of frozen or snow-covered ground will increase the loading of FC and TSS in the early stages of runoff; known as first-flush. The study examined the loading pattern from six sub-watershed under three management practices implemented in duplicates. The three practices included; *winter in-field bale grazing*, *manure spreading in fall*, and *control* (spring and summer grazing only). The study examined if the management practice on pasture affects the loading pattern of FC and TSS and whether the loading of FC and TSS exhibited a first-flush for all practices. The study also questioned whether the loading pattern of FC is similar to that of TSS. The main hypotheses tested are: 1) The loading pattern of FC is not identical to that of other suspended particles in the runoff, 2) Grazing practice and field conditions affect the temporal loading pattern, and first-flush might not be the only pattern.

4.3 Materials and Methods

Study Sites

Data were collected from six sub-watersheds located in the Pipestone Creek Watershed in the south-eastern corner of Saskatchewan, which is part of the Prairie Pothole Region. The Pipestone Creek flows into Manitoba, enters Oak Lake and forms part of the Souris River Basin. The region is characterized by long cold winters, short, hot summers, low precipitation, and clear skies (Ecological Stratification Working Group 1995). On average around 312 to 322 days a year have some hours of sunshine. Summer maximum temperatures are around 22°C higher than winter maximum and summer minimum temperatures are 28°C higher than winter minimum (McGinn 2010). The warmest month is July (18°C); the coldest January (-16°C). Extreme low temperatures in winter and extremely high temperatures in summer are also common. The region is also characterized by high wind speed, mainly in spring and fall. During winter, the blowing wind can transport and sublimate around 75% of snowfall in open fields which make snow depth highly heterogeneous (Fang *et al*. 2007).

Annual precipitation on the Canadian Prairie falls mainly as rainfall, and only one-third of the precipitation is attributed to snowfall, yet snowmelt runoff is the dominant source of runoff to streams on the Prairie. Unlike the localized summer rainfall events, snowmelt is more spatially extensive. According to Environment Canada's historical data for 1939-2010 from Broadview monitoring station, 27% of the annual precipitation occurs as snowfall. The average annual precipitation in the region is 431 mm with the highest precipitation occurring in June (83mm) and the lowest in February (13 mm). During winter season an average precipitation of 13-20 mm fall each month occurs as snowfall between the months of November and March.

The six sub-watersheds representing the study site are part of pasture land under permanent alfalfa operated by one producer (M). The sub-watersheds ranged in area from 0.5 to 2.3 ha and average slope of 5 to 6% with each sub-watershed representing natural drainage. For each sub-watershed, a 2-inch 45-degree trapezoidal flume was installed, with wooden berms to direct runoff flow into the flumes. Snowmelt runoff samples were collected at edge-of-field. Three grazing practices were implemented in duplicates: 1) winter in-field bale-grazing; 2) manure spreading in fall; and 3) spring and summer grazing only. The winter in-field bale-grazing treatment represents the practice of allowing the cattle to graze in the field during winter. The two sub-watersheds representing the *winter in-field bale grazing* are delineated as W1 and W2. The manure spreading in fall treatment represents the conventional manure management practice associated with wintering cattle in confinements during winter where manure piled in confined corrals during winter is spread on the field in the fall. Manure was collected from a concentrated feedlot operated by the same producer (M) and spread at a rate equivalent to the estimated dung expected to be deposited by the cows during winter. The two sub-watersheds representing the *manure spreading in fall* treatment are referred to as F1 and F2. The spring and summer grazing only treatment represents the conventional grazing practice where cattle graze in the field during spring and summer season only. The two sub-watershed representing the spring and summer grazing only; delineated as C1 and C2, are considered *control* fields. These fields did not receive any additional animal waste after the spring and summer grazing season.

Runoff Sample Collection

Runoff samples were collected over three years between the years 2011-2013. Runoff samples were collected every two hours, where possible, throughout spring snowmelt at edge-of-field. The samples were collected in plastic bottles using ISCO's continuous automated sampler with a flow rate data logger (ISCO model 6712, ISCO Inc.) Sample collection was coordinated as part of the Saskatchewan WEBs project. Therefore, sample collection followed the protocol of the Saskatchewan WEBs project which was designed primarily for evaluating nutrients loading. The plastic bottles used in sample collection were thoroughly washed with ultra-pure water between samples collection. Throughout the snowmelt runoff, 30 blank samples were analyzed to evaluate the effectiveness of the washing protocol in reducing cross contamination.

The results showed that the washing protocol did eliminate cross contamination during sample collection. After collection, samples were placed in a dark cooler and transported to the lab and analyzed within 24 hours of collection. Runoff samples were analyzed for FC and total particulate weight. Fecal coliform was enumerated by membrane filtration using 0.45 µm filters plated on MFC ager then incubated for 24 ± 2 h at 44.5 ± 0.2°C. At least three different volumes were analyzed and plated for each sample, where possible, to target colonies count less than 300 CFU. Plates with colonies number under the target count were recorded and expressed as CFU/100 ml. The protocol for preparation of different culture media, phosphate buffered dilution water, and enumeration procedures followed the Standard Methods for Examination of Water and Wastewater method 9222 D (APHA, 1998). The remaining volume of the collected sample was vacuum filtered with weighed, precombusted 0.7-mm glass fiber filters, equivalent to 0.45-mm nitrocellulose filters (Cade-Menun *et al*. 2013). Filters were then oven-dried at 30°C, and dry weights were determined. The difference in the weight of dried filters before and after filtration gives the total particulate weight.

Data Analysis

The concentrations of suspended particles and fecal coliform in each sample were compiled with flow data collected at 5-min intervals to calculate pollutant mass export for each sampling interval (k). The sampling interval represented the midpoint between two samples. For example, assume that snowmelt runoff started at 8:00 am and ended at 9:00 pm and four samples were collected; Sample 1 at 10:00 am, Sample 2 at 1:00 pm, Sample 3 at 3:00 pm and Sample 4 at 7:00 pm. The sampling interval for each sample will be as shown in the following table:

Table 8. Sampling interval estimation

Sample	Collection time	Sampling Interval	
		Start	End
Sample 1	10:00 am	8:00 am	11:30 am
Sample 2	1:00 pm	11:30 am	2:00 pm
Sample 3	3:00 pm	2:00 pm	5:00 pm
Sample 4	7:00 pm	5:00 pm	9:00 pm

The flow data and the concentration of TSS and FC at each sampling interval were used to calculate the cumulative volume (equations 2) and the cumulative mass (equations 3) at each sampling interval. For each sub-watershed, the cumulative values were then normalized by the total volume or total mass for each snowmelt runoff event. Then a plot of paired values $v(k_i)$ and $m(k_i)$ (mass-volume curve) is created and a bisector line at 45° is added to the graph. The bisector line represents uniform pollutant loading where pollutant concentration is constant during the runoff event. The location of the mass-volume curve in relation to the bisector line can graphically provide information on the temporal distribution of the pollutant loading during the runoff event (Shamseldin 2011). A pollutant loading is considered a first-flush when the mass-volume curve for that pollutant falls above the bisector line (Figure 4). If the curve is very close or equal to the bisector line, it indicates that the pollutant load is constant during the event. A pollutant load is considered delayed when the mass-volume curve falls below the bisector line. In this case, the bulk of the pollutant load is transported at the late stage of the runoff event.

$$\boldsymbol{v(k_i) = \frac{\sum_{i=1}^{i=n} Q_i \times ki}{Qt}} \quad \textbf{(2)}$$

$$\boldsymbol{m(k_i) = \frac{\sum_{i=1}^{i=n} Q_i \times k_i \times C_i}{Ct}} \quad \textbf{(3)}$$

Where $v(k_i)$ is the cumulative volume at the sampling interval k_i, where k_i is the i^{th} sampling interval, and n is the total number of the sampling intervals during the runoff event. Q_i is the flow volume during the i^{th} sampling interval, Q_t is the total volume of the runoff event. Where $m(k_i)$ is the cumulative mass at the sampling interval k_i, C_i is the concentration of the pollutant at the i^{th} sampling interval, and C_t is the total mass loading of the runoff event.

The percentage of the pollutant (TSS or FC) mass transported in the first 30% of the runoff event (denoted as F_{30}) is also evaluated for each sub-watershed for each snowmelt runoff event (Figure 4). A first-flush is considered when F_{30} is more than 0.3 or 30%. If F_{30} is more than 0.3, it indicates that more than 30% of the pollutant mass (TSS or FC) is transported during the first 30% of the runoff volume. The higher the value of F_{30}, the stronger the first flush effect.

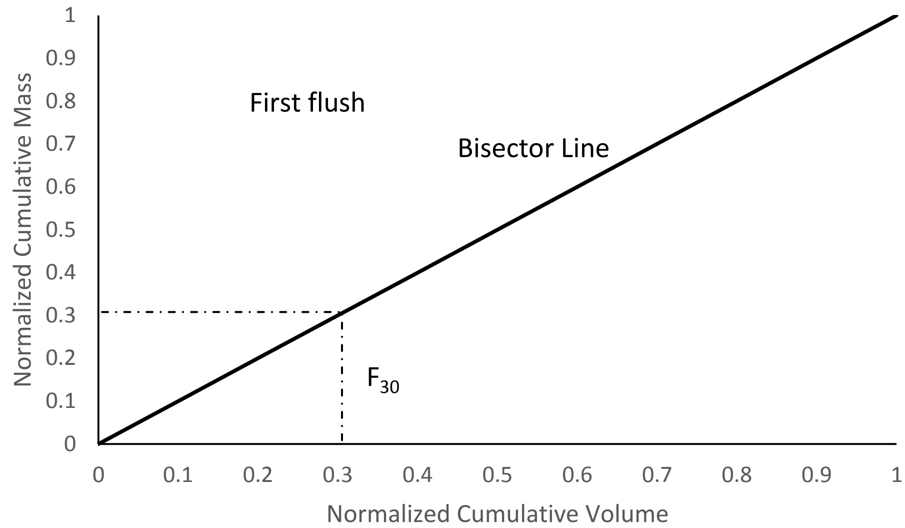

Figure 4. Visual presentation of first-flush

4.4 Results

The data were collected over three years (2011-2013) at edge-of-field from six sub-watersheds under three different treatments. The three treatments; *control* (C), *winter in-field bale grazing* (W), and *manure spreading in fall* (F), were each implemented in duplicate sub-watersheds. In the year 2011, only one sub-watershed (C2) was available as a control since the autosampler was not yet installed for sub-watershed (C1). In the year 2012, data was only available from one sub-watershed for each treatment. The autosampler was not yet installed for C1 and error with the autosamplers installed in W1 and F1, caused by ice formation, prevented the collection of flow data for W1 and F1. In the year 2013, the autosampler was installed for C1, and there were no issues reported with the other autosamplers. Therefore, the year 2013 was the only year that data were available from duplicate sub-watersheds for the each treatment.

In the year 2011, runoff started on April 8th and ended on April 21st. The two sub-watersheds W1 and F2 stopped flowing on April 13th. No flow data was available for F2 on April 8th. Runoff was interrupted on April 14th as the temperature dropped below zero. The fecal coliform mass-volume curves plotted for the different sub-watersheds were all well above the bisector line (Figure 5). The mass-volume curves showed a first-flush associated with FC loading with snowmelt runoff for all treatments. In general, the early stages of the snowmelt runoff transported more than 30% of FC load. The percentage of FC load with the first 30% of the runoff volume (F_{30}) varied among the different sub-watersheds with C2 and W1 recording the highest percentage (66%) as shown in **Error! Reference source not found.**.

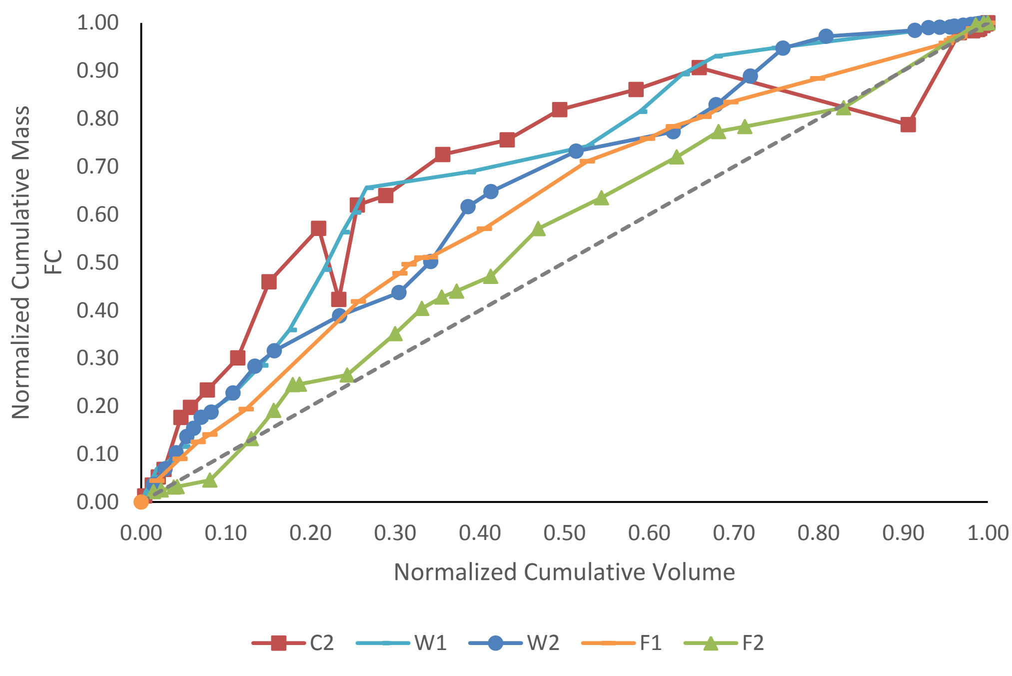

Figure 5. Normalized volume vs. normalized mass for fecal coliform in 2011

The TSS mass-volume curves plotted for the different sub-watersheds, for the year 2011, were all above the bisector line except for the one *control* sub-watershed C2 (Figure 6). The curves indicated that the early stages of the snowmelt runoff transported more than 30% of TSS load from the sub-watersheds under the winter in-field bale grazing (W1, W2) and the sub-watersheds under the manure spreading in the fall (F1, F2). However, the loading of TSS from the *control* sub-watershed C2 was relatively uniform throughout the snowmelt. In general, the percentage of TSS transported in the first 30% of snowmelt runoff (F_{30}) ranged between 48- 26% (Table 10).

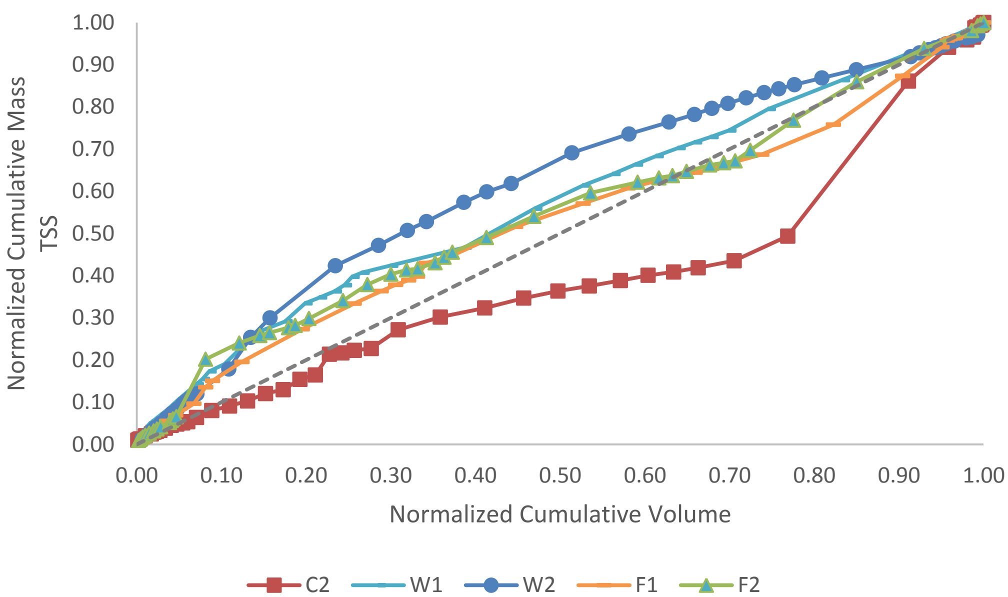

Figure 6. Normalized volume vs. normalized mass for TSS in 2011

In the year 2012, runoff started on March 12th and ended on March 16th. The fecal coliform mass-volume curves plotted for the two sub-watersheds (W2 and F2) were below the bisector line (Figure 7). However, the mass-volume curve for sub-watershed C2 was above the bisector line indicating a first-flush associated with FC loading with snowmelt runoff from the *control* field. The mass-volume curves for TSS showed a uniform loading for all treatments in the year 2012 (Figure 8). In general, the early stages of the snowmelt runoff transported between 6-38 % of FC load (**Error! Reference source not found.**) and between 25-30% of TSS (Table 10) during the snowmelt runoff event.

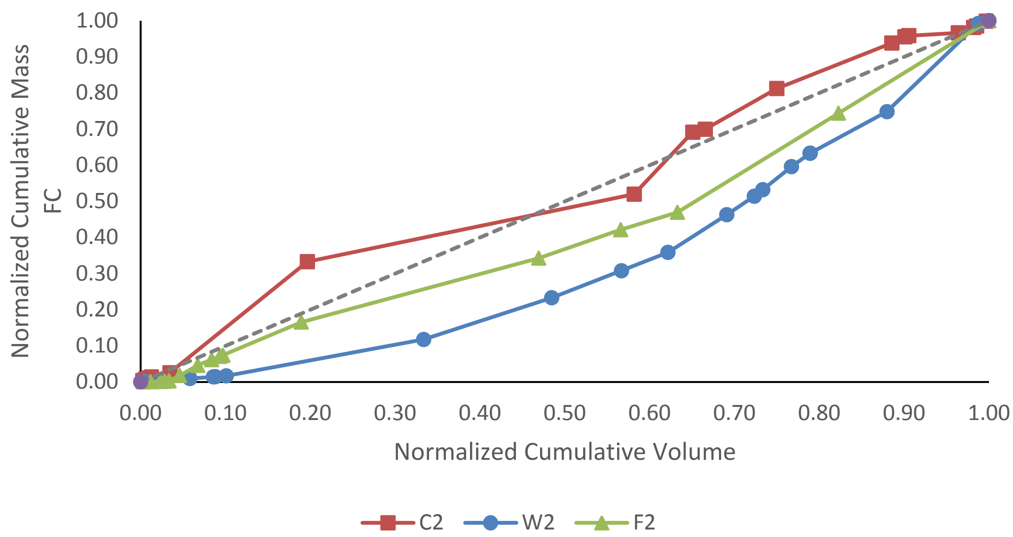

Figure 7. Normalized volume vs. normalized mass for fecal coliform in 2012

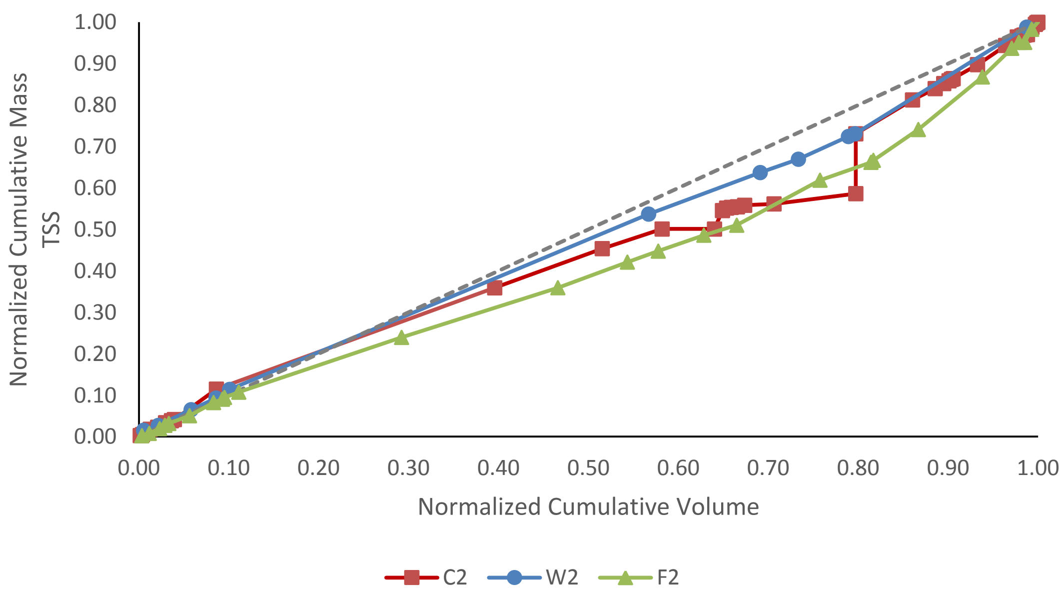

Figure 8. Normalized volume vs. normalized mass for TSS in 2012

In the year 2013, runoff started on April 26th and ended on May 3rd. The fecal coliform mass-volume curves were well above the bisector line for all sub-watershed except for C1 (Figure 9). The autosampler for C1 started collecting flow data on April 28th. Therefore there were no data available for C1 for the first two days of the snowmelt runoff. In general, the mass-volume curves indicated that the loading of FC exhibited a first-flush for all treatments with around 41-56% of FC load transported with the first 30% of the runoff volume (Table 9. The percentage of microbial mass transported in the first 30% of runoff (F30 values)

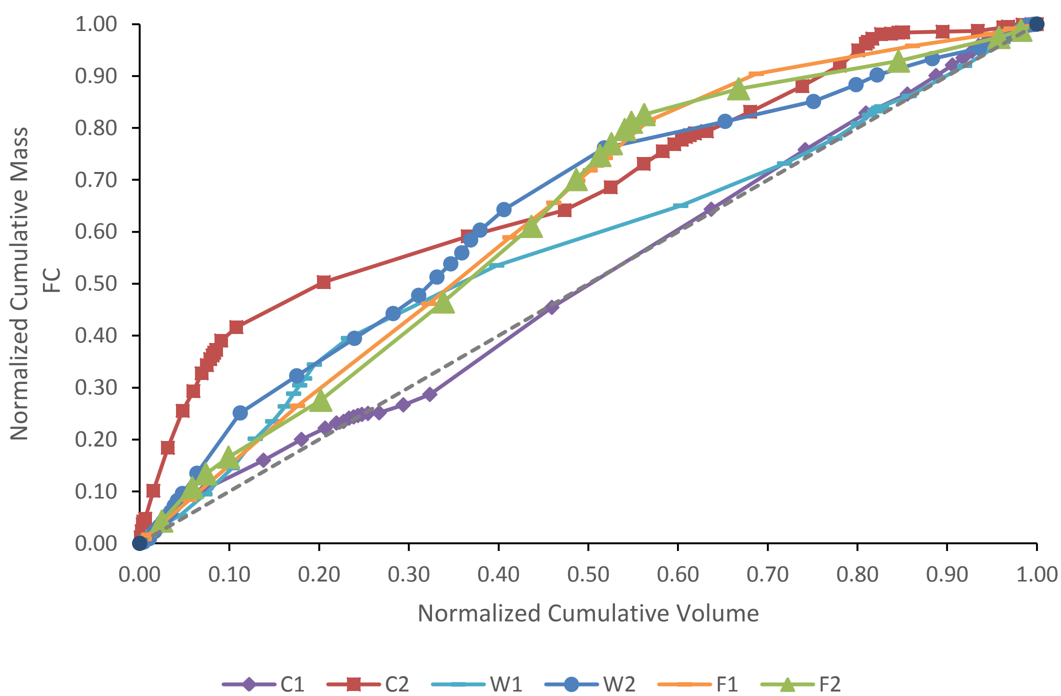

Figure 9. Normalized volume vs. normalized mass for fecal coliform in 2013

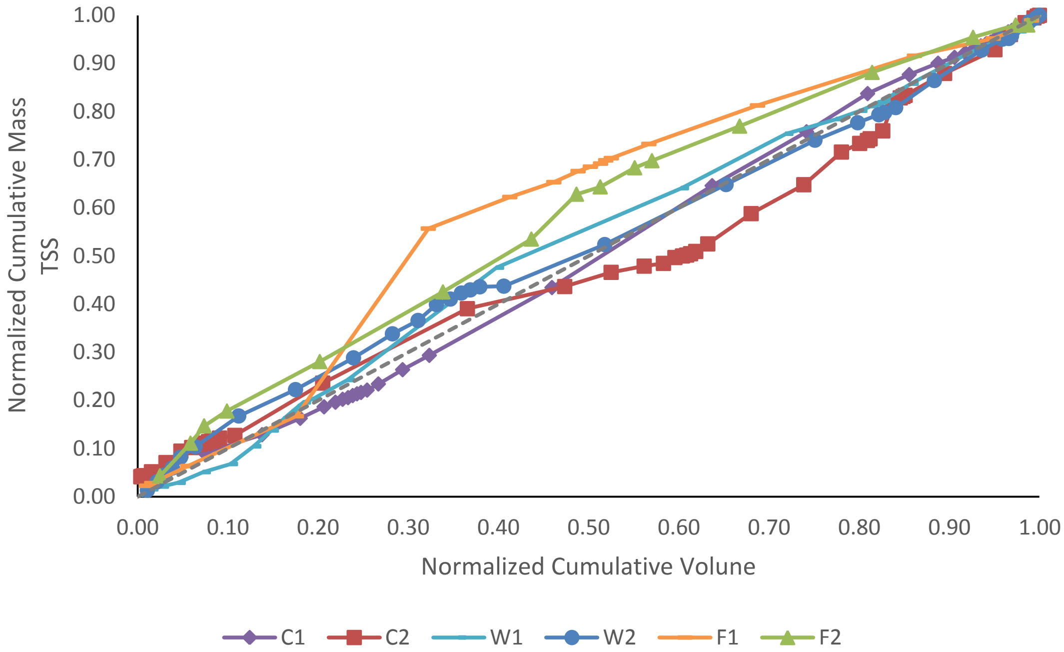

Figure 10. Normalized volume vs. normalized mass for TSS in 2013

Table 9. The percentage of microbial mass transported in the first 30% of runoff (F_{30} values)

	Control		Winter in-field bale-grazing		Manure spreading in fall	
	C1	C2	W1	W2	F1	F2
	FC	FC	FC	FC	FC	FC
2011	NA	66	66	44	47	35
2012	NA	38	NA	6	NA	24
2013	27	56	45	46	43	41

Table 10. The percentage of TSS transported in the first 30% of runoff (F_{30} values)

	Control		Winter in-field bale-grazing		Manure spreading in fall	
	C1	C2	W1	W2	F1	F2
2011	NA	26	42	48	37	40
2012	NA	28	NA	30	NA	25
2013	27	32	34	36	50	38

4.5 Discussion

The data collected at edge-of-field over the three years (2011- 2013) showed that the loading pattern of FC and TSS varied among the different treatment. The loading pattern of FC and TSS also varied among the years for the same treatment. The two pollutants; FC and TSS, do not always follow the same loading pattern. First-flush was observed in FC loading with snowmelt in the years 2011 and 2012. The first-flush was relatively strong where around 44 to 66% of the FC were transported in the first 30% of the runoff volume. However, in 2012 a delay was reported in FC loading. The years 2011 and 2013 recorded above normal precipitation and runoff started later in the spring (April) with the snowmelt interrupted by below freezing temperatures. On the contrary, the year 2012 reported below normal precipitation with early snowmelt starting before the official end of the winter season. This inter-annual variability in precipitation, snowmelt runoff regime, and ground conditions may explain this inter-annual variation in loading pattern and pollutant transport with snowmelt runoff (Su *et al.* 2011).

The bacteria in the animal waste material may exit the matrix with the flowing water that runs off over the surface of the waste or may exit the waste with the leachate leaving the waste. Blaustein *et al.* (2015) described the process by which microbes are released from animal waste to rainfall drop or irrigation. The process starts as the water from rain and irrigation hits the erodible surface of the animal waste, resulting in the suspension of the constituents of the surface. The suspended bacteria may erode and leave the waste system with the overland flow or leach downward through the waste matrix. Finally, the liquid is pressed out of the waste matrix and the transport of the microorganisms from the high concentration area to leachate starts taking place. Therefore, the release of microorganisms, including bacteria, to runoff, as well as the release of other animal waste constitutes, may be different based on their location within the waste matrix. *E. coli,* which consists of the majority of the fecal coliform indicator, is found to be more readily accessible to leachate than other fecal bacteria indicators like enterococci, which is expected to be associated with deeper dung aggregates (Guber *et al.* 2007). Several studies reported that *E. coli* have similar release patterns to that of dissolved chloride and inorganic surrogates (Guber *et al*. 2007, Blaustein *et al*. 2014, Muirhead *et al*. 2006).

All dung in the different fields in this study is expected to be old by the time the snowmelt runoff starts, but varies in age based on the management practice implemented. The dung in the fields under *winter in-field bale-grazing* is expected to be the youngest among all and it is the only dung that aged under the freezing winter conditions. The dung in the fields under *manure spreading in fall* is expected to be the oldest since it was collected the previous winter and piled until the fall. However, the dung in the *control* sites was deposited during the warm season and aged over the four seasons. The environmental conditions under which the dung ages are expected to affect the survival of bacteria in the dung differently. The Canadian Prairie is known for its cold winters, hot summers, and more than 2,400 hours of unobstructed sunshine annually (McGinn 2010). The bacteria in the old dung from the *control* fields, deposited during the grazing season in the spring and summer, is expected to enter the cold season after a long period of stress due to desiccation and UV exposure. UV exposure can be lethal to bacteria mainly in the outer part of the waste matrix. The bacteria in the surface of the dung may be inactivated due to desiccation and exposure to UV (Hodgson *et al*. 2009). Rehydration to the dry crust can take place after rainfall events, which may reactivate the bacteria in the upper layer of the dung matrix. However, the precipitation in the Prairie is mostly dominated by intense single-day summer storms with less frequent frontal rainfall events that may last for several days (Shook and Pomeroy 2012). The thick crust might limit moisture penetration deeper in the dung matrix under short rainfall events. The high wind speed of the Prairie can accelerate desiccation even after long and intense rainfall events (McGinn 2010). The protection that the dry crust can provide to bacteria from environmental factors can help support viable bacteria deep inside the dung matrix (Murihead *et al*. 2005). However, as desiccation continues, the interior matrix may not be able to support microbial activity and the bacteria may die or be inactivated. The dung deposited under freezing conditions is expected to retain its moisture content as it freezes in place. As the dung thaws, enough moisture content will be available to support the microbial activity as temperatures rise.

Earlier studies examining the release of bacteria to rainfall runoff suggests that the release pattern of microorganisms to runoff may depend on the dryness of the animal waste. In dry dung, the release of the microorganisms may be delayed to allow for the hydration of the dry dung

(Thelin and Gifford 1983). In a study where rainfall was applied over both fresh and aged cattle manure, Thurston-Enriquez *et al.* (2005) reported that approximately 75% of the applied water volumes were recovered in runoff coming from the fresh manure while only 50% was recovered from the water applied over the aged manure plots. The difference in water volume recovered might have been absorbed by the dry manure. Unlike rain events, the snowmelt can only start once the temperature in the snowpack reaches above zero temperatures and meltwater may be retained in the snowpack and released when the liquid water capacity is reached (Ferguson 1999). As the snowpack warms with rising temperatures, the frozen dung may thaw slowly, allowing time for dehydration before the actual snowmelt takes place. This slow process may allow enough time for the stressed or injured bacteria to reactivate slowly and be ready to leave the dung with the moisture leaving the manure matrix. This may explain the first-flush associated with bacteria release from the *control* fields where the old dung deposited during the conventional grazing season of spring and summer is expected to be the main source of microbial pollution in snowmelt runoff. The same pattern was observed in the *manure spreading in fall* fields. This may indicate that the bacteria in the old dung; not deposited during the cold season, may be activated and released to snowmelt runoff in a similar mechanism.

The years 2011 and 2013 recorded above normal precipitation and runoff started later in the spring (April) with the snowmelt interrupted by below freezing temperatures. On the contrary, the year 2012 reported below normal precipitation with early snowmelt starting before the official end of the winter season. In the years 2011 and 2013 the thick snow cover associated with above normal precipitation may have provided insulation to the bacteria in the dung from the repeated freeze-thaw cycles associated with the diurnal variations and temperature changes.

In 2012, however, the below normal precipitation did not provide enough snow cover and the bacteria may have been exposed to repeated freeze-thaw cycles. The bacteria leaving the warmth of the intestine to freezing temperatures may experience a cold shock that can affect its ability to survive. Freeze-thaw cycles can also have adverse effects on the survival of bacteria. The bacteria exposed to repetitive freeze-thaw cycle may become stressed and might not survive, however, bacteria that remains viable after repetitive freeze-thaw cycles may become more tolerant to subsequent freeze-thaw cycles which could enhance survival. This may explain the delayed pattern of FC loading reported in 2012 to allow enough time for the heavily injured bacteria to recover. The bacteria in the crust is expected to be the most vulnerable to the freeze-thaw cycles, whereas bacteria deeper inside the manure matrix may benefit from the protection provided by the crust against changes in temperatures. The bacteria in the older dung deposited during spring and summer may be more tolerant to freeze-thaw cycles as it is expected to have to enter the freezing season with a thick crust that provided protection to the bacteria deeper in the matrix. This may explain the first-flush of FC loading in snowmelt runoff draining the *control* fields and the delayed loading pattern of FC from the *winter in-field bale-grazing*.

The loading pattern for the total suspended particles in runoff was relatively more uniform than the loading pattern of FC, despite variations among the years. A first-flush in the years 2011 and 2013 for sites where animal waste was added to the field beyond the conventional grazing season. Fields under *manure spreading in fall* and *winter in-field bale-grazing* loaded more than 30% of total suspended particle load in the first 30% of the snowmelt runoff volume, reaching a maximum loading of 50% load in the first 30% volume in some cases. In the year 2012, a more constant loading pattern was observed where 25-30% of the load was transported with the first 30% volume of runoff. The *control* sites showed a constant load for all years where around 26-32% of the load transported with the first 30% volume of runoff. Particles from the extra animal waste were transported with runoff under snowmelt conditions over frozen or partially frozen soil in 2011 and 2013, resulting in a first-flush effect for TSS, whereas in 2012 the quick thaw of soil and the quick snowmelt resulted in delayed and more constant load. Tanasienko *et al*. (2011) examined suspended sediment in snowmelt runoff in three areas of southern West Siberia over multiple years with varying snow levels ranging from very low to very high. The study reported that fine particles <0.01 were mostly removed in the initial and final stages of runoff with higher loss observed in high snow years. The suspended particles from the fields where extra manure was added beyond the grazing season are expected to be mostly of an organic nature originating from the extra dung, and is expected to be of finer size. High volume snowmelt where infiltration capacity is limited by the freezing soil or freezing layer may favour the transport of manure particles with runoff in 2011 and 2013. In the year 2012 infiltration into unfrozen soil and the low runoff volume may have limited the transport of manure particles.

4.6 Conclusion and Recommendations

The results of this study indicate that the loading of pollutants from fields amended with animal waste beyond the grazing season, especially in animal wintering fields, can be dependent on precipitation levels and field conditions during snowmelt runoff. Animal wintering fields may transport most of its pollutant load of both microbial and suspended solids at the early stages of the snowmelt during high precipitation years. Loading of pollutants from fields where grazing takes place during the grazing season only are less dependent on the precipitation level and field conditions and do also exhibit first-flush in pollutant loading, especially for microbial loading. However, the loading pattern of fecal coliform was not similar to that of total suspended particles at all times. The loading of the living microorganisms is affected by the weather conditions, which affect the reactivation of bacteria to be released to runoff from the manure matrix. Management practices for first-flush is recommended to retain the runoff in the early stages of snowmelt (e.g. wetlands), and to reduce the concentration of pollutants reaching surface water during snowmelt runoff, which may reduce the risk to human health during the warm season when recreational activities and irrigation take place.

5 SURVIVAL OF FECAL COLIFORM IN COW DUNG IN THE CANADIAN PRAIRIES

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5.1 Abstract

Bacterial pollution of water, associated with livestock activities, depends on the ability of bacteria to survive and grow in feces after excretion. This study examines the survival of fecal coliform (FC) in artificial cowpats during winter under the cold climatic conditions of the Canadian Prairie, and compares it to the survival of FC during the spring and summer grazing season. The study also examines whether the conditions inside the cowpat can provide a shield for the microorganisms from fluctuations in temperatures during Prairie winters and examines changes in moisture content as the cowpats age. During winter, die-off started directly after deposition, while during spring, growth after deposition was reported. FC was no longer detected after 15 days during winter experiment. During spring, FC count reached a peak on day 3 and remained above initial levels for more than two weeks after deposition. The die-off coefficients for the first two weeks of the experiments were 0.35 log day^{-1} (R^2=0.7) for the winter experiment and (0.05 log day^{-1} (R^2=0.4) for the spring and summer experiment. Moisture content inside the cowpats froze in place during winter and remained around initial levels throughout winter. The snow-covered cowpats may limit the effect of diurnal fluctuations during winter, but the high

moisture content may not promote the survival of bacteria when combined with freezing temperatures. During spring/summer, high wind and low precipitation are expected to accelerate the desiccation of the cowpats where moisture content level can drop to less than 50% of the initials levels.

5.2 Introduction

Bacterial pollution of water is contingent on the ability of bacteria to survive and grow in dung after excretion. The concentration of indicator bacteria in runoff is affected by the number of viable bacteria in the dung (Muirhead *et al.* 2005). The age of the fecal deposit is the main factor related to bacteria die-off. Earlier studies revealed that indicator bacteria concentrations in runoff decreased with manure age, presumably due to mortality (Springer *et al.* 1983, and Thelin and Gifford 1983, Kress and Gifford 1984). The most common model used to simulate the die-off of bacteria in the environment is the exponential model (Pachepsky *et al.* 2006). This model is used to simulate the die-off of bacteria in such different environments as; stored manure, soil, land applied manure, groundwater, and surface water. The model assumes that the die-off starts directly after deposition and simulates die-off using the exponential equation:

$$C_t = C_0 \exp(-kt) \quad \textbf{(4)}$$

Where C_t is the number of bacteria at time t, C_0 is the number of bacteria at time 0, k is the first order die-off rate constant (day^{-1}), and t is the elapsed time (day).

Bacterial die-off does not follow the typical first order decay model under all conditions (Wang *et al*. 2004, Muirhead *et al*. 2006, Adhikari *et al.* 2007, Soupir 2008). Several studies reported growth after deposition (Kudva *et al*. 1998, Muirhead *et al.* 2005, Van Kessel *et al*. 2007). Living microorganisms are capable of adapting several survival strategies under different conditions. The decay equation, which is mostly used for chemical reactions, may therefore not represent bacteria die-off under all conditions. The fresh cow deposit resembles a batch experiment where all nutrients are available once and are not resupplied (Vaccari *et al*. 2006). Bacteria go through exponential growth within the intestine of the host. Once deposited, the growth may continue outside the host while environmental conditions that promote growth last. Where environmental conditions support bacteria growth outside the host, the growth phase may last for a few days, after which, the bacteria may enter a stationary phase where growth may stall, but the bacteria may remain viable.

Studies examining the survival of bacteria in feces were performed using naturally occurring cowpats (Buckhouse and Gifford 1976), manure samples (Wang *et al*. 2004), or artificial cowpats made from fresh deposits (Meays *et al*. 2005, Muirhead *et al*. 2005, Van Kessel *et al*. 2007, Sinton *et al.* 2007, Soupir 2008, Muirhead and Littlejohn 2009). The artificial cowpats were prepared from a mixture of feces of several animals to create a relatively uniform fecal deposit and provide valid replicate units (Meays *et al*. 2005).

Several studies examined the survival of bacteria in feces under field conditions (Buckhouse and Gifford 1976, Meays *et al*. 2005, Muirhead *et al*. 2005, Van Kessel *et al*. 2007, Sinton *et al*. 2007, Soupir 2008, Muirhead and Littlejohn 2009). Buckhouse and Gifford (1976) examined the survival of FC and TC in a fresh cowpat over 18 weeks extending from June to October on a semiarid watershed in southern Utah, USA. The study reported a decline in the FC numbers starting on the 9th week of the study, and on the 11th week for TC. Meays *et al*. (2005) examined the survival of *E. coli* in cowpats under four levels of solar exposure (0%, 40%, 80% and 100%) over 45 days during the months of July and August in the south-central interior of British Columbia, Canada. The study reported that although shading enhanced the survival, *E.coli* remained capable of replicating in the environment even under no shading. The effect of shading was only significant starting day 17 of the study. Muirhead *et al.* (2005) examined the changes in *E. coli* count in artificial cowpats over 30 days as part of a study focused on the erosion and transport of *E. coli* from cowpats under rainfall simulation in New Zealand. The study reported an increase in *E. coli* numbers in the cowpats in the first two weeks. Van Kessel *et al*. (2007) compared the survival of *E. coli* and FC under controlled temperatures in the lab (21.1, 26.7 and 32.2 °C) and under field conditions in both shaded and unshaded cowpats where the average temperature was 25.8°C. The study reported an increase in the concentrations of both FC and *E.coli* in the first week under the field and laboratory conditions before starting to decline. The study reported that shading slowed the die-off of E.coli and FC significantly. Sinton *et al.* (2007) examined the survival of *E. coli*, FS and *enterococci* over 150 days under different seasons of the southern hemisphere climate of New Zealand where temperatures ranged from 0.3°C to 46.9°C during the study period. The study reported an initial increase in the count of *E.coli* in the first few weeks under the different seasons except winter. Enterococci reported an initial increase in all seasons. Soupir (2008) examined the die-off of *E. coli* and enterococci in cowpats under the four seasons of the maritime climate of Virginia, USA where temperatures ranged from 6.22°C to 32.8°C during the study period. The study reported initial regrowth immediately or after few days during spring, summer, and fall for *E.coli*. However, during winter an initial decrease was reported followed by a mixed pattern of regrowth and decrease. Muirhead and Littlejohn (2009) examined the survival of *E. coli* in intact and disturbed cowpats under the four seasons of the southern hemisphere climate of New Zealand. The study reported an initial growth during summer, a decline during winter and a mixed pattern of increase and decline during spring and autumn.

In general, the examined studies indicated that growth after deposition is expected under warm temperatures. Growth could also take place under moderate temperatures. However, a decline is mostly expected under cold temperatures. Few studies examined the survival of bacteria at freezing temperatures (Adhikari *et al*. 2007, Kudva *et al.* 1998, Bach *et al.* 2005). However, these studies were performed under controlled conditions in the lab where die-off may be different from field conditions (Van Kessel *et al*. 2007). All of the available studies investigated the aging of cowpats and the survival of indicator bacteria under warm to moderate climates. The conditions under which these studies were performed is not directly comparable to the Canadian Prairie conditions.

There is a lack of studies on the aging of cowpats and the survival of bacteria in dung under the cold climate conditions of the northern hemisphere, especially under freezing winter conditions. The climate of the Canadian Prairie is known for long cold winters, short hot summers, and low precipitation (Ecological Stratification Working Group 1995). The Prairie is also known for long sunshine hours compared to other regions in Canada. During the spring and summer seasons, high wind speed can accelerate desiccation while UV exposure can be lethal to the microorganisms. During the cold and long winter of the Canadian Prairie, diurnal fluctuations in temperature are expected. The microorganisms are expected to be exposed to cold shock, freezing, and freeze-thaw cycles. All of these conditions will result in injury to the microorganisms and may even be lethal due to membrane or cell damage (Walker *et al*. 2006). In the Prairie, spring and summer are the main grazing seasons. During winter, cattle are mostly feed in confined corrals, and the collected dung is stockpiled and spread on field the next fall. Feeding cattle in field during winter is becoming a common practice on the Prairies due to the economic advantages it provides to producers over feeding cattle in confined corrals.

This study examines the survival of fecal coliform in artificial cowpats under the cold climate conditions of the Canadian Prairie during winter and the spring and summer grazing seasons. The study examines if the bacteria grow inside the cowpat after deposition during the spring and summer grazing season. The study also examines the changes in moisture content as the cowpat ages and examines whether there is a relation between FC concentrations in the cowpat and moisture content. The study also investigates if the bacteria inside the cowpat can survive the freezing temperatures during winter and the diurnal temperature fluctuations. The study also examines whether the conditions inside the cowpat can provide a shield to the microorganisms from diurnal temperature fluctuations during Prairie winters. The main hypotheses tested in this study are: (1): FC is expected to survive the cold winter, (2): The die-off of FC during the spring/summer seasons is different than die-off during the winter season. Initial growth is expected during the spring/summer seasons, while an initial decline is expected during winter, (3): The conditions inside the cowpats is expected to provide a shield for the bacteria from the expected diurnal fluctuations in temperature under the Canadian Prairie winter conditions.

5.3 Materials and Methods

Preparation of the cowpats

Dung material was collected from freshly excreted fecal pats from a beef cattle farm near Regina, Saskatchewan. In the winter, the dung was collected on January 5th, early in the morning after feeding. The cows fed on mixed grass alfalfa hay. In the spring, the dung was collected on May 16th from the same farm, later in the afternoon. The cows gave birth and were fed alfalfa which made the dung more watery compared to the dung collected during winter. The dung was collected in black garbage bags and kept cool during transport to the laboratory. The dung was used within 2 hours after recovery from the cows. The formation of the cowpats followed a similar procedure as was used in earlier studies (Thelin and Gifford, 1983, Wang *et al*. 1996, Soupir 2008, Sinton *et al.* 2007, Murrihead and Littlejohn 2009) with some modifications to the mixing method and the size of the pats.

The dung was mixed for fifteen minutes in a stand mixer (Cuisinart 7 Quart Brushed Chrome Stand Mixer, SM-70BCC). The homogenized mixture was then molded into cowpats using mini cake pans (4"X1.25" or 10.2×3.18 cm). Earlier studies prepared larger cowpats of 30 cm diameter, and 2 cm depth, which represents the average size of naturally occurring cowpats

(Sinton *et al*. 2007, Thelin and Gifford 1983). In this study, smaller molds were used to reduce the amount of fecal matter collected, while keeping the depth of the artificial cowpats at 2 cm.

Approximately 50 cowpats were prepared for each experiment and placed on the roof of the Classroom Building on the University of Regina campus. The cowpats were placed on top of a plastic tarp placed over wooden slats. In the winter, the cowpats were placed on top of a snow layer placed on top of the plastic tarp. During spring, the cowpats were placed directly on the wooden slats as it was not possible to keep the plastic tarp in place due to high wind. The cowpats were placed randomly on the available space. Five temperature probes with data loggers (HOBO U 12 Stainless Temp Data Logger, Onset Computer Corporation) were pushed to the middle interior of five cowpats (randomly selected) to record the temperature inside the cowpats (Figure 11).

Figure 11. Data Loggers inserted in the artificial cowpats

Samples analysis

Each cowpat was given a number, and five cowpats were selected for analysis each sampling event using a draw. The cowpats with the data loggers were analyzed at the end of the experiment. The cowpats were sampled more frequently during the first two weeks to monitor short-term survival. The cowpats were then sampled over more than 100 days to monitor long-term survival. The sampling schedule for the artificial cowpats during spring/summer and winter experiments is shown in Table 11.

Table 11. Sampling schedule during the spring/summer and winter experiments

Winter experiment		**Spring/summer experiment**	
Date	**Age of cowpat (days)**	**Date**	**Age of cowpat (days)**
January 5th	0	May 16th	0
January 6th	1	May 17	1
January 8th	3	May 19th	3
January 10th	5	May 21st	5
January 13th	8	May 24th	8
January 20th	15	May 31st	15
March 10th	64	June 15th	30
April 26th	111	September 8th	116

Each cowpat was analyzed for fecal coliform and moisture content. The dung was collected from a mixture of the outer crust and the interior of the cowpat to obtain a representative sample of the cowpat. The cowpats were not re-sampled, and any leftovers from the cowpats were disposed of. Sampling continued until fecal coliform was no longer detected. During the winter experiment, the frozen cowpats were allowed to thaw at room temperature for 3-4 hours before analysis. Any ice sticking on the frozen cowpat was carefully removed before thawing the cowpat to reduce the effect on moisture content.

For the enumeration of fecal coliform, eleven grams of the fecal matter was suspended in 99 ml of phosphate buffer solution. The bottle containing the fecal matter suspension was then shaken by hand for 10 minutes to enhance the physical dispersion of the bacteria (Soupir 2008). The fecal matter suspension was then allowed to settle for 5 minutes. Using a pipette, eleven ml of the supernatant fluid was serially diluted in 99 ml of phosphate buffer up to 10 dilutions. Fecal coliform was enumerated by membrane filtration on MFC ager (2 to 3 plates per dilution) and then incubated for 24 ± 2 h at 44.5 ± 0.2°C. The preparation of different culture media, phosphate buffer dilution water and enumeration procedures were performed according to the Standard Methods for Examination of Water and Wastewater Method 9222 D. Fecal Coliform Membrane Filter Procedure (APHA, 1998). Fecal coliform counts were reported as CFU/g.

Moisture content was determined gravimetrically and expressed on a wet basis. Three samples of approximately 10 grams were used in the analysis. The weight of each sample and the pan was recorded before and after drying. The samples were dried at 60°C for 24 hours. The samples were then cooled and weighed. The weight of water was calculated as the difference between the weight of the pan and sample before and after drying. The moisture content was then calculated using the following equation:

$$\boldsymbol{Moisture\ content} = \frac{\textbf{weight of water}}{\textbf{wet weight of sample}} \times \mathbf{100\%} \qquad \textbf{(5)}$$

Weather data

Data from the loggers was only available for the winter experiment as the loggers dropped out from the cowpats during the spring/summer experiment. Weather data was collected by the University of Regina (UR) meteorological station which is located directly above the experiment area (Figure 12). During the spring/summer experiment, weather data was compiled for daily cumulative rain, air temperature (min, max and average); and wind speed (max and average). During the winter weather data was compiled for air temperature only (min, max and average) to compare with the data from the loggers.

Figure 12. Location of UR Weather Station in relation to the experiment site

Statistical analysis

All statistical analyses were performed using R 3.3.2. The default statistical significance was set at 0.05 level unless otherwise noted. Normality of the data was evaluated using Shapiro-Wilks w test.

Indicator bacteria data

Fecal coliform count (CFU/g) was calculated based on dry weight then normalized by log-transformation. Die-off coefficients for the winter and spring/summer experiments were determined by linear regression of log-transformed FC data. Natural log transformation was also performed on the data to evaluate the fit of the indicator bacteria die-off to the first-order die-off model using the equation:

$$\ln C_t = \ln C_0 - kt \qquad \textbf{(6)}$$

Where C_t is indicator bacteria count at time t; C_0, initial indicator bacteria count; and k is the first-order die-off rate coefficient.

5.4 Results

Fecal coliform data

Initial mean concentrations of fecal coliform were 6.2×10^6 CFU/g dry weight for winter experiment and 3.1×10^5 CFU/g dry weight for spring/summer experiment. Initial moisture content was at 84% for the winter experiment and 85% for the spring/summer experiment.

During winter, a sharp decline in FC count was noticed starting day 1 and continued until FC was no longer detected at day 15 (Figure 13). Die-off coefficient for the first two weeks of the winter experiment is 0.35 log day^{-1} (R^2=0.7). FC was not detected in further analysis on day 64 and day 111.

During spring/summer, growth after deposition was observed starting on day 1 and continued to increase reaching a peak of 2.5 $\times 10^7$ CFU/g dry weight on day 3 before starting to decline (Figure 13). The FC count continued to be higher than the initial count even after 15 days and only dropped below the initial count to 2.3 $\times 10^5$ CFU/g dry weight on day 30. Die-off coefficient was estimated at 0.05 log day^{-1} (R^2=0.4) for the first two weeks.

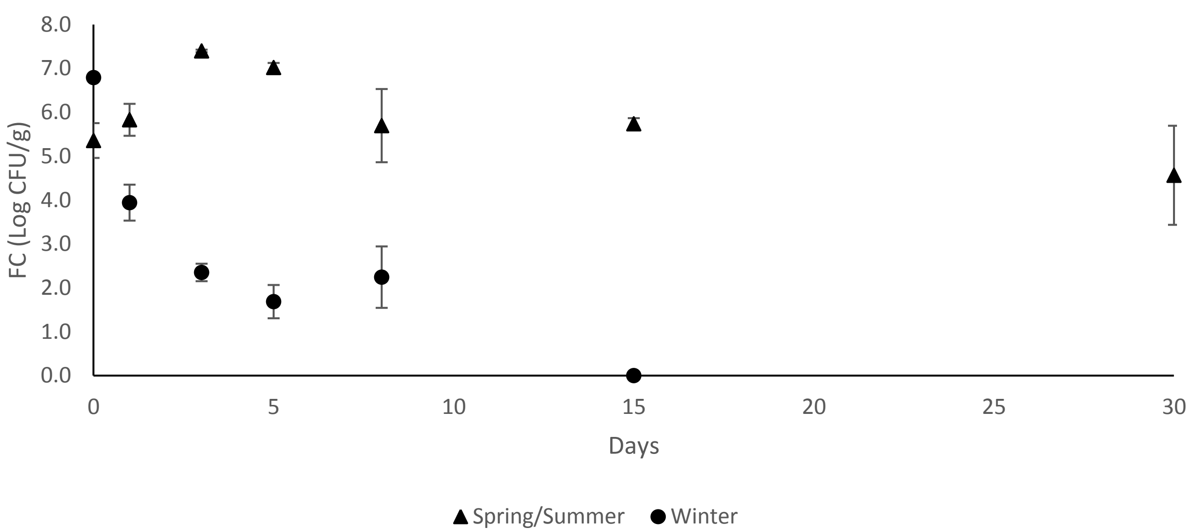

Figure 13. Changes in FC over days during spring/summer and winter experiments. Error bars are standard deviation

The data was further tested to examine the fit to the linear model. The results indicated that the die-off of FC during the spring/ summer experiment may not be approximated by the first-order die-off model where growth after deposition was recorded (r^2 = 0.2, Figure 14). In the winter experiment, an initial decline was observed, and the linear model may be used with caution to approximate FC die-off during winter (r^2=0.7, Figure 15).

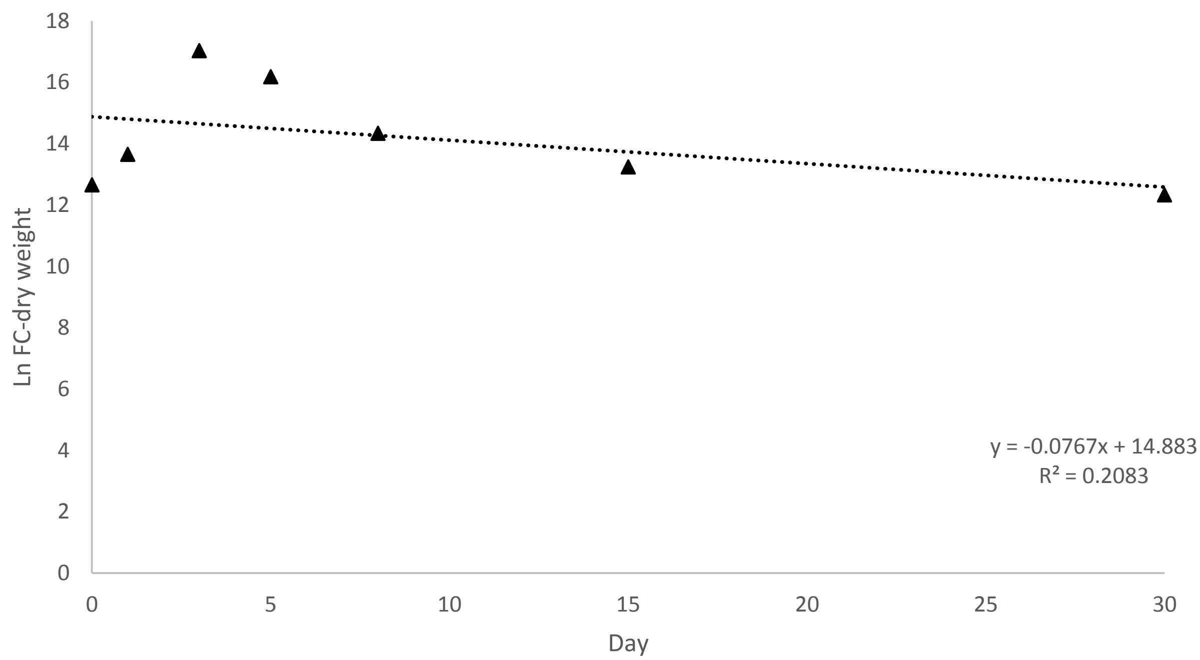

Figure 14. Evaluation of the data fit to the linear die-off model for spring/summer experiment

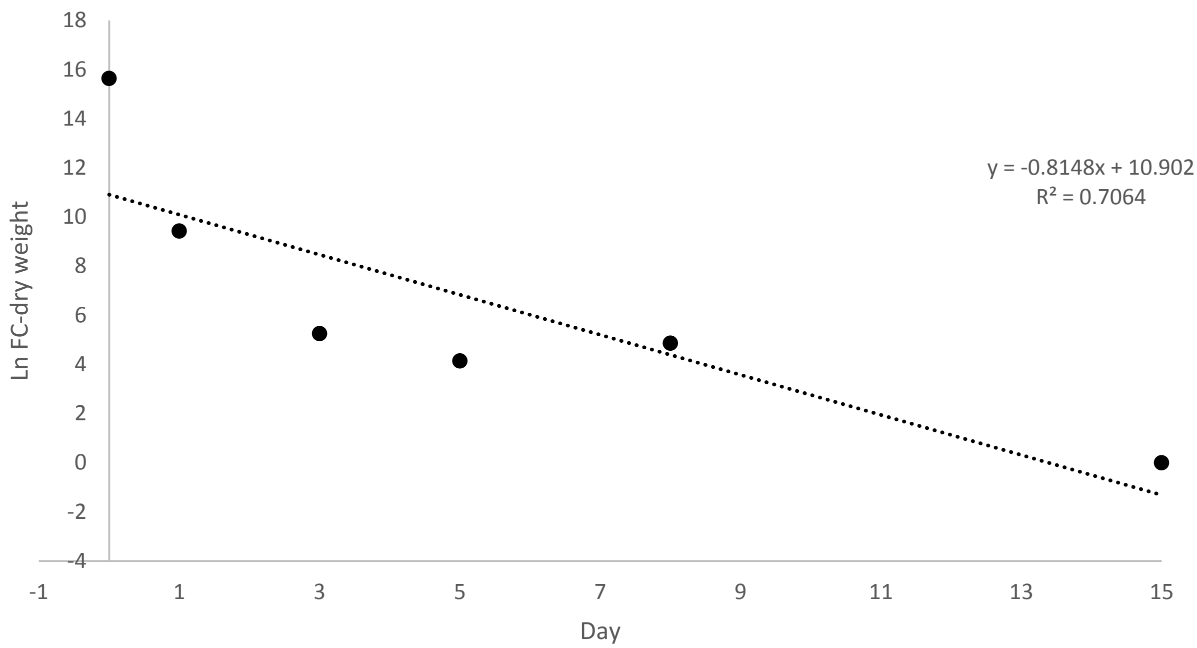

Figure 15. Evaluation of the data fit to the linear die-off model for winter experiment

Moisture content

During the winter experiment moisture content (MC) remained around initial levels throughout the experiment, with a slight drop to 79% on day 111 (Figure 16). During the sampling, the ice that attached to the cowpat was removed to minimize the effect on the moisture content in the cowpat. The cowpats remained moist throughout the winter experiment. During the spring/summer experiment, a drop in moisture in the cowpat was noticeable starting day 5. The upper crust dried faster and the inner core remained relatively moist. Moisture content declined over time to 48% on day 15 despite the cumulative rain of 13 mm during the 7 days preceding the sampling and 3.6 mm of rain on the day preceding the sampling. Moisture content then increased again to 71% on day 30 following several rain events. The relation between FC count and MC was examined using Pearson product-moment correlation coefficient (r). There was a weak positive correlation between moisture content and log FC count dry weight basis for both the spring/summer ($r = 0.4$) and winter experiment ($r = 0.3$).

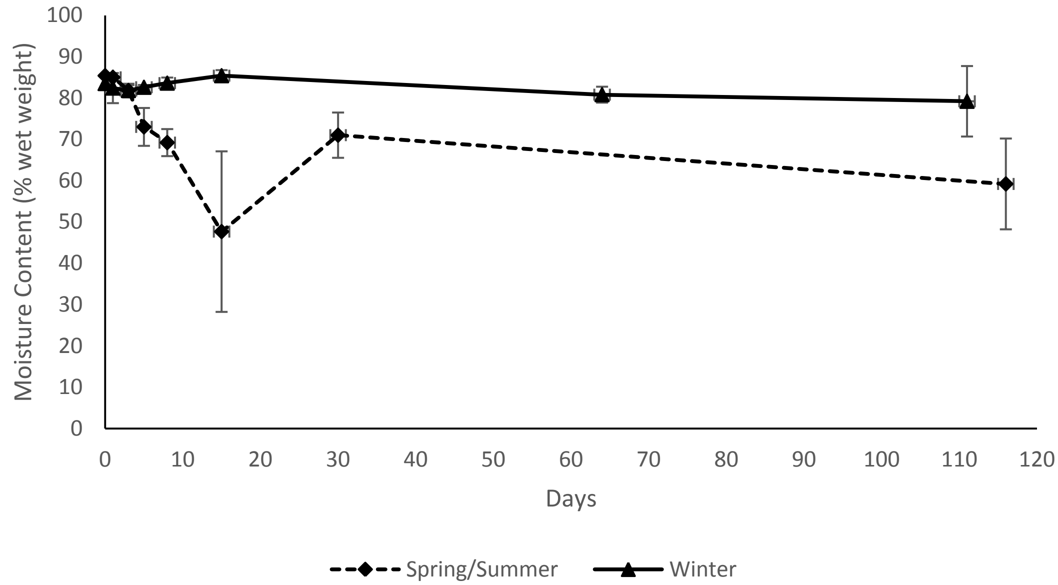

Figure 16. Change in moisture content during experiment. Error bar represent standard deviation with n=15

Temperature data

During the spring/summer experiment, average daily temperatures ranged from 14.7°C to 16.6°C with the lowest daily temperature recorded at 5.6°C and the highest temperature recorded at 25.5°C. During the winter experiment, the average daily temperature recorded in the first two weeks was -12°C. The lowest temperature recorded in the first two weeks was -27.7°C on January 20th (Day 15) and the maximum temperature recorded was -0.3 °C on January 7th (day 3). The lowest temperature recorded during the 111 days of the winter experiment was -31°C on January 31 (Day 26) and the maximum temperature recorded was 10.9 °C on April 26th (Day 111). The normality of the temperatures data collected from all loggers and the UR station was tested using Shapiro-Wilks test. In general, the results of the tests showed that the data do not follow a normal distribution.

Temperatures data collected from the five loggers, inserted inside the cowpats, and the UR station showed that all loggers reported higher temperatures compared to the UR station (

Table 12). In general minimum temperatures recorded by the loggers were 1.6 to 7.5°C higher than minimum ambient temperatures recorded by the UR station (Figure 17). Maximum temperatures recorded by the loggers were 0.5 to 1.9°C higher than maximum ambient temperatures (Figure 18), while average temperatures recorded by the loggers were 1.7 to 4.4°C higher than average ambient temperatures (Figure 19).

Table 12. The average difference between the loggers and the UR station

	Logger 1- UR Station	Logger 2- UR Station	Logger 3- UR Station	Logger 4- UR Station	Logger 5- UR Station
Minimum	7.2	7.5	1.6	4.3	3.8
Maximum	1.2	1.1	1.9	0.5	1.2
Average	4.4	4.4	1.7	2.6	2.6

The nonparametric Kruskal-Wallis rank test was used to examine whether there is a significant difference between the temperatures recorded by the loggers and the UR. The Kruskal-Wallis rank was used to compare three sets of data from all sources, minimum temperatures data, maximum temperatures data, and average temperatures data. The results of Kruskal-Wallis rank test for maximum temperatures showed that there is no significant difference between the maximum temperatures data collected from the different sources ($p=0.78>0.05$). However, the results of the Kruskal-Wallis rank test for minimum temperatures data and average temperatures data showed that there is a significant difference in the data with p-value approximates zero for both data sets ($p=0 < 0.05$). The null hypothesis was rejected, and the minimum temperatures data and the average temperatures data were further examined using the nonparametric test Mann-Whitney-Wilcoxon Test. The data from each one of the loggers was compared separately with the UR station data. The purpose was to identify if the data from the loggers was significantly different from the data collected by the UR station. The results showed that the minimum and average temperatures data collected by the five loggers, inserted inside the cowpats, were significantly different from the minimum and average temperatures data collected by the UR station.

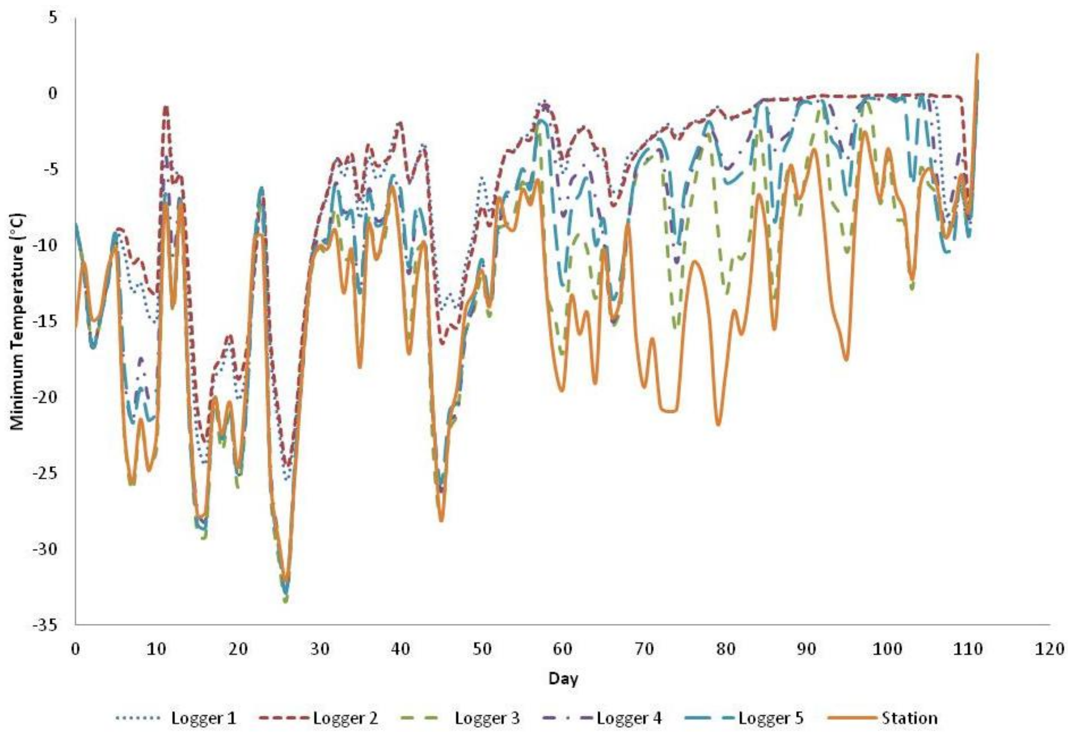

Figure 17. Plot of minimum temperatures during for loggers and UR weather station

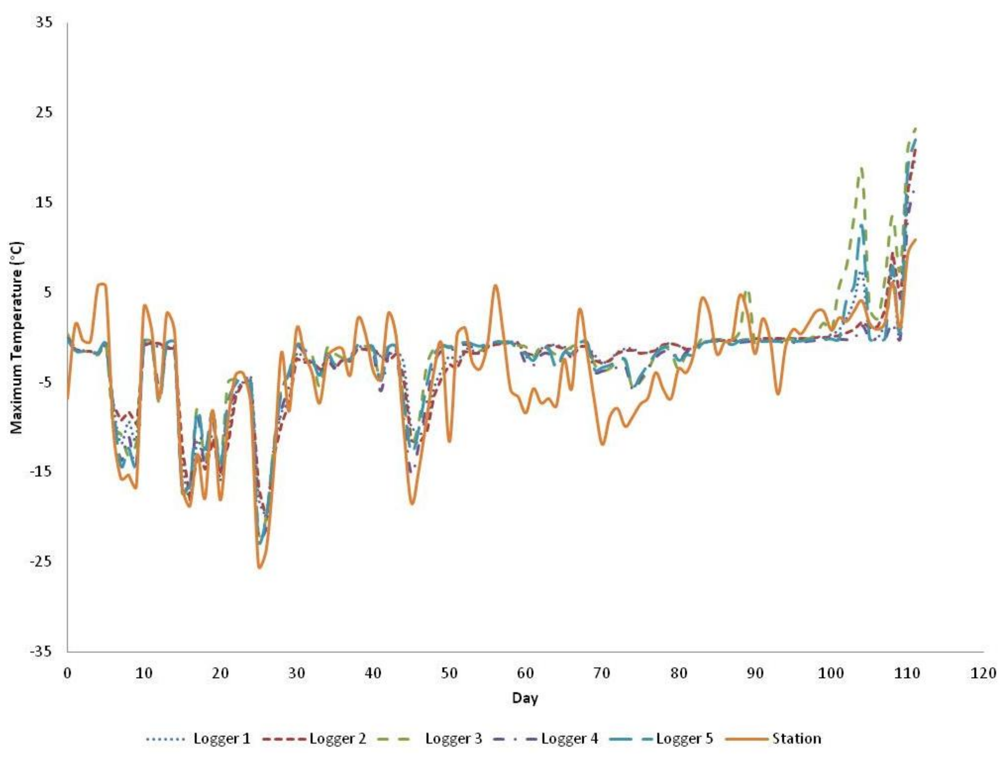

Figure 18. Plot of maximum temperatures for loggers and UR weather station

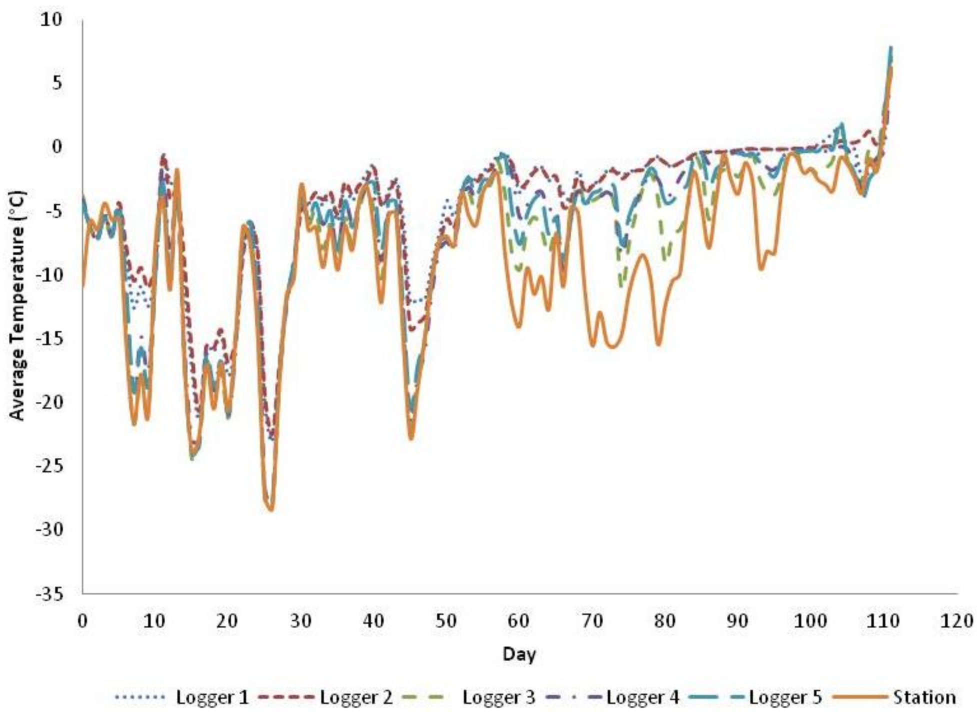

Figure 19. Plot of average temperatures for loggers and UR weather station

5.5 Discussion

The results of the spring and summer experiment reported growth after deposition that continued for three days followed by a decline. During the first week of the spring and summer experiment, the maximum daily temperature was above 20°C. According to Van Kessel *et al.* (2007) temperatures between 20°C to 35°C would be the best for post-deposit growth. Earlier studies under field conditions and in the lab reported initial growth after deposition under warm temperatures (Wang *et al.* 2004, Bach *et al*. 2005, Van Kessel *et al*. 2007, Sinton *et al*. 2007, Muirhead *et al.* 2005). In a lab experiment, Wang *et al*. (2004) reported initial growth of two orders of magnitude of *E. coli* and FC at 27°C. Under field conditions, Sinton *et al.* (2007), reported an initial increase of up to 1.5 order of magnitude for the three indicators of *E. coli*, FS, and enterococci, under the warm to hot seasons (summer, spring, and autumn). Bach *et al.* (2005)

reported a one-order of magnitude growth of *E. coli* O157:H7 at 22°C in the first 2 weeks of incubation. Kudva *et al.* (1998) reported an initial increase of 1 log in the first 24 hours of *E. coli* O157:H7 incubated at 23°C.

During the winter experiment, a sharp decline in FC count was observed starting day one and continued until FC was no longer detected at day15. Average temperatures during the first two weeks of the winter experiment were well below zero with few maximum temperatures reported around the freezing temperature. The average temperature recorded during the first two weeks of the experiment was -12°C, with temperature reaching as low as -27.7°C on day 15 of the experiment. Under winter conditions, initial die-off is expected and has been reported by several studies. Kudva *et al*. (1998) reported an initial decrease of 2 logs in the first 24 hours after inculcation under cold temperatures (-20°C, and 4°C) for *E. coli* O157:H7 in inoculated manure samples.

Changes in moisture content were observed as the cowpats aged. The data showed that moisture content almost did not change during the winter experiment. Moisture content was 83.5% as deposited and dropped to 79.2% on the last sampling day as the cowpat reached 111 days old. This indicates that the moisture inside the cowpats froze in place during winter. During spring/summer experiment moisture content dropped over time, reaching 47.7% on day 15 of the experiment. The drop in moisture content under warm temperatures has been reported in literature. Wang *et al*. (2004) reported changes in moisture content under different controlled temperatures with no added moisture and reported that the dung reached 55% moisture content level after 9 days at 4°C and 27°C.

In the current experiment, the reduction in moisture content was not linear; increase in moisture content was recorded after rain events. On day 30 of the experiment, moisture content increased to 71% after a few rain events. However, this was not the case at all times where moisture content dropped to 47.7% on day 15 despite being preceded by a few rainy days. Precipitation in the Prairie is mostly characterized by intense single-day summer storms (Shook and Pomeroy 2012). Rainfall events can rehydrate the dry crust. However, the low precipitation in the Prairie may not allow enough time for the crust to hydrate under the short length rainfall events. The thick crust may limit moisture penetration deeper in the cowpat matrix under short rainfall events. Frontal rainfall events that can last multiple days are less frequent; such events may provide enough time to hydrate the crust and penetrate into the cowpat matrix. The Prairie is also characterized by high wind speed, mainly in spring and fall, which accelerates desiccation (McGinn 2010). All of these factors may explain the continuous dryness of the dung, despite being preceded by rainfall events.

The Prairie is known for longer sunshine hours compared with other regions in Canada due to the continental air masses that bring clear skies to the Prairie. On average there are around 312 to 322 days a year that have some hours of sunshine. There are around 2,400 hours of unobstructed sunshine annually compared to other regions of Canada that have 1,200 to 2,000 hours of sunshine on an annual basis (McGinn 2010). The high UV exposure can be lethal to the bacteria, especially in the outer part of the cowpat matrix. During the spring/ summer experiment, FC was detected in the first 30 days of the experiment. However, toward the end of the experiment at day 116, FC was no longer detected as moisture content reached 59%. The bacteria in the surface of the old cowpats deposited during the spring and summer season may become inactivated due to desiccation and exposure to UV (Hodgson *et al.* 2009). The dry upper crust can provide protection for the bacteria from environmental stresses, and the inner matrix of the cowpat may contain viable bacteria for extended periods of time (Murihead *et al.* 2005). However, as the dry crust penetrates further into the cowpat matrix, moisture content that supports bacterial activities may become limited, and the bacteria may die or enter into inactive status and become undetectable using the current bacteria extraction and culturing techniques.

In freezing conditions, the combined effect of temperature and moisture content may have a different effect on the survival of indicator bacteria where survival is promoted with low moisture content. Adhikari *et al.* (2007) studied the survival of total coliform (TC) in soil contaminated with human and animal fecal pollution in Alaska. The study reported 66% survival of TC over a period of 170 days at subfreezing temperatures from -15°C to -28°C with relatively dry soil (24% moisture content). High moisture content inside the cowpat may have contributed to the accelerated die-off of bacteria under freezing conditions.

During the cold and long winter of the Canadian Prairie, diurnal fluctuations in temperature are expected, which may expose the microorganisms to cold shock, freezing, and freeze-thaw cycles. Fluctuating temperatures can affect the survival of bacteria. The exposure to freeze-thaw cycles can result in injury to the microorganisms and may even be lethal due to membrane or cell damage. Walker *et al.* (2006) examined the effect of slow freezing followed by slow warming on *E. coli* which was examined over 48 freeze-thaw cycles. The study reported that the numbers and the complexity of the community decreased with repeated freeze-thaw cycles. Colonies exposed to freeze-thaw treatment were smaller in diameter than unexposed colonies, which were 2.7 times larger. However, the bacteria that remained viable after the freeze-thaw treatment were >1,000-fold more tolerant to subsequent freeze-thaw cycles. Semenov *et al*. (2007) examined the effect of fluctuating temperatures, with three amplitudes (0, ±4, ±7 °C), on the survival of *E. coli* in inoculated steer manure. The study reported that the survival of bacteria significantly declined with increasing variation in daily temperature oscillations and was more pronounced with higher temperature oscillations.

In the current study, examination of the temperature inside the cowpats compared to air temperature during the winter experiment showed that the snow-covered cowpat provided a shield from temperature drop but did not warm the cowpat as the surrounding air warmed. Temperatures recorded inside the cowpats were higher than those recorded by the UR station. Snow cover provides an insulation effect which explains the difference in the minimum temperature reported by the loggers and the UR. However, the temperature inside the cowpats did not rise as quickly as the surrounding air temperature due to the reflection of sunshine by the snow which prevents the cowpats from warming up as the air warms.

5.6 Conclusion and Recommendations

The results of this study indicate that during the spring and summer grazing season, fresh cattle deposits may contain high concentrations of bacteria that exceed the as-deposited concentrations. As the cowpats age under the Canadian Prairie climate during the spring and summer, desiccation and UV exposure may kill or injure the bacteria which may not be detectable over the long term. Under the cold winter conditions of the Canadian Prairie, FC is not expected to be detectable in cowpats within two weeks after deposition. The snow-covered cowpats may limit the effect of diurnal fluctuations during winter, but the high moisture content within the cowpats that froze in place during winter may not promote the survival of bacteria when combined with freezing temperatures. The injured or stressed bacteria might not be detectable by culture methods but can remain viable and reactivate as environmental conditions changes. The risk of microbial pollution to surface water by the grazing animals can be high, and agricultural management practices that limit the cattle's access to water bodies or reduce runoff from grazing fields to surface water should be considered.

6 Conclusions and Recommendations

In-field bale-grazing during winter is becoming a common practice in the Canadian Prairie. Feeding cattle directly from bales distributed in the field can provide economic advantages to producers, as it eliminates the extra work associated with manure piling and storage during winter, and manure hauling and spreading on the field in the fall. Extra cost saving can also be realized by eliminating the need to transport the hay to the cattle feeding in confined corrals during winter. The economic advantages associated with in-field bale-grazing during the Prairie winter can be outweighed by the potential impact on the water quality of snowmelt runoff that provides the main source of runoff to streams. Cattle feeding in-field during winter will be depositing dung on snow-covered and/or frozen ground. Manure constituents can find their way to surface water leaving the field during snowmelt runoff. The impact on surface water can result from the transport of excess nutrients such as phosphorous and nitrogen, as well as the transport of harmful pathogens.

Studies found that FC count in runoff from grazing fields can be 5 to 10 time higher than that from ungrazed fields. Recreational water impacted by cattle feces can pose a similar risk to human health as recreational waters exposed to human sewage. Pathogenic microorganisms like *E. coli* O157:H7, Salmonella, *Giardia* spp, *Cryptosporidium* spp., *Campylobacter jejuni,* and hepatitis E virus are shed in animal feces. This risk to human health may extend beyond the runoff season as bacteria may settle in organic-rich sediment where it can survive longer than in the water column. The bacteria can then multiply and re-suspend in the water column during the warm seasons, posing risks to recreational uses and irrigation. Despite all, microbial pollution did not receive as much attention as pollution caused by the transport of nutrients.

The potential impact on the snowmelt runoff quality of *winter in-field bale-grazing* under the cold climate of Saskatchewan was evaluated as part of the Saskatchewan component of the Canada-wide Watershed Evaluation of Beneficial Management Practices (WEBs) program. This study was designed as part of the Saskatchewan WEBs project to support the evaluation of *winter in-field bale grazing* by providing information on fecal coliform (FC) loading with snowmelt runoff draining six sub-watersheds used as grazing pasture. Collection of snowmelt runoff samples followed the Saskatchewan WEBs project which was designed primarily for evaluating nutrients and sediment loading with snowmelt runoff. During snowmelt runoff, samples were collected every second hour using ISCO's continuous automated sampler with a flow rate data logger (ISCO model 6712, ISCO Inc.). Runoff samples were analyzed for FC and total particulate weight. Sub-samples of 100ml were further analyzed by molecular methods using qPCR to identify whether the bacteria detected in the samples come from bovine sources.

The study evaluated winter in-field bale grazing compared to the conventional manure management practice associated with feeding the cattle in confinement during winter, known by *manure spreading in the fall*. The main question asked by this study was whether feeding the cattle in the field during winter is expected to increase the microbial loading to surface water compared to feeding the cattle in confinement and spreading the manure on the field the next fall. The study also examined whether the extra dung added to the field past the spring and summer grazing season could increase the microbial loading during snowmelt runoff. The study did also evaluate the loading pattern of FC during snowmelt runoff compared to the loading pattern of the suspended particles (TSS) in the snowmelt runoff.

6.1 Conclusion

The data collected over the three years (2011-2013) showed that *winter in-field bale grazing* fields reported the highest flow-weighted FC count (2674 CFU/100ml) over the years. However, the mean flow-weighted FC count from the *winter in-field bale grazing* fields (497 CFU/100ml) was not significantly different (p=0.74>0.05) from the mean count from the *manure spreading in fall* fields (364 CFU/100 ml). The flow-weighted FC means count from the *winter in-field bale grazing* fields and the *manure spreading in fall* fields were significantly higher than the flow-weighted mean count from the c*ontrol* fields (110 CFU/100 ml). The results showed that microbial loading from fields receiving animal waste during spring and summer grazing season only could be well below the recreational water standards of 200 CFU/100ml. The excess animal waste added to the field past the spring and summer grazing season could increase the number of bacteria available for transport during snowmelt. The results also indicate that feeding the cattle in confinements during winter and spreading the collected manure on the field the next fall can lead to the same microbial pollution as that associated with the animal grazing in field during winter.

The number of indicator bacteria in the runoff is related to the number of bacteria inside the cowpat. The number of bacteria in the old cowpats from the *control* fields is expected to be lower than the number of bacteria in the freshly deposited cowpats. The number of bacteria in the old manure is supposed to be lower than that of the number of bacteria in the fresh cowpats.

Despite the low number of the bacteria count in the old manure, the number of bacteria in the runoff draining the *manure spreading in fall* fields was not significantly different than the number of bacteria in runoff draining the *winter in-field bale-grazing* fields. The results indicates that adding extra animal waste to the field beyong the spring and summer grazing season can increase the number of bacteria in snowmelt runoff, despite the age of the animal waste.

Precipitation levels during winter and field conditions during snowmelt runoff varied among the three years of the study. The years 2011 and 2013 reported above average precipitation levels and late snowmelt runoff. The year 2012, reported below average precipitation levels and early runoff. This variation between the years was reflected in the loading pattern of TSS and FC during snowmelt runoff. In general, the loading pattern of FC was not always similar to the loading pattern of TSS. The loading pattern of TSS from the *control* fields did not exhibit a first-flush and was mostly uniform over the years where around 26-32% of the TSS load transported in the first 30% of the snowmelt runoff. The loading of TSS from the *winter in-field bale grazing* fields and the *manure spreading in fall* fields did exhibit a first-flush in the years 2011 and 2013. The *manure spreading in fall* and the *winter in-field bale-grazing* fields loaded between 34-50% of TSS load in the first 30% of the snowmelt runoff volume in the years 2011 and 2013. However, in the year 2012, only 25-30% of the TSS load was transported with the first 30% volume of snowmelt runoff. The results showed that field receiving extra animal waste past the spring and summer grazing season could transport more than 30% of TSS load in the early stages of snowmelt runoff during high precipitation years. The results also showed that the loading pattern of TSS from fields receiving extra animal waste past the spring and summer grazing season could be dependent on precipitation levels and field conditions during snowmelt runoff.

The loading pattern of FC was different than the loading pattern of TSS. The loading pattern of FC exhibited a first-flush from the control fields over the years. Over the three years, the control fields transported around 38-66% of FC load in the first 30% of snowmelt runoff volume. However, the *winter in-field bale grazing* fields and the *manure spreading in fall* fields exhibited a first-flush in the years 2011 and 2013 with around 35-66% of FC load transported in the first 30% of snowmelt runoff volume. In the year 2012, the loading of FC from the *winter in-field bale grazing* fields and the *manure spreading in fall* fields were mostly delayed with only 6-24% of FC load transported in the first 30% of the snowmelt runoff volume. The results indicate that the loading pattern of FC is not similar to that of TSS especially from the *control* fields. However, the loading of TSS and FC from the fields that received animal waste past the spring and summer grazing season can exhibit a first-flush during high precipitation years.

The survival of FC in cattle dung was examined under the cold climate of the Canadian Prairie. Two outdoor experiments were set up during the winter (January-April) and during the spring (May-September). Around 50 cowpats were prepared from a mixture of animal feces and placed outside and the survival of FC and the changes in moisture content were observed over more than 100 days. Five temperature loggers were pushed inside five of the cowpats to monitor the temperature inside the cowpats and compare it to ambient temperature collected by the University of Regina weather station (UR station).

The results of the spring experiment showed that FC could grow outside the host under the climate of the Canadian Prairie. The maximum daily temperatures during the first week of the experiment were above 20°C which promoted growth after deposition. The growth of FC continued for the first three days then die-off started. However, FC count remained higher than the as-excreted count for more than two weeks. Die-off coefficient for the spring experiment was estimated at 0.05 log day^{-1} (R^2=0.4) for the first two weeks. The moisture content inside the cowpats dropped over time reaching 47.7% after two weeks. However, the reduction in moisture content was not linear, an increase in moisture content was recorded after rainy days. Rainfall events can rehydrate the dry crust, which may activate the bacteria. However, the low precipitation on the Prairie may not allow enough time for the crust to hydrate under the short-length rainfall events. The thick crust could limit moisture penetration deeper in the cowpat matrix during short rainfall events where the high wind speed can accelerate desiccation. FC was no longer detected on the last day of the experiment (day 116).

During the winter experiment, die-off started directly after deposition and continued until FC was no longer detected after two weeks. The die-off coefficient for the first two weeks of the winter experiment was estimated at 0.35 log day^{-1} (R^2=0.7). The moisture content almost did not change during the winter experiment. Moisture content was 83.5% as-excreted and dropped to 79.2% on the last sampling day as the cowpat reached 111 days old. The moisture inside the cowpat froze in place during the winter and the cowpats remained moist throughout the winter experiment. High moisture content inside the cowpat may have contributed to the accelerated die-off of bacteria under freezing conditions.

Temperatures recorded inside the cowpats were higher than those recorded by the UR station. However, no significant difference was identified between the maximum temperatures recorded inside the cowpats and the maximum temperatures recorded by the UR station. This may be due to the reflection of the sunshine by the snow, which prevents the cowpats from warming up as the air warms. Snow cover reflects solar radiation, which may explain some of the incidences where the air temperature was higher than the temperature obtained from the loggers. Snow cover also provides an insulation effect, which explains the difference in the minimum temperature reported by the loggers and the UR station. The results indicated that the snow-covered cowpats were shielded from temperature drops, but the cowpats did not warm as fast as the surrounding air.

The results of the molecular analysis showed that the Bovine marker was only detected in the *winter in-field bale grazing* samples. The Bovine marker was only detected in 60% of the *winter in-field bale grazing* samples over the years and was not detected in any of the samples in 2012. The AllBac marker was detected in samples collected from the different treatments at different percentages. The AllBac marker was detected in 90% of the *winter in-field bale grazing* samples, in 45% of the *manure spreading in fall* samples, and in 43% of the *control* samples. However, in the year 2012, the AllBac marker was only detected in *winter in-field bale grazing* samples and was not detected in any of the *control* or *manure spreading in fall* samples.

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the year 2012. The AllBac marker was detected in the samples collected from the different treatments at different percentages. The AllBac marker was detected in 90% of the *winter in-field bale grazing* samples, in 45% of the *manure spreading in fall* samples, and in 43% of the *control* samples. However, in the year 2012, the AllBac marker was only detected in the *winter in-field bale grazing* samples and was not detected in any of the *control* or the *manure spreading in fall* samples.

The results indicate that the currently available *Bacteroidales* markers may not be suitable to evaluate the grazing practices examined in this study. The persistence of the markers may prohibit the detection of pollution from older cowpats, which can limit the validity of the markers to evaluate the pollution from the old cowpats versus that from the freshly deposited cowpats. The Bovine marker used in this study was not able to detect bovine sources in any sample from the *manure spreading in the fall* fields and the *control* fields. The marker only detected bovine sources in the samples collected from the *winter in-field bale grazing* fields which contained younger cowpats compared to the other fields. All fields have fecal matter from a bovine source, either from the cowpats deposited by the cows during the grazing season or by the extra manure added in the fall. The general *bacteroidales* marker AllBac was detected in all sites in varying percentages over the years for all treatments, which indicated that the pollution is from fecal sources. However, the persistence of the *bacteroidales* markers may be affected by other environmental factors like temperature, pH, and light which explain the fact that the AllBac markers were not detected in any of the samples in 2012. The year 2012 recorded below average precipitation and early snowmelt compared to the years 2011 and 2013 which, on the contrary, recorded higher than normal precipitation and late start of snowmelt.

6.2 Recommendations

This study recommends that microbial pollution should be included in the evaluation of BMPs on pasture. Bacteria are expected to survive the cold winter and be available for transport to surface water with snowmelt runoff. The aging process and the cold temperatures may lower the number of bacteria in the cowpat but may not completely kill the bacteria. Cattle wintering fields and grazing fields receiving animal waste past the spring and summer grazing season may transport most of their pollution load of both microbial and suspended solids at the early stages of the snowmelt during high precipitation years. The loading of pollutants from fields receiving animal waste during the spring and summer grazing season only are less dependent on the precipitation level and field conditions but do also exhibit first-flush in microbial loading. Management practices that retain the snowmelt runoff in the early stages of snowmelt (e.g. wetlands) are recommended to reduce the concentrations of pollutants reaching surface water during snowmelt runoff especially during high precipitation years.

During the spring and summer grazing season, the fresh cattle deposits may contain high concentrations of bacteria that exceed the as-deposited concentrations. As the cowpats age, desiccation and UV exposure may kill or injure the bacteria. The injured or stressed bacteria might not be detectable by culture methods but can remain viable and reactivate as environmental conditions changes. The risk of microbial pollution to surface water by the grazing animals can remain high, and agricultural management practices that limit the cattle's access to water bodies or reduce runoff from grazing fields to surface water should be considered.

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8 Appendixes

8.1 Normality testing:

Treatments: C, W, F

> shapiro.test(C)

Data: C

W = 0.47615, p-value = 2.194e-08

Data: F

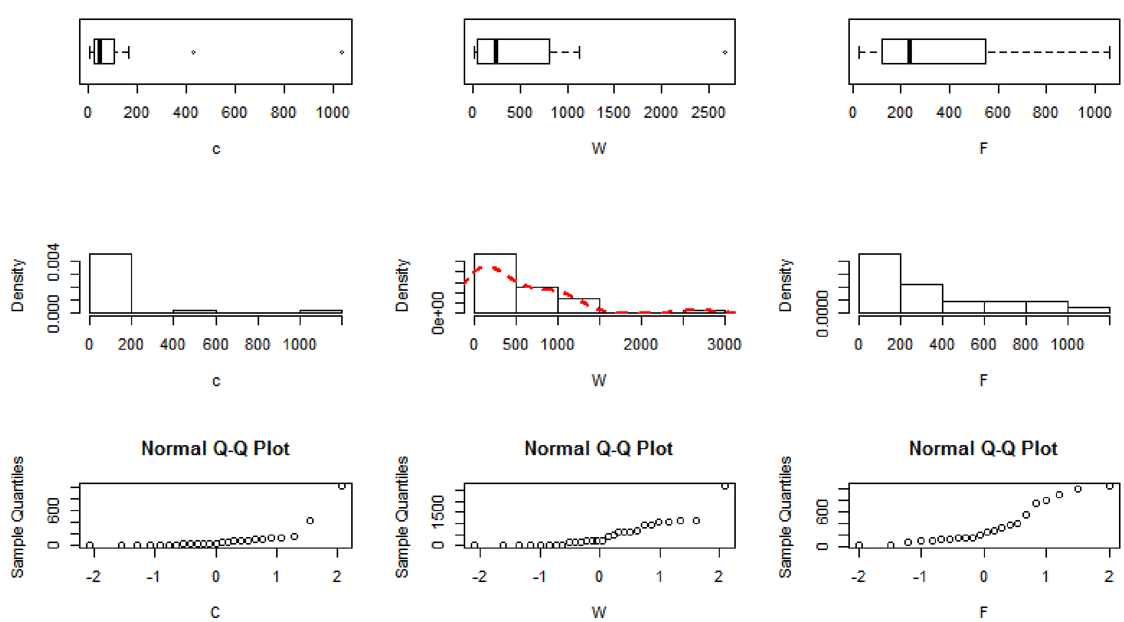
> shapiro.test(F)

W = 0.83989, p-value = 0.002261

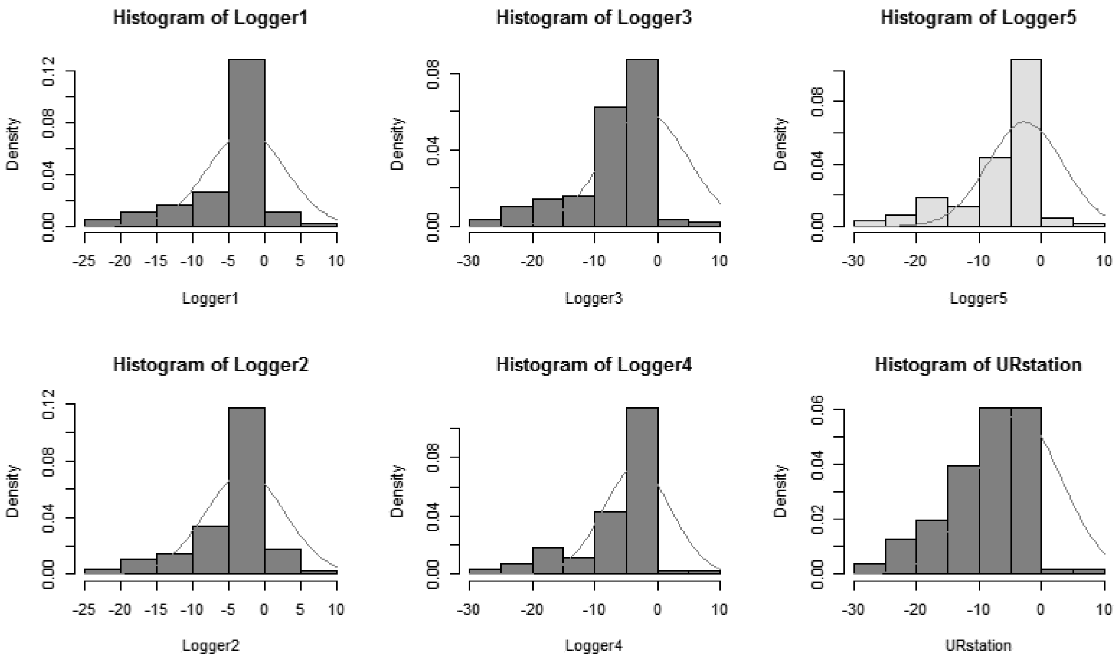
Data: W

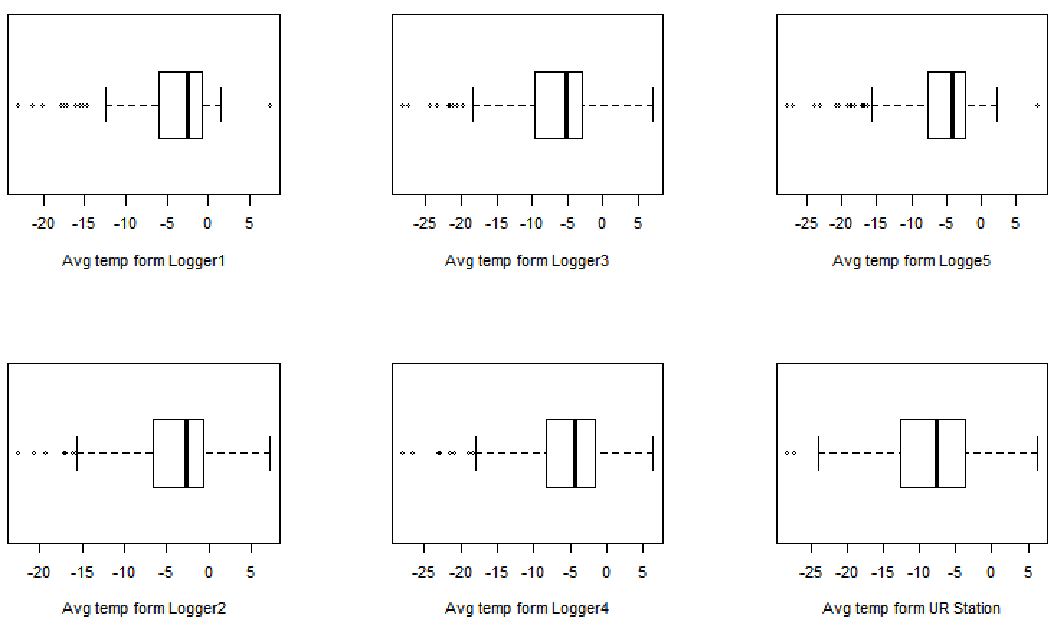
> shapiro.test(W)

W = 0.76601, p-value = 2.841e-05

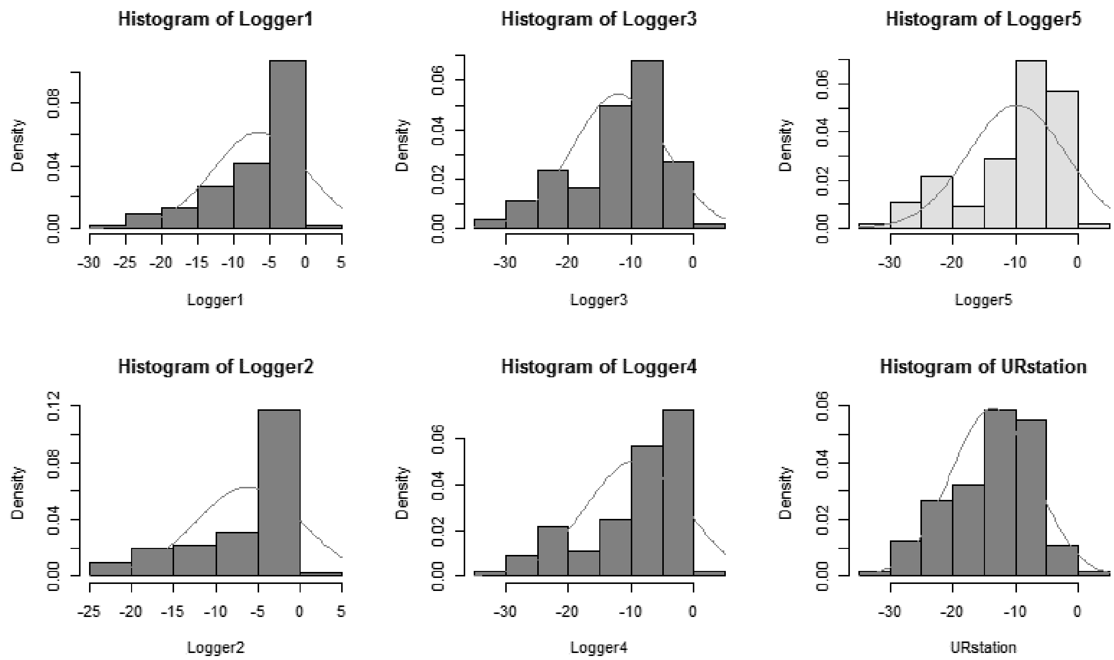


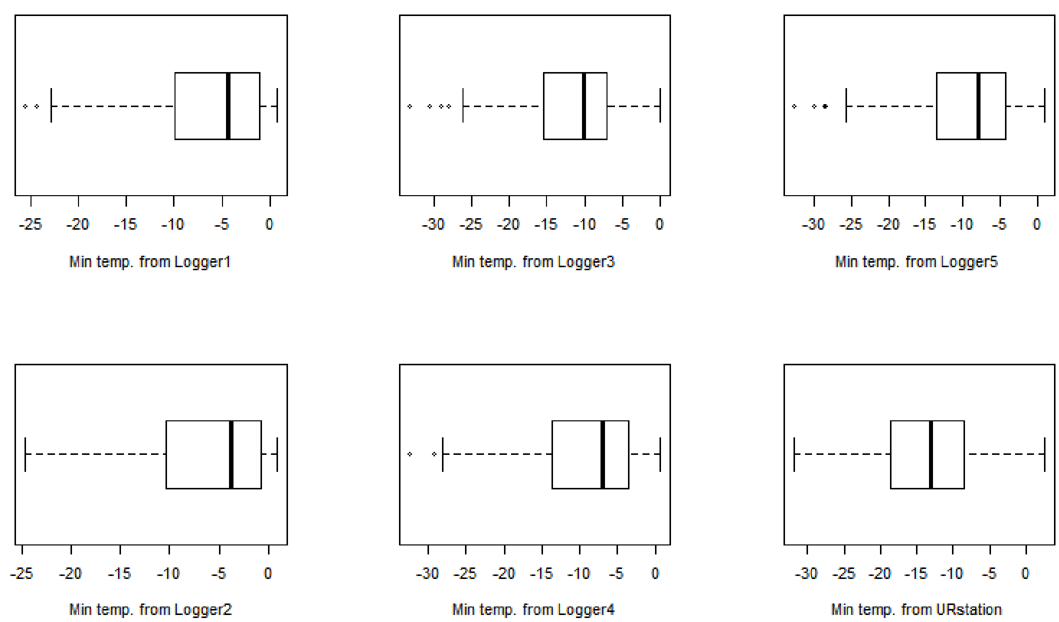
Average Temperatures



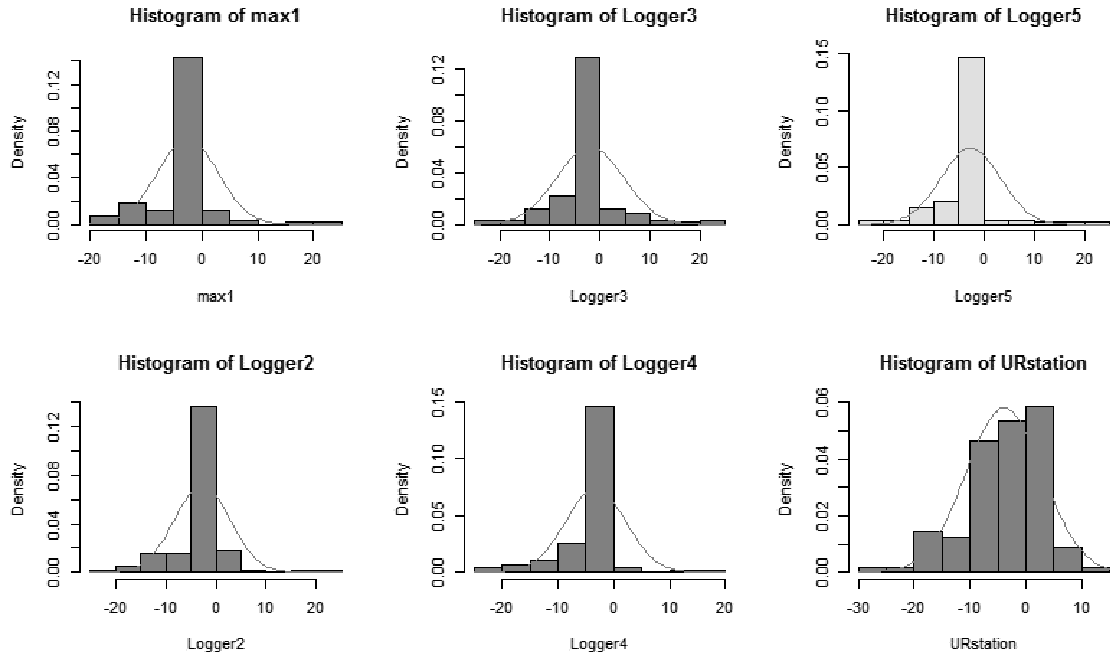


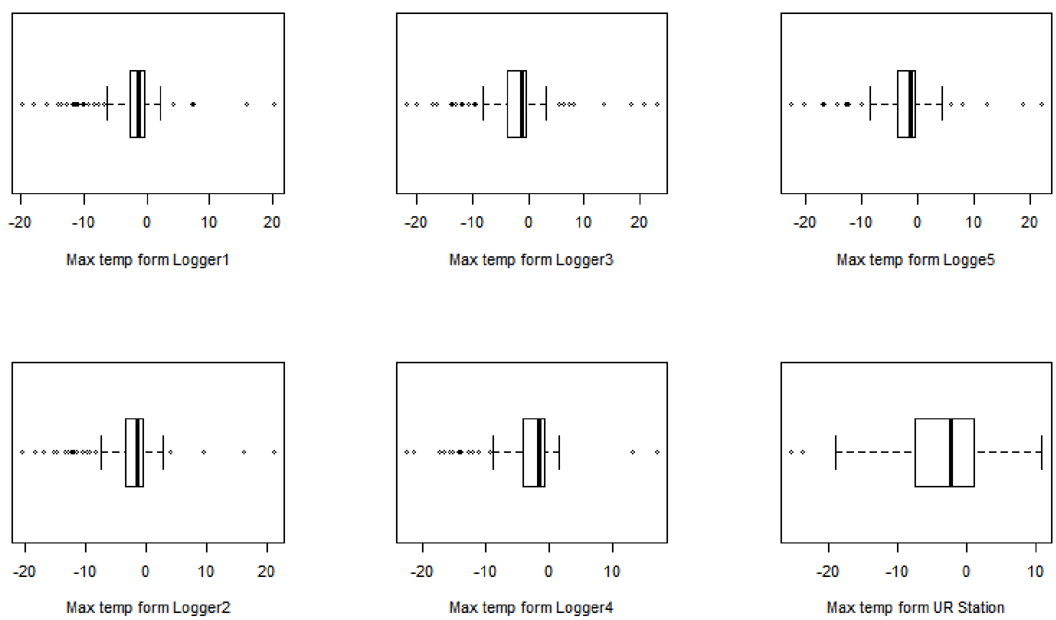
Minimum Temperatures





Maximum Temperatures





8.2 Temperature Data

Average Temperatures:

	Logger 1	Logger 2	Logger 3	Logger 4	Logger 5	UR station
Date	**Average**	**Average**	**Average**	**Average**	**Average**	**Average**
05-Jan-13	-4.38089	-4.10689	-3.74056	-4.094	-4.36522	-10.8729
06-Jan-13	-6.1705	-6.17821	-6.28154	-6.04296	-6.19683	-5.78972
07-Jan-13	-7.01217	-7.23608	-7.10717	-7.07129	-7.23854	-6.4834
08-Jan-13	-5.38271	-5.35483	-5.41792	-5.24058	-5.44058	-4.39278
09-Jan-13	-6.86996	-6.86479	-7.00138	-6.95217	-6.91971	-5.70597
10-Jan-13	-4.6423	-4.33308	-4.63571	-4.72933	-4.54567	-5.63956
11-Jan-13	-8.508	-7.88879	-14.6638	-11.6042	-11.2494	-15.6191
12-Jan-13	-12.5509	-10.4345	-21.6393	-19.0573	-19.148	-21.5516
13-Jan-13	-10.8921	-9.41821	-17.7058	-14.8716	-15.681	-17.7978
14-Jan-13	-12.4539	-11.0198	-20.5777	-17.4915	-18.6967	-21.0204
15-Jan-13	-9.75683	-8.83496	-9.97642	-9.7295	-9.77396	-7.41757
16-Jan-13	-0.79217	-0.67492	-3.29329	-1.66196	-2.73804	-3.91215
17-Jan-13	-2.74692	-4.04579	-11.0635	-8.30867	-10.4131	-11.1791
18-Jan-13	-3.22754	-3.33225	-3.41417	-3.44683	-3.31483	-1.80965
19-Jan-13	-11.0651	-9.24025	-16.6733	-16.0472	-17.0463	-17.113
20-Jan-13	-17.4774	-16.0864	-24.3668	-22.904	-24.0133	-23.86
21-Jan-13	-21.305	-20.713	-23.3402	-23.028	-23.0492	-22.6053
22-Jan-13	-15.5834	-15.581	-16.7265	-16.1994	-16.2798	-17.0903

23-Jan-13	-16.0485	-15.7388	-19.7765	-19.0424	-18.7555	-20.5174
24-Jan-13	-14.6938	-14.3043	-17.2315	-16.8568	-16.7688	-16.9441
25-Jan-13	-17.9206	-16.919	-21.144	-20.953	-20.7961	-20.6254
26-Jan-13	-15.2727	-14.9301	-15.0579	-15.7166	-15.261	-14.8907
27-Jan-13	-8.175	-8.5135	-6.71604	-7.73113	-7.01358	-6.30319
28-Jan-13	-5.75513	-5.82258	-6.55921	-6.17229	-5.87333	-7.58662
29-Jan-13	-8.32183	-7.89675	-13.2265	-11.4193	-12.3091	-14.3282
30-Jan-13	-20.1565	-19.3309	-27.5426	-26.6334	-27.161	-27.2883
31-Jan-13	-23.05	-22.5268	-28.287	-28.0406	-27.9508	-28.366
01-Feb-13	-17.2014	-17.1785	-18.3752	-18.4143	-18.1456	-18.6008
02-Feb-13	-11.8384	-12.0127	-12.1956	-12.3833	-12.2089	-11.6201
03-Feb-13	-9.00304	-9.06283	-9.50463	-9.13933	-8.82688	-10.2566
04-Feb-13	-4.61313	-5.014	-3.73992	-4.32954	-3.94179	-2.94576
05-Feb-13	-4.41365	-4.38779	-6.04733	-5.58763	-5.5335	-6.50326
06-Feb-13	-3.67663	-3.54679	-4.85492	-4.38829	-4.31642	-6.26861
07-Feb-13	-4.52513	-4.08525	-7.91892	-6.03279	-6.24513	-9.4048
08-Feb-13	-3.70396	-3.4765	-6.00588	-5.13338	-4.96633	-6.37014
09-Feb-13	-5.77092	-5.21371	-9.05508	-8.22358	-8.04496	-9.62889
10-Feb-13	-3.01304	-2.74013	-5.5065	-4.27304	-4.19142	-6.13944
11-Feb-13	-4.17808	-3.67646	-7.57458	-5.93192	-6.232	-8.06125
12-Feb-13	-2.9835	-2.91579	-4.76079	-4.31983	-4.33658	-4.0359

13-Feb-13	-1.99446	-2.552	-3.20458	-3.15271	-2.82288	-3.01778
14-Feb-13	-1.49638	-1.59638	-5.03229	-3.7565	-2.80588	-6.53736
15-Feb-13	-4.48275	-4.50454	-10.2653	-8.86088	-7.90383	-12.1931
16-Feb-13	-3.36117	-3.75558	-5.01925	-5.25296	-4.25417	-5.33792
17-Feb-13	-2.43758	-2.82433	-4.87729	-4.66958	-4.26538	-5.18972
18-Feb-13	-5.59067	-7.06917	-13.4569	-12.8488	-12.3809	-14.9493
19-Feb-13	-12.1538	-14.2293	-21.8599	-21.5013	-20.4859	-22.7835
20-Feb-13	-11.8512	-13.7698	-17.8098	-18.0532	-17.0948	-18.8642
21-Feb-13	-11.9361	-13.1878	-14.9342	-15.8653	-14.8422	-14.9199
22-Feb-13	-8.97221	-9.86308	-10.2485	-11.1275	-10.2599	-9.95479
23-Feb-13	-6.69533	-7.49008	-7.31954	-8.09933	-7.33425	-7.24694
24-Feb-13	-4.17308	-5.60217	-7.10308	-7.40042	-7.03725	-6.98972
25-Feb-13	-5.42821	-6.30296	-7.36833	-8.07333	-7.34083	-7.71743
26-Feb-13	-3.30917	-3.69288	-3.73625	-4.07592	-3.62471	-3.58424
27-Feb-13	-2.27496	-2.49046	-3.42033	-3.13667	-2.34888	-5.35917
28-Feb-13	-2.53671	-2.75242	-5.02792	-3.95158	-3.78613	-6.105
01-Mar-13	-1.86779	-2.04042	-3.06475	-2.70867	-2.55021	-3.4734
02-Mar-13	-1.36454	-1.68433	-2.88517	-2.60225	-2.43763	-2.54674
03-Mar-13	-0.63008	-0.88638	-1.09496	-1.15158	-0.95592	-2.05535
04-Mar-13	-0.50117	-0.6585	-3.69492	-0.71458	-0.66379	-8.38465
05-Mar-13	-1.3855	-1.30929	-8.46129	-2.27792	-5.16242	-12.499

06-Mar-13	-3.52121	-3.03308	-9.56425	-5.49279	-7.57175	-14.0185
07-Mar-13	-2.73533	-2.72038	-6.41542	-4.55988	-5.26117	-9.49326
08-Mar-13	-1.65792	-1.89733	-5.44229	-3.73913	-4.03358	-11.5261
09-Mar-13	-1.53063	-1.66138	-6.43342	-3.50275	-3.80196	-10.1505
10-Mar-13	-2.55929	-2.44321	-8.32958	-4.90667	-6.79617	-12.7072
11-Mar-13	-2.49342	-2.27121	-6.41775	-4.76813	-5.508	-6.74424
12-Mar-13	-3.60117	-4.66758	-9.66854	-9.60154	-8.78996	-10.9246
13-Mar-13	-4.01438	-4.19379	-5.81963	-5.74283	-5.55942	-4.74406
14-Mar-13	-1.82017	-2.34558	-2.81754	-3.444	-3.53904	-5.1209
15-Mar-13	-3.38746	-3.52458	-4.53213	-4.37958	-4.38325	-11.4781
16-Mar-13	-2.88713	-3.001	-4.15383	-4.06925	-3.5905	-15.5595
17-Mar-13	-2.56996	-2.63967	-3.8155	-3.90883	-3.34042	-12.9582
18-Mar-13	-1.80804	-1.97958	-3.23892	-3.5185	-2.749	-15.3508
19-Mar-13	-1.65888	-1.84563	-4.46708	-4.11525	-3.10088	-15.6449
20-Mar-13	-2.18192	-2.44546	-11.0833	-8.44404	-7.58488	-14.7048
21-Mar-13	-1.89417	-2.03363	-7.55667	-5.85942	-5.41496	-11.4567
22-Mar-13	-1.78121	-1.8015	-5.03933	-4.27138	-3.9165	-9.39382
23-Mar-13	-1.62829	-1.72163	-3.03342	-3.00742	-2.68275	-8.46292
24-Mar-13	-0.969	-1.04563	-2.01758	-2.06646	-1.60604	-10.366
25-Mar-13	-0.72525	-0.81804	-4.01758	-2.52038	-2.38958	-15.454
26-Mar-13	-1.23538	-1.197	-9.06267	-3.76704	-4.27404	-12.3849

27-Mar-13	-1.5365	-1.43771	-6.76358	-3.5435	-4.16821	-10.4415
28-Mar-13	-1.15783	-1.17783	-6.41771	-2.38783	-3.30888	-9.94757
29-Mar-13	-0.9475	-0.91221	-4.83829	-1.54088	-2.89654	-4.79979
30-Mar-13	-0.52463	-0.48954	-0.767	-0.59146	-0.60913	-1.91576
31-Mar-13	-0.35067	-0.36583	-1.81563	-0.43	-0.47317	-4.7341
01-Apr-13	-0.32383	-0.339	-5.61508	-1.32658	-3.07375	-7.88364
02-Apr-13	-0.32383	-0.35767	-2.31704	-1.51504	-1.66213	-4.02029
03-Apr-13	-0.318	-0.339	-1.74254	-1.39496	-1.30204	-0.56028
04-Apr-13	-0.2585	-0.304	-1.61379	-0.54329	-0.47346	-2.51833
05-Apr-13	-0.18733	-0.19921	-2.27788	-0.444	-0.40667	-3.64604
06-Apr-13	-0.15129	-0.10667	-1.29238	-0.47083	-0.59496	-1.2009
07-Apr-13	-0.12613	-0.088	-0.61975	-0.4405	-0.4545	-2.72993
08-Apr-13	-0.15588	-0.11808	-2.51942	-0.62567	-1.15596	-9.42576
09-Apr-13	-0.12479	-0.11117	-3.54129	-1.599	-1.80121	-8.14896
10-Apr-13	-0.17229	-0.13738	-3.6865	-1.73325	-2.27621	-8.29187
11-Apr-13	-0.1535	-0.12838	-2.20542	-0.75842	-0.91933	-3.25063
12-Apr-13	-0.10875	-0.102	-0.26783	-0.49767	-0.36817	-0.60278
13-Apr-13	-0.02992	-0.07283	-0.31513	-0.40083	-0.26083	-0.79632
14-Apr-13	-0.08771	-0.04833	-1.86463	-0.3285	-0.26667	-2.01458
15-Apr-13	0.03725	-0.02513	-1.63938	-0.27133	-0.206	-1.75375
16-Apr-13	0.218208	0.030417	-2.10292	-0.29117	-0.27483	-2.57104

17-Apr-13	0.611917	0.100792	-1.59296	-0.3145	0.4575	-2.93486
18-Apr-13	1.134083	0.179083	-0.95229	-0.28767	-0.12925	-3.42208
19-Apr-13	1.589542	0.529125	2.036583	0.040333	2.23625	-0.87062
20-Apr-13	-0.10425	0.400167	-0.95437	-0.20146	-0.80754	-1.30674
21-Apr-13	-0.14175	0.397333	-2.0845	-1.17633	-2.04038	-2.41632
22-Apr-13	-2.34883	0.68575	-2.59817	-3.31767	-4.08788	-3.60833
23-Apr-13	-1.38067	1.283708	-0.11879	-1.64329	-2.44963	-0.91472
24-Apr-13	-1.42783	0.2585	-1.15271	-0.86954	-1.51783	-1.91083
25-Apr-13	0.562917	2.040292	2.970333	-0.33367	2.141458	1.338904
26-Apr-13	7.3825	7.105125	7.086133	6.348208	8.003375	6.261181

Minimum Temperatures:

	Logger 1	Logger 2	Logger 3	Logger 4	Logger 5	UR station
Date	**Min**	**Min**	**Min**	**Min**	**Min**	**Min**
05-Jan-13	-8.63	-8.697	-8.63	-8.697	-8.597	-15.3
06-Jan-13	-11.505	-11.541	-11.469	-11.65	-11.433	-11.1
07-Jan-13	-16.367	-16.618	-16.534	-16.66	-16.576	-14.8
08-Jan-13	-14.826	-14.905	-15.064	-14.826	-14.945	-14.5
09-Jan-13	-12.049	-12.086	-12.123	-12.417	-12.269	-11.4
10-Jan-13	-9.235	-9.033	-9.679	-9.508	-9.337	-10.3
11-Jan-13	-10.441	-9.1	-22.371	-18.607	-18.032	-22.2
12-Jan-13	-13.05	-11.111	-26.136	-21.617	-21.667	-25.6
13-Jan-13	-12.454	-10.863	-21.716	-17.424	-19.377	-21.4
14-Jan-13	-14.628	-12.825	-24.654	-20.12	-21.518	-24.8
15-Jan-13	-14.984	-13.163	-23.198	-19.331	-20.883	-22.2
16-Jan-13	-1.015	-0.873	-7.155	-4.197	-6.674	-7.4
17-Jan-13	-5.079	-5.979	-14.392	-10.722	-13.965	-13.9
18-Jan-13	-5.511	-5.326	-7.64	-6.994	-7.122	-7.3
19-Jan-13	-14.826	-12.863	-21.617	-20.595	-21.567	-20.6
20-Jan-13	-22.781	-20.786	-28.989	-27.46	-28.34	-27.7
21-Jan-13	-24.378	-22.885	-29.121	-28.086	-28.533	-27.5
22-Jan-13	-18.832	-18.296	-20.547	-20.026	-20.073	-20.1
23-Jan-13	-18.12	-17.381	-23.515	-22.678	-22.729	-22.4

24-Jan-13	-16.534	-15.832	-21.174	-20.499	-20.883	-20.3
25-Jan-13	-20.167	-18.787	-25.902	-25.157	-25.214	-24.6
26-Jan-13	-18.252	-16.997	-20.931	-20.69	-20.69	-18.9
27-Jan-13	-11.397	-11.722	-10.058	-10.757	-10.127	-9.5
28-Jan-13	-6.325	-6.388	-7.608	-6.898	-6.706	-9.4
29-Jan-13	-17.167	-16.243	-26.136	-24.709	-25.556	-25.4
30-Jan-13	-21.966	-21.077	-30.619	-29.32	-29.927	-29.4
31-Jan-13	-25.498	-24.598	-33.218	-32.445	-32.674	-31.9
01-Feb-13	-22.016	-21.716	-24.654	-24.488	-24.105	-25
02-Feb-13	-15.954	-15.425	-17.813	-17.597	-17.424	-17.4
03-Feb-13	-10.792	-10.863	-11.722	-11.254	-11.326	-11.4
04-Feb-13	-8.297	-8.132	-10.092	-9.337	-9.508	-10
05-Feb-13	-6.674	-6.42	-10.511	-9.542	-9.782	-10.2
06-Feb-13	-4.439	-4.287	-7.478	-5.885	-5.885	-9
07-Feb-13	-5.449	-4.743	-10.934	-7.771	-8.497	-13.1
08-Feb-13	-4.378	-3.956	-10.898	-7.935	-7.738	-10.3
09-Feb-13	-8.231	-6.962	-17.424	-13.05	-13.126	-18
10-Feb-13	-4.227	-3.36	-8.364	-6.388	-6.706	-8.7
11-Feb-13	-5.574	-4.682	-10.651	-8.297	-8.663	-10.9
12-Feb-13	-4.987	-4.439	-9.576	-8.099	-8.297	-8.8
13-Feb-13	-3.242	-3.866	-6.388	-5.449	-5.388	-6.1

14-Feb-13	-1.871	-1.958	-9.989	-7.058	-6.294	-10.2
15-Feb-13	-5.823	-5.636	-16.078	-11.831	-11.397	-17.1
16-Feb-13	-4.439	-4.499	-10.336	-7.968	-7.251	-12.2
17-Feb-13	-3.39	-3.777	-9.954	-8.932	-9.235	-10
18-Feb-13	-9.405	-10.827	-23.303	-21.223	-21.077	-20.9
19-Feb-13	-14.12	-16.326	-28.086	-26.194	-25.671	-28.1
20-Feb-13	-13.163	-15.184	-22.218	-21.617	-20.931	-21.8
21-Feb-13	-14.12	-15.385	-21.028	-20.883	-20.167	-19.2
22-Feb-13	-11.218	-12.123	-15.791	-15.832	-15.345	-14.2
23-Feb-13	-9.134	-9.919	-14.275	-13.888	-13.505	-13.1
24-Feb-13	-5.542	-7.413	-12.013	-11.005	-10.898	-11.6
25-Feb-13	-7.836	-8.663	-14.589	-13.849	-13.543	-13.9
26-Feb-13	-5.542	-5.854	-8.965	-9.134	-8.663	-7
27-Feb-13	-3.836	-3.807	-8.63	-6.802	-6.515	-8.6
28-Feb-13	-3.717	-3.807	-7.836	-6.515	-6.674	-8.9
01-Mar-13	-2.859	-2.947	-5.791	-4.926	-4.926	-6.3
02-Mar-13	-2.537	-2.976	-6.93	-5.791	-6.231	-7.3
03-Mar-13	-0.732	-1.1	-2.044	-2.044	-1.814	-5.8
04-Mar-13	-0.563	-0.704	-13.05	-0.873	-2.16	-13.1
05-Mar-13	-2.16	-1.814	-14.746	-4.287	-8.764	-17.9
06-Mar-13	-5.233	-4.318	-16.997	-8.033	-12.602	-19.4

07-Mar-13	-3.449	-3.242	-10.441	-5.729	-7.673	-13.3
08-Mar-13	-2.276	-2.45	-9.202	-5.172	-6.642	-15.8
09-Mar-13	-2.305	-2.276	-10.336	-4.621	-5.667	-14.4
10-Mar-13	-3.926	-3.568	-13.466	-6.866	-9.954	-19
11-Mar-13	-4.408	-3.627	-10.058	-9.542	-8.264	-10.3
12-Mar-13	-6.357	-7.251	-15.184	-14.905	-13.428	-14.7
13-Mar-13	-6.357	-6.61	-14.159	-13.39	-12.269	-13.6
14-Mar-13	-4.076	-4.865	-8.63	-8.563	-8.43	-8.6
15-Mar-13	-3.866	-3.926	-5.388	-4.865	-4.926	-16.1
16-Mar-13	-3.271	-3.33	-4.621	-4.408	-3.866	-19.3
17-Mar-13	-2.742	-2.83	-4.106	-4.106	-3.449	-16.1
18-Mar-13	-2.392	-2.479	-3.717	-3.747	-3.006	-20.6
19-Mar-13	-2.015	-2.218	-11.254	-7.058	-4.621	-20.9
20-Mar-13	-2.83	-3.065	-15.669	-11.111	-9.919	-20.7
21-Mar-13	-2.247	-2.392	-9.713	-6.802	-6.262	-14.4
22-Mar-13	-1.842	-1.842	-6.483	-4.834	-4.439	-11.1
23-Mar-13	-1.756	-1.814	-3.836	-3.39	-3.065	-11.7
24-Mar-13	-1.299	-1.384	-2.8	-2.276	-1.814	-14.8
25-Mar-13	-0.873	-0.958	-8.63	-3.242	-3.568	-21.7
26-Mar-13	-1.613	-1.441	-13.201	-4.865	-5.791	-18.4
27-Mar-13	-1.67	-1.527	-10.511	-4.53	-5.698	-14.3

28-Mar-13	-1.299	-1.27	-10.863	-3.657	-5.202	-15.8
29-Mar-13	-1.043	-1.1	-9.303	-2.742	-4.834	-12.6
30-Mar-13	-0.676	-0.62	-2.218	-0.704	-0.732	-6.7
31-Mar-13	-0.395	-0.423	-6.388	-0.479	-0.591	-9
01-Apr-13	-0.339	-0.367	-13.428	-3.627	-8.397	-15.5
02-Apr-13	-0.339	-0.367	-7.155	-2.947	-4.106	-8.4
03-Apr-13	-0.339	-0.339	-4.682	-2.392	-2.742	-4.7
04-Apr-13	-0.283	-0.339	-8.066	-0.62	-0.817	-6.9
05-Apr-13	-0.227	-0.255	-4.499	-0.479	-0.507	-5.5
06-Apr-13	-0.171	-0.116	-2.334	-0.507	-0.76	-3.7
07-Apr-13	-0.143	-0.088	-0.817	-0.451	-0.507	-7.9
08-Apr-13	-0.171	-0.143	-6.866	-0.902	-2.947	-13.6
09-Apr-13	-0.143	-0.143	-7.869	-3.035	-3.747	-15.6
10-Apr-13	-0.199	-0.171	-10.406	-4.318	-6.738	-17.3
11-Apr-13	-0.171	-0.143	-6.515	-1.157	-2.131	-7.9
12-Apr-13	-0.143	-0.116	-0.395	-0.563	-0.423	-2.6
13-Apr-13	-0.088	-0.088	-1.527	-0.451	-0.283	-4.5
14-Apr-13	-0.143	-0.088	-7.187	-0.339	-0.395	-6.9
15-Apr-13	-0.088	-0.06	-4.137	-0.311	-0.227	-3.6
16-Apr-13	-0.06	-0.06	-8.198	-0.311	-0.507	-6.5
17-Apr-13	-0.116	-0.06	-8.43	-0.339	-0.283	-7.8

18-Apr-13	-0.06	-0.06	-12.788	-0.311	-6.01	-12.2
19-Apr-13	-0.116	-0.032	-4.957	-0.227	-0.171	-5.9
20-Apr-13	-0.143	-0.004	-5.979	-0.451	-3.39	-4.9
21-Apr-13	-1.043	-0.143	-6.674	-5.418	-7.673	-6
22-Apr-13	-7.381	-0.143	-9.713	-8.563	-10.371	-9.4
23-Apr-13	-8.764	-0.143	-7.673	-6.42	-10.058	-8.3
24-Apr-13	-5.979	-0.367	-6.294	-3.777	-5.264	-5.3
25-Apr-13	-9.202	-7.187	-8.563	-8.165	-9.269	-7.6
26-Apr-13	0.715	0.825	0.051	0.66	0.88	2.6

Maximum Temperatures:

	Logger 1	Logger 2	Logger 3	Logger 4	Logger 5	UR station
Date	**Max**	**Max**	**Max**	**Max**	**Max**	**Max**
05-Jan-13	-0.088	-0.088	0.356	-0.116	-0.255	-6.8
06-Jan-13	-1.413	-1.356	-1.498	-1.1	-1.47	1.4
07-Jan-13	-1.498	-1.527	-1.584	-1.498	-1.556	-0.3
08-Jan-13	-1.527	-1.613	-1.556	-1.441	-1.67	-0.4
09-Jan-13	-1.756	-1.871	-1.958	-1.785	-1.727	5.8
10-Jan-13	-0.732	-0.676	-0.732	-0.704	-0.704	5.7
11-Jan-13	-7.64	-7.381	-9.851	-8.597	-7.836	-10.3
12-Jan-13	-11.794	-9.269	-10.827	-13.696	-14.353	-15.7
13-Jan-13	-9.337	-8.297	-13.239	-11.076	-12.343	-15.3
14-Jan-13	-11.29	-9.713	-11.758	-14.12	-14.353	-16.6
15-Jan-13	-0.873	-0.958	-0.591	-0.704	-0.479	3.3
16-Jan-13	-0.676	-0.62	-0.507	-0.563	-0.395	0.9
17-Jan-13	-1.071	-0.676	-7.316	-5.449	-6.898	-6.8
18-Jan-13	-1.128	-1.27	-0.62	-0.817	-0.648	2.7
19-Jan-13	-1.1	-1.185	-0.62	-0.93	-0.591	0.7
20-Jan-13	-13.466	-12.343	-17.338	-16.576	-16.912	-16.4
21-Jan-13	-17.77	-18.296	-16.492	-17.295	-16.618	-18.8
22-Jan-13	-11.505	-12.863	-7.804	-8.898	-8.198	-13
23-Jan-13	-13.926	-14.628	-13.657	-14.353	-12.528	-17.9

24-Jan-13	-10.934	-11.397	-8.132	-9.303	-8.397	-8.1
25-Jan-13	-15.873	-15.184	-13.926	-15.628	-14.392	-18.1
26-Jan-13	-11.04	-12.086	-5.079	-8.43	-7.219	-9.3
27-Jan-13	-6.262	-6.483	-4.682	-6.136	-5.202	-4.2
28-Jan-13	-4.957	-5.141	-5.049	-5.018	-4.53	-4
29-Jan-13	-4.408	-4.804	-5.729	-5.11	-5.233	-7.6
30-Jan-13	-17.77	-16.786	-21.916	-22.371	-22.678	-25.4
31-Jan-13	-19.607	-20.309	-20.167	-21.272	-20.214	-23.7
01-Feb-13	-12.602	-13.315	-12.086	-12.602	-12.491	-14.7
02-Feb-13	-8.397	-9.679	-5.698	-8.132	-6.136	-1.7
03-Feb-13	-6.834	-7.219	-4.167	-5.357	-3.866	-8.2
04-Feb-13	-1.785	-2.392	-0.817	-1.157	-0.902	1
05-Feb-13	-2.45	-2.83	-1.527	-1.785	-1.356	-1.8
06-Feb-13	-2.683	-2.771	-1.986	-2.712	-2.305	-3.9
07-Feb-13	-3.866	-3.568	-5.511	-4.167	-4.227	-7.3
08-Feb-13	-2.102	-2.479	-1.185	-1.842	-1.842	-2.2
09-Feb-13	-3.301	-3.598	-1.842	-3.508	-3.006	-1.3
10-Feb-13	-2.421	-2.334	-2.305	-2.625	-2.508	-1.3
11-Feb-13	-2.334	-2.625	-2.45	-2.189	-2.421	-4.2
12-Feb-13	-1.071	-1.27	-0.732	-0.958	-0.845	2.1
13-Feb-13	-1.1	-1.299	-1.015	-1.413	-0.986	0.4

14-Feb-13	-1.1	-1.299	-1.584	-1.699	-1.1	-3.7
15-Feb-13	-2.073	-2.102	-4.621	-5.979	-4.834	-4.6
16-Feb-13	-1.986	-2.683	-0.789	-1.929	-1.128	2.7
17-Feb-13	-1.47	-1.814	-0.93	-1.527	-1.071	0.2
18-Feb-13	-2.8	-4.257	-7.122	-8.898	-7.187	-10
19-Feb-13	-10.058	-11.469	-11.794	-15.184	-12.788	-18.4
20-Feb-13	-10.023	-11.758	-9.439	-11.903	-10.162	-14.7
21-Feb-13	-9.235	-10.476	-3.242	-7.381	-5.357	-9.1
22-Feb-13	-5.574	-6.483	-1.242	-2.888	-1.929	-3.6
23-Feb-13	-3.627	-4.408	-0.873	-1.498	-0.986	-0.8
24-Feb-13	-2.073	-2.947	-1.015	-1.47	-1.157	-11.6
25-Feb-13	-2.654	-3.538	-0.704	-1.185	-0.902	0.3
26-Feb-13	-1.27	-1.584	-0.591	-0.789	-0.591	1.1
27-Feb-13	-1.441	-1.814	-0.732	-1.128	-0.76	-2.8
28-Feb-13	-1.584	-1.842	-1.498	-1.27	-0.986	-3.5
01-Mar-13	-0.958	-1.128	-0.817	-0.817	-0.676	-0.6
02-Mar-13	-0.732	-0.817	-0.451	-0.591	-0.507	5.8
03-Mar-13	-0.507	-0.704	-0.535	-0.648	-0.563	0.7
04-Mar-13	-0.479	-0.62	-0.591	-0.676	-0.535	-5.8
05-Mar-13	-0.563	-0.676	-0.93	-0.986	-1.47	-6.6
06-Mar-13	-2.131	-1.842	-1.071	-2.947	-1.871	-8.4

07-Mar-13	-1.785	-2.015	-2.16	-3.124	-2.566	-5.7
08-Mar-13	-1.242	-1.413	-0.958	-1.986	-1.27	-7.3
09-Mar-13	-0.845	-0.958	-1.413	-1.871	-1.242	-6.8
10-Mar-13	-0.845	-0.986	-1.929	-3.242	-3.419	-7.6
11-Mar-13	-1.043	-1.185	-1.556	-1.727	-1.699	-2.4
12-Mar-13	-1.213	-2.305	-1.071	-1.213	-1.814	-5.7
13-Mar-13	-1.015	-0.986	-0.507	-0.62	-0.591	3.1
14-Mar-13	-0.986	-0.986	-0.535	-0.648	-0.76	-1.6
15-Mar-13	-1.958	-2.508	-3.065	-3.836	-3.598	-7.2
16-Mar-13	-2.771	-2.859	-3.747	-3.777	-3.36	-11.9
17-Mar-13	-2.479	-2.537	-3.479	-3.598	-3.094	-8.8
18-Mar-13	-1.613	-1.756	-2.712	-3.36	-2.625	-7.9
19-Mar-13	-1.242	-1.441	-2.596	-3.242	-2.479	-9.9
20-Mar-13	-1.242	-1.47	-5.729	-5.854	-5.449	-8.8
21-Mar-13	-1.699	-1.756	-4.926	-4.651	-4.287	-7.4
22-Mar-13	-1.67	-1.727	-3.687	-3.39	-3.035	-6.7
23-Mar-13	-1.327	-1.413	-1.842	-2.247	-1.814	-3.9
24-Mar-13	-0.76	-0.817	-1.071	-1.613	-1.157	-5.8
25-Mar-13	-0.648	-0.732	-1.699	-1.641	-1.157	-6.8
26-Mar-13	-0.902	-0.958	-3.568	-2.596	-2.566	-3.5
27-Mar-13	-1.356	-1.327	-1.871	-1.67	-1.958	-3.9

28-Mar-13	-1.071	-1.128	-1.556	-1.299	-1.958	-1.7
29-Mar-13	-0.676	-0.62	-0.199	-0.732	-0.76	4.3
30-Mar-13	-0.395	-0.423	-0.143	-0.479	-0.423	2.9
31-Mar-13	-0.311	-0.339	-0.255	-0.395	-0.423	-1.8
01-Apr-13	-0.311	-0.311	-0.227	-0.395	-0.451	-0.5
02-Apr-13	-0.311	-0.339	-0.283	-0.902	-0.817	-0.2
03-Apr-13	-0.283	-0.339	0.66	-0.591	-0.395	4.7
04-Apr-13	-0.227	-0.283	5.591	-0.507	-0.283	2.7
05-Apr-13	-0.143	-0.116	-0.817	-0.423	-0.255	-1.8
06-Apr-13	-0.116	-0.088	-0.507	-0.423	-0.451	2.1
07-Apr-13	-0.116	-0.088	-0.423	-0.423	-0.395	-0.3
08-Apr-13	-0.116	-0.088	-0.535	-0.423	-0.451	-6.3
09-Apr-13	-0.088	-0.088	-0.311	-0.648	-0.479	-1
10-Apr-13	-0.088	-0.088	-0.199	-0.62	-0.479	0.9
11-Apr-13	-0.143	-0.116	-0.255	-0.591	-0.423	0.4
12-Apr-13	-0.088	-0.088	-0.199	-0.451	-0.283	1.6
13-Apr-13	0.135	-0.06	0.356	-0.367	-0.199	2.9
14-Apr-13	0.024	0.024	1.588	-0.311	-0.199	2.9
15-Apr-13	0.356	0.079	0.687	-0.227	-0.171	0.8
16-Apr-13	0.88	0.246	5.616	-0.227	-0.171	2.2
17-Apr-13	2.209	0.522	8.02	-0.283	4.272	1.8

18-Apr-13	4.22	0.907	13.57	-0.227	5.872	3
19-Apr-13	7.343	1.751	18.557	1.289	12.413	4.1
20-Apr-13	-0.088	1.071	3.274	-0.116	-0.143	1.6
21-Apr-13	-0.088	1.235	2.021	-0.171	-0.171	0.9
22-Apr-13	1.724	2.956	6.433	-0.171	-0.116	1.6
23-Apr-13	7.066	9.46	13.546	1.561	7.945	6.1
24-Apr-13	-0.032	4.089	7.444	-0.143	0.687	1.1
25-Apr-13	15.843	16.153	20.96	13.088	18.771	9.3
26-Apr-13	20.055	21.008	23.232	17.011	22.034	10.9