

BIOGEOCHEMICAL IMPLICATIONS OF ANIMAL MORTALITY BURIAL

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By

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ABSTRACT

Large scale livestock production facilities produce a variety of waste streams. Of those, animal mortality carcass disposal poses a significant threat to the environment depending on the chosen disposal route. Carcass burial is a common approach for both routine mortalities as well as large-scale catastrophic mortality events and has been studied in very limited detail. This study provided further assessment of carcass burial leachate and its inorganic chemical constituents and transport processes in the subsurface. The objectives of this thesis were to: (1) determine the likely geochemical impacts of the ionic strength of carcass burial leachate; (2) provide an assessment utilizing common numerical models to predict contaminant transport based on burial guidelines; (3) investigate existing burial sites for model validation; and (4) evaluate the effects at an existing site that the leachate had on indigenous microbiology and evaluate transport of microbes. These objectives were accomplished by laboratory testing, numerical modelling, and field investigations with the findings contained in four manuscripts that make up the bulk of this thesis. Major findings determined that the high ionic strength of the leachate at maximal concentrations (1.4 to 1.5 molar), lie in a grey area with respect to ion activity calculations for geochemical modelling and that ion activity calculations utilizing the Truesdell-Jones technique provided the best estimate of ion activity. Geochemically, speciation assessment indicated specific species of concern that can transport unattenuated and include ammonium sulfate compounds (up to 300 mg/L), phosphate and phosphoric acid compounds. Two-dimensional conservative and non-conservative (sorption) models and one-dimensional reactive transport models demonstrated plume evolution over a range of hydraulic conductivities, and predicted changes in ammonium retardation with subsequent developments of hard water plumes due to ion exchange reactions. Model validation was achieved by the investigation of two existing carcass burial sites in Saskatchewan and provided the first assessment of plume development and biogeochemical interactions by soil coring and pore water analysis. The site investigations concluded that ammonium and potassium in the leachate was adsorbed and exchanged for calcium and magnesium (as predicted by models). Biogeochemically, anaerobic microorganisms dominated the subsurface and were zoned based on geochemistry. The tools created and used for the microbiological aspects of this work can be useful in fingerprinting existing burial sites.

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DEDICATION

This thesis is dedicated to my parents Loren and Joanne for teaching me to work harder than the rest (but also to work “smarter” not “harder” – thanks Dad), to finish what I started, that what doesn’t kill me only makes me stronger, to never give up, and that life always goes on. And, to Douglas and David for being my best friends, biggest cheerleaders, and the ones not afraid to challenge me. Also, to Slate, Blair and Teagan; this thesis proves that anything you set your mind to is possible and that you should always chase your dreams, even if that means a derailment or two along the way – Aunt Dyan will always be there.

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LIST OF ABBREVIATIONS

1D	one dimensional
2D	two dimensional
α	adsorption constant related to the binding energy (L/mg)
A^0	angstrom
a_i	effective size of hydrated ion (A^0)
a_i^0	Truesdell-Jones ion size parameter
AA	atomic analyzer
AAFC	Agriculture and Agri-Food Canada
ABS	Acrylonitrile butadiene styrene
ADF	Agriculture Development Fund
AESB	Agri-Environment Services Branch
AI	avian influenza
APHIS	Animal and Plant Health Inspection Service of the USDA
ASABE	American Society of Agricultural and Biological Engineers
AU	animal units
β	maximum amount of solute that can be adsorbed by the solid (mg/kg)
b_i	Truesdell-Jones fit parameter
ρ_d	bulk density
BOD	biochemical oxygen demand
BSE	bovine spongiform encephalopathy
°C	degrees Celsius
C	concentration in solution (mg/L, activity or meq/L)
C_o	initial concentration
Ca^{2+}	calcium
c_i	molar concentration
Cl	chloride
CEC	cation exchange capacity (meq/100g)
CFIA	Canadian Food Inspection Agency
COD	chemical oxygen demand (mg/L)

Chao1	community richness
cpn60	chaperonin 60 sequence
CWD	chronic wasting disease
D	Simpson's Index
DNA	deoxyribonucleic acid
ϵ	dielectric constant
EM	electromagnetic
FAD	foreign animal disease
FAO	Food and Agriculture Organization of the United Nations
Fe^{2+}	iron
FMD	foot and mouth disease
GCL	geosynthetic clay liner
GW	groundwater
H'	Shannon Index
HCO_3^-	bicarbonate
I	ionic strength
γ_i	ion activity
ICP-AES	Inductively coupled plasma-atomic emission spectroscopy
ILO	intensive livestock operations
ISO	international organization for standardization
K	hydraulic conductivity (m/s)
K^+	potassium
K_D	Langmuir distribution coefficient (L/kg)
$K_{Na/I}$	ion selectivity coefficient (I is ion of interest)
kg	kilogram
λ	decay constant
L	liter
Lbs	pounds
M	molar
meq	milliequivalent

mg	milligram
mL	milliliter
mol	mole
Mg ²⁺	magnesium
MID	multiplexing ID's
MPa	megapascal
MRR	mark-release-recapture statistics
<i>n</i>	porosity
N	nitrogen
Na ⁺	sodium
NO ₃ -N	nitrate
NH ₄ ⁺ -N	ammonium as Nitrogen (-N)
NH ₃ -N	ammonia as nitrogen (-N)
NZ	New Zealand
OTU	operational taxonomic unit
P	phosphorus
Pa	pascal
PCR	polymerase chain reaction
PV	pore volume
PVC	polyvinyl chloride
R	retardation factor
rRNA	ribosomal RNA
S	concentration of ions on soil exchange sites
sff	standard flowgram format
SO ₄ ⁻²	sulfate
SK	Saskatchewan
SMA	Saskatchewan Ministry of Agriculture
θ	volumetric water content
T	temperature
TDS	total dissolved solids (mg/L)

TJ	Truesdell-Jones
UK	United Kingdom
US	United States
USDA	United States Department of Agriculture
USGS	United States Geological Survey
US EPA	United States Environmental Protection Agency
UT	universal target
WAHIS	World Animal Health Information System
WHO	World Health Organization
X	exchanger master species
XRD	x-ray diffraction
z_i	specific ion charge

Chapter 1: Introduction

1.0 Introduction

Animal husbandry is a common approach around the globe to meet basic work force needs as well as provide a source of protein for human consumption, and delivers other by-products such as leather, wool, milk, and eggs among others. Society no longer relies on hunting and gathering for food, therefore the domestication and subsequent intensification of livestock production has become paramount. An increasing global population provides strong correlation for increased livestock production to meet consumer demands, and as consumers become more wealthy, meat consumption also increases with average yearly consumption increases of approximately 5.1% (Daniel et al. 2011; Alexandratos and Bruinsma 2012). In the past century, animal husbandry has intensified significantly, with the majority of production now occurring on large scale farms, unlike the past. Globally, population numbers are estimated at 1.43 billion cattle, 1.87 billion sheep, 0.98 billion swine, and 19.6 billion chickens (Robinson et al. 2014). In North America alone, cattle populations in Canada and the United States for 2015 were approximately 12.2 million and 89.8 million head, respectively (USDA 2015). The distribution of approximately 100 million head in North America varies by state, with Texas housing over 10% of the total cattle population, and the province of Alberta with 50% of Canada's livestock population (Government of Canada 2014). The majority are located in the plains region from the south of Texas and north through Western Canada (USDA 2015).

The increasing growth in the livestock production sector also puts increasing pressure on the environment and surrounding natural resources. Livestock production practices include both intensive and extensive operations, with intensive operations confining the animals and bringing feed grown on arable land to them, while extensive operations generally refer to operations in which the animals graze pasture land. Each of these systems has advantages and drawbacks, but both are required to meet current demand. Environmental impacts of livestock production vary by production type and species and are under constant scrutiny in areas such as air quality and greenhouse gas emissions, land use management, nutrient cycles, and water quality (FAO 2016). Environmental impacts from many industrialized systems are typically size and location

dependent, meaning, the more concentrated the system is (i.e. increasing number of animal units (AU)), the greater chance for adverse impacts. For livestock systems, waste management at larger scales becomes increasingly difficult. Best practices in North America promote locations of intensive livestock operations (ILO) in vast areas of agricultural land use to help utilize the wastes produced from these facilities, generally in the form of fertilizer application to arable land which subsequently produces feed.

Water quality impacts are an important factor in livestock production, with water pollution from animal waste (manure) being paramount. The pathways of water pollution for intensive livestock operations include surface water runoff from the site and lands receiving manure application, direct deposit of fecal material into waterways, or groundwater contamination via leaching. The risk of contaminants leaching from established ILOs over a period of the last 40 years has been well studied; from transport beneath penning areas (Bennett 1975; Coote and Hare 1978; Betcher et al. 1996; Rosen 1996; Maule and Fonstad 2000; McCullough et al. 2001; Harter et al. 2002; Maule and Fonstad 2002; Rosen et al. 2004; Olson et al. 2005; Singleton et al. 2007), manure storage lagoons (Betcher et al. 1996; Arnold and Meister 1999; McMillan et al. 2000), surface runoff (Cho et al. 2000; Harter et al. 2001; Harter et al. 2002; Mallin and Cahoon 2003; Miller et al. 2004; Airaksinen et al. 2007), land applied manure (Harter et al. 2001; Harter et al. 2002; Koelsch 2005) and air quality (Sweeten et al. 1977; Sweeten 1991; Auvermann 2006; Bunton et al. 2007; Cole et al. 2008).

Nutrient migration to surface water and groundwater from ILOs in the form of nitrogen (N) and phosphorus (P) are typically the constituents of most concern along with organic matter, pathogens and hormones/antibiotics. Generally ammonium (NH_4^+) is attenuated by soil, while nitrate (NO_3^-) transports unattenuated and can be a significant problem when high concentrations occur in groundwater causing methemoglobinemia in infants (Comley 1945). Nitrate concentrations exceeding 10 mg/L $\text{NO}_3\text{-N}$ can cause a health risk to children less than 6 months of age and this concentration has been used as the maximum allowable concentration in drinking water by the World Health Organization in 1984, the United States Environmental Protection Agency in 1992 and by Health Canada in 1987. Human consumption is not the only risk from nitrate in water, at these same concentrations, nitrate in water can cause mortality in cattle (Kreiter and Jones 1975).

Nutrient migration due to animal wastes such as manure, aren't the only risks to water quality in all animal husbandry operations. The enormity of livestock production magnifies a variety of potential environmental and economic problems, one of which is carcass disposal. Routine mortalities as well as the potential for catastrophic mass mortality events, such as disease outbreaks or natural disasters, each pose a significant threat. Routine mortalities for livestock operations on average range from 1% to 15 % over all sectors, with approximately 3 billion pounds of material requiring disposal per year in the United States alone, not including any foreign disease outbreaks (Kelly and Janzen 1986; Loneragan et al. 2001; NABC 2004; Canfax 2013). Routine mortalities are disposed of through a variety of avenues and include: incineration, composting, rendering, landfilling, burial, anaerobic digestion, and thermal hydrolysis (NABC 2004; Cleary et al. 2010). Each disposal technique is employed across many sectors and are usually dependent on the size and networked capacity of the individual farm. For example, ILOs generally rely on services such as rendering to deal with their routine mortalities, where-as smaller operations typically dispose of carcass material on-farm via burial, composting or burning.

Public perceptions of livestock mortality burial and its risk to the environment are a major concern to regulators, policy makers and producers. In order for regulatory bodies to complete their due diligence and provide recommendations for burial sites, a full understanding of the risk of livestock burial to the environment is required through completion of studies such as this. Science based policy creation is key to providing sound regulations. That being said, burial in suitable areas of the US and Western Canada are still likely the best option in the event of a foreign disease outbreak and viable in select locations as a management option for slaughter waste. In the case of a foreign disease outbreak, transport to a disposal site may not be an option and burial will be necessary on site. Furthermore, if a significant number of animals are involved, burial again will likely be the only option as other options quickly become exhausted. Given this, the costs associated with not being allowed to bury due to public opposition, lack of knowledge of the risk associated with burial, lack of other available options such as rendering and/or the higher risk of other management options such as burning, are significant. The livestock industry, along with the state/provincial and federal governments, must take steps to complete thorough planning to ensure we can use burial as a mortalities and/or slaughter waste management option.

The economics associated with carcass disposal paint a dim picture. With burial being a simple solution, the costs of other options available for disposal during a mass mortalities event are one to several orders of magnitude higher than burial. If we don't prove that burial is a viable option, these significantly higher costs will be borne by the livestock producer, insurance agencies and/or the public. Economically speaking, mass livestock mortality events are expensive with respect to depopulation, decontamination and disposal. Saskatchewan Ministry of Agriculture (2012), and others (Bonhotal et al. 2002; Sparks Companies Inc. 2002; NRCS 2011), estimate the cost of burying mortalities on site to be \$0.01 to \$0.015 per pound. Burying with lining of the trenches increases the cost to approximately \$0.03 per pound. This value is well below the reported cost (approximately \$0.03 to \$0.06) of composting of the mortalities with land application of the compost if allowed (Bonhotal et al. 2002). Rendering is reported to double this value to \$0.06 to \$0.07 per pound without allowance for cost recovery from the rendered material. The implications of being able to bury for a mortality or disease control event at a typical 100,000 head cattle feedlot would be a cost saving of approximately \$1,500,000 between burial and burial with liners or up to \$4,500,000 for burial versus composting. In the event of a regional outbreak or mortality event, regulators must be able to assess the suitability of sites for unlined burial. Published work outlining the risk, or lack of risk, to groundwater resources from these activities is essential to the decision and could save millions of dollars in both funds and time.

Mitigating mass mortality carcass disposal risks must parallel an understanding of disease risks and transmission. Trends in disease transmission around the globe are affected by climactic conditions, civil unrest, disease vector drivers and trade (FAO 2015). Of the many zoonotic diseases, those of most concern for the livestock industry include foot and mouth disease (FMD), low pathogenic and highly pathogenic avian influenza (AI), anthrax, lumpy skin disease, and swine fever (CFIA 2010). Between 2004 and 2010, Canada suffered 5 zoonotic disease incidents in their livestock populations, the majority of which were avian influenza, with one instance of bovine spongiform encephalopathy (BSE) (CFIA 2010), three more outbreaks of AI and one case of confirmed BSE have occurred in subsequent years (CFIA 2016). FMD outbreaks have not occurred in North America since 1952 (CFIA 2012), while other portions of the world suffer from the inability to eradicate this highly transmissible virus. Countries such as Turkey and Brazil face endemics repeatedly, while others such as the United Kingdom have suffered through two

outbreaks in the last couple decades (2001 and 2007), with 2001 being catastrophic to their livestock industry (WAHIS Database 2016). In 2001, FMD ran rampant through Europe effecting France, the Netherlands, Ireland and Turkey (Junker et al. 2009). Asian countries have also suffered severe epidemics. Between 2000 and 2017, Korea suffered nine catastrophic FMD outbreaks, with 2011 being the most widespread and devastating to animal populations (Junker et al. 2009). Regionally, Asia suffered from over 60 various zoonotic disease outbreaks in 2016 alone, while the North America endured 14 epidemiological events in the same year (WAHIS Database 2016). Developing countries generally have higher disease instances than those of the developed world (WAHIS Database 2016).

While we have come a long way in providing epidemiological vaccination efforts for many diseases, instances still occur and catastrophes will still happen. Preparedness for these instances with planned disposal responses are necessary. Typically, for a catastrophic event in North America, a combination of disposal strategies are utilized depending on the type of disease, quantity of livestock to dispose of, applicable laws/regulations, and available resources (USDA APHIS 2012). Options generally include composting, burial, incineration, rendering and landfilling. Composting contains the pathogen on the premises but requires a large open space and carbon addition. Burial is an option in some locations pending environmental regulatory approval and is usually only permitted in geologically secure areas. Incineration is costly and limited to fuel availability, and is the method that generally causes the most public nuisance. Landfilling is usually an option for large quantities of carcasses depending on restrictions imposed by landfill managers. Of all the options, burial is typically the most cost effective and expedient option and the least understood with respect to environmental implications.

1.1 Study Rationale & Background Information

There is a significant lack of understanding of the subsurface environmental implications of animal carcass burial with very few studies in existence that examine these impacts. Of those that do exist, few portray a clear picture of understanding of the environmental impacts arising from a mass mortality event. Literature from the last decade include very few studies evaluating these subsurface impacts and do not necessarily provide a comprehensive review. In the majority of

current literature, impacts to the subsurface have been described based on reviews of three separate studies; Glanville (2000), Ritter and Chirnside (1995) and MacArthur and Milne (2002), and more recently the Master of Science work of Pratt (2009), that preceded and created the starting point for this thesis. More recently, leachate characterization studies for three separate species of livestock by Pratt (2009), and the extenuating circumstances surrounding zoonotic disease outbreaks throughout the world, South Korea has added to the scientific knowledgebase on impacts to the environment after their 2010 to 2011 FMD disaster. Their additions, along with others encompasses the small body of peer-reviewed scientific literature that exists on carcass disposal via burial and includes studies on: leachate characterization (Okanovic et al. 2009; Pratt 2009; Choi et al. 2013; Yuan et al. 2013; Kwon et al. 2014); characterization of microbiology (Hak et al. 2012; Pratt et al. 2012; Park et al. 2013; Kwon et al. 2014; Yang et al. 2015); survey of water quality and soil impacts surrounding burial sites (Hatzell 1995; Ritter and Chirnside 1995; Myers et al. 1999; Glanville 2000; Vane and Trick 2005; Kim et al. 2010; Kim and Kim 2012; Joung et al. 2013; Yuan et al. 2013; Kaown et al. 2014; Kim et al. 2014b; Park et al. 2014; Hseu and Chen 2016); modelling and assessing impacts (Scudamore et al. 2002; Cleary 2009; Gwyther et al. 2011; Jeong et al. 2012; Kim and Kim 2015); burial site post-construction management (Kim and Jeon 2014; Kim et al. 2014a; Kim and Pramanik 2015; Yoon et al. 2017); and cost analyses and policy litigation for future burial systems (Tromans 2002; Williams et al. 2009; Kim and Kim 2013; Lee 2013).

Most government regulatory agencies across many parts of the globe provide documents outlining carcass disposal options for routing mortalities. Jurisdictionally, burial is somewhat controlled depending on the quantity of carcasses, their mortality issue, and site-specific burial requirements. For North America, regulatory agencies such CFIA, AAFC, USDA, APHIS and others will provide guidelines and permitting for carcass disposal on a Federal level, with some states and provinces providing their own jurisdictional resources. Depending on each region's unique situation, local authorities will provide guidance and appropriate steps for disposal.

From a microbiological and geochemical standpoint, the effects of livestock carcass burial on the environment to date have not been satisfactorily evaluated. This thesis aims to tackle that challenge and provide answers to specific contaminant transport questions associated with livestock burial

as well as an assessment of possible microbiological factors connected to the burial of animal carcasses. This new information will enable evidence based policy creation and modelling to support site characteristics deeming potential burial sites as geologically secure (safe) for mass burial.

1.2 Research Objectives

The overall objective of this research project was to provide a body of work on the environmental implications of mass mortality carcass burial by utilizing past knowledge incorporating conservative and reactive transport models and validating this body of work by investigating existing burial sites. To do this, preliminary investigative work under Pratt (2009) M.Sc. program provided the first assessment of the chemical properties of livestock mortality burial leachate over two years of decomposition processes. Utilizing the information obtained from that study, it was soon realized that much information was missing to make proper assessments. Given the specifics of the concentration of constituents in burial leachate and the strength of ions in that leachate, more work was needed. Specifically, from a geochemical standpoint, are current thermodynamic geochemical mathematical models appropriate to use on this leachate? And given the leachate is now characterized, what implications will that have for contaminant transport through the subsurface and how might that affect subsurface processes from a microbiological standpoint. The specific research objectives/questions for this project are outlined below:

1. What are the likely impacts of the ionic strength of this leachate on the species of various ions present?
 - a. Is there a sensitivity to the method used to calculate geochemical thermodynamic properties?
 - b. What effects will these properties have as the leachate dilutes and moves through groundwater?
 - c. What effects will leachate chemistry have on soil sorption properties?
2. For typical Saskatchewan burial guidelines, what would we expect to see regarding contaminant transport beneath a burial trench?

- a. Are there impacts associated with burial trench spacing in a scenario involving large numbers of casualties requiring multiple burial trenches?
 - b. What impacts are observed from conservative versus reactive transport models?
3. Can objective 2 be validated with field investigations?
 - a. Two sites were investigated, one >50 years post burial and another >8 years post burial.
 - b. Geochemically speaking, what were the impacts at each location and are they similar to what was shown in the models and comparable to expected leachate chemistry?
4. What are the risks associated with pathogen transport and what effects does leachate have on indigenous microbiology beneath one of the existing burial sites?
 - a. Did pathogens transport?
 - b. What effect did leachate chemistry have on the subsurface microbiology?

1.3 Thesis Structure

This introduction is followed by five manuscripts (each manuscript addresses a set research question(s) outlined above) each manuscript is presented as a single chapter preceding the final concluding chapter. Each published manuscript is presented in its entirety as submitted or published with minor modifications made to accommodate the editorial guidelines provided by the College of Graduate and Postdoctoral Studies for manuscript-based dissertations, others under review are presented as submitted with minor editorial changes. The first manuscript (Chapter 2) titled “Speciation and Geochemical Implications of Carcass Burial Leachate”, was submitted for publication to the *Transactions of the ASABE* journal. This paper is a culmination of leachate chemistry data from Pratt (2009) and mathematical geochemical calculations to further describe the geochemical properties of the leachate as well as implications the specific set of chemistry has on these geochemical thermodynamic calculations and how that affects the chemical speciation of the leachate. This information provides insight on specific chemical properties to consider upon selection or approval of mass mortality burial site locations and their implication to transport

processes and reactions in the subsurface environment of soil and groundwater. Major findings of this assessment include the ability to calculate geochemical thermodynamic properties using the Truesdell-Jones activity calculation instead of a more advanced and less substantiated Pitzer equation for the chemistry of concern. This was then used to enhance the speciation of the mortality burial leachate to demonstrate specific ion species that may not have been expected and would allow the transport of unattenuated elements of concern such as ammonium, in the form of ammonium sulfate, or phosphorus in the form of orthophosphate or phosphoric acid.

The second manuscript (Chapter 3) titled “Determination of Distribution and Selectivity Coefficients for a Saskatchewan Till Using Simulated Livestock Mortality Leachate” has not been submitted for publication but is included in this thesis for context for further modelling efforts. This study was the first to assess ammonium sorption in a clay based soil at concentrations above 4000 mg/L. Commonly sorption is assessed for concentrations less than 100 mg/L and is predicted at that level to have high retardation factors. This study demonstrated the sorption effects at ionic strengths associated with livestock burial leachate and predicted ammonium sorption and distribution coefficients for other cations in solution. Sorption isotherms followed a Langmuir distribution with coefficients ranging from 1.5 to 0.003 L/kg at low and high concentrations, respectively. Ammonium selectivity coefficients increased with increasing ionic strength. Due to analytical issues in the laboratory, portions of this study remain incomplete. This study also aimed to assess changes in cation exchange capacities (CEC) due to the ionic strength effects of the leachate, it was shown that CEC of the soil changed as it was exposed to increasingly higher concentrations of ammonium based leachate. Errors in measurement were accounted for by performing an ion exchange mass balance and it was concluded that due to the laboratory methods used to measure both calcium and magnesium, the mass balance could not be closed appropriately. While this manuscript may not be suitable for publication due to errors associated with some of the measurements, useful ammonium sorption isotherm information was obtained, and since that is the ion of most interest and in high quantity, this specific dataset will be utilized in the modelling efforts that follow.

The third manuscript (Chapter 4) titled “Geochemical Modelling of Livestock Mortality Leachate Transport Through the Subsurface”, was submitted for publication to *Biosystems Engineering* and

is currently accepted pending final editorial review. This study provided a variety of conservative and reactive transport models based on burial pit guidelines for Western Canada. Leachate chemistry input data from these models was based on data generated by the first manuscript, with subsurface properties assumed for a typical Saskatchewan glacial till. This study provides clear imagery and a better understanding of reactive transport implications from this leachate as it moves through the subsurface, along with potential “worst-case” scenario implications. Models utilized include several two-dimensional models created in GeoStudio SEEPW/CTRAN in both a conservative and sorptive scenario for both single burial trenches and two multiple trench spacing scenarios. PHREEQC (USGS) was also used to further enhance this modelling effort by providing geochemical reactive transport solutions to leachate transport beneath the burial trench. Utilizing both modelling tools, a clearer picture on how leachate might move through the subsurface, how it might be attenuated, and plumes characteristics to look out for were key findings. For example, due to ion reaction processes involving the carcass burial leachate and subsurface soils, a hard water plume consisting of calcium and magnesium may be observed well ahead of the chloride or ammonium plume directly related to carcass burial. Ammonium sorption processes, given a soil with a cation exchange capacity of approximately 10 meq/100g will require 2 to 3 meters of depth to attenuate the majority of ammonium in the system. Up to 1% however, may still remain, which is a concentration higher than the drinking water limit. At sites properly suited for burial, transport of leachate after 100 years shows minimal risk to the environment.

The fourth manuscript (Chapter 5) titled “Geochemical Evolution and Leachate Transport Beneath Two Carcass Burial Sites: A Field Investigation”, was submitted for publication to the *Transactions of the ASABE*. This study involved the geochemical investigation of two existing carcass disposal sites in Saskatchewan and provides the first ever glimpse at subsurface impacts directly beneath and adjacent these sites and also provides a comparison to models produced in the previous chapter. The first site was utilized to dispose of elk euthanized from CWD. After nearly 7 years of burial at this site near Pierceland, SK, leachate transport of conservative ions such as chloride reached a depth of approximately 1.5 meters beneath the burial trench bottom. Ions of concern such as ammonium at that same site were attenuated within the first half-meter beneath the trench after approximately 7 years. The second site that was investigated arose from Canada’s only FMD outbreak in 1952 and was explored approximately 60 years post burial. This

site also demonstrated slow vertical movement of the carcass leachate to depths of 1 to 2 meters beneath the assumed pit bottom. Due to the presence of sand lenses at this site, lateral movement of up to 10 meters was observed for conservative ions. Ammonium at this site was attenuated to within a meter of the burial pit. Results from these field investigations demonstrate hard water fronts predicted by the geochemical models from the previous chapter with 100 to 1000 times greater than background concentrations of calcium and magnesium in front of the ammonium/potassium plume. While the 60 year old plume is somewhat complicated due to sand lenses present, they depict lateral movement of the hard water front of the plume consistent with the depths in which the sand lens was encountered while drilling. In the end, after 60 years of transport, even in the presence of a “less secure” site, the impact from burial at this location was minimal as the majority of ions of concern were attenuated appropriately. Similarly, for the younger site, attenuation and slow movement of ions have shown no significant environmental impact. It should be noted that both of these sites consisted of till strata with hydraulic conductivities in the range of 10^{-9} m/s or less, which would be considered relatively low but typical of tills found in Saskatchewan.

The fifth and final manuscript (Chapter 6) titled “Influence of Mass Burial of Animal Carcasses on the Types and Quantities of Microorganisms within a Burial Site”, has been published in the *Transactions of the ASABE* (Pratt et al. 2012). This study was the first of its kind to design a methodology to employ cpn-60 taxonomic profiling to an environmental sample (soil). The study was also the first to investigate microbial populations, their movement, and the effect leachate chemistry had on their ability to flourish in the subsurface. The quantities of microbes present was evaluated using quantitative PCR with the quantity of microbes present in the burial trench 2-5 times more than samples 2 meters below the burial trench. From the qualitative microbial analysis 5825 operational taxonomic units (OTUs) were identified in 13 core samples with community composition changing significantly with depth. Organisms associated with specific processes such as phosphate accumulation or sulfate reduction were categorized and related back to leachate processes occurring at similar depths. Anaerobic microorganisms were the dominant community at consolidated depth/location of animal burial.

The final chapter provides a summary of this body of work in its entirety as well as the successes of addressing specific research objectives. Recommendations for future research in this area as well as general discussion and comments are also provided.

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Chapter 2: Ion Activity and Speciation of Carcass Burial Leachate and Ionic Strength Impacts

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2.0 Preface

With the onset of crisis epidemiological events in livestock herds such as the 2001 Foot and Mouth Disease (FMD) outbreak in the United Kingdom, and the discovery of bovine spongiform encephalopathy (BSE) in Canadian cattle herds in 2003, the Canadian Food Inspection Agency (CFIA) pushed to generate evidence based information for regulatory action in the event of disease outbreaks or disasters. Through this mandate, the University of Saskatchewan, under Dr. Terry Fonstad, P.Eng., P.Ag., was awarded research grants through the Saskatchewan Ministry of Agriculture and Manitoba Livestock Manure Management Initiative to 1) determine the chemistry of leachate produced by decomposing carcasses in burial situations; and 2) to evaluate and determine the risk and impact of this leachate (both solution chemistry and microbiology) on the subsurface environment. To accomplish objective #1 a M.Sc. thesis (Pratt 2009), constructed livestock burial pits segregated by species (bovine, swine, poultry) and monitored leachate chemistry over two years of decomposition. Some of this work from Pratt (2009) is presented as part of this PhD manuscript to provide context to the continued analysis that was completed as part of this chapter. Upon completion of Pratt (2009), it was determined that in order to fully characterize and understand the geochemical impacts of livestock mortality leachate, this PhD project required further geochemical analysis of the dataset provided by Pratt (2009), specifically an assessment of ion activity based geochemical calculations. The ionic strength of the mortality leachate sits in a grey area with respect to thermodynamic calculations for ion activity. To assess this, it was imperative to re-evaluate the leachate chemistry from a more involved thermodynamic perspective. For readability and context, Chapter 2 presents how mortality burial leachate

chemistry was obtained and the concentrations over decomposition (first 2 years post burial) as seen in Pratt (2009) (specifically, sections 2.3.1, 2.3.2.2, 2.4.1 and 2.4.4) and was coupled with further thermodynamic geochemical assessment and validation of equations to be used for modelling efforts here forward (sections 2.3.2, 2.3.2.1, 2.4.3). Further analysis of this leachate included assessing ion activity changes over a series of dilution steps artificially created from maximal leachate values. This enabled the sensitivity analysis of specific ions of interest and how they were affected by which equation was used to calculate their respective activities. This work was imperative to inform future geochemical models, as an under or over estimation in this parameter may lead to significant over or under estimations in thermodynamic reactions, ion exchange and transport. For further context leading into future chapters of this thesis, initial PHREEQC speciation models (section 2.4.4) seen from (Pratt 2009) are also presented in this manuscript and explained in a more concise fashion due to the fact that the evaluation of ion activity parameters led to the original speciation model being still valid after this assessment.

2.1 Abstract

In the event of a mass livestock mortality situation, disposal routes such as burial are commonly chosen. The impact of burial on the environment could be substantial, but the composition of the leachate arising from a burial site prior to the onset of this study, has not been well documented. A study was performed to determine the chemical composition of leachate arising from animal mortalities in a burial setting. Three species of livestock were used: bovine, swine and poultry. Leachate collected from lined burial pits over two years of decomposition was analyzed for major and minor ions. Analysis indicated that livestock mortality leachate contains on average, concentrations of 12,600 mg/L of ammonium-N, 46,000 mg/L alkalinity (as bicarbonate), 2,600 mg/L chloride, 3,600 mg/L sulfate, 2,300 mg/L potassium, 1,800 mg/L sodium, and 1,500 mg/L phosphorus along with relative lesser amounts of iron, calcium and magnesium. Maximum concentrations of select samples had concentrations of ammonium-N and bicarbonate up to 50% higher than these average values. In comparison to earthen swine manure storages and landfills, the strength of the leachate was 2-4 times higher and therefore its impact to water resources could be greater. Following the study of livestock mortality leachate chemistry composition, the potential impacts of this leachate on the soil/water systems below a burial site were investigated. The ionic

strength of this leachate presents its own set of challenges. Basic modelling of ion activity utilizing the five most common activity coefficient equations (Debye-Hückel, Extended Debye-Hückel, Truesdell-Jones, Davies and Pitzer) were considered to assess the sensitivity of these methods on calculated ion activity as impacted by the significant ionic strength of the leachate. This was completed to further enhance modelling and speciation efforts. Based on these results, PHREEQC was used to assess chemical speciation of the leachate. The speciation of this leachate provides evidence of phosphate and sulfate compounds available for potential unattenuated transport. Understanding the geochemical implications of livestock mortality burial, will give scientists and regulators more information to perform risk analysis when considering mortality burial as a management option either routinely or during a mass mortality event.

2.2 Introduction

Increasing livestock populations and catastrophic large scale occurrences of mass mortality events due to natural disasters and disease outbreaks in North America provide potential for greater risks to the environment. Burial, incineration, composting and rendering are commonly employed methods to deal with carcass disposal (Engel et al. 2004; Cleary et al. 2010). Burial is a case which has not been studied in great detail (Ritter and Chirnside 1995; Glanville 2000; MacArthur and Milne 2002; MacArthur et al. 2003; CFIA 2006; Pratt 2009; Pratt and Fonstad 2009b; Pratt and Fonstad 2009a). Characterization of the leachate produced from mortality burial has been rarely investigated and fully understood. Characterization is required for scientists and regulators to better predict and evaluate risks involved with the transport of potential contaminants arising from a mortality burial site. The purpose of this study was to determine the chemical composition of the leachate released over a period of two years from livestock mortalities during decomposition for three species: bovine, swine and poultry.

Previous investigations involving livestock burial and subsequent soil and groundwater contamination focused on monitoring groundwater quality surrounding existing disposal sites, while information on the chemical constituents of the leachate is lacking (Ritter and Chirnside 1995; Glanville 2000). Glanville (2000) reported on the impact of livestock burial on shallow groundwater quality. He noted that proper disposal of livestock mortalities can be more difficult than manure management due to the rapid breakdown of the animal carcasses in the environment.

Glanville (2000) used two case studies to evaluate leachate, groundwater quality and contamination due to animal burial. A 1.8m deep pit containing 28,400 kg of turkey mortalities which demonstrated seasonally high water tables and low permeability demonstrated NH₄⁺-N concentrations up to 400 mg/L within 1 to 2 meters of the burial pit after 6 years of monitoring. The second case study consisted of engineered 1.2 m deep trenches containing 6 swine with a PVC liners and pumping systems to measure the duration of decay products utilizing lysimeter technology. This case study showed approximately 1350 liters of liquid were produced, equating to approximately 2,000,000 mg of nitrogen available to transport once decay is complete (Glanville 2000). Results from this work concluded that complete decay, even in well drained soils may take 2 years or more, and that significant groundwater contamination could occur in areas with high groundwater velocities.

Poultry disposal impacts on groundwater quality was examined adjacent to six existing disposal pits in Delaware (Ritter and Chirnside 1995). At the time of the study, the pits were still active with approximately 15 kg (33 lbs) to 25 kg (55 lbs) of dead birds added each week. To monitor the potential contamination, two to three monitoring wells were placed around the pits at a depth of 4.5 meters (15 ft). The bases of the pits were located within the water table for most of the year in sandy loam soil conditions. The potential for groundwater contamination at this site in those conditions was considered to be very high. This study concluded ammonia concentrations were high in two wells. Of the six pits, three of them demonstrated an increase in ammonia concentrations at a lateral distance of 12 m. In some cases the ammonia concentrations reached 366 mg/L and streptococci concentrations reached 209 per 100 mL in monitoring wells at distances of 6 meters. Over 80% of the groundwater samples near the pits did not contain fecal streptococci. One of the most relevant studies as compared to this paper is reported from the United Kingdom. MacArthur et al. (2003) studied leachate characteristics and virus survival arising from a foot and mouth mass burial site in Scotland. The study site consisted of six pits containing 400,000 livestock mortalities culled during April and May of 2001. The pits contained a mix of species, but the majority were culled sheep. They reported that the carcasses soon form an unstable mass of waste causing significant difficulties with burial. Due to bloating of carcasses and the uplift forces this creates, carcasses rise to the surface and rupture, potentially spreading pathogens in an aerosol form. To manage the leachate formed from the pits, longitudinal drains were installed and leachate

was collected in 40 ISO containers with 20,000 liter capacity each. Their calculations and measurements indicated approximately 4000 m³ of leachate was generated. Leachate sampling and analysis was performed and averaged values were reported from 199 samples of leachate, showing concentrations of ammonia-N, alkalinity, BOD & COD of 3294 mg/L, 9400 mg/L, 12700 mg/L and 20414 mg/L respectively for the time period. Due to the significant rain events during the burial phase, this leachate was likely impacted due to dilution effects from rainwater entering and pooling in the burial pit.

More recently, foot and mouth disease outbreaks around the world have caused significant environmental and economic impacts in countries such as South Korea and Taiwan (Ozawa et al. 2006; Kim et al. 2014; Hseu and Chen 2016). The FMD outbreak in South Korea in 2010 was catastrophic causing the cull and disposal of approximately 3.48 million animals via more than 4,500 on-farm burial sites (Park et al. 2012; Lee 2013), many of which proved to show adverse effects to the environment via subsurface and surface water contamination (Kim and Pramanik 2015; Kim and Kim 2015).

Very few studies give a complete analysis of unmixed or undiluted leachate resulting from the decomposition of buried animal carcasses. The objective of this study was to determine the leachate chemistry of bovine, swine and poultry so that risk analysis could be completed to determine the potential for mass mortality burial in various geological settings. Secondary objectives were to evaluate the leachate's geochemical composition and speciation and to provide comparisons on the effects of ionic strength on ion activity coefficients utilizing five common activity theories in solution through a series of dilution steps. This information can then be used to provide a clear starting point for future geochemical modelling for this leachate in soil and groundwater systems.

2.3 Materials and Methods

2.3.1 Field Experiment Set-up

Three burial pits were constructed in fine sandy clay soil southeast of Saskatoon, SK, Canada and loaded with livestock carcasses (Pratt 2009). Each pit was excavated to dimensions of 7 x 9 meters

and a depth of 2.5 meters, with a buffer zone of approximately 20 meters between each pit. The pits were completely lined with 40 mil polyethylene with a leachate collection system located at the bottom-center of the pit. (Fig. 2.1a) and were completely sealed by placing a 40 mil polyethylene cover on top of the carcass to ensure undiluted leachate samples. This cover also contained two ABS vent pipes to allow for gas transfer with the atmosphere. Approximately 0.9 m to 1.2 m of soil, previously excavated from each pit, was placed on top of the polyethylene cover and graded to direct rain water off of the mounded soil. One pit of each species: poultry, swine and bovine were evaluated over a period of time for pure, undiluted leachate chemistry.

Pit No. 1 (Poultry) was installed on day No. 1 and contained 1300 kg of chicken carcasses in an environment sealed from contact with soil and water. Pit No. 2 (Swine) was installed on day No. 8 and contained approximately 5900 kg of swine carcasses in a sealed environment. Pit No. 3 (Bovine), installed on day No. 8, contained approximately 3920 kg of bovine carcasses in a sealed environment.

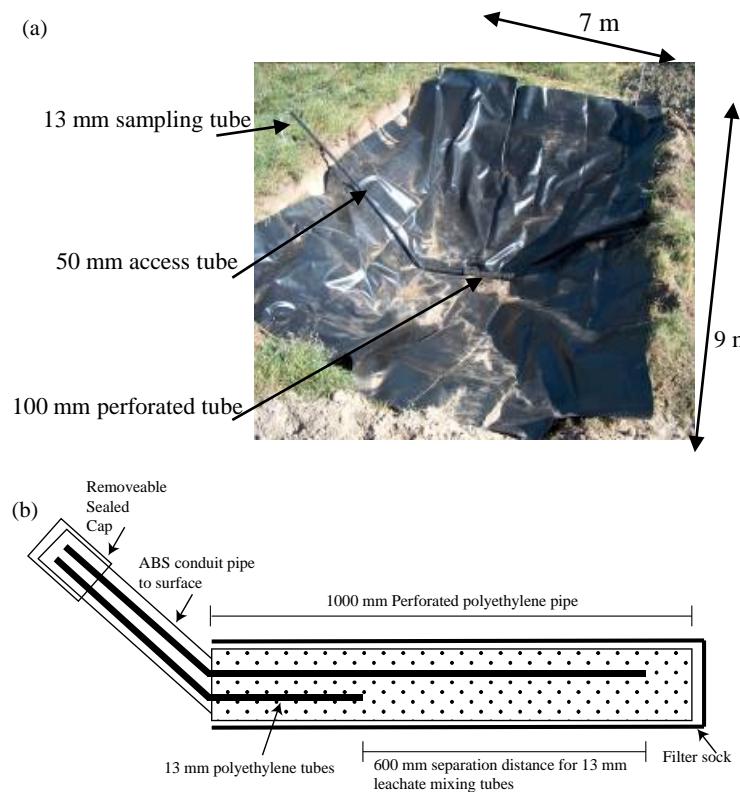


Figure 2.1 (a) Pit construction; (b) leachate collection system (Pratt 2009)

All pits contained a leachate collection system consisting of a 100 mm x 1 m perforated polyethylene pipe covered with a filter sock and placed within a sump in the bottom center of the pit on top of the polyethylene liner. This 100mm perforated pipe was attached to a 50 mm diameter ABS conduit pipe that extended to the surface. Inside the large diameter perforated pipe at the base of the pit, a sub-system of pipes were nested inside and consisted of two 13mm clear vinyl tubes with the inlet ends placed 600 mm apart running to the surface via a 50 mm ABS conduit pipe (Fig. 1b). The purpose of the dual lines was to provide a mechanism of mixing before the leachate sample was collected. Leachate was circulated from the near end of 100mm diameter chamber at the base of the pit, up to the peristaltic pump and pushed back down to the far end of the chamber without introducing any atmospheric air into the system.

Sampling was conducted periodically over a period of two years in order to observe trends in chemical composition as decomposition occurred. Leachate samples were drawn from the pits at two weeks after burial (day No. 8) for Pit No. 1 (poultry), and at two weeks for the remaining pits on day No. 21. Samples were then taken from all pits at 1 month, 2 months, 3 months; 9 months, 14 months and 25 months. Due to winter freeze-up, samples were not taken between the third and ninth month post-burial. Sampling was conducted with the use of a peristaltic pump connected to each 13 mm tube at ground surface. The pump was used to circulate fluid through the tubes for a period of 5 minutes. Once leachate was thoroughly mixed within the 100mm x 1m chamber, a 1 liter sample was drawn and stored at 4°C for transport to the lab. Samples were submitted to SRC Analytical Laboratories in Saskatoon for major and minor ion analysis. Chemical properties analyzed include: Ammonium by colorimetry, Bicarbonate and Carbonate by alkalinity, Chloride by colorimetry, Hydroxide, pH, Specific Conductivity, Alkalinity by ICP-AES, Nitrite-Nitrate Nitrogen by Colorimetry, Total Kjeldahl Nitrogen, Inorganic and Organic Carbon with the Dohrmann Phoenix 8000 Carbon Analyzer, and Aluminum, Barium, Beryllium, Boron, Cadmium, Calcium, Chromium, Cobalt, Copper, Iron, Lead, Magnesium, Manganese, Molybdenum, Nickel, Phosphorus, Potassium, Silicon, Silver, Sodium, Strontium, Sulfate, Sulfur, Titanium, Vanadium, Zinc, Zirconium by ICP-AES.

2.3.2 Geochemical modelling and analysis

Once leachate chemistry was obtained, it was imperative to evaluate the risk this specific leachate could have on the environment it may encounter. In order to do this, basic geochemical processes such as ion activity and ionic strength, speciation, and saturation indices were characterized utilizing numerical methods and PHREEQC (Parkhurst and Appelo 1999) software available from the USGS (United States Geological Survey, version 3.3.9).

2.3.2.1 Activity coefficient and ionic strength assessment

Activity coefficients have significant effects on thermodynamics, solubility and exchange in soil/groundwater interactions. The ionic strength of burial leachate is significant when compared to drinking water and dilute solutions. The leachate chemistry findings were evaluated based on ionic strengths of the solution through a series of dilution steps to determine which method of determining ion activity coefficients best represents ion activity of this solution at its most concentrated to its most dilute; makes its way through the subsurface. As solutions become increasingly concentrated, the distance between ions and water molecules diminishes, thereby ions come into contact with each other and interact via Coulombic effects (Langmuir 1997). Ionic strength (I) of the leachate was calculated as shown in (Eqn. 1) where z_i is the specific ion charge and c_i is the molar concentration of ion i (Lewis and Randall 1921).

$$I = \frac{1}{2} \sum_i z_i^2 c_i \quad (2.1)$$

The ionic strength of the solution is then used in the calculation of ion activity coefficients based on relative concentrations of each ion in solution among other parameters such as temperature, dielectrics, ion size, and fit parameters required for the different approaches used. Five common approaches for ion activity coefficient calculations were considered and included: Debye-Hückel (Debye and Hückel 1923), Extended Debye-Hückel (Harned and Owen 1958), Truesdell-Jones (Truesdell et al. 1974), Davies (Davies 1962) and Pitzer (Pitzer 1987) (Table 2.1). Due to the nature of the leachate, an assessment between available ion activity coefficient calculations was necessary to choose an appropriate thermodynamic model to assess chemical speciation.

Table 2.1 Equations available for calculation of ion activity coefficients in solution

Activity Coefficient Calculation	Equation	Ionic Strength Validity	Reference
Debye-Hückel	$\log \gamma_i = -Az_i^2 \sqrt{I}$	$< 10^{-2}$	Debye and Hückel, 1923
Extended Debye-Hückel	$\log \gamma_i = -Az_i^2 \left(\frac{\sqrt{I}}{1 + Ba_i \sqrt{I}} \right)$	$< 10^{-1}$	Harned and Owen, 1958
Davies	$\log \gamma_i = -Az_i^2 \left(\frac{\sqrt{I}}{1 + \sqrt{I}} - 0.3I \right)$	< 0.5	Davies, 1962
Truesdell-Jones	$\log \gamma_i = -Az_i^2 \left(\frac{\sqrt{I}}{1 + Ba_i^0 \sqrt{I}} \right) + b_i I$	< 1.5	Truesdell et al., 1974
Pitzer	$\log \gamma_i = z_i^2 f^\gamma + \sum_i D_{ij}(I)m_j + \sum_{ijk} E_{ijk} m_j m_k + \dots$	> 2	Pitzer, 1987
where:	z_i : charge of ion i a_i : effective size of the hydrated ion I : ionic strength $A = 1.82 \cdot 10^6 (\epsilon T)^{-3/2}$ $B = 50.3 (\epsilon T)^{-1/2}$ ϵ : dielectric constant of water T : Temperature in Kelvin b_i : fit parameter		

Each of these equations has specific scenarios and sets of limitations for its use. The Debye-Hückel (1923) equation was the first of its kind to describe Coulombic interactions of ions with the assumption that ion size is constant over varying ionic strengths and that there is no reaction between ions of the same charge. The extended version of the Debye-Hückel (Harned and Owen 1958) equation provided an increase in range of applicable ionic strengths by incorporating an additional term and effective ion size parameters which include aspect of the hydrated shell of the ion. The Davies (1962) equation was developed to further extend the range of ionic strengths of solutions to approximately 0.5 molar by eliminating ion size parameters and adding a linear relationship of $0.3I$ that accounts for increased ion pairing due to increased concentrations of dissolved ions. The Davies equation is commonly used due to its mathematical simplicity. Since the ion size parameter was removed, it is assumed that the same activity of ions of the same charge

will be consistent at a specific ionic strength. Many geochemical software suites base their thermodynamic databases from the Truesdell-Jones (Truesdell et al. 1974) theory. Due to the addition of ion size and fit parameters, accommodation of greater ionic strength solutions is possible. Similarly, the same is true for the Pitzer (1987) model; which can be applied to ionic strength solutions ranging from about 2-6 molar by taking into account binary and ternary ion interactions. Pitzer limitations include lack of ion interaction parameters needed for multicomponent solutions and estimation procedures are very limited to determine these parameters (Kim 1988). Many thermodynamic databases that include the Pitzer model also do not account for NH_4^+ (a problem for this study due to the leachate consisting predominantly of NH_4^+) (Plummer and Parkhurst 1990).

For this study, ionic strength was evaluated for the maximum concentrations observed in the mortality leachate using Equation 2.1. Activity coefficients were then calculated for each ion using all equations except Pitzer. Ion size parameters for the major ions in solution for the Debye-Hückel equations were described in Kielland (1937), while Truesdell-Jones ion size (a_i^0) and fit (b_i) parameters were taken from the thermodynamic database *wateq4f* (Truesdell et al. 1974). Ion activity coefficients were then compared against each other and a sensitivity analysis was performed to assess the applicability of each model on this type of leachate from concentrations ranging from 100% strength ($I=1.5 \text{ M}$) to 0.005% ($I=0.007 \text{ M}$).

2.3.2.2 Geochemical speciation

PHREEQC (Parkhurst and Appelo 1999) was used to characterize the leachate chemistry. This geochemical software is available from the USGS and has the capabilities to provide speciation and saturation indices for chemical solutions based on the user's choice of several thermodynamic databases. This software was used to calculate species distribution for all ions entered and saturation indices of the related precipitates and partial pressures of gasses. To provide a representation of the element species that can be present in the mortality leachate, an average value from 9 to 24 months post burial from all three species bovine, swine and poultry mortality leachate was used and are shown in Table 2.2. The thermodynamic database used to calculate the speciation comes from Parkhurst and Appelo (1999) and is titled phreeqc.dat. This analysis helped to estimate

what chemical species are present along with their respective activity in solution. The Truesdell-Jones equation was used to determine activity coefficients for this initial analysis.

Table 2.2 Average mortality leachate concentrations per species and total (Pratt 2009)

	units	Poultry	Swine	Bovine	Average
Bicarbonate	mg/L	39130	48470	50730	46100
Chloride	mg/L	2570	2380	2815	2600
pH		6.5	6.7	6.9	6.7
Total Alkalinity	mg/L	22500	39700	41600	34600
Ammonium as Nitrogen	mg/L	10400	13300	14100	12600
Nitrate + Nitrite Nitrogen	mg/L	2.3	3.1	3.8	3.1
Inorganic Carbon	mg/L	7700	9530	9950	9100
Organic Carbon	mg/L	79000	65000	68000	71000
Aluminum	mg/L	0.5	0.5	0.5	0.5
Calcium	mg/L	81	48	36	60
Copper	mg/L	0.9	1.7	0.6	1.1
Iron	mg/L	18	19	18	20
Magnesium	mg/L	79	17	18	40
Manganese	mg/L	0.5	0.1	0.1	0.2
Phosphorus	mg/L	1930	1515	1150	1500
Potassium	mg/L	2400	2400	2000	2300
Silicon, soluable	mg/L	20	24	26	20
Sodium	mg/L	1600	1700	2000	1800
Sulfate	mg/L	3970	3900	2900	3600
Sulfur	mg/L	1300	1300	965	1200
Zinc	mg/L	2.2	1.8	1.7	1.9

2.4 Results and Discussion

2.4.1 Leachate chemistry

Two years of leachate chemical analysis demonstrated chemical evolutions in the leachate and showed the following ions that were the most abundant and include: ammonium, bicarbonate, calcium, magnesium, iron, potassium, phosphorus, sulfate, sodium, and chloride. Each major ion is presented below in Figure 2.2. For a complete list of ions for each species at each sample date, the reader is referred to Pratt (2009).

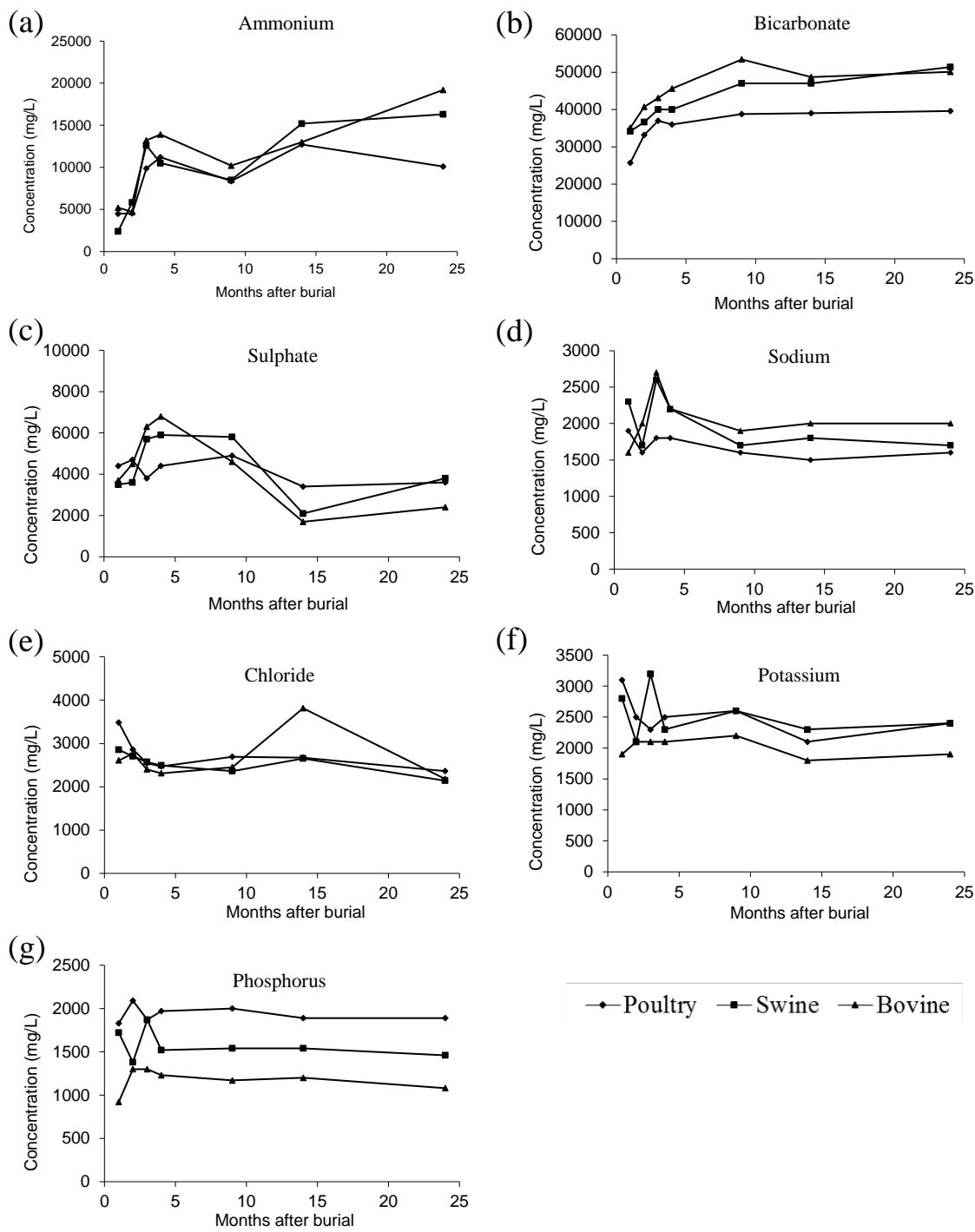


Figure 2.2 Concentrations of pure leachate: (a) Ammonium-N, (b) Bicarbonate, (c) Sulfate, (d) Sodium, (e) Chloride, (f) Potassium, (g) Phosphorus (Pratt 2009)

As the livestock carcasses decomposed, ammonium concentrations begin to rise (Figure 2.2a). In the early stages of decomposition, up to the first two months, ammonium concentrations were at their lowest of approximately 5,000 mg/L for each species. Ammonium continued in an upward trend for all sample dates except between four and nine months. At two years post burial, bovine had the highest concentration at 19,200 mg/L, which is consistent with a maximum ammonium value recorded at the mass mortality burial site in Scotland by MacArthur et al. (2003), with swine following closely at 16,300 mg/L and poultry having the lowest ammonium concentration at 10,100 mg/L. Average ammonium concentrations were 12,600 mg/L.

Since nitrogen is the third most abundant element in the body, the trend of Figure 2.2a likely follows the breakdown of different cellular systems in the livestock. The first period of sampling between 1 month and 2 months likely depicts the excretion of components in the digestive system such as urine and manure. The second period between 2 months and 4 months likely represents the breakdown of blood and liquids in the animal similar to human processes described by Vass (2001). Between 4 months and 14 months, it becomes harder to distinguish what might be occurring in the decomposition process. The breakdown of heavy proteins such as muscles and bone marrow is a possible route of more nitrogen appearing in the analysis at those time periods (Vass 2001; Tatsi and Zouboulis 2002).

Bicarbonate (HCO_3^-) concentrations during the sampling period of each species of livestock followed a similar trend (Fig. 2.2b). Poultry (Pit No. 1) started with a concentration of 25,700 mg/L and after two years had a concentration of 39,600 mg/L. Swine (Pit No. 2) had a concentration of 34,200 mg/L at the first sample date and ended with a concentration of 51,400 mg/L after two years of decomposition. Bovine (Pit No. 3) was similar to swine and started at 35,100 mg/L and increased to a peak concentration at nine months at 53,400 mg/L and decreased to 50,100 mg/L after 24 months. Average concentrations of bicarbonate from mortality leachate can be estimated at 46,000 mg/L. Poultry concentrations of bicarbonate could be expected to be up to 10,000 mg/L less than the average, while swine and bovine could be up to 4,000-6,000 mg/L above the average. These values are 3 times the median/average reported by MacArthur and Milne (2002) of approximately 14,000 mg/L, but half the maximum reported value of approximately 106,000 mg/L.

Sulfate (SO_4^{2-}) concentrations for each species follows the same general trend for the sample period (Fig. 2.2c). Poultry at the first sample date started with a concentration of 4,400 mg/L and increased slightly until the ninth month, after which concentrations decreased 3,600 mg/L at 24 months. Swine initially had a concentration of 3,500 mg/L and increased sharply for the first four months to 5,900 mg/L and decreased to 3,800 mg/L by 24 months post burial. Bovine reached the highest concentration at 6,800 mg/L within four months of burial and after 24 months had a concentration of 2,400 mg/L. Although there is variance throughout the sample dates, each species is similar within a year post-burial. An average sulfate concentration to expect in livestock mortality leachate is approximately 3,600 mg/L.

Each species analyzed presents similar concentrations of sodium (Fig. 2.2d) for all sample points. Bovine initially had the lowest concentration of sodium. After four months, sodium concentrations in bovine leachate reached 2,700 mg/L. Swine initially had the highest concentration of sodium at the first sample date at 1,700 mg/L. By the fourth month, the concentrations in the swine leachate followed the same trend as bovine with a concentration of approximately 2,200 mg/L and then decreased to approximately 1,700 mg/L for the remaining sample periods. Poultry began with a concentration of 1,900 mg/L, and slightly decreased to approximately 1,600 mg/L for the remainder of the sampling period. Average sodium concentrations expected from mortality leachate are approximately 1,800 mg/L.

Chloride concentrations for each species generally follow the same trend and have similar concentrations for all sample dates (Fig. 2.2e). Bovine reached the highest concentration at fourteen months with a chloride concentration of 3,810 mg/L. The results follow slightly different paths to the same end. An average chloride value for livestock decomposition leachate was considered to be 2,600 mg/L.

Potassium concentrations for each species did not fluctuate significantly during the 24 month sample period (Fig. 2.2f). During the entire sample period, bovine had the lowest average concentration and poultry and swine had a slightly higher concentration. The breakdown of proteins could be a major source of potassium production in the leachate (Vass 2001). The average

potassium value for the three species during the last three sample periods is 2,300 mg/L, with bovine approximately 25% lower than swine and poultry.

Phosphorous concentrations for each species generally follow the same trend of slight fluctuations in early sample dates to a leveled value by fourteen months (Fig. 2.2g). Poultry contained the highest amount of phosphorous with a maximum value of 2,090 mg/L. Swine had a maximum value of 1,870 mg/L and bovine the lowest at a maximum value of 1,300 mg/L. Differences in the values could possibly be explained by diet and biological attributes of the species. Poultry, in general have a significantly smaller frame than bovine and swine and, due to the large amount of feathers, have more protein structures that include phosphorous molecules (Okanovic et al. 2009). As for pure swine leachate, each sample date follows the same trend. Bovine also follow the same basic trend and are not statistically different. Average phosphorus concentrations range between 1,200 (bovine) and 1,800 mg/L (poultry). These values are significantly higher than those reported by MacArthur and Milne (2002) who reported an average value of 55 mg/L and a peak concentration of 476 mg/L.

Due to the early, rapid release of fluids from the livestock such as urine, digestive fluids and blood, higher concentrations for most ions are not achieved until 6-9 months post-burial. If livestock are buried in a soil with low hydraulic conductivity, a solution will be present for long periods of time, allowing the accumulation of higher concentrations due to the decomposition of the animal carcass. Therefore, the expected concentrations of livestock mortality leachate were averaged over the last three sample dates i.e. 9 months to 24 months post burial. Table 2.2 provides average concentrations expected per species and a total average which could be expected. Total alkalinity or bicarbonate is likely the value with the most uncertainty due to difficulties involved with titration of the original leachate solutions.

Livestock mortality leachate is relatively highly concentrated compared to other livestock solutions such as hog manure (Fonstad 2004), and landfill leachate (Thornton et al. 2001). Figure 2.3 shows the difference between the results of this study to other potential contaminants such as earthen manure storages, landfill leachate, and the Scotland burial site study, much of which has been exposed to precipitation and values reported are diluted. The results of this study show that

ammonium concentrations range from 2 to 4 times higher than hog manure storage lagoons and landfills, with the highest concentrations exceeding drinking water standards over 400 times. The threat occurs with this concentration when it reaches oxic conditions causing the ammonium to oxidize and form nitrate (Paul and Clark 1996). It is significant that the maximum ammonium concentration values found in this study match those values obtained from the mortality burial site in Scotland (MacArthur et al. 2003). Alkalinity in livestock mortality leachate is 60 times higher than drinking water standards and also exceeds concentrations in hog manure and landfill leachate by 20,000 mg/L. Alkalinity concentrations found at the Scotland site had a maximum value of 88,200 mg/L but average values were at approximately 12,000 mg/L. Average alkalinity concentrations from this study are nearly 20,000 mg/L higher than averages from Scotland. Many other constituents found in the mortality leachate also greatly exceed the concentrations found in manure storages and landfills and include sodium, sulfate, phosphorus, potassium and chloride. Calcium and magnesium present in the leachate was less than hog manure and landfill leachate. Iron is similar for all leachates, but its presence in solution is likely to complex with other ions or be adsorbed by the soil particles.

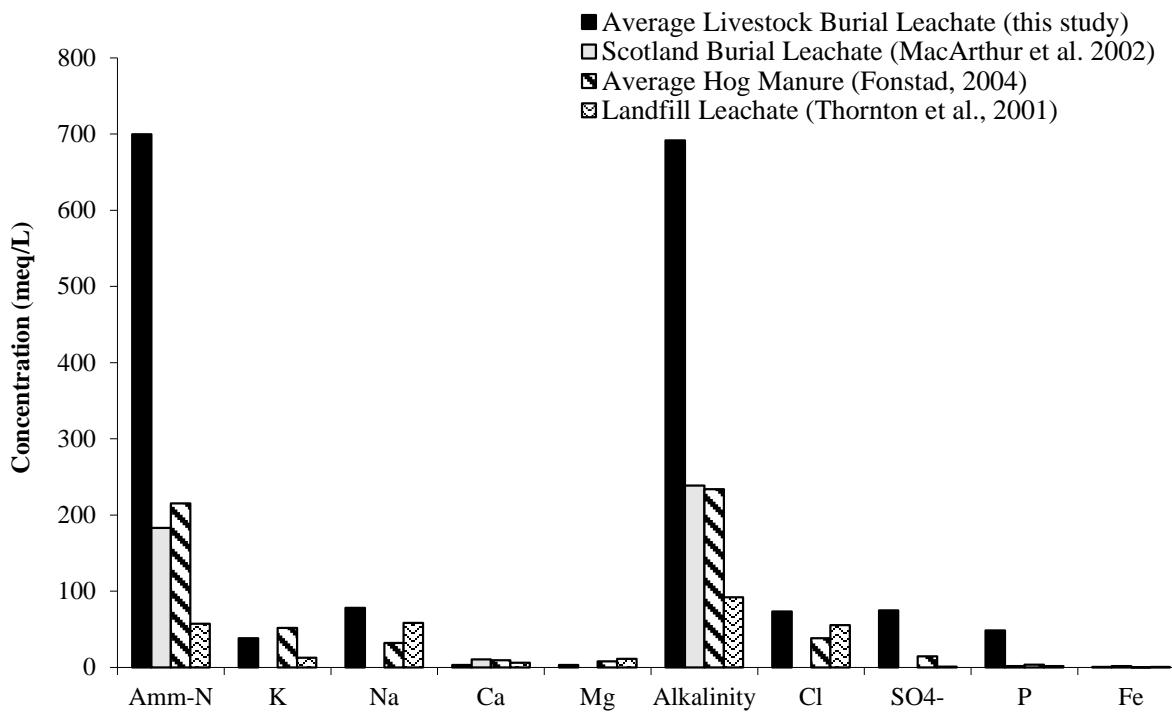


Figure 2.3 Comparison of carcass leachate to earthen manure storages and landfills (Pratt 2009)

2.4.2 Geochemical thermodynamic calculations and PHREEQC analysis

To simulate a worst-case scenario, chemistry results were analyzed for solution ionic strength and activity from the maximum value results reported for bovine (Pratt 2009). Maximum ionic strength was calculated using Equation 2.1 and a series of dilutions was performed from 100% incrementally to 0.005% of maximum values to find a range of leachate ionic strengths over which an analysis depicting behaviour of activity coefficients could be utilized. Geochemical speciations using PHREEQC (USGS) were then performed to provide a basis for future predictions of transport via geochemical modelling.

2.4.3 Ionic strength and activity of solution elements

Chemistry results for bovine were used at their maximum to perform a worst-case-scenario general analyses on the leachate chemistry. The ionic strength of the solution was calculated using major species components present in the leachate and produced an ionic strength upwards of 1.5 M (molar). As already stated, a series of dilution steps was performed on the chemistry results from

100% strength to 0.005% solution strength. This resulted in a range of ionic strengths from 1.5 M to 0.007 M. These dilution steps were then applied to four activity coefficient calculation equations (Debye-Hückel, Extended Debye-Hückel, Davies, and Truesdell-Jones) to help delineate appropriate theories with respect to ion activity coefficients to apply to future geochemical modelling efforts.

Results are depicted over a range of ionic strength dilution steps for most major cations found in the leachate. Cation results are important factors as they consist of the most significant portion of the chemical concerns found in the leachate (NH_4^+). Generally, most anions will transport through the subsurface unattenuated, so their results are not as significant with respect to ion exchange processes and attenuation and therefore not reported here. Figure 2.4 demonstrates the change in ion activity coefficients across a range of dilution scenarios for the bovine leachate. Since relative concentrations between species are consistent, it can be assumed that for the other two species (swine and poultry), these results would be similar at each ionic strength.

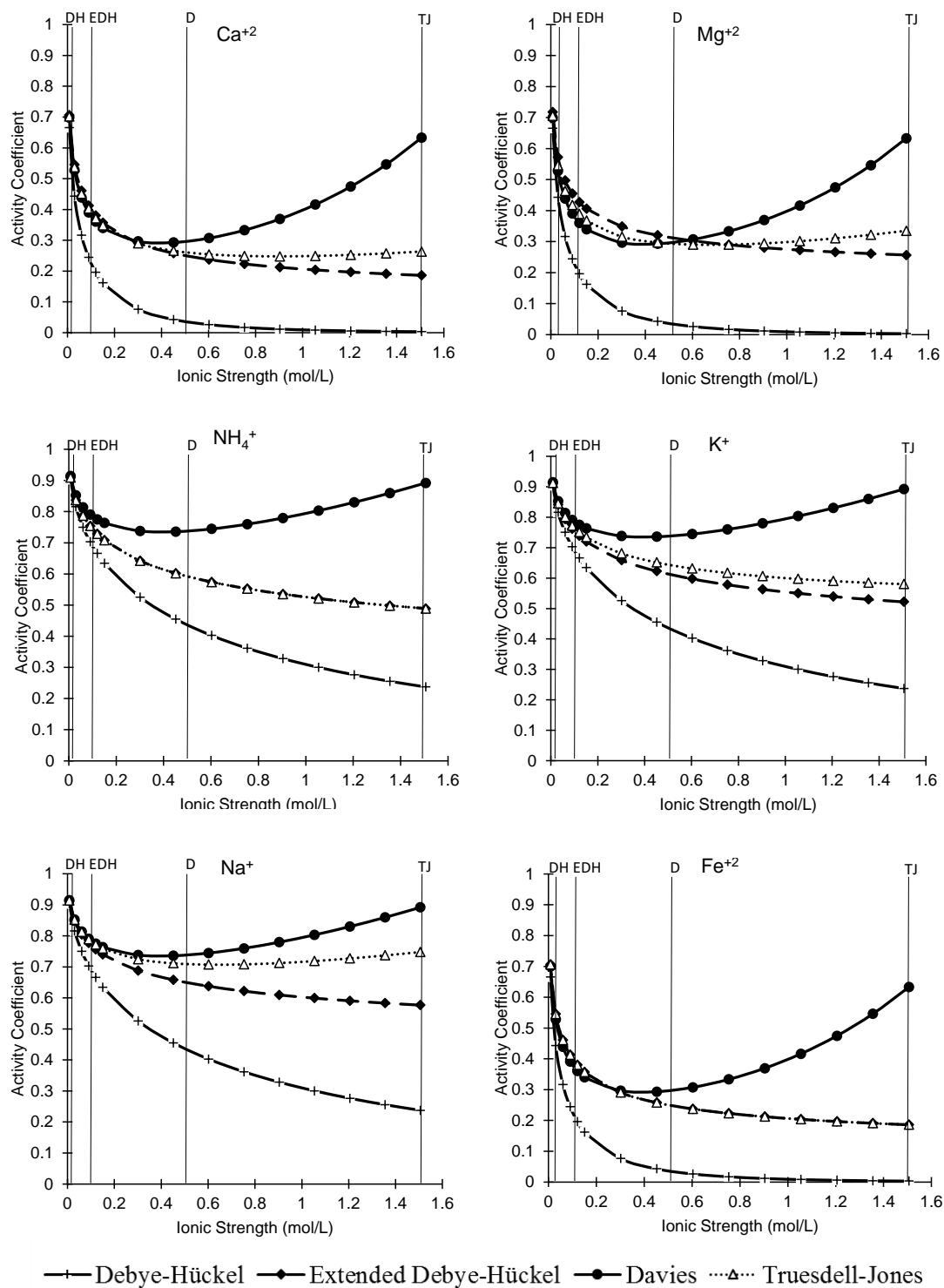


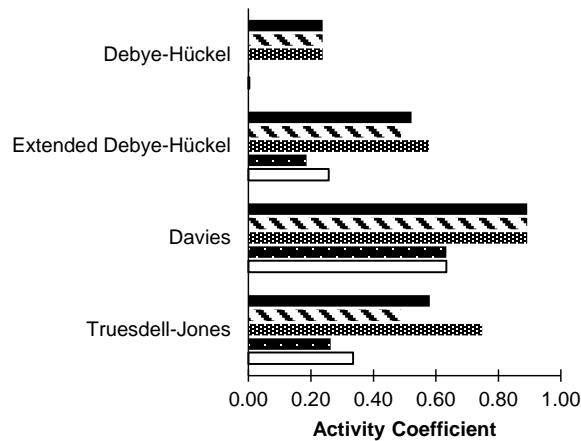
Figure 2.4 Activity coefficient comparison for leachate at varying ionic strengths with corresponding limits of applicability (vertical lines)

Figure 2.5 demonstrated effectiveness of each ion activity coefficient calculator at three specific ionic strengths in solution. The results shown depict similar trends as previously shown in the literature for salt based solutions (Lewis and Randall 1921). As a basis for analysis, it is generally accepted that as the ionic strength of a solution approaches zero (pure water), the activity coefficient approaches 1. Ion activity effects are also more pronounced for ions with a higher charge and smaller effective ionic radii. For example, Table 2.3 demonstrates atomic radii and hydrated radii for the most common ions in this leachate solution as well as their related parameters for evaluation based on the Truesdell-Jones equation. These parameters were determined for the Truesdell-Jones equation for each ion by fitting the equation to measured activities of pure salt solutions (Parkhurst 1990). Generally, ions with smaller hydraulic radii are thermodynamically preferred over larger ions as well as ions with higher valence being preferred over those with lower valence when it comes to complexation and exchange reactions. This is due to the fact that larger ions generally have less hydration energy (i.e. the distance between the center and the ion shell is greater, therefore the pull for hydration is lesser), therefore smaller, more highly charged ions bind more water molecules.

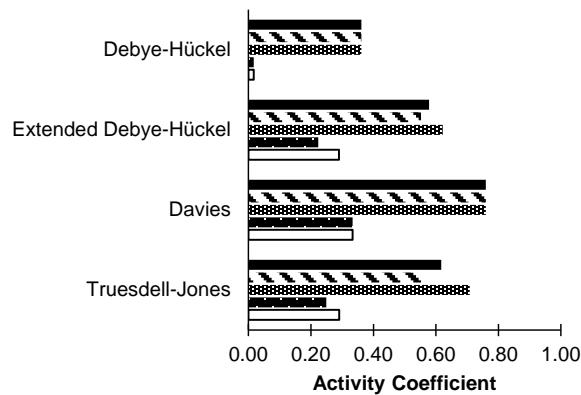
Table 2.3 Common cations in mortality leachate and associated atomic radii and hydrated radii in Angstroms (A^0) (Summarized from (Kielland, 1937; Truesdell et al., 1974; Conway, 1981; Parkhurst, 1990))

Ion	Atomic Radius (A^0)	Hydrated Radius (A^0)	TJ a_i^0 Parameter	TJ b parameter (A^0)
Ca^{2+}	1.4	6.0	4.86	0.15
Mg^{2+}	0.9	8.0	5.48	0.21
NH_4^+	1.5	3.3	2.50	0.10
Na^+	1.0	4.5	4.32	0.06
K^+	1.6	3.0	3.71	0.01
Fe^{2+}	1.0	6.0	5.08	0.16
Cl^-	1.9	3.0	3.71	0.08
SO_4^{2-}	2.6	5.5	5.31	-0.07
HCO_3^-	1.56	7.0	5.40	0.00

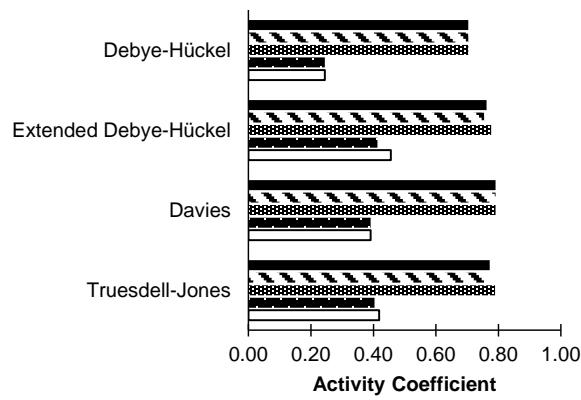
Activity Coefficient Sensitivity at 100% Ionic Strength



Activity Coefficient Sensitivity at 50% Ionic Strength



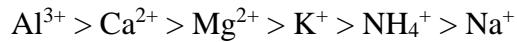
Activity Coefficient Sensitivity at 5% Ionic Strength



■ K ▨ NH₄ ♦ Na ■ Ca □ Mg

Figure 2.5 Ion sensitivity to activity coefficient calculations at three ionic strengths of bovine mortality leachate

More importantly, due to the high quantities of ammonium present in mortality leachate, preferential selectivity of ions on exchange sites in the soil will be encountered. Geochemical thermodynamics shows selectivity generally proceeds from the highest charged ions to the lowest in the following order although relative concentrations of each ion in the solution may impact this order (Fetter 1999):



As an example, McBride (1994) demonstrated that the attenuation of potassium and ammonium was effected by a change in the above lyotropic series for ammonium, whose replaceability on exchange sites was reduced when the percentage of monovalent cations in solution was less than 80% or when larger quantities of calcium were present on soil exchange sites. For mortality leachate, high concentrations of ammonium and potassium will thermodynamically exchange and sorb to soil particles in this order and allow the release of ions preferentially. This will also be affected by individual ion activities in solution. With increasing ionic strengths, activity coefficients will decrease dependant on charge. This is shown for each major ion in solution and helps to determine which ion activity calculation is appropriate for this leachate.

The original Debye-Hückel (1923) equation is reportedly appropriate for very dilute solutions (< 0.01M) before a breakdown occurs with a severe underestimation of ion activity. Figure 2.4 demonstrates this for all major cations found in mortality leachate. Much of this can be accounted for by the fact that $\gamma_i = f(I^{0.5})$ and the lack of an ion size parameter being included in the calculation, as ion size greatly assists in determining ion activity (Kielland 1937). Those equations that exclude these parameters (Debye-Hückel and Davies) tend to drift away from an appropriate ion activity coefficient at lower ionic strengths than those that include ion size parameters (Extended Debye-Hückel and Truesdell-Jones). This is more enhanced when comparing different charged ions (i.e. monovalent vs divalent ions); divalent ions show a more muted effect at all ionic strengths when compared to monovalent ions for the Debye-Hückel and Davies calculations. This agrees with the theory that ions with more charge energy count more in the calculation due to stronger electrostatic interactions with other ions. Much of the time, at higher ionic strengths, the Debye-Hückel solution shows an activity coefficient near zero for all divalent cations.

At very low ionic strengths (<0.03 M), all of the methods generally agree (within 5%) with each other. Commonly, the Davies equation is applied to the greatest variety of solutions since only the ion charge and concentration are needed to compute ion activity. At the highest ionic strengths, Truesdell-Jones and Extended Debye-Hückel provide similar results to each other for monovalent ions with the Extended Debye-Hückel providing a slight underestimation for both monovalent and divalent ions. The additional parameters included in the Truesdell-Jones equation slightly change the shape of the curves over the simulation of ionic strengths, with the a_i^0 parameter moving both the heel of the curve at low ionic strength and the “b” parameter at high ionic strengths, upwards. The incorporation of the “b” parameter into the Truesdell-Jones equation was developed from experimental mean salt single ion activity coefficients for most of the ions shown in Table 2.3. Since these coefficients were determined for single salt solutions, discrepancies are inherent for multicomponent and more concentrated solutions and may be greatly dependent on solution composition (Sparks 1998). The “b” parameter could be adjusted in models to match experimental data accordingly for multi-component non-dilute solutions. When observing sensitivity of individual cations respective of each method used to calculate activity coefficients (Fig. 2.5), at 100% ionic strength the Truesdell-Jones and Extended Debye-Hückel provided comparable values for potassium and ammonium monovalent cations. Activity coefficients between those same two methods differed from each other for divalent cations, while the Extended Debye-Hückel underestimated activity by 10% or greater.

The importance of determining an appropriate ion activity calculation comes more into play for additional geochemical analyses. Improperly utilizing activity coefficients may lead to severe underestimations in ion complexation reactions, solubility, and cation exchange/adsorption on soil particles. Underestimations in these reactions would then lead to less conservative estimations on leachate transport beneath a burial sites that may adversely impact the environment in an earlier timeframe. Results shown in Figures 2.4 and 2.5 demonstrate a good applicability of the Truesdell-Jones activity coefficient equation for thermodynamic calculations involving livestock mortality leachate. From this, future geochemical work in this study utilized the program PHREEQC as a basis for chemical speciation and complexation reactions as it is based on the Truesdell-Jones theory.

2.4.4 Speciation

Speciation of the mortality leachate chemistry produced the following results shown in Table 2.4. The calculated charge balance by PHREEQC on this solution was approximately eight percent. By adjusting alkalinity, the charge balance percentage could be lowered to less than one percent. Due to the nature of titration for determining alkalinity, it is possible for these concentrations in particular to have errors, therefore alkalinity was adjusted to achieve charge balance. To evaluate the placement of a burial pit in a location without a liner, typical soil and groundwater characteristics of the region were utilized for this analysis. The speciation was compared to a speciation of naturally occurring groundwater from Fonstad (2004). Groundwater concentrations used in this simulation originate south of Saskatoon, Saskatchewan in a glaciolacustrine deposit.

The results for phosphorus are consistent with that of Moore et al. (2002) showing that phosphorus is mainly present in the body in the form of phosphoric acids and at a neutral pH, it is proportioned approximately 60% and 40% as hydrogen phosphate and dihydrogen phosphate respectively. These phosphoric acids are then attracted to cations Fe^{2+} , Ca^{2+} , Mg^{2+} , K^+ and Na^+ consistent with findings from Stollenwerk (1996) and Weiskel and Howes (1992). The mortality leachate also shows oversaturation of hydroxyapatite and vivianite (Table 2.4) consistent with Zanini et al. (1998) as well as Stollenwerk (1996) and Weiskel and Howes (1992). Phosphorus present as phosphate compounds allows for unattenuated transport of phosphorus as well as ions bound to the phosphate due to the negative charge on the ion. Phosphoric acids are corrosive and available for unattenuated transport on their own, but are able to easily complex with metals in the soil therefore advancing the transport of metals through the system (Stollenwerk 1996).

Sulfate is found mostly in the form of ammonium sulfate, and sulfate (Table 2.4). It also forms ion complexes with Ca^{2+} , Mg^{2+} , K^+ and Na^+ similar to Hem (1992). Sulfate complexes such as KSO_4^- and NaSO_4^- allow for unattenuated transport of potassium and sodium as well as sulfate.

Table 2.4 PHREEQC Speciation of averaged livestock mortality leachate chemistry concentrations (Pratt 2009)

Species	Molality	mg/l	Activity	Species	Molality	mg/l	Activity
C(4)	8.67E-01			MgH2PO4+	6.68E-05	8.1	5.33E-05
HCO3-	6.89E-01	41997	4.50E-01	MgSO4	6.38E-05	7.7	8.10E-05
CO2	1.65E-01	7256	2.09E-01	N(-3)	9.56E-01		
NaHCO3	1.02E-02	859	1.30E-02	NH4+	9.34E-01	13080	4.88E-01
CaHCO3+	9.03E-04	91	5.90E-04	NH4SO4-	2.07E-02	2260	1.65E-02
MgHCO3+	8.67E-04	74	6.91E-04	NH3	1.10E-03	19	1.39E-03
CO3-2	5.80E-04	35	1.06E-04	Na	8.32E-02		
Ca	1.59E-03			Na+	7.15E-02	1644	5.13E-02
CaHCO3+	9.03E-04	91	5.90E-04	NaHCO3	1.02E-02	859	1.30E-02
Ca+2	4.15E-04	17	1.03E-04	NaSO4-	8.49E-04	101	6.77E-04
CaHPO4	1.72E-04	23	2.18E-04	NaHPO4-	4.86E-04	58	3.88E-04
CaSO4	4.25E-05	5.8	5.40E-05	P	5.15E-02		
CaH2PO4+	4.11E-05	5.6	3.28E-05	HPO4-2	2.99E-02	2869	3.87E-03
CaCO3	1.44E-05	1.4	1.83E-05	H2PO4-	2.01E-02	1944	1.25E-02
Cl	7.80E-02			NaHPO4-	4.86E-04	58	3.88E-04
Cl-	7.80E-02	2763	4.66E-02	KHPO4-	3.47E-04	47	2.77E-04
Fe(2)	3.81E-04			MgHPO4	2.97E-04	36	3.77E-04
FeHCO3+	2.57E-04	30	2.04E-04	CaHPO4	1.72E-04	23	2.18E-04
FeHPO4	5.52E-05	8.4	7.01E-05	S(6)	3.98E-02		
FeH2PO4+	3.56E-05	5.4	2.84E-05	NH4SO4-	2.07E-02	2362	1.65E-02
Fe+2	2.23E-05	1.2	4.54E-06	SO4-2	1.73E-02	1660	2.63E-03
FeCO3	9.08E-06	1.1	1.15E-05	KSO4-	8.50E-04	115	6.78E-04
FeSO4	1.67E-06	0.3	2.13E-06	NaSO4-	8.49E-04	101	6.77E-04
K	6.25E-02			MgSO4	6.38E-05	7.7	8.10E-05
K+	6.13E-02	2391	3.67E-02	CaSO4	4.25E-05	5.8	5.40E-05
KSO4-	8.50E-04	115	6.78E-04	Si	3.54E-04		
KHPO4-	3.47E-04	47	2.77E-04	H4SiO4	3.53E-04	34	4.49E-04
Mg	1.75E-03			Zn	3.25E-05		
MgHCO3+	8.67E-04	74	6.91E-04	Zn(CO3)2-2	1.81E-05	3.4	7.30E-06
Mg+2	4.38E-04	11	1.31E-04	ZnHCO3+	1.09E-05	1.4	8.67E-06
MgHPO4	2.97E-04	36	3.77E-04	ZnCO3	2.54E-06	0.3	3.23E-06

Nitrogen is found commonly in the leachate as ammonium (NH_4^+), with some ammonium sulfate salts forming along with small amounts of nitrate (Table 2.4). Due to the decomposition of livestock mortalities, nitrogen is leached in its most reduced state, ammonium, and without exposure to oxidizing conditions; the majority of nitrogen will remain as ammonium. Ammonium sulfate (NH_4SO_4^-) concentrations in solution are approximately 2,400 mg/L which is approximately 300 mg-N/L; the negative charge on this ion allows for the transport of unattenuated nitrogen. Chloride in leachate is present only in the form of Cl^- , consistent with Hem (1992).

Calcium in this solution is present as calcium-bicarbonate, calcium-phosphates, and calcium-sulfates, as well as some free calcium consistent with Hem (1992). Iron is mostly present as ferrous iron and the majority is complexed with phosphates and bicarbonate; while magnesium is complexed with bicarbonate, sulfate and phosphate with small amounts of free magnesium. Eighty percent of sodium in solution is available as free sodium, while the remainder is complexed with bicarbonate, sulfate and phosphate consistent with Hem (1992). Potassium is mostly present as free potassium, with small amounts of ion complexes with sulfate and phosphate.

It is observed that the speciated concentrations when back calculated to mg/L are at slightly higher values than the input. This is explained due to the assumption that the density of the leachate is the same as pure water, 1000 kg/m³. It appears that this assumption is incorrect, therefore causing the calculations to show slightly higher concentrations. It also could be explained by the activity of the water molecules in solution. Due to the solution's high ionic strength, many of the water molecules are bound with the ions in solution forming their hydrated radii. Due to this phenomenon, PHREEQC reports values in molality (mol/kg of water), therefore the concentration of active water in that kg of solution is less than 1 kg/L.

The activities of the ions in solution vary by one to two orders of magnitude. Important ion complexes in Table 2.4 are plotted in Figure 2.6 on a log scale to show activities for each species present in mortality leachate and compared to species present in a sample solution of groundwater from Fonstad (2004). Ammonium and bicarbonate are an order of magnitude higher than activities such as chloride, potassium and sodium in mortality leachate. In livestock mortality leachate, ions such as ammonium and bicarbonate have the highest activities and make up 97% and 80% of their respective complexes. These two ions account for approximately 81% of all equivalents in the solution. With respect to activities of species found in groundwater, bicarbonate activity is two orders of magnitude higher. The representative sample of groundwater used did not contain any ammonium. For the remaining ions shown in Figure 2.6, all are one to five orders of magnitude higher than natural groundwater. It is interesting to note the activities of ammonium sulfate and phosphoric acids are similar, but in regards to concentration, there is less ammonium sulfate in solution.

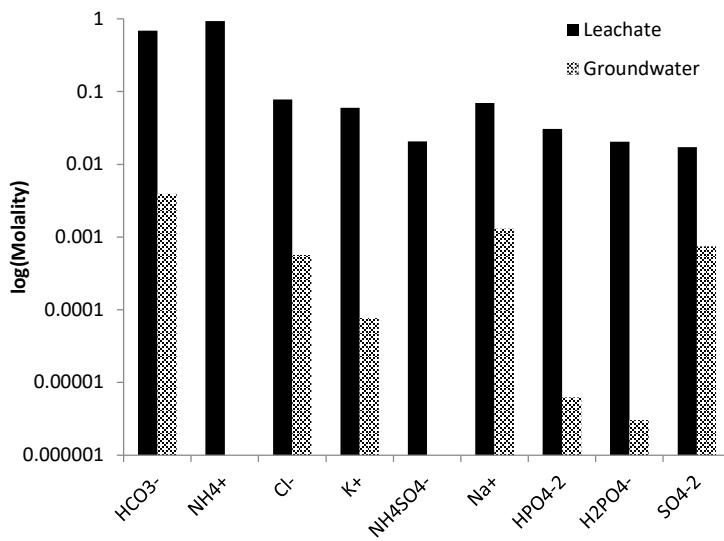


Figure 2.6 Activities of selected ions in livestock mortality leachate compared to groundwater
 (Pratt 2009)

2.5 Conclusion

The evaluation of leachate chemistry generated from livestock mortality burial site decomposition was described over a period of two years' time. Livestock mortality leachate contains on average (Table 2.2) concentrations of 12,600 mg/L of ammonium, 46,000 mg/L bicarbonate, 2,600 mg/L chloride, 3,600 mg/L sulfate, 2,300 mg/L potassium, 1,800 mg/L sodium, 1,500 mg/L phosphorus, along with small amounts of iron, calcium and magnesium. The pH of the leachate was near neutral. In comparison to earthen manure storages and landfills, the concentration of ions in the leachate was relatively higher.

Utilizing available geochemical calculations, the ionic strength of the leachate at its maximal values was found to be approximately 1.5 M. This ionic strength was then used in a stepwise dilution to calculate ion activity coefficients using four techniques. Results indicate the Truesdell-Jones technique is appropriately valid for this leachate and should be used in thermodynamic geochemical calculations here-forward. These results validated the use of the original PHREEQC speciation provided by (Pratt 2009) and provided estimates of phosphate compounds precipitating from solution, and phosphoric acids available for unattenuated transport. Due to the relatively high

concentration of ammonium and sulfates, significant concentrations of ammonium sulfate form, and due to the negative charge, will allow approximately 300 mg-N/L of unattenuated transport of nitrogen. Activities of ammonium and bicarbonate, sulfates, phosphates and other minerals are many orders of magnitude higher than concentrations present in naturally occurring groundwater.

2.6 Acknowledgements

We would like to thank the Saskatchewan Ministry of Agriculture and the Agricultural Development fund for project support and funding.

2.7 Transition Statement

Chapter 2 has established the geochemical attributes and provided calculations to come to speciation conclusions for a typical leachate that would be encountered during livestock carcass burial. This sets the stage for Chapter 3 in which models were created to assess in both a conservative and non-conservative manner the transport of this leachate into the subsurface.

2.8 Author Contributions

DP performed geochemical analysis and mathematical calcuations for ion activity coefficients as well as creating the geochemical PHREEQC speciations. DP also wrote the bulk of this manuscript with editing and comments provided by TF.

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Chapter 3: Determination of Distribution and Selectivity Coefficients for a Saskatchewan Till Using Simulated Livestock Mortality Leachate

Status: Unsubmitted

3.0 Preface

Chapter 2 provided a reiteration of Pratt (2009) coupled with further geochemical analyses on the thermodynamic properties and ion activities of the mortality leachate as measured in a controlled study. This chapter builds on the work done by Pratt (2009) by evaluating the effects this leachate may have on soil properties. The ionic strength of mortality leachate is high (up to an order of magnitude higher) compared to other liquid waste products such as manure lagoons and landfills, and the effects this chemistry may have on soil adsorption properties and ion exchange are unknown. In the past, few have evaluated ammonium sorption properties in soils (much of which were low concentrations <100 mg/L), and being that this specific leachate is laden with ammonium (>10,000 mg/L), it was imperative to assess these processes. To accomplish this, soil was taken from an existing livestock burial site for investigations described in Chapter 5 and 6 in this thesis; however, a background core was utilized for the evaluation of sorption properties described herein. This chapter forms the basis of the sorption equation that was applied to a new contaminant transport model developed in Chapter 4. Without this sorption equation for ionic strengths seen in this leachate, modelling efforts tend to under or overestimate ion sorption at concentrations of this level based on the input constant retardation factor. Earlier modelling by Pratt (2009) indicates that ammonium retardation is predicted to change based on concentration dilution along the flowpath. This manuscript has not been submitted for publication due to selected laboratory errors pertaining to Ca and Mg analyses. These errors were not significant to the ammonium sorption data and as a result, the data has been utilized in Chapter 4 and introduced for the first time herein.

3.1 Abstract

Batch tests following ASTM D4319 were performed on a Saskatchewan Floral Till taken from a background core near Pierceland, Saskatchewan to determine distribution and selectivity coefficients for varying concentrations of a laboratory simulated livestock mortality leachate at

concentrations never reported in the literature. Typical reported studies for ammonium distribution and ion selectivity involve concentrations orders of magnitude lower than those seen in livestock mortality leachate. To provide an assessment of these ion processes, a synthetic lab created mortality leachate with varied ionic strength from 0.1 to 0.8 and ammonium concentrations up to approximately 10,000 mg-N/L was utilized. Results in terms of distribution coefficients and ion selectivity coefficients were inconsistent with previous literature, but concentrations in this study were much higher than those studies reported. Sorption isotherms for ammonium demonstrated a distribution coefficient of 1.5 L/kg at low concentrations and 0.003 L/kg at high concentrations. Selectivity coefficients for ammonium increased with increasing ionic strength. Other ions in the simulated leachate such as potassium and sodium also showed competition for soil exchange sites. Calcium and magnesium analyses in the laboratory were inconsistent and results for each were uncertain due to high concentrations of alkalinity skewing titration analysis.

3.2 Introduction

In the event of large scale animal mortality events such as disease outbreaks, disposal of carcasses via burial is commonly carried out. In order to determine the risk of burying large quantities of animals, (Pratt 2009) carried out research on the decomposition of livestock and the leachate chemistry released during that process and concluded that high concentrations of NH_4^+ , HCO_3^- , Na , K , SO_4^{2-} and PO_4^{3-} are present in the leachate. Ammonium (NH_4^+) is the ion of most concern with an average concentration of 12,600 mg-N/L. Potassium, sodium, sulfate and phosphate follow with concentrations of 2,300 mg/L, 1,800 mg/L, 3,600 mg/L and 1,500 mg-P/L, respectively.

In order to evaluate the impact this potential contaminant would have on a site-specific basis, sorption isotherms and ion selectivity coefficients for the specific soil type should be determined. Previous work from Fonstad (2004), Mikołajkow (2003), Balci and Dinçel (2002), Ceazan (1989) and Valocchi et al. (1981), have performed sorption batch studies using laboratory tests or in-situ tests with varying concentrations of ammonium. None of these specific studies involved concentrations of ammonium above 4,000 mg-N/L or provided both ion selectivity coefficients as well as ammonium distribution coefficients. Table 3.1 provides a summary of the body of work that exists for ammonium.

Table 3.1 Distribution coefficients for NH₄⁺ from previous studies

	units	Balci & Dincel (2002)	Fonstad (2004)	Valocchi et al. (1981)	Mikolajkow (2003)	Ceanan (1989)
Peak NH ₄ ⁺ Concentration	mg L ⁻¹	4000	2000	100	50	25
CEC of Soil	meq 100g ⁻¹	19	7.0 - 12	10	0.1 - 20	0.5
Distribution Coefficient NH ₄ ⁺	L kg ⁻¹	19 - 40	0.01 - 3		7.42 - 152.5	0.08 - 0.4
Selectivity Coefficients						
K _{Na/NH4+}			0.01 - 1.0	0.22 - 0.68		
K _{Na/K}			0.18 - 0.4	0.07 - 0.11		
K _{Na/Ca}			0.07 - 0.79	0.29 - 0.57		
K _{Na/Mg}			0.3 - 0.85	0.54 - 0.94		

Fonstad (2004), performed batch tests to determine ion selectivity coefficients and sorption isotherms using liquid swine manure on various Saskatchewan soils, each with a maximum ammonium concentration of 2,000 mg-N/L. The results demonstrated Langmuir sorption isotherms for each ion studied. Distribution coefficients for ammonium from this study range from 0.03 to 0.8 L/kg for varying concentrations used in the batch test. This study also presented results for potassium, calcium, magnesium and sodium.

Mikołajkow (2003) studied the static migration of ammonium and nitrate ions in soils of the Kurpie outwash in Poland. This study produced Freundlich sorption isotherms and corresponding retardation factors for the site. This study was also performed using relatively low ammonium concentrations with a maximum value of 50 mg/L. Freundlich distribution coefficients for this study ranged from 7.42 L/kg in outwash sands to 152.51 L/kg in boggy soils.

Valocchi et al. (1981) performed laboratory batch experiments to simulate the injection of treated wastewater containing Na, Ca, Mg, K, and NH₄⁺ into the Palo Alto aquifer. Ammonium concentrations for this batch study did not exceed 100 mg/L. Selectivity coefficients were the product of this study, with K_{Na/NH4} varying from 0.22 during peak NH₄⁺ concentrations of 90 mg/L to 0.68 during low concentrations of 15 mg/L. Selectivity coefficients were also reported for Mg, Ca and K.

A study performed by Balci and Dincel (2002), determined ammonium ion adsorption rates on sepiolite for the use of cleaning up wastewaters from industrial areas. Their batch test findings

produced sorption isotherms with distribution coefficients ranging from 19 L/kg at maximum (4000 mg/L) ammonium concentrations to 40 L/kg at low (90 mg/L) concentrations.

The importance of determining sorption isotherms for potential contaminants aids in the determination of site specific retardation factors for the ions in consideration. The previous studies demonstrated the site-specific isotherms at much lower ion concentrations than those arising from an animal mortality burial site. The evaluation of burial risk to a specific site relies on complete knowledge of site characteristics including soil properties, geology, hydrology and land use. Soil properties, coupled with geology are important factors utilized for modelling contaminant transport risks, without proper evaluation of these properties, severe over or under estimation of transport may occur. Each potential burial site should be evaluated for ion selectivity and ammonium distribution, as these factors will play a large role in ion adsorption processes in modelling efforts.

The objective of this study was to evaluate the distribution and selectivity coefficients of a background core at an existing livestock burial site. This burial site is located 20 km south of Pierceland, Saskatchewan, and contains many trenches filled with culled elk from a chronic wasting disease (CWD) disposal in 2001. Soil cores were taken from this site in October 2008 and included background samples which were used for this study to determine sorption isotherms and ion selectivity coefficients for this site to aid in further transport research.

3.3 Materials and Methods

Batch tests were performed on a Saskatchewan Floral Till to determine adsorption isotherms and ion selectivity coefficients for an existing livestock burial site. ASTM Standard D4319 (ASTM D4319-93 2001) was conducted to produce distribution coefficients using soil cored from a depth of 4.5 m taken from a background hole at the Pierceland CWD site (Pratt and Fonstad 2017a). Two batch tests were performed as a replicated process. Batch test one and two were prepared by measuring 50 g of oven dried soil into 10 plastic containers. To achieve a 4:1 solution to soil ratio, 200 mL of solution was added to each container, with each container receiving a stepwise dilution of the simulated mortality leachate solution ranging in concentrations from 100% to 10% of initial values.

The solution added to each container was comprised of a synthetic livestock mortality leachate that was created in the laboratory using a combination of distilled tap water and ammonium bicarbonate, sodium chloride, potassium chloride, calcium carbonate, magnesium sulfate and sodium sulfate. Phosphate and organic carbon were left out of the simulated leachate. Once the solution and stepwise dilutions were prepared and added to the soil containers, they were placed on a shaker table at 20°C for seven days. The containers were inverted twice per day. On the seventh day, the containers were removed from the shaker table, and the solutions were vacuum filtered and stored at 4°C until analysis was completed. The soil remaining after each sample filtration was then dried at 104°C for 48 hours. This dried soil was then used to perform extraction analysis to determine adsorbed ions and CEC.

To determine the amount of ions sorbed for each test, two separate extraction procedures were performed. Extraction of nitrogen (in the form of ammonium) from the soil was conducted using methods from Bigham (1996). Fifty mL of 2M KCl solution was added to 5 g of soil and shaken for 1 hour. After shaking, the solution was filtered and filtrate stored at 4°C. To extract the remaining ions from the soil, methods from Jones (1992) were employed. A 1M solution of ammonium acetate was created and 25mL was added to 5 g of soil and shaken for five minutes. The sample was filtered and the filtrate was stored at 4°C until analysis. Ions extracted were summed to determine cation exchange capacity.

Laboratory analyses of the filtrate was performed at the University of Saskatchewan College of Engineering Environmental Engineering Lab. Total alkalinity analysis was determined by titration, ammonium by steam distillation, nitrate/nitrite and sulfate colorimetrically, calcium and magnesium determined by hardness titration, potassium and sodium by flame photometer and chloride by titration.

Sorption isotherm analysis was performed using the results of the extractable and exchangeable ion analysis. Due to the high concentration of solutions, a Langmuir type analysis was used. Plots were created using analytical data with quantity on exchange sites S versus concentration in solution C . Fetter (1999) discussed a Langmuir distribution on soils having a finite number of

exchange sites, therefore when all the exchange sites are filled, the soil will no longer adsorb ions from solution. The equation for a Langmuir sorption isotherm is as follows:

$$\frac{C}{S} = \frac{1}{\alpha\beta} + \frac{C}{\beta} \quad [3.1]$$

where:

α = adsorption constant related to the binding energy (L/mg)

β = maximum amount of solute that can be adsorbed by the solid (mg/kg)

By plotting C/S versus C , a linear relationship is expected to result, where β is the reciprocal of the slope and α is the reciprocal of the intercept multiplied by β . The retardation factor for an ion in solution can then be calculated on a site specific basis if the porosity (n) and bulk density (ρ_d) of the soil are known. The retardation factor R determined from a Langmuir distribution from Fetter (1999) is:

$$R = 1 + \frac{\rho_d}{n} \left(\frac{\alpha\beta}{(1+\alpha C)^2} \right) \quad [3.2]$$

While the distribution coefficient K_D can be calculated from:

$$K_D = \left(\frac{\alpha\beta}{(1+\alpha C)^2} \right) \quad [3.3]$$

Distribution coefficients are important, but ion selectivity coefficients in relation to other ions are imperative in the understanding of ion exchangeability and selectivity and is generally dependent on the concentration in the soil solution. Equivalent fractions, (β_I) were used to calculate selectivity coefficients with respect to sodium. To calculate the equivalent fractions for each ion, the equivalents of each ion sorbed is divided by the total equivalents of all cations (cation exchange capacity). Once equivalent fractions are determined, the selectivity coefficients for each ion with respect to one ion (usually Na^+) can be calculated as demonstrated for the exchange of a monovalent cation for a divalent cation:

$$K_{A/B^{2+}} = \frac{[B^{2+}]^{0.5}}{[A^+]} \frac{\beta_A}{\beta_B^{0.5}} \quad [3.4]$$

Concentrations of ions *A* and *B* are in the form of ion activities. Activities in this study were calculated using PHREEQC (Parkhurst and Appelo 1999) and the Truesdell-Jones equation. Once activities were determined, equivalent fractions were calculated for each ion using experimental data. Selectivity coefficients were calculated in reference to Na^+ .

3.4 Results and Discussion

3.4.1 Sorption Isotherms

Langmuir sorption isotherms were evaluated for two replicate batch tests, each consisting of varying concentrations of simulated synthetic livestock mortality leachate. Langmuir isotherms were chosen due to the fact that at higher solution concentrations, the ions of interest are expected to reach maximum adsorption. Langmuir sorption isotherms for ammonium (Fig. 3.1) show a linear trend with good correlation (R^2) of 0.96 and 0.98. Batch 1 and Batch 2 produced similar results and their corresponding α and β values are 0.003 L/mg and 909 mg/kg respectively. The distribution coefficient for ammonium concentrations of 10,000 and 100 mg-N/L was then calculated and resulted in 0.003 and 1.56 L/kg respectively.

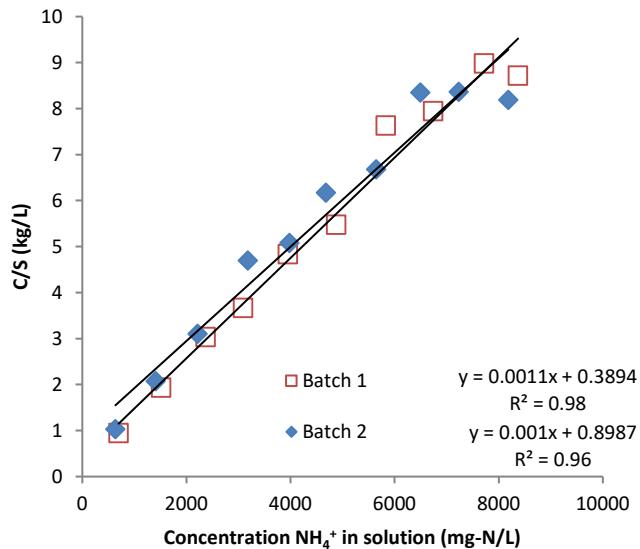


Figure 3.1 Langmuir sorption isotherms for NH_4^+

The α and β are calculated from the equation of the linear line shown in Figure 3.1, using equation 1, and subsequently, these values can then be used to calculate retardation factors at known concentrations given site specific conditions such as bulk density and porosity. For comparison sake, C/S values were computed via numerical analysis based on α and β found in this study for a range of concentrations from 10 to 10,000 mg/L as compared to those from the Kelvington site from Fonstad (2004) and are presented in Figure 3.2. For further analysis on the effect of concentration on retardation factors, the α and β were then used to calculate the change in retardation factors at the same concentrations. Retardation factors for both this study and the Kelvington study used an assumed bulk density of 1.7 g/cm³ and a porosity of 0.35. The soil types for both this study and the Kelvington site of Fonstad (2004) were both glacial tills, so results were expected to be somewhat similar. Fonstad (2004)'s work presented ammonium-N concentrations that topped out at approximately 2000 mg/L, while this study extended that range to nearly 10,000. The figures were extrapolated to demonstrate what results could be expected in Fonstad (2004) soil based on that soil's α and β values obtained as compared to concentrations up to 10,000 mg/L similar to this study. Retardation of ammonium for both soils is very similar, with retardation taking place only at concentrations lower than 2000 mg/L, at concentrations higher than this, retardation factors are constant at 1, meaning the ion is moving similar to chloride through the system until it is dilute enough to show retardation.

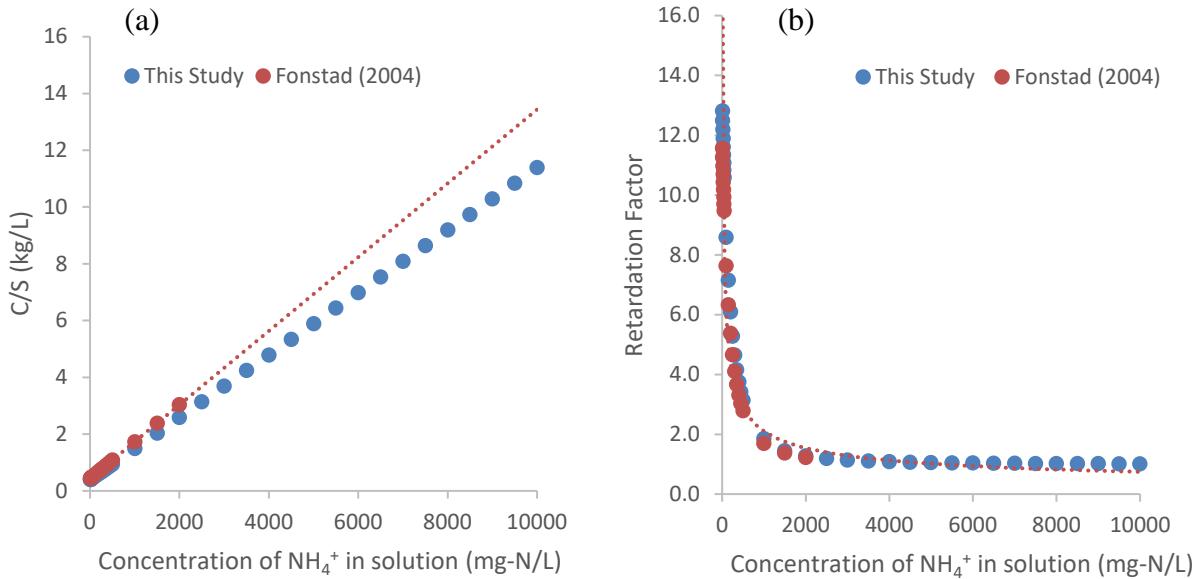


Figure 3.2 (a) Numerical analysis of C/S for this study versus Fonstad (2004); (b) calculation of retardation factors for this study versus Fonstad (2004)

Sodium isotherms shown in Figure 3.3 also produced similar results from Batch 1 and Batch 2 with a linear trend and correlation (R^2) of 0.71 and 0.76. Maximum absorption values (β) of 714 mg/kg for Batch 1 and 555 mg/kg for Batch 2 indicate approximately 2.3 to 3 meq/100g of sodium is being adsorbed by the soil. The corresponding α for Batch 1 and 2 are 0.0007 and 0.001 L/mg. Sodium is well known as the ion that has limited adsorption ability due to thick hydration shell and single charge, which limits its abilities to compete with other ions in solution for exchange sites (Appelo and Postma 2005). Therefore, even though there are fairly high concentrations of sodium in livestock mortality leachate, it will have a relatively small retardation factor.

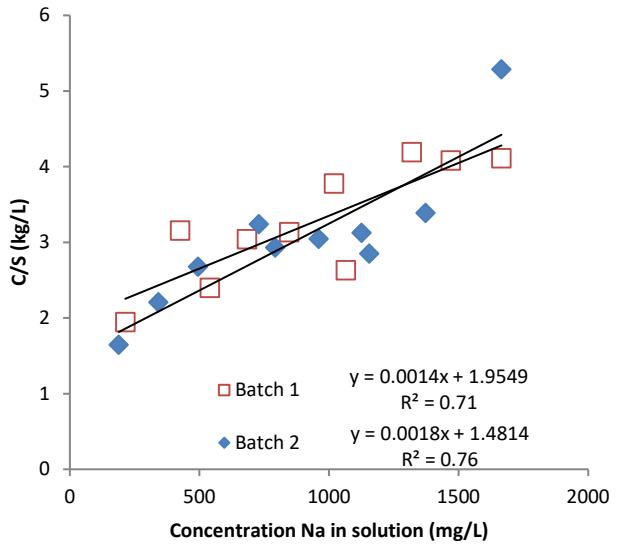


Figure 3.3 Langmuir sorption isotherms for Na^+

Figure 3.4 demonstrates sorption isotherms for potassium from Batch 1 and 2. The sorption isotherm produces a linear graph with correlation and similar results for both batch tests. Maximum sorption for potassium (β) for batch 1 and 2 is 769 and 666 mg/kg respectively. Values determined for α in Batch 1 and 2 were 0.003 and 0.0045 L/mg respectively. A gradual increase in adsorption of potassium is noticed as the concentration of potassium in solution is increased. Potassium adsorption starts to become constant as solution concentrations exceed 1,000 mg/L.

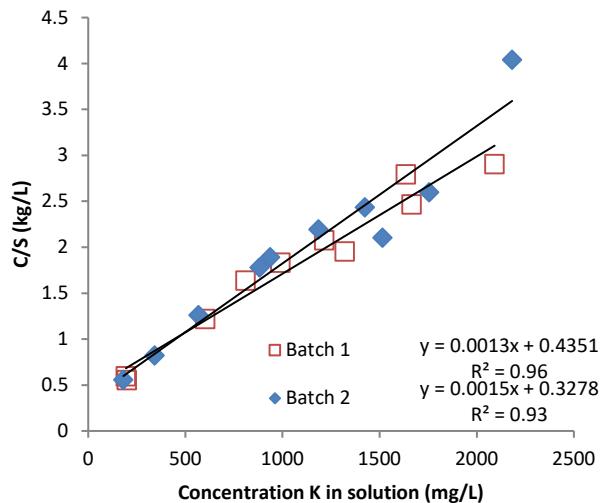


Figure 3.4 Langmuir sorption isotherms for K^+

The sorption isotherm plots for calcium and magnesium (Fig. 3.5 and 3.6) are inconsistent between batch tests and show poor correlation. The analyses of calcium and magnesium in the laboratory presented difficulties for all samples. Due to the high amounts of alkalinity in the leachate, standard methods of titrating for total hardness and calcium hardness were inadequate. End points for the titrations were undistinguishable and therefore the corresponding calculations for total calcium and magnesium are likely unreliable. No other methods of analysis or funding for these two ions were available at the time of this paper.

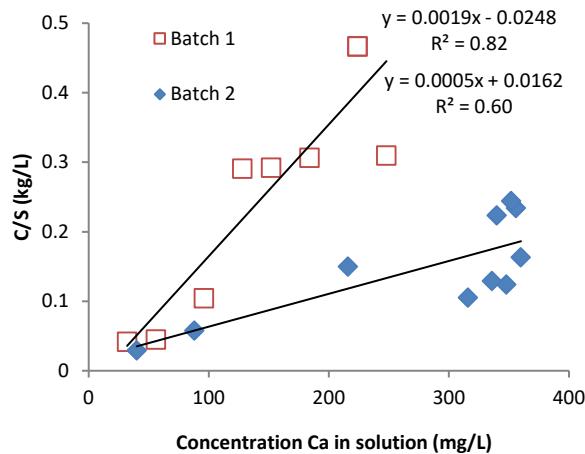


Figure 3.5 Langmuir sorption isotherms for Ca^{2+}

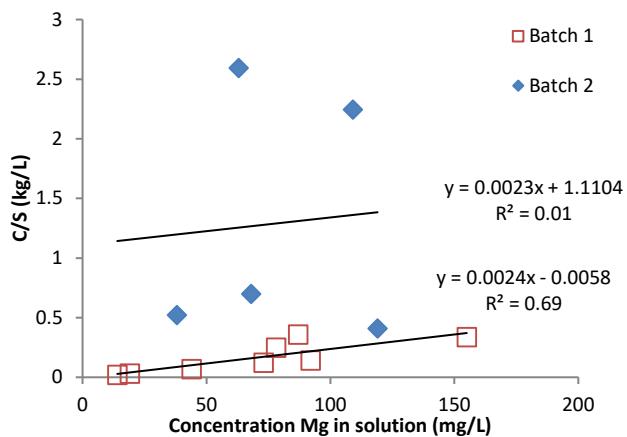


Figure 3.6 Langmuir sorption isotherms for Mg^{2+}

3.4.2 Selectivity Coefficients

Determination of ion selectivity coefficients is important to understand the potential competition between ions on the soil exchange sites. Previous studies demonstrated ion selectivity coefficients for low ionic strengths. The determination of ion selectivity coefficients at high ionic strengths is important for livestock mortality burial to allow modelling of the long-term impact of solutions of these ionic strengths.

To calculate these coefficients, equivalent fractions of each ion are determined from the cation exchange capacity and the amount sorbed of each ion. Once equivalent fractions were determined, the ion selectivity coefficients were calculated with respect to sodium. Due to the similarity in results from batch one and two, selectivity coefficients for only Batch 1 will be discussed. Fonstad (2004) initially presented a correlation between the ratio of monovalent to divalent cations in solution and the selectivity coefficient. As a comparison, results from this study were also plotted as selectivity coefficients versus the ratio of monovalent to divalent cations in solution and for reference, for this particular leachate, increasing $1+/2+$ ratio is also increasing ionic strength. A best fit line was determined for each ion and a simple model was created to calculate exchange coefficients from the best fit line. Microsoft Excel solver was used to determine selectivity coefficients from measured activities of ions in solution by calculating for selectivity coefficients while minimizing the difference between modelled and experimental equivalent fractions. This method produced a model to duplicate data determined experimentally.

Selectivity coefficients for ammonium (Fig. 3.7), show an increase in selectivity with increasing ammonium concentrations in solution similar to Fonstad (2004). Selectivity coefficients at low concentrations are 0.6 and for high concentrations, 2.5. These results are inconsistent with Valocchi et al. (1981), which found a decrease in ammonium selectivity with its increasing concentration in solution.

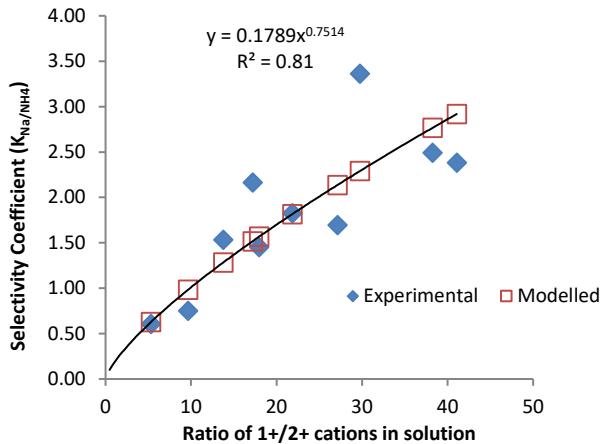


Figure 3.7 Selectivity coefficients for ammonium

To show a visual comparison, Figure 3.8 demonstrates a compilation of data from Fonstad (2004) and results from this study. Plots of the ammonium selectivity coefficient versus ionic strength are very different between the two studies. Fonstad (2004) shows increasing selectivity coefficients up to an ionic strength of approximately 0.1 and then a constant selectivity coefficient from 0.1 to 0.2. This study demonstrates increasing selectivity coefficients for increasing ionic strength from 0.1 to 0.8.

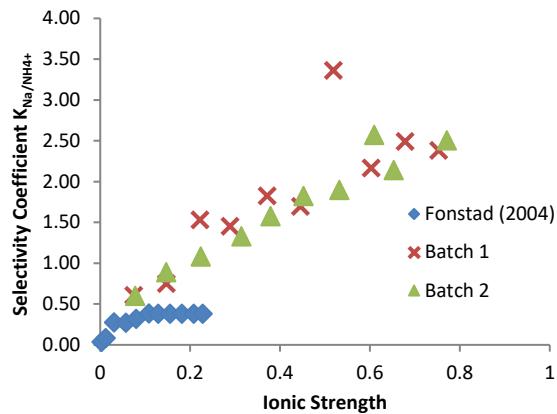


Figure 3.8 Ammonium selectivity coefficient comparison

Potassium also shows an increase in the selectivity coefficient with increasing potassium concentrations in solution (Fig. 3.9). These results are inconsistent with the cited literature. Potassium selectivity coefficients from this study range from 0.15 at lower, to 0.7 at high ratios of

monovalent to divalent cations. Due to the high ionic strength of the leachate, more competition for exchange sites is occurring. Cited literature performed their studies with ionic strengths less than 0.2. The leachate in this study has an ionic strength of approximately 0.8. There is limited documentation in the literature for ion selectivity and sorption for ammonium solutions at these high ionic strengths.

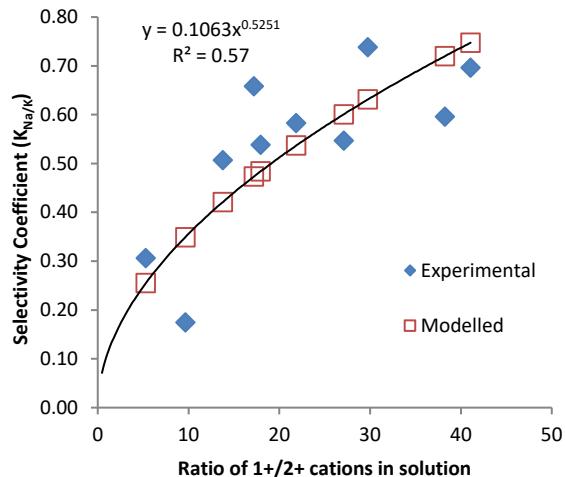


Figure 3.9 Selectivity coefficients for potassium

Calcium selectivity coefficients in Figure 3.10, does not have a good fit to the model. Calcium selectivity in comparison to results from Fonstad (2004) are comparable in that they fall within the range found in that study and are inconsistent with the range of selectivity coefficients found by Valocchi et al. (1981). Increasing ratios of 1+/2+ cations show decreasing calcium selectivity coefficients. Again, due to problems determining calcium as well as magnesium in the laboratory, these results have some uncertainty.

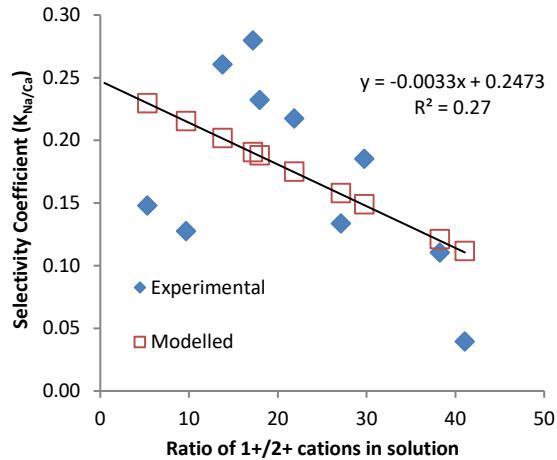


Figure 3.10 Selectivity coefficients for calcium

Magnesium selectivity coefficients (Fig. 3.11) also fall within the range of selectivity determined by Fonstad (2004), but are slightly lower than those found by Valocchi et al (1981). With increasing ratios of 1+/2+ cations, the selectivity coefficient for magnesium decreases. Magnesium selectivity coefficients for this study range from 0.4 at low ionic strength to 0.1 at high ionic strength.

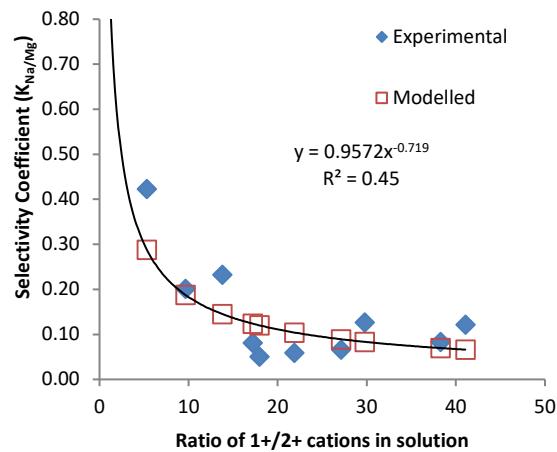


Figure 3.11 Selectivity coefficients for magnesium

3.4.3 Cation Exchange Capacity and Mass Balance

After all batch tests were complete and ion extractions were performed, an interesting trend was observed with respect to the cation exchange capacity of the soil. Figure 3.12 demonstrates this phenomenon. As ionic strength of the solution increased, the ion adsorption of the soil also increased. The cation exchange capacity of the soil prior to any batch test was 11 meq/100g. Post-batch, the CEC was found to be 17 meq/100g for the most concentrated batch test. Fonstad (2004) as well as others have hypothesized that not all exchange sites are available at different ionic strengths. They indicated that calcium adsorption may increase as ionic strength increases to a point at which it then starts to release. Standard exchangeable ion methods may not account for this. The results found in this study demonstrate that for higher ionic strength solutions, more exchange sites become available. It is also possible that the methods used to extract ions from the soil to determine CEC may not be adequate. In either case, the CEC gains 6 meq/100g from 10% dilution to 100% dilution and ammonium, sodium and potassium concentrations on exchange sites increase as well. It is also important to note that the concentration of ammonium acetate used in the extraction method is comparable to the concentrations of ammonium used in the batch tests. Since ammonium acetate extractions are used to replace all exchangeable cations, it could be assumed that for the high ionic strength batch solutions, the ammonium in those solutions is replacing cations on the soil surface.

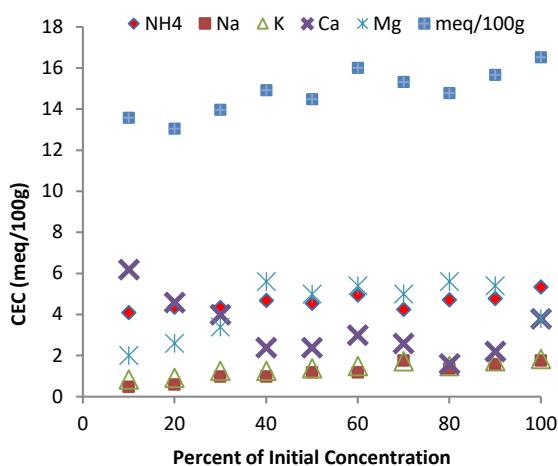


Figure 3.12 Variable cation exchange capacity

Experimental errors for this study are quantified by performing a mass balance for ions gained and lost. Figure 3.13 demonstrates for each dilution set, the amount of cations gained (positive side) and the amount of ions lost (negative side) on the soil exchange sites. For nearly all dilutions, 4 meq/100g of ammonium was sorbed onto the soil, along with varying amounts of magnesium, sodium and potassium. Totaling the amounts of ions sorbed does not equal the amount of ions lost from the soil. This unbalanced exchange of mass could arise from lack of analysis for other cations released such as barium, lithium, iron, etc. Further analysis on other trace metals could close the mass balance.

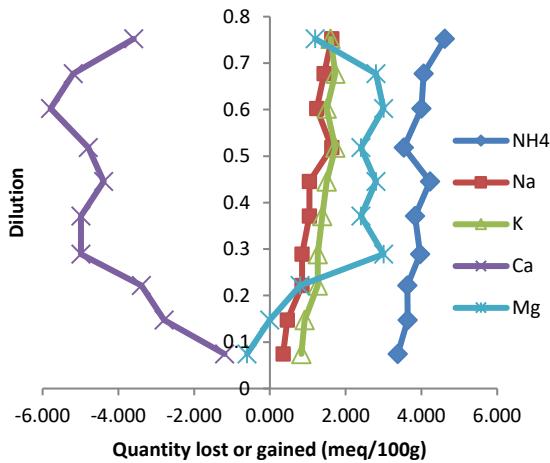


Figure 3.13 Ion exchange mass balance

3.5 Conclusion

The batch tests performed on the Saskatchewan Floral Till for this study produced results somewhat inconsistent with previous literature. Concentrations used in this study were on a completely different magnitude. The high ionic strength of livestock mortality leachate greatly effects the ion exchange and sorption of those ions onto the soil. Sorption isotherms were determined to be Langmuir distributions with ammonium showing a distribution coefficient of 1.5 L/kg at low concentrations and 0.003 L/kg at high concentrations. Selectivity coefficients for ammonium with respect to sodium also increase for increasing ionic strengths. Potassium found in mortality leachate also competes for ammonium for exchange sites and shows a selectivity coefficient that also increases with increasing ionic strength. Retardation factors for ammonium

at concentrations above 2000 mg/L were approximately 1, meaning dilution must occur in mortality leachate to a level below this before retardation of the ion would be seen in this system. Calcium and magnesium data for this study do not provide an accurate measure of the respective ion exchange and sorption behavior because of laboratory limitations. Cation exchange capacity appears to follow a variable trend in soils exposed to increasing strength solutions. This phenomenon is an area that needs further research.

Mass balance of gained and lost cations shows further analysis is key on this soil type to appropriately determine selectivity coefficients. Another ion other than calcium must be displaced from exchange sites, for the soil to be able to sorb the ions in solution.

3.6 Acknowledgements

This work was funded in part by Saskatchewan Agriculture and Food/Agricultural Development Fund. Special thanks to Doug Fischer in the Environmental Engineering Laboratory for his guidance on methods of analysis and allowing me to work in his lab.

3.7 Transition Statement

To bridge the knowledge gap on transport processes for high concentrations of ammonium as seen in Pratt (2009) as well as Chapter 2 of this thesis, and to better inform modelling efforts, this study was a necessary component to determine ion sorption processes at concentrations never before reported in the literature. This study was then utilized in the following chapter in a contaminant transport model including sorption processes. By utilizing the ion sorption parameters evaluated here, modelling efforts provide a closer estimation of transport time/distances due to the incorporation of site specific data related to ion processes evaluated with this chemistry set.

3.8 Author Contributions

D.P. designed the study, performed the batch test in the laboratory and performed all sample chemical analyses. D.P. also prepared and evaluated the analytical dataset, model and wrote the manuscript. T.F. provided critical feedback on analysis, as well as provided edits and comments on the manuscript and contributed to the text in later iterations.

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Chapter 4: Geochemical Modelling of Livestock Mortality Leachate Transport Through the Subsurface

Status: Accepted (*in press*)

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4.0 Preface

Chapter 2 and 3 provided further insight on the geochemical issues associated with mortality burial leachate. As part of the initial evaluation of leachate transport, Pratt (2009) presented preliminary models created in both CTRAN and PHREEQC to assess hydraulic conductivity changes in the subsurface as well as evaluate burial trench spacing requirements and provide a preliminary assessment of reactive transport utilizing assumed basic soil properties and model defaults. Upon completion of the M.Sc. project (Pratt 2009), it was apparent that more work was needed from a modelling standpoint due to instabilities and a better assessment of sorption properties and parameters. Model stability in the older versions of the models shown in the M.Sc. thesis (Pratt 2009) was improved upon and new, more stable models (utilizing newer software versions and better control of boundary conditions and model properties) were created in CTRAN for part of this manuscript. Based on the results from Chapter 2, the original PHREEQC reactive transport model is also presented here for comparison to the new modelling efforts arising from the work done in Chapter 3. The new CTRAN model was created based on additional work which was done in the laboratory to predict ammonium distribution coefficients (at concentrations orders of magnitude higher than any in current literature) for ion exchange at average concentrations documented from mortality leachate (Chapter 3). These sorption isotherms were utilized in this new CTRAN model for ion sorption processes utilized advection-dispersion equation and were created with data from (Fonstad 2004) and work done in Chapter 3. While some of the work in Chapter 4 is a combination of M.Sc. work (Pratt 2009) already done (portions of sections 4.3.1,

4.4.1, 4.4.2) and new modelling efforts (portions of sections 4.3.1 and 4.4.2) based on further laboratory investigations on the adsorption/exchange properties of ammonium, the reader of a published manuscript would benefit from seeing all scenarios and would provide better context for new modelling scenarios.

4.1 Abstract

Leachate chemistry arising from carcass disposal burial pits may pose a significant threat to the environment. Based on the results of previous studies, models were created to assess these impacts. Simple 2D models using CTRAN were created to evaluate the impacts of three orders of magnitude changes in hydraulic conductivity representative of glacial tills common to Western Canada, as well as impacts of burial trench/pit spacing on the subsurface for a conservative ion. Sorption properties were included in one scenario to provide insight on transport and sorption for ammonium ions based on published distribution coefficients. The conservative and sorption models for the mid-range hydraulic conductivity indicated transport up to 10 m after 100 years with concentrations at 40% and 1% of initial values respectively for the conservative and sorption model. The CTRAN sorption model estimates ion sorption, but does not account for ion exchange effects with soil properties, therefore, PHREEQC was used to also provide a geochemical contaminant transport model including ion exchange reactions occurring along the flow path. The PHREEQC model demonstrated elevated concentrations of a calcium and magnesium plume forming in front of an ammonium plume due to ion exchange occurring on the soil particles. After 50 years of transport, ammonium concentrations were approximately 4% of initial values in a soil with a cation exchange capacity (CEC) of 10 meq [100g]⁻¹. Modelling demonstrated high concentrations of ammonium, orthophosphate, sulfate and other ions in mortality leachate where transport and precipitation of these ions could occur beyond 100 years.

4.2 Introduction

Carcass disposal, whether for routine mortality or the ever-feared mass mortality events caused by foreign animal diseases (FAD), catastrophes, or natural disasters, is a significant concern to the agricultural sector. Carcasses are generally disposed of in one of a variety of methods including burial, incineration, composting, anaerobic digestion and rendering. On-farm burial is common

throughout North America for day-to-day losses and generally poses little threat to the environment (Gwyther et al. 2011). In the event of mass mortality events, burial is the disposal route studied in limited detail, and one that may cause significant environmental impacts to soils and surface/groundwater (Ritter and Chirnside 1995; Glanville 2000; MacArthur and Milne 2002; MacArthur et al. 2003; CFIA 2006; Pratt 2009). Characterisation of the leachate produced from mortality burial was determined (Table 4.1) and evaluated for its geochemical characteristics and species (Pratt 2009; Pratt and Fonstad 2017). This characterisation was the first of its kind to fully describe major and minor ions and metals in mortality leachate produced through the burial of chickens, pigs and cattle and was a basis for future work in this area. The information from that study has enabled scientists and regulators to understand and evaluate risks involved with the establishment of a mortality burial site. The purpose of this study was to provide both conservative and reactive contaminant transport models to evaluate the impacts this relatively high strength leachate may have on soil and groundwater systems beneath carcass burial pits or trenches.

Table 4.1 Chemical concentrations in three species of mortality burial leachate (poultry, pigs, bovine) (Pratt, 2009)*

	units	Poultry	Pigs	Bovine	Average
Bicarbonate	mg/L	39130	48470	50730	46100
Chloride	mg/L	2570	2380	2815	2600
pH		6.5	6.7	6.9	6.7
Total Alkalinity	mg/L	22500	39700	41600	34600
Ammonium as Nitrogen	mg/L	10400	13300	14100	12600
Nitrate + Nitrite Nitrogen	mg/L	2.3	3.1	3.8	3.1
Inorganic Carbon	mg/L	7700	9530	9950	9100
Organic Carbon	mg/L	79000	65000	68000	71000
Aluminum	mg/L	0.5	0.5	0.5	0.5
Calcium	mg/L	81	48	36	60
Copper	mg/L	0.9	1.7	0.6	1.1
Iron	mg/L	18	19	18	20
Magnesium	mg/L	79	17	18	40
Manganese	mg/L	0.5	0.1	0.1	0.2
Phosphorus	mg/L	1930	1515	1150	1500
Potassium	mg/L	2400	2400	2000	2300
Silicon, soluable	mg/L	20	24	26	20
Sodium	mg/L	1600	1700	2000	1800
Sulfate	mg/L	3970	3900	2900	3600
Sulfur	mg/L	1300	1300	965	1200
Zinc	mg/L	2.2	1.8	1.7	1.9

* Values are averages over the first 24 months after burial for each species.

Mortalities were buried in Saskatchewan, Canada within HDPE lined pits to produce pure leachate samples.

A small body of work exists on field evaluations surrounding and beneath existing livestock burial sites. Much of these works were small-scale routine mortality disposal systems or constructed research disposal pits (Ritter and Chirnside 1995; Glanville 2000). There is minimal information regarding environmental contamination issues from mass-mortality animal disposal via burial. Of those that exist, the most recent are from Korea. Four catastrophic foot and mouth disease (FMD) and avian influenza (AI) outbreaks have occurred in Korea since 2000, with the worst in late 2010 to early 2011, in which burial was the most common disposal route for 3.48 million animals in 4,583 on-farm burial sites (Ozawa et al. 2006; Wee et al. 2008; Lee 2013; Park et al. 2013). Due to the rapid spread of disease and inability to expediently find suitable burial sites, significant environmental impacts have been observed including surface and groundwater contamination, soil pollution and nuisance gas emissions (MOE 2010; Lim et al. 2012; Kim and Pramanik 2015). High

risk sites were monitored and deemed defective, requiring remediation and treatment. Concentrations of elevated Ammonia-N was observed in 24% of monitoring sites at concentrations up to 2593 mg L⁻¹ (Park et al. 2012).

Many of the literature reports of the past have been largely focused on poultry disposal with limited information available for a small number of potential groundwater contaminants such as nitrate, ammonia, chloride and faecal pathogens (Hatzell 1995; Ritter and Chirnside 1995; Myers et al. 1999; Glanville 2000). Glanville (2000) reported on the impact of livestock burial on shallow groundwater quality and used two studies to evaluate groundwater quality and contamination from a 1.8 m deep pit containing 28,400 kg of turkey mortalities and a second study of engineered 1.2m deep trenches containing 6 pigs with a PVC liner and pumping system to measure duration of decay products. The first case study monitored groundwater quality from monitoring wells placed around the pit with reported seasonal high water tables between 0.3 m and 0.9 m below ground level. Monthly samples of groundwater were completed for 15 months and periodically at 20 and 40 months. For the second case study of pigs buried in trenches, leachate was measured to determine the mass, concentration and duration of decay products. Contaminant migration was also monitored for this study by eight monitoring wells.

For the two case studies, elevated levels of biochemical oxygen demand (BOD) at 230 mg L⁻¹, ammonia-nitrogen (NH₄⁺-N) at 403 mg L⁻¹, nitrate at 4.8 mg L⁻¹, total dissolved solids (TDS) at 1527 mg L⁻¹, and chloride (Cl) at 109 mg L⁻¹ were found in monitoring wells down gradient within 1 m of the burial site. It was suggested that elevated chloride levels may be the best indicator of burial related groundwater contamination (Glanville 2000). Although his studies involved more of a groundwater contamination aspect of leachate characteristics, the concentration of contaminants found in the groundwater adjacent to the pits were elevated enough to cause concern. He also noted that in lightly loaded burial trenches, in a well-drained soil, complete decay may take two years or more. His results showed groundwater contamination at distances of 1 – 2 m from the pit; this could be more concerning in areas of high groundwater velocities or vertical movement.

In contrast, Ritter and Chirnside (1995) evaluated the impact of poultry mortality disposal on groundwater quality near six existing disposal pits in Delaware. These burial pits were for routine

mortalities and, at the time of the study, the pits were still actively loaded with approximately 15 kg to 25 kg of dead birds each week. Potential contamination was monitored in piezometers at depths of 4.5 m around the pit. Due to site conditions and geology, the bases of the pits were located within the water table for most of the year in sandy loam soils. The potential for groundwater contamination at this site in those conditions was considered to be very high. They found high ammonia concentrations (366 mg L^{-1}) in two of the monitoring wells up to 12 m away and streptococci concentrations of 209 per 100 mL at 6 m away.

Other studies from Hatzell (1995) and Myers et al. (1999) demonstrated similar results. Myers et al (1999) reported elevated concentrations of nitrate (up to 39.7 mg L^{-1}) and faecal pathogens generally less than 20 per 100mL in monitoring wells. Hatzell (1995) reported slightly increased concentrations of nitrate (2 mg L^{-1} above the median for the area) near a Florida chicken disposal pit, along with elevated nitrate concentrations near litter stockpiles and fields receiving heavy applications of fertiliser. The study observed no real indication of groundwater contamination due to the carcass disposal pits. For both of these studies, it was determined that other site-wide practices (manure stockpiles, etc) could have influenced both nitrate and faecal pathogen indicators in groundwater samples.

Other large scale outbreaks with basic information available on environmental impacts include the FMD outbreak in the United Kingdom in 2001 and the FMD outbreak in Taiwan in 1997. The UK outbreak generated a cull of over 1 million sheep and other livestock. Much of the disposal was performed via engineered burial pits. Of the most relevant, MacArthur et al. (2003) studied leachate characteristics and virus survival arising from a mass burial site in Scotland. The study site consisted of six engineered pits containing 400,000 livestock mortalities culled during April and May of 2001. To manage the leachate formed from the pits, longitudinal drains were installed and leachate was collected in 40 ISO containers with 20 m^3 capacity each. Approximately 4000 m^3 of leachate was generated; sampling and analysis gave average values from 199 samples of leachate, with concentrations of ammonia-N, alkalinity, BOD and COD of 3294 mg L^{-1} , 9400 mg L^{-1} , 12700 mg L^{-1} and 20414 mg L^{-1} respectively. Due to significant rain events during the burial phase, this leachate was probably diluted by rainwater entering and pooling in the burial pit. Groundwater contamination was monitored around these disposal pits with minor localised

contamination occurring within 5 m of the east pit edge that stabilised in 2006 and has since declined (MacArthur 2006). In the instance of the burial pits in Taiwan, 3.8 million culled pigs were disposed of, the majority by burial in large municipal landfills (80%) (Hseu and Chen 2016). Environmental monitoring plans were put in place to assess environmental damages accrued after the event. More than two years of monitoring took place at four locations around the country in various geologic settings. Generally, downstream wells showed elevated TDS ($2,000 \text{ mg L}^{-1}$), chloride (60 mg L^{-1}), ammonium-N (1.3 mg L^{-1}), and sulfate (270 mg L^{-1}) concentrations as compared to upstream wells for the majority of locations (Hseu and Chen 2016). One site in particular was located in a more granular, gravelly medium and presented the most contamination and most water samples exceeded Taiwan drinking water standards, and therefore requiring disinfection and treatment prior to drinking.

A common measure of success of a carcass disposal site is its geological security. For sites such as those in Korea, Taiwan or marginal locations such as the Delaware peninsula, the geologic subsurface was a detriment that would generally not meet mass disposal site requirements. Taiwan suffered from seasonally high water tables, Delaware's issues included high hydraulic conductivities and high sand content and Korea, shallow depth to fractured bedrock. In North America (USA and Canada), much work has been done to assess requirements for disposal sites and minimum geologic parameters outlined to ensure environmental protection (NABC 2004; CFIA 2006; St. John & Associates 2008; USDA APHIS 2012; CFIA 2017; Saskatchewan Ministry of Agriculture 2017). Although these guidelines vary by state or province, they generally agree on similar themes. In Canada, on-farm burial is generally an option in all provinces for routine mortalities at farms which have less than 300 animal units (AU) (Cleary et al. 2010; Province of Alberta 2014). Provinces such as Saskatchewan and Nova Scotia require government-approved dead animal management plans (Province of Nova Scotia 2011; Saskatchewan Ministry of Agriculture 2017). Standard practices throughout Canada for routine, on-site burial of carcasses are summarised as follows with variances by province: minimum earth cover of 0.6 to 1.0 m; minimum distance from pit or trench bottom to groundwater of 0.6 to 4.0 m; minimum distance to watercourse 15 to 100 m; minimum distance to well of 30 to 300 m; maximum mass per burial pit of 700 to 2500 kg (Freedman and Fleming 2003; Cleary et al. 2010). In the event of a mass mortality event, most developed countries require government regulation and control of the

situation. Pre-emptive steps have been carefully thought out prior to the onset of a mass disposal situation, and implementation plans exist in some countries. Of importance, location selection for mass burial sites is a critical step in the process and should be identified prior to any need (Dolan and Koppel 2005). A few guidelines exist around the world to assist with burial site selection criteria and environmental protection based on geology, hydrogeology, topography and pit construction characteristics (Loehr 1972; The Animal By-Products (Scotland) Regulations 2003; Dolan and Koppel 2005; CFIA 2006; Cleary et al. 2010; MOE 2010; Government of Alberta 2011; USDA APHIS 2012)

Geology of a potential site is an important factor in site selection suitability to ensure minimal impact to the surrounding environment. Acceptable soil types include those with the ability to limit groundwater flux/movement and naturally attenuate chemical components of the leachate such as clays and silts. Generally, sites higher in clay content are likely to have lower permeability than sites with higher sand contents. Low permeability soils are critical to reduce or slow the movement of leachate over time, thereby protecting water resources. Geological sites with higher potential for transport include those with highly fractured or unconsolidated material as well as those with shallow depths to bedrock and are generally unfavourable and should be selected with extreme caution (Beal et al. 2005; Williams et al. 2009). Geological requirements for a secure site include areas with permeability less than $1 \times 10^{-9} \text{ m s}^{-1}$ with soils high in clay content and high ion exchange capacities (Government of Alberta 2011; USDA APHIS 2012; Saskatchewan Ministry of Agriculture 2017). Typically, sites that would meet minimum guidelines should have aspects similar to those required for compacted clay liner or natural undisturbed clayey barrier systems whose target hydraulic conductivities are less than $1 \times 10^{-9} \text{ m s}^{-1}$, ensuring a diffusion-dominated system eliminating advective processes (Rowe et al. 1995; Benson et al. 1999). Soil properties for these types of containment systems include the presence of clay minerals (<0.002 mm diameter) at minimum contents between 15 and 20% by mass, plasticity indexes greater than 7% and a minimum cation exchange capacity (CEC) greater than 10 meq [100 g] $^{-1}$ (Rowe et al. 1995). Areas to avoid include those subject to instabilities, embankments of rivers/lakes/oceans, highly fractured subsurface materials, active fault lines, and regions of highly soluble rocks and highly permeable bedrocks.

From a hydrogeology standpoint, the protection of surface and groundwater is crucial. Avoidance of watercourses, drinking water wells, rivers/lakes/oceans and areas of high advective subsurface flows are paramount. High flow areas will contribute to faster movement of leachates and siting a burial pit in the water table may exacerbate this problem. Pit locations in well drained soils with deep water tables are less sensitive than those in poorly drained soils with high water tables. Alongside this, topography can play an important role in leachate transport. Flat land (slopes less than 2%) are preferred over sloped land since drainage gradients are generally slower on flat land, while hilly land may also erode and expose the burial pit potentially triggering surface water contamination issues (Williams et al. 2009).

Pit construction also plays an important role in leachate transport into the subsurface particularly regarding cumulative contamination issues in areas involving multiple burial trenches or pits. In the USA, (USDA 2001) estimated the volume of 0.92 m^3 to accommodate a mature cattle carcass whereas this value was 1.53 m^3 in AU and NZ estimated by the Agriculture and Resource Management Council of Australia and New Zealand (Nutsch and Spire 2004). Pit or trench locations, and spacing in the event of multiples, will most likely depend on site specific characteristics in geology. Geologically secure burial sites would eliminate risk to groundwater resources due to natural geological protection, which generally consist of sites with moderate to high clay contents (preferably with $\text{CEC}>10\text{ meq [100 g]}^{-1}$), meet minimum regulated depths to the water table, have hydraulic conductivities less than $1 \times 10^{-9}\text{ m s}^{-1}$, and lie in unfractured zones (Saskatchewan Agriculture 2005). The suggested ratio of the pit volume to carcass volume could be 4:1 for up to two layers of large animals and 2:1 for up to three layers of medium or small-sized animals (Mukhtar 2012). Pits that must be located in geologically unsecure areas should be engineered systems with liners and with leachate collection and treatment systems well thought out. In geologically secure locations, pit construction could be as simple as excavation, filling and capping at sites that meet minimum hydrogeological natural clay barrier requirements. Geotechnical testing at potential sites will help in the determination of pit construction parameters and whether lining systems such as impermeable barriers via geosynthetics, or constructed compacted clay liners should be applied (Lake et al. 2008). Synthetic liner requirements include compatibility of the liner with the geologic material it comes into contact with, as well as any pit boundary limitations and the ability to resist weathering and puncturing. As a generic standard for

other potential contaminants, Albano et al. (2011) recommended the use of synthetic liners with a minimum thickness of 0.3 mm; and compacted clay liners constructed with a minimum thickness of 0.015 m and a hydraulic conductivity of no greater than $1 \times 10^{-9} \text{ m s}^{-1}$.

While these recommendations from around the world somewhat coincide with each other from a regulatory standpoint, no studies exist that have provided any sort of numerical model to evaluate potential impacts of changing geological conditions and flow on leachate movement and contaminant transport beneath carcass burial sites. The objective of this study was to quantify the effects both conservatively and non-conservatively on leachate transport beneath burial trenches both spatially in the event of multiple trenches and vertically in the context of reactive and non-reactive transport along a vertical flow path. This modelling will aid in the development of risk analysis for geologically secure burial sites and help to determine the extent of contamination for geologically sensitive areas in the event of a liner breach.

4.3 Materials and Methods

4.3.1 Modelling methods

Two modelling scenarios were completed to evaluate conservative (non-reactive) effects of multiple burial trenches side-by-side as well as a single burial trench including reactive transport phenomenon. In Western Canada, carcass burial sites are typically constructed as trench systems approximately 2 m wide at a depth of 4 m dependent on site geology and depth to water table (Jansen 2012). These dimensions are commonly chosen for mass mortality events due to their ease of construction with standard, readily-available construction equipment such as tracked excavators and backhoes. These were also the dimensions chosen for the modelling scenarios presented in this paper. It was estimated that a trench with these dimensions, for every 1 m of trench length, approximately 1 m^3 (depth of 0.5m x 2m width) of leachate is free to drain from the trench (CFIA 2006; Pratt 2009). Utilising these dimensions and parameters, two numerical models were created, one in CTRAN/SEEPW (GeoStudio) and one in PHREEQC (USGS).

4.3.1.1 Non-reactive modelling using CTRAN

For the evaluation of non-reactive and sorption process transport and the effects created by various trench spacings, two dimensional models were created in GeoStudio's SEEPW/CTRAN. The GeoStudio software package solves the two-dimensional advection-dispersion equation based on the finite element technique which discretises the area under investigation into elements connected by nodes (Geo-Slope International 2008). Concentrations anywhere in an element are expressed in terms of the element's nodal concentrations:

$$C = \langle N \rangle [C] \quad [4.1]$$

where C = concentration in the element; $\langle N \rangle$ = a matrix of interpolating (or shape) functions and $[C]$ = the vector of nodal concentrations.

Using this approach, the finite element equation is derived from:

$$R(c) = \theta D \frac{\partial^2 C}{\partial x^2} - q \frac{\partial C}{\partial x} - \lambda K_d \rho_d - \left(\theta + \rho_d \frac{\partial S}{\partial C} \right) \frac{\partial C}{\partial t} \quad [4.2]$$

where: $R(c)$ = concentration of finite element; θ = volumetric water content; D = coefficient of hydrodynamic dispersion; q = flux of water = $n * v_x$; K_d = sorption coefficient; λ = decay coefficient; ρ_d = bulk density of the soil; and S = adsorption.

The solution to the finite element equation is a function of time and the integration is performed by a finite difference approximation scheme (Geo-Slope International 2008). The user can either use a Backward Difference Approximation, or a Central Difference Approximation. To solve for nodal concentrations at a certain time increment, CTRAN needs to know the nodal concentrations at the start of the time increment, therefore, the initial conditions of the problem must be known for a solution to the transport equation.

Models for this study were constructed with specific properties which were selected to simulate a worst-case scenario that met, at a minimum, government-regulated burial requirements in Canada, with one model exceeding the permeability standard by an order of magnitude. Each model assumed saturated conditions i.e. the bottom of the burial trench is in the water table, a non-ideal

scenario, but may be generally acceptable if subsurface geology allows for minimal permeability. To enable a comparison of permeability, the CTRAN models were run three separate times with a hydraulic conductivity variance between each. This provides a simulation for soil hydraulic conductivity changes from a moderately permeable glacial till to a low permeable glacial till (Milligan 1967; Legget 1976) (typical of subsurface conditions across much of the prairies of Canada). Other model parameters were also assumed for a glacial till and include 0.3 for porosity (Legget 1976; Saskatchewan Agriculture 2005), $9.5 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$ for the coefficient of molecular diffusion, hydraulic conductivities of $1 \times 10^{-10} \text{ m s}^{-1}$, $1 \times 10^{-9} \text{ m s}^{-1}$ and $1 \times 10^{-8} \text{ m s}^{-1}$ (representative of a low (Lake and Rowe 2005), medium and moderately permeable glacial till (Legget 1976)), and a dispersivity of 0.1 m. An initial concentration of $10,000 \text{ mg L}^{-1}$ (based on a rounded value of average ammonium concentrations for ease of numerical assessment) was assumed at the trench bottom with a finite contaminant depth of 0.5 m (1 m^3 of leachate per metre of trench) simulated by adding a concentration per time equation to the trench bottom boundary condition based on the hydraulic conductivity being assessed. CTRAN models were run incrementally in 10 year time spans up to 100 years.

Models were run both with and without adsorption parameters to simulate the effects soil properties may have on the retardation of the contaminant. Fonstad (2004) and others (Valocchi et al. 1981; Ceazan 1989; Balci and Dinçel 2002; Mikołajkow 2003; Pratt 2010) have studied the effect of ammonium concentration on the sorption properties of soil by performing sorption batch studies using laboratory tests or in-situ tests with varying concentrations of ammonium mixed with soil in order to provide ion selectivity coefficients ($K_{A/B}$ with A corresponding to the reference ion and B corresponding to the ion of interest) for each of their respective soils. In multicomponent ionic solutions, ion selectivity may change due to relative concentrations, valence, solvation, and van der Waals forces; and can be determined using multi-dilution sorption experiments. None of these specific studies involved concentrations of ammonium above $4,000 \text{ mg-N L}^{-1}$, however, those from Fonstad (2004) would be most applicable here and are coupled with values into the higher range determined for a similar soil by (Pratt 2010). Table 4.2 summarises distribution coefficient and selectivity coefficient results determined in these studies. The retardation factor for an ion in solution can then be calculated on a site specific basis if the porosity (n) and bulk density (ρ_d) of the soil are known. The retardation factor R, determined from a Langmuir distribution from

Fetter (1999) is shown in Equation 4.3, while the distribution coefficient K_D can be calculated from Equation 4.4.

$$R = 1 + \frac{\rho_d}{n} \left(\frac{\alpha\beta}{(1+\alpha C)^2} \right) \quad [4.3]$$

where: C = the concentration of the ion, and α and β = coefficients derived from sorption experimental results.

$$K_D = \left(\frac{\alpha\beta}{(1+\alpha C)^2} \right) \quad [4.4]$$

Table 4.2 Summary of ammonium selectivity coefficients

		units	This Thesis	Fonstad (2004)	Balci & Dincel (2002)	Valocchi et al. (1981)	Mikolajkow (2003)	Ceazan (1989)
Peak NH_4^+ Concentration		mg L^{-1}	10000	2000	4000	100	50	25
CEC of Soil		meq 100g^{-1}	12	7.0 - 12	19	10	0.1 - 20	0.5
Distribution Coefficient NH_4^+		L kg^{-1}	0.003 - 1.5	0.01 - 3	19 - 40		7.42 - 152.5	0.08 - 0.4
Selectivity Coefficients								
$K_{\text{Na}/\text{NH}_4}$			0.1 - 2.5	0.01 - 1.0		0.22 - 0.68		
$K_{\text{Na}/\text{K}}$			0.15 - 0.7	0.18 - 0.4		0.07 - 0.11		
$K_{\text{Na}/\text{Ca}}$			0.04 - 0.25	0.07 - 0.79		0.29 - 0.57		
$K_{\text{Na}/\text{Mg}}$			0.1 - 0.4	0.3 - 0.85		0.54 - 0.94		

To evaluate a mass mortality scenario in which carcass quantities would be excessive and require multiple burial trenches, it was also imperative to evaluate the effects of placing multiple trenches side-by-side. In the event of a mass mortality scenario, this would be a common potential solution dependent on the number of livestock and available space for burial. Assuming the same trench dimensions as above, two spacing scenarios were performed to observe the effects of trench spacing on the respective zone of influence from leachate. Five (5) m and 10 m trench spacing were simulated in a conservative manner (no adsorption), at a moderate permeability value of $1 \times 10^{-9} \text{ m s}^{-1}$ (the minimum regulatory standard for site hydraulic conductivity) and a constant vertical

gradient equal to one. Model setup for all three configurations (single burial trench and multiple burial trench spacing) are shown in Figure 4.1.

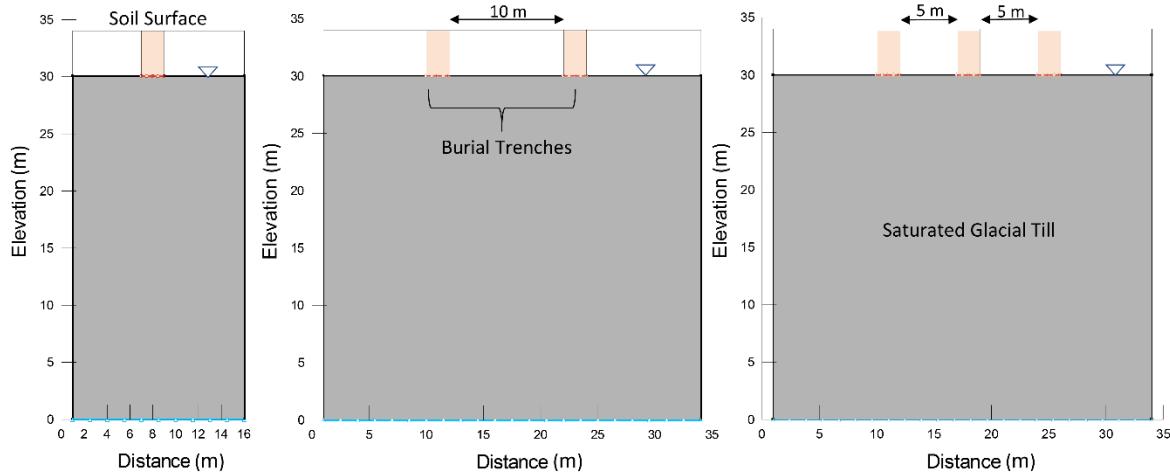


Figure 4.1 CTRAN model set-up for three scenarios, single trench, and two multiple trench spacings (10 m and 5 m separation distance)

4.3.1.2 Reactive Transport Model

While CTRAN models provide some insight to conservative and absorptive properties involved in contaminant transport, they leave out many of the geochemical reactions along the pathway. In order to accommodate this, a second software package was used to assess some of these processes. PHREEQC (freeware from the USGS) was used for transport modelling including geochemical thermodynamics, and soil interactions including ion exchange. The basic equation employed by PHREEQC was the advection-reaction-dispersion equation. Cation exchange equilibria were calculated using PHREEQC's thermodynamic databases, which are based on the Gaines-Thomas convention of using equivalent fractions for activities and the form: $\text{Ca}^{2+} + 2\text{X}^- = \text{CaX}_2$ (where X- is the exchanger master species) for the chemical reactions (Parkhurst and Appelo 1999). Due to the flexibility of the software code, other exchange equations can be used. The default activity coefficient for an exchanger species is 1.0, but PHREEC will allow for the use of the Davies, extended Debye-Hückel or WATEQ Debye-Hückel (Truesdell-Jones) activity coefficient for thermodynamic calculations. Given the magnitude of the ionic strength of the solution used as the

leachate input from Table 4.1 (> 1), it was decided to model exchange using the default Truesdell-Jones coefficients. Pratt and Fonstad (2017) demonstrated differences in activity coefficient calculations for ions present in livestock mortality leachate. Appropriately, the Truesdell-Jones equation yielded the best corresponding ion activity coefficients of approximately 0.6 for most monovalent ions and 0.3 for most divalent cations of concern. The PHREEQC model presented here utilised the Truesdell-Jones activity coefficients. The assumption, therefore, will favour the monovalent cations on exchange sites to some degree.

Transport is solved using a finite difference technique, while the chemical reaction term is calculated separately for each time step (Parkhurst and Appelo 1999). The transport simulation involved a 1-D column defined by a series of cells with the same pore volume. Cell length and time steps were defined to provide the velocity of water moving through the cells. A time step was simulated with an upwind scheme with concentrations calculated at the cell centre.

The PHREEQC 1-D column was simulated as follows. The column consisted of 50 cells, each 0.2 m in length for a total column length of 10 m. Dispersivity and diffusion coefficients were assumed to be 0.1 m and $9.5 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$, respectively. Cation exchange was also incorporated into the model with a conservative CEC value of 10 meq [100g] $^{-1}$. The column was equilibrated with groundwater using analytical data from a livestock facility site in Saskatchewan (Fonstad 2004) shown in Table 4.3. Once equilibration was complete, transport of leachate was performed utilising average livestock mortality leachate concentrations from Table 4.1. The volume of leachate transported was equivalent to 0.14 pore volumes or approximately 0.5 m depth of free solution (simulating 1m 3 of solution per 1m length of trench). To evaluate further transport of the leachate, transport of sequential volumes of fresh groundwater equal to 0.14 pore volumes of the column were completed up to 0.56 more pore volumes. Each 0.14 pore volume transport increment equated to roughly 15 years of time for a total time assessment of 75 years.

Table 4.3 Chemical composition of a background groundwater sample from a livestock facility site in Saskatchewan* (Fonstad, 2004)

	units	Concentration
pH		7.4
Ca	mg/L	52
Mg	mg/L	25
Na	mg/L	29
K	mg/L	66
NH4-N	mg/L	0.6
Alkalinity	mg/L	253
Cl	mg/L	29
SO4	mg/L	82
P	mg/L	0.37
Fe	mg/L	0.55
Mn	mg/L	0.36
Si	mg/L	6
Sr	mg/L	0.48

*Geology at the site was a lacustrine deposit consisting of interbedded sands and silts with a CEC of approximately 8 meq/100g.

4.4 Results and Discussion

4.4.1 Non-reactive transport modelling

As an initial step towards modelling livestock burial leachate transport, a numerical transport model in its most simplistic form was used to simulate non-reactive transport of leachate beneath a typical mass disposal burial trench. The first series of models shown here depict transport of a non-reactive ion at an initial concentration of 10,000 mg L⁻¹ from the bottom of a burial trench for three different subsurface permeability scenarios (Figure 4.2). By increasing the hydraulic conductivity of the soil by three orders of magnitude, transport distances subsequently were amplified with each increasing increment of hydraulic conductivity. For example, for the highest permeability, transport depths at the 10-year timespan indicated peak concentrations of up to 7% of the initial value reached a depth of approximately 6 to 7 m with advection dominated flow and wide (relative to pit dimensions) horizontal and vertical spreads. In scenarios with higher hydraulic conductivities, advection/dispersion dominated processes in two dimensions, create a larger

horizontal spread of the plume which corresponds to lower concentrations down the plume's centreline relative to the lower permeability scenarios. The Canadian standard for burial site hydraulic conductivity ($K = 1 \times 10^{-9} \text{ m s}^{-1}$) (CFIA 2006) is shown in the middle of Figure 4.2 and demonstrates up to 5 m of vertical transport in the first 10 years, however, not all leachate has left the burial pit at that time step. By 50 years, the peak concentrations from that scenario has transported approximately 5 to 6 m with lower concentrations tapering to 10 m. The lowest hydraulic conductivity scenario produced results demonstrating a very secure burial site. Transport depths after 100 years for this scenario were approximately 2 m, with the highest concentrations just beneath the pit bottom. This simulation presents an ideal scenario from a contaminant transport and site security point of view, keeping in mind that these models are conservative in nature and are demonstrating transport of a non-attenuated or non-sorbed ion, i.e. similar to chloride. Ions that generally have a positive charge would be attenuated and transport distances would be less for those ions, but their exchange or adsorption would probably produce a hard water front ahead or at the front of the plume as demonstrated later in this paper.

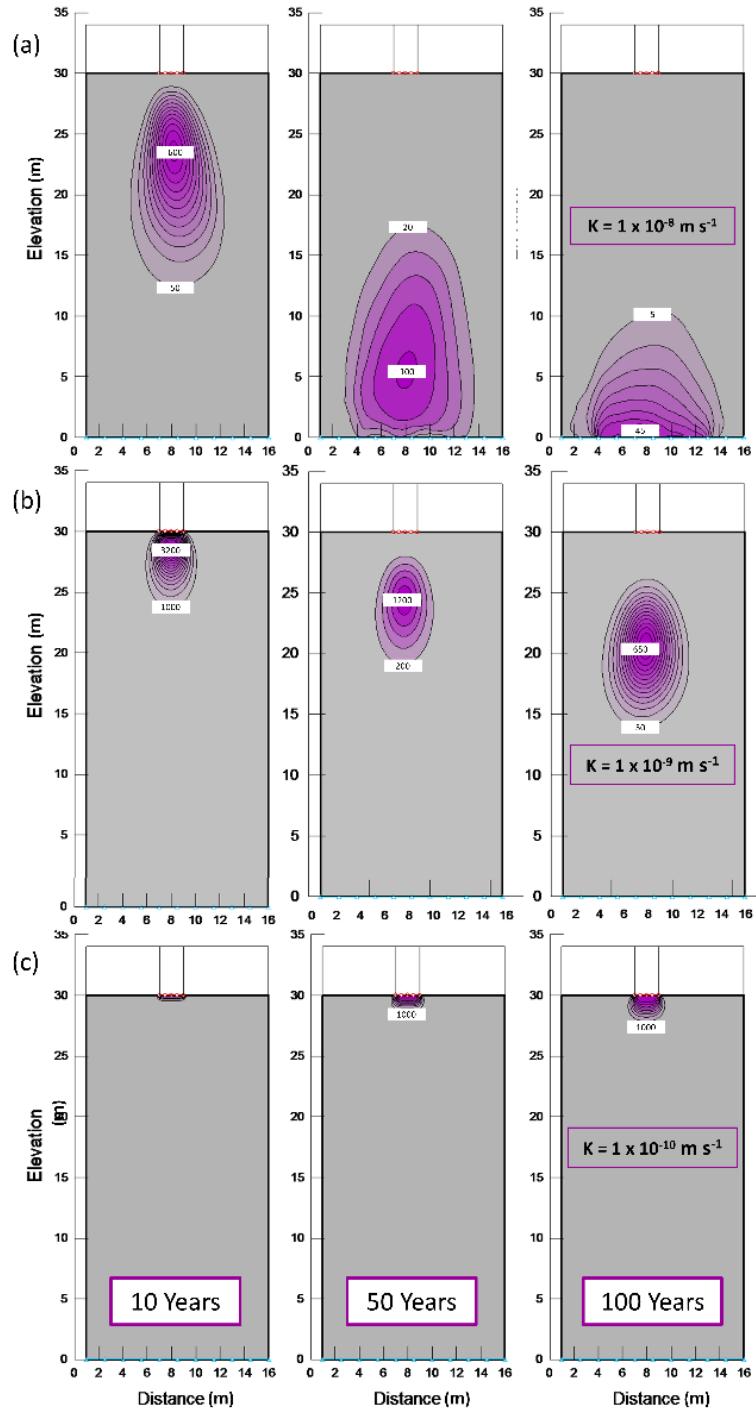


Figure 4.2 Modeled impacts of hydraulic conductivity on leachate transport after 10, 50 and 100 years for three scenarios of typical glacial till values a) $K = 1 \times 10^{-8} \text{ m s}^{-1}$, b) $K = 1 \times 10^{-9} \text{ m s}^{-1}$, c) $K = 1 \times 10^{-10} \text{ m s}^{-1}$. Plume concentrations in mg L^{-1} are shown for the center and edge of each

plume

The effect of multiple burial trenches side by side over 10, 50 and 100 years are shown in Figure 4.3. Two trench spacings were used in these models: 5 and 10 m and $K = 10^{-9} \text{ m s}^{-1}$. The top half of Figure 4.3 shows modelling results for 10 m spacing between burial trenches. At fifty years, the plumes are separated by approximately 8 m. After 100 years of transport, the plumes associated with each burial trench in this spacing scenario do not contact each other, and their concentration fronts are still approximately 5 m apart. For the 5 m spacing scenario (bottom half of Figure 4.3), within fifty years, each burial trench's leachate plume is becoming close to combining with that from the trench beside it. After 100 years the trenches' plumes have joined and produced one large plume with approximately a 10 m spread in the vertical direction. A 1 to 2-m-thick zone of approximately 1% to 5% of initial concentration exists in-between the pits after 100 years. The risk associated with joining plumes determines the zone of influence of the burial site, and the goal would be to eliminate the possibility of these plumes joining to maximise soil sorption processes. In order to expose the burial leachate to its maximum potential for adsorption onto soil particles, a significant separation distance of at least 10 m should be in place for regulatory purposes.

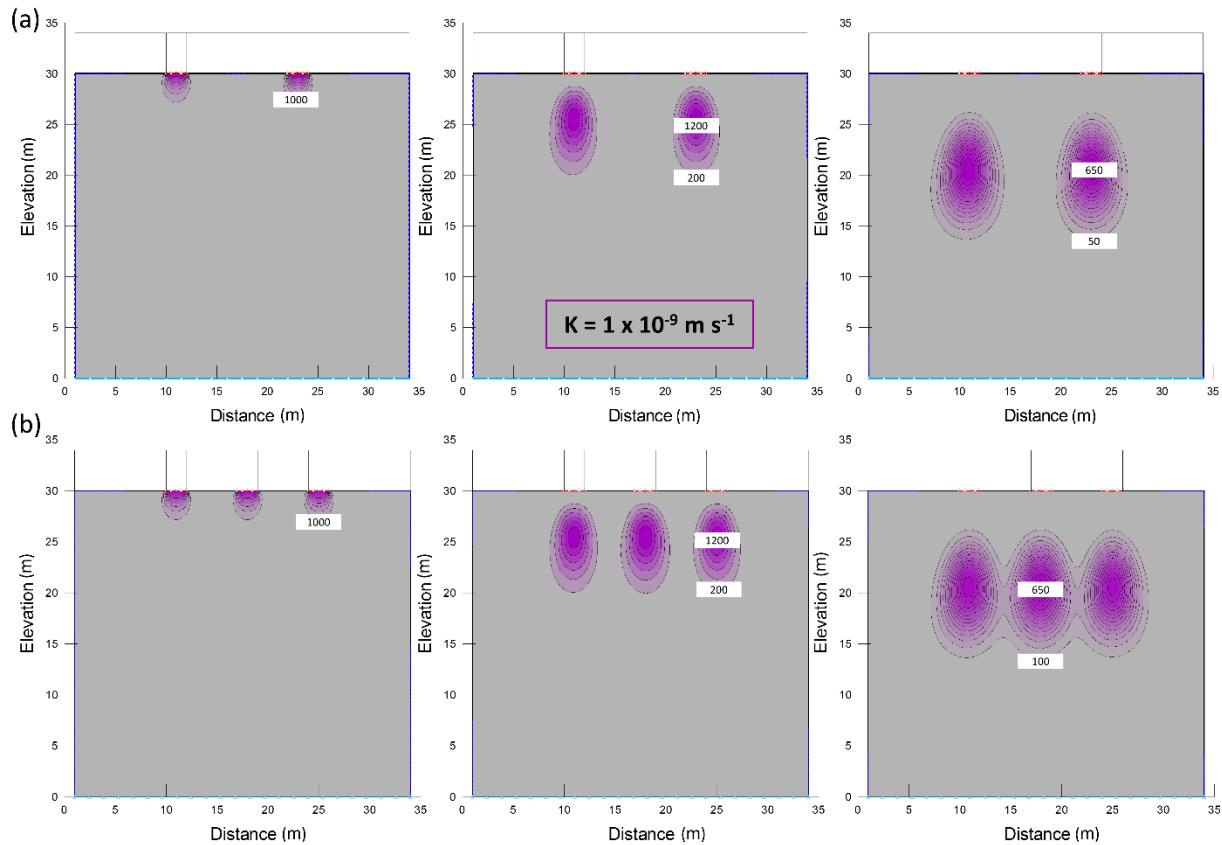


Figure 4.3 Multiple trenches scenario at two trench spacing (10 meters and 5 meters)

Unreactive transport models are useful tools to evaluate conservative movement of contaminants. In Western Canada, the soils available for burial sites would generally have higher clay contents and provide sorptive attributes. CTRAN was also used to evaluate the effects of ion sorption on transport distances over time. As a comparison, Figure 4.4 demonstrates results from the sorption model as compared to the conservative model for the same permeability (the minimum standard in Canada, $1 \times 10^{-9} \text{ m s}^{-1}$). At each time step, the figure shows the concentration profile at that time step in two ways: a 2D representation of the plume as well as a 1D vertical concentration gradient with depth (presented as a ratio of computed concentration versus initial concentration (C/C_0)). The top half of the figure shows an unreactive conservative tracer with no attenuation or geochemical interactions/evolutions, while the bottom half includes soil adsorption properties with sorption values from Fonstad (2004) and Pratt (2010). For the conservative model at 20 years post burial, approximately 25% of the initial concentration had reached a depth of 3 m. By 100 years,

nearly 10% of initial values had migrated to a depth of 10 m below the burial trench, similar to models produced using comparable parameters and clay attributes for a leachate lagoon from Lake and Rowe (2005). Due to the boundary conditions and properties assumed and chosen for this model, transport is dominated in the vertical direction, i.e. no lateral advective flow was included, only transverse and longitudinal dispersion values. If transverse advection was included, at some point beneath the burial trench we would see a change in direction of plume migration. This is concerning for a multitude of reasons. Selection of burial sites in areas without strong lateral flow systems, such as those with subsurface shallow aquifers fed by a series of sand lenses, should be avoided. Once these systems are introduced beneath the burial site, strong lateral flows may take the 40% of initial concentration values found 100 years later and transport it laterally in a more expedient fashion. By increasing or decreasing permeability, we either expedite or slow the process shown in Figure 4.4, similar to findings demonstrated in Figure 4.2.

While the conservative model is useful for studying tracer transport, in real-life scenarios soil properties such as adsorption play a large role in the rate at which contaminant moves through the subsurface. Sorption properties were included in the same model to show the significant effect this has on transport of ammonium. The bottom half of Figure 4.4 shows 20 year intervals up to 100 years of absorptive transport of ammonium beneath a burial trench. It is immediately noticeable that the model produces dissimilar transport depths over time when all factors (except for sorption) stay the same, showing significant retardation of the ammonium ion. When comparing the 20 year sorptive model to the conservative model, dispersion and diffusion allow for a migration depth that is less than half the distance, and substantially less contaminant (less than 1 percent at 2.5 m). After 20 years onward to 100 years, plume profiles are dissimilar between the two models, with the sorption model showing significant retardation in the transport of ammonium due to sorption processes. By 100 years, soil sorption processes have sorbed the bulk of the ammonium, while a plume is still present, it exists in concentrations of less than 1% of initial values at depths below 10 m from the bottom of the burial trench. At that concentration ($>100 \text{ mg L}^{-1}$), nitrogen levels (if conversion to nitrate or nitrite succeeds) still exceed standards for drinking water ($>10 \text{ mg L}^{-1}$), but are less concerning than initial values (United States EPA 1995). The bulk of the contaminant load is captured in the first 2 m of soil beneath the burial pit due to ion adsorption. What this model doesn't show are the many other effects caused by the sorption of ammonium. With sorption in

the natural environment comes desorption or ion exchange. Ammonium ions will replace other ions through the competitive ion exchange processes which this model does not account for.

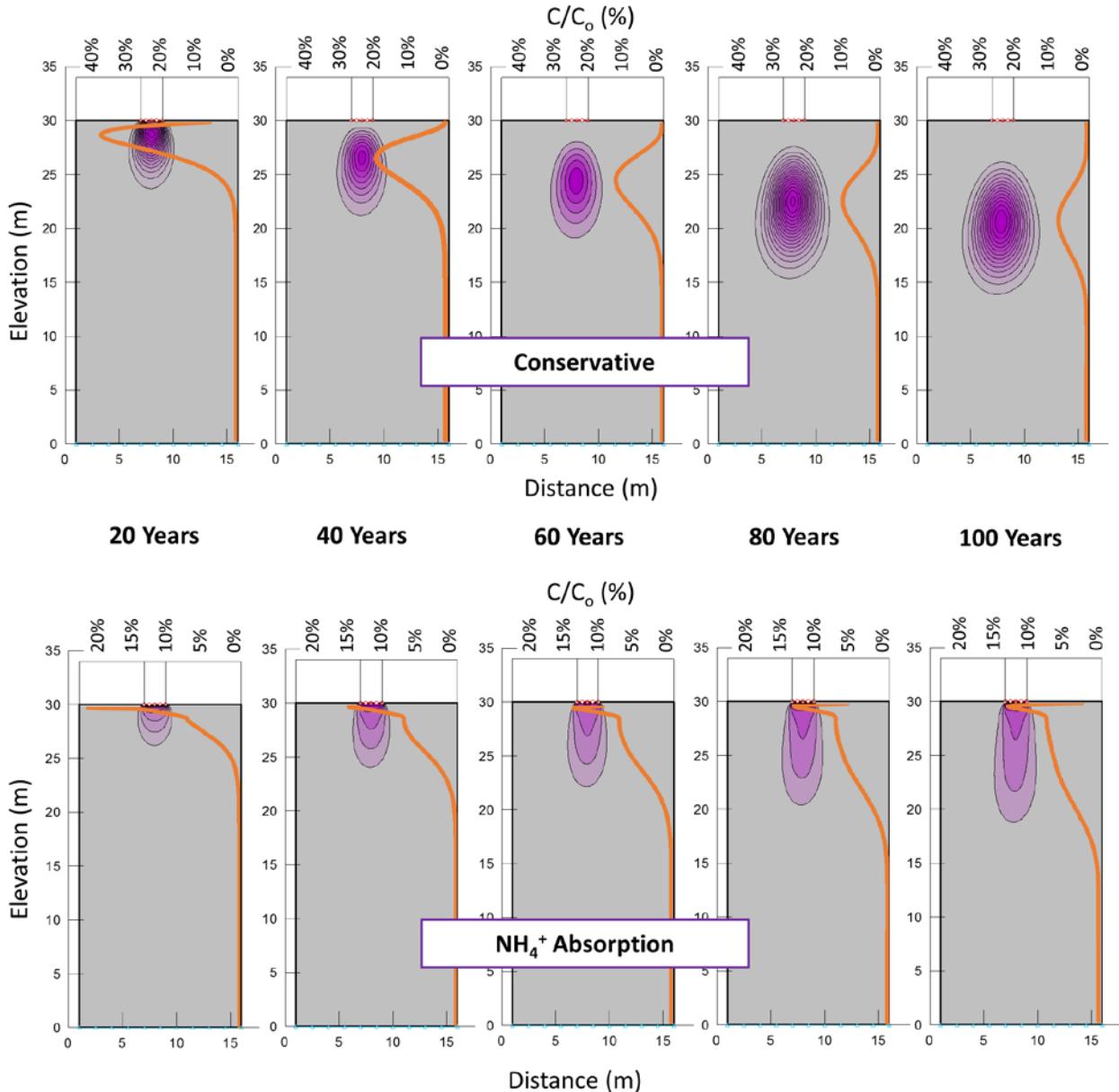


Figure 4.4 CTRAN results for conservative and sorptive models at 5 intervals in time overlain by concentration vs depth profiles from the model down the plume centerline at each time interval presented as concentration ratio C/C_0

4.4.2 Reactive transport modelling

To evaluate potential geochemical reactions and ion exchange occurring beneath the burial trench during transport, PHREEQC was used to simulate transport of livestock mortality leachate through a column of moderately permeable glacial till soil. The transport simulation involves a 1-D column defined by a series of cells with the same pore volume. The 10 m column was equilibrated with groundwater from Table 4.3 initially, after which a 0.14 column pore volume of livestock mortality leachate solution was applied to the top of the column. This is a volume equivalent to approximately 0.5 m depth of fluid also corresponding to approximately 15 years of time (hydraulic conductivity of approximately $1 \times 10^{-9} \text{ m s}^{-1}$). Groundwater was then again applied to the top of the column in 0.14 pore volume increments up to 0.56 pore volumes (each increment equates to approximately 15 years). The results at each pore volume/time increment can be seen in Figure 4.5a-e. Results shown here also indicate a change in the retardation factor for ammonium over the course of the model, indicative of variable ion selectivity due to concentration changes. Retardation factors after the first groundwater infiltration were approximately 1.4 for ammonium compared to chloride, and by the last infiltration model, retardation increased. Others have found similar results for other ions in contact with soil solutions, with the greatest implication being the importance of determining site specific distribution coefficients for specific contaminant sets (United States EPA 1999; Selim and Sparks 2001; Mikołajkow 2003).

Figure 4.5a shows an initial concentration front of calcium and magnesium (indicators of water hardness) leading the plume due to cation exchange occurring with ammonium and potassium on the exchange sites. This hard water concentration front is approximately 1 m ahead of the ammonium front. Bicarbonate, sodium and chloride show unattenuated transport. This trend continues once groundwater is added on top of the column model after the draining of the mortality leachate. Figure 4.5b shows 0.14 pore volumes of groundwater applied on top of the burial site and allowed to transport after the leachate. This figure shows ammonium retardation and rising concentrations in the calcium/magnesium front. Bicarbonate concentrations are slightly decreasing; this could be caused by bicarbonate ions precipitating with calcium and magnesium. Figure 4.5c shows 0.28 pore volumes of water added to the column. The hard water front is then

slightly decreasing in concentration. The attenuation of ammonium is still occurring and bicarbonate is also decreasing in concentration. The same is occurring in Figures 4.5d and 4.5e.

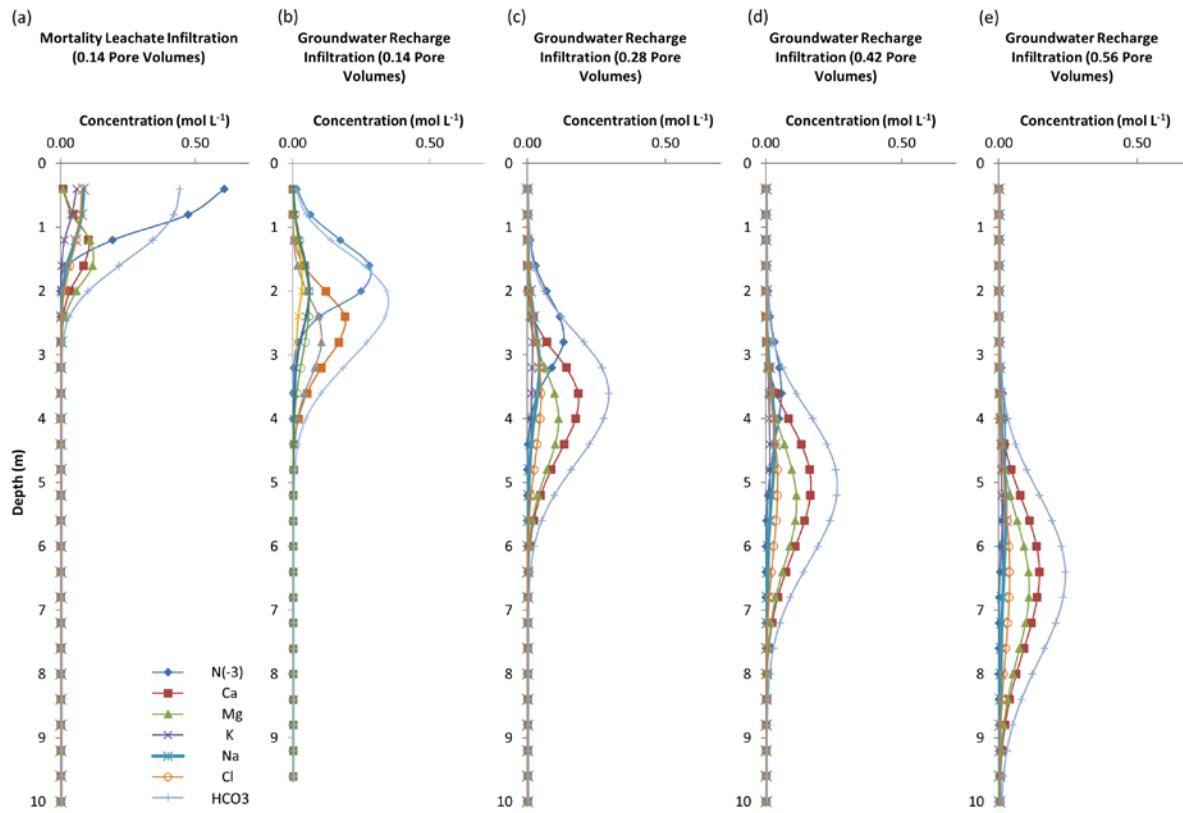


Figure 4.5 Reactive transport modelling: (a) Mortality leachate 0.14 pore volumes infiltration into column (b) Groundwater 0.14 pore volumes infiltration into column on top of leachate; (c) Groundwater 0.28 pore volumes infiltration into column on top of leachate; (d) Groundwater 0.42 pore volumes infiltration into column on top of leachate; (e) Groundwater 0.56 pore volumes infiltration into column on top of leachate *Note: Concentrations of each ion are presented as a total sum of all species containing that element. (Pratt 2009)

The 22 most abundant species of ions involved in the mortality leachate transport are shown in Figure 4.6; ammonium and bicarbonate being the most abundant cation and anion respectively. During the transport intervals, ammonium decreases in concentration and was four percent of initial concentration after 0.56 pore volumes of fresh groundwater transport. Sodium and potassium also show significant drops in concentration as well as phosphoric acid. Increases in

sulfate concentrations can be attributed to the high amount of sulfate in the leachate as well as the concentration of sulfate present in the groundwater.

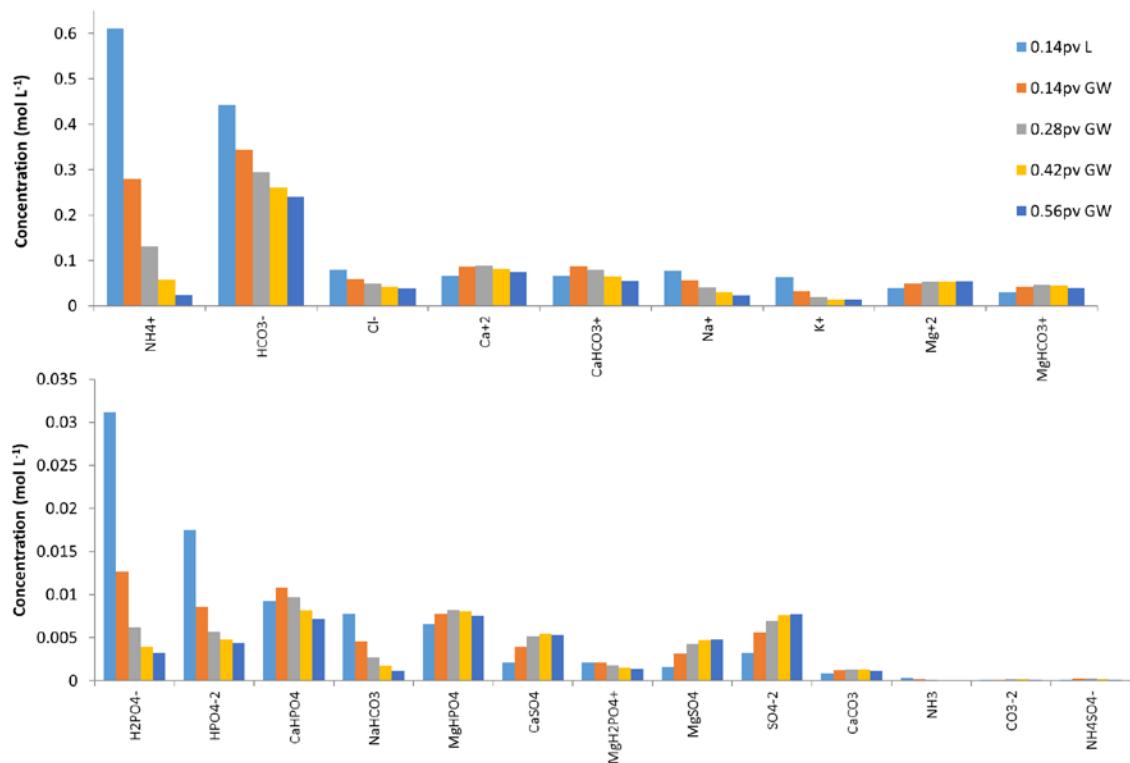


Figure 4.6 Most abundant species or ions involved in the transport process at each transport step
(Pratt 2009)

Because of increasing sulfate concentrations to match background conditions, it can be expected that sulfate minerals such as CaSO₄ and MgSO₄ will increase in concentration until the sulfate concentration has reached its saturation limit. Increases in calcium and magnesium concentrations can be attributed to ion exchange, as ammonium was trading places with these ions on soil exchange sites. Due to the release of these ions, their concentrations in solution were increasing and they were binding with other ions such as phosphoric acid, bicarbonate, and sulfate. The speciation results of the leachate show potential threats from ammonium transport due to the formation of ammonium sulfate (NH₄SO₄), phosphoric acid and sulfate compounds in solution.

4.5 Conclusion

Subsurface transport of livestock mortality leachate in areas that are not geologically secure (high hydraulic conductivity, low CEC, fractures, etc) poses a significant threat to the environment as seen in countries around the world after large scale catastrophic mortality events. This study has provided insight and better predictions of the contaminant transport issues arising from such sites. Multiple models are presented to assess both conservative and geochemically reactive transport features for sites typically found in the Western Canadian Prairies. Features such as hydraulic conductivity (ranging from $1 \times 10^{-8} \text{ m s}^{-1}$ to $1 \times 10^{-10} \text{ m s}^{-1}$), soil sorption parameters and ion exchange coefficients were selected, based on typical glacial tills for the area. They are important to the overall predictive model and can greatly impact transport distances. Three separate models were created in GeoStudio's CTRAN (single trench, 5 m trench spacing and 10m trench spacing) to evaluate in 2D the subsurface transport phenomenon. A range of hydraulic conductivities typically measured for the region were varied for each of the 2D models. The greatest transport impacts were observed for the highest hydraulic conductivity, which was one order of magnitude higher than the Canadian standard for hydraulic conductivity at a mass burial site. The lowest hydraulic conductivity produced results that demonstrated a very secure burial site. Sorption parameters were also included for ammonium as a comparison to the conservative models and demonstrated a decrease in the concentrations observed in the plume. For that scenario, less than one percent of the initial ammonium concentration was observed at depths less than 10 m beneath the bottom of the burial trench after 100 years of transport, as compared to 40% of initial concentrations in the conservative model. The assessment of burial trench spacing showed that, in order to expose the burial leachate to its maximum soil adsorption potential, a significant separation distance of at least 10 m should be in place.

While sorption and conservative models are useful tools for visualising and quantifying transport processes, they do not have the ability to account for all ion exchange and geochemical properties of the subsurface. To help assess some of these properties and their effects on transport, a model was also created in PHREEQC. This transport model used a conservative CEC of 10 meq [100 g]¹ and demonstrated a highly-concentrated calcium and magnesium plume forming in front of the ammonium plume, suggesting ion exchange and attenuation of ammonium. After 0.5 m of leachate

was infiltrated into the column, and another 0.5 m of water was added on top, the plume reached a depth of approximately 2 m at a concentration slightly less than the initial input. After 0.56 pore volumes (2 m of water, or 75 years), the most concentrated portion of the plume reached a depth of 7 m with concentrations of bicarbonate and calcium/magnesium near 0.3 and 0.2 mol L⁻¹ (18,300 mg L⁻¹, 8000 mg L⁻¹, and 4900 mg L⁻¹), respectively, the majority of the ammonium at this interval had been attenuated in the soil. After approximately 50 years, ammonium concentrations were nearly 4% of the initial values, indicating that high concentrations still remained and were able to undergo geochemical thermodynamic reactions with other ions in solution.

Livestock mortality burial in the Western Canadian Prairies at sites that fit within government regulated parameters, as modelled in this study, present little threat to their surrounding environments. If soils are present with sufficient clay content, low permeability, moderate to high CEC, and low groundwater gradients (both lateral and vertical), leachate movement will be minimal, and adsorption onto soil particles will keep surrounding areas safe. In areas that do not meet requirements for burial, site investigations should proceed and further modelling should be done based on those site specific parameters to assess the risk in that particular area.

4.6 Acknowledgements

The authors would like to thank the Saskatchewan Ministry of Agriculture's Agriculture Development Fund (ADF) for project funding.

4.7 Transition Statement

Two modelling tools were created here to assess geochemical impacts beneath livestock burial sites. This information provided a useful tool to evaluate plume migration, geochemical interactions and possible plume attributes that could be routinely tested for diagnosis of plume development surrounding a burial site. The next chapter provides the first ever investigation of multiple burial sites across Saskatchewan and evaluated plume development and transport properties at two sites. The investigation presented in the next chapter provides a useful tool for model validation using existing burial sites.

4.8 Author Contributions

D.P. designed the study, prepared and executed the geochemical models and performed the model analysis. D.P. wrote the manuscript, T.F. provided edits and comments on the manuscript and contributed to the text in later iterations.

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Chapter 5: Geochemical Evolution and Leachate Transport Beneath Two Carcass Burial Sites: A Field Investigation

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5.0 Preface

The previous chapters of this thesis have evaluated the geochemical aspects of mortality leachate and utilized that information to create both conservative, sorption and reactive transport models to predict leachate impacts in the subsurface. Modelling efforts are generally scrutinized until validation efforts have been made. In order to validate the applicability of our models and be able to provide evidence based policy and burial regulations, site investigations are crucial. Site investigations can provide useful tools in the assessment and validity of modelling scenarios and provide real-world examples of contaminant impacts to the subsurface. This chapter provides the first ever field and laboratory coupled investigation of multiple burial sites across Saskatchewan by evaluating plume development and transport properties at two regionally different sites. This field investigation provides indications of burial site contaminant transport characteristics for two sites that would generally be deemed acceptable for livestock burial during a catastrophic event. It is studies such as this, that during these catastrophic scenarios, will help ease public perceptions, mitigate risk, and provide value for regulatory framework done in this arena.

5.1 Abstract

This study presents the first complete investigation utilizing soil coring for evaluating the geochemical evolution of leachate plumes beneath existing livestock burial sites. The objective of the study was to broaden our understanding and provide evidence based resources for minimizing the environmental impacts of mass mortality carcass burial. Pre-existing livestock burial sites were selected for a detailed analysis of contaminant transport beneath and surrounding the sites in an attempt to determine the risk to soil and groundwater. This analysis entailed soil coring of the

site along with specific ion and solution extraction analyses on the soil cores to provide a detailed 2-D picture of leachate movement around and below these burial sites. The first site, located near Pierceland, SK, was used in 2001 to bury euthanized elk potentially suffering from chronic wasting disease (CWD). The soil cores were taken seven years post burial and the extent of leachate transport, upon analysis of the soil cores, was 1 meter to 1.5 meters of vertical transport of anions (Cl, Alkalinity) as well as some cations arising from ion exchange reactions (Ca and Mg). Ammonium ions were attenuated near the bottom and first meter beneath the bottom of the trench. There was no indication of lateral movement of ions at this site. The second site, located near McLean, SK was used in 1952 to bury carcasses of culled livestock during an emergency depopulation effort for disease control measures during Canada's only foot and mouth disease outbreak. This site was cored nearly 60 years post burial and demonstrated vertical leachate movement that was relatively slow over the 60 years with movement of up to 1 meter to 2 meters. Due to the presence of sand lenses in and around the burial pit, movement of up to 10 meters horizontally of anions, such as Cl and bicarbonate, was discovered. Ammonium ions were indicated within the confines and immediate soil surrounding the burial pit. Both sites demonstrated plume characteristics consistent with previous geochemical models and both showed little impact to the immediate surrounding environment. This would appear to indicate that burial site selection characteristics were appropriately determined and that many parts of Saskatchewan are suitable for mass quantities of livestock carcass burial in the event of catastrophic events such as disease outbreaks or natural disasters.

5.2 Introduction

Canada's livestock industry provides economic and social stimulus to its economy and the size of the industry (approximately 30M head of cattle, pigs and sheep; 684M poultry (Government of Canada 2014)) puts the nation at risk in the event of large-scale disease outbreaks or catastrophic events. Diseases such as avian influenza (AI), foot and mouth disease (FMD) and all transmissible spongiform encephalopathies (TSE, BSE or CWD) are documented to occur in many places throughout the world (WAHIS Database 2016). Due to the nature of these diseases in particular, they generally require immediate management and culling of livestock that can be, or are affected, within a certain radius of the outbreak. Management of disease outbreaks require containment

plans to prevent further spread as well as disposal plans to mitigate culling operations. Common disposal options for animal carcasses include burial or landfilling, incineration, composting, rendering, alkaline hydrolysis, anaerobic digestion and lactic acid fermentation (Engel et al. 2004). Of those methods, the most commonly utilized in North America include burial, composting, rendering and incineration with many recommended methods and alternatives dictated by government regulations such as the United States Environmental Protection Agency (US EPA) or the Canadian Food Inspection Agency (CFIA). In the event of a disease outbreak or natural disaster, the need to dispose of large quantities of livestock mortalities will arise. In areas of intensive livestock operations, the number of animals to be euthanized has potential to deem the most common methods of disposal, unacceptable due to the likelihood of exceeded capacity. Rendering and incineration are viable alternatives, but their daily capacities are easily overwhelmed. While composting presents another alternative, the supply of adequate carbon sources could be insufficient, especially in certain geographical regions. Burial is the most likely choice for many of these events and is the least understood with respect to environmental implications (Pratt 2009; Kim and Kim 2012). There is currently a lack of knowledge of the transport of contaminants beneath burial sites.

5.2.1 Leachate Chemistry and Potential Impacts to Groundwater

Previous research on the complete inorganic chemical characterization of carcass leachate by burying carcasses of various types in plastic lined pits and sampling leachate from the pits for chemical analysis was performed by Pratt (2009) and indicated that livestock mortality leachate contains, on average, after two years of decomposition, concentrations of 12,600 mg/L of ammonium-N, 34,600 mg/L alkalinity (as bicarbonate), 2,600 mg/L chloride, 3,600 mg/L sulfate, 2,300 mg/L potassium, 1,800 mg/L sodium, 1,500 mg/L phosphorus along with relative lesser amounts of iron, calcium and magnesium. Maximum values for the major ions were up to 50% higher than the average in some instances. The pH of the leachate was near neutral. In comparison to earthen manure storages and landfills from Fonstad (2004), the chemical composition of the leachate was 5-10 times higher. Others have analyzed quality and quantity of leachate produced from carcass burial, including those from FMD disposal sites in the United Kingdom, (Glanville

2000; MacArthur et al. 2003; Cleary 2009; Yuan et al. 2013). Table 5.1 shows the results of Pratt (2009) as compared to other studies characterizing portions of carcass leachate.

Table 5.1 Summary of the characteristics of carcass decomposition leachate as measured by five studies (units are in mg/L except pH) and include Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), organic carbon, Ammonia-N or Total Kejeldal Nitrogen (TKN), Total Dissolved Solids (TDS), Phosphorus, Chloride, Sulfate and pH

Constituent	Pratt (2009)*	MacArthur et al (2003)**	Glanville (2000)**	Cleary (2009)**	Yuan et al (2013)+
BOD	-	12700	230	-	-
COD	-	20414	-	-	95333
Organic Carbon	64000-81000	-	-	-	27158
NH ₄ ⁺ , Ammonia-N or TKN	11000-19200	3294	403	-	14640
TDS	-	-	1527	845	-
Phosphorus	990-1890	55	-	-	3.06
Chloride	2600	486	109	46	2614
Sulfate	3600	-	-	56	-
pH	6.7	6.9	-	-	-

*Averages
**Influenced by rainwater
+Maximums

Along with the limited literature available on carcass leachate composition, there is limited published literature characterizing the environmental implications of burying livestock. A few studies exist using experimentally constructed sites, as well as in-place burial sites, and monitoring of the groundwater quality around them (Ritter and Chirnside 1995; Glanville 2000; MacArthur and Milne 2002; Scudamore et al. 2002; MacArthur et al. 2003). Many of the early investigations focused on monitoring groundwater quality surrounding existing mortality disposal sites (Glanville 2000; Ritter and Chirnside 1995). These studies gave a basic analysis of potential contaminant transport into groundwater systems for the most common disposal of routine on-farm mortalities and small, one-time load burial pits. Average livestock losses occurring on site are generally not considered a threat to ground and surface water bodies in most provinces and states; however, when dealing with a mass mortality event, the sheer number of culled animals to be

disposed of requires a more careful evaluation of disposal sites and the risks involved (Engel et al. 2004; Saskatchewan Agriculture & Food 2005).

One of the first studies to evaluate the impact of livestock carcass disposal on groundwater quality took place in Delaware by Ritter and Chirnside (1995). Their study placed monitoring wells at a depth of 4.5m around the perimeter of routine (continuous use) mortality carcass disposal pits with weekly loadings of 15kg to 25 kg of poultry. These pits were located in areas of sandy loam soils and high water tables, meaning the bottoms of many of the disposal pits were located below the groundwater table for most or all of the year. By using this method of disposal, the potential for groundwater contamination was relatively high. Monitoring of these pits occurred from 1987 to 1990 and samples were drawn every 4 to 8 weeks and analysed for ammonia, nitrates, fecal coliforms and fecal streptococci. Their study concluded that ammonia-N ($\text{NH}_3\text{-N}$) concentrations were high in two wells. Of the six pits, three of them demonstrated an increase in ammonia concentrations at a lateral distance of 12 m. In some cases, the ammonia-N concentrations reached 366 mg/L and streptococci concentrations reached 209 mg per 100 mL. In all monitoring wells, fecal coliform and fecal streptococcus concentrations were low and only detected 20% of the time during sampling. Bacterial contamination of groundwater by the disposal pits was reportedly low. They concluded that more groundwater contamination could occur in the event of a large-scale mortality event due to higher mass loading of the burial system.

Glanville (2000) studied two methods of burial disposal (pits with poultry and trenches with swine) and the impacts each had on shallow groundwater quality. Monitoring wells were constructed at varying depths and distances from each burial location and monitored routinely. Neither case study demonstrated contamination more than a meter or two from the site within the sampling period (up to 6 years), but this situation could easily be changed in areas with high water tables and high groundwater velocities. Elevated levels of biochemical oxygen demand (BOD) at 230 mg/L, ammonia-nitrogen ($\text{NH}_4\text{-N}$) at 403 mg/L, total dissolved solids (TDS) at 1527 mg/L, and chloride (Cl) at 109 mg/L were found within the burial site and at a distance of 1 meter to 2 meters downgradient. Glanville (2000) also concluded that localized contamination may persist for a decade or more in wet soil with a high seasonal water table and low groundwater flow velocity.

Even in lightly loaded burial trenches constructed in well-drained soil, complete decay may take two years or more.

In 2001, an FMD outbreak in the United Kingdom required euthanization of nearly 1.3 million animals, with many disposed of using burial in pits of varying scale (Scudamore et al. 2002). The most documented burial site from this outbreak was located in the Birkshaw Forest and contained over 400,000 carcasses in six disposal pits. Three of the first pits constructed were unlined due to time constraints, but the remaining three were constructed with geo-synthetic clay liners (GCL) and all pits possessed a leachate collection system (MacArthur et al. 2003). The burial sites were commissioned in April 2001 and not capped until June 2001. During the first two months of pit filling, rainwater was allowed to enter the pits. To keep pollution at a minimum, leachate was collected and sent to a treatment plant. For the entire outbreak, surface and groundwater chemistry were monitored closely around all disposal sites. Impacts to streams and rivers were reported at 212 total incidents resulting from leachate spills and contamination along disposal routes, with only one incident of a category 1 which cause major damage to the aquatic ecosystem and 11 category 2 incidents which cause significant damage to the aquatic ecosystem (Environment Agency 2001). Since disposal routes were the most common source of environmental contamination, the disposal sites themselves, such as those in the Birkshaw Forest, showed very few incidents. Groundwater contamination was continuously monitored through boreholes located at varying distances and depths from the burial sites. Data up to 2006 showed very localized groundwater contamination of ammonia (4.2 mg/L) on the east side of the Birkshaw site (within 5 meters of the burial pit) indicative of minor localized contamination that has been stabilized and is now on the decline (MacArthur 2006). Monitoring has suggested, that due to the high quality of surface and groundwater at this site, the engineered pit system and leachate collection has been successful and are effective carcass management strategies in the event of a disease outbreak (MacArthur 2006). According to the Environment Agency (2002), water companies have found no contamination of public water supplies surrounding any of the other burial sites.

More recently, FMD outbreaks in South Korea in 2010 to 2011 have devastated their swine populations as well as significantly impacted the environment surrounding many of the disposal sites. During this specific outbreak, more than 4 million animals were culled and buried in more

than 4000 burial pits (Kim and Kim 2012). Due to haste, lack of manpower and supplies, and accidents, many of these burial pits were poorly constructed in ill-suited locations and have leaked. Groundwater was monitored at high risk sites weekly for the first six months and quarterly for the duration. Because of this monitoring, many of the burial sites have been deemed defective, requiring treatment and/or remediation (Kim et al. 2014). Park et al. (2012) evaluated ammonia-N concentrations in over 300 monitoring wells surrounding specific, at-risk burial sites and concentrations of up to 2593 mg/L was observed. Of the wells monitored, 24% showed higher than 10 mg/L ammonium-N, >100 mg/L chloride and more than 800 uS/cm electrical conductivity (Park et al. 2012). Many of the sites showing high concentrations in monitoring wells were excavated and the material moved to more environmentally secure locations. Due to the downturn this outbreak caused for the agricultural economy of Korea and their dire need to prevent groundwater contamination, government agencies have rallied and created research groups and facilities to further study treatment and disposal options as well as deal with existing sites that are causing environmental problems (MOE 2010; Joung et al. 2013; Kim 2015; Kim and Pramanik 2015).

5.2.2 Study Objectives

Much of the research to date has focused on accidental releases from burial sites and their immediate effect on the surrounding environment in high risk locations. Little is known regarding the long-term implications a burial site may have on geochemistry at particular sites. Preliminary modelling done by Pratt (2009) and Pratt and Fonstad (2017) provided insight as to transport processes that potentially could be occurring below a mass mortality site with ammonium attenuation and subsequent release of ions such as calcium and magnesium, creating a substantial hard water plume. In areas with higher clay contents, indicators of plume migration ahead of leachate due to cation exchange may be calcium and magnesium or other cations released from the soil as the leachate plume migrates. These results are similar to plume characteristics at earthen hog manure storage sites found by Fonstad (2004). Carcass disposal sites for mass-mortality events are unique when compared to agricultural routine mortality disposal sites or earthen manure storage (EMS), where-by the routine mortality site or EMS is constantly loaded, while the mass burial site could be considered a longer-duration finite source leached during the decay process of

the carcasses. While mass burial sites only have a specific mass of contaminants available for transport, the concentrations of elements, such as nitrogen, found in this leachate are cause for concern (Table 5.1)(Pratt 2009). As a result of this, the need for further research into plume migration and ion adorption and exchange along the process, arose. Such information on the leachate source characteristics is important for modeling purposes, but further research is required to verify contaminant plume development occurring beneath burial sites. The research objective in this study was to determine the fate and transport potential of contaminants from livestock mortalities burial sites using soil coring of pre-existing burial sites of different ages. Of particular interest is the geochemistry during transport and plume development as quantifying these transport properties will enable better planning and execution of carcass disposal during routine or mass mortality events.

5.3 Methods

To evaluate contaminant transport beneath existing carcass disposal sites, a field investigation of two existing sites in Saskatchewan was undertaken. This investigation was the first to provide a critical evaluation of contaminant transport processes beneath carcass disposal pits by coring through the pit/trench concluding with subsequent pore water and soil property analyses. Meeting the objective of this field investigation will enable the validation of geochemical modeling efforts (Pratt 2009; Pratt and Fonstad 2017) that have shown attenuation of ions of concern as well as the subsequent release of ions involved in the exchange process as well as the effect soil properties and mineralogy may have on ion processes and plume migration as compared to previous work in the field. Careful planning and execution were required for the completion of this investigation and is detailed below.

5.3.1 Site Selection

Sites were screened and selected in collaboration with Agri-Environment Services Branch of Agriculture and Agri-Food Canada (AESB of AAFC) based on location and potential geology, age, number of animals buried and accessibility. Site screening was performed to eliminate sites where only a few animals (<20) were buried, eliminate those that were located in clay shale (due to natural high salt concentrations), to include those that had documentation of approximate

pit/trench location to delineate dimensions and to include those where drilling rig access and land owner permissions were available. Of the potential sites identified in the province of Saskatchewan, two were selected for investigation. Both sites were continuously cored for complete soil profiling on a transect. The first site, near Pierceland, Saskatchewan, was used to bury approximately 3000 elk that had been euthanized during a CWD control exercise approximately 8 years prior to this sampling program. Burial guidelines followed early management plans laid out by Saskatchewan Agriculture and Food (2000). The Pierceland CWD site was located on pastureland and was easily accessible, therefore installations of nested piezometers were left behind in a few selected boreholes for future research and monitoring. The Pierceland CWD site consisted of a series of multiple burial trenches 2 m wide and approximately 4 m deep, with lengths ranging from 10 to 20 meters. The two trenches chosen for the study were approximately 10 meters apart (Figure 1).

The second site, located near McLean, Saskatchewan, was a FMD burial pit constructed and filled in 1952. The site was used to bury approximately 40 head of dairy cattle and multiple sides of butchered beef culled during a Foot and Mouth Disease (FMD) control measure (Sellers and Daggupaty 1990; Briere 2001). This site provided a unique opportunity to study carcass leachate contaminant transport at a 58-year-old site. Site screening began with electromagnetic inductive equipment surveys (EM) to try to locate edges of the burial pits and trenches. These surveys were typically unsuccessful in locating burial pits for this study. At the CWD site, burial trenches were found due to ground settlement, while auger drilling was performed in a grid pattern at the FMD site to provide location information of the burial pit prior to soil coring (burial pit was located initially using scent and further delineated using a Hach ammonium test kit on soil pore water extracted while drilling). More stringent analysis and preparation was taken at the FMD site including conversations with microbiologists and government biosecurity officials on the risks associated with the FMD virus, survivability and transmission (deemed not a risk due to age of the burial site) due to the nature of the material buried at the site. Both sites have an approximate average yearly precipitation of 400 mm, with the majority falling as rain during the summer months. Figure 5.1 shows the location of the sites relative to one another. Location A is the Pierceland CWD site and Location B is the McLean FMD site; site specific drilling configurations are also shown. More detailed methodology for each site is described below.

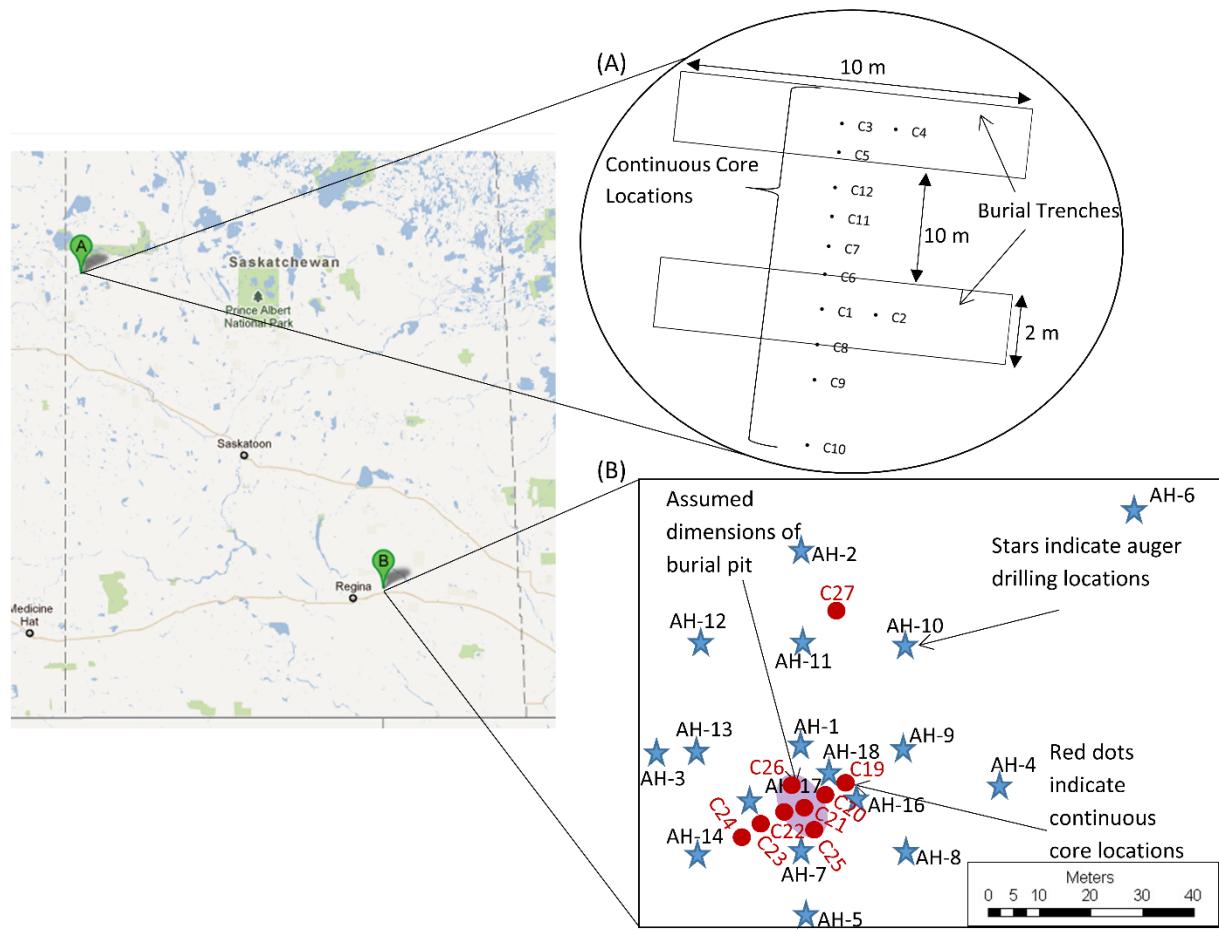


Figure 5.1 (A)Locations of Pierceland CWD Site (not to scale, dimensions approximate) and (B) McLean FMD Site and site specific coring locations; “C” indicates coring locations, “AH” indicates auger test holes

5.3.2 Soil Coring

Soil properties and pore water chemistry versus depth at each location were characterized through continuous core sampling from ground surface to a depth of 10 meters below grade. Sampling at the site was completed by retrieving undisturbed soil cores in 70 mm by 700 mm sealed plastic tubes (100% sampling (i.e. all soil from each hole was taken for analysis) at varying intervals dependent on site geometry using a cable-tool drilling rig. Core holes left behind were sealed with bentonite to eliminate preferential flow paths in and around the site. Two transects were drilled through the pit area with each transect being perpendicular to the other. This allowed not only

potentially unaffected soil samples, but samples of the carcass material within the profile of the pit itself. The Pierceland CWD site drilling layout is shown in Figure 5.1a, and the McLean FMD site drilling layout shown in Figure 5.1b. All soil sample tubes were immediately, upon retrieval from the cable tool drilling rig, capped, sealed and stored below 4°C using generator powered freezers onsite and during transportation. Samples were then stored at a constant -10°C once they arrived at the laboratory. Soil sample analysis was performed at the University of Saskatchewan, with select samples, as required, sent to AGAT Laboratories Ltd. (Alberta, Canada) for analysis of soil clay and bulk mineralogy by x-ray diffraction (XRD). AESB provided basic geotechnical characterization of the soil samples.

5.3.3 Pore Water Extraction and Analysis

Soil pore water was extracted via high pressure squeezing of samples taken at specific intervals (25 to 50 cm) in each soil core. Pore water extractions followed similar protocols and equipment setup as described in the literature (Howes 1985; Jahnke 1988; Manning 1993; Böttcher et al. 1997; Turrero 2006) but was scaled up to squeeze 25 samples concurrently. Böttcher et al. (1997) discussed the effects on solutes by varying the pressure applied to the squeezer. This work suggested to apply pressures up to 100 MPa (15,000 psi), but in doing so, would expect to see slight increases in concentrations of ions in the pore fluid. Others have suggested maximum pressures up to 58 MPa (7,900 psi)(Howes 1985; Manning 1993). For this project, 75 to 100 gram samples were initially squeezed to a pressure of 27 MPa (4,000 psi); if less than 5 mL of water was obtained at this pressure, the pressure was increased to 41 MPa (6,000 psi) (pressures were chosen in order to eliminate pressure effects on solute concentrations). All squeezing and preliminary analysis of water samples was completed in a cold room at 4°C. As soon as the water sample was collected from the squeezers, it was analyzed for pH (IQ Scientific Instruments Model IQ125), electrical conductivity (ATI Orion 128), and alkalinity (Hach Al-DT). The sample was then frozen at -20°C for preservation until ion analysis by atomic absorption spectroscopy (Varian AA240 AAS) for major cations and SmartChem discrete analyzer (Westco Scientific Instruments, Inc.) for major anions and ammonium.

5.4 Results and Discussion

5.4.1 Pierceland CWD Site

Surficial geology at the Pierceland site was dominated by glacial till with the top 2 m of soil being grey-brown in color, medium plasticity (10% to 20% as based on methods presented by Burmister (1949)), and moderate stiffness (2 to 4.5), which was evaluated upon sample retrieval with pocket penetrometer (Humboldt-H 4195). Below 2 m and up to 10 m, the till was grey, stiff to very hard with a few cobbles and traces of oxidation. In a few of the core locations (C2 and C11), a thin sand layer (0.3 m) was present at approximately 6.25 m below grade. Piezometer installations were installed after coring and showed initial water levels after installation across the site ranging from 3 m to 7.5 m below grade. Core locations located within the confines of the burial trench were comprised of fill material and organics to a depth of approximately 4 meters, the exact depth of the burial pit was unknown but assumed to be approximately 3.75 to 4 m depth due to drilling geotechnical information and constituents/consistency of the soil and presence of decayed carcass material. A bulk soil sample from the background core location (C10 for FMD site, C27 for FMD site) indicated an average ($n=5$) cation exchange capacity (CEC) of approximately 12 meq/100g. Soil mineralogy results produced via XRD analysis indicated a 20% clay fraction dominated by kaolinite (41%), illite (50%) and quartz (9%) with bulk mineralogy made up of quartz (51%), potassium feldspar (27%) and dolomite (13%). Plume development at this site indicated vertical site hydraulic conductivities somewhere between 1×10^{-9} m/s and 1×10^{-10} m/s, estimated by chloride plume transport depth for the time interval, meeting and exceeding hydraulic conductivity site requirements (1×10^{-9} m/s) for mass livestock burial in Saskatchewan.

Pore water chemistry results for the Pierceland CWD site are presented in Figure 5.2. This 2-D contour plot representation of individual ion concentrations provides a picture of ion movement and concentration with depth in 2-D space (a vertical slice between cores C3 and C10) and a visual representation of plume development. Each plot shows the burial trench locations and depth profile by use of shaded box overlays. These figures distinctly show attenuation of ammonium, sodium and potassium within the confines of the trench, while ion exchange reactions resulting from ammonium, sodium and potassium attenuation produced a front of calcium and magnesium ions

at nearly consistent depth of transport similar to the anions (alkalinity and chloride). Chloride transported the furthest below the burial trench to a depth below ground surface of approximately 5.25 m, with calcium and magnesium trailing it at depths of approximately 4.25 meters below the surface; these findings are consistent with initial geochemical transport models done by Pratt (2009) and Pratt and Fonstad (2017). It was expected that ion exchange would dominate plume development in this system due to the presence of clays in the glacial till material. This phenomenon was observed here with the development of the calcium and magnesium plume at concentrations significantly higher than what would naturally be found in carcass leachate (7,000 mg/L versus 60 mg/L respectively for each ion); this result is similar to the concentrations expected from initial geochemical modeling done by Pratt and Fonstad (2017). Those models indicated that in a soil with a CEC of approximately 10 meq/L, a 5-fold increase in Ca and Mg concentrations at the head of the plume would be present due to ion exchange reactions. Discrepancies could be explained by higher CEC values at this specific site, coupled with different soil initial chemistry. Other ion concentrations observed at the site were at concentration levels similar to those expected at a burial site. Alkalinity, chloride, potassium and sodium concentrations observed in the pore water in, and directly below the burial trench, were consistent with concentrations observed in carcass leachate (Pratt 2009).

Additional plume concentration information can be found in the supplemental figures (Figures 5.4-5.6). These figures characterize concentration versus depth profiles for each test-hole location. Cores C1, C2, C3 and C4 were taken directly through the middle of the burial trench and provide the most detailed characterization of vertical ion movement. Supplemental Figure 5.4 revealed a hardness plume (calcium and magnesium) up to approximately one meter below the bottom of the trench, while attenuation of ammonium has slowed its movement significantly and has induced the movement of a hard water front consisting of calcium, potassium and magnesium due to ion exchange in the soil. Concentrations of alkalinity as high as 45,000 mg/L were recorded in cores C2, C3 and C4. Cores adjacent to the burial trenches (Supplemental Figures 5.4-5.6, cores C5-C9, C11-C12) showed little leachate impact; the cores on the very edges of the trenches (C5, C6, C8) show a slight increase in alkalinity and a slight increase in Ca and Mg concentrations. It was noticed that background concentrations of alkalinity, at approximately 7 meters, was approximately 10,000 mg/L (Supplemental Fig. 5.6, C10). Higher background concentrations of

sodium and chloride were also found in this zone, indicating a potential zone of differing geologic material.

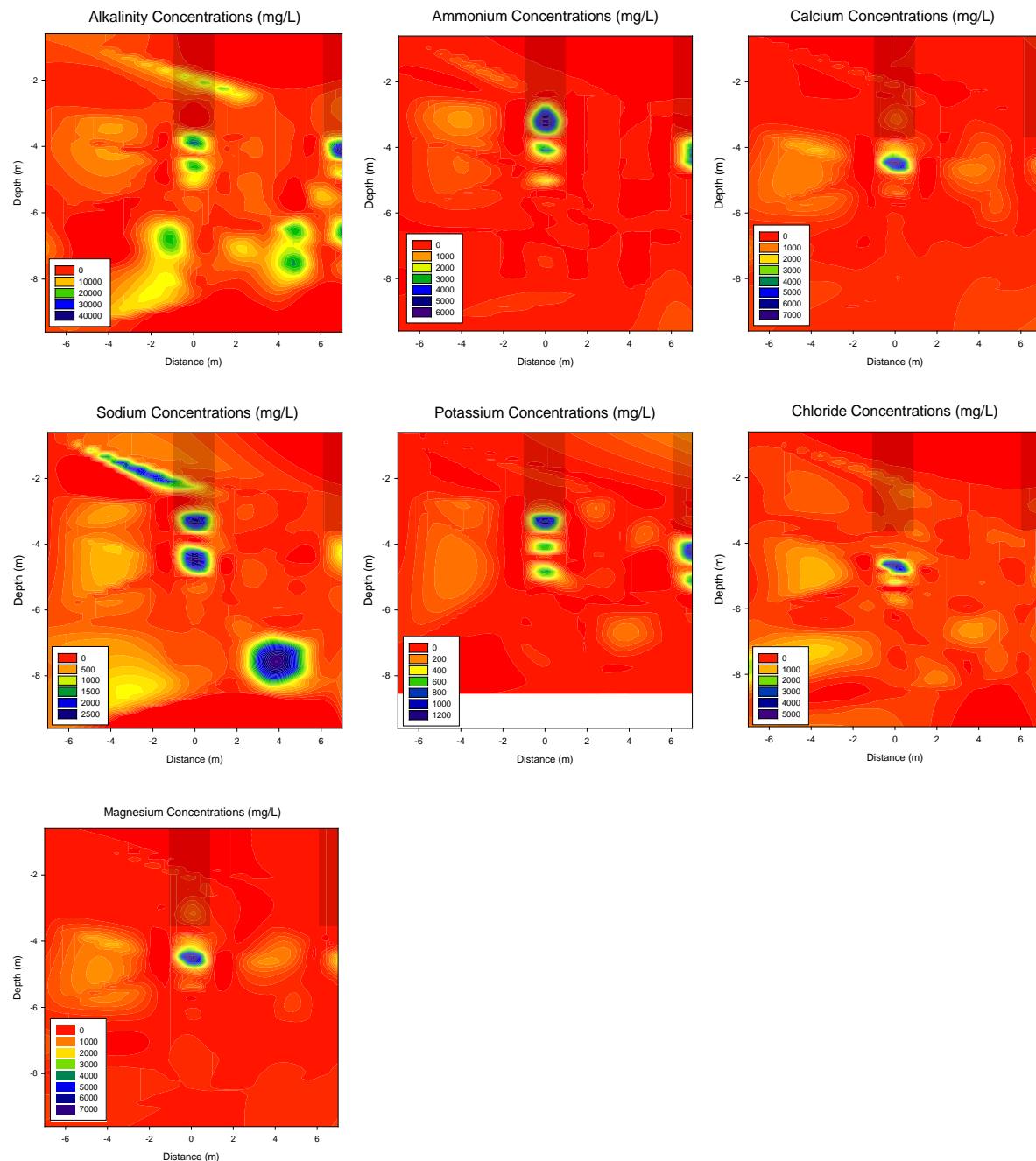


Figure 5.2 Alkalinity, ammonium, calcium, sodium, potassium, chloride and magnesium concentrations across the CWD site cross-section (shaded area indicates trench location)

5.4.2 McLean FMD Site

Surficial geology at the McLean FMD site was dominated by glacial till with the top 1 m to 2 m of soil comprised of soft, slightly sandy, organic material with characteristics of oxidation. Below 2 m, the soil was characterized as glacial till, more clay dominated and stiff to hard (2 to 4.5 range on pocket penetrometer) with characteristics of oxidation and also included some cobbles and limestone pebbles and an average CEC of 10 meq/100g (n=5). Soil mineralogy results produced via XRD analysis were comprised of one sample in the sand lens and another for the clay layer. The sand lens showed a 5% clay fraction dominated by kaolinite (12%), illite (34%), smectite (14%), with the remainder of the clay fraction consisting of small amounts of quartz, plagioclase feldspar, potassium feldspar, calcite and dolomite. Bulk mineralogy was dominated by quartz (58%), plagioclase feldspar (22%), potassium feldspar (15%) and calcite (5%). The sample from the clay layer produced the following mineralogy: analysis indicated a 19% clay fraction dominated by smectite (30%), illite (27%), kaolinite (17%), chlorite (8%); bulk mineralogy was made up of quartz (53%), plagioclase feldspar (13%), potassium feldspar (4%), calcite (6%) and dolomite (23%). A 0.75 m thick, very wet sand lens was present at a depth of approximately 6.5 m in borehole C22, with other smaller lenses in boreholes east and south of the burial pit. The water table was located at approximately 3.5 m below ground surface. Core locations located directly inside the burial pit were comprised of fill material to a depth of approximately 3.5 meters, the exact depth of the burial pit was unknown but assumed to be approximately 3.5 m due to drilling geotechnical information and constituents/consistency of the soil and presence of adiopocere tissue. This site was unique in its dimensions and scale. The burial pit was relatively small (a few meters by a few meters) and was only directly contacted by two of the soil cores (C21 and C26) as shown in Figure 5.1b, contact with the pit was assumed due to presence of hair, organics and adiopocere tissue. Cores C20 and C22 demonstrated a slight odor at approximately 3 m depth, and showed no signs of burial remnants as seen in C21 and C26. Another interesting component of this site was the existence of a “compost” or “manure” layer approximately 0.5-0.75 meters below the surface. The layer was approximately 0.25-0.5 meters thick over the entire site, indicative of past manure application strategies for soil amendment.

Similarly, to the Pierceland CWD site, pore water chemistry results for each core location are shown in Supplemental Figures 5.7-5.12 as concentration (mg/L) versus depth (m) profiles for each test-hole location. A more visual representation of the concentrations of each ion found across the 2-dimensional cross section can be seen in Figure 5.3. Core C21 and C26 were the two cores taken directly through the burial pit. The ion concentration profiles shown in the Supplemental Figures for cores C21 and C26 appear to demonstrate vertical movement of the chloride ion to a depth of approximately 5 meters at concentrations ranging from 1800 mg/L to 3200 mg/L with background concentrations at that depth of 1000 mg/L. Visually we see this in the 2-D plots as well. Utilizing the 2-D plots, plume migration for most constituents are consistent in direction with each other. Ammonium impact from the burial pit shows attenuation within the base of the pit at concentrations of approximately 3800 mg/L. Potassium concentrations of 9300 mg/L were observed in C26 at a depth of 4.5 m, these concentrations are significantly higher (approximately 4 times higher) than what would be expected from carcass decomposition leachate (approximately 2300 mg/L based on Pratt (2009)). Alkalinity was the highest in C26 at 27600 mg/L peaking at a depth of 5 m, consistent with the depth of chloride transport, with background alkalinity between 2000 and 3000 mg/L. Alkalinity observed here was slightly below expected concentrations (~34600 mg/L) based on results observed by Pratt (2009). A hardness plume was observed at this site consisting of calcium and magnesium. In C26, concentrations of these ions were approximately 1300 mg/L at 5 m, also consistent with chloride transport, similar to the Pierceland CWD site.

The depth of the burial pit was approximately 3.5-4 meters; therefore, conservative ion movement of approximately 1-2 meters was observed in the two cores penetrating directly through the pit. Directly beneath the burial pit the soil was a very stiff to hard till to approximately 5.75 meters, where a sand lens was located. The two-dimensional contour plots taken across the cross section (Figure 5.3), show movement of chloride, sodium and potassium horizontally, at depths consistent with this sand lens. The sand lens was more predominant in C26 than in C21 and very wet with confined aquifer attributes. Cores adjacent to the burial pit showed little impact from ammonium and potassium, but slight horizontal impact from alkalinity and chloride, indicating conservative movement of ions. Figure 3 shows apparent pulses of chloride and sulfate movement that may be attributed to groundwater table fluctuations or spring freshet events due to snowmelt, however

significant cation movement associated with this is negligible (i.e. ion exchange reactions kept ions of concern attenuated) while a hard water plume also follows this flow path to a lesser extent. Due to the nature of glacial tills in Saskatchewan (high clay content, stiff and highly compacted (Milligan 1967; Legget 1976)), it is not surprising that the majority of cations found in the burial leachate have been absorbed and exchanged on soil particles and that the majority of the contaminants have been held in the first 2 meters beneath the burial pit. These findings are consistent with models produced by Pratt and Fonstad (2017). Even when we exclude the lateral movement component at this site, significant geochemical impacts due to 60 years of transport from this burial pit are negligible, as the ions of interest are attenuated. The anisotropy at this site would present challenges if gradients were high and CEC was low and movement was in the direction of a usable water source in the event that the pit was much larger and contained a higher quantity of carcass material. In this instance, due to pit size and other site aspects such as a 500 meter separation distance between the pit and any surrounding structures, water bodies and livestock facilities; would largely eliminate any threat.

It was also important to note that the organic manure/compost layer throughout the land area of the site (from nutrient addition via manure based fertilizer for crop cultivation) has demonstrated an effect on ion concentrations closer to the surface. This is apparent on the contour plots. Assuming this organic layer was manure which was land applied from the dairy that existed 60 years ago, we can conclude that there will be a chloride plume along with smaller amounts of nitrogen, and alkalinity and potassium near the surface consistent with findings from manure management studies (Sommerfeldt et al. 1973; Mooleki et al. 2002; Olson et al. 2005; Petersen et al. 2007). This was seen in nearly all plots of ion concentrations for this site (Supplemental Figures 5.6-5.12, cores C19-C27). Movement and impact from this organic layer was apparent and located approximately 2 m below surface.

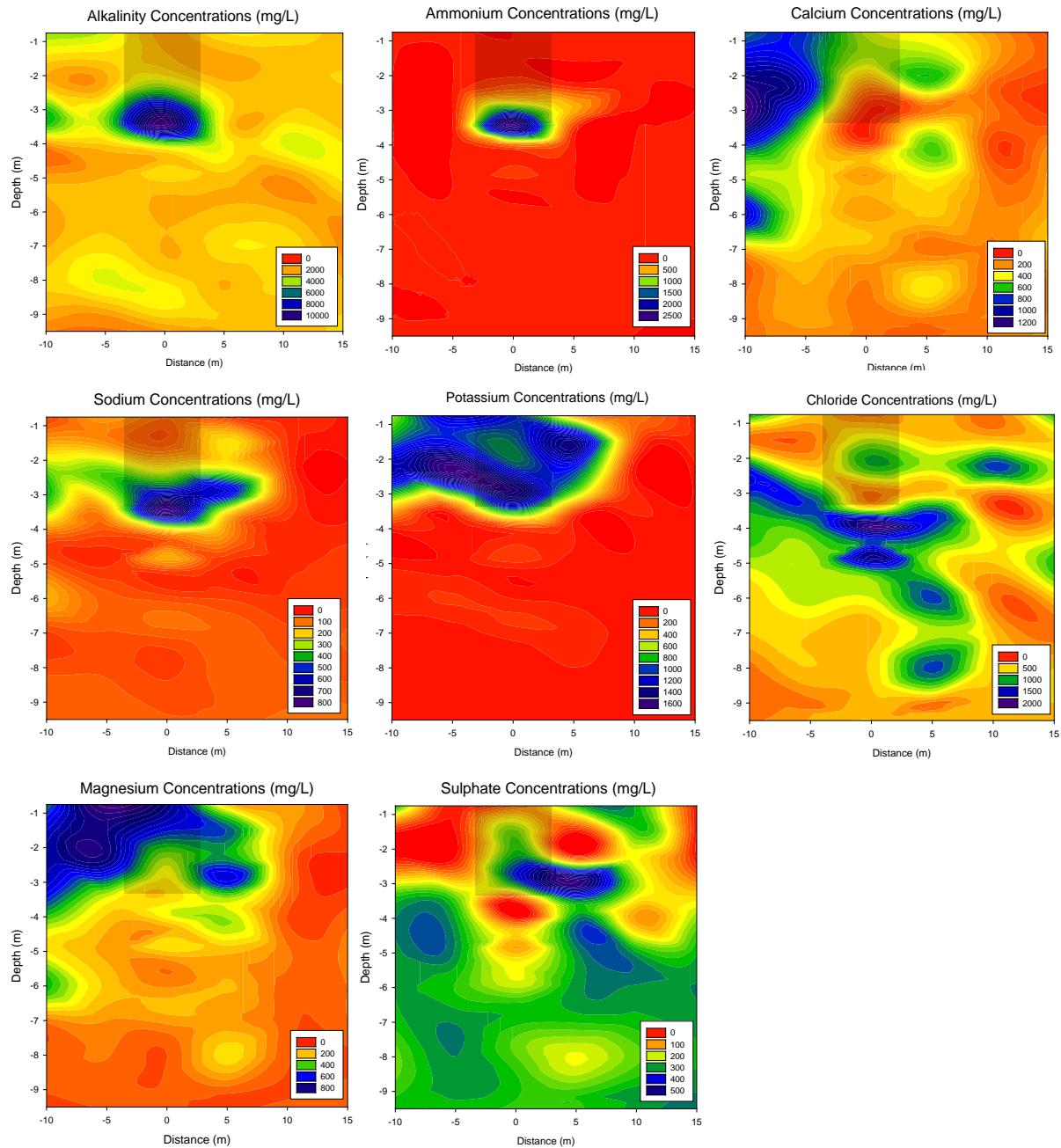


Figure 5.3 Alkalinity, ammonium, calcium, sodium, potassium, chloride, magnesium, and sulfate concentrations across the FMD disposal area cross-section

5.4.3 Transport as compared to others

In comparison to what others have found in close proximity to carcass burial sites, those in this study are somewhat different, particularly the Pierceland CWD location. The uniqueness of the soil characteristics (stiff glacial till, high clay content, soil thickness below the pit); these sites provided us with a “best case” scenario where livestock could potentially be buried without long-term implications to groundwater. The McLean FMD site provided an interesting assessment of leachate movement over decades as compared to much of the literature. Even over many decades, the constituents of most concern do not appear to have transported a significant distance from the pit and appear to have been attenuated by soil adsorption and ion exchange. Those ions present in the plume that have migrated were generally formed based on ion exchange reactions or conservative ions such as chloride and alkalinity, which have significantly diluted over time and distance.

This study is significantly different to studies originating from South Korea. Soil types and depths to bedrock differed greatly between our study and burial pits across Korea from the 2009-2010 FMD outbreak. The Korean outbreak showed geological differences and their effect on leachate transport and plume characteristics (Kaown et al. 2014) and the importance of sites meeting stringent requirements for carcass disposal. Sites that were less secure, proved problematic sooner with respect to leachate transport and groundwater contamination (Lim et al. 2012; Kaown et al. 2015). The uniqueness of the Korean FMD incident was that many animals were buried in geologically unsecure locations and impacts to groundwater were seen in short time frames due to highly permeable sandy-loam soils, shallow depths to bedrock and improper pit construction (Ahn 2012). These studies showed that the conversion of ammonium to nitrate due to nitrification processes was extremely slow, demonstrating that ammonium is greatly attenuated and while nitrate exceeds drinking water standards, concentration levels are still relatively low. Park et al. (2014) also discovered that agricultural lands in general demonstrated similar impacts to groundwater as carcass burial sites in Korea. They also concluded that while there was little movement of ammonium at distances between 0 and 100 m from burial pits, chloride and nitrate showed transport up to 100 m from the pit location as compared to background levels but these levels may also be contributed to by the agricultural land surrounding the sites.

Comparing this study to earlier studies on mortality carcass disposal in North America (Ritter and Chirnside 1995; Glanville 2000), concentrations of some ions observed here were much greater in zones directly beneath the burial pit. Glanville (2000), concluded that chloride and elevated BOD are good indicators of leachate effects on groundwater in a burial system. Similarly, our study showed that elevated levels of calcium and magnesium would also be excellent early indicators (in some cases before chloride) of contaminant migration from carcass burial sites, also demonstrated from geochemical models by Pratt and Fonstad (2017). Ritter and Chirnside (1995) found ammonia contamination at distances of up to 12 meters from a routine mortality disposal pit for poultry. Unlike our study, this pit was constantly loaded, weekly, and located within soils with high hydraulic conductivity. Soils high in sand content generally present challenges for burial due to low CEC, and high groundwater flow rates, causing more rapid movement of contaminants, and in this instance, aerobic conditions. Aerobic conditions may expedite the conversion of ammonium to nitrate, which would then transport through the system more freely and is a higher risk to water bodies.

From a mineralogy standpoint, both sites investigated here each present their own unique set of geologic materials with some similarities and differences. The McLean FMD site showed two different materials i.e. a stiff clay with a subsurface sand lens throughout the entire site, while the Pierceland CWD site contained a fairly consistent clay based till through the profile. Transport depths vertically for each site were similar, concentrations however, were different. These differences vary due to the age of the site, horizontal transport conditions, differences in cation exchange capacities and differences in parent materials. Mineralogy at the McLean FMD site showed a high proportion of smectite based clays throughout the site. Smectite generally has a net negative charge that is less pH dependent than other clay types and has a relatively high active surface area making them a good clay for cation adsorption. Their surfaces are generally balanced by Na, Ca, Mg with cation exchange capacities ranging from 25 to 100 meq/100g (Odom 1984). Other clays found at the McLean FMD site include illite and kaolinite. Illite is known for being one of the most common, non-expanding clay mineral produced from the decomposition of micas and feldspars (a common bulk soil property at this site) with interlayer structures consisting of predominately potassium and in general, have a cation exchange capacity ranging from 20 to 30 meq/100g (Carter 1993). Kaolinite, another common clay mineral with CEC of 3 to 15 meq/100g,

has a negative surface charge controlled by pH, lack of isomorphous substitution, and broken edges (Ma and Eggleton 1999). The combination of clay minerals found at the McLean FMD site and their parent soil materials, makes this site acceptable for livestock burial due to the clay attributes that would limit ammonium transport in terms of cation exchange capacities (measured at 10 meq/100g), and soil mineralogy.

Plume development at livestock burial sites will be controlled by parent materials, clay mineralogy, and the abundance of exchangeable cations. The relative abundance from greatest to least, of exchangeable cations in clay minerals are as follows: Ca^{2+} , Mg^{2+} , H^+ , K^+ , NH_4^+ , and Na^+ (Grim 1968). For a typical site such as either of the two investigated here (CEC of approximately 10 meq/100g), assuming a bulk density of soil of 1,600 kg/m³, it can be estimated that for a typical mortality leachate depth of 0.5 meters (1m³ of leachate per metre length of 2 m wide trench or 0.5 m³ of leachate per square meter of trench) in a trench based system, it would take approximately 2.2 meters of soil per square meter of trench to fully adsorb/exchange with just the ammonium (at concentrations of approximately 12000 mg/L) in the leachate. Assuming the lyotropic series of ion exchange isn't stationary, the constant change in concentration gradients through the adsorption process may mean that ammonium ions will be adsorbed first, but once they've reached a specific concentration, may not be as competitive on ion exchange sites, in which potassium or other cations will compete for the same sites. Concentrations observed in the McLean FMD site plume are less than those observed at Pierceland CWD site, likely due to dilution effects over time and lateral transport through the sand lens. Concentrations of exchanged ions such as calcium and magnesium are 50 to 90 percent less at the McLean FMD site as compared to those in the plume at the Pierceland CWD site. Unequivocally, the Pierceland CWD site showed high concentrations of both calcium and magnesium, in nearly equal amounts in the plume that had developed. Soil properties at this site were predominantly kaolinite and illite clays with parent materials of quartz, potassium feldspar and dolomite. High concentrations of calcium and magnesium in the plume are consistent with the presence of dolomite ($\text{CaMg}(\text{CO}_3)_2$), naturally assuming the clays in its presence would be predominately filled with calcium and magnesium due to parent material breakdown over millennia, making them the primary ions available for cation exchange. Dolomite dissolution properties demonstrate that it dissolves in the presence of acidity, but is a slow process and is generally more stable than its similar mineral, calcite (Palache et al. 1951). This may also

explain some of the excess concentrations of calcium, magnesium and increased alkalinity observed at the Pierceland CWD site due to mineral dissolution of dolomite in the presence of slightly acidic pore water most likely mediated by the presence of phosphoric acids in the leachate.

5.5 Conclusions

This study provided the first field investigation on previously constructed burial sites located in glacial tills to aid our understanding of carcass burial impacts to the environment in the short-term (<10 years) as well as longer-term (>50 years). The Pierceland CWD site had Soil properties that would be typical for most burial locations regulatory agencies would choose for geologically secure disposal. The McLean FMD site from 1952, due to lack of information at the time and using the information we know now, was not placed on a preferred geologically secure area due to the sand lenses and higher water tables encountered in and around the site. The redeeming feature at this site was the very stiff glacial till immediately below the burial pit, minimizing vertical movement of ions; however, lateral movement of ions of limited concern was apparent.

The Pierceland CWD site was used in 2001 to bury euthanized elk suffering from chronic wasting disease (CWD). The soil cores were taken from this site in 2008 and the extent of leachate transport upon analysis of the soil cores was 1-1.5 meters of vertical transport of anions (Cl, Alkalinity) as well as some cations arising from cation exchange reactions (Ca and Mg). There was no lateral movement of ions at this site. The second site, located near McLean, SK was used in 1952 to bury carcasses affected by Canada's only foot and mouth outbreak. This site was cored in 2010, nearly 60 years post burial. Leachate movement vertically at this site was very slow over that period of time, showing movement of 1-2 meters. Due to the presence of sand lenses in and around the burial pit, movement of up to 10 meters horizontally of anions such as Cl and bicarbonate was shown. Ammonium ions were attenuated within the confines and immediate soil surrounding the burial pit.

Results from this study have helped to understand the impacts that have occurred with respect to leachate transport beneath burial sites. For the Pierceland CWD site, appropriate land was chosen for burial, as movement in 8 years was minimal, with the ions of concern still attenuated immediately beneath the burial pit. This same phenomenon was also observed at the McLean FMD

site, ammonium ions were attenuated within the confines of the burial pit and in immediately surrounding soil, with no impact to surrounding soil cores. The hard water plumes created by the ion exchange reactions from ammonium at each of these sites will be the first to reach groundwater systems in the long-term and are of less concern regarding contamination than the attenuated ions.

5.6 Acknowledgements

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5.7 Transition Statement

This chapter provided the first that we know of in the world site assessment for geochemistry beneath two carcass burial pits/trenches. Much information was obtained to further validate modelling efforts, but little was still known with respect to potential microbiological aspects. The next chapter presents a microbiological investigation beneath the Pierceland site and present both qualitative and quantitative analysis of the species of microbes present as well as those that are flourishing due to chemical impacts.

5.8 Author Contributions

D.P. designed the study, collected the samples and conducted pore water extractions and analysis. D.P. wrote the manuscript, T.F. provided edits and comments on the manuscript and contributed to the text in later iterations.

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5.10 Supplemental Figures

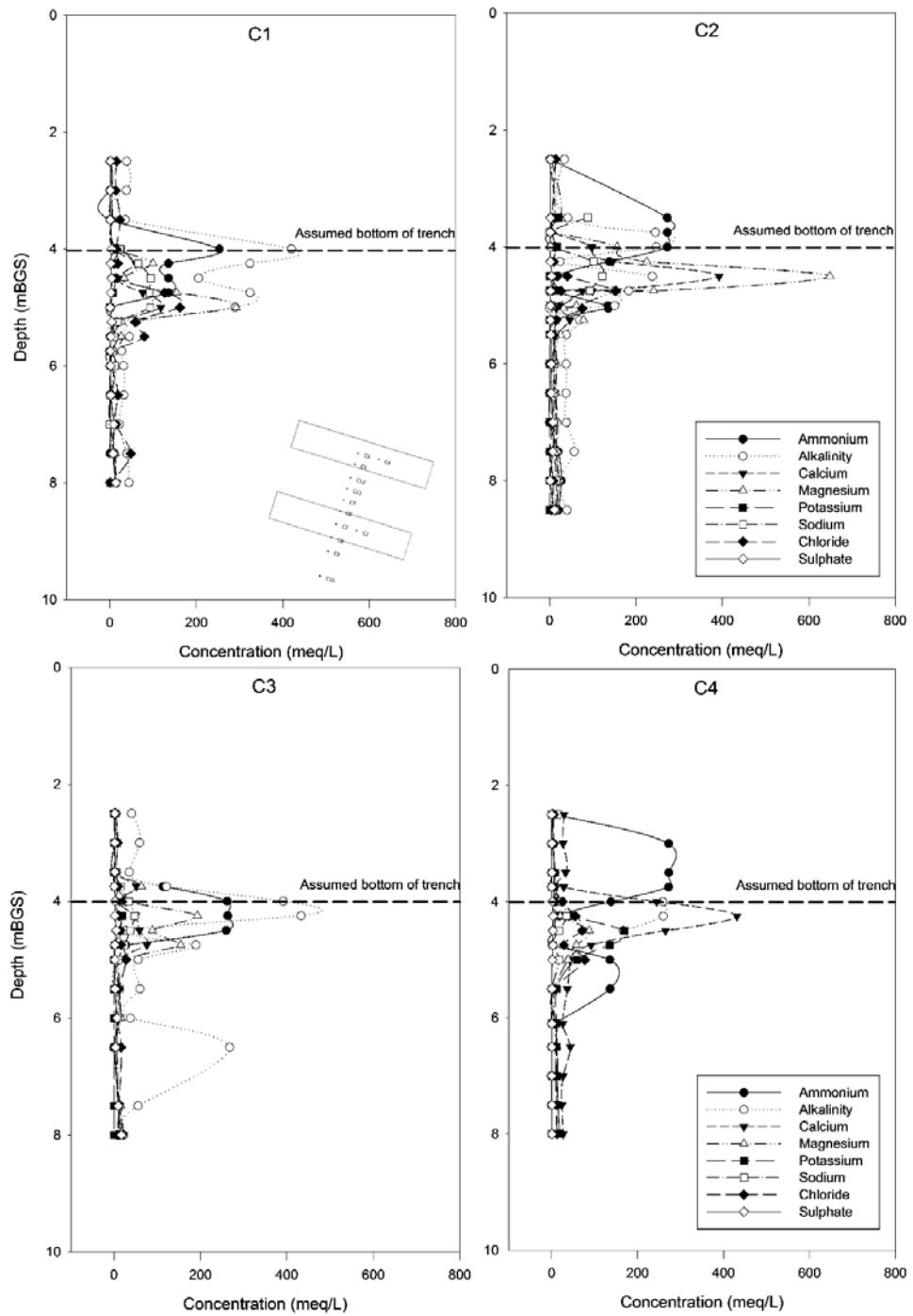


Figure 5.4 Supplemental Figures C1, C2, C3 and C4

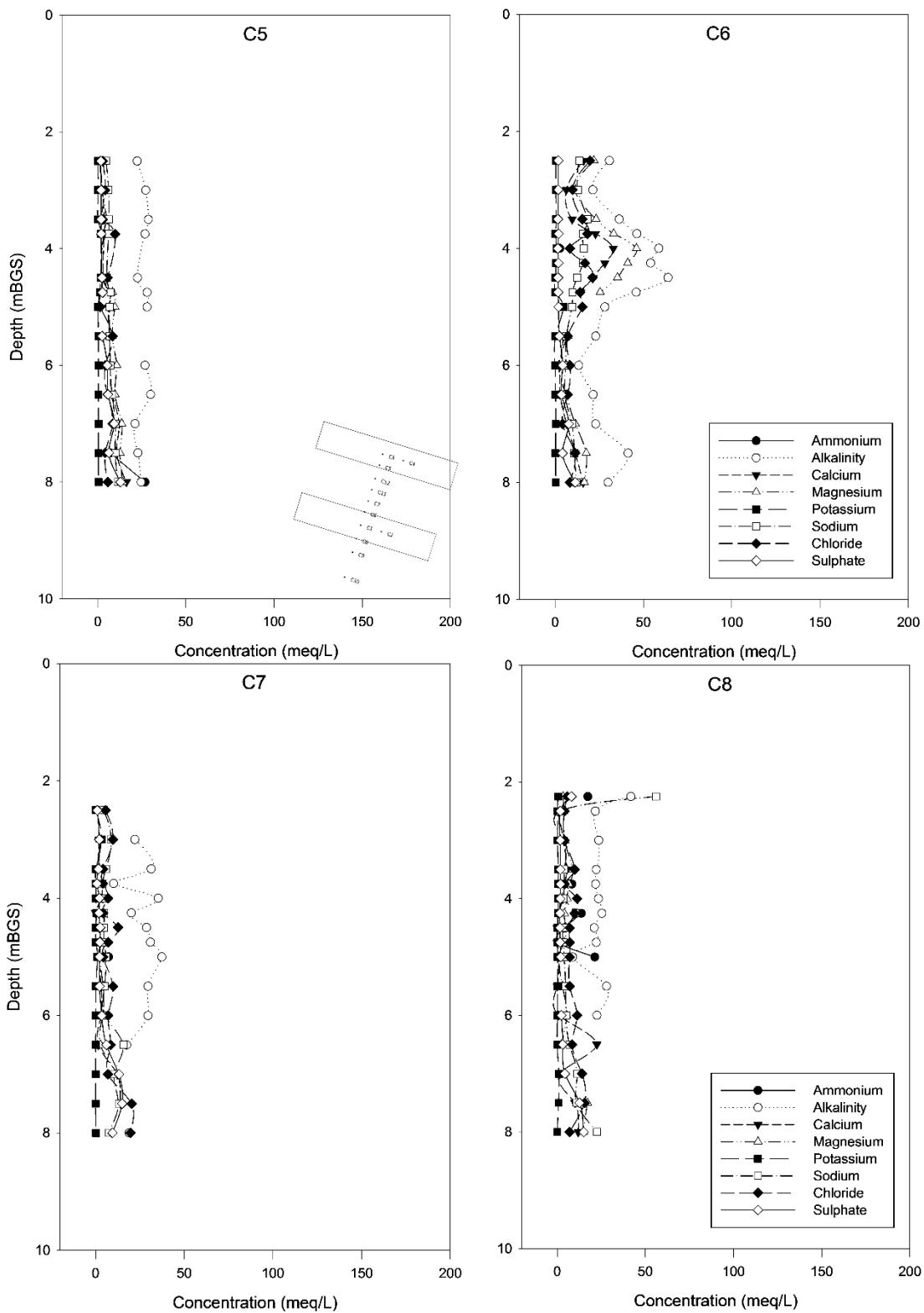


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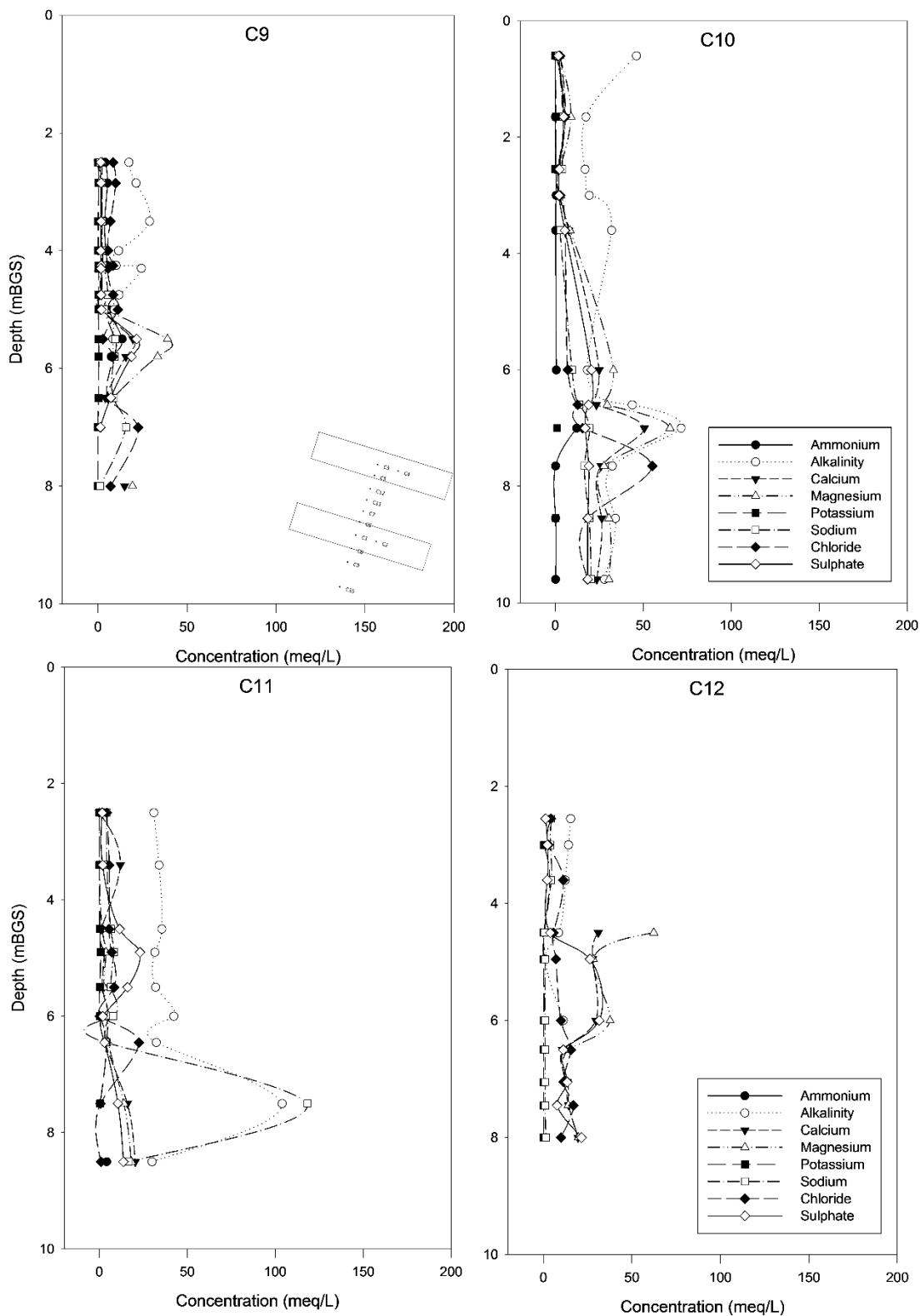


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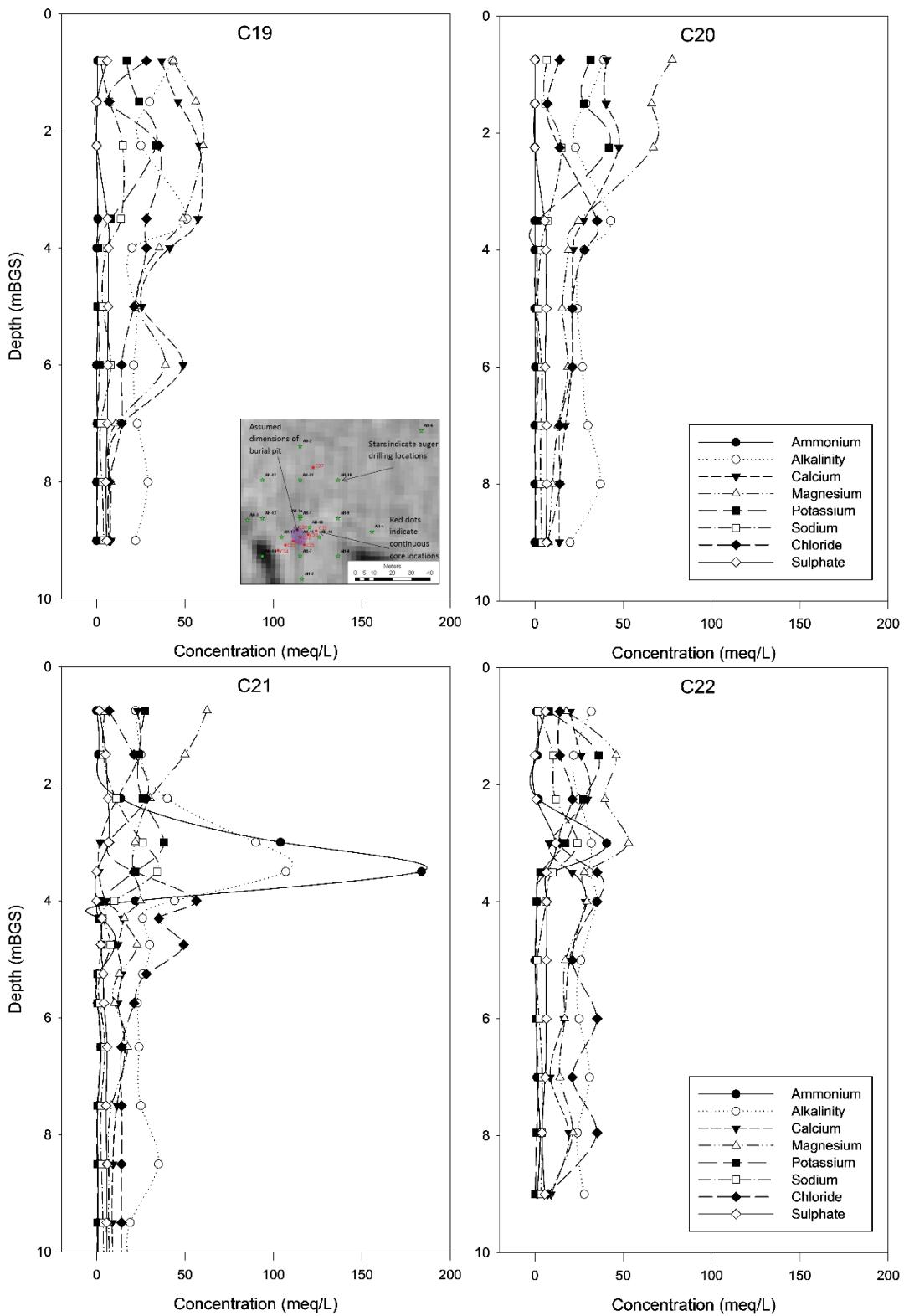


Figure 5.7 Supplemental Figures C19, C20, C21 and C22

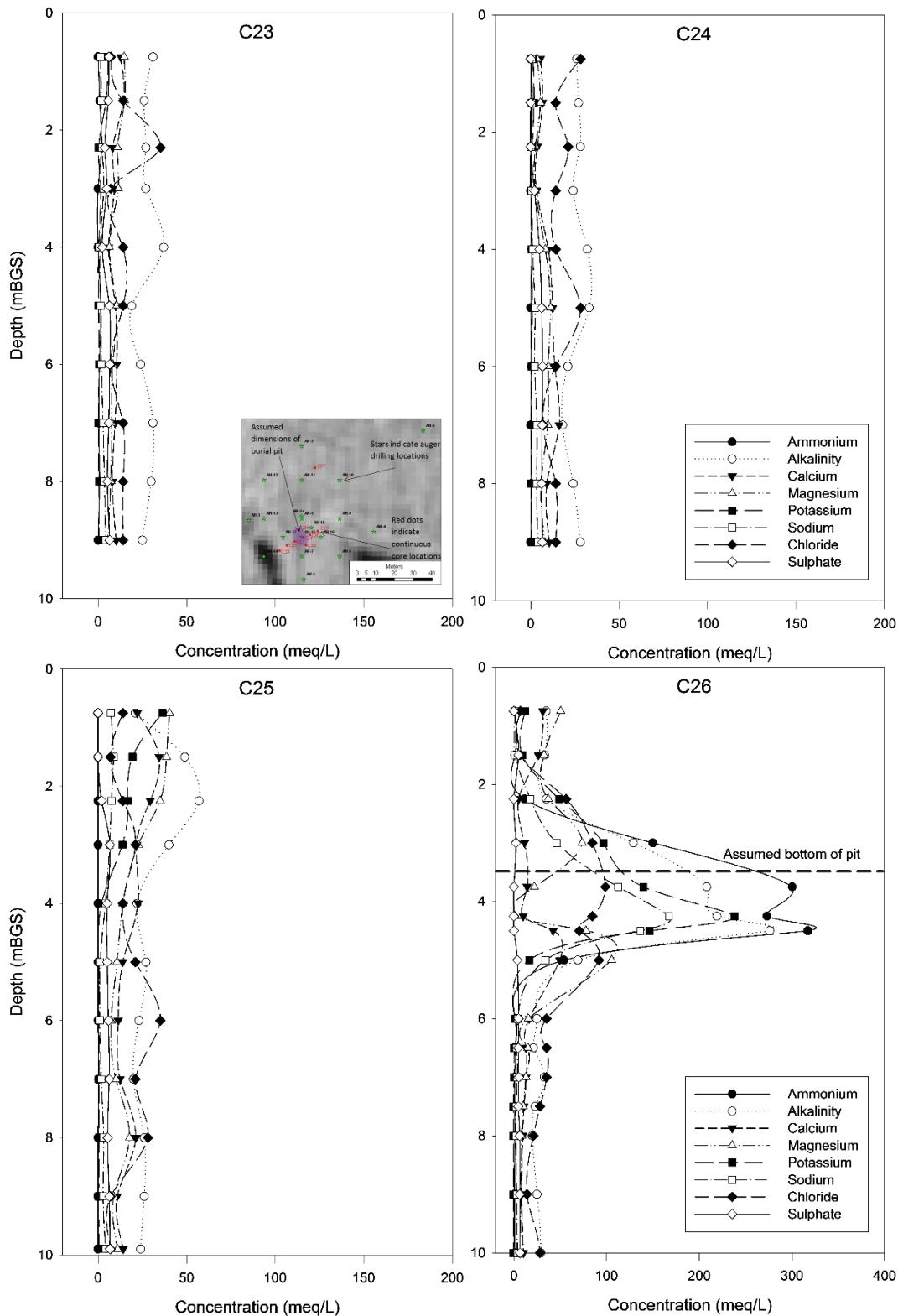


Figure 5.8 Supplemental Figures C23, C24, C25 and C26

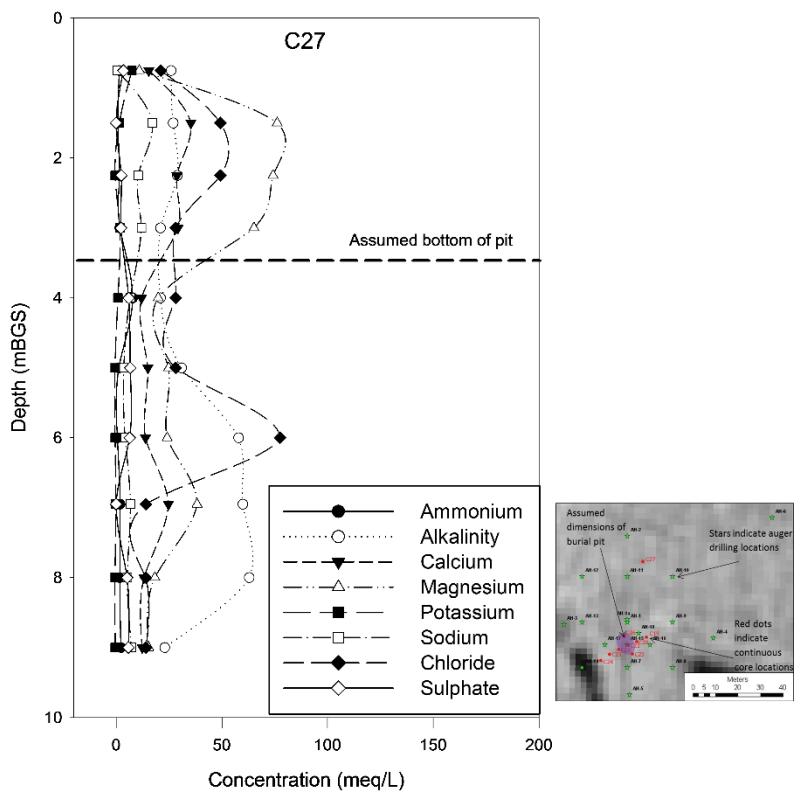


Figure 5.9 Supplemental Figure C27

Chapter 6: Influence of Mass Burial of Animal Carcasses on the Types and Quantities of Microorganisms Within a Burial Site

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6.0 Preface

In the past 5 years, much work has been done to assess chemical impacts to the environment by both this thesis, and others abroad in regions where FMD and avian influenza outbreaks have decimated livestock populations requiring mass burial for disposal. A common theme among most studies is the assessment of groundwater and surrounding soils for chemical contamination. A common question that always arises upon discussion of carcass disposal is pathogen transmission in the subsurface. From a microbiological standpoint, the survivability of many pathogens in the subsurface can range from days to years depending on the microorganism (virus, bacteria, spore, etc.). Also, due to the unique decomposition chemistry, questions arose about whether or not, indigenous microbes present in the soil would flourish or change depending on the extent of leachate contamination and how the leachate might affect the population. This chapter presents a microbiological investigation beneath the Pierceland site and presents both qualitative and quantitative analysis of the species of microbes present as well as those that are flourishing due to chemical impacts. This work involved the design and use of a new technique for DNA extraction in soils, being the first to assess soil microorganisms based on the cpn-60 amplification technique as well as providing the first taxonomic classification of deep soils impacted by a potential contaminant.

6.1 Abstract

Microbial communities were characterized through a depth of 10 m in and near an eight-year-old burial site that was implemented as a control measure for cases of chronic wasting disease among wild elk. Quantitative PCR based on the 16S rRNA-encoding gene showed that the burial trench had significantly (2 to 5 orders of magnitude) more bacterial 16S rRNA-encoding genes g⁻¹ soil within and up to 2 m below the burial site compared to a nonburial control core sample at the same depths. Topsoil and depths below the burial site (>6 m) showed similar quantities of 16S rRNA genes for both cores. Furthermore, when microbial communities were examined by cpn60 universal target amplification and pyrosequencing, 5825 operational taxonomic units (OTU) were found at a variety of abundances in all of the 13 core samples that were analyzed. Taxonomic analysis indicated that the overall community composition changed considerably with increasing depth, and that the burial core community was distinct from that of the control core at all depths. Organisms associated with phosphate accumulation, nitrogen fixation, and ammonium oxidation were found in highest abundance near the surface of the burial core (up to 2.5 m), while organisms associated with ammonification were found at the burial depth, consistent with an increase in ammonium concentration in pore water. Sequences from organisms associated with dissimilatory metal reduction were concentrated just below the burial depth (4.5 to 5.5 m). Anaerobic microorganisms dominated the microbial community at the burial site (3.75 m). The approaches described in this study provided distinct sequences that can be used to develop molecular assays for organism tracking and overall fingerprinting of microbes associated with burial sites. These data can be helpful in site evaluation and may help in the selection of future burial sites.

6.2 Introduction

In the event of a disease outbreak such as foot and mouth disease (FMD), avian influenza (AI), or chronic wasting disease (CWD), as well as many others, the need to dispose of livestock mortalities is likely to arise. Within areas of intensive livestock operations, the number of dead stock and euthanized animals has great potential to confirm most methods of disposal as unacceptable due to processing time and loading requirements. Burial is the most likely choice for many of these events but is also least understood with respect to environmental implications. Pratt (2009) characterized leachate for three species of livestock burial pits and showed that concentrations of

ammonium, bicarbonate, phosphorus, and sulfate are 5 to 10 times higher than concentrations found in liquid swine manure storages. The difference between a mass livestock disposal site and manure storage is that the latter is constantly loaded, while the burial site is a one-time contaminant load. Even though the burial site has a specific amount of contaminant available for transport, the concentrations found in the leachate are cause for concern.

Many factors can affect the transport of mortality leachate, such as soil type, hydraulic properties, and microbial communities. The microbial community in soils beneath burial sites could affect the transport and chemical evolution of the mortality leachate (McCarthy and Zachara 1989). More importantly, the survival and transport of microbes and/or viruses from the carcasses into the underlying soil and groundwater has yet to be determined. Little is understood regarding microbial transport from a burial site. The types of microbes in soils such as sulfate reducers, nitrate reducers, as well as those present in the burial pit (e.g. enteric bacteria) will affect the leachate by changing its chemical composition and altering its transport properties. These microbes may also transport through the soil along with the leachate. Cases in which carcasses with disease are disposed of via burial could potentially result in the transport of pathogens and thereby become a potential point source of pathogens, causing further disease outbreaks. For example, the recent FMD outbreak in South Korea in 2010-2011 resulted in a stamping out protocol of nearly 3.5 million livestock, most of which were culled in less than four months and buried at more than 4,500 sites by digging pits (Ahn 2012). Not only is leachate transport a concern in an instance such as this, but also survivability and transport of pathogens. In order to investigate the microbial ecology of a livestock burial site, background or control soil samples must be analyzed in parallel with samples taken directly through the mortality pits. After analysis of the respective soil cores, the changes in the microbial community of the mortality pit can be quantified and evaluated as changes relative to the background or control sample.

Understanding the physiology and ecology of microorganisms at contaminated sites is important in order to fully assess leachate attenuation and remediation properties of a site. For example, in a carcass disposal site, the carcasses themselves present their own inoculating microbiota, but the microbiota in the soil they come into contact with may determine the characteristics of the leachate as well as the natural attenuation of that leachate. Traditionally, soil microbial communities have

been analyzed by culture in a laboratory setting. The difficulty involved in obtaining cultures (Zengler et al. 2002) has hampered the analysis of microbial communities in deep soil cores. It is estimated that only 0.01% to 0.1% of all bacteria in soil have been isolated in pure culture (Chapelle 1993). Some of the difficulties involved with culturing deep soils include replication of conditions sustainable for culture growth, as culture conditions are generally biased toward specific organisms (Connon and Giovannoni 2002). In addition, many of the viable cells present in soil can only be grown in their natural environment and therefore may not provide visual growth on plates (Xu et al. 1982; Eilers et al. 2000).

Recently, culture-independent techniques have been applied to assess more fully soil microbiota. Isolating soil microbial DNA through the lysis of cells and subsequent purification may reveal the presence of unculturable microorganisms, allowing a more complete characterization of the soil microbiota than by culture methods (Abulencia et al. 2006; Liles et al. 2008). Most previous studies have examined microbial communities by sequencing the 16S rRNA-encoding genes, but some studies suggest that using a chaperonin-60 (cpn60) target provides higher taxonomic resolution with similar overall taxonomic profiles (Schellenberg et al. 2009). This higher resolution is very beneficial in determining the microbial communities in and around a livestock burial site. The cpn60 target is universal in eukaryotes and bacteria, and an extensive reference database is available for classification (Hill et al. 2004). The length of the cpn60 universal target (cpn60 UT), 549 to 567 bp, coupled with the high resolution it provides makes it an ideal molecular target for the application of next-generation sequencing methodologies (Schellenberg et al. 2009). The cpn60 UT is suggested as a barcode marker for bacteria (Links et al., 2012) with a barcode gap that exceeds that of any of the many 16S rRNA-based targets that have been described. It also provides the additional advantage of profiling the eukaryotic as well as prokaryotic component of microbial communities, which gives a more complete picture of the microbial community under analysis.

Our objective of this study was to describe the microbial communities associated with a burial site created as a control measure for chronic wasting disease. A secondary objective was to relate the bacterial genera observed at various depths to the chemical profiles obtained from pore water within the core samples. Microorganisms can play a significant role in subsurface evolution of

leachates involved in contaminant transport and remediation efforts, and it is important to implement and advance modern technologies and methods to assess microbial communities present in affected and unaffected environmental sites (Azadpour-Keeley et al. 2009). Not only will this aid our understanding of the impacts that microbial communities can have on subsurface conditions, but it can also help to improve upon remediation designs as well as to make science-based decisions with respect to policy creation and regulation of burial sites.

6.3 Materials and Methods

6.3.1 Sample Sites and Coring

A livestock burial site located 20 km south of Pierceland, Saskatchewan, Canada, containing approximately 3000 elk carcasses disposed of in 2001 as a chronic wasting disease control measure was selected for sampling. The carcasses were buried in trenches of varying dimensions. The two trenches utilized in this study were dug approximately 2 m wide and 3.5 to 4 m deep and did not contain a liner or impermeable membrane. The site was located approximately 1 km from the closest residence and waterway. Soil cores were taken continuously from the surface to approximately 10 m below grade (Fig. 6.1) using a cable tool drilling rig equipped with a 750 mm drive sampling barrel. Cores were taken in clear cellulose acetate butyrate tubes (62 mm inner diameter, 750 mm length, 13 tubes per hole), which were capped and stored at -20°C. In order to prevent cross-contamination between core locations, the drilling equipment was sterilized with 5% sodium hypochlorite between each hole. Cores C1, C4, and C10 were dissected at various intervals (C1 at 0.75, 1.5, 2.5, 3.75, 4.5, 4.8, 5.5, 6.5, 7.5, and 8.5 m; C10 at 0.75, 2.5, 3.75, 4.5, 5.5, 6.5, and 8.5 m; C4 at 2.25 and 4.5 m) for complete DNA extraction, with one sample per interval for analysis. Differing depths were selected due to the variable depth of carcass burial as well as site topography. These cores, as well as others present in Figure 6.1 were used in subsequent leachate transport studies (Pratt and Fonstad 2012). Precautions were taken during drilling and dissection of the soil cores to avoid contamination with surface microbes. During dissection, samples used for DNA extraction were cut out from the center of the soil core in a sterile setting, eliminating contact with the exterior of the soil core to avoid cross-contamination, and then placed into a sterile container prior to extraction.

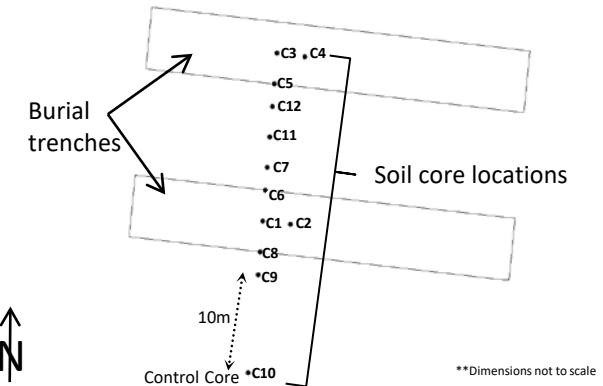


Figure 6.1 CWD disposal site core locations. The rectangles indicate the locations of the burial trenches. Carcasses were buried at a depth of approximately 3 to 4 m.

6.3.2 Chemical Analysis of Soil Core Pore Water

To analyze potential plume development, soil pore water was extracted via a custom high-pressure squeezing device (University of Saskatchewan, Saskatoon, Saskatchewan, Canada) at specific intervals in each soil core. Pore water extractions followed a hybridization of similar protocols as found in the literature (Böttcher et al. 1997; Howes 1985; Jahnke 1988; Manning 1993; Turrero 2006) and is described below. For this methodology, approximately 100 cm³ samples of soil were initially squeezed to a pressure of 27 MPa (4,000 psi); if less than 5 mL of water were obtained at this pressure, the pressure was increased to 41 MPa (6,000 psi). All squeezing and analysis of water samples was done at 4°C. As soon as the water sample was obtained, it was analyzed for pH (Hach H135, Loveland, Colo.), electrical conductivity (ATI Orion 128, Boston, Mass.) and alkalinity (Hach AL-DT, Loveland, Colo.) and then frozen at -20°C for preservation until ion analysis by atomic absorption (Varian SpectraAA 220, Walnut Creek, Cal.) for cations (Ca, Mg, Na, K), colorimetric determination for nitrate (Hach DR4000V, Loveland, Colo.), and by SmartChem discrete analyzer (Unity Scientific, Brookfield, Conn.) for anions (Cl and SO₄⁻) and ammonium, all following standard methods (Franson, 2005).

6.3.3 Extraction of Microbial DNA from Soil Cores

DNA was extracted from 10 g of each soil core sample using an UltraClean Mega Soil DNA Isolation Kit (Mo-Bio Laboratories, Carlsbad, Cal.). After extraction of DNA present in the soil, extensive washing was performed to remove PCR inhibitors using the UltraClean Mega Soil Kit.

Nucleic acids were precipitated with 3 M NaOAc and 95% ethanol, and then 50 µL of 10 mM Tris buffer at pH of 8 was added. DNA concentration was determined in each sample using a Qubit HS dsDNA Quantification Kit (Invitrogen, Grand Island, N.Y.).

6.3.4 Determination of Total Microbial 16S rRNA-Encoding Genes by Quantitative PCR

Quantitative PCR (qPCR) amplification using primers SRV3-1 and SRV3-2, which amplify nucleotides 330 to 533 of the 16S rRNA-encoding gene (Lee et al. 1996) (numbering according to *E. coli* 16S rRNA sequence) was performed using a standard consisting of a plasmid containing an insert corresponding to nucleotides 11 to 536 of the 16S rRNA-encoding gene from an uncultured human gut-derived bacterium. Aliquots of the extracted DNA were amplified with SsoFast EvaGreen Supermix (Bio-Rad Laboratories, Hercules, Cal.) with primers at 400 nM each. Amplification conditions were as follows: 95°C 3 min (1×), 95°C 15 s, 62°C 15 s, and 72°C 15 s (30×). Data collection was set at the extension step. Following amplification, a melt curve was generated consisting of 55°C to 95°C with an increment of 0.5°C s-1 and data collection for 5 s. Results were corrected to a scale of counts g-1 soil by considering the dry weight of soil used for extraction and the template volume used for qPCR. In order to evaluate the potential effects of PCR inhibition on the quantitative results, six different dilutions (1:1, 1:2, 1:5, 1:10, 1:20, and 1:50) of DNA product were amplified and compared, as described by Dumonceaux et al. (2006). Average 16S rRNA-encoding gene counts, corrected for dilution, were calculated for each dilution. The standard deviations of these averages produced the inherent error of total rRNA genomes per gram of dry soil of each sample for both the background core and the core through the burial trench.

6.3.5 Preparation of Samples for Pyrosequencing

DNA samples from selected soil core samples were prepared for taxonomic profiling by pyrosequencing. To prepare these samples, DNA was amplified using PCR with platinum Taq polymerase (Invitrogen) and PCR primers targeting the cpn60 universal target (cpn60 UT) (Hill et al. 2002). Universal cpn60 UT amplification primers were modified with multiplexing IDs (MIDs), generating amplicons with MIDs on both ends of the PCR product, as described by Schellenberg et al. (2009) and Schellenberg et al. (2011). Equimolar concentrations of gel-purified, MID-tagged

amplicons were pooled prior to ligation of emulsion PCR/sequencing adaptors and pyrosequencing with titanium chemistry using manufacturer's protocols (Roche). Sequences were generated from both ends of the PCR products.

6.3.6 Pyrosequencing Data Analysis

Roche 454 binary output files in standard flowgram format (sff) were separated by MID to generate a dataset corresponding to DNA templates extracted from each core for taxonomic analysis, as described previously (Chaban et al. 2011). Data from all libraries were combined and assembled into operational taxonomic units (OTUs), as described by Links et al. (2012), in cDNA mode with a minimum match length of 137 and a minimum match identity of 90. Primer sequences were removed from the resulting sequences (isotigs) using SeqClean (<http://sourceforge.net/projects/seqclean/files/>). These sequences were clustered at 100% identity using cd-hit (Li and Godzik, 2006) to produce a set of non-redundant OTUs (Links et al. 2012). These OTUs corresponded to the observed set of complete or nearly complete cpn60 UT sequences from all of the samples. The abundance of each OTU in each library was determined by tracking all of the sequences that assembled or were collapsed into each non-redundant OTU. Abundance data were exported into formats suitable for use in other analytical tools using PERL scripts written for that purpose.

6.3.7 Statistical Analysis of Microbial Communities

Microbial community statistics, including Chao1, Simpson's index (D), and the nonparametric Shannon index (H') (Hill et al. 2003), were calculated using mothur (Schloss et al. 2009) with input corresponding to OTU frequencies for each sample. Good's coverage estimates and rarefaction analysis was also performed for each sample using mothur.

6.3.8 Taxonomic Profiles

Community taxonomic profiles were generated from raw pyrosequencing read data by performing BLASTx analysis of each read and mapping the results to a nonredundant peptide version of cpnDB (version cpnDB_nr20120523; downloadable at www.cpndb.ca) using MEGAN version 4.61.4 (Huson et al. 2007).

6.3.9 Functional Categorization

An additional classification of the OTUs was carried out using a variant of the RDP classifier (Wang et al. 2007). Using the RDP classifier codebase, a custom Bayesian classifier was trained using data from cpnDB. Each OTU was classified using the cpn60 Bayesian classifier with a confidence cutoff of 0.8. Based on reports in the literature, each OTU that had a robust classification using the Bayesian classifier was grouped into broad functional categories (SI table 6.4). Genera with broad functionality (e.g., *Lactobacillus* and *Pseudomonas*) were not assigned to a functional category, regardless of their abundance in the core samples.

6.3.10 Phylogenetic Analysis

The cpn60 UT sequences from reference strains were retrieved from cpnDB and aligned with full-length assembled OTU sequences from the pyrosequencing data using ClustalW. Phylogenetic trees were constructed using the neighbor-joining method (Saitou and Nei 1987) with bootstrapping using 500 replicates. Distances were calculated using the maximum composite likelihood method (Tamura et al. 2004). Alignments were performed and trees were calculated using MEGA v5.05 (Tamura et al. 2007).

6.4 Results and Discussion

6.4.1 Total Estimated Bacterial 16S rRNA-Encoding Genes in Each Core Sample

Within the burial core (C1), the highest bacterial 16S rRNA gene counts were observed at the burial depth (approximately 3 to 4 m), with 2 to 5 orders of magnitude more bacterial 16S rRNA genes g⁻¹ soil sample at that depth than at any other, and bacterial 16S rRNA gene counts decreased steadily with increasing depth beyond 3.75 m (fig. 6.2). In contrast, the control core (C10) 16S rRNA gene count was similar to the burial core at the surface but decreased with depth until 4.5 m, below which it remained relatively constant. The 16S rRNA gene count at burial depth (3.75 m) was 5 orders of magnitude greater in the burial core compared to the control core (fig. 6.2); this is readily explained by the very high organic load caused by the deposition of the carcasses and suggests that active decomposition was still occurring in this burial site eight years

after the CWD control measure was implemented. Under degradative conditions, microbial DNA from nonviable organisms would be expected to be degraded rather quickly (Xu et al. 2009).

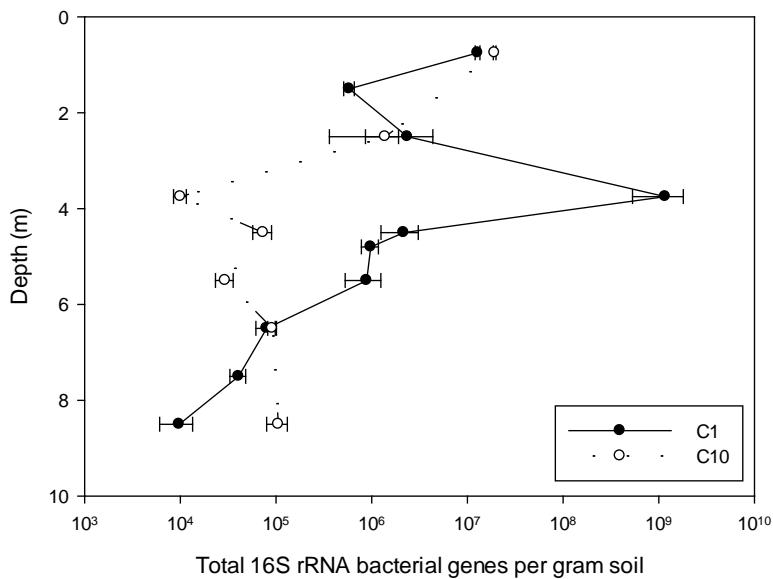


Figure 6.2 Total bacterial 16S rRNA-encoding genes per gram of soil for burial core (C1; solid line) and control core (C10; dashed line).

6.4.2 Pyrosequencing of Selected Soil Core Samples

DNA extracted from all of the burial trench (C1) depths along with two from C4 (2.25 and 4.5 m) and one sample from the control core (C10, 5.5 m) was used as a template for cpn60 UT PCR and analyzed by pyrosequencing to profile the microbial communities in each sample. In total, 452,646 usable reads were generated from all cores (Table 6.1). Supplemental information on all samples is available in the Appendix. We determined that these microbial communities were represented by 5825 OTUs that were present at a variety of abundances in each of the core samples. Rarefaction plots and Good's coverage estimate were calculated for each sample to determine the completeness of sequencing coverage. The mean coverage for all samples, excluding the 8.5 m sample from C1 that produced only 202 reads, was 0.960 with a range of 0.908 (C1, 1.5 m) to 0.996 (C1, 3.75 m) (table 6.1 and SI fig. 6.11).

Table 6.1 Soil core samples subjected to deep sequencing for microbial community analysis and numbers of reads associated with each core.[a]

Core	Depth (m)	Total Reads	OTUs	Good's Coverage
C1	0.75	43605	12895	0.986
C1	1.5	13043	5949	0.908
C1	2.5	94953	57419	0.992
C1	3.75	52973	34721	0.996
C1	4.5	32613	23252	0.982
C1	4.8	82458	56609	0.994
C1	5.5	20649	12163	0.995
C1	6.5	21042	9715	0.981
C1	7.5	7130	3623	0.959
C1	8.5	202	97	0.804
C4	2.25	17546	8036	0.949
C4	4.5	17431	8753	0.969
C10	5.5	49001	34758	0.995
Totals:		452646	267810	

[a]See figure 6-1 for core identities. Elk carcasses were buried at a depth of approximately 3 to 4 m. Good's coverage estimate of 1.0 indicates complete coverage (Good 1953; Schloss et al. 2009).

6.4.3 Overview of Microbial Communities

Community diversity statistics were calculated for each microbial community that was analyzed. Chao1 (Chao 1984) estimates richness using nonparametric estimators based on the mark-release-recapture (MRR) statistics. This estimate shows that in a diverse community, the probability of a species being seen more than once is small (Hughes et al. 2001). Chao1 estimates also seem to correlate with sample size, in that they tend to underestimate community richness at low sample size. Within burial core C1, community richness (Chao1) increased with depth from the soil surface (0.75 m) to reach a maximum at 2.5 m, after which the richness decreased and remained roughly constant until 4.8 m. Below this depth, the richness decreased further (fig. 6.3). Simpson's index (D) shows the probability that two randomly selected sequences will be from the same OTU (Hill et al. 2003; Simpson 1949). Simpson's index demonstrates the evenness of the community: D = 1.0 indicates an uneven community (all composed of the same OTU), and D = 0 indicates an

even community (not as dominated by one OTU). Community evenness (Simpson's index) was greatest between 1.5 and 3.75 m and decreased sharply just below the burial depth (4.5 to 4.8 m) before increasing again at 5.5 m (fig. 6.3). The microbial community at 4.8 m was the least even of all the communities. The Shannon index (H') is one of the most commonly used means to determine population diversity, but it gives more weighting to rare OTUs than to those that are abundant (Shannon and Weaver 1963). The Shannon index was highest in the 1.5 to 3.75 m samples (fig. 6.3). The community that could be considered the most complex (highest richness and evenness) was the 2.5 m depth in the burial core. This was reflected in the Shannon index, which was highest in this sample (fig. 6.3 and table 6.2).

Comparing burial core C1 to control core C10 at the same depth (5.5 m) revealed that the two communities had the same richness but markedly different evenness; the burial core microbial community was substantially more even than the control core (table 6.2). The high organic load in the burial core may have encouraged the relatively even outgrowth of microbial species compared to the control core. The microbial community in burial core C4 was more even than in C1 at a depth of 4.5 m, although the C1 community was somewhat richer than the C4 community at this depth (table 6.2 and fig. 6.3).

Table 6.2 Community descriptive parameters in the control core (C10) and burial cores (C1, C4).

Core	Depth (m)	Simpson's		Shannon
		Index (1/D)[a]	Chao1[a]	Index (H')
1	5.5	14.40 (13.98- 14.84)	674.54 (613.07- 765.73)	3.745
10	5.5	2.66 (2.64-2.68)	763.63 (621.52- 980.96)	1.489
4	4.5	18.75 (18.02- 18.75)	948.47 (865.35- 1062.66)	4.245

[a] Simpson's evenness index and the Chao1 richness index are shown with 95% confidence intervals.

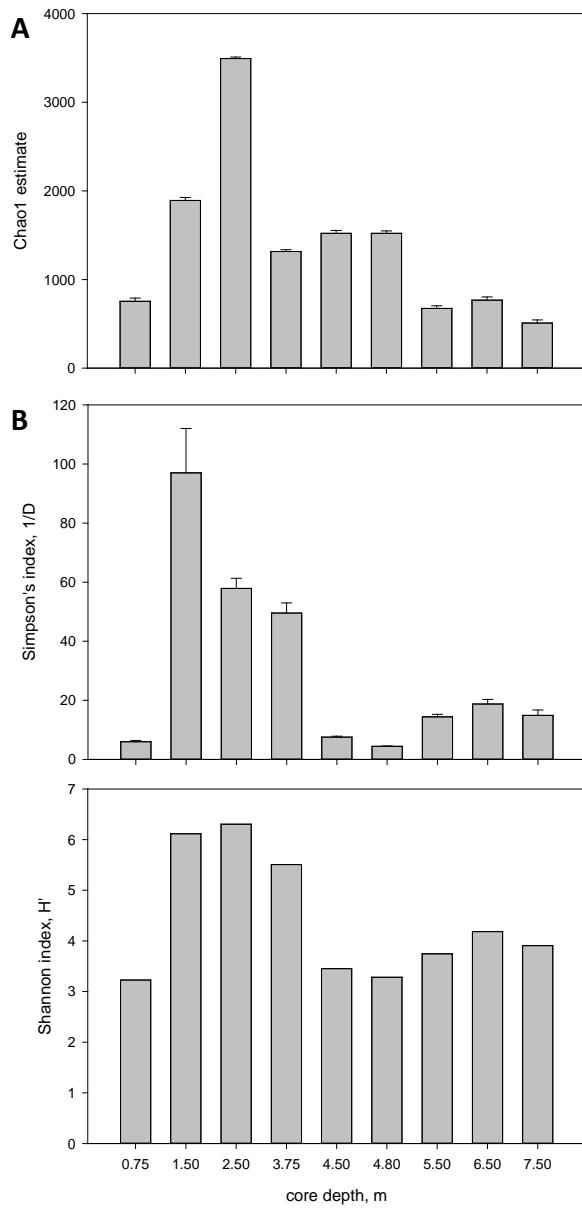


Figure 6.3 Microbial community descriptive statistics for burial core (C1): (a) Chao1 community richness (results are shown $\pm 95\%$ confidence intervals), (b) Simpson's evenness index (1/D; results are shown $\pm 95\%$ confidence intervals), and (c) the Shannon index (H').

6.4.4 Taxonomic Profiles of Microbial Communities in the Soil Cores

Taxonomic overviews of each soil core microbial community were generated using MEGAN, which maps the results of BLASTx analysis of each OTU and its corresponding frequencies to the

NCBI taxonomy tree (Huson et al. 2007; Mitra et al. 2009). This analysis showed that the composition of the C1 burial core microbial communities changed remarkably with depth; for example, the surface community (0.75 m) showed a predominance of Lactobacillaceae, Nitrosporaceae, and Gemmatimonadaceae, while the taxon abundances in the core just slightly deeper (1.5 m) were somewhat more evenly distributed and more taxa were represented (Supplemental Information (SI) fig. 6.6). At the depth of the burial site (3.75 m), where bacterial abundance was highest (fig. 6.2), anaerobic microorganisms including Bacteroidales and Clostridiales were strongly represented (SI fig. 6.8). The burial depth sample also contained a variety of eukaryotic cpn60 UT sequences including fungal sequences, plant sequences (possibly from decaying plant matter), and a few poorly classified, possibly animal-derived sequences (SI fig. 6.8). Although elk (*Cervus elaphus*) is not currently represented in cpnDB, and it is tempting to speculate that some of these sequences may have derived from the decaying elk carcasses, it is difficult to say with certainty if any of these sequences may have been from the buried animals. Nevertheless, the detection of eukaryotic sequences is a unique advantage that is provided by cpn60 UT compared to 16S rRNA-encoding genes, which are restricted to the detection of bacterial sequences.

Comparing the taxonomic distribution of microorganisms at the same depth (5.5 m) in the burial (C1) and control (C10) cores revealed that these communities were highly distinct from each other, suggesting that the composition of the burial core community was influenced by the presence of the organic load from the decaying animals (fig. 6.4). In particular, the microbial community of the control sample, which had a similar Chao1 richness to the burial sample at the same depth (table 6.2), was strongly represented by Lactobacillaceae, while the burial sample at 5.5 m had far more taxa detected; this taxonomic difference was reflected in the increased evenness determined for the burial vs. control sample at 5.5 m (table 6.2).

When the two burial cores were compared at similar depths, it was apparent that the communities in the two burial sites were also quite distinct from one another. For example, above the burial depth (2.5 m for C1 and 2.25 m for C4), a range of taxa were represented in both cores, but C1 was more strongly represented by Nitrosomonadales, Gemmatimonadales, and Deltaproteobacteria, while C4 showed more sequences classified as Chlamydales, Chloroflexales,

Sphaerobacterales, and Alteromonadales (SI fig. 6.12). This suggests that, in the two burial cores, somewhat different taxonomic arrays of bacteria were available for the decomposition of the corpses. These differences were reflected in the 4.5 m samples for both cores, which were just below the burial sites; many of the same taxa were more strongly represented in C4, while C1 was comparatively strongly represented by Desulfuromonadales and Burkholdariales (SI fig. 6.13).

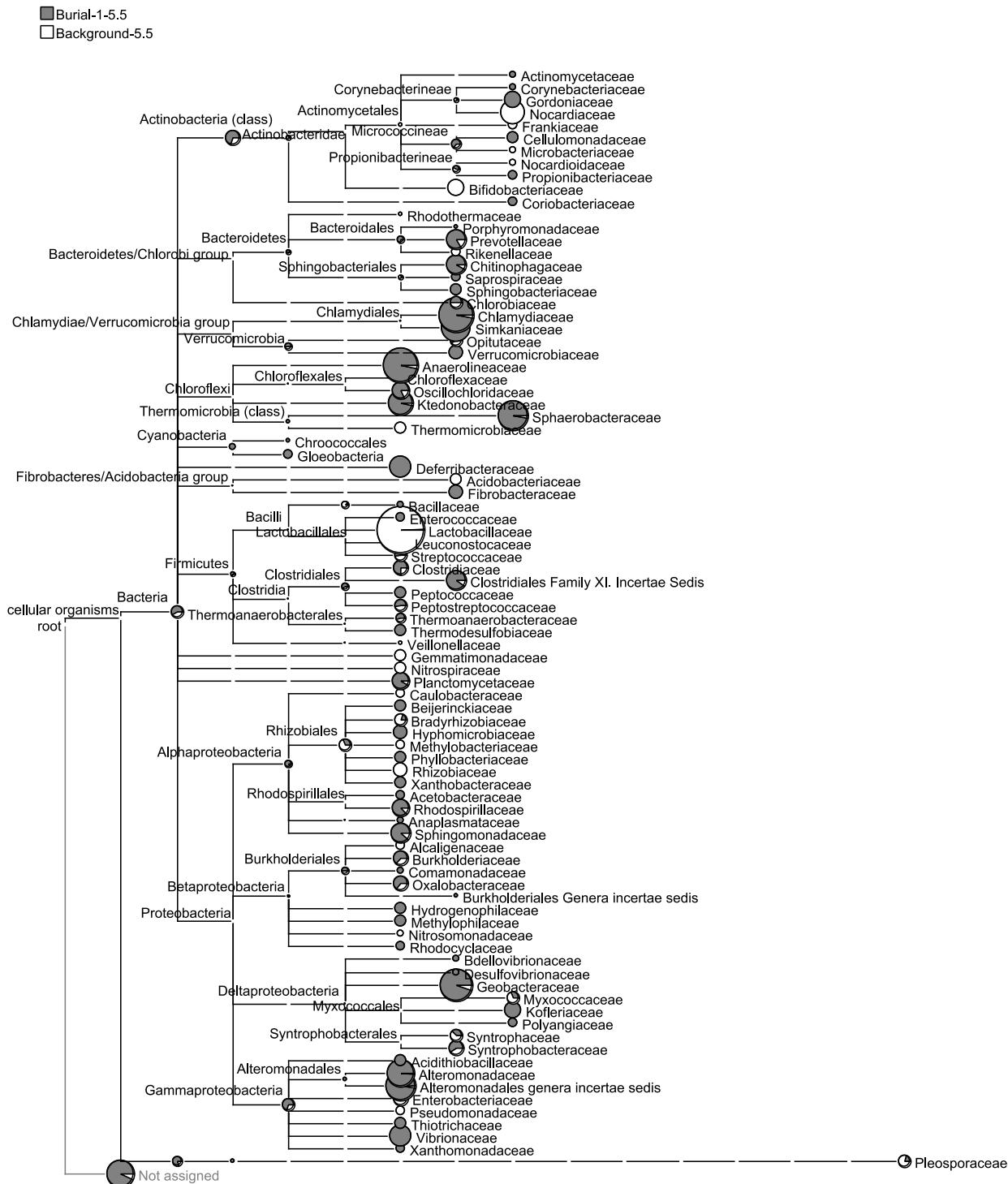


Figure 6.4 Family-level taxonomic profiles of microbial communities from control (white) and burial (gray) cores at 5.5 m below grade; circle size reflects read abundance.

6.4.5 Functional Classification of OTUs

Assigning taxa to functional classes based on their known metabolic activities can be difficult, since no evidence of functionality exists beyond sequence similarity to organisms with known functionality. For this reason, we assigned probabilities to the taxonomic classification of each OTU using the cpn60 UT-based Bayesian classifier and restricted our analysis to OTUs with a strong probability (at least 0.8) of being correctly classified. With this restriction in place, only 575 of the 5825 OTUs were confidently classified to the genus level (10%), which is consistent with other studies that show a large number of difficult-to-classify sequences from soil samples (Nacke et al. 2011; Werner et al. 2012). We further restricted our analysis to genera for which published evidence could be found for the functionality of organisms within that taxonomic class (table 6.4); as a result, certain genera such as *Lactobacillus* and *Pseudomonas*, despite being well represented within confidently classified OTUs, were excluded from this analysis. Nevertheless, some interesting patterns emerged. Within the burial core, we noted a tremendous increase in organisms associated with ammonification and putrefaction at the burial depth (table 6.3). In particular, a large number of OTUs classified as *Pusillimonas* sp. (family Alcaligenaceae) were noted at this depth (table 6.3 and SI fig. 6.8); these organisms are known to accumulate putrescene, a nitrogenous compound associated with decaying corpses (Lee et al. 2010). Very few OTUs classified as *Pusillimonas* sp. were observed immediately above or below the burial depth, suggesting that these organisms were closely associated with the decaying corpses and did not migrate far from the burial site. In addition to organisms associated with putrefaction, a total of 1392 OTU counts were most closely related to *Acidaminococcus* sp. (fig. 6.5), although they fell below the confidence level of 0.8 for genus assignment. These organisms are known to be involved in ammonification (Eschenlauer et al. 2002), producing ammonium by deamination of amino acids. The peak in sequences derived from organisms associated with these early steps in the decay of protein-rich organic material was closely associated with a strong peak in ammonium in the pore water (table 6.3).

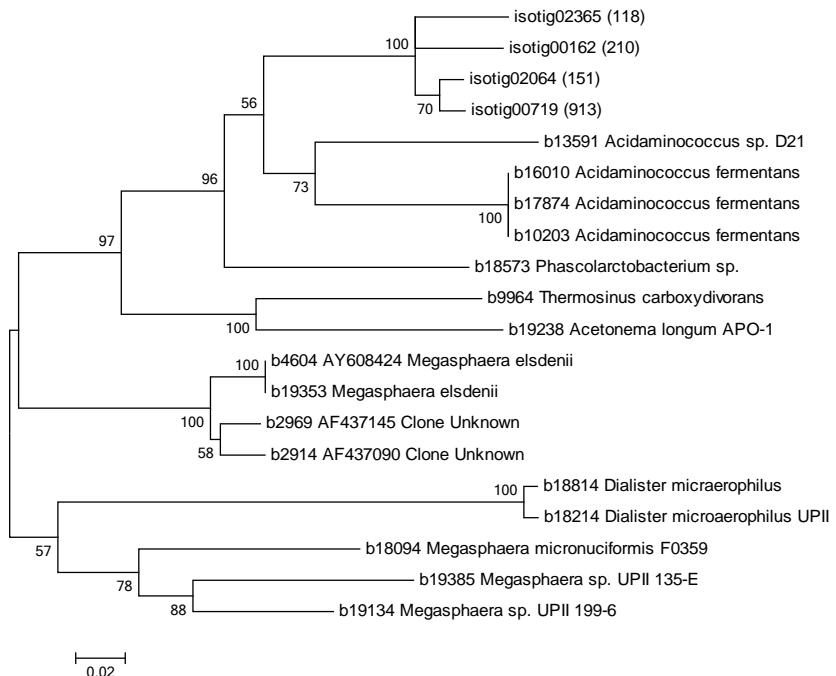


Figure 6.5 Phylogenetic analysis of OTUs related to Acidaminococcus (order Selenomonadales) that were observed at least 100 times at the burial depth (3.75 m) in core 1. OTUs are identified by their isotig numbers, and the number of times each OTU was observed in this sample is indicated in parentheses. Reference sequences are identified by their GenBank accession numbers and/or cpnDB IDs.

Microorganisms associated with nitrogen fixation and denitrification were concentrated closer to the surface, predominantly in the 0.75 m core sample with few OTU counts observed below this depth. Furthermore, OTUs derived from microorganisms classified as Nitrobacter and Nitrospira, which are associated with nitrite oxidation (table 6.4), were concentrated in the core samples up to 2.5 m below grade, with almost no counts observed below this depth. The levels of ammonium were comparatively low in the pore water from these samples (table 6.3), which suggests that ammonia-oxidizing organisms may have been active in these samples. Consistent with this, OTUs classified as Nitrosospira, organisms that are known to oxidize ammonia aerobically (SI table 6.4), were concentrated in the 2.5 m core sample (table 6.3). Sequences assigned to other genera associated with aerobic ammonia oxidation, such as Nitrosomonas (Otawa et al. 2006), even at low confidence levels, were absent from all core samples. Nitrate levels were low in the shallowest core samples, and nitrite was undetectable in all samples despite the high levels of ammonium at

the burial depth (table 6.3), suggesting that nitrite oxidation and denitrification were actively occurring. The concentration of OTUs from microorganisms associated with aerobic ammonia oxidation in the core sample just above the peak in ammonium concentrations suggests that nitrification was occurring in the aerobic zone above the burial depth, while at the burial depth ammonium was likely produced from the organic material by microorganisms associated with ammonification. The abundance of OTUs derived from anaerobic microorganisms at the burial depth where ammonium concentrations were highest suggests that ammonium may have been oxidized at that depth through an anaerobic (anammox) pathway (Nannipieri and Eldor 2009). Consistent with this suggestion, organisms that were taxonomically assigned to the order Planctomycetales, in which anammox organisms are commonly classified (Jetten et al. 2005; Pathak et al. 2007), were observed in the C1 samples from the surface to the burial depth (SI figs. 6.6 to 6.8), albeit at comparatively low counts.

Table 6.3 Functional class categorization of reads that were classified to the genus level with high confidence (Bayesian classifier score ≥ 0.80).[a]

Functional Class	Counts of Functionally Classified OTU in Core at Depth (m)					
	0.75	1.5	2.5	3.75	4.5	4.8
Ammonification/deamination; putrefaction	3	16	9	3233	63	16
Aerobic ammonia oxidation	0	0	3190	4	11	1
Nitrite oxidizing	348	389	437	1	7	1
Denitrification	41	0	5	0	0	0
Dissimilatory metal reduction	1	0	9	9	6017	10749
Phosphate accumulation	290	226	915	3	69	18
Plant growth promotion; nitrogen fixation	549	60	26	2	2	2
Chemical Analysis of Pore Water in Core at Depth (m)						
Functional Class	0.75	1.5	2.5	3.75	4.5	4.8
NH_4^+ (mg L ⁻¹)	6	8	26	3819	248	101
SO_4^{2-} (mg L ⁻¹)	68	72	84	77	233	76
NO_3^- (mg L ⁻¹)	3	5	0.7	12	35	4
NO_2^- (mg L ⁻¹)	0	0	0	0	0	0

[a] Functional classes were assigned to the genera observed in each core according to the known properties of the genus (SI table 6.4). Read counts were scaled to the median library size (26828) for all cores. Chemical analysis of pore water from corresponding core samples is also shown.

Organisms associated with phosphorus accumulation, mostly assigned to the genus *Gemmamimonas*, reached a peak at 2.5 m below grade and then became much less abundant (table 6.3). This can be accounted for by the association of phosphorus uptake by plants in the root zone, since phosphate-accumulating organisms play a large role in the solubilization, mineralization, and plant uptake of phosphorus in surface soils (Bünemann et al. 2010; Khan et al. 2009).

A large number of sequences was also observed from organisms associated with metal ion reduction, principally *Geobacter* spp., immediately below the burial site. This is suggestive of a reducing environment produced by the organic load from above. Certain species of *Geobacter* are iron-reducing microorganisms that oxidize organic material to carbon dioxide (Lovley et al. 1989). Sulfate levels were highest in the 4.5 m sample and dropped off sharply immediately below this depth (table 6.3). While organisms classified within the order Desulfuromonadales were well represented in these cores, by far the most abundant organisms within this order were classified as *Geobacter* spp., especially *G. bemandjiensis* (SI figs. 6.9 and 6.10). This suggests that the role of sulfate-reducing bacteria may have been fulfilled by *Geobacter* spp. in these soil core samples.

6.5 Conclusion

Recent advances in sequencing technology along with concomitant improvements to data analysis procedures have greatly facilitated the nearly complete profiling of microbial communities associated with a variety of environments. In the case of livestock disposal sites, it is clear that the organic load caused by the deposition of the carcasses influenced both the bacterial load and the composition of the microbial communities in these areas, but also that different burial sites can have distinct taxonomic profiles associated with the sites. Quantitative PCR based on the 16S rRNA-encoding gene showed that the burial trench had significantly (2 to 5 orders of magnitude) more bacterial 16S rRNA-encoding genes g-1 soil within and up to 2 m below the burial site compared to a nonburial control core sample at the same depths. Taxonomic analysis indicated that the overall community composition changed considerably with increasing depth, and that the burial core community was distinct from that of the control core at all depths. Organisms associated with phosphate accumulation, nitrogen fixation, and ammonium oxidation were found in highest abundance near the surface of the burial core (up to 2.5 m), while organisms associated with ammonification were found at the burial depth, consistent with an increase in ammonium

concentration in pore water. Sequences from organisms associated with dissimilatory metal reduction were concentrated just below the burial depth (4.5 to 5.5 m). Anaerobic microorganisms dominated the microbial community at the burial site (3.75 m).

The OTU assembly approach described in this work provides distinct sequences that can be used to develop molecular assays for tracking transport and population dynamics of organisms associated with burial events, and can provide an overall fingerprint of the microbes associated within a given site. This can provide information useful in retrospective site evaluation and, combined with the analysis of chemical and physical data and its correlation to biological functionality, may help in the selection of burial sites for future disease mitigation interventions. It has been demonstrated here that subsurface microbial communities provided a response to the burial of carcasses, and this is seen by the changes in diversity and structure of the community. Small changes in the microbial communities beneath burial sites can have implications with regard to contaminant transport and consequences to the environment. By utilizing and improving upon modern molecular methods, we can enhance the ability to measure the responses and in turn provide science-based evidence for burial site regulation and policy creation to protect water resources and the environment.

6.6 Transition Statement

Chapter 5 provided the last link to the puzzle with respect to the evaluation of subsurface transport of leachate and microbe populations beneath two carcass disposal sites. This is the final scientific study portion of this thesis. Chapter 6 will provide concluding remarks and future recommendations.

6.7 Acknowledgements

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6.8 Author Contributions

D.P. designed the study, collected the samples and conducted pore water and microbial extractions. T.D. and M.L. assisted with metagenomic data compiling and MEGAN analysis. D.P. wrote the manuscript, T.F., M.L. and T.D. provided edits and comments on the manuscript and contributed to the text in later iterations.

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6.10 Supplemental Information

This supplemental information includes family-level phylogenetic trees created with MEGAN for core C1 at various depths (figs. 6.6 to 6.11), rarefaction curves for the cores analyzed (fig. 6.11), comparative phylogenetic trees for various samples (figs. 6.12 to 6.14), and a functional class categorization of assignments including the appropriate references (table 6.4).

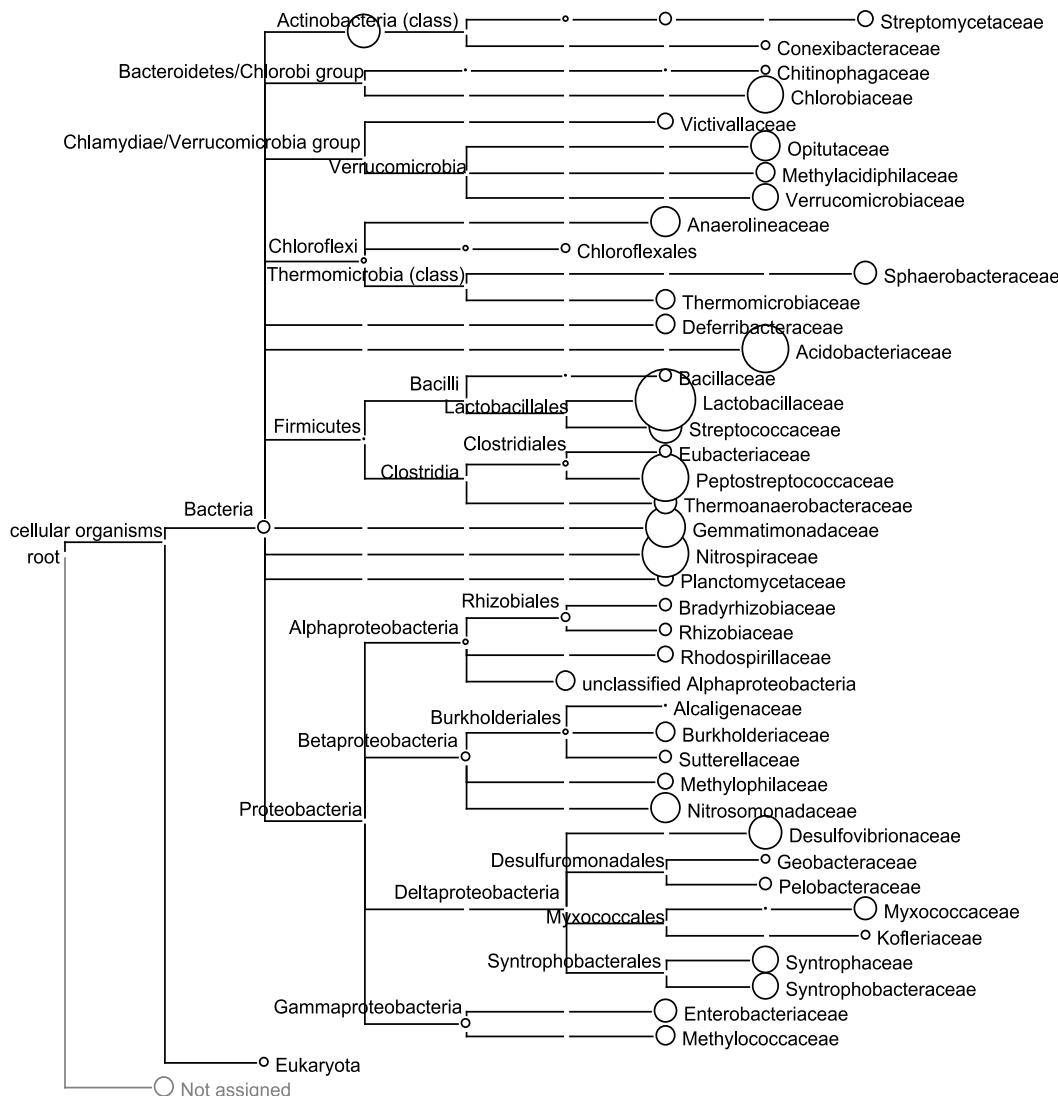


Figure 6.6 Family-level taxonomic profile of burial core C1 at 0.75 m.

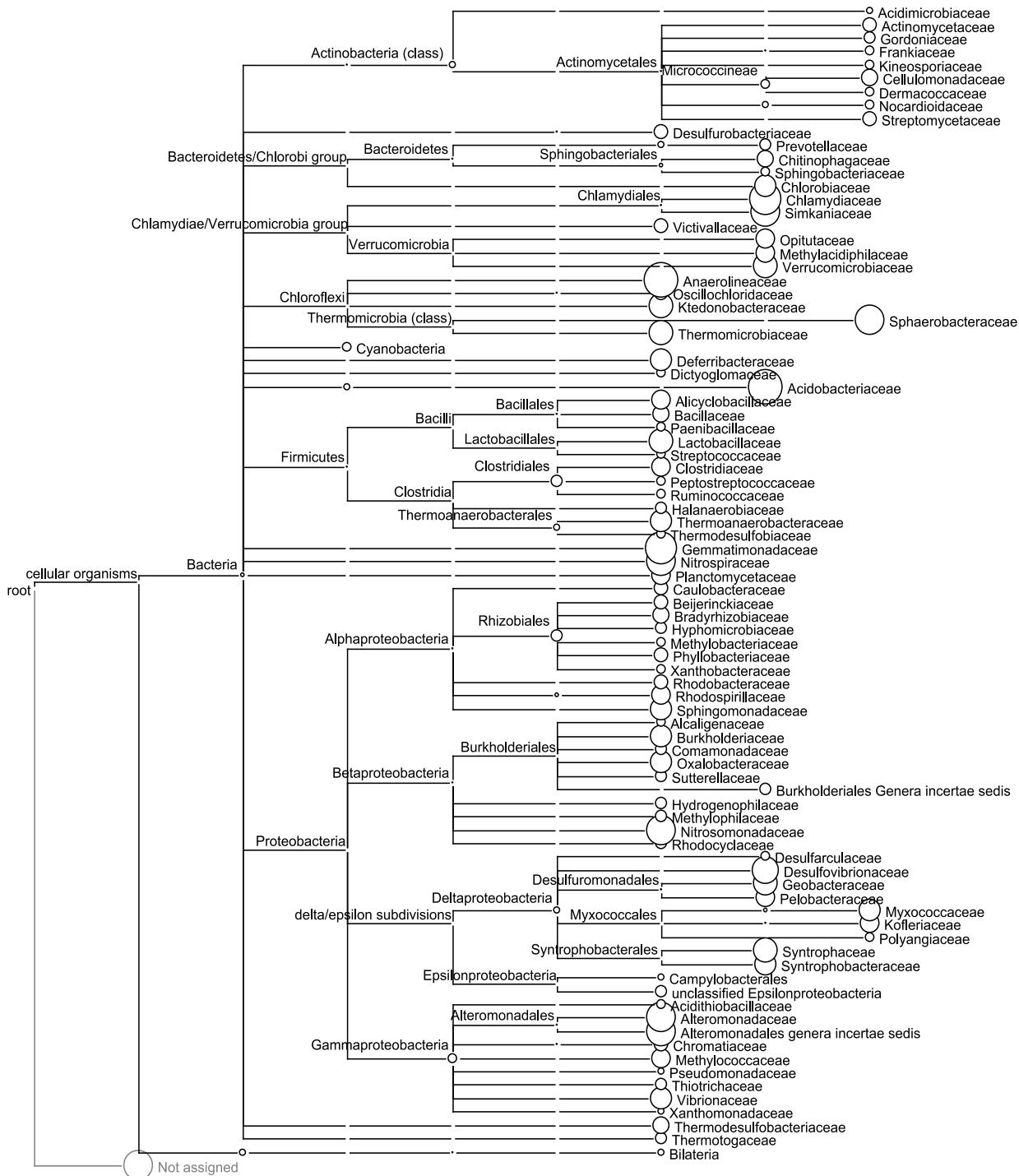


Figure 6.7 Family-level taxonomic profile of burial core C1 at 1.5 m.

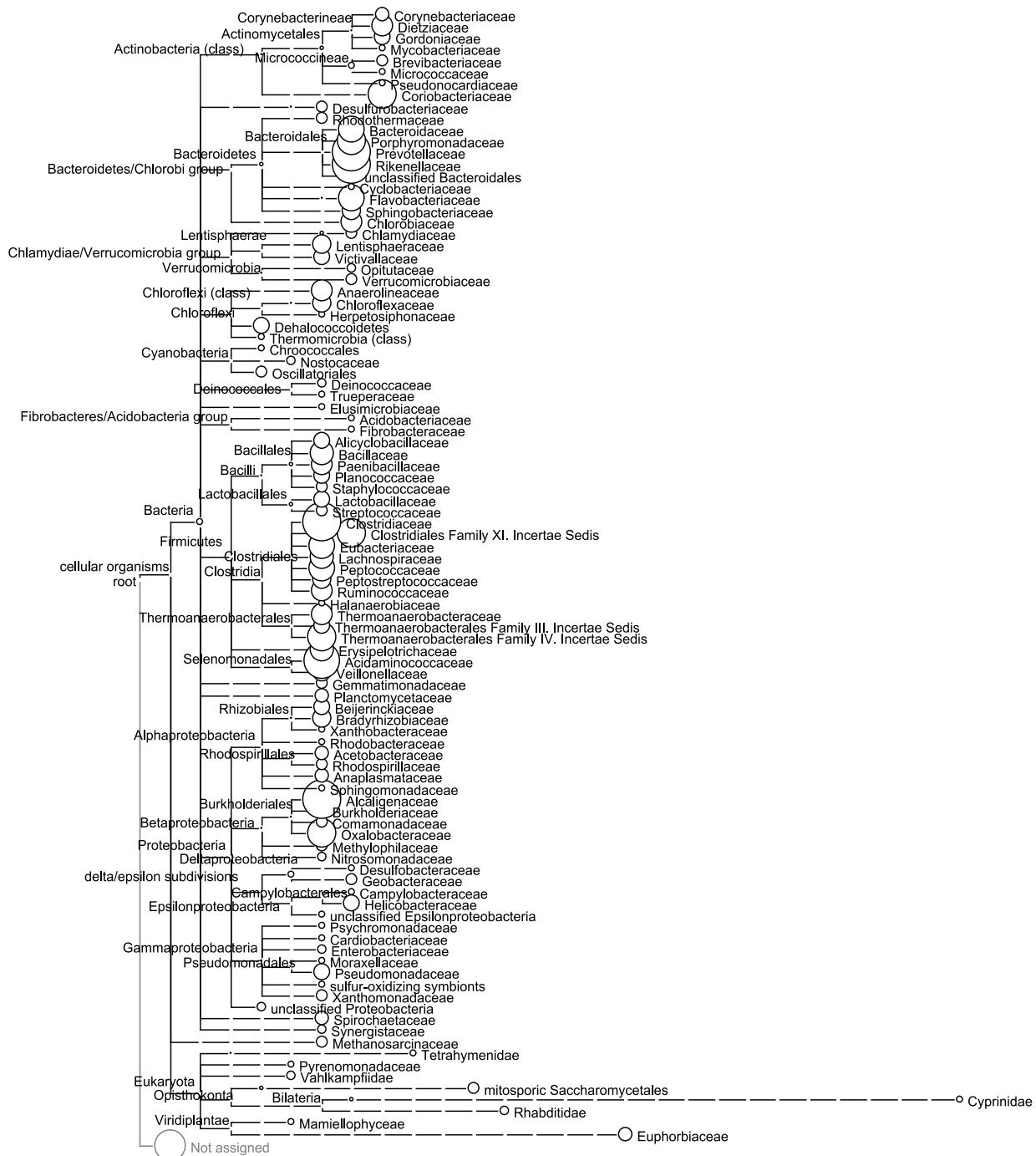


Figure 6.8 Family-level taxonomic profile of burial core C1 at 3.75 m (burial depth)

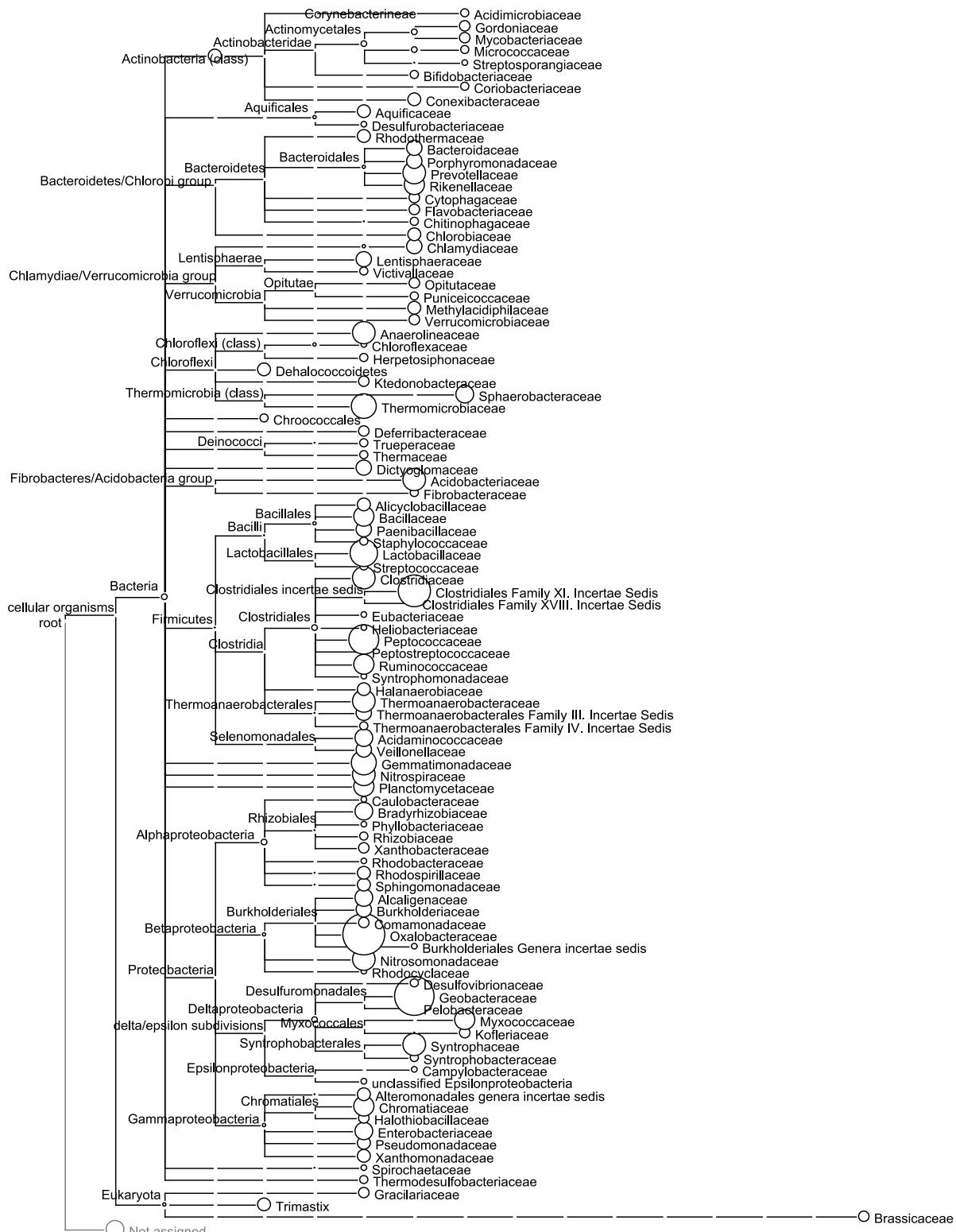


Figure 6.9 Family-level taxonomic profile of burial core C1 at 4.5 m (burial depth).

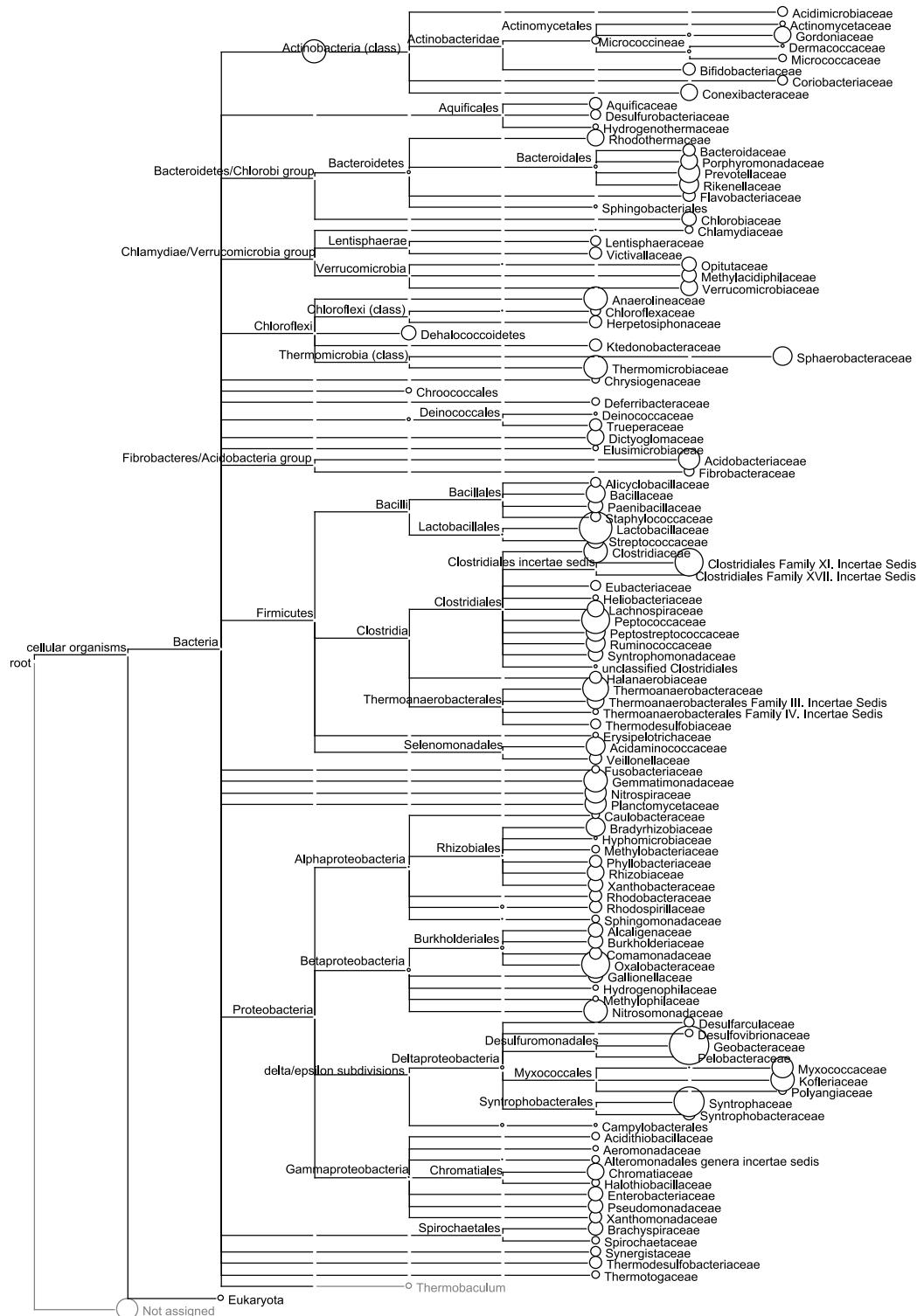


Figure 6.10 Family-level taxonomic profile of burial core C1 at 4.8 m (below burial depth)

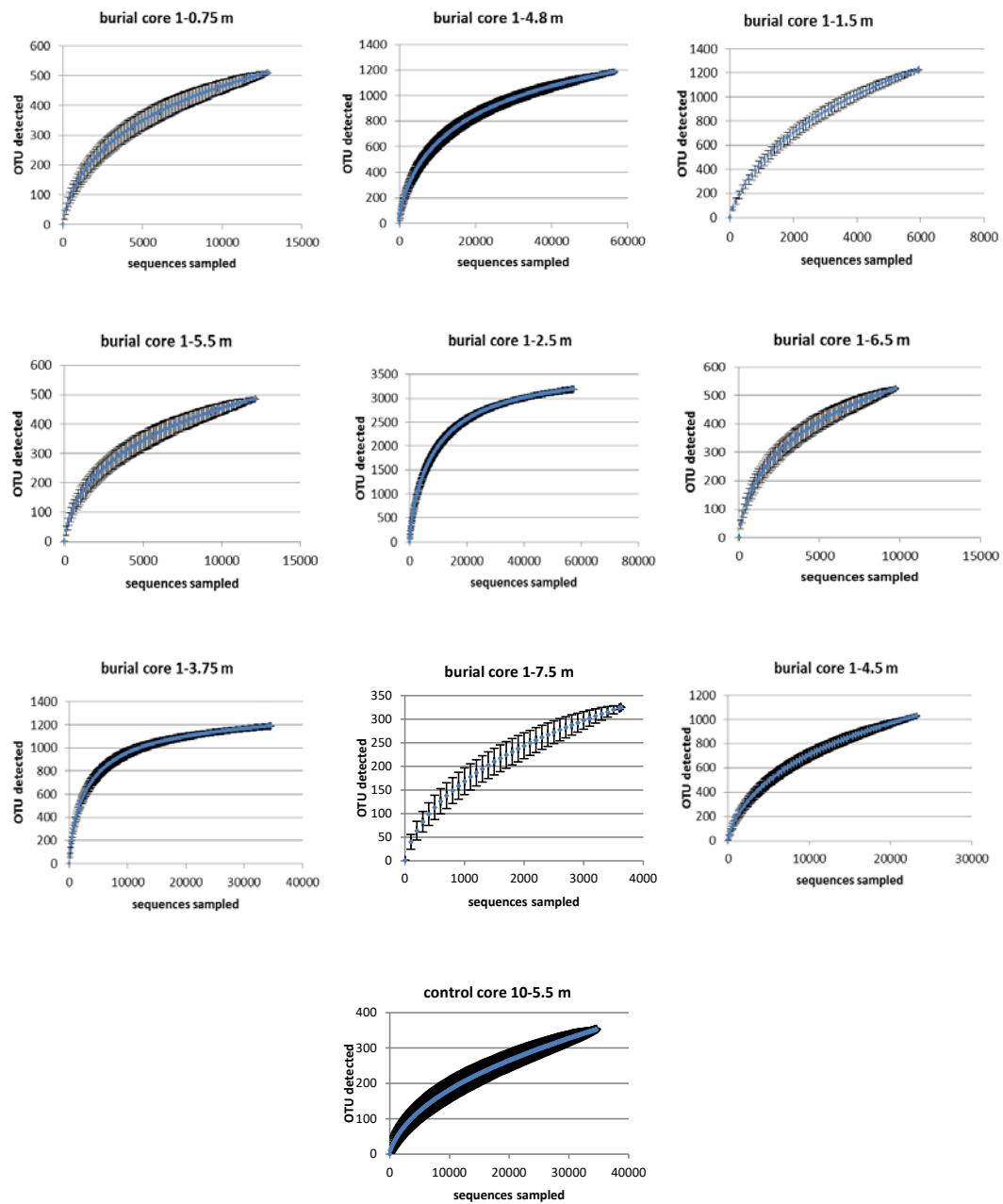


Figure 6.11 Rarefaction curves for cores analyzed at the 2001 CWD disposal site

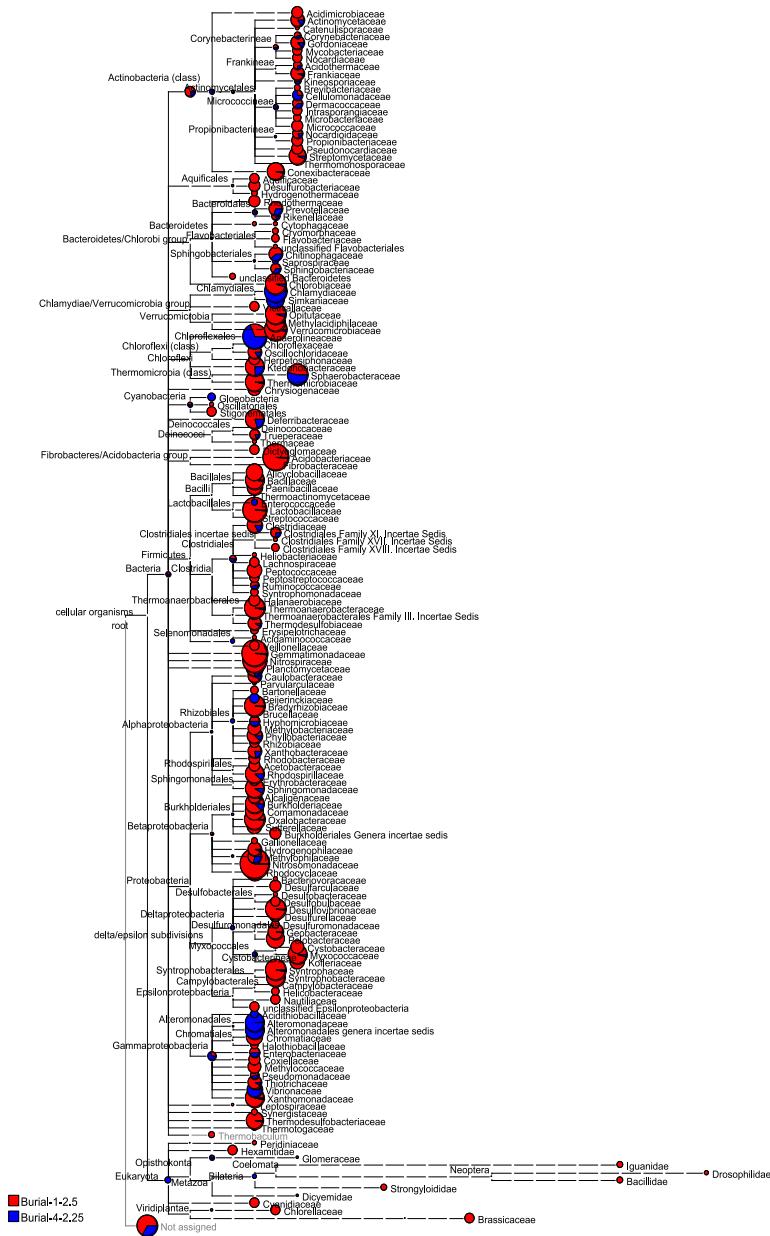


Figure 6.12 Core 1 vs. core 4 (burial cores) at 2.5 and 2.25 m: taxonomic profiles from MEGAN

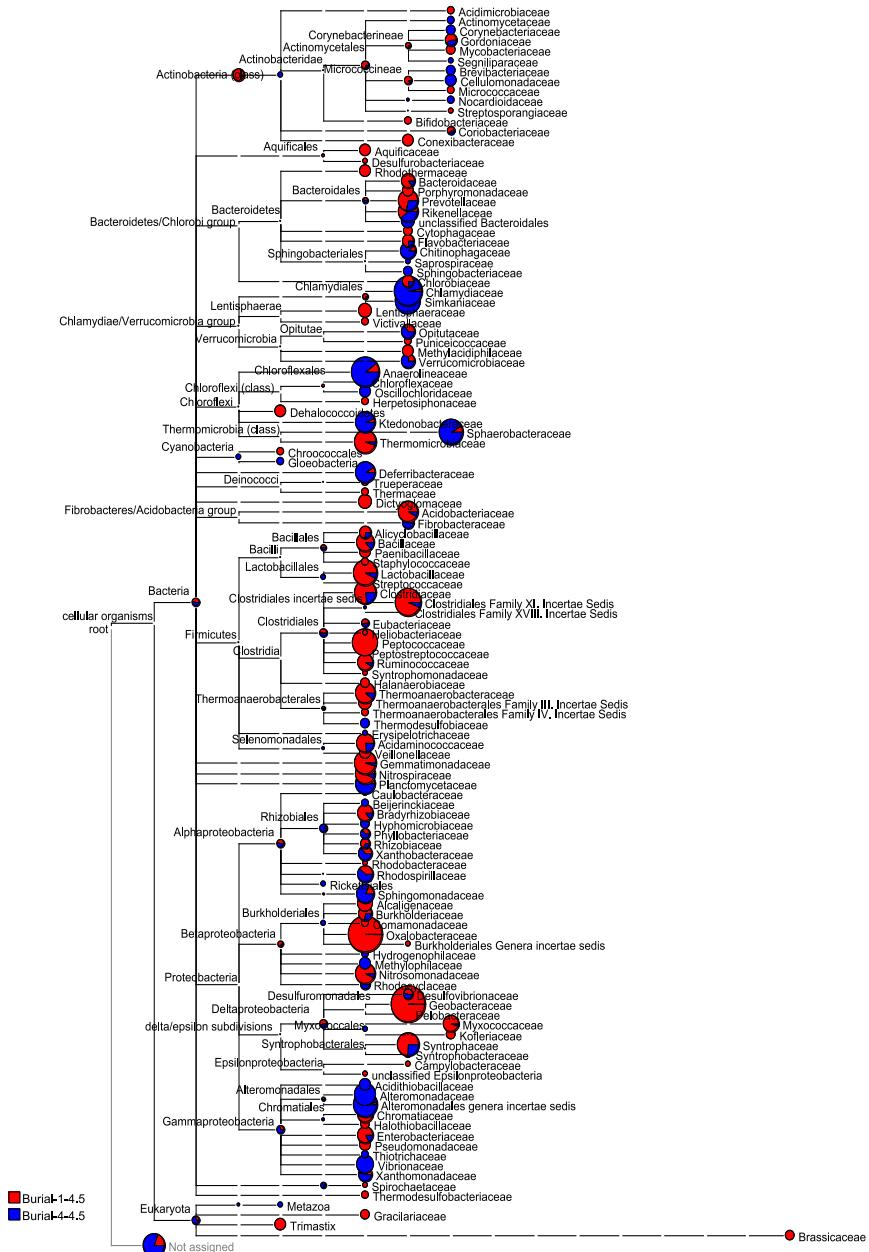


Figure 6.13 Core 1 vs. core 4 (burial cores) at 4.5 m: taxonomic profiles from MEGAN

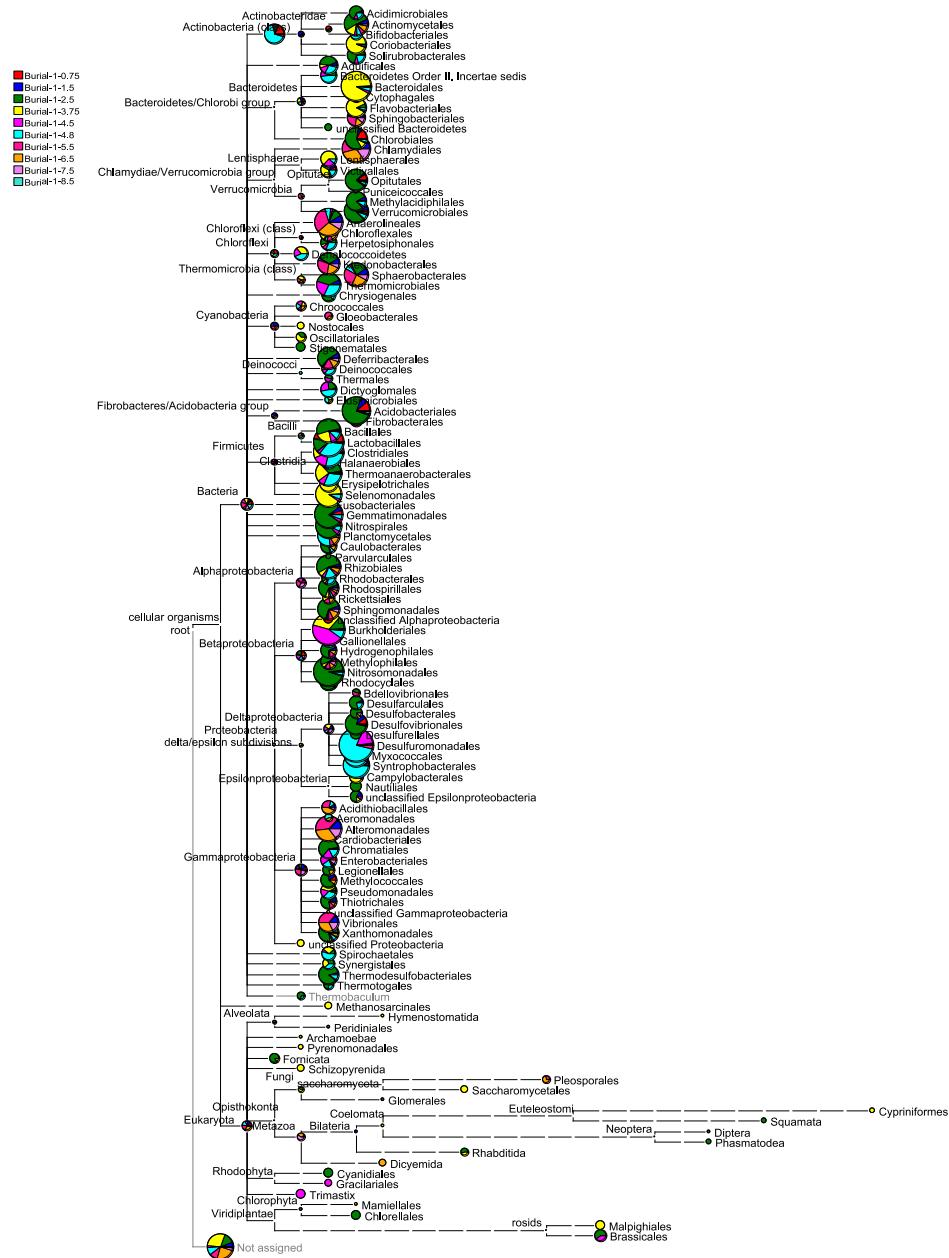


Figure 6.14 Core 1 (burial core) at all depths: order-level taxonomic profiles from MEGAN

Table 6.4 Functional class categorization. Assignments were made as described in the Materials and Methods section

Functional Class	Representative Genera Found in Core Samples	References
Dissimilatory metal reduction	<i>Geobacter</i> , <i>Anaeromyxobacter</i> , and <i>Shewanella</i> <i>Acidimicrobium</i> <i>Rhodoferax</i>	Aklujkar et al. (2010), Nevin et al. (2005), Plymale et al. (2011), Lovley (1993) Adams et al. (2007) Gault et al. (2011)
Phosphate accumulation	<i>Gemmatus</i> <i>Acidovorax</i> <i>Dechloromonas</i>	Zhang et al. (2003) Ren et al. (2007) Crocetti et al. (2000)
Plant growth promotion and nitrogen fixation	Free living in soil: <i>Azospirillum</i> Free living in soil: <i>Xanthobacter</i> and <i>Aminobacter</i> Symbiotic: <i>Rhizobium</i> , <i>Bradyrhizobium</i> , <i>Azorhizobium</i> , <i>Allorhizobium</i> , <i>Sinorhizobium</i> , and <i>Mesorhizobium</i> <i>Herbaspirillum</i> <i>Agrobacterium</i> <i>Novosphingobium</i> and <i>Sphingomonas</i>	Bashan and Holguin (1997) Sawada et al. (2003) Hayatt et al. (2010) Bazhanov et al. (2010), Monteiro et al. (2008) Willems (2006) Islam et al. (2010)
Denitrification	<i>Ochrobactrum</i> <i>Lutiella</i> <i>Thiobacillus</i> <i>Oligotropha</i> <i>Hyphomicrobium</i> <i>Achromobacter</i>	Lee and Park (2009) Weber et al. (2006) Torrentó et al. (2010) Puig et al. (2011) Yamaguchi et al. (2003) Ryuda et al. (2011)
Ammonia oxidizing	<i>Nitrosospira</i>	Norton et al. (2008)
Nitrite oxidizing	<i>Nitrobacter</i> and <i>Nitrospira</i>	Xia et al. (2011)
Ammonification/deamination	<i>Acidaminococcus</i>	Eschenlauer et al. (2002)
Putrefaction	<i>Pusillimonas</i>	Lee et al. (2010)

Chapter 7: Conclusion

7.0 Discussion and Conclusions

The overall objective of this research project was to provide a body of work on the geoenvironmental implications of mass mortality carcass burial by utilizing past knowledge, incorporating conservative and reactive transport models, extending previous bodies of work and validation through investigation of existing burial sites both from a chemical and biological standpoint. To accomplish this, preliminary investigative work under the M.Sc. program of Pratt (2009) had provided the first assessment of the aqueous geochemical properties of livestock mortality burial leachate over two years of decomposition processes. At the culmination of that project, it was soon realized that the whole story wasn't being portrayed from a chemical thermodynamic standpoint, proving more questions to our modelling scenarios than answers. Given the specifics of the concentration of constituents in burial leachate and the strength of ions in that leachate, more work was needed. Specifically, from a geochemical standpoint, an evaluation of the current thermodynamic geochemical mathematical models was merited, as well as site investigations to corroborate our models, and also an assessment of microbiological aspects of carcass burial and the leachate's effect on in-situ microbes and the potential transport of bacteria from the carcasses themselves.

Utilizing the information obtained on carcass leachate constituents from Pratt (2009), Chapter 2 described this further and explored the thermodynamic questions associated with this specific strength of leachate on the evaluation of ion activity coefficients using five common approaches. This helped to determine the most applicable mathematical model to utilize for geochemical modelling and speciation of ions. Pratt (2009) found that, on average, chemical concentrations of constituents in mortality burial leachate contains 12,600 mg/L of ammonium, 46,000 mg/L bicarbonate, 2,600 mg/L chloride, 3,600 mg/L sulfate, 2,300 mg/L potassium, 1,800 mg/L sodium, 1,500 mg/L phosphorus, along with lesser amounts of iron, calcium and magnesium. While the ratio of constituents in mortality leachate are similar to sewage waste (Gloyna 1971; Polprasert 1989) or livestock manure storages (Fonstad 2004; Marino et al. 2008), relative concentrations in this case, are much higher at 2.5 to 5 times higher for major ions such as ammonium, sodium,

bicarbonate and sulfate and 6 to 12 times higher for phosphorus, iron, and copper on a meq/L basis (Figure 2.3) . Concentrations in the range of mortality leachates have ionic strengths of between 1.3 and 1.5, which generally lie between thermodynamic models that are commonly utilized for geochemical calculations of ion activity and contaminant transport modelling.

Utilizing available geochemical calculation tools, the ionic strength of the leachate at its maximal values was found to be approximately 1.5 M. A stepwise dilution was applied to maximal leachate chemistry values to provide a basis for the calculation of ion activity coefficients using four techniques (Debye-Hückel, Truesdell-Jones, Davies, and Extended Debye-Hückel), a fifth technique (Pitzer) was not included due to the lack of reaction information for NH₄⁺ (the leachate's main constituent of concern). Results from these models indicate the Truesdell-Jones technique is appropriately valid for this leachate type and could be used in thermodynamic geochemical calculations here-forward (Figure 2.4). The ionic strength stepwise dilution showed sensitivity in two areas of the ion activity coefficient curve, with the Extended Debye-Hückel appearing to show slight underestimation of activity for both monovalent and divalent cations by up to 10%, which was rectified with the inclusion of the "b" parameter of the Truesdell-Jones calculation method. Finding realistic thermodynamic activity calculators is important for further geochemical work involving this leachate, if ion activity coefficients are improperly utilized, under or over estimations in complexation, sorption, solubility and ion exchange can lead to poor predictions of geochemical processes and transport. This could have potentially significant implications in areas where geological properties are marginal for burial, but necessary to eliminate a disease threat.

The thermodynamic ion activity calculation results were then incorporated into PHREEQC, which was employed for speciation of the leachate. Speciation results (Figure 2.4) indicated phosphate compounds precipitating from solution, as well as phosphoric acids becoming available for unattenuated transport. Due to the relatively high concentration of ammonium and sulfates, significant concentrations of ammonium sulfate form, and due to the negative charge, will allow approximately 300 mg-N/L of unattenuated transport of nitrogen in that complexed form. In the event that the ammonium sulfate dissociates, ammonium will then be available for sorption or conversion to nitrate. It is imperative that the burial pits stay anaerobic, as the majority of nitrogen in the system would be forced to remain as ammonium unless in the presence of annamox bacteria

(Van de Graaf et al. 1995), which would be unlikely in deep soils. However, if the system were to become aerobic with appropriate pH conditions (>9.3), ammonium would then be present as ammonia and the nitrification/denitrification processes would advance and convert to nitrate and nitrite. Microbially mediated nitrification may also occur in the event pH remained below 9.3 to convert ammonium to nitrate. This conversion could make nitrate concentrations excessively high, and if appropriate microbial communities and oxygen are present, the speed at which this conversion happens would overwhelm the groundwater system with concentrations well above drinking water standards (Joo et al. 2006). Once the conversion to nitrate/nitrite happens, these ions can then easily move through the soil/groundwater systems unattenuated (Tindall et al. 1995; Nolan et al. 1997; Molénat and Gascuel-Odoux 2002).

PHREEQC was also incorporated to provide a comparison of leachate solution ion activity to a typical groundwater it may come into contact with. The groundwater chosen was from a surficial sand aquifer and had a relatively low ionic strength (Fonstad 2004) when compared to the mortality leachate chemistry. Activities of ammonium and bicarbonate, sulfates, phosphates and other minerals are many orders of magnitude higher than those in the groundwater (Figure 2.6). Concentration of ions in groundwater may be indicative of the soil/bedrock system in which the water originates, giving us information on the ions that may or may not be present on soil exchange sites (Domenico and Schwartz 1990, reprinted 1998). Typically, cations on soil particles will exchange with those found in the mortality leachate. Lyotropic series effects based on ionic strength and the quantities of each ion present, may be dependent on soil type and in-situ ion concentrations (McBride 1979; McBride 1994). In general, lyotropic series of ion exchange tends to preferentially absorb highly charged ions first, but can be altered depending on solution concentration. In mortality leachate, the highest concentration is ammonium, a monovalent cation, that typically, would be the last to absorb ahead of sodium. Since concentrations of monovalent ions in this specific solution are greater than 99% of the total cations in solution and ammonium represents over 80% of the total cations present in solution, it is predicted that ammonium will be preferentially exchanged first due to concentration effects, similar to findings from McBride (1994). If ammonium is preferentially absorbed before potassium, calcium and magnesium, the ions originally on the exchange sites, typically mainly calcium and magnesium in Saskatchewan tills, would be released into solution at the forefront of the plume. As a result, those ions,

specifically calcium and magnesium, would be a good indication of plume migrations if their concentrations are higher than those in naturally occurring groundwater in the area. As the leachate moves through the system and becomes diluted, the lyotropic series may then reverse and those ions first exchanged would be reabsorbed if soil exchange or absorption sites are available.

Chapter 2 provided a closer look on the speciation and chemical properties of mortality leachate. From a modelling standpoint, standard thermodynamics using the Truesdell-Jones equation for the calculation of ion activities was proven sufficient. However, this isn't the only modelling conundrum that is associated with a leachate of this type. Since the ionic strength of the leachate is remarkably high, it was unknown what effects the high concentrations of ammonium would have on ion sorption processes for both ion selectivity and ion distribution coefficients in soil. Past literature on ammonium sorption in soils is sparse (Valocchi et al. 1981; Balci and Dinçel 2002; Mikolajkow 2003; Fonstad 2004), and none existed at concentrations seen in mortality leachate. To evaluate these effects, a standard ASTM batch test assessment was performed to determine the impact a synthetically created mortality leachate might have on ammonium sorption properties in soil for incorporation into future modelling efforts seen in Chapter 4. Soils used for this study came from a background core location at the Pierceland burial site described in Chapter 5. This batch test produced Langmuir sorption isotherms for concentrations of ammonium up to approximately 10000 mg/L (Figure 3.1), which resulted in distribution coefficients of 0.003 and 1.56 L/kg for the high end (10000 mg/L) and low end (100 mg/L) respectively. Data obtained from the Pierceland site closely matched data from the Kelvington soils (similar in type to the Pierceland soil) seen in Fonstad (2004) and when those soils were extrapolated, sorption and retardation curves closely match between the two soils (Figure 3.2). These results demonstrate that concentrations above approximately 2000 mg/L ammonium, suggest a retardation factor of 1 (little to no retardation), while concentrations decreasing below 2000 mg/L show increasing retardation (i.e. the lower the concentrations, the higher the retardation). This point is reiterated later in Chapter 4 when looking at retardation of ammonium in a reactive transport model. Drawing conclusions for some of the other ions in the synthetic mortality leachate proved difficult due to laboratory analysis issues. Due to the high amounts of alkalinity in the leachate, data for calcium and magnesium are unreliable due to titration standard methods. It may be beneficial in future

analysis utilizing laboratory techniques similar to these that calcium and magnesium be treated as a combined datapoint “CalMag.”

Ion selectivity coefficients were also evaluated in Chapter 3 to test the affinity of each ion for ion exchange in soils. This common phenomenon is controlled by factors such as ion charge, ion size, ion hydrated radius and total solution concentrations. Generally, ion selectivity coefficients have been published in numerous studies summarized by (Bruggenwert and Kamphorst 1979) and may be useful in certain scenarios, but for this specific scenario, due to the high concentrations of a monovalent ion in particular, published results may deem insufficient. Fonstad (2004) and others (Vanselow 1932; Gaines and Thomas 1953) suggested that ion selectivity for multi component solutions are not actually reaction constants, but are variables effected by solution concentrations, ratio of 1+/2+ ions and other factors. In the case of this study, the simple batch test has reiterated that statement, as ion selectivity is not constant and changes with both solution concentration and the ratio of monovalent to divalent cations in solution. Due to changing selectivity and distribution coefficients, this will in turn have an effect on ion retardation and contaminant transport processes provided in Chapter 4.

Using the tools and new information obtained from the first portion of this study (Chapters 2 and 3), and typical burial guidelines for Saskatchewan, it was imperative to produce informative models to provide predictive information on transport properties for several scenarios (Chapter 4). Common guidelines in Saskatchewan and much of Canada, where it is considered safe to bury livestock mortalities, suggest utilizing trenches approximately 2 m wide and 4 m deep. Using this information, coupled with minimum hydraulic conductivity guidelines for burial sites, models were produced that evaluated contaminant transport 3 ways: (1) conservatively, (2) including attenuation only, and (3) geochemical ion exchange. Conservative tracer models are a useful tool to predict the speed at which a plume might travel and where it might end up. They don't, however, account for any retardation, due to such processes as ion exchange, that would typically occur along a flow path for this type of leachate. For this specific instance, they provide a useful tool to evaluate the transport of anions. Results from the conservative model at three different hydraulic conductivities indicated a significant change in plume development and transport distance, expected with a three order of magnitude change in hydraulic conductivity (Figure 4.2). Others

have modeled clay barrier systems with similar soil properties and hydraulic conductivities as the lowest investigated here (1×10^{-10} m/s) and demonstrated minimal ion movement, similar to findings from this study, of transport of less than 0.5 meters in 10 years (or 1.5 meters in 100 years) (Lake and Rowe 2005). Plume development for the highest hydraulic conductivity (1×10^{-8} m/s) was rapid and spread to distances of 15 meters below the trench bottom in only 10 years. For sites that meet hydraulic conductivity requirements for burial (1×10^{-9} m/s), plume spread was approximately 6 meters in 10 years.

Non-conservative models used in this study via GeoStudio's CTRAN, approach subsurface transport by only accounting for the retardation of one specific ion, in this instance the ammonium ion was chosen for incorporation into the models including retardation. Langmuir sorption curves were generated in the lab (Chapter 3) for ammonium concentrations at ionic strengths greater than 0.5, with values less than 0.5 being taken from the literature based on a similar soil type in Saskatchewan (Fonstad 2004). As a comparison, the models including ammonium attenuation (Figure 4.4) demonstrated a significant decrease in ammonium concentrations as compared to the conservative model at similar depths. Less than one percent of ammonium remained in solution at depths less than 10 meters beneath the bottom of the burial trench after 100 years of transport for a system that met minimum hydraulic conductivity requirements (1×10^{-9} m/s). Problems associated with modelling in this manner include the lack of ion exchange and geochemical thermodynamic processes. When concentrations of a contaminant are high (similar to the leachate described here), it is predicted that as ion exchange and absorption occur, ions initially present on the soil exchange sites, will then end up in solution, producing a leading edge on the contaminant plume. Models only including retardation do an excellent job at predicting the attenuation of ions on the soil and concurrent plume migration based on that single ion, but lack pertinent information on the full geochemical process.

Complete geochemical models should incorporate both the contaminant transport functions as well as a full suite of geochemical thermodynamic calculations at each point in the model. Modelling scenarios for this study initially presented in Pratt (2009) also utilized PHREEQC (USGS) to produce a 1-D contaminant transport model with a similar soil parameterization as the conservative model but with the inclusion of ion exchange properties and full strength leachate inputs followed

by a series of additions of groundwater. Results from this model are indicative of a change in retardation or variation in ion selectivity with soil exchange sites over the course of the model (Figure 4.5). For example, over the course of the model, retardation factors for ammonium increased from 1.4 to nearly 2 demonstrating the isotherm process seen in Chapter 3 where ammonium at high concentrations acts more conservatively than at low concentrations. Complexation reactions and speciation of ions also changed throughout the transport process, with the majority of sulfate in solution increasingly complexing with magnesium and calcium ions (most likely coming from those desorbed from soil exchange sites) and phosphorus in the form of phosphate complexing with calcium, magnesium and sodium. Ammonium sulfate was found and transported in amounts of 1-3 percent of initial ammonium concentrations and tended to slightly increase as more ammonium was sorbed to soil particles (Figure 4.6). This model is a useful tool to predict ion adsorption and desorption properties for site-specific scenarios. The inclusion of ion exchange theory and additional soil properties provide a better assessment of the contaminant transport potential of specific leachates.

Modelling scenarios also investigated the addition of multiple side-by-side trenches for instances where mortality numbers would be considered extreme, requiring burial of thousands of carcasses. This scenario would be common in areas of intensive livestock operations during disease outbreaks or natural disasters. In the event of an outbreak, immediate culling and disposal would most likely be necessary. Some US states and Canadian provinces require new intensive livestock operations to prepare mortality management plans prior to facility start-up that include site investigations and plans for a potential mortality event (Manitoba Conservation 1998; Saskatchewan Agriculture 2005; CFIA 2006; Minnesota Department of Agriculture 2008; Province of Alberta 2014; USDA-APHIS 2015). Sites deemed geologically secure (have enough clay content, low hydraulic conductivity, unfractured with appropriate geology, and meet water table and surface water distances) for ILO's would likely require a multiple trench burial scenario. To assess subsurface impacts of this, two trench spacings (5 m and 10 m) were modeled to predict potential plume migration and influences. At a trench spacing of 5 meters, impacts from the plume between trenches were shown to merge after 50 years of transport (Figure 4.3). At a trench spacing of 10 meters, plumes did not come into contact with each other at 100 years of model duration. For planning purposes, in sites that are capable of trench burial, trenches should be spaced at a

minimum of 10 meters apart. Spacing them in this manner gives the leachate enough soil to come into contact with for ion exchange and adsorption of ammonium, therefore further minimizing subsurface risk. In unique situations, transport due to higher horizontal hydraulic permeability than vertical, may serve to allow plumes from adjacent trenches to combine within shorter timelines and therefore greater distances between trenches should be considered.

Model validation is an important aspect of conceptualization of processes and providing scientific evidence that our ability to predict subsurface impacts via numerical modelling are on target. To accomplish this, two site investigations of existing carcass burial sites was performed and were described in Chapter 5. This study provided the first reported field investigation that we could find of soil coring of sites representative of good burial locations both through and adjacent to the burial pits or trenches, which propels us towards a better understanding of carcass burial impacts to the environment in the short-term (<10 years) as well as longer-term (>50 years). Two sites were selected in Saskatchewan that followed general burial procedures from their respective eras, one located near Pierceland, SK (commissioned in 2001), the other near McLean, SK (commissioned in 1952). The Pierceland site consisted of multiple 4 m deep x 2 m wide trenches, some spaced approximately 10 m apart, some closer than 10 m. Soil properties at that site would be typical for most burial locations that regulatory agencies would choose for geologically secure disposal. The McLean site utilized a single pit design versus the typical trench style used today. Both sites were continuously cored through two cross-sections and the extent of leachate transport was determined via pore water extraction and ion analysis.

Core from the Pierceland site were taken in 2008 and the extent of leachate transport upon analysis of the soil cores was 1-1.5 meters of vertical transport of anions (Cl, Alkalinity) as well as some cations arising from cation exchange reactions (Ca and Mg) (Figure 5.2). There was no lateral movement of ions at this site. The second site, located near McLean, SK was used in 1952 to bury carcasses affected by Canada's only foot and mouth outbreak. This site was cored in 2010, nearly 60 years post burial. Leachate movement vertically at this site was very slow over that period of time, showing movement of 1-2 meters (Figure 5.3). Due to the presence of sand lenses in and around the burial pit, movement of up to 10 meters horizontally of anions such as Cl and bicarbonate was shown. Ammonium ions were attenuated within the confines and immediately

adjacent soil surrounding the burial pit. Due to lack of information at the time of commissioning and using the information we know now, the McLean site was not placed on a preferred geologically secure area due to the sand lenses and higher water tables encountered in and around the site. The saving grace at this site was the very stiff glacial till immediately below the burial pit, minimizing vertical movement of ions, however, lateral movement of ions was apparent.

Comparing these results to those that were modeled earlier in this study, plume development at the Pierceland site demonstrated that site hydraulic conductivities were similar or even better than (less vertical movement) modelling scenarios utilizing the burial site requirement of 1×10^{-9} m/s. Plume development demonstrated the hard water (calcium and magnesium) plume ahead of the ammonium plume at both locations, indicating that this may be a good indicator for plume migration before nitrogen becomes apparent (Fonstad 2004; Chang and Donahue 2007). While the sites differed slightly in subsurface geology, both provided adequate protection of groundwater resources around them and had enough clay content to adsorb a majority of the ammonium present in the burial trench/pit and demonstrate a good example of a best-case scenario for mortality burial locations.

Results from these site investigations have helped to understand the impacts that have occurred with respect to leachate transport beneath burial sites in Saskatchewan. We know from past work with ion exchange, that those ions may not be held to soil exchange sites permanently, but their release to the environment will be slow, therefore their potential impact to groundwater will be minimized (Lumbanraja and Evangelou 1994; Kithome et al. 1998; Rinas 2011). The hard water plumes created by the ion exchange reactions from ammonium at each of these sites are good initial indicators of plume development and will be the first to reach groundwater systems in the long-term and are of less concern regarding contamination than the attenuated ions from a chemical standpoint. For other agricultural wastes such as manure storages, others have shown attenuation of ammonium beneath storage lagoons within approximately one meter of soil at the lagoon base (DeSutter et al. 2005). While the concentrations of ammonium at these sites are significantly less than those found in mortality leachate, the constant volume available for seepage is greater (Ham and DeSutter 1999; DeSutter et al. 2005). Some other lagoon assessments in Iowa provide indication that over 50% leak at a rate greater than 1.6 mm/day; plumes under this scenario could

become large rather quickly (Simpkins et al. 2002). In sites less geologically secure, an indication of a hard water plume in monitoring wells could provide an accelerated signal for clean-up plans or remediation actions that may need to be taken.

The last portion of this study, and the most unique, involved the investigation of microbial profiles, diversity and populations beneath the Pierceland burial site (Chapter 6). Much concern has arisen on the ability for disease transport and flourishing of microbial communities in and around livestock burial sites. Due to the high ionic strength of mortality leachate, it was predicted that the amount of specific ions present will have an effect on indigenous soil microbiology. The composition and dynamics of a microbial community in environmental samples such as soil, sludge, or water can be extremely diverse. Many microorganisms are biogeochemically and environmentally significant with much of our knowledge about these organisms coming from their isolation in pure cultures. It is estimated however that only approximately 0.01 to 0.1% of all bacteria in soil have been isolated in pure culture (Chapelle 1993). In order to better predict what is happening in the environment, there is a need to obtain quantitative information about microbial numbers, growth and activity in the environment. The ability to measure the diversity and population of these samples until recently has been extremely difficult due to the reliance of culture based techniques. There are many methods that exist to gain a greater understanding of the biogeochemical role of different microorganism and typically several methods must be combined to determine their properties; the two most important being culture-based methods and metagenomic analysis. Due to the fact that most soil samples to date are un-culturable (Zengler et al. 2002; Zengler 2008), it was imperative to employ metagenomics techniques to answer the microbiological questions arising from mortality disposal.

Utilizing common metagenomics tools, this study was the first to incorporate the *cpn60* taxonomic profiling method on soil samples. Previous to this study, the *cpn60* methodology had only been applied to samples taken from human or animal tissues (Hill et al. 2005; Dumonceaux et al. 2006; Desai et al. 2009; Schellenberg et al. 2009). Others have routinely used 16s rRNA methods on environmental samples to delineate microbial populations (Chachkiani et al. 2004; Desai and Madamwar 2007; Schloss and Westcott 2011; Kaown et al. 2014; Yang et al. 2015). The *cpn60* gene is universal in eukaryotes and eubacteria and has an extensive reference database available

for classification (Hill et al. 2004). The length of the *cpn60* universal target (549-567 bp), coupled with the high resolution it provides, makes it an ideal molecular target for the application of next-generation ultra-high throughput sequencing methodologies (Schellenberg et al. 2009). Advantages of using metagenomics include the ability to assess previously uncultured organisms. One problem with this technique is its inability to distinguish viable cells from dead cells. This distinction is under development and could be important in making conclusions about the microbial community and its metabolic capabilities (Council 2007). Metagenomics is also expensive and data analysis is difficult, but becoming easier as the technology progresses. Typically, in environmental samples, the abundance of species varies; using metagenomics on these samples may not capture those species that are the least abundant. Applying metagenomics to environmental samples is increasing in popularity, and large amounts of information can be obtained using this technique, but it is also important to look at the bigger picture. Metagenomics alone cannot directly determine what is going on in an environmental sample; physical and chemical aspects of the sample should also be assessed to draw conclusions from. Sequencing alone is limited by databases and defined functional groups for each sequence in the database. By analyzing more than just genomics, this will enable us to draw clearer conclusions to help better characterize the sample and its processes.

For this study, the *cpn60* metagenomics analysis method was amended and applied to soil samples to produce a complete profile of microorganisms through the burial pit and below soil profile as well as a 16S rRNA quantitative population assessment via qPCR. These methods were able to quantify and qualify the types and species of bacterial organisms present at depth in cores located both through the burial trench and background soils at the same site. Results demonstrated that the organic load caused by the deposition and decomposition of the livestock carcass influenced both the bacterial load and the composition of the microbial communities in these areas, but also that different burial sites can have distinct taxonomic profiles associated with the sites. Quantitatively speaking, the burial trench evaluated had significantly more (2 to 5 orders of magnitude) bacterial 16S rRNA-encoding genes per gram of soil within and up to 2 m below the burial site compared to a nonburial control core sample at the same depths (Figure 6.2). Composition of the bacterial community also changed with depth and was significantly different than communities associated with the control core at all depths investigated. Microbial populations were compared side-by-side

to soil pore water characteristics to provide more breadth for a thorough explanation of community characteristics (Table 6.3). For example, at depths associated with carcass placement (3.75 m), anaerobic microorganisms such as *Bacteroides*, *Clostridiales* and *Acidaminococcus* sp. dominated the microbial community, generally organisms associated with ammonification (Eschenlauer et al. 2002). This would be expected due to the anaerobic aspects of the burial trench and the high concentrations of ammonium found at the base of the burial trench. Other organisms associated with phosphate accumulation, nitrogen fixation, and ammonium oxidation were found in highest abundance near the ground surface of the burial trench to depths up to 2.5 meters. Dissimilatory metal reducing organisms were found just below the burial trench bottom (4.5 to 5.5 m), indicating a reducing environment produced from the organic load above it. At similar depths, sulfate concentrations in the pore water was high, which was strongly correlated to the presence of sulfate reducing organisms such as *Desulfuromonales* and dominated by *Geobacter* spp., which is assumed to have taken over the sulfate reduction role in this system.

The ability to fingerprint microorganisms in contaminated and non-contaminated sites will aid our ability to further describe burial impacts to the environment. This study's approach provides distinct sequences that can be used to develop molecular assays for tracking transport and population dynamics of organisms associated with burial events, and can provide an overall fingerprint of the microbes associated within a given site. The study demonstrated that carcass burial had an influence on the types of microbes present in soil as well as the flourishing of native species dependent on pore water chemistry composition (Figure 6.4). Microbial changes in burial sites may then implicate aspects of contaminant transport over time by their ability to alter the speciation and composition of pore water chemistry.

Since publication of Pratt et al. (2012), others have investigated microbiological aspects of carcass burial sites, specifically in South Korea after the 2010 FMD disaster (Hak et al. 2012; Joung et al. 2013; Kaown et al. 2014; Yang et al. 2015; Choi et al. 2017; Kim et al. 2017). Many of these studies employed the more common 16S rRNA sequencing or provided only quantitative PCR analysis of samples. Few studies exist that provide evidence for microbial community composition (although rather sparse in their datasets) of livestock carcasses undergoing decomposition (Howard et al. 2010; Yang et al. 2015). Yang et al. (2015), assessed bacterial communities in leachates of

decomposing pig carcasses of varying degrees (1 week to 14 weeks) via 16S rRNA sequencing and found that bacterial communities changed greatly over the decomposition period and may be based on pH changes over the process and suggest that these changes may induce shifts in soil microbiota (similar to what was found in this study). Of the studies that do exist on microbial populations and livestock burial, Kaown et al. (2014) would be the most similar and comparable to this study. They assessed aquifer microbial populations using 16s rRNA pyrosequencing to determine whether there was a microbial shift in aquifers effected by leachate from carcass disposal sites in Korea. Findings are similar to this study in that microbes found in affected areas were of similar type to the microbes found beneath the Pierceland burial trench (Bacteroides, Lactobacillae, Clostridiales, etc.) and they concluded that due to microbiological and chemical effects seen in specific burial sites, more care should be taken prior to disposal for appropriate site selection.

This final study provided the first utilization of the *cpn60* method to environmental soil samples, and far exceeded expectations in its ability to provide adequate DNA sequences for both qPCR and metagenomics analysis via pyrosequencing. It is still one of the most thorough investigations of microbiological populations in and around a livestock burial site. Through studies such as this, we can utilize and improve upon modern molecular methods, and enhance our ability to measure responses of the environment to potential pollutants. Through this work, we can then in turn provide science-based evidence for burial site regulation and policy creation to protect water resources and the environment.

7.1 Study Limitations & Recommendations for Future Work

The findings of this study have provided an evaluation of livestock mortality burial leachate, its potential transport through modelling, model validation via site investigations, and the first fingerprint of microbiological characteristics, communities and quantities at a burial site. Due to the nature of this study and the significant costs involved with doing research like this, many limitations exist and should be discussed. In a perfect research scenario, sample replication is critical for statistical analyses to draw solid conclusions based on datasets and averages created from the replication efforts. The first chapter of this thesis describes a procedure in which only one sample was taken from each burial pit and analyzed at each time stamp. To accommodate any

discrepancies that might have arisen due to lack of replication, it was imperative that all samples were given a full analysis to include all major and minor cations, anions and metals. This is important for a variety of reasons. The chemistry of the burial leachate was unknown, so a full sweep of analyses was critical for a more complete understanding of this solution. Due to the lack of replication, datasets were validated by utilizing common geochemical tools such as ionic balance calculations with requirements that ion balance would only be acceptable at percentages of less than 10% error (typical dilute samples would have an error acceptability of much less), and any error encountered less than the 10% threshold was generally rectified by an adjustment of alkalinity. Alkalinity tends to change rapidly due to oxygenation effects and sampling and analysis induced effects and would be the most likely cause of small discrepancies between sampling date and analysis date. Additionally, due to the magnitude of the alkalinity of these solution, samples were diluted significantly and, due to the nature of the samples, there was significant colour/turbidity that may have added some error to alkalinity titrations. . Similar problems were encountered during the site investigation efforts. It is quite expensive to continuously core multiple locations in the same zone to have enough core for multiple replication efforts at each depth of interest. During the soil squeezing process at each depth, pore water extraction provided quantities of water less than 50 mL, and was only enough volume for the chemical analyses presented in Chapter 5. In an ideal scenario, multiple cores from the same location/depth would be necessary to provide enough expelled pore water for a complete statistical analysis of variance in that data set. Ion charge balance was also utilized on the data generated from the site investigation utilizing the same acceptability error as the leachate study. From a microbiology standpoint, many statistical analyses were performed utilizing common tools available for that particular dataset. Replications were performed on the quantitative assessment of microbial DNA found in the burial core and background core. Replication was not performed on the pyrosequencing analysis, however, many pre-analyses procedures provided strong confidence in our DNA recovery, purification and amplification processes, all tested multiple times and in a variety of ways prior to 454 pyrosequencing submission.

Due to increasing livestock production, the risks associated with carcass management will also be growing along-side this. Future work with regards to carcass disposal is paramount, not only for burial, but other disposal routes. For burial specifically, pathogen fate is a common public concern,

and this work and the work of others has demonstrated that leachate does indeed transport as well as microbes. If microbes are able to transport, concerns of disease transmissibility due to geologically questionable burial sites should be addressed, specifically for pathogens of interest with high environmental survivability. Not only are pathogens of concern, but also livestock pharmaceuticals. It is known that pharmaceuticals from human consumption place risks on water bodies when discharged, similarly, livestock pharmaceuticals may present a risk as well, and their transport properties through the subsurface at a minimum should be evaluated. As the world becomes more developed and both human and livestock populations increase, further assessment of disposal impacts due to population density and nearby geology, as well as future climate regimes, should be evaluated.

In the past decade, with the onset of this work, the information obtained from Pratt (2009) and this study, government policies have been updated and scientific based evidence of burial impacts have been utilized to layout the framework for disposal plans across much of North America (Cleary et al. 2010; USDA-APHIS 2015) and Korea (MOE 2010). While this study has demonstrated the effects burial would have on sites deemed acceptable under current regulation, more work should be done in areas of questionable geology. Some large scale animal feeding operations exist in areas where burial would be a marginal choice for disposal. Alternative options such as incineration, composting or rendering, in the event they become overwhelmed, would need a burial back-up plan in place. More research would be beneficial for these areas in the design and construction of an engineered burial site, based on trenches or pit systems that utilize local materials. This may be as simple as researching soil and geotechnical aspects of the area and formulating a plan to meet minimum hydraulic conductivity requirements and minimum CEC requirements by utilizing local soils and compaction, or implementing a geomembrane liner. Little work has been done of the effect of mortality leachate on geomembrane liners (Lake et al. 2008), these systems may be a critical component to the mitigation of carcass burial pollution on the environment in many areas. Specific issues seen in Korea, such as tearing and puncturing of liners, created widespread pollution and produced significant challenges in their mortality management plans under duress events.

In the end, this study has provided many more questions on whether or not sites may actually be

deemed suitable for carcass burial in unlined pits or trenches. The USDA EIS (USDA 2015) indicates that the top two disposal practices in the event of an animal health emergency will be unlined burial and air curtain burning in trenches. There has been little regard in that EIS as to the implications burial may have on the environment, especially in regions with intensive livestock operations. Due to this, it would be critical to perform geotechnical site investigations and significant efforts should be made in regional geochemical/hydrological modeling for carcass burial suitability to take place in regions such as the high plains (TX, OK, NE, CO) in the areas of the most intense concentrations of livestock. It's areas such as this that will suffer the most in the event of a disease outbreak such as FMD. Pre-preparation efforts should be focused, discussed and evaluated prior to any emergency actually occurring. Current regulations should also be updated to include these specifics as well as provide other suggestions and tools for burial efforts depending on scale and location, whether that is an assessment of soil properties to ensure high CEC, minimum clay content and low gradients or a stockpile requirement of burial liner materials or other design scenarios that would achieve the same result for environmental protection that would be able to be expediently implemented in the event of an animal health emergency.

7.2 References

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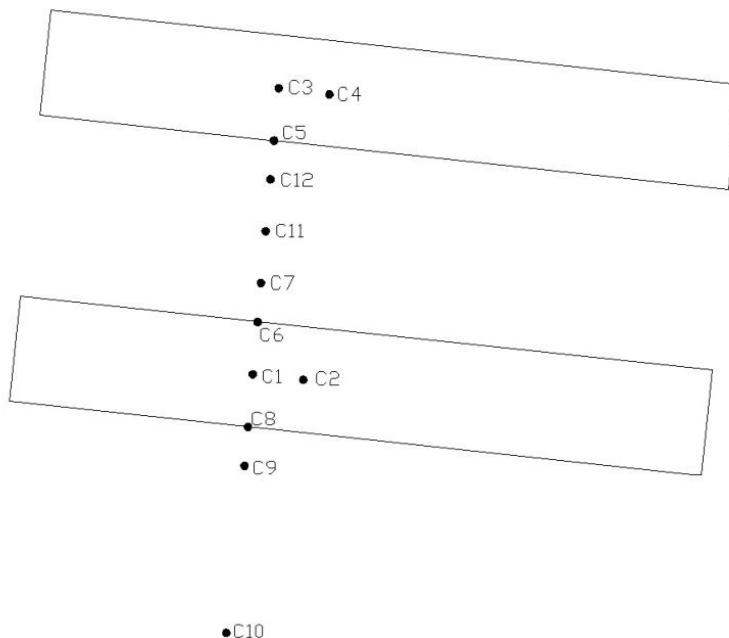
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APPENDIX

Pierceland CWD Site Drilling Logs and Miscellaneous Information



Core #	Easting	Northing	Accuracy (m)
C1	12U 0582801	6004356	4
C2	12U 0582807	6004357	4
C3	12U 0582803	6004367	4
C4	12U 0582802	6004367	4
C5	12U 0582801	6004366	4
C6	12U 0582801	6004356	4
C7	12U 0582802	6004360	4
C8	12U 0582802	6004353	4
C9	12U 0582802	6004353	4
C10	12U 0582803	6004347	4
C11	12U 0582801	6004358	4
C12	12U 0582801	6004362	4

Figure A.1 Pierceland CWD burial site GPS coordinates

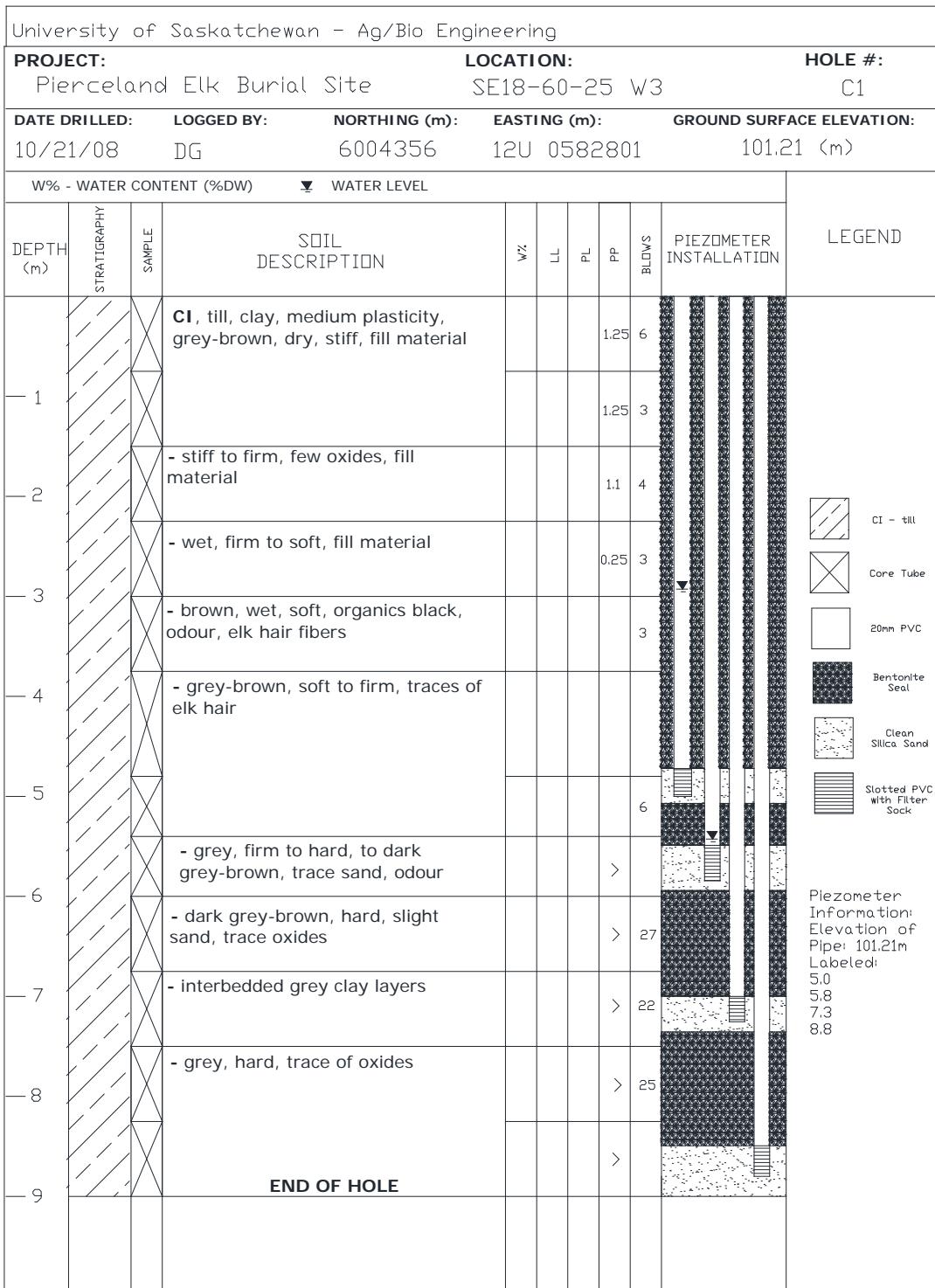


Figure A.2 Drill logs Pierceland C1

Figure A.3 Drill logs Pierceland C2

PROJECT:		LOCATION:				HOLE #:				
Pierceland Elk Burial Site		SE18-60-25 W3				C3				
DATE DRILLED:		LOGGED BY:	NORTHING (m):	EASTING (m):	GROUND SURFACE ELEVATION:					
10/22/08		DG	6004367	12U 0582803	101.17 (m)					
W% - WATER CONTENT (%DW) ▼ WATER LEVEL										
DEPTH (m)	STRATIGRAPHY	SAMPLE	SOIL DESCRIPTION	W%	LL	PL	PP	BLOWS	PIEZOMETER INSTALLATION	LEGEND
1			CI, till, clay, medium plasticity, grey-brown, dry, firm, fill material					0.7	6	
1			- soft					0.25	2	
2			- very soft					5		
3			- very soft, trace oxides					0	5	
3			- grey, stiff, slightly silty					1.25	5	
4			- grey-brown, very soft, elk hair, bone, strong odour, wet, organics, bottom of pit ~ 4.3m, water seeping					>	5	
5			- grey, stiff, slight odour					1.25	6	
6			- stiff to very stiff, trace oxides					3.25	27	
6			- dark grey-brown, hard, trace oxides					>	26	
7								>	38	
8								>	37	
9								>	40	
END OF HOLE							 SM - Silty Sand			
							 CI - till			
							 Core Tube			
							 20mm PVC			
							 Bentonite Seal			
							 Clean Silica Sand			
							 Slotted PVC with Filter Sock			
							 Piezometer Information: Elevation of Pipe: 101.17m Labeled: 5.8 7.3 8.8			

Figure A.4 Drill logs Pierceland C3

University of Saskatchewan - Ag/Bio Engineering															
PROJECT: Pierceland Elk Burial Site	LOCATION: SE18-60-25 W3				HOLE #: C4										
DATE DRILLED: 10/23/08	LOGGED BY: DG	NORTHING (m): 6004367	EASTING (m): 12U 0582802	GROUND SURFACE ELEVATION: 101.11 (m)											
W% - WATER CONTENT (%DW)  WATER LEVEL															
DEPTH (m)	STRATIGRAPHY	SAMPLE	SOIL DESCRIPTION			W%	LL	PL	PP	BLOWS	PIEZOMETER INSTALLATION	LEGEND			
1			CI , till, clay, medium plasticity, grey-brown, stiff to firm, fill material						0.7	5					
2			- firm						0.5	3					
3			- firm to very soft						0.2	8					
4			- very soft						0	5					
5			- dark grey, soft, organics, strong odour							3					
6			- grey-brown, soft to stiff, strong odour, wet, organics, oxides, traces of water							18					
7			- grey-brown, stiff, slight odour							15					
8			- grey, firm to stiff, slight odour						1.0						
9			- dark grey-brown, stiff to hard, few oxides												
			- grey, very stiff												
			- grey, very stiff to hard						>	40					
END OF HOLE															
												No Piezometer Installation			

University of Saskatchewan - Ag/Bio Engineering											
PROJECT:			LOCATION:			HOLE #:					
Pierceland Elk Burial Site			SE18-60-25 W3			C5					
DATE DRILLED:	LOGGED BY:	NORTHING (m):	EASTING (m):	GROUND SURFACE ELEVATION:							
10/23/08	DG	6004366	12U 0582801	100.91 (m)							
W% - WATER CONTENT (%DW) ▼ WATER LEVEL											
DEPTH (m)	STRATIGRAPHY	SAMPLE	SOIL DESCRIPTION	W%	LL	PL	PP	BLOWS			
			CI , till, clay, medium plasticity, grey-brown, very stiff					25			
1			- stiff				1.75	12			
2			- stiff to very stiff				2.25	14			
3			- stiff, few cobbles				1.5	15			
4											
5			- dark grey-brown to grey, stiff, few cobbles				1.25	20			
6			- grey, stiff to firm, cobbles				0.75	20			
7			- grey, stiff to hard, few oxides				>	35			
8			- grey, hard, water at 7.45m				>	34			
9			- grey, hard, trace water at 8.25m				>	33			
			- grey, hard, few cobbles				>				
END OF HOLE											
PIEZOMETER INSTALLATION											
LEGEND											
 SM - Silty Sand  CI - till  Core Tube  20mm PVC  Bentonite Seal  Clean Silica Sand  Slotted PVC with Filter Sock											
No Piezometer Installation											

Figure A.6 Drill logs Pierceland C5

University of Saskatchewan - Ag/Bio Engineering									
PROJECT:		LOCATION:				HOLE #:			
Pierceland Elk Burial Site		SE18-60-25 W3				C6			
DATE DRILLED:	LOGGED BY:	NORTHING (m):	EASTING (m):	GROUND SURFACE ELEVATION:					
10/24/08	DG	6004356	12U 0582801	101.25 (m)					
W% - WATER CONTENT (%DW)  WATER LEVEL									
DEPTH (m)	STRATIGRAPHY	SAMPLE	SOIL DESCRIPTION	W%	LL	P _L	P _P	BLWS	PIEZOMETER INSTALLATION
1			CI, till, clay, medium plasticity, grey-brown, dry, soft - soft to stiff			0.3	7		
2			- stiff			1.5	5		
3			- stiff to very stiff, trace oxides			1.25	8		
4			- very stiff, grey, slight odour, few cobbles			2.5	17		
5			- grey, very stiff to firm, trace oxides, very slight odour			1.5			
6			- firm to hard, few cobbles, trace oxides			2.25			
7			- grey, hard, few oxides			>			
8			- grey, hard to stiff, trace oxides			1.25	36		
9			- stiff to hard			>	37		
			- grey, hard, few oxides			>	1.75		
END OF HOLE									
Legend									
 SM - Silty Sand  CI - till  Core Tube  20mm PVC  Bentonite Seal  Clean Silica Sand  Slotted PVC with Filter Sock									
Piezometer Installation Top of pipe at 101.25m Labeled: 8.8 7.3 5.8									

Figure A.7 Drill logs Pierceland C6

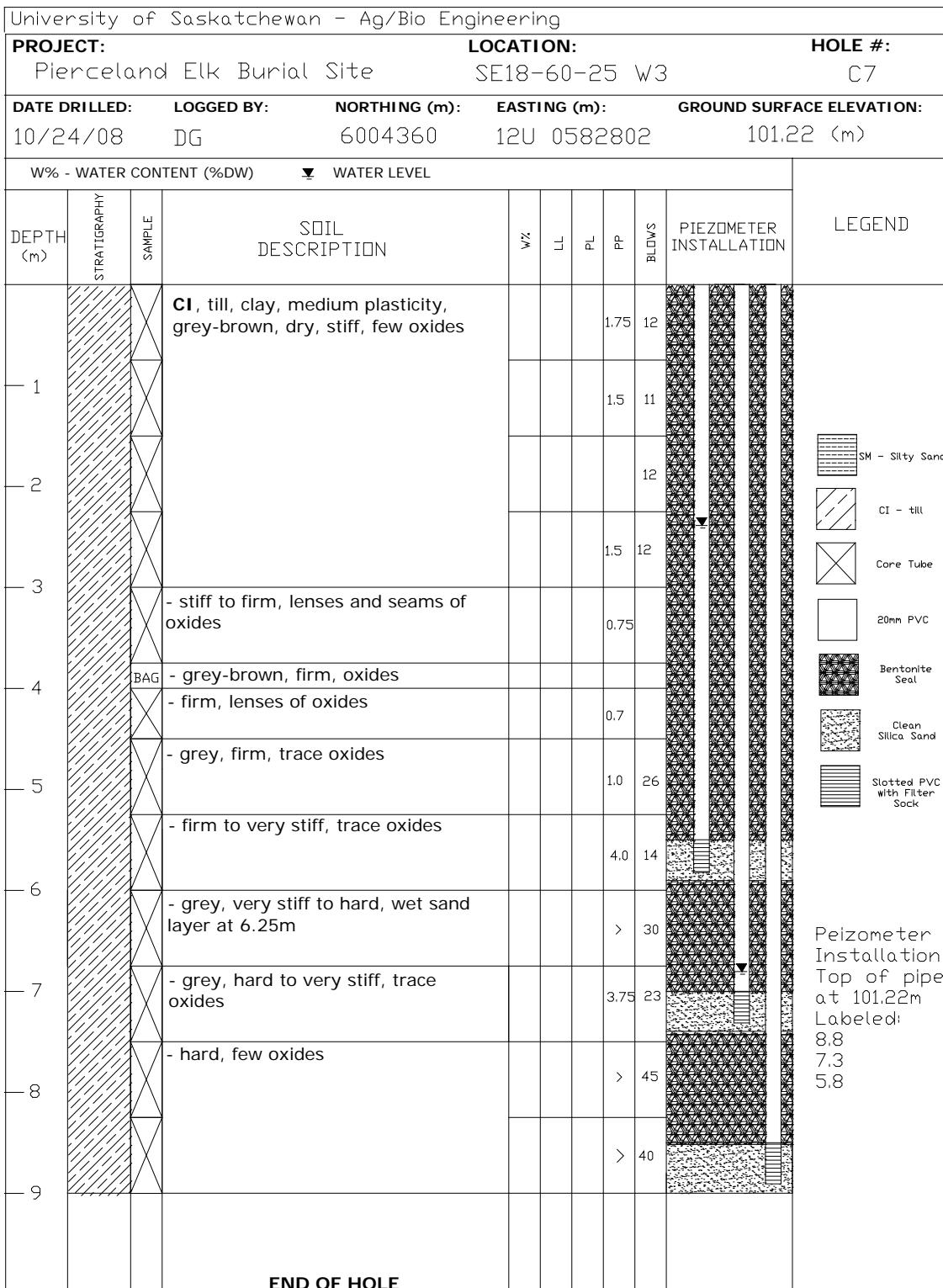


Figure A.8 Drill logs Pierceland C7

University of Saskatchewan - Ag/Bio Engineering								
PROJECT:			LOCATION:			HOLE #:		
Pierceland Elk Burial Site			SE18-60-25 W3			C8		
DATE DRILLED:	LOGGED BY:	NORTHING (m):	EASTING (m):	GROUND SURFACE ELEVATION:				
10/24/08	DG	6004353	12U 0582802	100.70 (m)				
W% - WATER CONTENT (%DW) ▼ WATER LEVEL								
DEPTH (m)	STRATIGRAPHY	SAMPLE	SOIL DESCRIPTION	W%	LL	PL	PP	BLDS
			CI , till, clay, medium plasticity, grey-brown, dry, very stiff, slightly sandy				2.2	10
1			- stiff , lenses of oxides				2.0	10
2			- stiff , oxide lenses				1.5	11
3			- firm, oxides				0.75	13
4			- firm , lenses of oxides				0.5	7
5		BAG	- firm to stiff, trace oxides				0.5	9
			- grey, stiff, oxides, grey layers				2.0	
			- grey, very stiff, oxides, trace water at 6.0m				2.25	
6			- dark grey-brown, hard, cobbles				>	
7							>	
8			- grey, very stiff				3.25	30
9			- grey, hard, pebbles				>	37
							>	47
END OF HOLE								
LEGEND								
No Piezometer Installation								
 SM - Silty Sand  CI - till  Core Tube  20mm PVC  Bentonite Seal  Clean Silica Sand  Slotted PVC with Filter Sock								

Figure A.9 Drill logs Pierceland C8

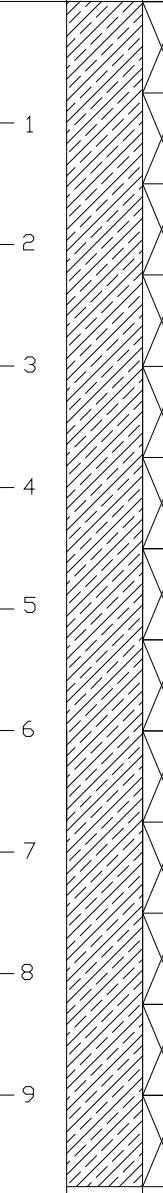
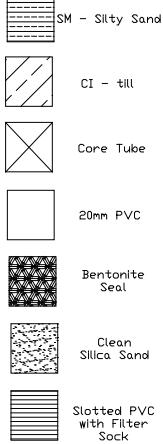
University of Saskatchewan - Ag/Bio Engineering										
PROJECT: Pierceland Elk Burial Site	LOCATION: SE18-60-25 W3				HOLE #:	C9				
DATE DRILLED: 10/25/08	LOGGED BY: DG	NORTHING (m): 6004353	EASTING (m): 12U 0582802	GROUND SURFACE ELEVATION: 100.70 (m)						
W% - WATER CONTENT (%DW) ▼ WATER LEVEL										
DEPTH (m)	STRATIGRAPHY	SAMPLE	SOIL DESCRIPTION	W%	LL	P _L	P _P	BLDS	PIEZOMETER INSTALLATION	LEGEND
1			CI , till, clay, medium plasticity, grey-brown, dry, stiff, - very stiff , lenses of oxides					2.0	10	
2			- very stiff , cobbles, rock at 2.05m					2.2	14	
3			- stiff to firm, oxides					2.0		
4			- firm , lenses of oxides					0.75	13	
5			- firm to stiff, trace oxides					0.6	16	
6			- grey, stiff to very stiff					1.0	12	
7			- dark grey-brown, very stiff to hard, oxides					1.0	13	
8			- grey, hard, wet sand seam at 7.5m					3.75	13	
9			- hard, oxides, slightly sandy					>	30	
										No Piezometer Installation
END OF HOLE										
										
										

Figure A.10 Drill logs Pierceland C9

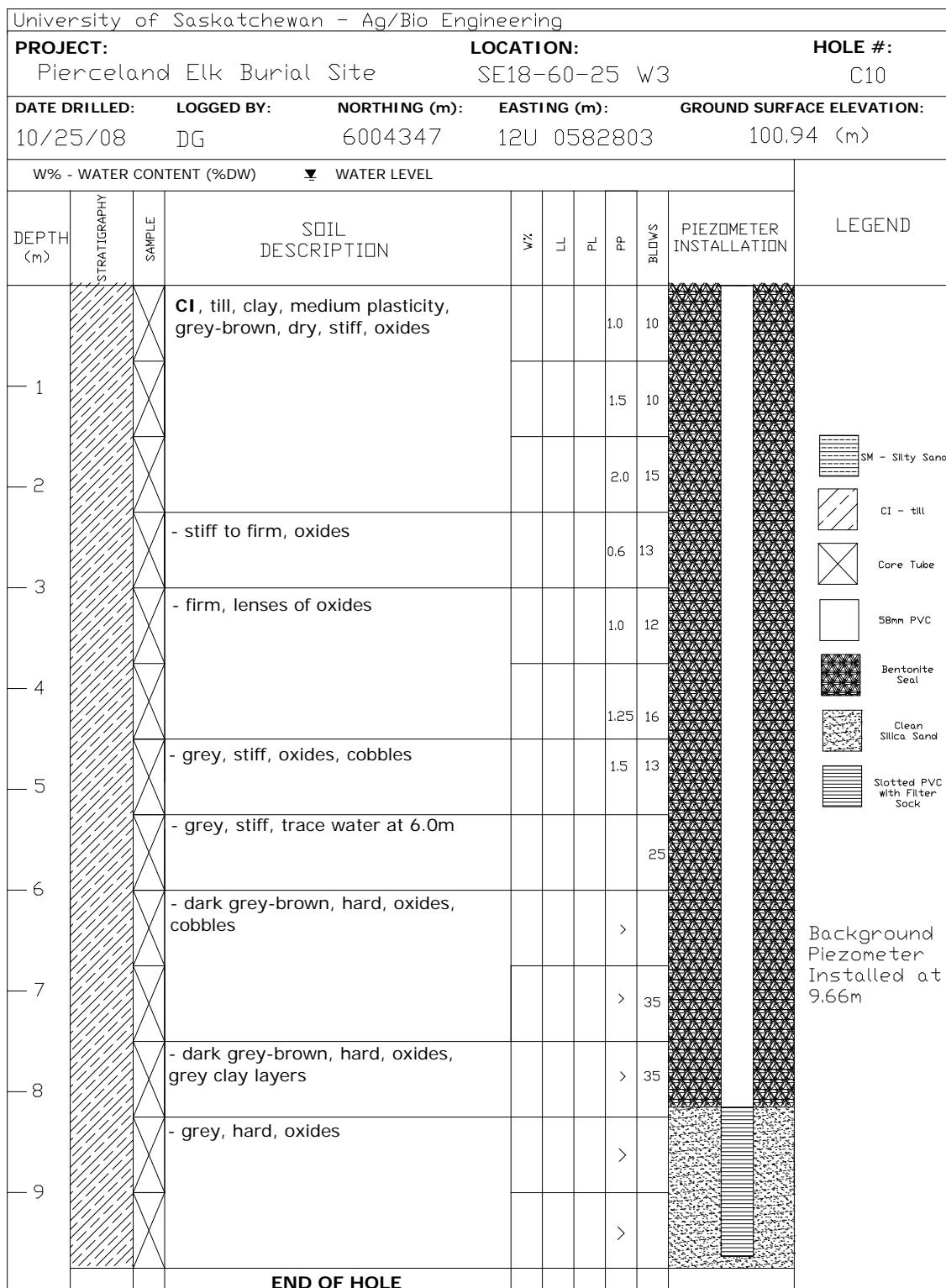


Figure A.12 Drill logs Pierceland C11

University of Saskatchewan - Ag/Bio Engineering											
PROJECT:			LOCATION:			HOLE #:					
Pierceland Elk Burial Site			SE18-60-25 W3			C12					
DATE DRILLED:	LOGGED BY:	NORTHING (m):	EASTING (m):	GROUND SURFACE ELEVATION:							
10/26/08	DG	6004362	12U 0582801	101.16 (m)							
W% - WATER CONTENT (%DW)  WATER LEVEL							LEGEND				
DEPTH (m)	STRATIGRAPHY	SAMPLE	SOIL DESCRIPTION		W%	LL	PL	PP	PIEZOMETER INSTALLATION		
1			CI , till, clay, medium plasticity, grey-brown, dry, stiff, oxides					1.75	10		
2			- rock at 2.15m					1.75	8		
3			- Limestone Rock					1.5			
4			- grey-brown, stiff to firm, oxides					0.75	15		
5			- stiff, oxides					1.25	22		
6		BAG	- dark grey-brown to grey, stiff to firm								
7			- grey, stiff, cobble								
8			- olive-grey, hard, oxides					>			
9			END OF HOLE					>	37		
								>	43		
										No Piezometer Installation	

Figure A.13 Drill logs Pierceland C12

University of Saskatchewan - Ag/Bio Engineering

PROJECT:		LOCATION:		HOLE #:						
Pierceland Elk Burial Site		SE18-60-25 W3		C13						
DATE DRILLED:		LOGGED BY:	NORTHING (m):	EASTING (m):	GROUND SURFACE ELEVATION:					
11/4/08		DG	6004424	12U 0582863	101.58 (m)					
W% - WATER CONTENT (%DW)  WATER LEVEL										
DEPTH (m)	STRATIGRAPHY	SAMPLE	SOIL DESCRIPTION	W%	LL	PL	PP	BLDS	PIEZOMETER INSTALLATION	LEGEND
1			CI, till, clay, medium plasticity, grey-brown, dry, firm, oxides - firm to soft, fill material				0.5	7		
2			- soft, alluvial, black, organics, odour				0.2			 SM - Silty Sand
3			- grey-brown, hard, black organic layers, odour				1.5	7		 CI - till
4			- stiff, oxides, organic layers, slight odour				4.0	17		 Core Tube
5			- grey-brown to grey, stiff, seams of oxides				2.0	18		 58mm PVC
6			- grey, stiff, oxides				1.25	14		 Bentonite Seal
7			- grey, stiff to firm				1.2	21		 Clean Silica Sand
8			- grey to olive-grey, stiff to hard				0.9	21		
9			- olive grey, hard				>	40		
			END OF HOLE							No Piezometer Installation

Figure A.14 Drill logs Pierceland C13

University of Saskatchewan - Ag/Bio Engineering											
PROJECT:			LOCATION:			HOLE #:					
Pierceland Elk Burial Site			SE18-60-25 W3			C14					
DATE DRILLED:	LOGGED BY:	NORTHING (m):	EASTING (m):	GROUND SURFACE ELEVATION:							
11/4/08	DG	6004426	12U 0582864	101.66 (m)							
W% - WATER CONTENT (%DW) ▼ WATER LEVEL											
DEPTH (m)	STRATIGRAPHY	SAMPLE	SOIL DESCRIPTION		W%	LL	PL	PP			
							BLWS	PIEZOMETER INSTALLATION			
1			CI , till, clay, medium plasticity, grey-brown, dry, firm				0.5	7			
1			- soft, fill material				0.2	6			
2							0.2	5			
3			- grey-brown, soft to stiff, organics, black, odour								
3			- stiff to hard, oxides, black organic layers, strong odour				2.0	18			
4		BAG	- grey-brown, stiff, seams of organics, elk parts, oxides, odour								
4			- grey-brown to grey, very stiff to stiff, oxide lenses				1.25				
5			- grey, stiff, oxide lenses				1.1	17			
6			- grey to olive-grey, stiff to hard, oxides				>	19			
6			- olive grey, hard				>	50			
7							>	60			
8							>	62			
9							>	50			
END OF HOLE											
LEGEND											

University of Saskatchewan - Ag/Bio Engineering								
PROJECT:			LOCATION:			HOLE #:		
Pierceland Elk Burial Site			SE18-60-25 W3			C15		
DATE DRILLED:	LOGGED BY:	NORTHING (m):	EASTING (m):	GROUND SURFACE ELEVATION:				
11/5/08	DG	6004429	12U 0582865	101.44 (m)				
W% - WATER CONTENT (%DW) ▼ WATER LEVEL								
DEPTH (m)	STRATIGRAPHY	SAMPLE	SOIL DESCRIPTION	W%	L	PL	PP	BLOWS
								PIEZOMETER INSTALLATION
1			CI , till, clay, medium plasticity, grey-brown, dry, stiff, oxides - stiff to firm, fill material				1.75	10
2			- grey-brown, firm, oxides				1.0	12
3			- firm to stiff, seams of oxides				1.0	9
4			- grey-brown, stiff, seams of oxides				0.75	10
5			- grey-brown to grey, stiff, oxides				1.5	12
6			- grey, stiff to firm, oxidized sandy joints				1.6	13
7			- grey, firm to hard, oxides				1.0	20
8			- grey, hard				0.5	17
9							>	40
							>	33
							>	43
							>	35
END OF HOLE								
No Piezometer Installation								

Figure A.16 Drill logs Pierceland C15

University of Saskatchewan - Ag/Bio Engineering										
PROJECT:			LOCATION:			HOLE #:				
Pierceland Elk Burial Site			SE18-60-25 W3			C16				
DATE DRILLED:	LOGGED BY:	NORTHING (m):	EASTING (m):	GROUND SURFACE ELEVATION:						
11/5/08	DG	6004431	12U 0582866	101.58 (m)						
W% - WATER CONTENT (%DW)  WATER LEVEL							LEGEND			
DEPTH (m)	STRATIGRAPHY	SAMPLE	SOIL DESCRIPTION		W%	LL	PL	PP		
								BLOWS		
								PIEZOMETER INSTALLATION		
1			CI , till, clay, medium plasticity, grey-brown, dry, firm				0.75	8		
1			- firm to very soft, fill material			<		7		
2			- soft to firm, oxides					0.5		
3			- grey-brown, firm to stiff, black organics, strong odour							
3			- organic, black to grey-brown, firm, organic layers, elk hair, strong odour, water at 3.75m							
4			- grey-brown, stiff, seams of organics, slight odour, oxide lenses					12		
5			- dark grey-brown to grey, stiff, oxides, layers of organics, odour				1.0	12		
5			- grey, stiff to firm, oxides				0.75	18		
6			- grey, firm,							
7	BAG		- olive grey, hard, cobbles				>			
7	BAG						>			
8							>			
9							>			
END OF HOLE										
No Piezometer Installation										

Figure A.17 Drill logs Pierceland C16



Figure A.18 Pierceland site photos facing West



Figure A.19 Pierceland site photos facing South (top) and West (bottom)



Figure A.20 Pierceland drilling photos

McLean FMD Site Drilling Logs and Miscellaneous Information

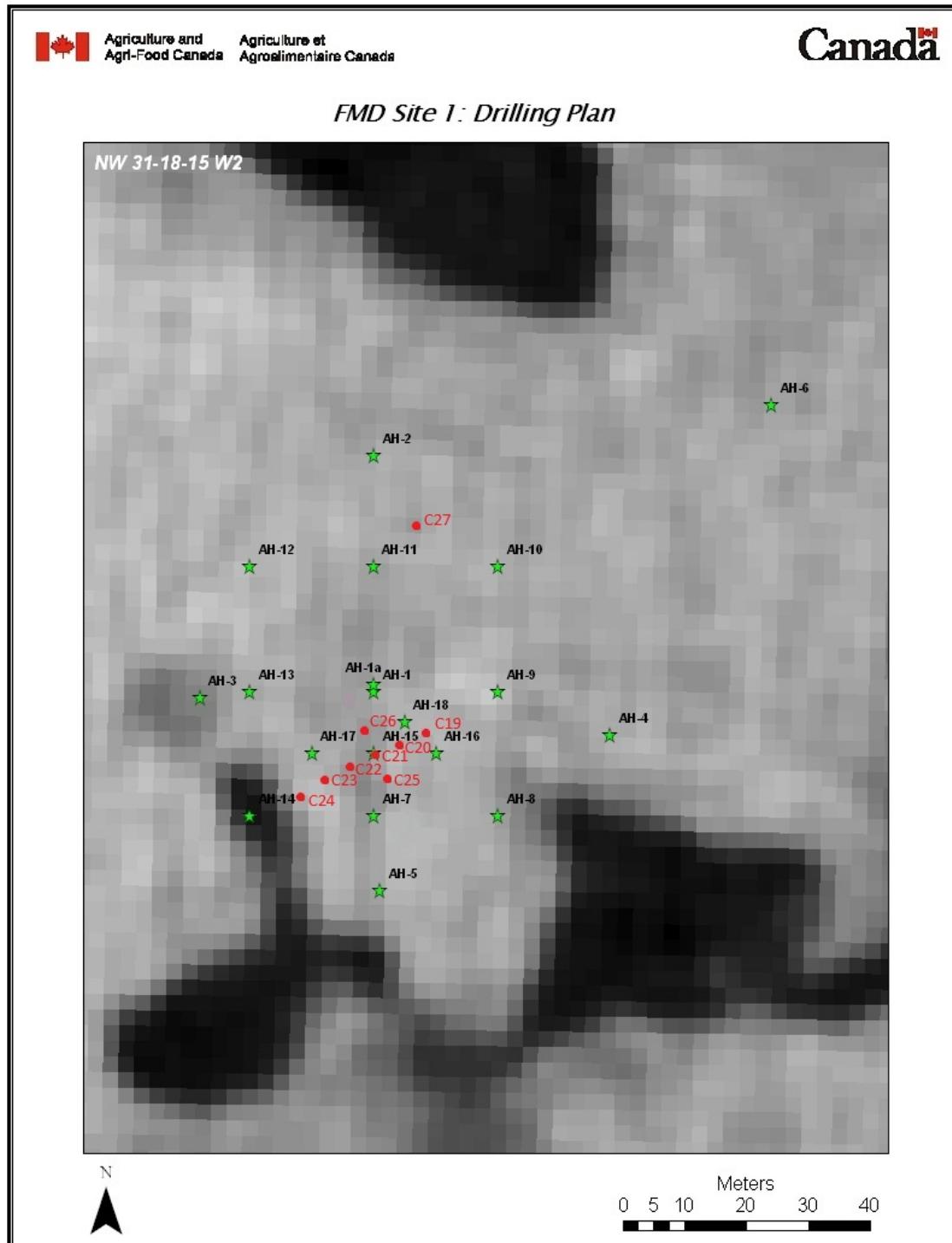


Figure A.21 McLean site aerial sampling location diagram

Hole #	Easting	Northing	Accuracy (m)
C19	13U 0566569	5602362	4
C20	13U 0566567	5602361	4
C21	13U 0566560	5602361	3
C22	13U 0566559	5602357	3
C23	13U 0566561	5602356	3
C24	13U 0566547	5602350	3
C25	13U 0566561	5602352	4
C26	13U 0566561	5602364	4
C27	13U 0566572	5602391	3

Figure A.22 McLean site GPS coordinates of sampling locations

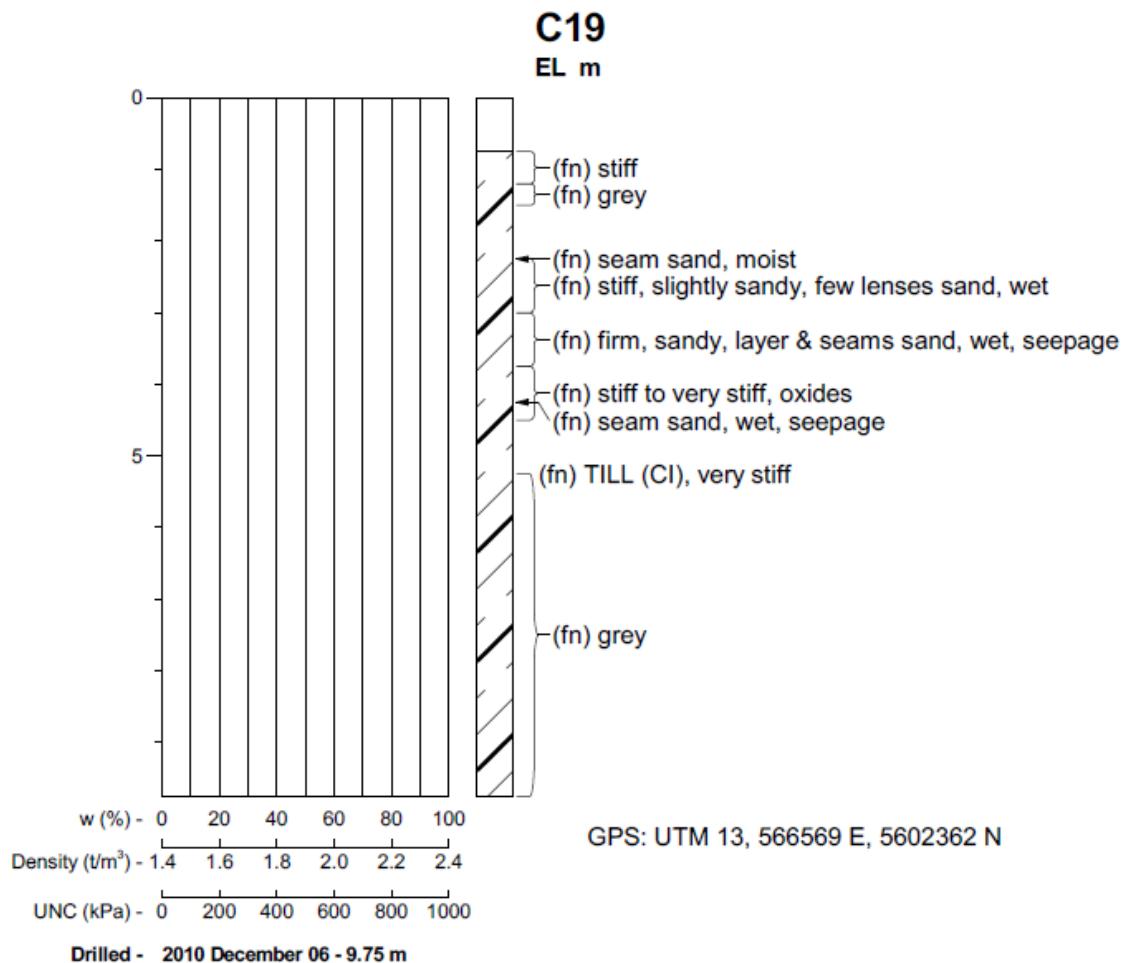


Figure A.23 Drill logs McLean C19

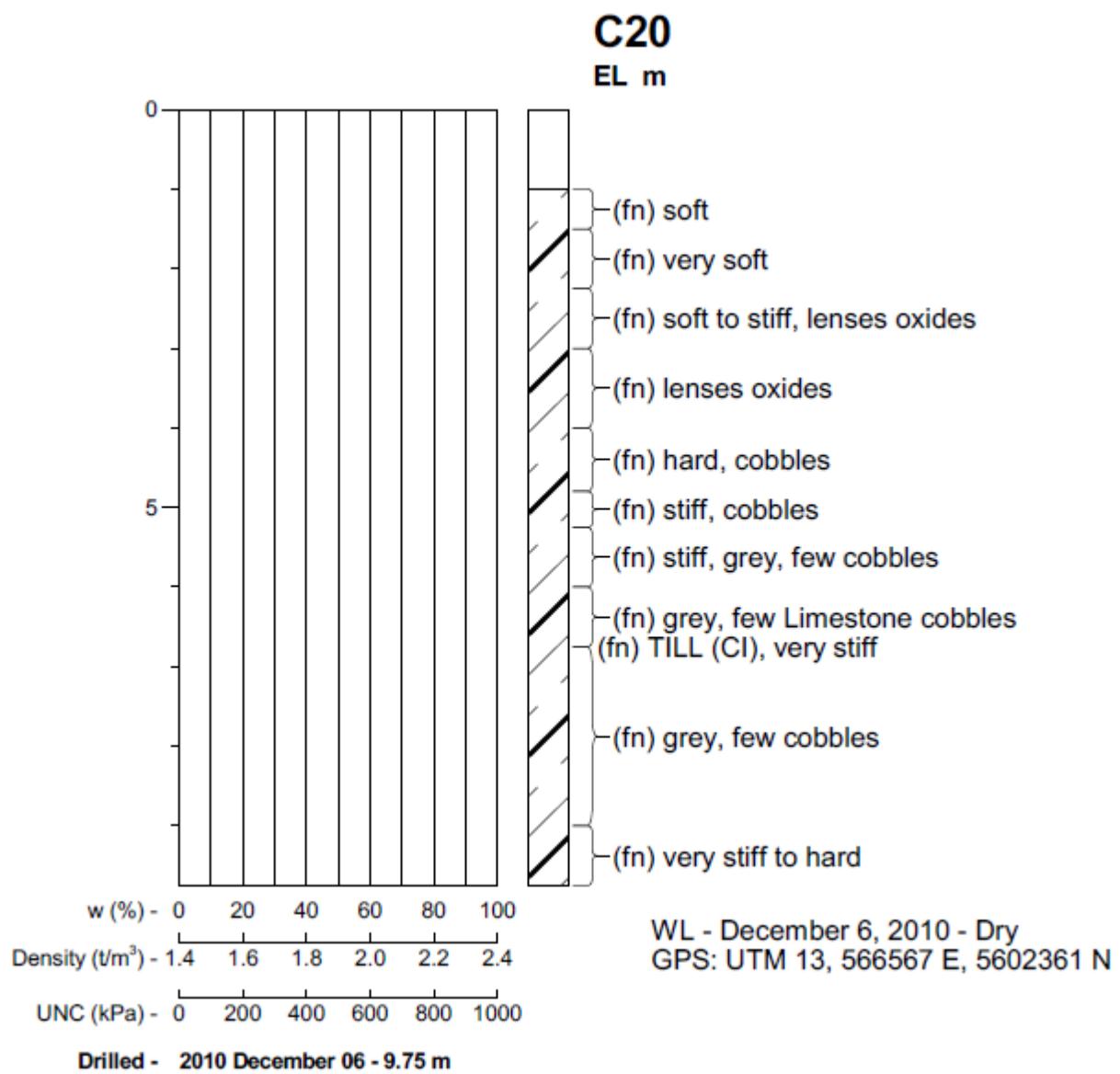


Figure A.24 Drill logs McLean C20

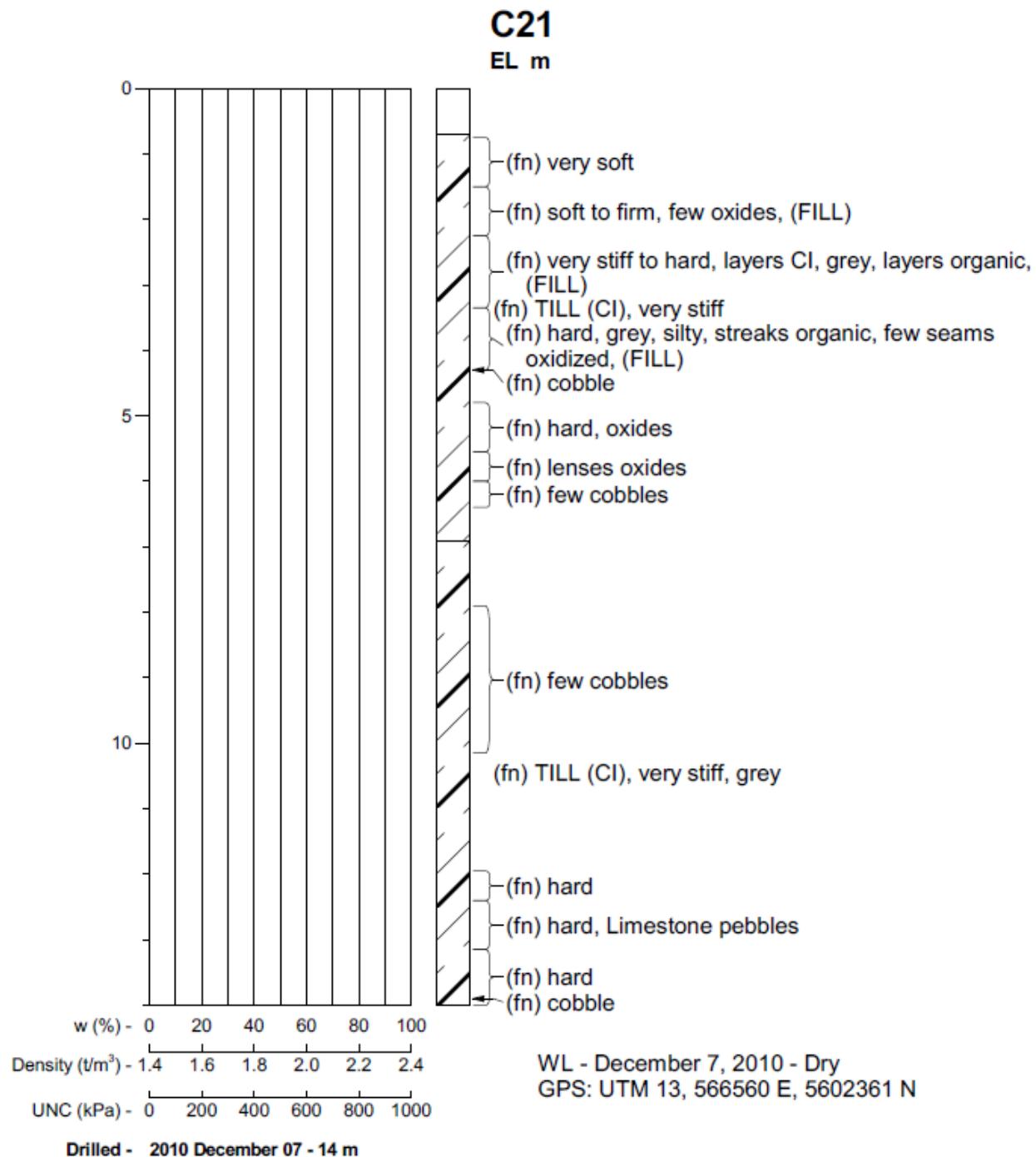


Figure A.25 Drill logs McLean C21

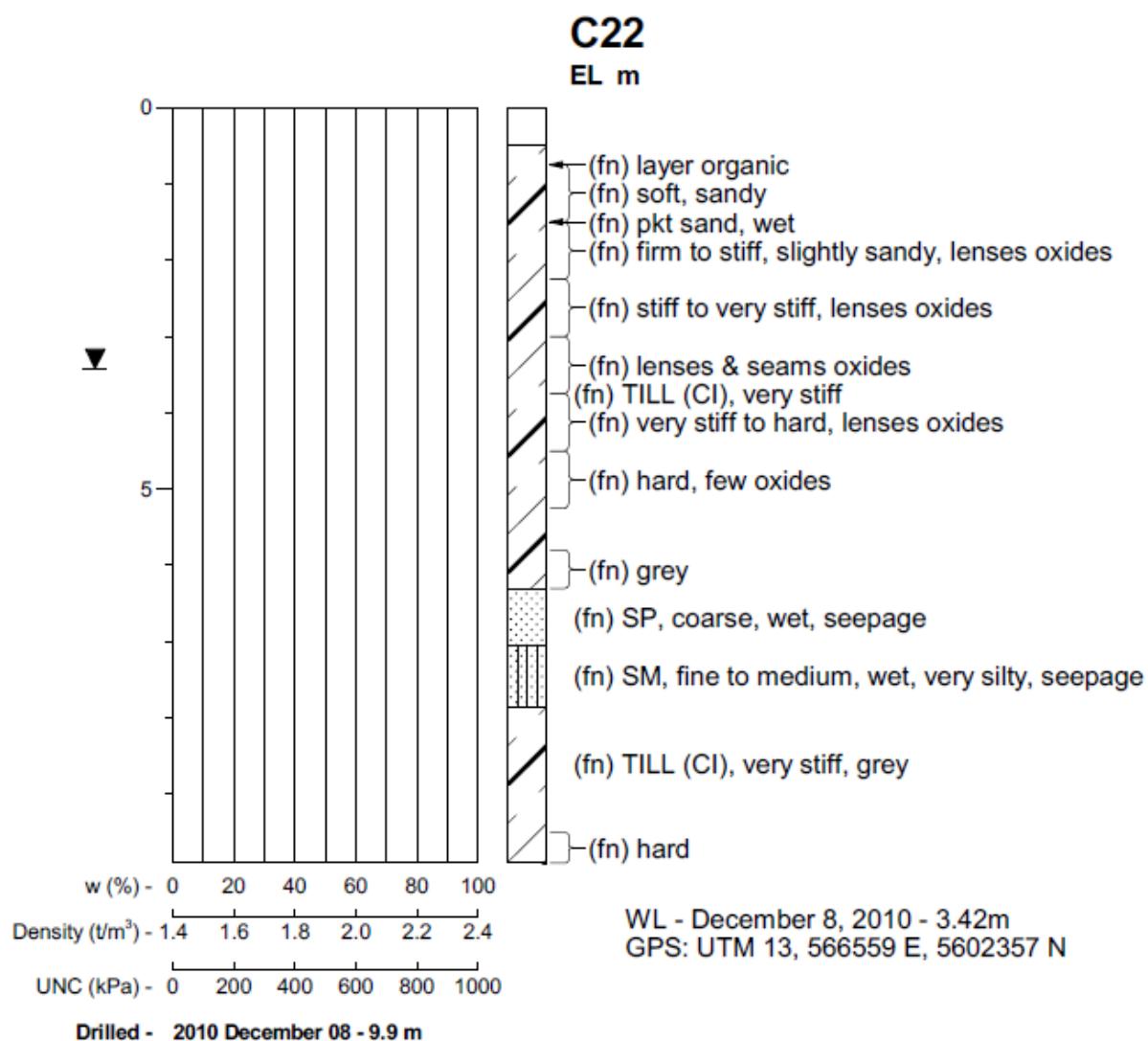


Figure A.26 Drill logs McLean C22

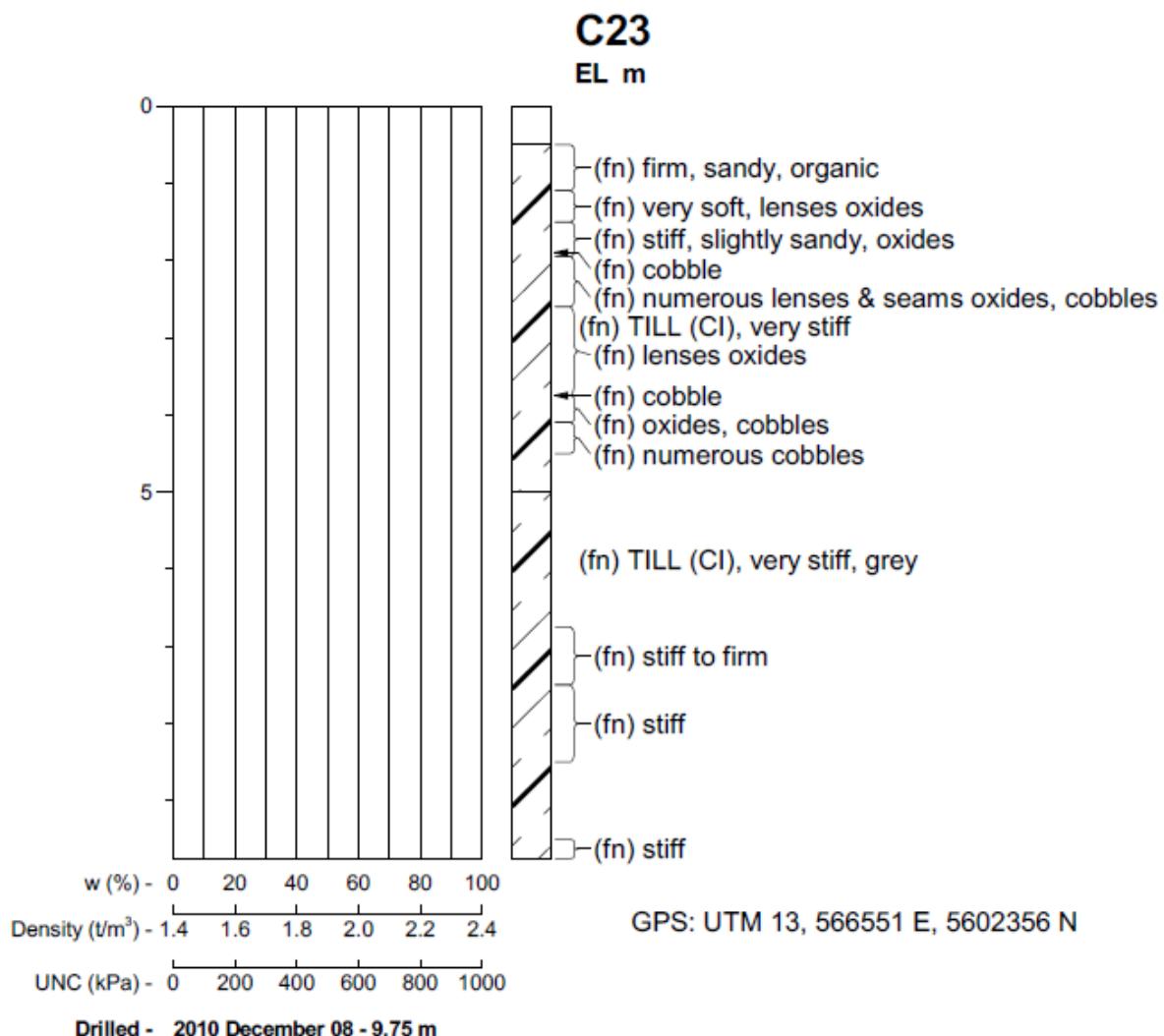


Figure A.27 Drill logs McLean C23

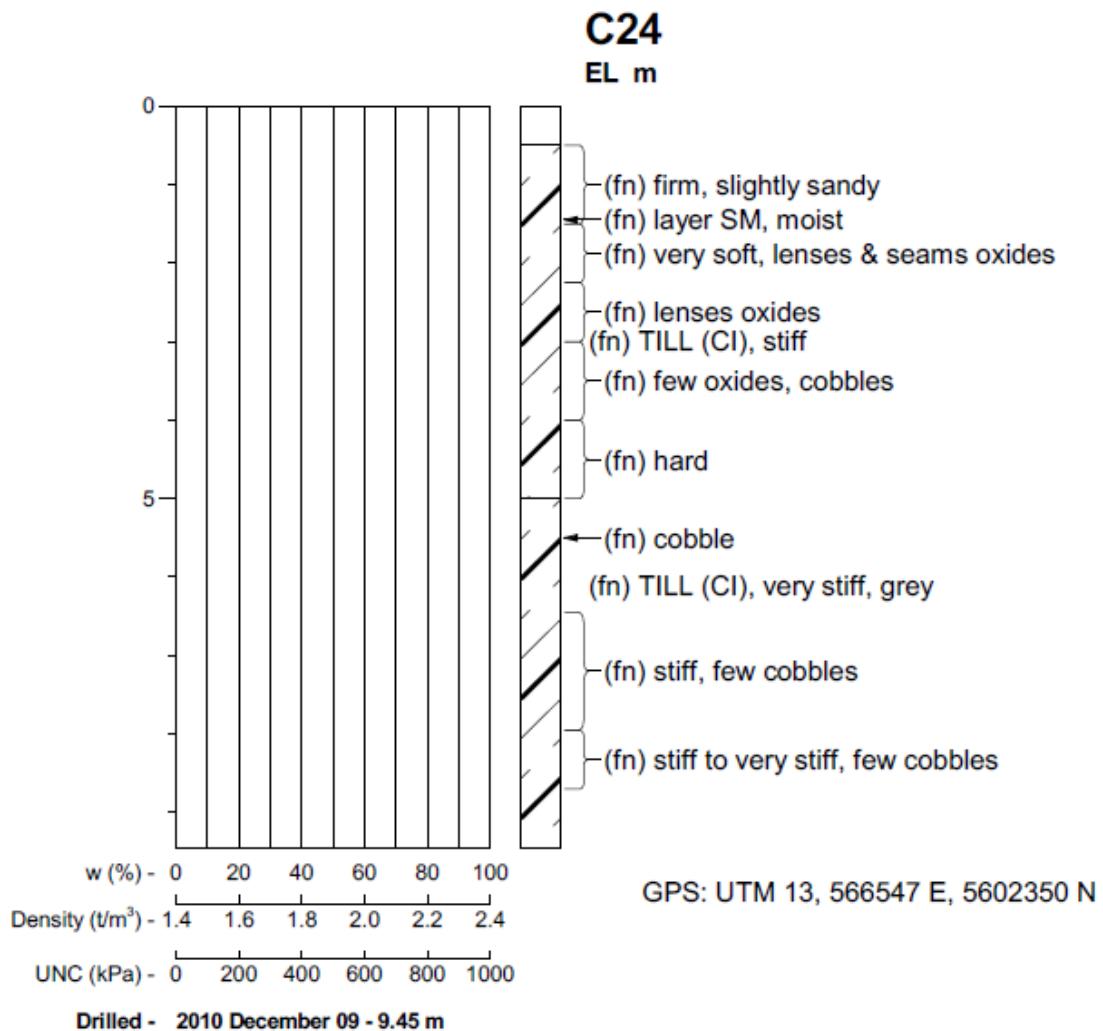


Figure A.28 Drill logs McLean C24

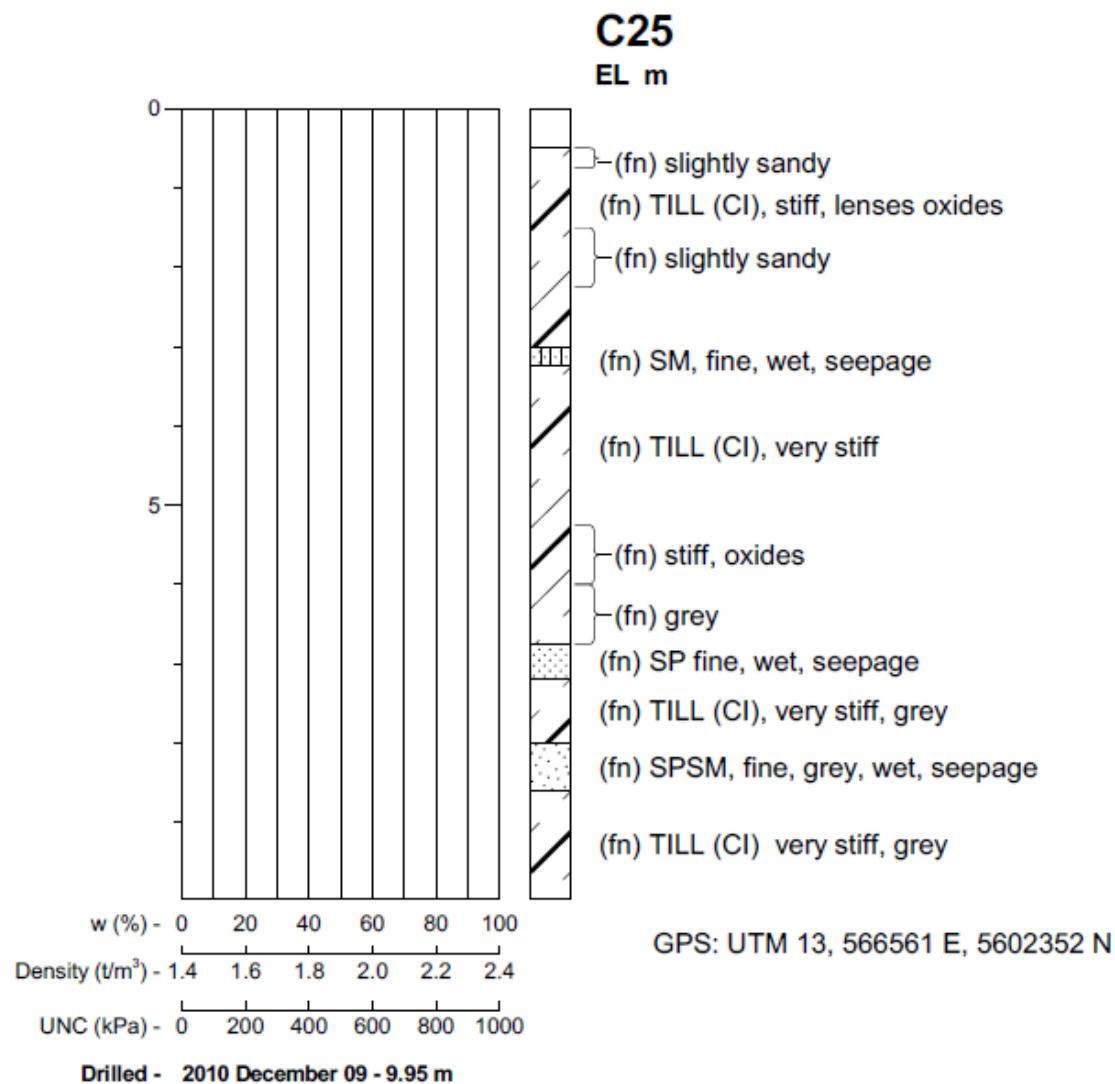


Figure A.29 Drill logs McLean C25

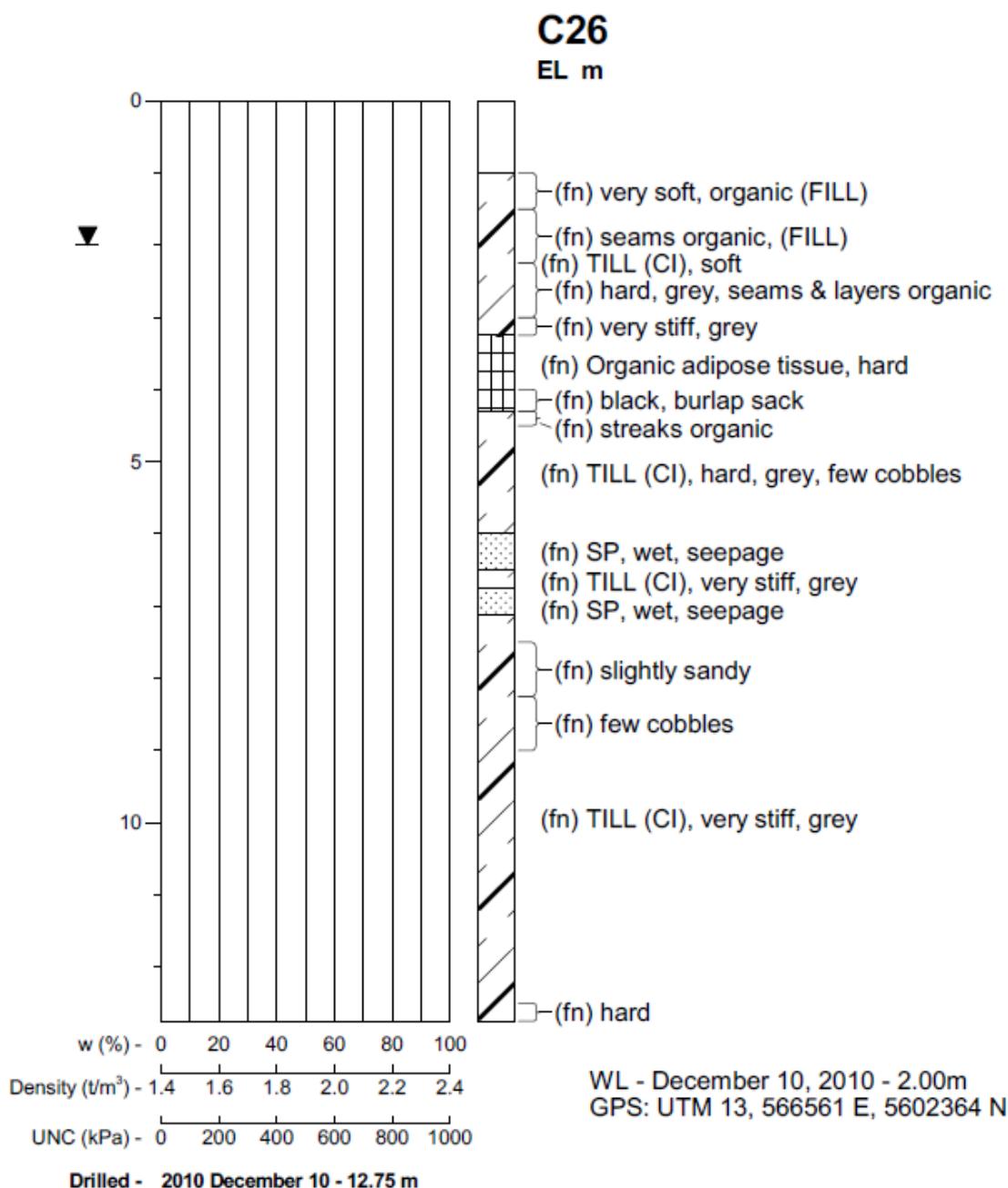


Figure A.30 Drill logs McLean C26

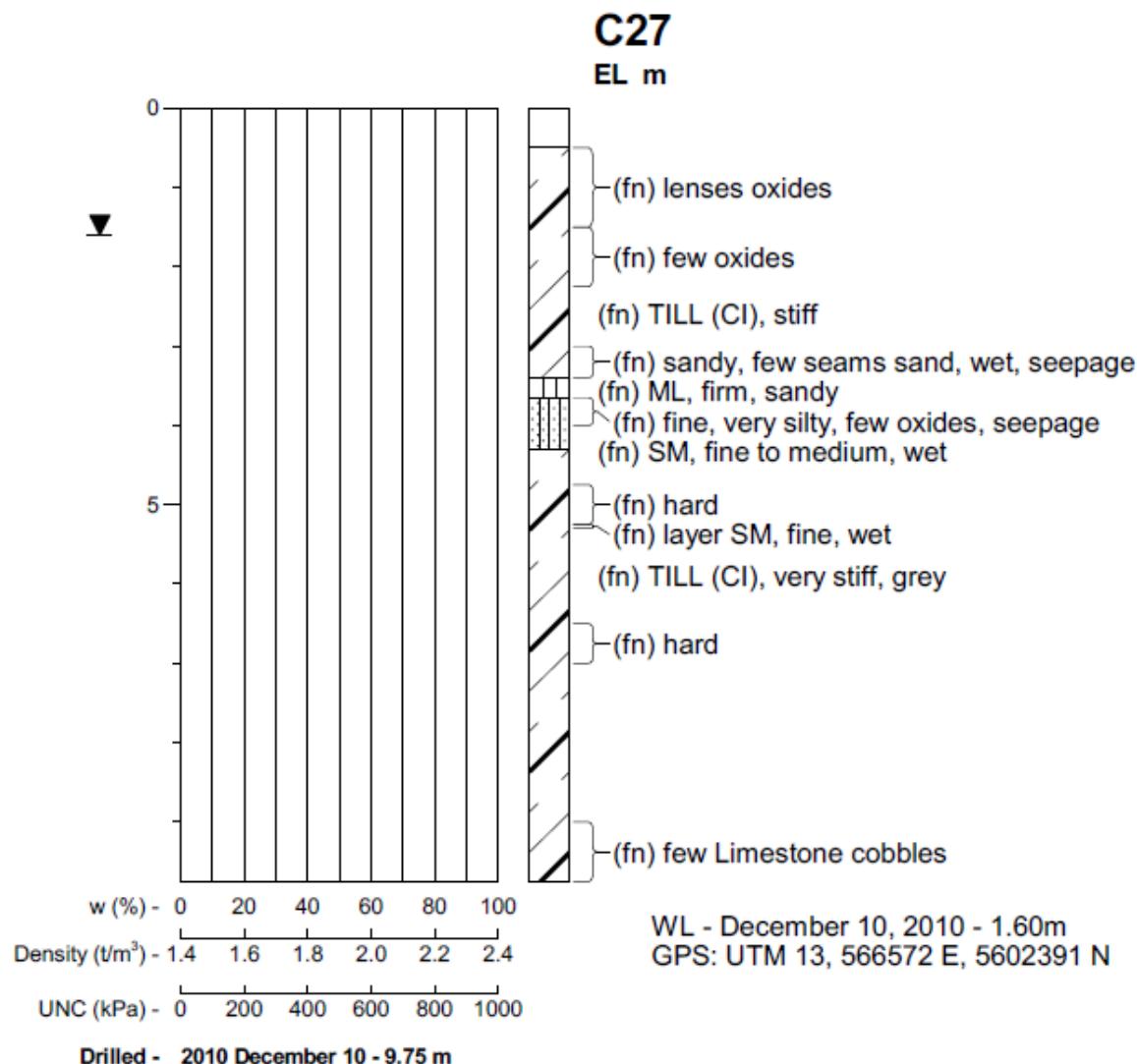


Figure A.31 Drill logs McLean C27



Figure A.32 McLean Site drilling photos of sampling



Figure A.33 McLean Site drilling photo