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Indicators of Bacterial Pathogens in Groundwater and Well Water Supplies

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Indicators of Bacterial Pathogens in Groundwater and Well Water Supplies" submitted by Jennifer Oliphant in partial fulfillment of the requirements for a degree of Master of Science.

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ABSTRACT

Recent health issues related to microbial groundwater pathogens has highlighted the need for safe, easy and fast assessment of groundwater samples for microbial contamination. Currently, Membrane Filtration (MF) is the most recognized analytical technique for enumeration of bacterial indicators. Fecal and total coliforms were most commonly measured, although fecal streptococci and *E. coli* (both specific members of the total and fecal coliform groups) are becoming more frequently used. This investigation consists of three studies related to the collection and analysis of groundwater samples for coliform indicators, and the assessment of problems associated with assessing groundwater contamination in consideration of health risks.

A well chlorination study was conducted to assess the efficacy of shock chlorination for the remediation of private drinking water wells with persistent coliform contamination suspected to be from private sewage disposal systems in the Hamlet of Bragg Creek, Alberta, Canada. Coliform positive samples were detected within two days after chlorination at one location, within two weeks at a second contaminated well site, and were still coliform negative after four months at the third site. The rapid recovery of coliforms in two of the three contaminated wells suggests the coliforms found in these domestic supplies are likely due to the continual loading of septic-system impacted groundwater. In these cases, shock chlorination is not an effective remediation strategy.

Groundwater monitoring wells at two study areas were sampled over an 11-month period to assess bacterial contamination due to manure and sludge amendment to soils as a means of fertilization. The groundwater monitoring wells were located in a shallow sand aquifer (<2m to the water table) in southern Alberta in a region with a high density of intensive livestock operations north of Lethbridge, and in the region immediately southwest of the City of Calgary where most of the city's sewage sludge is applied to farm fields. Total and fecal coliforms were consistently found in 3 of 5 treatment wells sampled in the Lethbridge region. No significant coliform contamination was found at the sludge-amended (Calgary) wells sites with the exception of one well (CSA2-2) where low coliform levels were usually present. Microbial identification of the coliform colonies revealed that many of the coliforms belong to the *Enterobacter* and *Klebsiella* groups. *E.coli* and *Salmonella spp.* were not detected. Atypical bacteria were almost exclusively *Pseudomonas spp.* The higher general bacterial and coliform concentrations found in Lethbridge wells are probably due to the considerably higher groundwater vulnerability in the region.

The ability of high concentrations of atypical bacteria from manure-impacted groundwater to inhibit coliform growth on membrane filters (MF) using standard M-Endo media plates was investigated using bacterial spiking. Inhibiting atypical morphologies were also identified and most were found to be strains that occur naturally in soil and water or are associated with agricultural crop disease, and are not directly associated with manure. The high

concentrations of atypicals found in the manure-impacted groundwater (up to $2.5x10^4$/mL) may be related to the high nutrient levels and suggest that these bacteria can maintain significant populations in manure-impacted groundwater.

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DEDICATION

This work is dedicated to my parents, Mark and Sharron Oliphant whose unwavering faith, everlasting patience, and financial support made this possible.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS, ABBRVIATIONS AND NOMENCLATURE

ABBREVIATIONS:

Acute Gastrointestinal Illness	AGI
American Public Health Association	APHA
Bonnybrook Wastewater Treatment Plant	BWTP
Colony Forming Units	CFU
Carbon	C
Degrees Celsius	°C
Electron Microscopy	EM
Fecal Coliform Bacteria	FC
Free Living Bacteria	FLB
Lethbridge Northern Irrigation District	LNID
Heterotrophic Plate Count	HPC
Hydrogen Peroxide	H_2O_2
Inertial Foot Valve	IFV
Intensive Livestock Operations	ILO
Litres	L
Temperature	Temp
Total Coliform Bacteria	TC

CHAPTER ONE: INTRODUCTION AND LITERATURE REVIEW

Historically, water originating from the subsurface was thought to be clean and acceptable to drink without disinfection or other treatment. This attitude was reflected in the inequitable regulations pertaining to the treatment of water coming from the surface or from the ground. Until recently the treatment of groundwater was poorly regulated in Canada and the U.S., compared to regularly monitored surface water supplies. As land use and farming practices evolve and intensify, the occurrence of illnesses related to consumption of untreated groundwater has increased. Those who drink groundwater that has not been disinfected are at an increased risk of infection and disease from pathogenic microorganisms (Macler and Merkle, 2000).

Well water constitutes the sole water source for many geographic regions such as Prince Edward Island and the Yukon Territory which are solely dependent on groundwater for all municipal drinking water supplies (Hess, 1981). It is estimated that 26% of Canadians (Hess, 1981) and approximately 50% of the U.S. population rely on groundwater as their principle source of potable water (Todd, 1980). Of the 100 million people currently served by groundwater-based public water systems in the United States, and the approximately 20 million on private wells, up to half of the wells may show evidence of fecal contamination (Macler and Merkle, 2000).

Estimates of illness caused by contaminated groundwater in the United States range from 2,038 people in a year (Barwick et al., 2000), to 750,000 and even 5.9 million per year (Macler and Merkle, 2000). During 1997 and 1998, 17 outbreaks were reported in the U.S. associated with drinking water. Of the 17 outbreaks, 15 were linked to groundwater sources (Barwick et al., 2000).

Contamination of groundwater due to pathogenic microorganisms is generally believed to result from migration or introduction of fecal material into the subsurface (Gosselin et al., 1997). Primary sources of fecal contamination of health concern to humans include wastewater from private septic systems and animal feces originating from agriculture operations (Gosselin et al., 1997; Macler and Merkle, 2000).

Pathogenic organisms identified in groundwater supplies consist of bacteria, protozoa, and viruses. One group of intestinal bacteria, the coliform bacteria, has historically been used as an indication of fecal contamination (Yates and Yates, 1988). Coliform bacteria can include organisms such as *Escherichia coli (E. coli)*, *Salmonella* and *Shigella*. Protozoa such as *Cryptospiridium spp.* and *Giardia spp.* have also been of recent concern to water treatment professionals. Viruses are unregulated in Canada (Health Canada, 2002) and are still poorly understood.

Most organisms of fecal origin are transmissible via a fecal-oral route of exposure (Macler and Merkle, 2000). The possible microbial illnesses that result from infection vary with the type of organism and vary markedly in their severity.

The predominant recognized illness is generalized Acute Gastrointestinal Illness (AGI), resulting in fever, nausea, diarrhea, and/or vomiting (Macler and Merkle, 2000). Elderly persons, children, infants and immunocompromised individuals are the most susceptible to severe infections resulting from these pathogenic organisms.

Certain strains of bacteria have been known to produce a much more significant effect on individuals than their closely related counterparts. *E. coli* O157:H7 is a virulent and deadly organism that produces a Shiga toxin as a by-product of its metabolism. Epidemiological data from a majority of human infections traceable to the consumption of *E. coli* O157:H7 confirm cattle as the principle reservoir of this bacterial strain (Armstrong et al., 1996). The toxin produced from *E. coli O157:H7* is associated with serious human alimentary infection characterized by bloody or watery diarrhoea which may, particularly in immunocompromised individuals be complicated by life-threatening haemolytic uraemic syndrome (O'Brien and Kaper, 1998).

A outbreak of *E. coli* O157:H7 reported in Walkerton, Ontario in May 2000 was traced to contaminated groundwater from a well used for the local drinking water supply. At least seven deaths have been attributed to the consumption of the contaminated water. Inconsistency in the chlorination systems used for disinfection has been the suspected reason for the contamination found in the distribution system (O'Connor, 2002).

Evidence of antibiotic resistant strains of pathogenic bacteria found in the ambient environment is also cause to further understand the health risks associated with consumption of contaminated drinking water. McKeon et al (1995) showed the presence of coliforms in groundwater supplies that were resistant to many antibiotics commonly used in veterinary practices, and routinely supplemented to livestock feed. In addition, multiple-antibiotic resistance, meaning resistance to more that one type of antibiotic, is common to many of these antibiotic-resistant strains.

Canadian Drinking Water Guidelines

In Canada, the maximum acceptable concentration (MAC) for coliforms in drinking water is zero organisms detectable per 100mL (Health Canada, 2002). Because coliforms are not uniformly distributed in water and are subject to considerable variation in enumeration, conditions must be set to decide whether water samples are considered to be in compliance with the coliform MAC. These conditions have recently been revised. The microbiological parameters issued in the 1999 version of the Canadian Drinking Water Guidelines were as follows (Health Canada, 1999).

1. No sample should contain more than 10 total coliform organisms per 100mL, none of which should be *E. coli* or thermotolerant (fecal) coliforms.
2. No consecutive sample from the same site should show presence of coliform organisms.
3. For community drinking water supplies:
   a) Not more than one sample from a set of samples taken from the community on a given day should show the presence of coliform organisms
   b) Not more than 10% of the samples based on a minimum of 10 samples should show the presence of coliform organisms.
4. Numerical guidelines for viruses and protozoa are not proposed at this time. It is desirable, however, that no human enteric viruses or viable protozoa (e.g. *Cryptosporidium spp.*, *Giardia spp.*) be detected.

These parameters were changed in April 2002. The new microbiological guidelines are much more explanatory in nature than the previous 1999 version. The new guidelines also seem to make it easier for the general public to recognize if samples exceed the microbiological MAC.

Drinking water samples must now satisfy the following conditions to be considered potable (Health Canada, 2002):

In Public Drinking Water Systems

1. No sample should contain *Escherichia coli. E. coli* indicates the possible presence of enteric pathogens that may adversely affect human health. If *E. coli* is confirmed, the appropriate agencies should be notified, a boil water advisory should be issued, and corrective actions taken.
2. No consecutive samples from the same site or not more than 10% of samples from the distribution system in a given calendar month should show the presence of total coliform bacteria. The ability of total coliforms to indicate the presence of faecal pollution is less reliable than *E. coli.* However, this group of bacteria is a good indicator of quality control. The presence of total coliforms does not necessarily require an issuance of a boil water advisory, but corrective actions should be taken.

In Semi-public and Private Drinking Water Supplies

1. No sample should contain *E. coli.* As stated above, the presence of *E. coli* indicates faecal contamination and the possible presence of enteric pathogens; therefore the water is unsafe to drink. If *E. coli* is detected, a boil water advisory should be issued, and corrective actions taken.
2. No sample should contain total coliform bacteria. In non-disinfected well-water, the presence of total coliform bacteria in the absence of *E. coli* indicates the well is prone to surface water infiltration and therefore at risk of faecal contamination. In disinfected water systems, the presence of total coliform bacteria indicates a failure in the disinfection process. In both disinfected and non-disinfected water systems, total coliform detection may also indicate the presence of biofilm in the well or plumbing system. The degree of response to the presence of total coliform bacteria and the absence of *E. coli* may be site specific and can vary between jurisdictions. Numerical guidelines for viruses and protozoa are not proposed at this time. It is desirable, however, that no human enteric viruses or viable protozoa (e.g. *Cryptosporidium spp.*, *Giardia spp.*) be detected.

These guidelines focus more on the detection of *E. coli* rather than the much more generalized and commonly analyzed fecal coliform bacteria, because *E. coli* is a more likely indicator of faecal contamination (Raina et al, 1999). Analysis for

E. coli is becoming much easier and reliable with new analytical products and rapid detection media available on the market today.

Although these guidelines are suggested thresholds for microbial contaminants in drinking water, the provinces individually set their own guidelines and regulations for their region. For example the Ontario Drinking Water Objectives (ODWO) are similar, but require that total coliform levels not exceed 5 cfu/100mL rather then the 10 cfu/100mL guideline proposed by Health Canada.

Water-borne enteric parasites such as *Giardia spp.* cysts and *Cryptosporidium spp.* oocysts are also associated with fecal contamination (Ong et al., 1996; Brush et al., 1998). These protozoa enter the environment along with the feces of infected farm animals, and then enter watercourses through overland transport or via infiltration through highly permeable soils (Brush et al., 1998). The most common human health effect produced from these two species of parasites is chronic diarrhea (Macler and Merkle, 2000).

Certain qualities of a cyst are important to consider when describing protozoan transport in groundwater. Cysts and oocysts are a dormant stage in the life cycle of a protozoan, which protects the organism against adverse changes in the environment. The development of a cyst, called encystation, is marked by the presence of a thick cell wall, and reduction in metabolic activity to a very low level (Prescott, 1996).

Firstly, the ability for cysts to survive harsh environmental conditions over long periods of time increases their viability. The longer a cyst remains viable the further it may be transported within the subsurface. In comparison, coliform bacteria may be inactivated by temperature, pH or nutrients long before a cyst is stressed by these same factors (Prescott, 1996).

Secondly, cysts serve as a means of transfer between hosts in parasitic species. Although the exact stimulus for excystation (escape from cysts) is unknown, it is generally triggered by a return to favorable environmental conditions. Cysts of parasitic species usually excyst after ingestion by the host (Prescott, 1996).

Lastly, although the development of a cyst may seem to favor its ability to be transported long distances in groundwater, the relatively large size of the organism (1-5 μm) may hinder its movement. However there are no real data pertaining to the transport and survival of these microsporidia in groundwater. Reliable methods used for identification and quantification still offer limited capability (LeChevallier, 1999).

The presence of coliphages, enteroviruses, and other virus types is also of importance when considering public health relating to the consumption of fecal contaminated drinking water. Viruses are obligate intracellular parasites, ranging in size from 20 to 200 nm. Human enteroviruses are so named because they replicate within the intestinal tracts of the human mammal (Yates and Yates,

1988). These viruses are shed from the human intestinal tract along with other fecal material.

Human enteroviruses comprise 71 known types. Although they are easily removed (>99.9%) by conventional water treatment, certain types of enterovirus are now recognized as the leading cause of acute fevers among young children and infants and the most common cause of aseptic meningitis in developed countries (LeChevallier et al., 1999). These viruses are almost always transmitted via the fecal-oral route of exposure and can remain viable in groundwater for more than nine months (Deborde et al., 1998b).

The contribution of viral contamination to the overall state of drinking water supplies has caused many researchers to attempt the quantification of certain virus types as an indicator of fecal contamination. Coliphages monitored in a high school septic effluent plume for 10 months showed a strong relationship between coliphage concentration and the septic system use during the school year, as well as a strong drop in coliphage concentration with distance from the septic discharge (Deborde et al., 1998a). These results suggested that coliphages were sensitive indicators of fecal contamination. Since the testing for viruses as indicators of contamination is costly, time consuming and hard to reproduce (Deborde et al., 1998a), easily identifiable organisms such as fecal coliforms, or more specifically, *E. coil* are used extensively.

Well Contamination and Susceptibility

The typical groundwater well consists of a fine stainless steel cylindrical screen and a pump, which draws water through the screen to the surface-delivery system. The screen and usually the pump are situated below the level of the groundwater and are expensive to install and repair (Ralph et al., 1995).

In the past, much of the research into the presence of microorganisms in water wells has focused on iron bacteria. "Iron bacteria" is a general term that refers to a number of different and diverse bacterial species. These types of bacteria are generally limited to the well environment and are associated with the iron oxides that occur there (Ralph, 1995). Iron oxides occur when a well is pumped, the temporary draw down in the water table produced from pumping allows oxygen to enter the subsurface and iron to oxidize (Ralph, 1995). Iron bacteria have an economic impact through their slime-forming by-products and incidental harboring of corrosion-causing bacteria such as mesophilic sulfate-reducers.

Past Studies of Bacteria in Groundwater and Water Wells

Many studies pertaining to the occurrence of bacterial contaminants in domestic wells have been conducted in North America over the last decade (Table 1.1). Results obtained from these studies have shown a common link between high-intensity agriculture and Intensive Livestock Operations (ILO), well construction characteristics, and the likelihood of bacterial well contamination (Conboy and Goss, 1999; Conboy and Goss, 2000; Macler and Merkle, 2000).

Table 1.1: Summary of Recent Studies Pertaining to Coliform Prevalence in Domestic Drinking Water Wells.

Year	Location	Researchers	n	Coliform Positive Wells (%)
Spring 1990	Kings Co, NS	Reynolds et al., 1990	102	34*
1991-1992	Ontario	Goss et al., 1998	1292	34▫
Fall 1992	Huron Co, Ont	Fleming, 1992	301	34*
1993	Ontario	Ag. Canada, 1993	1300	34*
1994-1995	Nebraska	Gosselin et al., 1997	1808	15□
Spring 1996	Ontario	Conboy and Goss, 1999	300	48*
Summer 1997	Ontario	Conboy and Goss, 1999	300	49*
June 1997	Zimbabwe	Conboy and Goss, 1999	148	95*
1997	Alberta	Fitzgerald et al., 1997	857	8*
1997	Argentina	Marteau et al., 1998	62	49°
Fall 1999	Bragg Creek	ENSC 502,U of C	81	20*/15▫

Note: * ≥5 total coliforms/100mL and/or ≥1 fecal coliform/100mL (Ontario Drinking Water Objective)
▫ ≥10 total coliforms/100mL and/or ≥1 fecal coliform/100mL (Canadian Water Quality Guideline)
□≥1 coliform bacteria/100mL
°≥1 *E.coli* bacterium

Gosselin et al (1997) studied the domestic well water quality of 1,808 rural wells in Nebraska, a state whose economy is based mainly on agriculture. As well as containing nitrate-nitrogen and pesticides exceeding the drinking water standards allowed for this state, coliforms were found to be present in 15% of wells sampled statewide (Table 1.1). Results from the microbial analysis of the study emphasized the effect of well construction on bacterial water quality. Of the open-jointed casings (concrete, brick, or tile), more than 43% showed evidence of coliform contamination. Conversely, only 12% of PVC and steel-cased wells were found to contain coliforms.

Conboy and Goss (1999) further emphasized the concern regarding illnesses caused by the consumption of bacteriologically contaminated groundwater in rural areas. In domestic drinking water samples taken from over

300 wells in Southern Ontario, 48% exceeded the provincial Ontario Drinking Water Objective (ODWO) for total or fecal coliforms in spring and 49% in summer (Table 1.1). These same researchers sampled wells located in Zimbabwe in June 1997, during the dry season. In this region 95% of wells were found to exceed the ODWO and 59% had fecal contamination (Table 1.1). This comparison of soil moisture and type, well construction and land management showed that a significant percentage of bacteria of fecal origin found in rural wells originated from animal manure.

Vectors of Bacterial Well Contamination

Contaminant sources, such as field-applied manure, livestock pens, and septic systems are generally located at or near the surface. In order for microorganisms originating from these sources to occur in the well environment, a migration pathway from source to well must be established. Many studies have found that groundwater samples taken from certain wells consistently exceed the water quality guidelines, whereas groundwater samples taken from other wells do not (Conboy and Goss, 1999; Goss et al., 1998; Raina et al., 1998).

There are many features of a well and location that may produce a natural susceptibility to contamination (Conboy and Goss, 2000). Shallow dug or bored wells, located in sites where there is a thin soil profile or shallow water table, were found to be most vulnerable to contamination. Older wells, wells situated

in limestone, dolostone, clay and loam were found to be at the highest risk of contamination.

A major contributor to drinking water quality is the integrity of the well itself (Mackler and Merkle, 2000). Old or improperly placed or dug wells may allow water to enter the well directly form the surface (short-circuiting). Wells sited close to feedlots and exercise yards, and farms that utilized manure spreading for fertilizer have been correlated with increased fecal contamination in their drinking water (Goss et al., 1998). Higher microbial concentrations were found in wells located on farms which manure was spread one month prior to spring sampling (Conboy and Goss 2000). In many cases this time period would have been prior to snow melting and spring thawing. They suggest that this farming practice may contribute to the poor water quality presented in the results. This relationship seems to suggest that infiltration from snowmelt may be a major vector in the transport of microbes from the surface to the water table.

Differentiating between fecal contamination originating from different sources is difficult (Hagedorn, 1978), as feces produced by any warm-blooded mammal, once degraded in the subsurface environment, are virtually similar. Attempts using fecal coliform-fecal streptococci ratios have not been successful in distinguishing different pollution sources (Brion and Lingireddy, 1999). Antibiotic resistance has been used to distinguish agricultural bacterial contamination from rural (Whitlock et al., 2002). In addition, neural network

analysis (Brion and Lingireddy, 1999) and DNA technology such as PCR and subsequent RNA sequencing enable some well-equip scientists to seek out bacteria that can only be found in human intestines (Kreader, 1995), thus allowing for better estimations of contamination sources.

It is clear large volumes of animal manure, or high densities of private sewage disposal systems in one area can contribute to the degradation in water quality in the immediate area. However, the strong relationships found between sources of contamination and water quality in all the above-mentioned studies suggests that the residence time of fecal bacteria in groundwater is relatively short. Transportation within the subsurface for long distances over extended periods of time seems unlikely.

Bacterial Species in Groundwater, Abundance and Distribution

Many studies have been conducted for the purpose of identifying the types of bacterial species native to aquifer sediments and well water samples. Although various species have been identified in many groundwater studies, metabolic, morphological, and genetic properties of dominant strains seem to be similar throughout. Important mechanisms of control include inter-specific competition and metabolic diversity allowing opportunistic, genetically stable species to best adapt to changing groundwater conditions.

A study conducted in northern Germany exposed packets of sandy sediments to groundwater for 12 weeks at depths of 10 and 20m. Both sterilized

and non-sterilized sediments were exposed. Growth of natural populations on both sediment types was similar at the end of the study period (Hirsch and Rades-Rohkohl, 1990). Presumably natural groundwater species are able to maintain an active and increasing community within the aquifer. Bacterial abundance, enumerated using plate count methodology, found most samples to contain 10^7-10^8 bacteria per gram of dried sediment (Hirsch and Rades-Rohkohl, 1990). Colony counts arising from a study on an Oklahoma floodplain were collected from the shallow sediments (<6m) above and below the water table at the study site. The sediment samples were then examined both bacterial using rich media (viable cell counts), as well as light and electron microscopes (total cell counts). The reported a range of bacteria isolated using rich media was 10^2 to 10^6. However actual cell counts using microscopic analysis found the range to be on the magnitude of 10^6 for most samples (Balkwill and Ghiorse, 1985). Total cell counts seem to be the most consistent and highest values reported in this study and thus are likely the most accurate enumeration technique.

Aeromonas hydrophila was the most dominant and persistent bacterial strain observed during a 4 yr study on water from a 38m deep water well (Kuhn et al., 1997). Although many other strains were identified, their presence in the well seemed to be more transient. The genetic instability of this strain may allow better adaption to the well environment, increasing the competitive advantage of *A. hydrophila* over time (Kuhn et al., 1997).

Of more than 500 colony isolates grown on low-nutrient agar were identified from two continually-pumping drinking water wells (151m and 185m depth) over a two-year period, gram-negative, rod-shaped, non-motile species were the most prevalent in the resulting samples (Stetzenbach et al., 1986). *Acinetobacter spp.* compromised 70% and 37% of the total species composition of the two different wells.

Investigations in shallow sediment samples (1-5m depth) on an Oklahoma floodplain used Electron Microscopy (EM) to identify bacterial species in aquifer sediments. This study concluded that aerobic, nutritionally versatile species that are able to survive in a low nutrient environment without forming resting cells, dominated the bacterial population (Balkwill and Ghiorse, 1985). Facultatively anaerobic bacteria and microeukaryotes were also present in significant concentrations.

Bacterial Survival and Transport in Groundwater

Well water contamination requires the transport of sufficient amounts of viable bacteria through the substratum. Environmental factors controlling bacterial survival and transport are thus relevant to the migration of pathogenic bacteria from contamination source to groundwater and water wells. These factors can act on the bacteria to affect the viability of the organism over time and space. For example, on the most basic level, more conducive environmental conditions will allow bacteria to survive for longer periods; and be transported further within the subsurface, with a higher potential for well contamination downgradient of the source.

Under favorable conditions enteric bacteria may be able to survive within the subsurface for an indefinite amount of time. However, the subsurface environment does not usually favor the survival of introduced microorganisms (Pavelic et al., 1998). Enteric bacteria have not evolved to live in groundwater and so survival for long time periods allowing for the contamination of wells downgradient of the pollution source are restricted to situations and conditions allowing for their persistence.

The major controls influencing bacterial survival in groundwater have not been well explored. These can include microbial growth, death, starvation, predation, filtration, and chemotaxis (Peterson and Ward, 1989). This may be partly because subsurface bacteria are highly diverse, and each species is affected more or less by specific environmental conditions. Major controls of

bacterial survival include the same parameters that are relevant to bacterial survival in any other environment; temperature, pH, and nutrients including organic matter. Lower temperatures appear to be most favorable for prolonged survival of enteric bacteria (Yates and Yates, 1988). In addition, predation (Kinner et al., 1998), competition from other microbes, and life cycle dynamics may also contribute to the length of time bacterial species are able to survive in the groundwater environment.

The ability of a bacterium to survive for long periods, as well as its ability to be transported within a groundwater aquifer may be affected by pH. Neutral pH values are most favorable to bacterial survival and growth (Yates and Yates, 1998). Bacteria and soil particles tend to have negatively charged surfaces, which cause repulsive forces that may hinder sorption onto soil colloids. In certain cases reduced pH in soil bacteria may decrease the repulsive charge with consequentially increased bacterial attachment to soil particles (Yates and Yates, 1998). However, a study conducted in a saturated environment exploring the effect of pH on bacterial attachment did not find any significant relationship (Harvey and Metge, 2000).

Land-use in the region of groundwater recharge may be one influence on bacterial ability to survive and persist in the subsurface (Dodds et al, 1996; Johnson et al., 2003). This may be some distance away from and unrelated to the groundwater monitoring system. Land-use patterns can and do affect all the above-mentioned factors contributing to microbial contamination, including the

species of microbes responsible for contamination. For example, a highly manured agricultural field will increase nutrient availability, which would allow for the survival of more bacteria for longer periods.

The movement of bacteria once introduced into an aquifer is affected by abiotic and biotic controls. Factors that aid in the movement of microorganisms are the concentration of dissolved nutrients in the groundwater, and the groundwater movement itself. Factors hindering the movement of bacteria can usually be classified into two categories, sorption and filtering.

Coliform bacteria are approximately 0.2 to 10 μm in length. The transport or filtering of bacteria through porous media depends on the size and shape of the cell itself; more mobile bacteria having a smaller overall surface area, length, and width (Weiss et al., 1995). Bacterial filtering can be defined as the preferential removal of large bacteria by straining through soil particles and organic matter colloids. We would assume that soils with small pore sizes or larger organic macromolecules are more efficient in filtering large bacteria.

The effect of pore size on the filtering of bacteria is additionally compounded by increased advection. Advection is a term used to describe the transport of a substance through an aquifer solely due to the groundwater movement (Appela and Postma, 1994). Larger overall pore size within the aquifer allows groundwater velocity to increase, this in turn will increase transportation through advection as well as decrease inactivation by filtering. Hagedorn et al (1978) observed these effects when studying the movement of *E.*

coli and *Streptococcus faecalis* introduced into pits of two different depths, one of which had a higher clay content than the other. Both organisms traveled two to ten times farther in the silty loam soil than in the silty clay loam.

Sorption, the electrostatic attraction between bacterial cells and soil surfaces, exerts a major influence on the transport of microorganisms through porous media (Yates and Yates, 1988). Studies have shown that variables such as ionic strength and pH of the solution influence bacterial sorption through their effect on charge density and electrostatic repulsion (Hendry et al., 1997; Fontes et al., 1991). Increasing the strength of the ionic solution may increase cell attachment to sorption sites within the soil matrix. When the ionic solution of sand soil columns was increased using hydrogen peroxide (H_2O_2), bacterial transport rates in sand soil columns decreased, possibly by the removal of inorganic C, unblocking inorganic exchange sites (Lindqvist and Bengtsson, 1995).

There are two main kinds of sorption to consider; reversible and irreversible. Reversible sorption is transient whereas irreversible involves the permanent sorption of bacteria to a particle surface. Both types of sorption reactions can be described by a first-order kinetic relationship (Hendry et al., 1997). Thus, omitting any other bacterial process, the further a set concentration of bacteria move through porous media the less the dissolved concentration will be as bacteria are sorbed onto particle surfaces.

The effect of groundwater velocity (advection) on bacterial transport further emphasized the importance of surface chemistry and sorption as a control on transport rates. The rate of transport through silica sand was measured for two different types of bacteria, *Klebsiella oxytoca* and *Burkholderia cepacia* (Hendry et al., 1999). The breakthrough curves for each bacterial type in these column experiments concluded, that although water velocity affects bacterial transport rates, the effect is highly dependent on the type of bacteria used. Differing sorption rates were a greater influence on transport velocity than the conductive force.

Many attempts have been made to accurately model the movement of bacteria within an aquifer (eg. Pang et al., in press; Sinton et al., 1997; Reddy and Ford, 1996). The majority of models encountered in the literature entail some variation of the advection-dispersion model modified to include relevant bacterial processes using phenomenological coefficients. As mentioned previously, advection is the transportation of the microorganism caused by the movement of groundwater. Bacterial dispersion can be described as the dilution of microorganisms in flowing water by physical forces (Appelo and Postma, 1994). For example, if a known concentration of bacteria were introduced into an aquifer at a specific point, the organisms would tend to spread out from the source, in the direction of flow. This spread is mainly due to the fact that the organisms must move around the in situ soil particles in order to follow the hydraulic gradient.

The advection-dispersion equation (Peterson and Ward, 1989) was modified specifically for bacteria and can only roughly describe bacterial contamination from "point-sources". Estimating bacterial transportation in areas where contamination sources are not as well defined, such as agricultural settings, is severely restricted. Although this equation considers the importance of dispersion, diffusion, sorption, and advective flow on bacterial transport, it fails to consider other important aspects that may influence microbial survival in the subsurface, such as reproduction and death.

If pathogenic bacteria associate to a high degree with sediment particles within the aquifer, then a problem arises in obtaining an adequate representation of the groundwater when sampling. In the past, when turbid water was obtained from a monitoring well, the suspended solids were presumed artifacts of groundwater collection and not present or mobile in the subsurface (Backhus, 1993). Consequently, samples were filtered using 15 or 30 nm polycarbonate membrane filters to remove suspended solids. It now seems possible that at least a portion of the suspended solids removed by filtration are actually mobile in the aquifer under natural groundwater flow conditions (Backhus, 1993). This mobility in suspended solids may increase transport rate particle-associated microbes within the aquifer as well as help to maintain the viability of the microbes themselves. This gives relevance to the practice of pre-filtering turbid samples before analysis for coliforms.

It seems intuitive that higher availability of easily accessible nutrients such as dissolved organic carbon (DOC) would also be favorable for bacterial growth. The dynamics between nutrients associated with soil particles and those dissolved in the groundwater may strongly affect the amount of free-living bacteria (FLB) as opposed to bacteria sorbed to the surface of soil particles. If dissolved nutrients are abundant, the amount of mobile bacteria may increase, thus increasing subsurface transportation. A strong correlation ($r=0.80$, $p<0.05$, $n=26$) between DOC and FLB was found in a plume of sewage contaminated groundwater in Cape Cod, MA (Harvey and Barber, 1992). A significant correlation between specific conductance and FLB ($r=0.75$) was observed in the same plume where specific conductance was thought to reflect the degree of dilution of the sewage effluent by groundwater. However, the degree of association between the sediment and groundwater bacteria seems to be strong. Up to 100% of the groundwater bacteria, and up to 50% of the fecal coliforms taken from in situ wells from a karst aquifer were found to be attached to suspended sediments at various times (Mahler et al., 2000).

It is clear from the arguments made in this thesis that particle size greatly influences the mobility or retardation of subsurface bacteria (Backhus, 1993). As well, the dynamics between sorbed and FLB influence the likelihood of bacterial transmission in the subsurface, but are not well explored (Harvey and Barber, 1992). Further study is needed on the effect sorption has on the viability of an organism as opposed to freely mobile bacteria. Contributions of dissolved

nutrients, sorbed nutrients and the temporal distribution and type of contamination interact in a complex manner to produce the observed effects on groundwater pathogens. These interactions must be carefully examined and understood in order to allow maximum protection from waterborne disease outbreaks.

This study investigates potential groundwater contamination in areas expected to be impacted by fecal material though both point-source contamination (septic systems) and non-point sources (manure and sludge application to land). Bacterial contamination was assessed using coliform analysis along with other relevant biotic and abiotic parameters. In addition, this study includes a paper on the problems with assessing coliforms in certain groundwater supplies. Atypical or non-coliform growth on plates may bias results and underestimate coliform contamination.

Sample Collection and Analysis Methodologies

Currently there are no government regulations regarding the protocol for field sampling of monitoring well or drinking water wells for coliforms or other pathogenic microbial indicators. The United States Environmental Protection Agency (USEPA) has recently proposed a "Ground Water Rule" that acknowledges the need for monthly sampling of source groundwater for systems that serve 25 or more individuals, as well as Sanitary Surveys and Hydrogeologic Sensitivity Assessments (USEPA, 2002). Although this proposed rule was implemented to protect groundwater users against exposure to fecal indicators, methodology regarding the sampling for microorganisms is not included. Simply put, the objectives of sampling from any given well should be to recover a representative sample of FLB present in the aquifer water. Thus adequate pumping of the well to remove standing water is necessary. Also, care must be taken to ensure excess sediment located on the bottom of the well is not stirred and integrated in with the water sample.

The natural abundance of environmental microflora throughout the environment necessitates the need for sampling techniques that shield against soil or even air-born bacteria from entering the sampling equipment during the sampling event. Traditionally, groundwater monitoring wells have been sampled using two types of equipment, an Inertial Foot Valve (IFV) or a peristaltic pump (PP). In either situation it is important to sterilize all components of the sampling equipment that come into contact with the water sample. Initially the

feasibility of sterilizing equipment in the field with 70% ethanol was tested. The peristaltic pump used much less ethanol. In addition, the Tygon® flexible plastic tubing used in the peristaltic pump could be sterilized in the autoclave prior to sampling events and stored in individual bags to minimize environmental contact until use. The stiff tubing used with a IFV did not have these benefits and was therefore discontinued. Tubing was cut to length according to well depth and was used to sample one well only; this was done to ensure cross-contamination would not occur between the individual wells. In addition, advantages to the peristaltic pump were noticed because the pumping rate could be set at a low and constant rate which minimized the turbidity of the resulting sample and thus the bacteria that may be sorbed to the surface of those particles. Also, the incoming sample could be easily attached to a flow-thru cell to analyze dissolved oxygen (DO), electrical conductivity (EC) and other parameters of interest. After measuring the well parameters this tubing can then be moved to inert, autoclavable polypropylene bottles for sample collection. Field situations can be very windy and blow surface soil into the sample, a small mouth bottle was found to be best for shielding the sample from atmospheric contamination. The use of field, trip and equipment blanks were employed to ensure the methodology was sound. This method was found to be cost effective and feasible for repetitive sampling of specific wells, which was the case for all the investigations in this study. The exact methodology used in each of the investigations can be found in their respective chapters.

Standard Methods for the Examination of Water and Wastewater (American Public Health Association (APHA), 1988) outlines two methods for the quantification of coliforms in wastewater. The most probable number (MPN) also called multiple-tube fermentation (MTF) method #9221, as well as the direct plate count or membrane filter method #9222. A detailed discussion of these two methods is discussed in Chapter 4. In addition to coliforms, Heterotrophic Plate Counts (HPC) were assessed for most samples in these investigations. The methodology for HPC analysis can also be found in Standard Methods #9215.

Nitrate, chloride and phosphate analysis was conducted using both Technicon Autoanalyser [Industrial Method Nos. #100-70W-B (Nitrate), #94-70W (Phosphorus), #99-70W (Chloride)], or using a HACH DR2000 and DR2010 spectrophotometers depending on the study. Appropriate standard curves were generated for each analyte. In the detection limits were 0.05mg/L for all nutrient analysis.

Data obtained from bacterial plate counts cannot be analyzed using traditional statistical methods. The use of varying dilutions of the source water as well as the intrinsic variability of the plate count data produces highly positively skewed distribution. Data sets can rarely be transformed to normalize data. Using data obtained from plate counts include "non-detects" which cannot be considered zero. It can only be assumed that these samples have concentrations less than the detection limit, which may change depending on the amount of atypical bacteria within the sample. The intrinsic variability of

bacterial plate counts must be considered when evaluating data obtained to better interpret influencing factors and the quality of the data obtained. Due to distribution of these results coliform data is generally limited to temporal graphs or correlations.

CHAPTER TWO: EFFICACY OF ANNUAL BACTERIA MONITORING AND SHOCK CHLORINATION IN WELLS FINISHED IN A FLOODPLAIN AQUIFER

Introduction

Coliform bacteria are one of the most frequently exceeded water quality criteria in surveys of rural well water quality (Briggins and Moerman, 1995; Gosselin et al., 1997; Goss et al., 1998; Tuthill et al., 1998; Macler and Merkle, 2000). Private well water quality is usually a homeowner's responsibility. Water quality sampling frequencies vary widely, but annual monitoring is commonly recommended. In many instances, water quality sampling of private well water is only conducted when properties are sold. When bacterial contamination is detected, shock chlorination is usually recommended, followed by immediate testing, and then subsequent resumption of annual monitoring. Shock chlorination was historically developed to remediate well clogging from iron bacteria (Driscoll, 1986), but is now routinely used to disinfect wells and distribution systems with suspected coliform contamination (Buchanan et al., 1998). Shock chlorination implicitly assumes that the origin of contamination is from the well, and is not an aquifer problem.

It is usually not clear if the coliforms found in well water originate from colonies previously established within the water well and/or associated distribution system, or if the bacteria result from the continuous loading of bacteriologically contaminated groundwater. If coliforms have been introduced to the well directly or by means of poor well design, the bacteria may be able to

survive and reproduce within the well system, resulting in long-term well water impacts if untreated. In this case, shock chlorination should effectively reduce exposure unless colonies are re-introduced and re-established. Shock chlorination would not result in a reduction of coliforms for a significant period of time if the bacteria continually enter the well from the groundwater zone (i.e. through the well screen).

Given the prevalence of coliform contamination of water wells, it is important to evaluate current practices related to private well sampling and monitoring, and the use of shock chlorination as a remediation strategy, particularly in vulnerable hydrogeologic settings. In the context of long-term potable groundwater supplies, it is also important to discern whether bacterial contamination originates from within and/or outside of wells. This study monitored coliform levels in selected private well supplies over a six-month period to evaluate the utility of annual water well sampling. It also investigated the applicability of shock chlorination to remediate coliform impacts in water wells.

Study area

The hamlet of Bragg Creek, Alberta (population ~ 500) is largely developed on the alluvial aquifer of the Elbow River which flows from the Eastern slopes of the Rocky Mountains to the City of Calgary where it enters into the Bow River. Its median monthly discharge ranges from about 14 $m^3 s^{-1}$ (494 $ft^3 s^{-1}$) in

June to 2 $m^3 s^{-1}$ (71 $ft^3 s^{-1}$) in January (Beers and Sosiak, 1993). The floodplain or alluvial aquifer consists of sands and gravels with estimated hydraulic conductivities ranging from 10^{-3} to 10^{-4} m s^{-1} ($10^{-3.5}$ to $10^{-4.5}$ ft s^{-1}; Meyboom, 1961), and is about 600m wide at Bragg Creek. Private wells and septic systems in the Hamlet service about 200 residences. Well water impacts from septic system effluent have long been identified. Four independent water well surveys have reported coliform bacteria in 9.6 to 39 percent of the wells (Table 2.1). Significant correlations between most constituents of septic system effluent (including chloride, coliforms, and nitrates; ENSC, 2000) indicate that septic systems are the main source of the drinking water contamination in the hamlet.

Table 2.1: Percentage of Wells in Bragg Creek Exceeding the Canadian Drinking Water Guidelines for Various Criteria in Four Previous Water Surveys. Note: Each study represents different sampling locations and there is limited overlap between years.

Criteria	Drinking Water Objective	Wells Exceeding Canadian Drinking Water Guideline for Specified Study (%)			
		1975/6[1] n=71	1982-84[2] n=143	1998[3] n=23	1999[4] n=103
Fecal Coliform	0 cfu/100mL	5.6*	15.3*	15.8	12.3
Total Coliform	0 cfu/100mL	9.6*	24.3*	31.6	39.0
Chloride	250 ppm	1.4	2.7	-	2.5
Fluoride	1.5 ppm	0	0	-	1.3
Nitrate Nitrogen	10 ppm	2.7	7.2	-	4

* Estimate was made using Most Probable Number (MPN) methodology rather than a direct colony count
[1, 2]Alberta Environmental Protection (1976, 1984); [3]Calgary Health Authority, unpublished data;
[4] ENSC502, 1999.

Groundwater impacts are invariably observed when septic systems are used in hydrogeologically vulnerable settings, with long narrow plumes typically emanating from individual tile distribution systems (LeBlanc, 1984; Robertson et

al., 1991; DeSimone and Howes, 1998). The Bragg Creek setting is particularly vulnerable to well water impacts from septic systems. It is hydrogeologically similar to that of a septic study in Frenchtown, Montana where rapid virus transport was observed and minimum coliform attenuation expected (DeBorde et al., 1998a, 1998b, and 1999). Also well water impacts by bacteria are typically more severe when septic systems are used in regions with high residential densities (Tuthill et al., 1998) and when shallow dug wells are used (Wireman and Job, 1998; Conboy and Goss, 2000; Francy et al., 2000). Both of these criteria apply to Bragg Creek.

Materials and Methods

Well Monitoring

Data were collated from two well monitoring programs. The University of Calgary monitored well water from six homes (four wells with consistent coliform contamination and two consistently uncontaminated wells). The sampling methodology in this program is the same as that described for the well chlorination study, and sampling was conducted at least every four weeks. These data were complemented with data from a voluntary sampling program offered to Bragg Creek homeowners by the Calgary Health Region (CHR). The CHR provided gratis bacterial analyses for Bragg Creek residents on a biweekly basis. Homeowners conducted the sampling, with total and fecal coliforms (TC

and FC, respectively) and total heterotrophic plate counts (HPC) were enumerated at the Provincial Lab in Calgary, Alberta for the CHR program.

Shock Chlorination Study

The five wells in the shock chlorination study included three experimental wells (E1 to E3; all with persistent coliform contamination and all chlorinated in this study) a negative control (NC; persistent coliform contamination, but not chlorinated) and positive control (PC; uncontaminated and chlorinated). The positive control was included to evaluate if chlorination of an uncontaminated well influenced coliform concentrations for any reason.

In a 1999 well water sampling program (Table 2.1), contamination was generally related to well depth, with deep wells greater than ~20m (66ft) being free from contamination associated with septic effluent (ENSC, 2000). The wells selected for the well chlorination study were all shallow dug wells, lined with large diameter steel culverts (Table 2.2).

Table 2.2: Well Characteristics and Amount of $Ca(OCl)_2$ used for Chlorination.

Well ID	Well Depth m (ft)	Well Diameter m (ft)	Water Depth m (ft)	$Ca(OCl)_2$ needed to produce 1000 ppm in Well Chlorination Procedure Kg (lbs)
PC	3.7 (12.1)	1.2 (3.9)	0.6 (2.0)	1.05 (23.10)
NC	2.6 (8.5)	0.8 (2.6)	0.9 (3.0)	Not chlorinated
E1	3.7 (12.1)	1.2 (3.9)	0.6 (2.0)	0.87 (19.14)
E2	5.2 (17.1)	0.5 (1.6)	1.5 (4.9)	0.45 (9.90)
E3	3.7 (12.1)	1.5 (4.9)	1.2 (3.9)	2.49 (54.78)

Well chlorination was conducted on Nov 22 for wells PC and E1, and Nov 23 for E2 and E3. A modified procedure was required because of the large well diameters (Buchanan et al., 1998). Briefly, calcium hypochlorite was dissolved in

a small amount of water and then added directly to each well and mixed such that a concentration of 1000 ppm as Cl^- was reached in the standing water in the well bore (Table 2.2). An additional 1000L (220gal) of 1000 ppm Cl^- was subsequently added to the well, causing the chlorinated water to seep into and around the gravels surrounding the well casing. Every part of the distribution system including toilets, washing machines and showers were run until chlorine could be detected by smell before the disinfection period to ensure the decontamination of all parts of the distribution system. (Buchanan et al., 1998). The chlorinated water was subsequently allowed to remain in the well and distribution system for an 18-24 hour disinfection period before the system was flushed.

Sample Collection and Analysis

Water samples were collected in sterile 500mL (18oz) polypropylene bottles from taps that by-passed any treatment (i.e. water softeners, charcoal filters, etc.). The water was run through the tap for 5-10 minutes until physical parameters stabilized to ensure the water sampled originated from the well bore. Temperature, dissolved oxygen (DO) and electrical conductivity (EC) were measured in a flow-through cell at the time of sampling. The water samples were then stored at 4°C (39°F) for less than 24 hours before bacterial analyses were conducted. Total and fecal coliform were quantified by standard membrane filtration, and HPC by direct plate counts (APHA, 1998). The Alberta Provincial Health Lab conducted analysis of samples taken between Nov 22nd and

Dec 31st, 2000, in addition to the gratis samples from the CHR study. All other samples were analyzed at the University of Calgary.

The uncontaminated and chlorinated control well (PC) was sampled regularly for the length of the entire study. Monitoring of the non-chlorinated, negative control (NC) was discontinued on Dec 27, 2000 after the well bore was accidentally drained and subsequently replenished with treated imported water. The three experimental wells (E1 to E3) were monitored for six months following chlorination.

Results and Discussion

Correlations of Well Water Parameters

Correlations were conducted to provide information on whether coliform and heterotrophic bacteria were associated with septic system effluent. Septic system plumes are characterized by increased EC, increased temperature and decreased DO (Wilhelm et al., 1994). Although pair-wise correlations were conducted between all measured well water parameters (temperature, EC, DO, HPC, TC, and FC), only statistically significant relationships are reported (Table 2.3). Fecal coliforms were most dependent on septic system-related parameters, while total coliforms correlated with EC, but not with DO or temperature. Total heterotrophic bacteria were not correlated with any of the septic system effluent-related parameters. In addition, all three bacterial types measured showed strong positive correlations with each other (Table 2.3). These correlations

suggest fecal coliform were most related to septic system effluent, and HPC the least related.

Table 2.3: Correlations observed between Total Coliform, Fecal Coliform, Heterotrophic Plate Counts, Dissolved Oxygen, Electrical Conductivity, and Temperature. Only significant correlations are reported.

Variable	By Variable	Spearman Rho Probability (p>IrhoI)*
HPC	Total Coliform	0.0001
HPC	Fecal Coliform	0.0014
Total Coliform	Fecal Coliform	<0.0001
Temperature	Fecal Coliform	0.0007
Dissolved Oxygen	Fecal Coliform	0.0005(-)
Electrical Conductivity	Total Coliform	0.0009
Electrical Conductivity	Fecal Coliform	0.0006
Electrical Conductivity	Temperature	<0.0001

*Non-normal data distribution required the use of a non-parametric, Spearman Rho, measure of association
Note: All relationships were positive unless indicated by (-), which is a negative correlation

Well Monitoring Study

Temporal variation in water wells was significant, but consistent differences in the degree of bacterial contamination were observed (Figure 2.1; Table 2.4). For example, low total coliform concentrations were only occasionally observed and fecal coliforms not detected in the PC well, while E1 consistently had greater than 100 cfu/100mL of total coliform and consistently tested positive for fecal coliform. These monitoring data were used to select which wells should be chlorinated, and which wells should be used as controls in

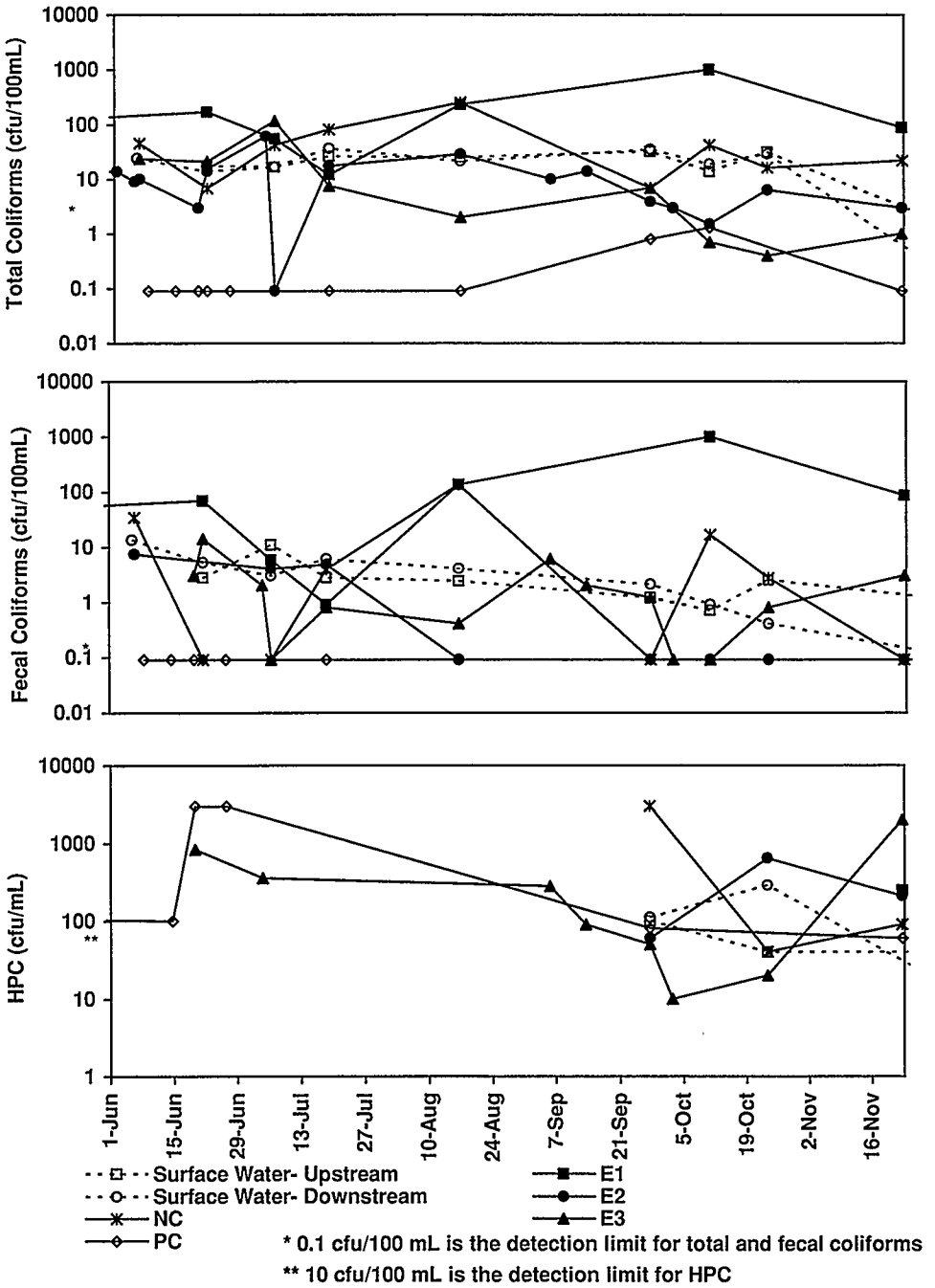


Figure 3.1: Coliform Concentrations in Shallow, Dug Wells in Bragg Creek (June-Oct, 2000)

the shock chlorination study (Table 2.4). Although total coliform water quality standards for single samples are ambiguous (i.e. detections in single samples are permissible, but not in consecutive samplings), no fecal coliforms are acceptable in drinking water. If one considers a detection limit of <1 cfu/100mL for fecal coliform based on the typical sampling size of 100mL, three of the six wells monitored alternately passed and failed the fecal coliform rule over the course of the monitoring study. In these cases, annual monitoring of well water quality would clearly be insufficient.

Table 2.4: Average Bacteriological Concentrations of Individual Well Water Sampling Locations in Bragg Creek, Alberta (June – Nov).

Site ID	Average Total Coliform cfu/100mL [SD, n]	Average Fecal Coliform cfu/100mL [SD, n]	Averaged Heterotrophic Plate Count cfu/mL [SD, n]	Chlori-nated	Use of Sampling Location in Study
PC	0.2 [0.4, 14]	0 [0, 14]	1098 [1477, 6]	Yes	Positive Control
NC	57.0 [76.1, 9]	21.7 [44.4, 9]	569 [1422, 4]	No	Negative Control
E1	225.7 [350.7,7]	187.2 [361.9, 7]	250 [0, 1]	Yes	Experimental
E2	18.3 [33.4, 11]	2.0 [2.8, 10]	376 [251, 5]	Yes	Experimental
E3	12.5 [14.9, 17]	2.2 [3.7, 17]	1592 [3706, 10]	Yes	Experimental

Shock Chlorination Study

No significant changes in temperature, dissolved oxygen, or electrical conductivity were observed between pre- and post- shock chlorination sampling (data not presented). Coliform bacteria in the well with the highest pre-chlorination coliform concentrations (E1) recovered at the fastest rate, with pre-chlorination levels of both total and fecal coliforms (~200 cfu/100 mL) reached

within a week of chlorination (Figure 2.2). Well E2 did not test positive for fecal coliforms until 21 weeks after chlorination. Although E3 tested positive for fecal coliforms in three sampling events between two and four weeks after chlorination, it remained negative for much of the study, testing positive again at the end of the 21 weeks of monitoring.

Heterotrophic bacteria recovered to pre-chlorination levels (10 cfu/mL to 100 cfu/mL) in all three wells at a similar and relatively fast rate (two to three weeks; Figure 2.2). This suggests the heterotrophic bacteria are more related to biofilms developed in the well and distribution system than they are to the influx of bacteriologically contaminated groundwater. Biofilms are gel-like slimes comprised of natural bacterial communities that typically provide bacterial protection from disinfection. They grow on most surfaces including commonly used materials in water well and distribution systems (Momba et al., 1998). Water that passes through vessels with biofilms can accumulate bacteria by shedding bacterial "clumps" off a mature biofilm (McMath et al., 1999) which can contain both heterotrophic and coliform bacteria (LeChevallier, 1999). The relatively rapid rate of heterotrophic bacteria recovery may be related to their presence in and protection by biofilms in the well and/or distribution systems. This is consistent with the lack of correlation of HPC with any of the septic system-related parameters (i.e. EC, dissolved oxygen, and temperature).

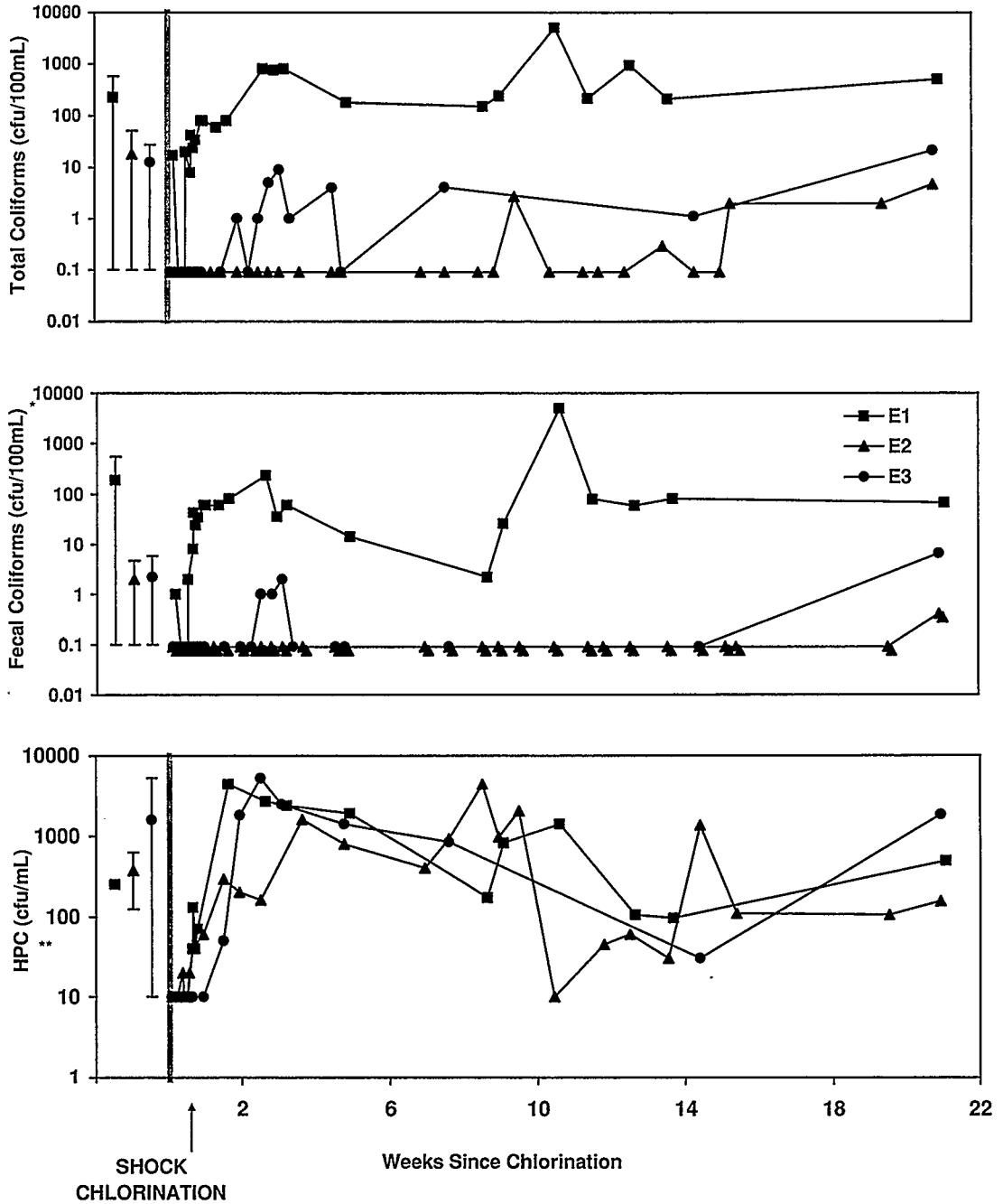


Figure 3.2: Coliform and HPC Concentrations of Shallow, Dug Wells in Bragg Creek following Shock Chlorination. Average pre-chlorination concentrations and standard deviations (May to November, 2000) are indicated on the left hand side.

The recovery of fecal coliform in all three wells suggests that pathogens can survive for long enough time periods to facilitate their transport through the aquifer. The elimination and subsequent recovery of bacterial populations in water samples taken just a few days following chlorination in E1 most likely can be attributed to the continual-loading of groundwater contaminated from upgradient septic systems.

The reason for the initially lower degree of contamination in wells E2 and E3 and the longer time periods for fecal coliform to re-appear after chlorination are not clear. There may be only a slight intersection between the capture zones of E2 and E3 and septic system plumes, and the degree of intersection may vary with seasonal changes in water table elevation and groundwater flow direction. Alternatively, the return of coliforms in these wells may be due to slow re-growth of coliform colonies previously established within the well or well casing, and perhaps protected by biofilms within the well and distribution system.

The chlorinated, positive control well (PC), was free of coliform contamination for every sampling event following chlorination and showed no artifacts caused by chlorination of an uncontaminated well. The negative control well (NC), which did not test positive for fecal coliform during the 6 month monitoring period, intermittently tested positive for a few sampling events before sampling at this site was discontinued on Dec 27, 2000 (data not shown). This behavior is consistent with the variability observed in the monitoring study and may be due to seasonal changes in groundwater flow direction.

Conclusions

There is reason for concern over well sampling, monitoring, and remediation practices in hydrogeologically vulnerable areas that depend on private well supplies and septic systems. Three of the six wells monitored in this study alternately tested positive and negative for fecal coliform over the course of the monitoring study. This variability in coliform concentrations over time may cause annual or even quarterly well monitoring programs to be inadequate and inappropriate in this setting. Potable water supplies from such wells require continuous disinfection (i.e. 99.99% or 4-log pathogen reduction) for use.

The general assumption that shock chlorination will eliminate pathogenic bacteria from a well for a significant amount of time is not supported by the findings of this paper. The amount of time observed for coliform reappearance after shock chlorination was related to pre-chlorination coliform concentrations observed in the monitoring study, with the highly contaminated well being re-contaminated in the shortest time period. All three wells returned to pre-chlorination bacterial levels within two to 21 weeks after chlorination. Although shock chlorination is effective at decreasing bacterial concentrations over very short time periods, it cannot be considered an effective remediation strategy for the elimination of coliform contaminated wells in the alluvial floodplain environment studied here.

The pattern of re-appearance of heterotrophic bacteria observed within two weeks of chlorination for all three experimental wells, in combination with

their apparent lack of correlation with other septic system-related parameters (temperature, DO, and EC), suggests heterotrophic bacteria are present as biofilms within water wells and their associated distribution systems and are not directly related to contaminated groundwater. The relatively rapid heterotrophic bacterial recovery suggests the biofilms are resistant to chlorination. It is also possible that biofilms may shield pathogenic bacteria from disinfection by shock chlorination.

CHAPTER THREE: PATHOGENS IN GROUNDWATER FROM LAND APPLICATION OF MANURE AND SEWAGE SLUDGE

Introduction

Manure and sludge application to land are common practices that are not stringently regulated. There is little knowledge about their groundwater impacts despite the fact that coliforms are typically detected in 30 to 50% of domestic water well surveys (Macler and Merkle, 2000; Conboy and Goss, 1999; Gosselin et al., 1997; Table 1.1). Coliforms are used as indicators of pathogenic bacteria. Total and fecal coliforms [including *E. coli*] continue to be the most widely used bacteriological indicator for drinking water quality in Canada (Health Canada, 1999) and the United States (USEPA, 2000). Coliforms, even at low concentrations, are particularly important when assessing drinking water quality.

Although the impact of manure and sludge application on groundwater quality in agricultural areas has been the subject of recent concern in Canada (Macler and Merkle, 2000) there are few applied field studies. In studies and surveys that have been conducted a high percentage of waterborne disease outbreaks have been associated with groundwater supplies (e.g. 15 of 17 events reported in the U.S. in 1997 and 1998; Barwick et al., 2000). Recent events in Walkerton, Ontario attributed the deaths of seven citizens to *E.coli* O157:H7 found in the local water supply. This event produced much attention and highlighted the potential seriousness posed by microbially contaminated groundwater.

The City of Calgary currently disposes of its sewage sludge by land application. An ongoing monitoring program, conducted by the Wastewater Division, measures heavy metals in soils under fields receiving sewage sludge, but does not monitor for groundwater or soil pathogens.

There is also a significant amount of intensive livestock activity in southern Alberta. Although the manure produced from these activities is ideally applied to land at agronomic rates (i.e. as a fertilizer rather than as a waste product); this is often economically unfeasible in larger operations due to high transport costs. Although manure application rates are now regulated in Alberta according to crop needs (National Resources Conservation Board, 2002), there is little information on the microbiological impacts on underlying aquifers. Groundwater wells located in close proximity to feedlots and exercise yards, as well as farms that utilized manure spreading for fertilizer, have been correlated with increased fecal contamination of well water (Goss et al., 1998).

This study assesses the concentration of selected nutrients and bacterial species in the groundwater of two distinct study areas within Alberta; one where soils receive sludge and a second where cattle-manure application is common. In addition to assessing the concentrations of nutrients, total and fecal coliforms, and general heterotrophic bacteria, specific identification of selected representative colonies were further assessed for identification to the species level.

Methods and Materials

Study Areas

The sludge-amended study area is located in the vicinity of the City of Calgary's sludge lagoons on the southeastern rural outskirts of Calgary, AB near the town of Shepard, AB. Groundwater samples from this site were collected from five groundwater monitoring wells located around the town of Shepard, AB. This area has a moderate level of agricultural activity and is the primary area that receives sewage sludge application as a means of soil amendment for agricultural land due to its proximity to the City of Calgary sludge lagoons. The three Calgary Sludge ('CS') wells (CS1, CS2 and CS3) are located on the downgradient edge of fields that have received sewage sludge application once per annum for a minimum of three consecutive years, previous to the year 2001 (pers. comm Ted Tatum, 2001). Two additional wells (CNSP and CNSF) were used as reference sites and are located in areas that have not received sludge application during the past ten years. All wells located at the sludge-amended study location are comprised of PVC tubing (5 cm O.D.) with various screen lengths, located between 1.5 to 8.0m below the water table (Table 3.1). In some instances, sampling at certain wells was discontinued as a result of low sampling yields and/or dry wells.

Table 3.1: Well Location, Construction and Geologic Records from wells at Calgary Sludge Amended and Lethbridge Manure Amended Study Sites

Well Nest	Well #	Screen Depth (m) Top	Bottom	Geologic Log Depth (m)	Sediment Description	Location (Md-Ts-Rg-Sc-Qs)
CALGARY SLUDGE-AMENDED SITES						
CSA1-	1	3.6	5.2	0.8	sandy silt	W4-23-29-13-NW
	2	9.1	10.6	1.5	silty clay	
				4.2	clay till, medium brown, slightly moist with occasional stones	
				5.0	clay till, Iron staining, brown grey	
				5.2	sand stringer	
				6.1	clay till, medium brown, slightly moist with occasional stones and coal fragments	
				9.8	clay till, dry with bedrock fragments and white weathered inclusions	
				10.6	shale, light grey, dry	
CSA2-	1	3.0	3.9	0.3	topsoil	W4-22-28-14-NW
	2	9.1	10.3	1.1	fined grained clay, medium brown, occasional stones	
				5.6	silty clay till with iron deposits, occasional mottling and many sand fractures	
				5.8	sandstone	
				7.6	silty clay till with iron deposits, few sand fractures and many rocks	
				9.1	sandy silt, medium grained till	
				9.7	silty clay till, light grey/brown, slightly moist and few rocks	
				10.2	sandy silt, medium grained till	
				10.3	silty clay till with iron deposits, few sand fractures and many rocks	
CSD-	1	4.9	10.6	0.3	topsoil	W4-23-29-11-NW
				10.6	sandy clay, brown, some rocks	
CNSA-	1	5.8	7.3	3.3	clay, medium brown, oxidized iron, CaCO3 flecks, pebbles	W4-23-28-09-SW
	2	4.5	6.1	4.2	silty sand, medium brown, medium grained with gravel	
				5.5	sand, medium brown, medium grained, moist	
				6.1	sand, medium brown, medium grained, very moist, few bedrocks	
				6.8	sand medium to dark brown, occasional coal and pebbles (2-3mm diam)	
				7.3	shale bedrock, light brown	
CNSP-	1	1.5	5.4	1.2	brown silt and gravel	W4-23-29-11-NW
				5.8	clay till, brown, pebbles, Fe stains and salty streaks	
				6.1	sand stringer	
				7.6	till with bedrock fragments	
				8.5	silty till, brown	
				9.1	till and weathered bedrock	
				10.0	silty shale, grey	

Legend
C-Calgary
S-Sludge Amended Site
NS-No Sludge Application (>10yrs)
A-Agricultural Field
D-Domestic Well
P-Adjacent Calgrow Sludge Ponds

Table 3.1: Well Location, Construction and Geologic Records from wells at Calgary Sludge Amended and Lethbridge Manure Amended Study Sites, Continued.....

Well		Well		Screen Depth (m)	Geologic Log	Location (Md-Ts-Rg-Sc-Qs)
Nest	#	Top	Bottom	Depth (m)	Sediment Description	
LETHBRIDGE MANURE-AMENDED SITES						
LMA1-	2	0.5	3.6	4.2	sandy loam, course grained, oxidized, fluvial	W4-11-20-2-NW
	7		5.3	5.5	sandy clay, medium grained, lacustriane	
				6.1	loamy sand, course grained, oxidized, fluvial	
				7.0	sandy clay loam, medium grained, reduced, lacustrine	
				8.4	loamy sand, course grained, reduced, fluvial	
				11.6	clay and sandy clay, course grained, reduced, fluvial	
				14.6	sandy clay loam, reduced, till	
				31.5	clay loam, dense, reduced, till	
				45.0	clay loam, reduced, till	
				54.8	stoney layer, reduced, till	
				58.2	clay loam, dense, reduced, till	
				59.4	Judith river sediment	
LMA2-	2	1.0	4.5	0.9	sandy loam, course grained, fluvial	W4-11-20-15-NE
				4.6	silty clay, fine grained, oxidized, fluvial	
				6.1	sandy clay, medium grained, reduced, lacustrine	
				12.2	sandy clay loam, reduced, till	
LMA3-	2	4.5	5.5	5.5	silty clay, fine grained, oxidized, fluvial	W4-11-20-11-NE
	3	5.5	6.5	6.5	sandy clay, medium grained, reduced, lacustrine	
LMF1-	1	0.5	2.3	1.0	sandy clay, medium grained, oxidized, lacustrine	W4-11-20-10-SW
	2	2.9	3.5	2.5	clay and sandy clay, fine grained oxidized, lacustrine	
				4.5	silty clay, fine grained, oxidized, lacustrine	
				6.0	clay and sandy clay, fine grained oxidized, lacustrine	
				9.1	clay, fine grained oxidized, lacustrine	
				10.2	clay, oxidized, till	
				10.7	clay loam, reduced, till	
LMF2-	1		6.1	1.5	salts, coal, oxidized, top-soil stripped and replaced with fill	W4-08-21-34-SE
				3.0	till, course grained	
				6.0	till, sandy clay, fine grained	
LMF3-	1		6.1	1.8	salts, top soil stripped and replaced with fill	W4-08-21-34-SE
				6.1	sand, course grained, oxidized	
LNMA-	3	3.2	3.5	1.3	sandy clay loam, medium grained, oxidized, lacustrine	W4-11-19-7-NE
				3.3	silty clay, medium grained, oxidized, lacustrine	
				4.6	clay and silty clay, fine grained, oxidized, lacustrine	
				5.5	sandy clay loam, medium grained, reduced, lacustrine	
				6.1	sandy clay, medium grained, reduced, lacustrine	
				7.6	clay, fine grained, reduced, lacustrine	
				9.1	sandy clay, medium grained, reduced, lacustrine	
				16.8	clay, fine grained, reduced, lacustrine	
				22.9	sandy clay, reduced till	

Information for table obtained from well logs made during well instillation (Calgary), and (Rodvang et al., 1998; Olson et al., 2002) (Lethbridge).

Legend
L-Lethbridge
M-Manure Amended Site
NM-No Manure Application
A-Agricultural Field
F-Adjacent Feedlot

In areas where two wells were drilled adjacent to one another, numbers are appended to the well label. The shallowest well is labeled with the number one (i.e. CS1-1 vs. CS1-2 which is deeper). All wells were drilled by Beck Drilling with a 6" auger rig in Octctober 2000 (Table 3.1) except for one domestic well (CSD) and a groundwater monitoring well that was installed by Stanley Associates Engineering Ltd. in March 1989 (CNSP).

The depth of the water table in the Calgary study area is generally 6-10m below the ground surface. The shallow sediments consist of silty and clayey moraine-till (Meyboom, 1961) extending to an average depth of approximately 15m. The till is underlain by the Paskapoo formation of alternating shale and sandstone (Beers and Sosiak, 1993).

Groundwater samples from the manure-amended sites were collected from six monitoring wells installed by Alberta Agriculture, Food and Rural Affairs (Rodvang et al., 1998) located within 15km northwest of Lethbridge, Alberta within the LNID. The monitoring wells were installed in the fall and winter of 1993/1994 (LMA sites), and Spring of 1996 (LMF sites). This area is one of several irrigation districts neighboring the Oldman River and is home to one of the highest densities of intensive livestock operations in Canada (Statistics Canada, 2002). A shallow, unconfined aquifer (with the water table located 1-5m below the soil surface) overlies the eastern part of this region. Three of the monitoring wells are situated downgradient of irrigated agricultural fields, two of which receive annual fall applications of cattle manure (LMA1, LMA2) and one

reference well (LNMA). One was located adjacent to a privately owned feedlot (LMF1) while two monitoring wells are located within the Lethbridge Research Centre's Experimental Feedlot (LMF2 and LMF3, Table 3.1). Silty sand sediments extend from the soil zone to a few meters below the water table (Rodvang et al., 1998). All wells at this site are comprised of PVC tubing (5 cm O.D.) with 1 meter long screened intervals located 0.5-3 meters below the water table (Table 3.1). In some instances, sampling at certain wells was discontinued as a result of low sampling yields and/or dry wells.

Groundwater samples were collected monthly for both study areas, with the exception of June and July where sampling was conducted bi-monthly in attempt to obtain recently recharged groundwater associated with spring runoff and snowmelt. Monitoring of the sludge-amended sites commenced on November 7th, 2001 and continued until July 9th, 2002. The manure-amended sites were sampled from October 22nd, 2001 to July 2nd, 2002.

Field Methods

Groundwater monitoring wells were sampled using a peristaltic pump and dedicated sampling tubing for each individual well to minimize the possibility of cross-contamination between wells. Tubing was sterilized by autoclaving prior to sampling events and wrapped individually until used at the well site. Care was also taken to minimize contamination of the monitoring wells, sampling apparatus, and sample containers by airborne or surface-soil associated microorganisms. Tubing was kept in plastic bag until moment of use and care

was taken to keep the tubing away from possible contaminate sources (e.g. soil, dusty wind). Trip and field blanks were taken during each sampling event to ensure adequate sterilization procedures during sampling.

After initial water level measurements were taken using a standard water-level tape, one bail volume of sampling water was bailed at most sampling sites. At two of the monitoring wells (CS1-2 and CS2-2) 5L only were bailed before sampling because the peristaltic pump was only able to pump very slowly due to the depth to the water table in these wells. During the winter months the water would freeze in the pump tubing before the prescribed amount of water was bailed from the well. In these cases water samples were collected before tubing and water could freeze. After bailing, sampling water was pumped into a flow-through cell in which electrical conductivity (EC), dissolved oxygen (DO), and water temperature were measured. Subsequently, 1L samples were collected in autoclaved sample bottles and were stored at 4° Celsius for a maximum of 24h prior to bacterial analysis. Samples were also collected in acid-washed bottles and frozen in the laboratory for chemical analysis at a later date.

Laboratory Methods

Nitrate, chloride and phosphate were analyzed at the University of Calgary using colorimetric methods. All samples taken prior to March 2002 were analyzed for nitrate and chloride on a Technicon Autoanalyser [Industrial Method Nos. #100-70W-B (Nitrate), #94-70W (Phosphorus), #99-70W (Chloride)]. Samples taken after March 2002, as well as all of the phosphate samples, were

analyzed using HACH DR2000 and DR2010 spectrophotometers. Appropriate standard curves were generated for each analyte. HACH detection limits were <0.05 mg/L for all nutrient analysis.

Total, fecal and atypical coliforms were enumerated using standard membrane filter (MF) techniques (Section 9222; APHA, 1988). For total and atypical bacterial analysis, filtered samples were grown on *m-Endo* media, *m-FC* was used for fecal coliforms. Atypical coliforms were enumerated after 48 hours (instead of the standard 24 hours used for coliforms) to allow adequate incubation time for small colonies to become visible. To estimate the quantity of live heterotrophic bacteria in the samples, the heterotrophic plate count (HPC) method was used with *tryptone glucose yeast media* (Method 9215A; APHA, 1988).

Fecal coliforms colonies from the MF tests taken after November 15th, 2001 were isolated to obtain pure colonies for further identification (media was not available for these test previous to this date). Colonies were looped from the MF plates and streaked onto HPC agar. Isolated and pure colonies were subsequently inoculated by loop into appropriate liquid media and tested to determine if they were *Escherichia coli* or *fecal streptococci* (Method 9221F and Method 9230B respectively; APHA, 1988).

Selected representative morphologies of total and fecal coliforms as well as different morphologies of atypical colonies were isolated from the MF plates for identification to the species-level using the BIOLOG® microbial identification

system. BIOLOG® provides a probable match with a database of bacteria based on their various carbon substrate utilization characteristics. Cultures obtained using the stated methodology are transferred into the BIOLOG® Micrologging™ system and analyzed using the associated Microbial Identification Software™

Results and Discussion

Groundwater from all sampling sites showed seasonal changes in temperature over the course of the monitoring period. These changes were similar between the Calgary and LNID sites (Figure 3.1). All the parameters measured over the course of the study are summarized in Table 3.2.

The water temperature, DO and EC values were similar for the wells in both the Calgary and Lethbridge study areas (Figure 3.1, Table 3.2). Dissolved oxygen measurements were much more variable depending on the sampling location and time of year (Figure 3.1). Wells situated within feedlots (specifically LMF1 and LMF3) showed a consistent decline in EC over the monitoring period. The geochemistry of this well water may have been affected by pen cleaning or maintenance at this site.

Comparison of nitrate, chloride and phosphate levels between the wells located in both study areas show higher nutrient impact in the wells situated in the manure-amended wells as compared to the sludge-amended wells (Figure 3.2; Table 3.2).

Table 3.2: Summary Parameters Measured in Groundwater from Wells at a Manure Amended (Lethbridge) and a Sludge Amended (Calgary) Study Areas.

	Temperature (°C)	Dissolved Oxygen (mg/L)	Electrical Conductivity (mS/cm)	HPC (cfu/mL)	Total Coliforms (cfu/100mL)	Fecal Coliforms (cfu/100mL)	Atypical Coliforms (cfu/100mL)	Nitrate (mg/L)	Chloride (mg/L)	Phosphate (mg/L)
			Manure	*Lethbridge*	*Site*					
Average	**7.9**	**2.63**	**3.78**	**14 633**	**13 708**	**13 495**	**73 012**	**30**	**282**	**0.23**
SD	2.1	2.08	2.05	37 564	50 1000	51 310	269 450	43	241	0.34
Max	12.2	8.60	14.00	221 000	250 000	230 000	1 666 000	243	714	1.63
Min	5.0	0.30	1.03	29	>1	>1	77	0.4	10	<0.05
N	54	55	49	49	53	53	52	47	43	44
#N exceeding MAC	none	n/a	n/a	39	9	4	n/a	none	none	none
			Sludge	*Calgary*	*Site*					
Average	**7.0**	**2.49**	**3.18**	**4 470**	**192**	**2**	**7 849**	**5**	**22**	**0.10**
SD	2.1	1.58	2.21	12 534	448	2	14 416	9	17	<0.05
Max	15.0	8.54	7.58	98 000	1440	5	100 000	4	44	0.31
min	4.0	0.91	0.0012	<10	<1	<1	2	0.70	8	<0.05
N	52	60	57	63	64	64	54	52	44	44
#N exceeding MAC	none	n/a	n/a	41	8	6	n/a	none	none	none
Health Canada MAC	<15	n/a	n/a	10	10**	<1	n/a	45	250	n/a

MAC-Maximum allowable concentration
n/a-no federal or provincial guidelines
*aesthetic guideline
**one test >10 total coliforms or two consecutive tests having >1 total coliform exceeds MAC

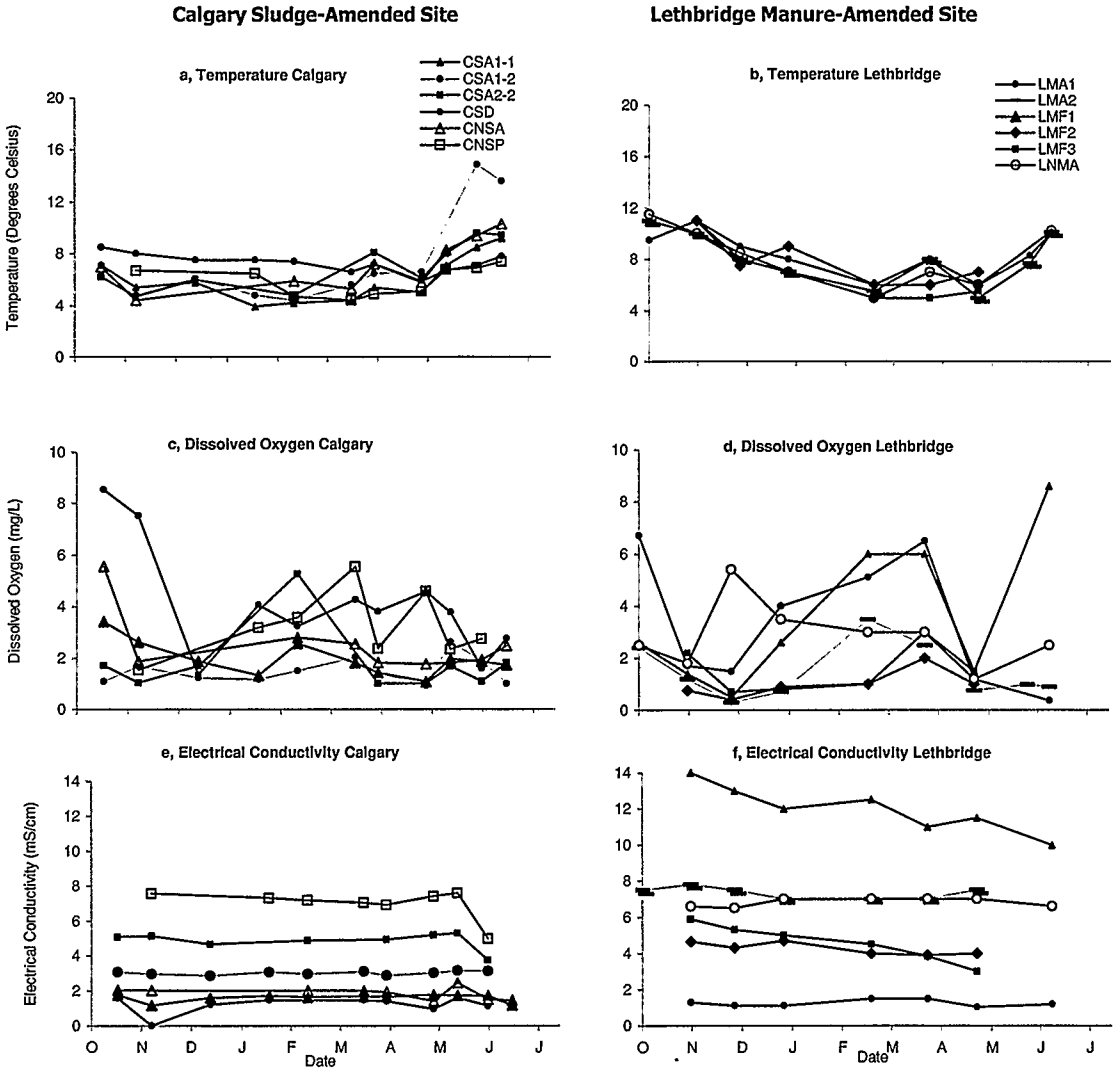
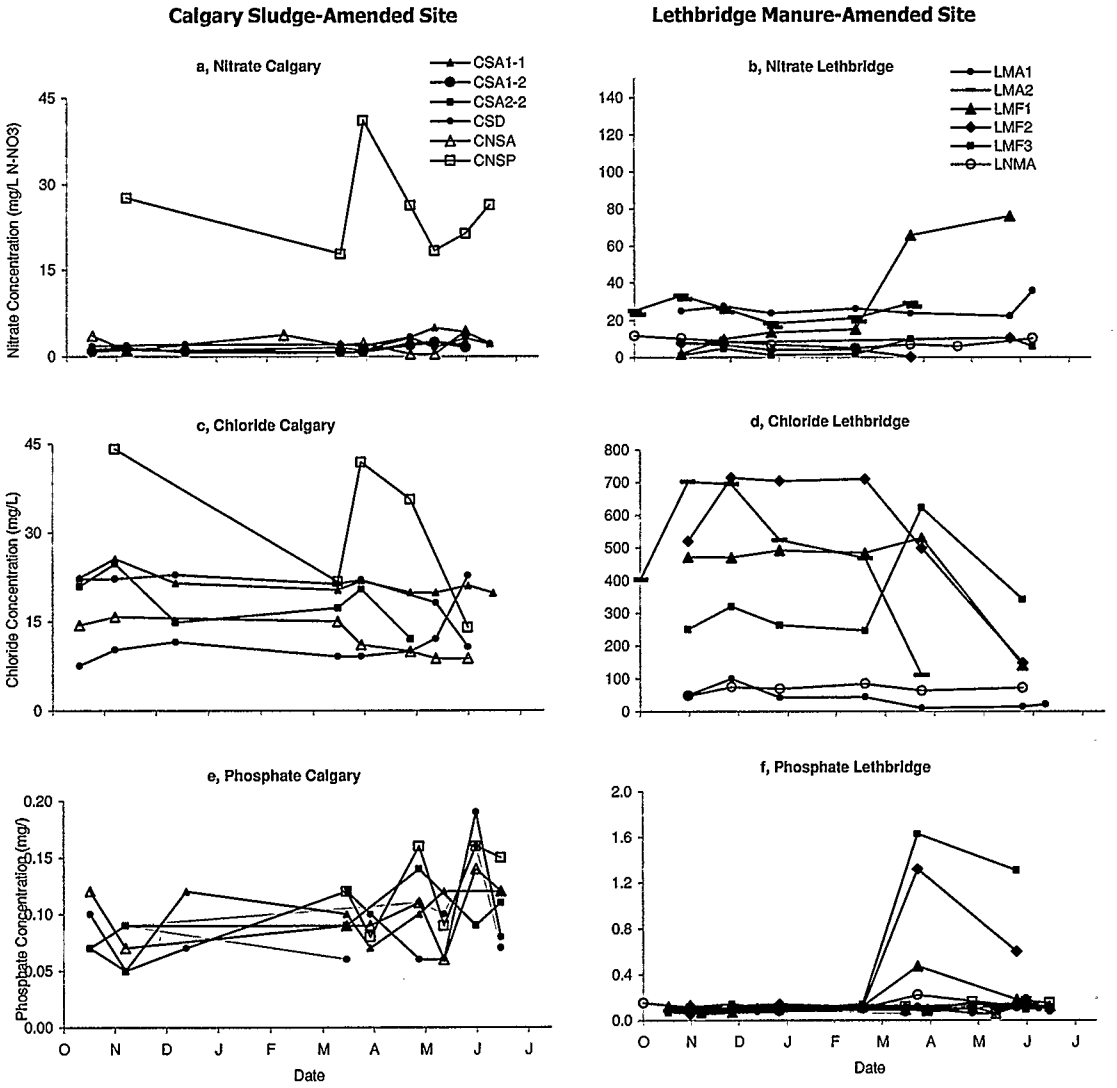


Figure 3.1: Physical measurements taken using Flow-through cell at a well sites at manure-amended (Lethbridge) and sludge-amended (Calgary) Study Areas.



*HACH detection limit is 0.05mg/L, all measurments exceeded this concentration

Figure 3.2: Nitrate, chloride and phosphate concentrations at well sites at manure-amended (Lethbridge) and sludge-amended (Calgary) Study Areas.

The average nitrate concentration of all the samples collected in the manure study area was 29.7 mg/L (SD=42.5, n=47), with 9% of the samples exceeding the Canadian drinking water maximum allowable concentration (MAC). The average concentration of nitrate from all the sludge samples was 5.0 mg/L (SD=8.7, n=52). Interestingly, the only sample to exceed the MAC for nitrate was obtained from a reference well (CNSP; Table 3.2). This particular well is located directly downgradient of the Shepard sludge ponds, but is not adjacent to a feild receiving sludge application.

Microbiological assessment of water quality was comprised of three separate parts. First, each sample was assessed for coliform (Figures 3.3 and 3.4, Table 3.2), atypical and HPC bacteria (Figures 3.5 and 3.6, Table 3.2). Selected samples containing total or fecal coliforms were further tested for the possibility that they may be of the *E.coli* or *F. streptococcus* group. No coliforms from these tests were found to be positive for either of these bacterial strains. Lastly, selected coliforms and atypical colonies were analyzed with BIOLOG® for microbial identification (Table 3.3).

Low-level coliform contamination was found at the sludge-amended (Calgary) wells sites (Figure 3.3). Average total coliform concentrations for the sludge-amended samples (including reference wells) averaged 192 cfu/100mL, the fecal coliform average was 2 cfu/100mL (Table 3.2). With the exception of one well (CSA2-2), coliform levels were below detection limit for most wells

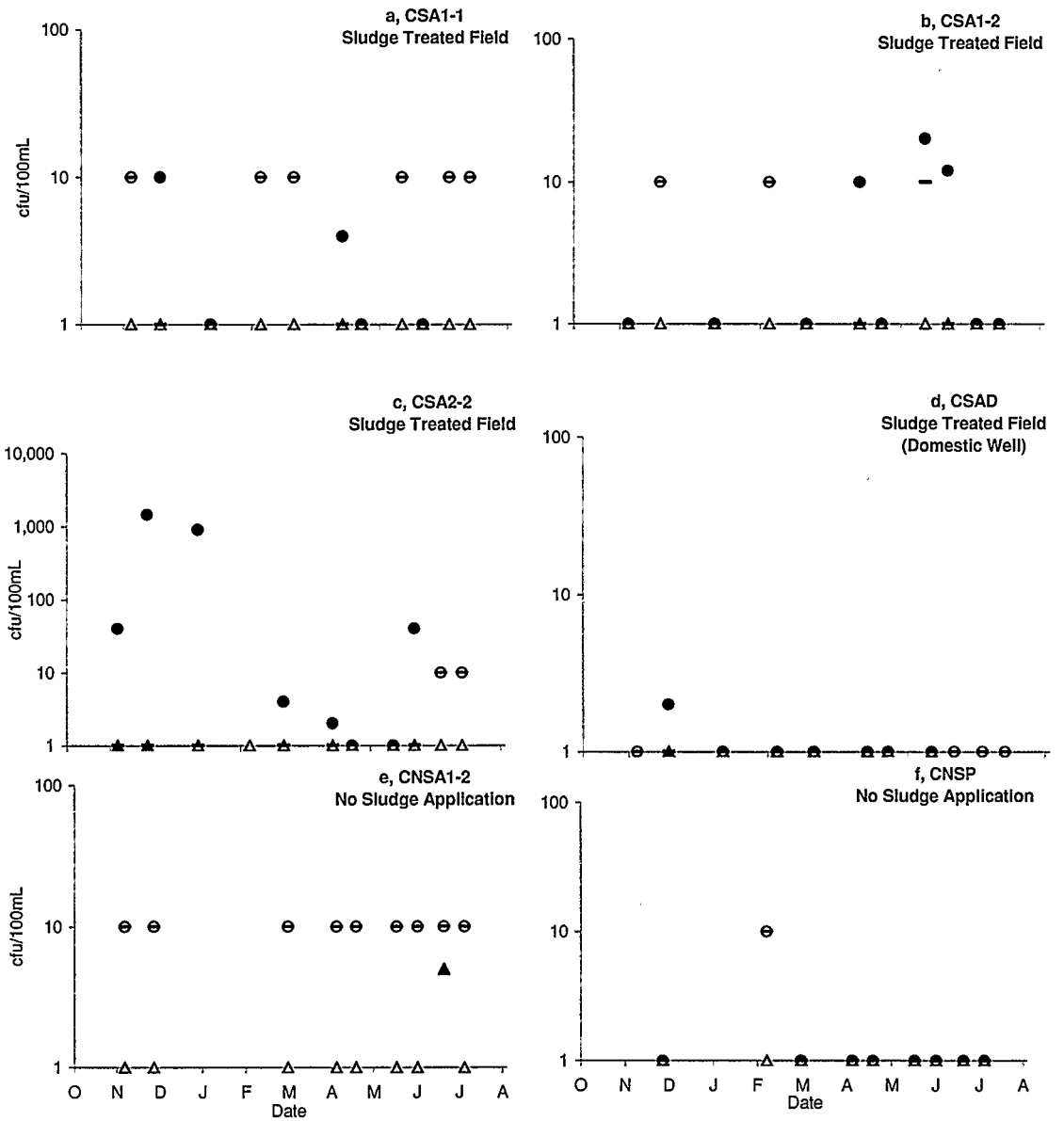


Figure 3.3: Total and Fecal Coliform Concentrations of Groundwater Samples taken from Sludge Ammended Sites, Shepard, AB.

Closed circles indicate TC concentration. Dash indicates the detection limit for TC for each sampling event. Open circle coinciding with dash indicates a non-detect for TC. Solid triangles indicate FC concentration above detection limit (Detection limit for FC was <1/100mL for all sampling events). Open triangles indicate FC non-detects.

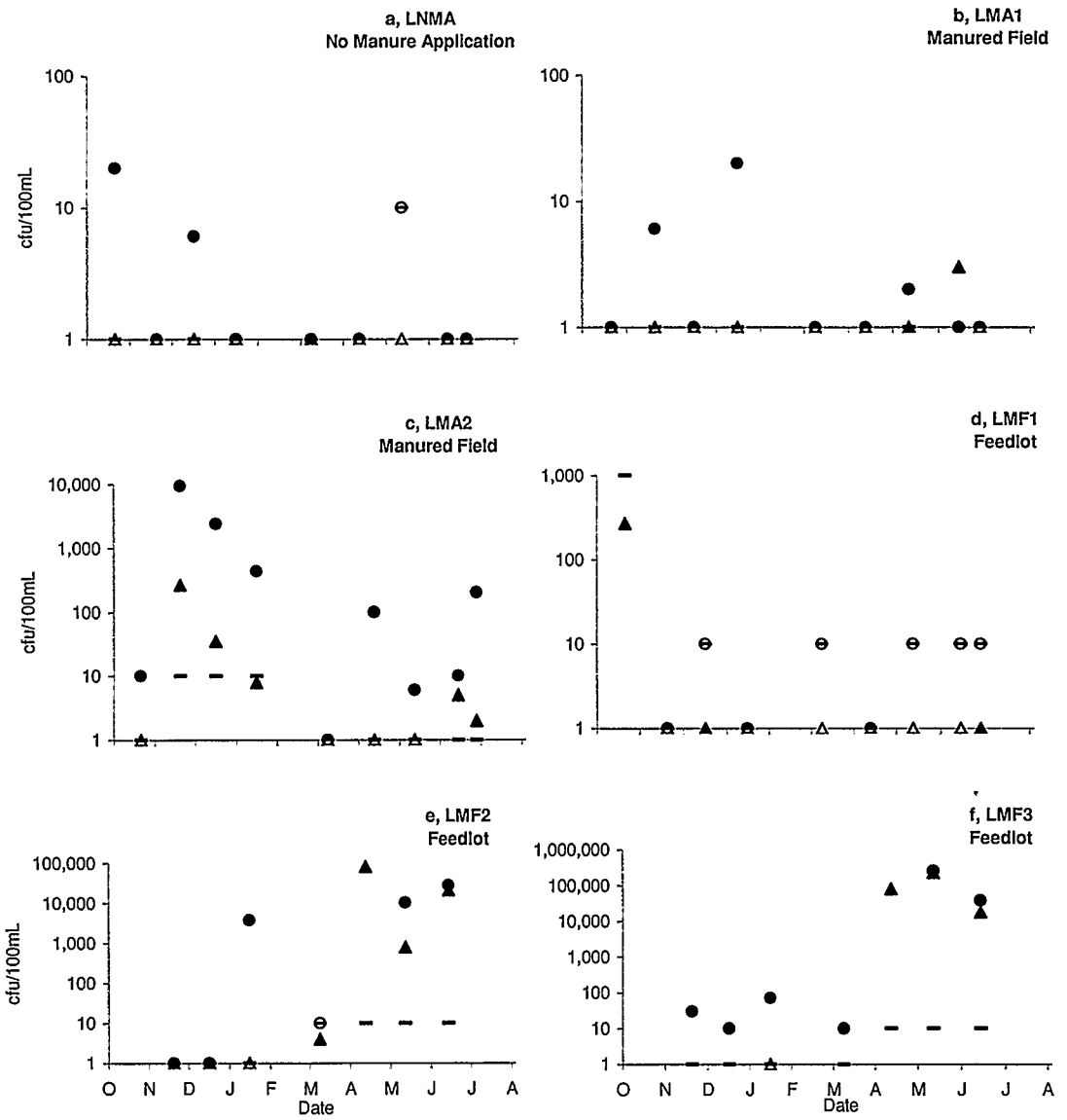


Figure 3.4: Total and Fecal Coliform Concentrations of Groundwater Samples taken from Manure Amended Sites, LNID, AB.
Closed circles indicate TC concentration. Dash indicates the detection limit for TC for each sampling event. Open circle coinciding with dash indicates a non-detect for TC. Solid triangles indicate FC concentration (Detection limit for FC was <1/100mL for all sampling events). Asterisks indicate atypical concentration. Crosses indicate atypical concentrations too numerous too count for the associated sampling event.

Table 3.3: Bacterial Strains Isolated from Groundwater Wells near Calgary and in the Lethbridge Northern Irrigation District, Alberta. Information obtained from appropriate papers (sited in Krieg and Holt, 1984).

Biolog ID	Isolated from Calgary or LIND	Colony Morphology (on MF Plates)	Gram Reaction	Respiration	Nitrate-Nitrite Reduction	Growth Range (C)	Isolated from
Enterobacter aerogenes	Calgary & LNID	Coliform	-	Facultative Anaerobe	Yes	25-40 (35 optimal)	Soil/ Water/ Sewage/ Dairy Products
Klebsiella pnemoniae	LNID	Coliform	-	Facultative Anaerobe	Yes	N/A	N/A
Enterobacter agglomerans	LNID	Coliform	-	Facultative Anaerobe	Yes	20-30 (some 37)	Plants/Seeds/Vegetables/ Water/Soil/Food/Human or Animal
Staphylococcus warneri	Calgary	Coliform	+	Strict Aerobe	N/A	~37	Treated Water/Human Skin/ Diseased Rainbow Trout
Areomonas echila	Calgary & LNID	Atypical	-	Facultative Anaerobe	Yes	4-37	European Freshwater Eels
Pseudomonas asplenii	Calgary & LNID	Atypical	-	Strict Aerobe	N/A	7-45 (28 optimal)	Diseased Plants or Cultivated Mushrooms
Pseudomonas corrugata	Calgary & LNID	Atypical	-	Facultative Anaerobe	Yes	37 not 41	Tomato Pith Necrosis
Pseudomonas fluorescens (Biotype F)	Calgary & LNID	Atypical	-	Strict Aerobe	Yes	<4 - >41 (27 optimal)	Soil/Water/Spoiled Food (Meat and Dairy)/ Diseased Plants
Pseudomonas cocovenenans	LNID	Atypical	-	Strict Aerobe	Yes	6-41 (30 optimal)	Soil/ Fermented Corn Meal/ Fermented Coconut Food/ Deteriorated White Fungus
Alcaligenes xylosoxydans	LNID	Atypical	-	Strict Aerobe	Yes	20-37	Dairy Products/ Intestinal Tracts of Vertebrates/ Rotten Eggs/ Other Natural Foods
Brevibacterium otitidis	LNID	Atypical	+	Strict Aerobe	No	20-30 or 30-40 depending on strain	Cheese/ Fish/ Skin
Pseudomonas putida (Biovar A)	Calgary	Atypical	-	Strict Aerobe	No	~27	Plant Rhizobacterium
Pseudomonas testosteroni	Calgary	Atypical	-	Strict Aerobe	No	~30	Soil
Aquaspirillium dispar	Calgary	Atypical	-	Strict Aerobe	Yes	20-40 (30 optimal)	N/A
Vibrio logi	Calgary	Atypical	-	Facultative Anaerobe	Yes	4-25	N/A

during all sampling events, and comparable to values obtained from the reference sites in this area. Well CSA2-2 had consistently elevated total coliforms levels (up to 1,440 cfu/100mL) but did not show significant levels of fecal coliforms (≤1 for all samples, Figure 3.3c). This may suggests that the total coliforms found may most likely be native to soil and not originate from a fecal source. Microbial identification found the total coliforms from both the Calgary and Lethbridge sites to be mainly from the *Enterobacter* and *Klebsiella* groups (Table 3.3). These bacterial strains are found in human and animal sludge and in manure, but can also be found naturally in soil and water (Buchanan and Gibbons, 1975). Fissures and cracks in till surrounding the well may aid in the transport of soil-borne coliforms into the subsurface (Mawdsley et al., 1995; Stoddard et al., 1998).

Both total and fecal coliform concentrations in the wells situated in the LNID were significant (Figure 3.4, Table 3.2). The reference (LNMA) well had moderately low levels of both total and coliforms (Figures 3.4a). One feedlot well (LMF1) and one well located downgradient of a manured-field (LMA1) were comparable to the reference well for coliform levels. LMA2 also located downgradient of a manured field and both wells located on the Alberta Agriculture Experimental Feedlot (LMF2 and LMF3) had total coliform concentrations exceeding 10,000 cfu/100mL and fecal coliform levels from 200-200,000 cfu/100mL depending on the well and time of year (Figure 3.4e and 3.4f).

Atypical and heterotrophic bacterial plate counts were used to gain a general understanding of the magnitude bacterial populations of each well. In general, atypical concentrations were an order of magnitude higher in the Lethbridge study than Calgary (Table 3.2; Figures 3.5 and 3.6).

Atypical bacteria were almost exclusively *Pseudomonas spp.* (Table 3.3). The fact that *Pseudomonas spp.* were found in every well tested (reference and test wells included) may show that this species of bacteria may be prevalent in many groundwater supplies. This species has been mentioned in the past as comprising a significant portion of the bacterial community when identifying well-water bacteria (Stetzenbach et al., 1986). The metabolic diversity of *Pseudomonas spp.* may contribute to their ability to survive in a variety of microenvironments.

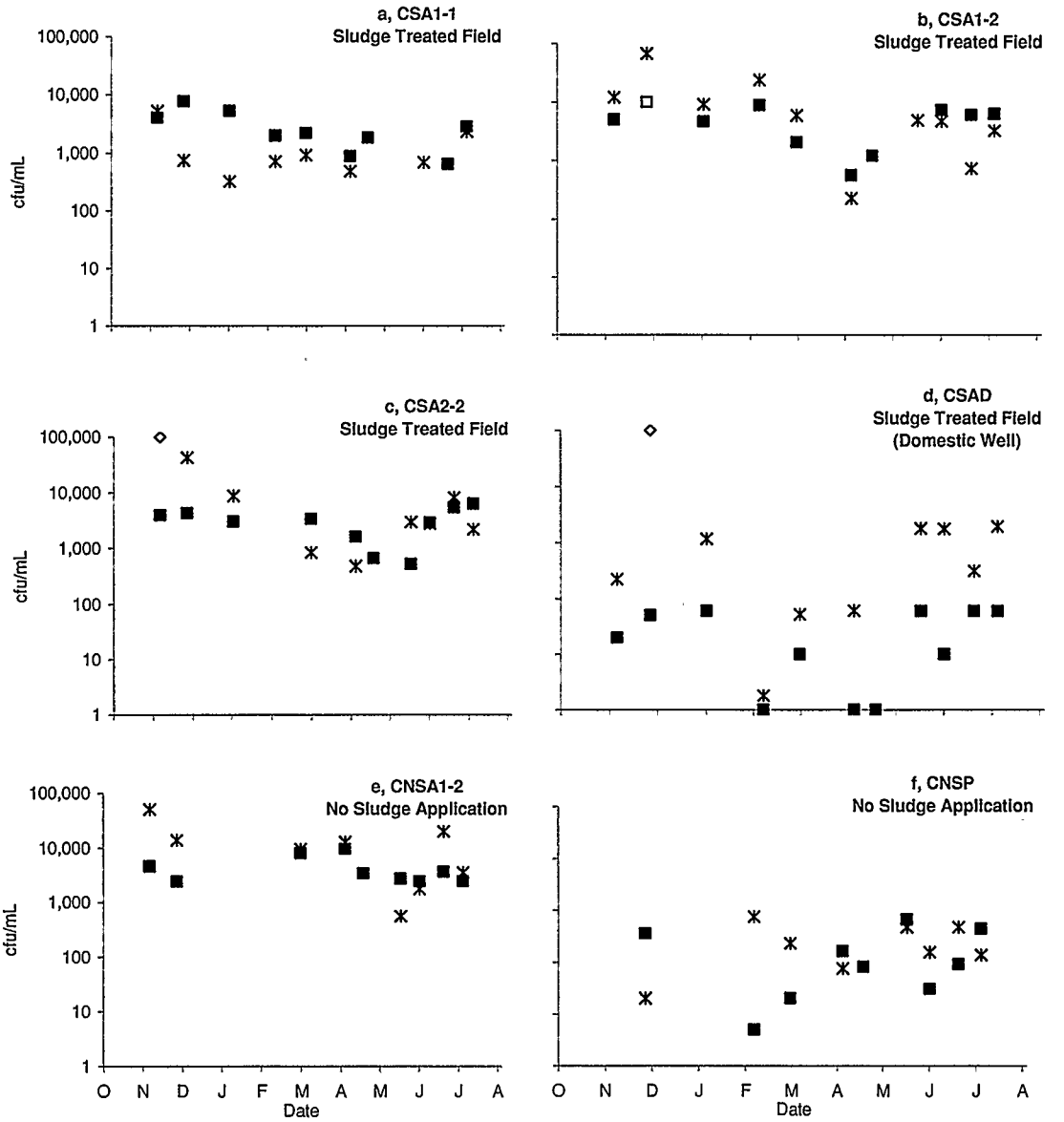


Figure 3.5: Atypical Coliform and Heterotrophic Bacteria Concentrations (HPC) of Groundwater Samples taken from Sludge Ammended Sites, Shepard, AB.

Closed squares indicate HPC concentration. Open Squares age estimates of HPC that were Too Numerous Too Count (TNTC). (Detection limit for HPC was <10/mL, HPC values below 10 were non-detects). Asterisk indicate Atypical concentration. Open diamonds indicate estimates of TNTC atypical concentrations.

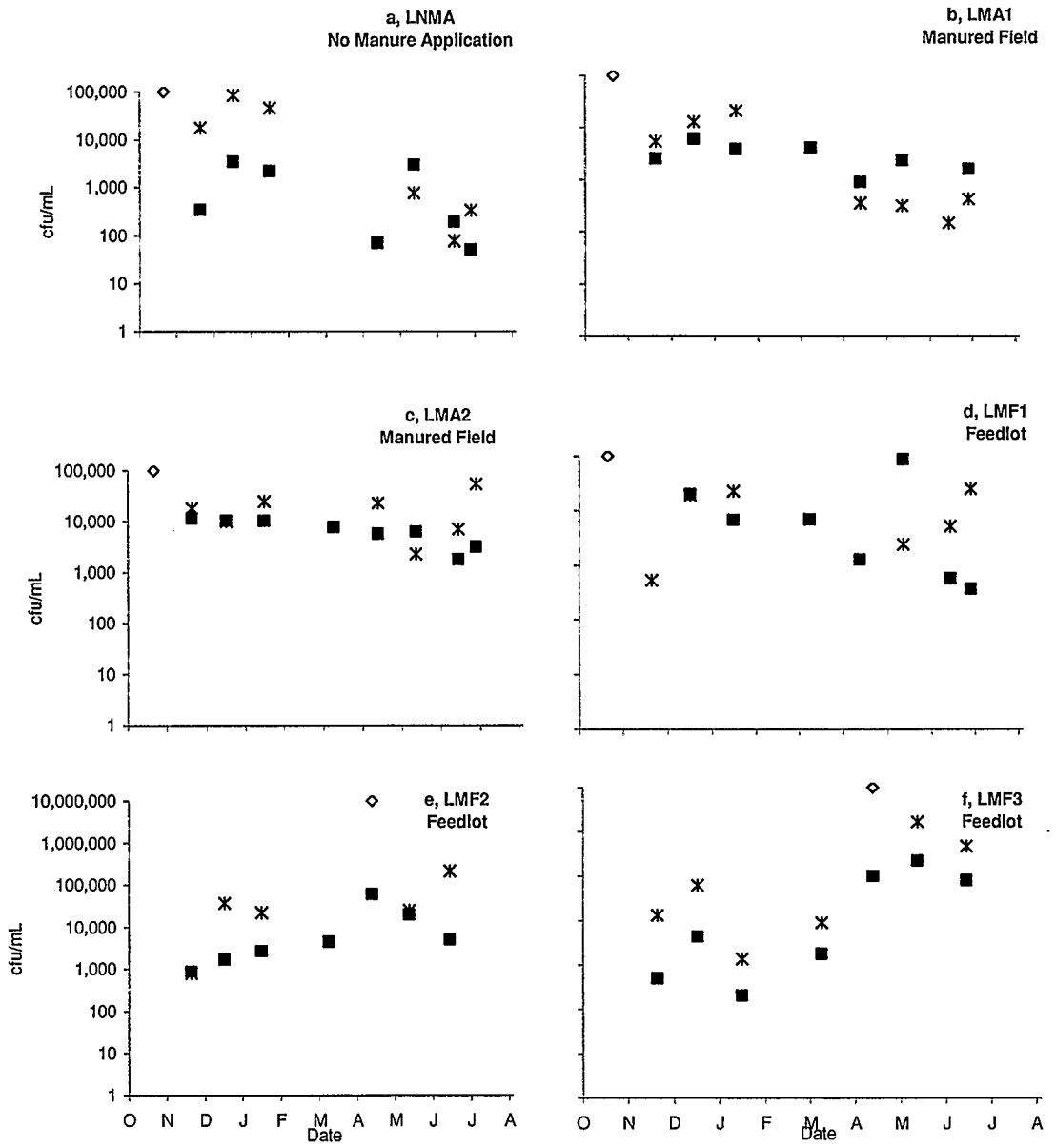


Figure 3.6: Atypical Coliform and Heterotrophic Bacteria Concetrations of Groundwater Samples taken from Manure Ammended Sites, LNID, AB.

Closed squares indicate HPC concentration. Open Squares are estimates of HPC that were Too Numerous Too Count (TNTC). (Detection limit for HPC was <10/mL, HPC values below 10 were non-detects). Asterisk indicate Atypical concentration. Open diamonds indicate estimates of TNTC atypical concentrations.

Conclusions

Overall, lower concentrations of nutrients and bacteria were found in the Calgary sludge amended study area as compared to the Lethbridge manure amended sites (Table 3.2). These results may likely be attributed to the relative groundwater vulnerability to contamination of each area. The water table at the manure-amended site is much shallower than Calgary, with an unconfined silty sand aquifer. This correlates to a relatively short vadose zone residence time and consequently relatively fast transport to groundwater. The shallow sediments in the Calgary sludge application area are comprised of fractured clayey till which has a much lower matrix permeability (the fracture permeability is difficult to estimate). The lower permeability combined with the deeper water table (6-10m) in the Calgary region lend the groundwater a lower vulnerability.

No significant coliform contamination was found in the Calgary study wells, although a significant level of general heterotrophic bacteria does exist (Table 3.2; Figures 3.3 and 3.5). All natural waters contain a certain level of indigenous bacterial populations, and it is possible that the heterotrophic bacteria levels measured in these wells is reflective of these native communities (Ghiorse and Balkwill, 1983). These results suggest that the practice of sludge amendment to the land in this area is not impacting the local underlying groundwater, and are supported by both the low nutrient and chloride levels observed for the wells in this area (Figure 3.2) and the similar concentrations of total, fecal, heterotrophic and atypical coliform concentrations observed between

the reference and treatment wells (Figures 3.3 and 3.5). In contrast high levels of both coliforms and heterotrophic bacteria were found in most wells at the Lethbridge study area (Table 3.2, Figures 3.4 and 3.6). Wells located within or near feedlot pens had the greatest concentrations of nutrient concentration (Figure 3.2), coliforms (Figure 3.4) and heterotrophic and atypical coliform levels (Figure 3.6).

Results from the microbial identification of total coliforms and fecal coliforms indicated that the vast majority of coliforms found in the groundwater are of the *Enterobacter* and *Klebsiella* groups (Table 3.3). The lack of positive results for both *Escherichia coli* and *fecal streptococci* , indicate that the majority of coliform bacteria found in these samples most likely originate from the indigenous community and may not be directly related to sewage sludge or manure land application. However, the high concentrations of coliforms found wells in the LNID, is still a matter of concern if these waters are to be used as a potable supply. Shallow sand aquifers underlying areas treated by manure and sludge could be at significant risk of bacteriological impacts from soil application of sludge or manure. Regular monitoring and modified practices regarding land-amendment of materials containing coliform organisms may reduce these risks.

CHAPTER FOUR: TOTAL COLIFORM ANALYSIS BY MEMBRANE FILTRATION OF MANURE-IMPACTED GROUNDWATER: NONCOLIFORM INHIBITION AND IDENTIFICATION

Introduction

Coliform bacteria were among the first drinking water parameters promulgated (Sayre, 1988). Assessment of the microbiological quality of water relies on accurate enumeration of coliforms in all types of water supplies. Coliforms, even at low concentrations, are particularly important when assessing drinking water quality. The bacterial quality of groundwater supplies in rural agricultural areas is under increasing scrutiny (Macler and Merkle, 2000). Groundwater wells sited close to feedlots and exercise yards, as well as farms that spread manure for fertilizer have been correlated with increased fecal contamination (Goss et al., 1998). Total and fecal coliforms (including *Escherichia coli*) continue to be the most widely used bacteriological indicator for drinking water quality in Canada (Health Canada, 1999) and the US (United States Environmental Protection Agency, 2000).

Membrane filtration (MF) using M-Endo medium is a widely accepted method for total coliform quantification in waters (APHA, 1998). Potential limitations to this method have been suspected when significant heterotrophic populations exist, but have not been demonstrated (Geldrich et al., 1972; Clark, 1980; Standridge and Defino, 1982; Burlingame et al., 1984). Untreated groundwater supplies in particular can contain high concentrations of non-

coliform bacteria which are able to grow on coliform media (Franzblau et al., 1984; Standridge and Sonzogni, 1988; Shirey and Bissonnette, 1997).

Noncoliforms, also called atypical colonies, are organisms capable of growth on MF plates that do not produce the target reaction. Interfering atypical colonies are rarely identified but include *Pseudomonas aeruginosa, Areomonas hydrophila* (Burlingame et al., 1984; Brion et al., 2000), *Acinetobacter calcoaceticus* (Franzblau et al., 1984), *Pseudomonas fluorescens, Morganella morganii,* and *Proteus vulgaris* (Shirey and Bissonnette, 1997). Noncoliform overgrowth on MF plates can also create an atypical response in certain coliforms by inhibiting sheen producing capabilities. For example, *Citrobacter* and *Enterobacter* species have been shown to produce this false-negative response (Franzblau et al., 1984).

Although anaerobic incubation of MF plates can improve coliform recovery (Franzblau et al., 1984), many coliforms colonies are unable to produce a characteristic sheen if grown under anaerobic conditions (Standridge and Sonzogni, 1988). However, the increased amount of false-negative coliforms colonies caused by this modified method is near or equal to the amount of increased recovered coliform colonies (Shirey and Bissonnette, 1997).

The most-probable-number (MPN) technique (also referred to as the multiple tube fermentation (MTF) method) for quantitatively assessing coliforms is also used in the bacteriological monitoring of water supplies. This method is also subject to interference by noncoliform bacteria (Lupo et al., 1981; Seidler et

al., 1981). At low coliform densities (<200cfu/ 100mL), MF is superior to MPN because of the high amount of statistical variation produced by the MPN method (Hsu and Williams, 1982). In attempts to remediate the amount of false-negatives produced by the MF technique on overgrown plates, this study suggested that swabs from overgrown plates be inoculated at 37°C into brilliant green bile broth (BGB) to verify the presence of coliforms. Samples were considered coliform positive if growth and gas production was observed within 48h. Although this verification technique is useful in the qualitative assessment of coliforms in overgrown cultures, it does not allow for the quantitative assessment of coliform concentrations. The swab inoculation technique and the anaerobic technique have been shown to yield similar percentages of coliform positive samples (Standridge and Sonzogni, 1988).

Recent investigations into the bacterial quality of groundwater in an intensive agricultural area in Southern Alberta have resulted in similar inhibition problems with regards to total coliform enumeration. In some instances countable plates using the MF procedure were not achieved due to noncoliform overgrowth. Preliminary investigations into anaerobic incubation by the authors did not significantly decrease the recovery of atypical colonies, or increase coliform recovery of the rural groundwater supplies used in this study (unpublished data) and was therefore judged unsuitable for quantifying coliform concentrations in this particular groundwater supply.

Bacterial enumeration at sites used in this study yielded concentrations between 0.34 and $2.5x10^4$ cfu/mL, with a mean of $2.5x10^3$ cfu/mL (S.D.= $5.4x10^3$, n = 32). Total coliform concentrations ranged between 0 and $9.2x10^2$ cfu/mL with a mean of 45 ± $1.8x10^2$ cfu/mL. In any given sample, non-coliform colony densities were usually 10 to 1000 times greater than the recovered total coliform densities. When samples were diluted to the degree that atypical inhibition did not occur, total coliform concentrations were consequently too low to be accurately assessed.

Fecal coliforms were also preliminarily assessed for atypical inhibition complications although no significant inhibition was apparent. The combination of rosalic acid found in m-FC media in combination with higher incubation temperatures (44.5°C rather than 35°C) is intolerable to most potentially interfering colonies. For these reasons fecal coliform inhibition was not investigated in this study.

This study investigates the hypothesis that non-coliform bacteria suppress total coliform growth on standard M-Endo media. The suppression of coliform bacteria on MF plates has been implicated in many studies where there are high atypical concentrations. In all cases suppression has been suggested only because of decreased coliform recovery as non-coliform densities increase, however no study has properly shown this inhibition effect using quantification. This study uses the method of "bacterial spiking" of known amounts of coliform bacteria to demonstrate and quantify the degree of inhibition caused by atypical

bacteria present in rural groundwater supplies. It also compares inhibition from atypical bacteria originating from groundwater with inhibition in treated sewage effluent that generally contain little to no atypical bacteria. In addition, this study seeks to identify interfering noncoliform bacteria to the species level.

Methods and Materials

Groundwater samples were taken from monitoring wells located approximately 9 miles northwest of Lethbridge, Alberta, Canada within the Lethbridge Northern Irrigation District (LNID). The LNID is one of several irrigated land blocks neighboring the Oldman River and is home to one of the highest densities of intensive livestock operations in Canada. The wells sampled in this study are located on an irrigated field that receives fall applications of cattle manure. Groundwater nutrients in the LNID reflect the effect of high manure application over long periods of time. The average groundwater concentration of nitrate is 30 mg N /L (S.D. = 16, n = 88), and total phosphorus is 0.22 mg/L (S.D. = 0.51, n = 88[19]). Silty sand sediments extend from the soil zone to a few meters below the water table, which is approximately 2m below the ground surface. The wells are constructed of PVC (5 cm O.D.) with 1m long screened intervals located 0.5 - 3 meters below the water table. Groundwater monitoring wells were sampled using a peristaltic pump and silicon tubing. Aseptic techniques were used to mitigate cross-contamination between well samples and to minimize the possibility of airborne or surface-soil associated

bacteria from entering into the sampling apparatus and containers. Tubing was sterilized by pumping 75% ethanol for a minimum of two minutes, and subsequently rinsed by pumping 1L of sterile, distilled water prior to sampling. Sample tubing was inserted into the well casing with care taken to prevent ground-surface contact. Field blanks were taken at each sampling event to demonstrate that cross contamination did not occur.

Since the moderately low aquifer hydraulic conductivity ($\sim 10^{-7}$ m/s) precluded purging of multiple standing wellbore volumes prior to sampling, a modified sampling technique was used (Sanders, 1998). Two to three liters (one well volume) of water were pumped from the shallowest part of the water column to remove water directly exposed to the atmosphere. After purging, the tubing was inserted deeper into the water column and 1 L of water was pumped into sterile bottles.

Wastewater was collected from the Bonnybrook Wastewater Treatment Plant in Calgary, AB. Samples were taken post-treatment but before UV disinfection. After collection both groundwater and wastewater were stored at 4°C until analysis, which was conducted within 24 hours of sample collection. Water samples for the inhibition experiment were collected in November of 2000.

Inhibition Estimation

Atypical and coliform densities were quantified in "spiked" and "unspiked" groundwater samples and sewage effluent using 0.45µm membrane filters (APHA, 1998). The extent of coliform inhibition by atypical bacteria was

estimated by bacterial "spiking" (Figure 4.1). The "spikes" consisted of coliform bacteria (*Enterobacter aerogenes*) isolated from groundwater collected from the same well in a previous sampling event. Isolated coliforms were cultured in a 1/10 dilution of liquid *tryptone glucose yeast media* to a concentration of approximately 10^9 cfu/mL. To produce acceptable plate counts when spiking one mL of the coliform culture, the culture was then diluted with sterile water to a concentration of 10^2 cfu/mL.

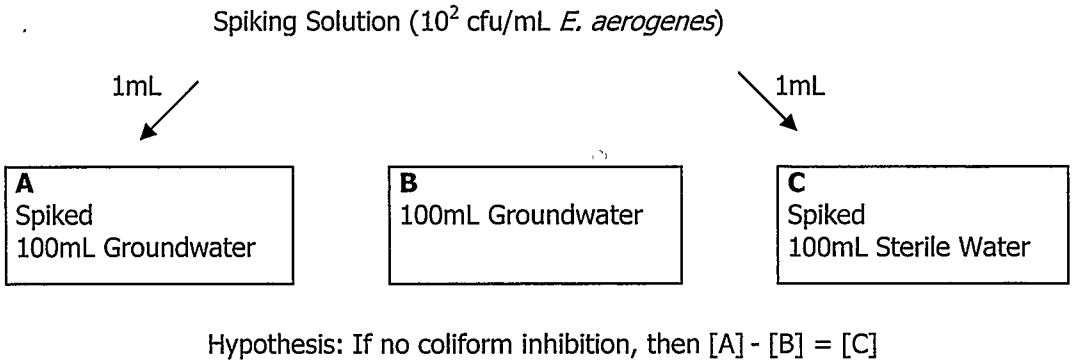


Figure 4.1: Methodology for Estimation of Bacterial Inhibition in Groundwater Samples by Bacterial Spiking Method.

Groundwater samples (100mL) and sterile water (100mL, autoclaved and deionized) aliquots were spiked with one mL of the coliform culture. The spiked samples were subsequently analyzed and enumerated. Unspiked groundwater samples were also filtered to provide an enumeration of groundwater coliforms that would be detected in the absence of the spike (Figure 4.1). If no inhibition occurred in the groundwater, the concentration of coliforms in the spiked groundwater [A], minus the unspiked groundwater [B] concentration, would equal the concentration of coliforms in the "spiked" sterile water [C]. The same

methodology was then used for sewage effluent collected from the Bonnybrook Wastewater Treatment Facility (which was known to have low atypical coliform concentrations relative to groundwater samples). After filtering, membranes were transferred to plates containing M-Endo media, and incubated at 35°C for 24 hours prior to total coliforms enumeration (APHA, 1998).

Bacterial Identification

Thirteen commonly occurring atypical bacteria, and two representative coliform bacteria appearing on the MF plates were selected based on colony morphology and streaked by loop onto a 1/10 dilution of *tryptone glucose yeast agar* (APHA, 1998) to assess purity. Plates were incubated for three days at room temperature. Individual colonies were then transferred to *sheep blood agar* and incubated at room temperature for an additional two days. After the first day of incubation on *sheep blood agar*, cultures were gram stained. On the second day the cultures were classified according to their oxidase reaction. These cultures were then transferred by sterile swab into inoculation media for subsequent transfer into BIOLOG™ Microlog™ system and associated Microbial Identification Software for bacterial identification.

Results and Discussion

Bacterial Inhibition

The concentration of non-coliform colonies in the groundwater samples collected in this study were $6.8x10^4$ cfu/mL (SD = $1.6 x10^4$, n = 10). The results of the bacterial inhibition experiment (Table 4.1) indicate that 46% of the coliforms added to the 100mL groundwater samples were not recovered by the MF method ($t=-0.51$, $p<0.05$, $df=14$, one-tailed, two-sample t-test). In contrast, no significant inhibition was detected for the coliforms added to the sewage effluent samples ($t=-1.99$, $p>0.05$, $df=6$, one-tailed, two-sample t-test).

The calculated 46% inhibitory effect of coliforms when spiked into groundwater demonstrated in this study suggests that an unmodified MF method for manure-impacted groundwater would consistently underestimate the actual amount of coliforms present in any given sample. Coliform underestimation may have health implications when assessing the microbial water quality of domestic wells located in rural, agriculturally intensive areas. The development of a modified method to detect indicator bacteria in groundwater supplies where high concentrations of atypical bacteria are present is needed. Development of this method would increase the ability to identify the possible environmental impacts created by intensive agricultural activities on groundwater supplies.

Table 4.1: Direct Plate Counts from Bacterial Spike Experiment.

Experiment	Sample Name (Letters refer to Fig 1)	Sample Contents*	Average Total Coliforms counted (SD, n)
Inhibition by Groundwater	Spiked Sterile Water (A)	1 mL CC	62.0 (7.2, 8)
	Unspiked Groundwater (B)	100 mL GW	0.1 (0.3, 10)
	Spiked Groundwater (C)	1 mL CC + 100 mL GW	33.4 (16.2, 15)
Inhibition by Effluent	Spiked Sterile Water (A)	1 mL CC	99.7 (6.1, 6)
	Unspiked Sewage Effluent (B)	1 mL SE	23.4 (1.5, 7)
	Spiked Sewage Effluent (C)	1 mL CC + 1mL SE	116.3 (13.4, 7)

*CC = coliform culture, GW = groundwater, SE = sewage effluent

Bacterial Identification

Of the thirteen representative atypical bacteria morphologies isolated from the MF plates and classified using the BIOLOG™ method, seven distinct bacterial strains were identified. These consisted of *Aeromonas echila, Pseudomonas asplenii, Pseudomonas corrugata, Pseudomonas fluorescens* (Biotype F), *Alcaligenes xylosoxydans, Burkholderia cocovenenans,* and *Brevibacterium otitidis.*

Two representative coliforms types isolated from the groundwater samples were identified as *Pantea dispersa (Enterobacter agglomerans)* and *Enterobacter aerogenes* (note that *Enterobacter aerogenes* was the strain isolated and cultured for use in the bacterial spiking process). The repeated identification of *E. aerogenes* from independent sampling events suggests it may be associated with fecal contamination. This particular bacterial strain may have adapted to survive in a high-nutrient groundwater environment, which may allow it to persist longer than other fecal-associated coliforms. It may therefore occur in groundwater samples at a higher frequency than other associated fecal coliforms.

The reason for the large abundance of non-coliform, thermotolerant bacteria is unclear. Many of the bacteria seem to be associated with soil, water, seeds, fungus, agricultural plants and their associated diseases (Table 4.2). All of the atypical bacteria were identified as gram-negative, oxidase-positive rods, with the exception of *Brevibacterium otitidis,* which was gram-positive, oxidase-negative and variable in shape, showing both rod and cocci morphologies. In addition, all of the atypical bacteria isolated were either strict or facultative aerobes, and all were capable of nitrate-nitrite reduction (Krieg and Holt, 1984). The capability of nitrate reduction in all of the isolated atypical bacteria is relevant given the elevated nitrate levels found in the groundwater of the LNID.

Table 4.2: Identification and Origin of Classified Bacteria from Manure-Impacted Groundwater

Bacteria Name	Bacterial Type	% Biolog Match	Origin*
Aeromonas echila	Atypical	99	Treated Water, Human Skin, European Freshwater Eels
Pseudomonas asplenii	Atypical	65	Diseased Plants or Cultivated Mushrooms
Alcaligenes xylosoxydans	Atypical	94	Intestinal Tracts of Vertebrates, Dairy Products, Rotten Eggs, Other Natural Foods
Pseudomonas corrugata	Atypical	93	Tomato Pith Necrosis
Pseudomonas fluorescens (Biotype F)	Atypical	98	Soil, Water, Diseased Plants, Spoiled Food (Meat and Dairy)
Burkholderia cocovenenans	Atypical	99	Soil, Fermented Corn Meal, Fermented Coconut Food, Deteriorated White Fungus
Brevibacterium otitidis	Atypical	100	Cheese, Fish, Skin
Enterobacter aerogenes	Coliform	99	Soil, Water, Sewage, Dairy Products
Pantea dispersa (Enterobacter agglomerans)	Coliform	90	Plants, Seeds, Vegetables, Water, Soil, Food-stuffs, Human or Animal Origin

* Bacterial origins obtained from appropriate papers sited in (Krieg and Holt, 1984)

Two of the atypical bacteria identified (*Aeromonas echila* and *Brevibacterium otitidis*) have not been previously associated with groundwater or soil environments (Table 4.2). Although *Alcaligenes xylosoxydans* is a strict aerobe and not considered a coliform, it has been shown to reside within the intestinal tracts of animals, and is a known decomposer. This is the only non-coliform isolated in this study that has a direct association with manure.

Most of the atypical bacteria inhibiting coliform growth are naturally occurring soil and plant organisms, and do not appear to originate from manure.

The high concentrations in which these organisms are found suggest that once they are introduced into a "nutrient-rich" groundwater environment, they are able to survive and proliferate. Under these conditions, traditional coliform enumeration using the MF technique will consistently underestimate the true concentration of coliforms, if present.

CHAPTER FIVE: CONCLUSIONS

The individual studies in this paper point to a variety of concerns regarding the collection and analysis of indicators for pathogenic microorganisms in groundwater and well-water supplies. Chapter Two outlined the need for repeated and regular monitoring for microbial indicators at all times of year. Shifts in groundwater flow, direction, surface-groundwater inputs, temperature, or even land-use patterns could all possibly contribute to spatial and temporal variations in the amount of coliforms or pathogenic indicators in groundwater wells at all times of year. In addition, the possibility of natural heterotrophic biofilms within the well systems potentially allow for the survival and sloughing of small levels of pathogenic bacteria within the water delivery system after exposure to fecal contamination. This may cause bacterial indicators to increase during times or seasons thought of as low-risk for coliform contamination.

In Chapter Three the potential impacts of non-point source manure and sludge application to land and the occurrence of pathogenic indicators from monitoring wells located within these areas was discussed. Theory in the introduction state that most bacteria sorb to the surface of soil particles within the soil matrix, thus infiltration of affected groundwater would greatly decrease the concentration of bacteria found in the groundwater. Both chapter Three and Four find that shallow, unconfined aquifers as well as areas with increased matrix permeability appear most susceptible to fecal contamination.

Chapter three also identified the colonies that were able to survive on the total coliform media, whether they exhibited the traditional sheen produced by total coliforms, or non-sheen atypical colonies. The coliforms obtained from this study were found to be of *Enterobacter* or *Klebsiella* species. Specific media designed to isolate *Escherichia coli and fecal streptococci* contained no positive results. Non-coliforms were mainly identified as *Pseudomonas spp.* and may be either indigenous bacteria found naturally in the groundwater or may be certain opportunistic strains that originate from sewage and sludge and are able to survive in the groundwater for extended periods of time. In any case, these atypical bacteria exibit similar metabolic characteristics to coliform bacteria and thus create problems in the isolation and analysis of pathogenic indicators.

In chapter four, the need for enhanced selective methods in the analysis of total coliforms from well-water samples was illustrated. The ability of many non-coliform bacteria originating from groundwater samples to produce colonies on total coliform m-Endo media interferes with the proper quantification of sheen producing coliforms because of the increased competition in a limited nutrients environment (i.e. media plates). Fecal pollution indicators must produce reliable and consistant results if used for groundwater application, and current analysis methods are inadequate.

In conclusion, viable and effective sampling and assessment methods need to be further developed and standardized for use by both public and private communities to asses potential for microbial contamination in groundwater. If

effective and accurate quantification of coliform data is achieved, it may allow for better comparisons of microbial data in areas of differing contamination types and hyrdogeological characteristics.

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APPENDIX ONE: RAW DATA

CHAPTER TWO: EFFICACY OF ANNUAL BACTERIA MONITORING AND SHOCK CHLORINATION IN WELLS FINISHED IN A FLOODPLAIN AQUIFER

House Letter	Time (M/D/Y Hr)	Time (Day Value)	Time From to Chlorination (Weeks)	Lab HPC (cfu/1mL)	HPC Value (cfu/1mL)	TC (cfu/100 mL)	FC (cfu/100 mL)	Temp (C)	DO (mg/L)	EC (mS)
PC	11/1/90 12:00	33178.50	-524.93			<1	<1			0.02
PC	9/1/98 12:00	36039.50	-116.21	350	350	<1	<1			
PC	12/4/99 12:00	36498.50	-50.64			<1	<1	6.40	6.33	
PC	6/8/00 12:00	36685.50	-23.93			<1	<1			
PC	6/14/00 12:00	36691.50	-23.07	100	100	<1	<1			0.66
PC	6/19/00 12:00	36696.50	-22.36	>3,000	3000	<1	<1			
PC	6/21/00 12:00	36698.50	-22.07			<1	<1	9.20	6.66	0.70
PC	6/26/00 12:00	36703.50	-21.36	>3,000	3000	<1	<1			0.02
PC	7/6/00 12:00	36713.50	-19.93			<1	<1	8.00	6.69	0.02
PC	7/18/00 12:00	36725.50	-18.21			<1	<1	8.20	6.86	0.72
PC	8/16/00 12:00	36754.50	-14.07			<1	<1	9.80	3.15	0.02
PC	9/27/00 12:00	36796.50	-8.07	80	80	<1.8	<1	11.20	5.06	
PC	10/10/00 12:00	36809.50	-6.21			1.3	<1	8.60	6.43	
PC	11/22/00 13:00	36852.54	-0.07	60	60	<1	<1			
PC After	11/23/00 10:00	36853.42	0.06	<10	10	<1	<1			
PC After	11/24/00 10:15	36854.43	0.20	<10	10	<1	<1			
PC After	11/25/00 9:00	36855.38	0.34	<10	10	<1	<1			
PC After	11/25/00 22:00	36855.92	0.42	<10	10	<1	<1			
PC After	11/26/00 10:30	36856.44	0.49	<10	10	<1	<1			
PC After	11/27/00 10:30	36857.44	0.63	<10	10	<1	<1			0.74
PC After	11/27/00 19:30	36857.81	0.69	<10	10	<1	<1			0.01
PC After	11/28/00 8:30	36858.35	0.76	<10	10	<1	<1	10.10	7.57	0.02
PC After	11/30/00 14:45	36860.61	1.09	11,000	11000	<1	<1	6.40	7.36	
PC After	12/7/00 12:30	36867.52	2.07	<10	10	<1	<1	6.70	6.92	0.02
PC After	12/11/00 12:45	36871.53	2.65	3,300	3300	<1	<1			0.68
PC After	12/15/00 12:30	36875.52	3.22	3,100	3100	<1	<1	6.10	5.91	0.65
PC After	12/19/00 12:40	36879.53	3.79	2,300	2300	<1	<1	7.00	5.57	0.68
PC After	12/23/00 0:00	36883.00	4.29					9.50	6.06	0.64
PC After	12/27/00 9:55	36887.41	4.92	460	460	<1	<1	5.40	7.71	0.08
PC After	12/31/00 0:00	36891.00	5.43					5.40	7.88	
PC After	1/11/01 10:20	36902.43	7.06			<1	<1	5.70	8.35	0.74
PC After	1/16/01 11:00	36907.46	7.78	890	890	<1	<1			1.45
PC After	1/29/01 12:30	36920.52	9.65	755	755	<1	<1	9.10	4.57	0.66
PC After	2/11/01 12:00	36933.50	11.50			<1	<1	10.40	7.92	0.69
PC After	2/26/01 19:00	36948.79	13.68	290	290	<1	<1	8.60	6.55	0.00
PC After	4/9/01 20:15	36990.84	19.69	560	560	4.1	<1.4	7.30	10.60	2.18
PC After	5/22/01 12:30	37033.52	25.79	55	55	11.3	<1	5.00	9.36	1.50
NC	6/6/00 12:00	36683.50	-24.29			45.7	34.6	12.90	7.50	0.87
NC	6/21/00 12:00	36698.50	-22.14			6.8	<1	13.30	6.40	0.88
NC	7/6/00 12:00	36713.50	-20.00			41.9	<1	14.10	5.95	1.72
NC	7/18/00 12:00	36725.50	-18.29			81.7	3.9	14.20	6.30	1.02
NC	8/16/00 12:00	36754.50	-14.14			25<1	136	14.60	2.78	2.36
NC	9/27/00 12:00	36796.50	-8.14	>3,000	3000	6.7	<1	15.80	6.24	4.50
NC	10/10/00 12:00	36809.50	-6.29			42	16.4	14.60	6.20	
NC	10/23/00 12:00	36822.50	-4.43	40	40	16.4	2.7	14.90	5.06	
NC	11/23/00 10:30	36853.44	-0.01	90	90		<1			2.36
NC	11/23/00 15:00	36853.63	0.02	180	180	22	2			1.72
NC	11/24/00 20:00	36854.83	0.19			<1	<1	14.60	6.20	

NC	11/25/00 12:00	36855.50	0.29	100	100	1	1	14.60	2.78	
NC	11/25/00 21:00	36855.88	0.34	60	60	1	<1			
NC	11/26/00 12:15	36856.51	0.43	60	60	<1	<1			1.80
NC	11/26/00 23:00	36856.96	0.49	100	100	<1	<1			
NC	11/27/00 11:00	36857.46	0.57	90	90	<1	<1	14.90	5.06	2.50
NC	11/27/00 21:15	36857.89	0.63	60	60	<1	<1			
NC	11/29/00 15:15	36859.64	0.88			7	1	16.60	6.51	
NC	11/29/00 22:00	36859.92	0.92			<1	<1			1.60
NC	12/4/00 11:15	36864.47	1.57	20	20	1	1			1.53
NC	12/7/00 13:00	36867.54	2.01	10	10	<1	<1	14.20	6.68	
NC	12/15/00 12:00	36875.50	3.14	40	40	<1	<1	14.40	7.21	1.44
NC	12/17/00 0:00	36877.00	3.36			<1	<1			1.51
NC	12/19/00 12:15	36879.51	3.72	30	30	<1	<1	14.10	7.23	1.67
NC	12/23/00 12:00	36883.50	4.29					14.60	6.62	2.10
NC	12/27/00 9:45	36887.41	4.84	20	20	<1	<1	15.10	6.50	
NC	12/31/00 12:00	36891.50	5.43					14.50	7.21	0.99
NC	4/19/01 12:00	37000.50	21.00	1,845	1845	162	6.9			0.82
NC	5/22/01 13:00	37033.54	25.72	15	15	76	<1.4	8.82	9.60	
E2	11/1/99 12:00	36465.50	-55.50			26	6.4	8.90	4.05	0.59
E2	5/30/00 12:00	36676.50	-25.36	360	360	23	4			0.68
E2	6/6/00 12:00	36683.50	-24.36			23.7	7.5	8.20	8.21	0.68
E2	6/21/00 12:00	36698.50	-22.21			21.1		5.70	5.11	0.67
E2	7/6/00 12:00	36713.50	-20.07			114.7	4	6.00	5.11	0.64
E2	7/18/00 12:00	36725.50	-18.36			7.5	4.8	6.60	3.42	0.84
E2	8/16/00 12:00	36754.50	-14.21			2	<1	7.90	1.66	0.58
E2	9/27/00 12:00	36796.50	-8.21	60	60	6.9	<1	8.10	3.36	0.59
E2	10/10/00 12:00	36809.50	-6.36			<1.7	<1	8.10	3.70	
E2	10/23/00 12:00	36822.50	-4.50	640	640	<1.4	<1	7.50	5.37	0.68
E2	11/23/00 10:00	36853.42	-0.08	210	210	1	<1			
E2 After	11/24/00 9:30	36854.40	0.06	<10	10	<1	<1	5.70	5.11	0.68
E2 After	11/24/00 17:45	36854.74	0.11			<1	<1			
E2 After	11/25/00 10:45	36855.45	0.21	<10	10	<1	<1	6.00	5.11	
E2 After	11/26/00 12:00	36856.50	0.36	20	20	<1	<1			0.67
E2 After	11/26/00 19:30	36856.81	0.40	10	10	<1	<1			
E2 After	11/27/00 10:00	36857.42	0.49	<10	10	<1	<1	6.60	5.42	
E2 After	11/27/00 19:10	36857.80	0.54	20	20	<1	<1			
E2 After	11/28/00 11:30	36858.48	0.64	40	40	<1	<1			
E2 After	11/29/00 6:45	36859.28	0.75			<1	<1			0.58
E2 After	11/29/00 18:00	36859.75	0.82			<1	<1			
E2 After	11/30/00 14:30	36860.60	0.94	60	60	<1	<1	6.00	5.45	
E2 After	12/2/00 10:00	36862.42	1.20			<1	<1			0.01
E2 After	12/4/00 10:50	36864.45	1.49	290	290	<1	<1			
E2 After	12/7/00 12:00	36867.50	1.93	200	200	<1	<1	6.10	5.48	0.26
E2 After	12/9/00 16:45	36869.70	2.24			<1	<1			
E2 After	12/11/00 12:00	36871.50	2.50	160	160	<1	<1	5.40	6.00	
E2 After	12/13/00 9:15	36873.39	2.77			<1	<1			0.53
E2 After	12/15/00 13:50	36875.58	3.08			<1	<1			1.07
E2 After	12/19/00 11:50	36879.49	3.64	1,600	1600	<1	<1	5.30	6.70	
E2 After	12/23/00 12:00	36883.50	4.21					5.10	6.60	0.55
E2 After	12/25/00 14:00	36885.58	4.51			<1	<1			0.55
E2 After	12/27/00 10:30	36887.44	4.78	800	800	<1	<1	5.10	5.76	
E2 After	12/31/00 12:00	36891.50	5.36					4.70	6.55	0.99
E2 After	1/5/01 11:00	36896.46	6.07					5.10	6.26	
E2 After	1/11/01 10:07	36902.42	6.92	400	400	<1	<1	5.20	7.20	
E2 After	1/16/01 0:00	36907.00	7.57	915	915	<1	<1	4.70	7.61	1.27
E2 After	1/22/01 11:00	36913.46	8.49	4,390	4390	<1	<1			0.79
E2 After	1/25/01 13:00	36916.54	8.93	970	970	<1	<1	4.40	6.83	0.76

E2 After	1/29/01 11:00	36920.46	9.49	2,045	2045	2.7	<1	4.70	6.90	0.11
E2 After	2/5/01 2:00	36927.08	10.44	<10	10	<1	<1	4.90	6.42	0.20
E2 After	2/11/01 12:00	36933.50	11.36			<1	<1	3.70	6.57	0.72
E2 After	2/14/01 12:00	36936.50	11.79	45	45	<1	<1	3.70	5.60	0.69
E2 After	2/19/01 12:00	36941.50	12.50	60	60	<1	<1	3.80	5.92	0.69
E2 After	2/26/01 17:35	36948.73	13.53	30	30		<1	3.90	6.57	0.83
E2 After	3/4/01 17:20	36954.72	14.39	1,345	1345	<1	<1	7.30	6.64	0.98
E2 After	36959.71	36960	15.10			<1	<1	5.10	6.65	0.83
E2 After	3/11/01 17:11	36961.72	15.39	109	109	2.00	<1	3.70	6.98	
E2 After	4/9/01 17:32	36990.73	19.53	105	105	2.00	<1	3.30	8.10	0.94
E2 After	4/19/01 12:00	37000.50	20.93	153	153	4.70	<1.4			0.90
E2 After	5/22/01 11:45	37033.49	25.64	3,060	3060	<1	<1	3.20	6.37	
E3	12/4/99 12:00	36498.50	-50.79			12	<1.8	7.20	8.28	
E3	6/1/00 12:00	36678.50	-25.07	12,000	12000	14	<1			0.68
E3	6/5/00 12:00	36682.50	-24.50	280	280	9	<1			
E3	6/6/00 12:00	36683.50	-24.36			1<1	><1.5	6.60	9.39	
E3	6/13/00 12:00	36690.50	-23.36							0.59
E3	6/19/00 12:00	36696.50	-22.50	830	830	3	3			
E3	6/21/00 12:00	36698.50	-22.21			15.4	14	8.40	5.60	0.82
E3	7/4/00 12:00	36711.50	-20.36	360	360	61	2			0.78
E3	7/6/00 12:00	36713.50	-20.07			<1	<1	8.70	8.85	0.86
E3	7/18/00 12:00	36725.50	-18.36			17.3	<1.8	8.70	7.46	
E3	8/16/00 12:00	36754.50	-14.21			28.8	<1.4	11.60	4.10	
E3	9/5/00 12:00	36774.50	-11.36	280	280	1<1	6			0.78
E3	9/13/00 12:00	36782.50	-10.21	90	90	14	2			
E3	9/27/00 12:00	36796.50	-8.21	50	50	3.9	1.2	9.90	7.60	0.74
E3	10/2/00 12:00	36801.50	-7.50	10	10	3	<1			0.79
E3	10/10/00 12:00	36809.50	-6.36			1.5	<1	13.70	7.82	
E3	10/23/00 12:00	36822.50	-4.50	20	20	6.4	<1.8	9.00	7.81	0.96
E3	11/23/00 13:00	36853.54	-0.07	2,000	2000	3	3			
E3 After	11/24/00 10:00	36854.42	0.06	<10	10	<1	<1	8.40	5.60	
E3 After	11/25/00 11:00	36855.46	0.21	<10	10	<1	<1			
E3 After	11/25/00 19:45	36855.82	0.26	<10	10	<1	<1			
E3 After	11/26/00 12:00	36856.50	0.36	<10	10	<1	<1			0.88
E3 After	11/26/00 21:45	36856.91	0.42	<10	10	<1	<1			
E3 After	11/27/00 10:30	36857.44	0.49	<10	10	<1	<1	6.60	9.39	0.83
E3 After	11/27/00 23:00	36857.96	0.57	<10	10	<1	<1			
E3 After	11/28/00 11:30	36858.48	0.64	<10	10	<1	<1	6.80	6.80	
E3 After	11/29/00 8:10	36859.34	0.76			<1	<1			0.06
E3 After	11/29/00 20:30	36859.85	0.84			<1	<1			
E3 After	11/30/00 14:45	36860.61	0.94	<10	10	<1	<1	9.60	8.13	0.78
E3 After	12/4/00 10:30	36864.44	1.49	50	50	<1	<1			
E3 After	12/7/00 12:40	36867.53	1.93	1,800	1800	1	<1	12.60	8.05	0.90
E3 After	12/9/00 15:20	36869.64	2.23			<1	<1			
E3 After	12/11/00 12:15	36871.51	2.50	5,200	5200	1	1	9.10	8.77	0.87
E3 After	12/13/00 13:50	36873.58	2.80			5	1			
E3 After	12/15/00 12:10	36875.51	3.07	2,500	2500	9	2	7.70	8.92	
E3 After	12/17/00 13:50	36877.58	3.37			1	<1			0.87
E3 After	12/25/00 16:47	36885.70	4.53			4	<1			0.84
E3 After	12/27/00 10:48	36887.45	4.78	1,400	1400	<1	<1	9.20	8.49	
E3 After	12/31/00 12:00	36891.50	5.36					7.60	8.89	1.21
E3 After	1/16/01 0:00	36907.00	7.57	835	835	4.1	<1	6.50	9.26	
E3 After	3/4/01 17:01	36954.71	14.39	30	30	1.1	<1	5.10	8.99	
E3 After	4/19/01 12:00	37000.50	20.93	1,840	1840	21.5	6.5			0.74
E1	11/27/99 12:00	36491.50	-51.64			22	1<1.4			0.69
E1	6/21/00 12:00	36698.50	-22.07			171	69.4	7.50	5.80	0.57
E1	7/6/00 12:00	36713.50	-19.93			54.8	5.8	10.20	4.65	0.64

E1	7/18/00 12:00	36725.50	-18.21			12	<1.9	10.30	5.44	0.73
E1	8/16/00 12:00	36754.50	-14.07			232	136	10.00	2.65	
E1	10/10/00 12:00	36809.50	-6.21			>10	>10	10.10	3.43	0.74
E1	11/22/00 14:30	36852.60	-0.06	250	250	>88	88			
E1 After	11/25/00 3:00	36855.13	0.30	<10	10	<1	<1	7.50	5.80	
E1 After	11/25/00 11:00	36855.46	0.35	<10	10	<1	<1			0.64
E1 After	11/26/00 4:30	36856.19	0.46	10	10	<1	<1			
E1 After	11/26/00 10:25	36856.43	0.49	<10	10	<1	<1	7.00	2.65	
E1 After	11/26/00 11:30	36856.48	0.50	<10	10	20	2			
E1 After	11/27/00 10:00	36857.42	0.63	40	40	>8	8			
E1 After	11/27/00 12:30	36857.52	0.65	130	130	>42	42			1.03
E1 After	11/27/00 23:30	36857.98	0.71	40	40	>24	24			
E1 After	11/28/00 11:00	36858.46	0.78	70	70	>34	34	7.00	3.27	
E1 After	11/29/00 14:30	36859.60	0.94			>80	>60			
E1 After	11/29/00 16:00	36859.67	0.95			>80	>60			
E1 After	11/29/00 22:15	36859.93	0.99			>80	>60			
E1 After	11/30/00 12:00	36860.50	1.07							
E1 After	12/2/00 12:00	36862.50	1.36			>60	>60			
E1 After	12/2/00 16:00	36862.67	1.38			<1	<1			1.04
E1 After	12/4/00 11:00	36864.46	1.64	4,400	4400	>80	>81			
E1 After	12/11/00 11:45	36871.49	2.64	2,700	2700	>80	230	7.90	2.60	0.95
E1 After	12/13/00 13:00	36873.54	2.93			750	35			0.93
E1 After	12/15/00 13:00	36875.54	3.22	2,400	2400	>80	>60	7.50	2.45	0.91
E1 After	12/27/00 12:00	36887.50	4.93	1,900	1900	180	14	7.50	3.21	
E1 After	12/31/00 12:00	36891.50	5.50					6.80	3.61	1.27
E1 After	1/22/01 11:30	36913.48	8.64	170	170	150	2.2	6.10	7.86	1.43
E1 After	1/25/01 13:30	36916.56	9.08	815	815	240	25.5	10.50	2.40	2.32
E1 After	2/5/01 2:30	36927.10	10.59	1,400	1400	>50	>50	12.10	2.47	1.34
E1 After	2/11/01 12:00	36933.50	11.50			0.5	79	11.70	2.90	1.55
E1 After	2/19/01 12:00	36941.50	12.64	105	105	930	58	7.80	1.95	
E1 After	2/26/01 17:50	36948.74	13.68	95	95	210.00	80	10.00	1.78	0.00
E1 After	4/19/01 12:00	37000.50	21.07	485	485	500	66.9			0.00
surface	6/21/00 12:00	36698.50	-22.14			17.8	2.8	9.60	10.00	0.00
surface	7/6/00 12:00	36713.50	-20.00			16.6	1<1.9	11.50	9.55	0.00
surface	7/18/00 12:00	36725.50	-18.29			26.2	2.8	15.60	8.22	0.00
surface	8/16/00 12:00	36754.50	-14.14			25.2	2.4	12.30	4.75	0.00
surface	9/27/00 12:00	36796.50	-8.14	100	100	32.5	1.2	7.00	11.00	0.00
surface	10/10/00 12:00	36809.50	-6.29			13.9	<1.7	7.00	10.14	0.00
surface	10/23/00 12:00	36822.50	-4.43	40	40	31.6	2.5	5.00	11.02	0.00
surface	12/7/00 12:10	36867.51	2.00	40	40	1	1	1.00	13.72	0.37
surface	1/16/01 12:00	36907.50	7.71	25	25	8.9	<1	0.10	13.72	0.00
surface	5/22/01 11:30	37033.48	25.71	47	47	14.8	3.5	8.20	10.30	0.00
surface	6/6/00 0:00	36683.00	-24.36			24.4	13.5	13.50	9.05	0.00
surface	6/21/00 12:00	36698.50	-22.14			14	5.2	12.20	9.53	0.00
surface	7/6/00 12:00	36713.50	-20.00			16.8	3	11.50	9.38	0.00
surface	7/18/00 12:00	36725.50	-18.29			36.2	6	13.90	8.60	0.00
surface	8/16/00 12:00	36754.50	-14.14			21.5	4	12.60	4.78	0.00
surface	9/27/00 12:00	36796.50	-8.14	110	110	35.2	2.1	6.90	10.50	0.00
surface	10/10/00 12:00	36809.50	-6.29			19	<1.9	6.60	10.45	0.00
surface	10/23/00 12:00	36822.50	-4.43	290	290	29.3	<1.4	5.00	11.16	0.00
surface	12/7/00 12:45	36867.53	2.00	10	10	<1	<1	1.00	13.73	0.39
surface	1/16/01 12:00	36907.50	7.71	>10	10	14.4	<1	0.10	13.62	
surface	5/22/01 12:00	37033.50	25.71	2,195	2195	19.4	2.4	8.90	9.92	

CHAPTER THREE: LAND APPLICATION OF MANURE AND SEWAGE SLUDGE

Well Nest	Well #	Well Depth (m)	Date (d-m-yr)	Code S- sampled, NS- not sampled NW-dry well	Water Level (m)	HPC (cfu/mL)	TC (cfu/ 100mL)	FC (cfu/ 100mL)	Atypical (cfu/100mL)	Temp (C)	DO (mg/L)	EC (mS)	NO3 (mg/L)	Cl (mg/L)	PO4 (mg/L)
LMA1	2	3.62	22-Oct-01	S	3.72		<1	<1	100,000	9.5	6.7				
(9-7)	7	5.28	21-Nov-01	S	4.42	2,540	6	<0.4	5,400	11	1.7		24.9	48.4	0.12
	7	5.28	18-Dec-01	S	4.45	6,120	<1	<1	13,000	9	1.5		27.4	99.0	0.07
	7	5.28	17-Jan-02	S	4.43	3,840	20	<0.4	21,000	8	4		23.7	41.7	0.07
	7	5.28	12-Mar-02	S		4,060	<1	<1					26.1	43.0	0.13
	7	5.28	16-Apr-02	S		880	<1	<1	350				23.5	9.7	0.12
	7	5.28	16-May-02	S		2,340	2	1	312						
	7	5.28	18-Jun-02	S	2.48	30	1	3	145	8.3					0.14
	7	5.28	02-Jul-02	S	2.45	1,570	<1	<1	420	10.1	0.37	1.192			0.10
LMA2	2	4.54	22-Oct-01	S	3.61		10	<1	100,000	11	2.4		24.8	402.3	0.06
(23-2)	2	4.54	21-Nov-01	S	3.65	11,520	9,400	264	18,200	10	1.2		33.3	700.7	0.20
	2	4.54	18-Dec-01	S	3.67	10,240	2,400	35	10,200	8	0.3		26.3	694.8	0.12
	2	4.54	17-Jan-02	S	3.67	10,240	435	8	25,000	7	0.7		18.2	522.6	0.11
	2	4.54	12-Mar-02	S		7,840	<1	<1					21.0	467.7	0.11
	2	4.54	16-Apr-02	S		5,700	100	<0.4	23,000				29.0	109.5	
	2	4.54	16-May-02	S		6,330	6	<0.4	2,300						
	2	4.54	18-Jun-02	S	3.24	1,800	10	5	7,140	7.7	0.99				0.17
	2	4.54	02-Jul-02	S	3.09	3,165	200	2	53,700	10.1	0.90	12.5			0.15
LMA3	2		22-Oct-01	NS											
(MLS#27)	2		21-Nov-01	NS											
(27-2)	3		18-Dec-01	S		1,300	<1	<1	4,700						0.14
	2		17-Jan-02	NS									49.2	83.7	
	2		12-Mar-02	NS									44.1	51.5	0.08
	2		16-Apr-02	S		550	<1	<1	306				23.0	35.9	0.17
	2		16-May-02	NS											
	2		18-Jun-02	NS											
	2		02-Jul-02	Ns											
(27-3)	3		22-Oct-01	NS											
	3		21-Nov-01	NS											
	3		18-Dec-01	S		490	<1	<1	690				35.5	45.9	0.08
	3		17-Jan-02	NS											
	3		12-Mar-02	NS											
	3		16-Apr-02	S		470	<1	<1					17.5	45.8	0.21
	3		16-May-02	NS											
	3		18-Jun-02	NS											
	3		02-Jul-02	NS											
LMF1	1	2.13	22-Oct-01	S	2.03		OG	270	100,000	12	2.6				
(8-2)	2	3.53	21-Nov-01	S	2.06	20	<1	<1	520	11	1.4		16.4	4701	0.13
	2	3.53	18-Dec-01	S	2.38	20,000	<10	1	19,000	9.5	0.5		1.7	469.5	0.07
	2	3.53	17-Jan-02	S	2.54	6,760	<1	<0.4	23,000	7	2.6		9.7	490.7	0.11

	2	3.53	12-Mar-02	S		6,890	<10	<1					13.3	482.2	0.12
			16-Apr-02	S		1,280	<1	<1					14.9	528.2	0.47
			16-May-02	S		88,400	<10	<1	2,400						
	1	2.27	18-Jun-02	S	1.52	570	<10	<1	5,170						0.18
	1	2.27	02-Jul-02	S	1.14	370	<10	1	25,400	12.2	8.60	9.97			
LMF2			22-Oct-01	NS											
(#5)			21-Nov-01	S	1.89	885	<1	1	810	11	0.75		10.3	519.5	0.05
			18-Dec-01	S	1.68	1,710	1	1	37,000	7.5	0.4		7.7	714.1	0.12
			17-Jan-02	S	1.80	2,700	3,800	<0.4	22,200	9	0.9		6.6	704.0	0.14
			12-Mar-02	S		4,520	<10	4					3.8	709.6	0.11
			16-Apr-02	S		60,000	TNTC	TNTC	10,000,000				4.2	498.7	1.32
			16-May-02	S		19,800	10,200	780	25,000						
			18-Jun-02	S		5,000	27,950	20,600	209,000						0.60
			02-Jul-02	NS											
LMF3			22-Oct-01	NS											
(#10)			21-Nov-01	S	2.41	495	30	23	13,090	11	2.2		0.9	250.1	0.11
			18-Dec-01	S	1.88	4,360	10	3	61,000	8	0.7		4.7	319.8	0.14
			17-Jan-02	S	2.09	200	70	<0.4	1,360	7	0.8		1.3	262.8	0.08
			12-Mar-02	S		1,780	10	1	8,900				1.8	246.8	0.14
			16-Apr-02	S		100,000	TNTC	TNTC	10,000,000				9.8	622.0	1.63
			16-May-02	S		221,000	250,000	230,000	1,660,000						
			18-Jun-02	S		80,000	38,000	17,900	472,000						1.31
			02-Jul-02	NS											
LNMA	4		22-Oct-01	S	2.37		20	<1	100,000	11.5	2.5				0.15
(13-3)	3	3.45	21-Nov-01	S	2.46	340	<1	<0.4	17,600	10	1.8		26.7	47.9	0.11
	3	3.45	18-Dec-01	S	2.52	3,425	6	<0.4	84,000	8.5	5.4		24.8	730.1	0.11
	3	3.45	17-Jan-02	S	2.53	2,165	<1	<0.4	46,000	7	3.5		37.8	68.4	0.08
	3	3.45	12-Mar-02	S			1	<1					49.5	83.6	0.10
	3	3.45	16-Apr-02	S		70	<1	<1					6.8	62.8	0.22
	3	3.45	16-May-02	S		2,980	<10	<0.4	770						
	3	3.45	18-Jun-02	S	1.80	190	<1	<1	77						
	3	3.45	02-Jul-02	S	1.83	50	<1	<1	330	10.2	2.49	6.6			0.12
Lab Blank			18-Dec-01			50	<0.4	<0.4							
			17-Jan-02			5	<0.4	<0.4							
			12-Mar-02			<10	<0.4	<0.4							
			16-Apr-02			10	<0.4	<0.4							
			16-May-02				<0.4	<0.4							
			18-Jun-02			<10	<0.4	<0.4							
			02-Jul-02			<10	<0.4	<0.4							
Field Blank			18-Dec-01			10	<0.4	<0.4							0.05
			17-Jan-02												0.07
			12-Mar-02												
			16-Apr-02			10	<0.4	<0.4							
			16-May-02				<0.4	<0.4							
			18-Jun-02			15	<0.4	<0.4							0.07
			18-Jun-02			10	1	<0.4							
			02-Jul-02												0.06

Well Nest	Well #	Well Depth (m)	Date (d-m-yr)	Code S-sampled, NS- not sampled NW-dry well	Water Level (m)	HPC (cfu/mL)	TC (cfu/100mL)	FC (cfu/100mL)	Atypical for graph	Temp (C)	DO (mg/L)	EC (mS)	NO3	Cl-	PO4
CSA1	1	6	07-Nov-01	S	1.40	4,000	<10	<10	5,300	7.1	3.41	1.748	1.3	22.3	0.07
(CS1-a)			28-Nov-01	S	1.40	7,725	10	<1	740	5.4	2.62	1.146	1.5	25.5	0.05
			03-Jan-02	S	1.40	5,240	10	<1	320	5.8	1.86	1.59	1.1	21.5	0.12
			08-Feb-02	S	1.35	1,965	<10	<1	710	3.9	1.31	1.69			
			04-Mar-02	S	1.30	2,190	<10	<1	910	4.2	2.57	1.621			
			08-Apr-02	S	1.25	870	4	<1	480	4.4	1.82	1.68	1.6	20.3	0.10
			22-Apr-02	S	1.25	1,830	<1	<1		5.4	1.42	1.65	1.1	21.8	0.07
			21-May-02	S	1.25	98,000	<10	<1		5.0	1.06	1.752			0.10
			05-Jun-02	S	1.25	640	<1	<1	688	7.1	1.95	1.74			0.12
			24-Jun-02	S	1.55	2,855	<10	<1		8.5	1.85	1.711			
			09-Jul-02	S	1.50	18,200	<10	<1	2,330	9.2	1.73	1.181			0.12
	2	11.3	07-Nov-01	S	6.30	5,120	<1	<1		7.1	1.08	3.05	0.8	22.2	
(CS1-b)			28-Nov-01	S	6.30	TNTC	<10	<0.4		4.8	1.68	2.97	1.2	22.1	0.06
			03-Jan-02	S	4.70	4,660	<1	<1		6.1	1.22	2.85	1.0	22.8	
			08-Feb-02	S	5.70	8,900	<10	<1		4.8	1.16	3.07			
			04-Mar-02	S	4.80	2,080	<1	<1		4.4	1.49	2.97			
			08-Apr-02	S	5.80	570	10	<1		5.6	2.02	3.08	0.8	21.3	0.09
			22-Apr-02	S	5.90	1,210	<1	<1		6.5	1.4	2.86	0.8	22.0	
			21-May-02	S	5.80	17,080	20	<1		6.6	0.91	3.01			0.11
			05-Jun-02	S	5.90	7,300	12	<1			2.61	3.14			0.10
			24-Jun-02	S	6.90	6,125	<1	<1		14.9	1.89	3.11			0.16
			09-Jul-02	S	5.85	6,400	<1	<1		13.6	0.99				0.07
CSA2	1	4.5	07-Nov-01	NW	None										
(CS2-a)			28-Nov-01	NW	None										
			03-Jan-02	NW	None										
			08-Feb-02	NW	None										
			04-Mar-02	NW	None										
			08-Apr-02	NW	None										
			22-Apr-02	NW	None								0.8	20.5	0.08
			21-May-02	NW	None										
			05-Jun-02	NW	None										
			24-Jun-02	NW	None										
			09-Jul-02	NW	None										0.31
	2	10	07-Nov-01	S	5.75	3,920	40	1	100,000	6.2	1.71	5.07	1.3	20.9	0.07
(CS2-b)			28-Nov-01	S	6.40	4,340	1,440	1	42,900	4.7	1.03	5.14	1.6	24.7	0.09
			03-Jan-02	S	5.80	3,035	900	<1	8,800	6.0	1.69	4.66	0.7	14.8	
			08-Feb-02	NS											
			04-Mar-02	S	6.80	3,430	4	<1	840	4.8	5.26	4.88			
			08-Apr-02	S	6.60	1,640	2	<1	480				0.7	17.3	0.09
			22-Apr-02	S	6.75	670	<1	<1		8.1	1.01	4.92	0.8	20.5	
			21-May-02	S	6.60	530	<1	<1	3,000	6.1	1.02	5.18			0.14
			05-Jun-02	S	5.70	2,930	40	<1	2,780	8.0	1.67	5.29			
			24-Jun-02	S	6.60	5,490	<10	<1	8,255	9.6	1.08	3.73			0.09

			09-Jul-02	S	5.70	6,400	<10	<1	2,200	9.5	1.82				0.11
CSAD	1	10.7	07-Nov-01	S	?	20	<1	<1	220	8.5	8.54	1.58	1.9	7.6	0.10
			28-Nov-01	S	?	50	2.0	1	100,000	8.0	7.53	0.001	1.9	10.2	0.05
			03-Jan-02	S	?	60	<1	<1	1,160	7.5	1.35	1.208	2.1	11.5	0.07
			08-Feb-02	S	?	<10	<1	<1	2	7.5	4.06	1.456			
			04-Mar-02	S	?	10	<1	<1	52	7.4	3.24	1.47			
			08-Apr-02	S	?	<10	<1	<1	62	6.6	4.27	1.477	2.1	9.1	0.12
			22-Apr-02	S	?	<10	<1	<1		7.2	3.81	1.394	1.8	9.2	0.10
			21-May-02	S	?	60	<1	<1	1,800	5.9	4.57	0.936			0.06
			05-Jun-02	S	?	10	<1	<1	1,740	6.7	3.77	1.6			0.06
			24-Jun-02	S	?	60	<1	<1	314	7.1	1.58	1.124			0.19
			09-Jul-02	S	?	60	<1	<1	1,955	7.8	2.76				0.08
	2	8.1	07-Nov-01	S	1.60	4,540	<10	<1	50,050	7.0	5.56	2.03	3.5	14.4	0.12
(CNSA-b)			28-Nov-01	S	1.60	2,420	<10	<0.4	13,900	4.4	1.87	2.01	1.0	15.8	0.07
			03-Jan-02	NS											
			08-Feb-02	NS											
			04-Mar-02	S	1.45	7,880	<10	<1	9,500	5.9	2.79	2.02			
			08-Apr-02	S	1.60	9,500	<10	<1	12,500	5.3	2.54	1.98	3.8	15.0	0.09
			22-Apr-02	S	1.60	3,350	<10	<1		7.2	1.82	1.91	1.9	11.1	0.09
			21-May-02	S	1.40	2,680	<10	<1	550	5.8	1.76	1.401			0.11
			05-Jun-02	S	1.55	2,410	<10	<1	1,750	8.3	1.81	2.44			0.06
			24-Jun-02	S	2.15	3,630	<10	5	19,450	9.4	1.93	1.495			0.14
			09-Jul-02	S	1.55	2,400	<10	<1	3,500	10.3	2.48	1.43			0.12
CNSP	1	4.4	07-Nov-01	NS											
			28-Nov-01	S	0.50	355	<1	<1	20	6.7	0.99	7.56	27.6	44.1	
			03-Jan-02	NS											
			08-Feb-02	S	0.40	5	<10	<1	740	6.5	1.53	7.3			
			04-Mar-02	S	0.30	20	<1	<1	230	4.7	3.18	7.18			
			08-Apr-02	S	0.40	160	<1	<1	75	4.4	3.58	7.04	17.8	21.7	0.12
			22-Apr-02	S	0.50	80	<1	<1		4.9	5.56	6.92	41.1	41.9	0.08
			21-May-02	S	0.56	670	<1	<1	470	5.1	2.37	7.41			0.16
			05-Jun-02	S	0.72	30	<1	<1	154	6.8	4.59	7.58			0.09
			24-Jun-02	S	0.59	90	<1	<1	474	6.9	2.33	4.98			0.16
			09-Jul-02	S		440	<1	<1	137	0.6	2.74	9.66			0.15
F.Blank			28-Nov-01	S		<10	<1	<1							
			03-Jan-02	NS											
			08-Feb-02	S		20	<1	<1							
			04-Mar-02	S		<10	<1	<1							
			08-Apr-02	NS											0.06
			22-Apr-02	S		5	<1	<1							0.06
			21-May-02	S		4,680	<1	<1	7,120						0.07
			05-Jun-02	S		<10	<1	<1							0.05
			24-Jun-02	S		<10	<1	<1							0.06
			09-Jul-02			25	<1	<1							

CHAPTER FOUR: ATYPICAL INHIBITION

Well Coliforms

Well ID	Total Coliforms (cfu/100mL)
LF1-1	1
LF1-1	0
LF1-1	0
LF1-1	0
LF1-1	0
LF1-1	0
LF1-1	0
LF1-1	0
LF1-1	0
Average	0.10
SD	0.32

Atypical Densities

Dilution	Atypical cfu on plate	cfu/100mL culture
1.0E-01	78	7.8E+04
1.0E-01	84	8.4E+04
1.0E-01	50	5.0E+04
1.0E-01	61	6.1E+04
Average	68	6.8E+04
SD	16	1.6E+04

Culture Densities

Dilution	cfu on plate	cfu/100mL culture	~TC in culture (cfu/100mL)
1.0E-07	64	6.4E+10	*6.2E+10*
1.0E-07	50	5.0E+10	
1.0E-07	62	6.2E+10	
1.0E-07	75	7.5E+10	
1.0E-07	61	6.1E+10	
1.0E-07	61	6.1E+10	
1.0E-07	57	5.7E+10	
1.0E-07	67	6.7E+10	
Average	62.125		
sd	7.26	7.3E+09	

Innoculated Samples

Well ID	cfu in 100mL well sample	cfu innoculated into sample	cfu expected	actual cfu on plate	% Inhibtion
LF1-1	0.10	62.13	62.23	38	38.9
	0.10	62.13	62.23	49	21.3
	0.10	62.13	62.23	42	32.5
	0.10	62.13	62.23	30	51.8
	0.10	62.13	62.23	29	53.4
	0.10	62.13	62.23	15	75.9
	0.10	62.13	62.23	0	100.0
	0.10	62.13	62.23	48	22.9
	0.10	62.13	62.23	0	100.0
	0.10	62.13	62.23	42	32.5
	0.10	62.13	62.23	47	24.5
	0.10	62.13	62.23	39	37.3
	0.10	62.13	62.23	34	45.4
	0.10	62.13	62.23	43	30.9
	0.10	62.13	62.23	45	27.7
Average				33.4	46.3

SD of actual
16.18994397
t-test
Actual<61.60
-1.990741911
df
14
t crit (one-tailed)
1.761
ts>tcrit, reject null
SIGNIFICANT DIFF
Average of 46% Inhibtion

SEWAGE CONTROLS

Coliform Culture Densities

Dilution		cfu/100mL culture
1.0E-07	100	1.0E+11
1.0E-07	96	9.6E+10
1.0E-07	91	9.1E+10
1.0E-07	103	1.0E+11
1.0E-07	109	1.1E+11
1.0E-07	99	9.9E+10
Average	*99.7*	*1.0E+11*
SD	6.121002097	

Effluent Coliform Densities

Dilution	cfu on plate	cfu/100mL culture
1.0E-02	25	2.5E+05
1.0E-02	23	2.3E+05
1.0E-02	25	2.5E+05
1.0E-02	21	2.1E+05
1.0E-02	24	2.4E+05
1.0E-02	24	2.4E+05
1.0E-02	22	2.2E+05
Average	*23.4*	*2.3E+05*
SD	*1.5*	*1.5E+04*

Innoculated Samples

Average cfu in 10-7 culture	Average cfu in 10-2 effluant	(10-7+10-2) cfu expected	(10-7+10-2) actual cfu on plate	Comparison (expected-actual)
99.70	23.40	123.10	137	13.9
99.70	23.40	123.10	112	-11.1
99.70	23.40	123.10	96	-27.1
99.70	23.40	123.10	124	0.9
99.70	23.40	123.10	125	1.9
99.70	23.40	123.10	108	-15.1
99.70	23.40	123.10	112	-11.1
Average			116.2857143	
SD			13.42527822	

SD of actual
13.42527822
t-test
Actual<123.10
-0.507571285
df
6
t crit (one-tailed)
1.943
ts<tcrit, accept null
NO SIGNIFICANT DIFF

APPENDIX TWO: PERMISSION TO INCLUDE PREVIOUSLY PUBLISHED ARTICLES

From: "Jill Ross" <jross@ngwa.org>
To: <psyancegirl@shaw.ca>
Cc: "'Linett Adell'" <ladell@ngwa.org>
Subject: RE: Permission to Publish GWM&R article as part of MSc thesis
Date: Monday, June 23, 2003 2:11 PM

Dear Jennifer,

You have our permission to include the article mentioned below as a chapter in your thesis. Please include the full citation in your thesis (Ground Water Monitoring & Remediation 22, no.4: 66-72). I trust that you will be contacting the other authors to get their permission. We cannot assume the responsibility for this.

Sincerely,

Jill Ross/ Director of Publications

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601 Dempsey Rd.
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<http://www.wellowner.org/>
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calgary health region

Timothy Lambert PhD., MSc.
Manager Environmental Health Risk Assessment and Management
4th Floor, 1509 Centre Street SW
PO Box 4016 Station C
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Healthy Communities

October 21, 2003

Jennifer Oliphant

Re: Efficacy of Annual Bacteria Monitoring and Shock Chlorination in Wells finished in a Floodplain Aquifer, GWM&R.

You have my permission to use the above publication as part of your fulfillment for your master degree from the University of Calgary.

Timothy Lambert

----- Original Message -----
From: Cathy Ryan
To: Jennifer Oliphant
Sent: Tuesday, October 21, 2003 7:41 PM
Subject: GWMR 22(4):66-77

Jennifer Oliphant has my permission to republish the "Efficacy of Annual Bacteria Monitoring and Shock Chlorination in Wells finished in a Floodplain Aquifer" (originally published in Groundwater Monitoring and Remediation, 22(4):66-77) in her Master's thesis in Civil Engineering at the University of Calgary.

Cathy Ryan

Associate Professor, Ph.D., P.Geol, P.Eng
Department of Geology and Geophysics
University of Calgary, Calgary, Alberta, T2N 1N4, Canada
Phone: (403)220-2793
Fax: (403) 284-0074
e-mail: cyan@ucalgary.ca
http://www.geo.ucalgary.ca/ryan.htm

----- Original Message -----
From: achu@ucalgary.ca
To: Jennifer Oliphant
Sent: Wednesday, October 22, 2003 9:25 AM
Subject: Re: Permission to Publish GWM&R article as part of MSc thesis

Jennifer,

I give permission for the article entitled: Efficacy of Annual Bacteria Monitoring and Shock Chlorination in Wells finished in a Floodplain Aquifer pulished in Ground water Monitoring and Remediation to be published in your MSc thesis. Regards,

Angus Chu
Associate professor
Department of Civil Engineering
The University of Calgary