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WATER QUALITY ASSESSMENT IN THE SANTA FE RIVER: TRACKING POLLUTION SOURCES VIA QUANTITATIVE POLYMERASE CHAIN REACTION ANALYSIS

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THESIS

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ABSTRACT

Microbial contamination affects many water bodies in the United States and pathogens associated with contamination pose a threat to human health. While the nation's lakes, streams and rivers have been monitored for decades, many still do not meet the requirements of the 1972 Clean Water Act. Due to the number of pathogens that occur in water bodies, it is not feasible to directly monitor all of them. Instead of testing for a plethora of pathogens, it is standard practice for water divisions to monitor fecal indicator bacteria (FIB) as a proxy to determine water quality. There are significant flaws, however, with this approach, including the poor correlation of FIB with many significant pathogens and, most importantly, the inability to identify the sources of contamination.

The City of Santa Fe Water Division monitors FIB in the Santa Fe River but cannot determine the source of contamination when tests come back positive. In this thesis, microbial source tracking is used on water samples from five different locations along the river to provide insight into the quality of the Santa Fe River water and determine the sources of contamination. Water from each site was tested for human, dog, bird, beaver and/or ruminant genetic markers and identified through the terminal restriction length polymorphism of the 16S ribosomal RNA gene. FIB were detected at all sites at either low, medium or high concentrations. The information from this thesis aids the Santa Fe Water Division in complying with the United States Environmental Protection Agency stormwater discharge permit requirements by informing their best management practices. Future microbial source tracking will allow the city to create a water quality baseline for the Santa Fe River and allow water quality progress to be quantified and verified.

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ABBREVIATIONS AND ACRONYMS

BDD	Buckman Direct Diversion
BMPs	Best Management Practices
CWA	Clean Water Act
DNQ	Detected, Not Quantified
DO	Dissolved Oxygen
EPA	Environmental Protection Agency
FIB	Fecal Indicator Bacteria
FWPCA	Federal Water Pollution Control Administration,
IPCC	International Panel on Climate Change
IWRM	Integrated water resource management
ml	Milliliters
MS4s	Municipal Separate Storm Sewer Systems
MST	Microbial Source Tracking
NMED	New Mexico Environment Department
NOIs	Notice of Intent
NPDES	National Pollutant Discharge Elimination System
OSE	Office of the State Engineer
qPCR	Real-time Polymerase Chain Reaction
SWMP	Stormwater Management Program

CHAPTER ONE

INTRODUCTION

The southwestern United States is experiencing a water crisis. The per capita quantity of available water has declined due to population growth and industrial development, which have also led to decreased water quality. Municipal water resource managers across the region are challenged to provide sufficient clean water to meet the demands of residential, commercial, and industrial users. The majority of the water supply for the Southwest is snowmelt (Diaz and Anderson 2016). The supply from melting snow however, can vary extensively depending on the proportion of precipitation that falls as snow. In years with limited snowfall, this can translate into less water being available for storage and transport in southwestern reservoirs. A reduction in water quantity can also negatively impact the water quality. Human activity and development are adding to the complexity of the water crisis by contaminating the already scarce resource.

In New Mexico, there are a small number of water courses available to provide potable water to the public; few have perennial flow. The Santa Fe River in Santa Fe is an intermittent stream that runs periodically throughout the year, usually during summer months. It courses through developed, urban environments where it gathers water from streets, buildings, parking lots and residential yards during storm events. The river is regularly used for livestock watering, recreational activities and irrigation, mostly via diversion into acequias (irrigation canals). The New Mexico Heritage Preservation Alliance recognizes the Santa Fe River is under distress and has named it as one of the state's twelve most endangered places in 2007. The advocacy group American Rivers, based in Washington D.C., also named it one of America's Most Endangered River in 2007 (City of Santa Fe 2016).

The river is regularly tested for *E. coli* which acts as a potential fecal indicator bacteria (FIB). Water divisions test for the presence or absence of FIB such as total coliform or *E. coli* because they act as proxies for human health risk. While testing for these bacteria is common practice among water divisions nationwide, the information provided by the tests only provide proof of contamination, but no information about the potential sources of that contamination. A newer analytical technique, microbial source tracking (MST), promises to provide better information to water managers. MST is an innovative technique that has emerged within the last decade to determine sources of contamination in water. It utilizes real-time quantitative polymerase chain reaction (qPCR) to identify specific microorganisms that are strongly associated with particular human and animal hosts and therefore can be used to determine fecal contamination by specific hosts (Field and Scott 2015). MST methods can identify "who" is the source of pollution while older, traditional methods can only tell "if" and "when" contamination occurs (Astrom et al. 2015, Boehm et al. 2006, Boiteau et al. 2009, Nshimyimana et al. 2017).

The City of Santa Fe currently makes assumptions about the sources of positive FIB tests. These assumptions are based on the surroundings of the sample sites. However, because environments are not isolated, there are multiple potential sources that cannot be differentiated using traditional water quality testing methods. The specific information provided by MST testing will be helpful to municipal water managers by enabling them to stop any contamination occurring in a timelier and more effective manner and it will allow them to track changes in specific pollutants throughout the year. It will also benefit public health by providing water managers with the vector information necessary to determine the cause of a waterborne illness outbreak.

Stormwater runoff is the leading source of water pollution, including microbial contaminant pollution (Ghane et al. 2016). High concentrations of polluted runoff can cause societal, ecological and economic concerns because they are a public health risk (Ghane et al. 2016). To decrease the public health and environmental risks caused by contaminated waters, the City of Santa Fe must comply with the Federal 1972 Clean Water Act and obtain federal permission to discharge pollutants into municipal storm sewer systems (MS4s). The MS4s in Santa Fe generally drain into the Santa Fe River. To obtain the necessary permit, the Stormwater Management Division of the city must provide a plan to reduce and control pollutants going into the river.

This thesis aims to determine the sources of fecal contamination in the Santa Fe River, which is an important question the City of Santa Fe must answer to develop a plan for compliance with state and federal water quality requirements. This will be accomplished utilizing the MST method real-time polymerase chain reaction (qPCR) on water samples from the Santa Fe River at five different sites to test for human, dog, bird, beaver and/or ruminant genetic biomarkers. In identifying and quantifying contamination sources, the Santa Fe Water Division will have a baseline for water quality. The MST results will also provide the objective evidence necessary for the City of Santa Fe to make sound water management decisions. This research will support efforts to improve best management practices (BMPs), instruct future regulations and educate the City of Santa Fe and the public.

The background for this project places the Santa Fe River in the greater Santa Fe watershed and discusses its significance as one of the city's primary water sources. The literature review explores three topics related to the primary research of this thesis, watershed management, the impact of a southwestern climate on water resources as well as traditional and recent water quality testing methods. The research design section details the methods used to select sample sites and biomarkers, collect water samples and describes the MST methodology performed by Source Molecular. The ensuing discussion identifies trends in the results, project limitations, and provides recommendations to better fulfill water quality requirements.

CHAPTER TWO

BACKGROUND

The quality of Santa Fe River water is important to study because the river is one of very few water resources in the city. The river lies within the Santa Fe Watershed (City of Santa Fe 2015). A watershed is defined as an area of land where all the water that falls and drains in the given area goes to a common outlet such as a reservoir, river or mouth of a bay (United States Geological Survey 2015). The Santa Fe Watershed is part of the larger Rio Grande Watershed that encompasses 116.6 million acres (City of Santa Fe 2016). Throughout the 1800s, heavy grazing and logging as well as homesteading was prevalent in the upper watershed (Santa Fedia 2012). By the early 1900's the area was depleted of trees and most vegetation, which led to significant erosion that contaminated the water. The upper Santa Fe Watershed became closed to the public in 1932 by order of the Secretary of Agriculture due to the contamination concerns caused by human activity (Santa Fedia 2012).

However, there were initially four reservoirs built on the Santa Fe River between 1881 and 1943. Stone Dam was the first and stored 25 acre-feet of water. Acre-feet is a common metric used in water resources. One acre-foot covers one acre (43,560 square feet) with water one foot deep (Duris and Reif 2015). This is about 326,000 gallons (Duris and Reif 2015). In 1904 Stone Dam filled with sediment from a flash flood and never stored water again. The second reservoir was Two-Mile Dam constructed in 1893, holding up to 387 acre-feet. However, in 1994 it was deemed structurally unsafe and destroyed. McClure Reservoir is upstream of Nichols Reservoir and the larger of the two remaining reservoirs. The storage capacity of McClure Reservoir is 3,255.6 acre-feet while Nichols Reservoir has a capacity of only 684.2 acre-feet (Gonzales 2009). Watershed runoff is stored in the reservoirs, then released to the Santa Fe River, assorted irrigation channels called acequias or the Canyon Road Water Treatment Plant from which it is distributed to residents. The Santa Fe River watershed is classified as a category 1 watershed (Grant 2002). It is classified a category 1 because it provides 40 to 50 percent of the City of Santa Fe's water supply from the Sangre de Cristo mountains east of the city and is thus in urgent need of continued restoration (Grant 2002).

During times of drought, the reservoirs are drawn down to the point where they need to be augmented by wells pumping groundwater to provide the water for Santa Fe. However, the amount of pumping has depleted the supplying aquifers. The city also receives a small percentage, 5-6%, of water from the San Juan Chama project. These problems prompted Santa Fe and the surrounding county to undertake the Buckman Direct Diversion Project in order to ensure a readily available sustainable drinking water source for the city should something jeopardize use of the McClure or Nichols Reservoirs.

The Buckman Direct Diversion Project (BDD), completed in 2010, consists of a diversion structure, a sediment removal facility, two raw water booster stations, a water treatment plant, 11 miles of underground raw water pipeline, another pipeline to pump water to the Las Campanas community, two treated water pump stations, and eight million gallons of raw water storage (Buckman Direct Diversion 2015). BDD pumps water from the Rio Grande to the processing facilities and eventually distributes the water once it is clean. The diversion is capable of pumping 15 million gallons per day, the expected maximum water demand of Santa Fe County and the City of Santa Fe (Buckman Direct Diversion Project n.d). The water also goes to replenishing wells that were being depleted due to the increase in demand and inability of McClure and Nichols Reservoirs to accommodate the pre-BDD increased demand. The BDD is allowed to divert by law 8,730 acre-feet per year from the Rio Grande (Buckman Direct Diversion Project n.d.). That water is shared between the Santa Fe County, the City of Santa Fe and the Las Campanas community, a residential area just outside the city. Water quality is generally high but after it is diverted it is processed through the Buckman water treatment plant that has a nine-step water treatment process.

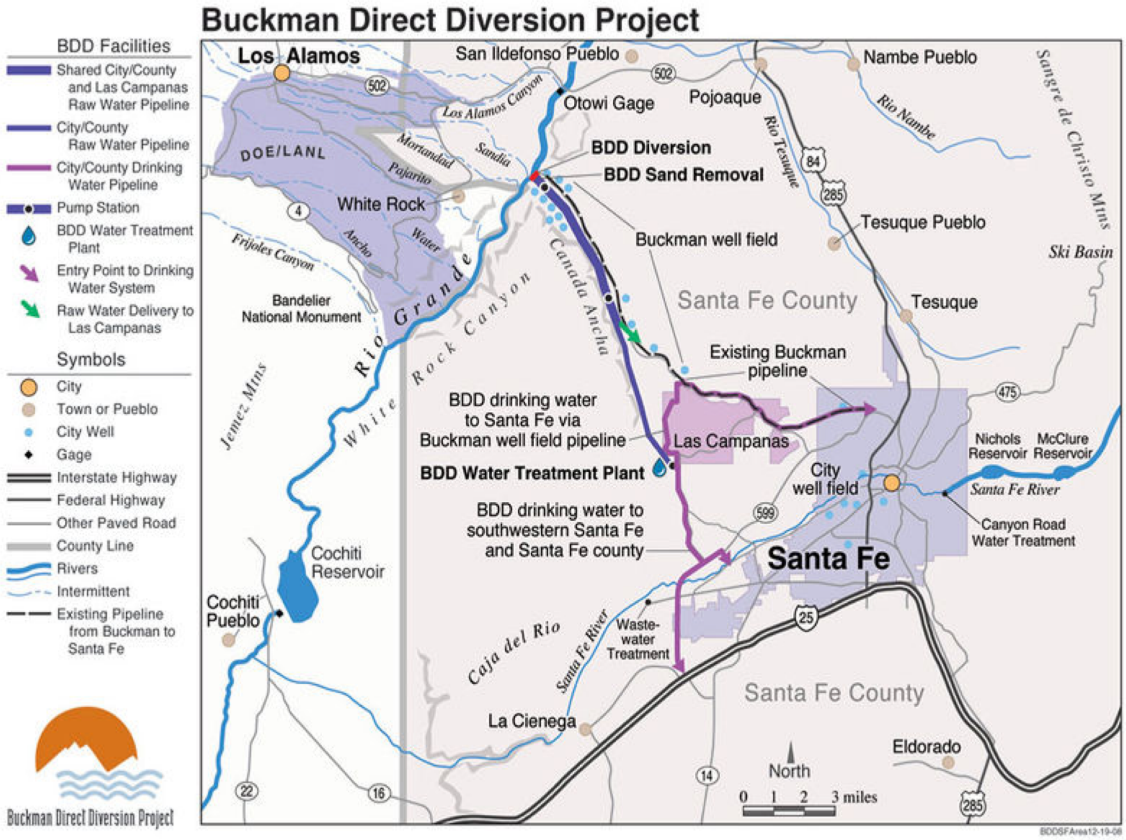

Figure 1. Map of Buckman Direct Diversion Project lay out, sources and final recipients. (Source: http://www.santafedia.org/wiki/index.php?title=File:Buckman_diversion.jpg)

CHAPTER THREE

LITERATURE REVIEW

3.1 Introduction

The following literature review explores the connections between watershed management, climate and water scarcity to better demonstrate the need for water contaminant testing along the Santa Fe River in Santa Fe New Mexico. The first section explains the evolution of watershed management and the variables that make setting a standard management protocol challenging. It also examines environmental and human impacts on water quantity and quality. The second section demonstrates the importance of water in the Southwest due to the area's arid climate. It details how growing populations and continuous droughts have put stress on water resources and watershed management. It also reviews alternative water sources and the consequences of utilizing those sources. The third and final section of the review focuses on water quality testing. It examines traditional water quality testing and the potential health consequences of contamination. It also discusses the crucial role stormwater runoff plays in the contamination of watercourses. The section concludes with a summary of microbial source tracking and the potential this new method has for contaminant identification and mitigation in the future.

3.2 Watershed Management

Water's role in everyday life cannot be overstated because water fuels every aspect of life. It is essential in food and energy production, ecosystem health, livelihoods and sanitation (Ganoulis, J. 2006, O'Lear et al. 2013, Figure 3).

Approximately 1.6 billion people currently live in countries with water scarcity and in two decades this will double (World Bank Group 2016). Climate change has created variability in rainfall patterns and caused more extreme temperatures, leading to shorter rainy seasons and longer dry seasons. The spatial distribution of runoff, which many countries rely on, will also become more uneven across the globe (World Bank Group 2016). The decreased water supplies negatively impact economies, health and migration. This is most often seen in periphery developing countries however, complacency in developed core countries is one of the most significant threats to water longevity because readily accessible water that is thought to be guaranteed can lead to poor planning, contamination or waste. Watersheds provide a framework to manage water resources in the United States and successful watershed management strategies will have to include a range of scales in decision-making and the environment. A variety of variables impact watersheds but too often watershed management is focused more on water quantity than quality, especially in the arid southwest. Studies such as this thesis are important because large quantities of water can mean very little if the water quality is not suitable for use.

Watersheds have been used as the fundamental spatial unit of analysis for natural and human landscapes since water resources entered the American policy and planning agenda (National Research Council 1997). Watershed management is defined as the "plans, policies, and activities used to control water and related resources and processes in a given watershed" (National Research Council 1997). Federal agencies used watersheds as a cornerstone when planning the nations waterways in the 1920's (National Research Council 1997). During that time, much of the research on watersheds emphasized enhancing knowledge on water quantity and its movement. It was not until the 1940's that water quality control entered the watershed management agenda (National Research Council 1997). By the 1960's, watershed management programs were widespread under the guidance of the Federal Water Pollution Control Administration (FWPCA) (National Research Council 1997). With the creation of the FWPCA, research expanded to include pollution identification and transport instead of focusing solely on water quantity. In 1972, sweeping amendments to the Federal Water Pollution Act were passed by Congress (Environmental Protection Agency 2017). The goal of these amendments, also known as the Clean Water Act (CWA), were to protect fishable, drinkable and swimmable waterways by maintaining the chemical, physical and biological integrity of the waterways (Environmental Protection Agency 2017). The CWA currently provides guidelines on the management of pathogenic organisms, nonpoint sources, hazardous substances, wetland protection and ecosystem restoration. It has data for watershed managers on the harmful levels of different contaminants and the potential adverse health effects these contaminants have on human and aquatic life. The effectiveness of the guidelines varies however, because of the numerous factors influencing a watershed.

Applying elements of watershed management across all watersheds small-scale or large-scale is difficult because of the complex social, economic, and environmental setting that is any watershed. The scale of the information should determine the scale of the decision-making. The properties within a watershed are not necessarily additive so transferring knowledge between watersheds of varying scales is challenging. Integrated, holistic, ecosystem-based management is the goal of watershed managers but it is difficult to achieve with enough certainty that is often demanded in policymaking. Integrated water resource management (IWRM) is currently the accepted standard internationally for watershed management (The United Nations n.d.). It is a place-based holistic approach that values on-the-ground experience of practitioners. It integrates natural, social and economic factors that are shared in a geographic area. The focus is on hydrologically defined areas rather than areas defined by political boundaries. IWRM relies on the conviction that none of the factors influencing the watershed, chemical, biological, physical and economic, can be altered without influencing water quality (James 2009). Due to their interconnectedness, all factors must be considered when implementing new water management policies. The IWRM approach creates a flexible framework that strives for interagency coordination, public involvement and consideration of the interaction between physical, biological and social systems to preserve a healthy system (James 2009).

While the IWRM is widely accepted, watershed management is still struggling with the fragmentation of authority. There are multiple agencies that play a role in water resources but they are not necessarily working towards the same goal. At the federal level in the United States, the EPA determines and assesses water quality for recreational and consumption use under the Safe Drinking Water Act and the Clean Water Act (National Research Council 1997). The U.S. Army Corps of Engineers examines wetland preservation, flood control and navigation while the

Natural Resources Conservation services are responsible for soil erosion and the U.S. Fish and Wildlife Service is responsible for the health of aquatic and adjacent terrestrial communities (National Research Council 1997). They are competing for federal money and often disagree on the appropriate approach to managing their branch of water resources. This same problem is mirrored at the state and local level as well and has been an impediment to IWRM and thus the overall quality of water.

Human activities on all spatial scales have cascading effects on water quality and quantity. Water quantity and quality are not mutually exclusive because the quantity of water available is closely linked to the quality of that same water which can limit its use. The literatures demonstrate that when water levels rise, pollutants are generally diluted and water quality improves (Tecle and Neary 2015 and Lind and Davalos-Lind 2002). Alternatively, increasing water levels often occur because of storm events which introduce new pollutants into the watercourse from runoff thus worsening the water quality (Gelt 1998). Documented water quality problems due to decreasing water quantity include shallow water algal blooms, declining native fish, increases in toxic substances and infestation of water plants (Lind and Davalos-Lind 2002). Humans are over-exploiting the resource and causing declining volumes thus water quality issues are likely to become more prevalent in the future.

The surrounding media and activities in any watershed will determine the quantity and quality of the water entering and flowing through the watershed. Watersheds can easily be altered by activities such as mining, agriculture, or urban development. However, the interventions water managers use to protect watersheds also present a threat to the overall quality of the watershed (James 2009). For example, a common management practice is to reduce the suspended solid load in a water body to increase water quality (Gelt 1998 and Peters and Meybeck 2000). However, a decrease in solid load will increase the transparency of the water which with enough residence time could lead to eutrophication (Dahlegren et al. 2004). Therefore, before any restoration plans are enacted all surrounding factors must be considered to reduce management risks.

Municipalities are taking steps to more actively manage their watersheds in the face of climate change but in doing so may be undermining their water quality. A change in watershed management practices could lead to a change in the source of the primary water supply. Water supply is dependent on forest health and resilience to catastrophic forest fires. In regions like the Southwest that have arid climates, the understory vegetation in a forest is usually grass and shrubs that are easily susceptible to fire (Tecle and Neary 2015). Vegetation and top soil slow the movement of water so it can infiltrate the soil and be taken up by plants (Gelt 1998). Prescribed burns, used to protect these areas from wildfire, impact the soil chemistry and kill much of the vegetation. Fires also lead to hydrophobicity which slows the rate of water infiltration in soil and causes an increase in surface water movement (Tecle and Neary 2015). Rainfall after burns quickly flows from the burned areas into reservoirs picking up significant amounts of sediment, debris and chemicals along the way and negatively impacting reservoir water quality (Tecle and Neary 2015 and Peters and Maybeck 2000). Water managers must also be cautious of legacy sediment (James 2009). Legacy sediment can contain vast reservoirs of toxic biologic or geologic material (James 2009). Chemically after a forest fire, there is often an increase in the production of macronutrients, micronutrients, biological demand in the reservoirs and a decrease in oxygen level (Tecle and Neary 2015). These changes will create zones of hypoxia or eutrophication in the watershed. Sediment carrying toxic material that is in the hyporheic zone, the region just beneath or alongside a body of water where mixing of surface water and deeper water occurs, is of greater concern because those contaminants will be easily transferred to water supplies and aquatic organisms (James 2009). Sediment at a greater depth will likely be away from ecological activity and points of water extraction. Thus, when sampling it is important to keep the sampling location and depth in mind.

Watershed management is being revisited and scrutinized today because of the growing concern surrounding climate change. The National Weather Service organizes its climatic, precipitation and drought data according to climate regions that correspond to watershed boundaries (National Research Council 1997). Watershed management will have to evolve as global change continues and management efforts will have to be highly specific to a region and the local context. Given these changes, it is paramount to understand waters role in different climates and how global change is expected to change the availability of water.

3.3 Water Resources in the Southwest

Due to the arid climate, water has played a significant role in shaping the economic activities and overall lifestyle of the Southwest. The stability of the region relies on the predictability of its climate. To grasp the future of watershed management in New Mexico, it is crucial to understand the regions climate and how it is projected to change in the coming years. With this knowledge and that of the local geographic and cultural conditions, water managers, to the best of their ability based on the data, must accurately and reliably plan for the future. The best management practices (BMPs) that will need to be enacted will change the geography of not just water in the southwest but also the population.

Climate modelers and scientists are predicting climate change will make many parts of the Southwest hotter and significantly drier, thus making water an even more limited and valuable resource (Garfin et al. 2014). Based on the International Panel on Climate Change (IPCC) A2 emissions scenario, regional annual average temperatures are projected to rise 2.5 F to 5.5 F by 2041-2070 and 5.5 F to 9.5 F by 2070 – 2099 (Garfin et al. 2014). Even with the IPCC B1 scenario, where global emissions are significantly reduced, temperatures are still projected to rise significantly. For the B1 emissions scenario temperatures are projected to increase from 2.5 F to 4.5 by 2041-2070 and from 3.5 F to 5.5 F by 2070-2099 (Garfin et al. 2014). Researchers assert temperature increases will be fairly uniform throughout the region but there is less continuity in modeled precipitation trends. Currently, portions of the Southwest are experiencing precipitation increases while others are experiencing decreases (Garfin et al. 2014). The regional disparity in precipitation in the Southwest is expected to continue, making prediction challenging. While all states in the Southwest can expect increases in temperature, these states must also prepare for potential decreases and/or increases in precipitation that are unlikely to be consistent spatially through time.

The increases in temperature and variations in precipitation will ultimately determine winter snowpack. Winter snowpack and the subsequent spring melt off is key to New Mexico and southwestern water supplies. It is also key to New Mexico's water quality because higher snowmelt will translate into large quantities of water that will dilute pollutants. Due to temperature increases over the last 50 years, there has been less late-winter precipitation and earlier, and at times more rapid, spring snowmelt and subsequent runoff occurring in many parts of the Southwest (Garfin et al. 2014). For example, stream flow totals for the Rio Grande River were between 5% and 37% lower from 2001 to 2010 than the 20th century average flows for the river (Garfin et al. 2014). Snowpack is also impacted by dust from lowland drying due to reoccurring drought. Dust blown from the lowlands to the higher elevations accumulates on the snow. The new darker color of the snow causes the area's albedo to decrease and therefore the amount of the sun's radiation absorbed by the surface increases (Skiles and Painter 2017). The increase in radiation absorbed increases the temperature, thus causing or accelerating snow melt evaporation, and possible sublimation (Skiles and Painter 2017).

Along with increased rates of snowmelt, rising temperatures and precipitation variation in the Southwest make the area more prone to wildfire. Wildfire will not only damage the ecosystem of a watershed but also contribute a substantial amount of sediment and pollutants to the water thus reducing its quality. The arid climate leaves the southwest particularly prone to wildfires due to drought, insect infestation and accumulation of woody and grass fuels (Cook et al. 2015). Westerling, A.L. et al. (2006) found that earlier spring snowmelt and longer summer dry seasons increased wildfires and produced longer fire seasons. The rising temperatures coupled with periodic drought have also caused massive tree death across the Southwest. The die off of trees has the potential to increase erosion and runoff, dumping unwanted debris into reservoirs and other water systems.

The rising temperatures globally will also cause water temperature to rise in streams, lakes and reservoirs (Environmental Protection Agency 2017). An increase in water temperature will decrease the levels of dissolved oxygen that plants and animals rely on and thus decrease the diversity and health of the watercourse (Environmental Protection Agency 2017). Warmer water temperatures also promote the growth and reproduction of diseases such as legionella, campylobacteriosis and cholera (Physicians for Social Responsibility 2014). Additionally, an increase in soil temperature will lead to more nitrogen mineralization, increased enzymatic activity in the soil that will lead to more nitrogen availability thus raising the nutrient load in water bodies (Delpla, I et al. 2009). Along with a decrease in snowmelt there is expected to be an increase in intense rain events which leads to runoff that washes sediments, nitrogen, pesticides, herbicides and disease pathogens into watercourse (Rehana and Mijumdar 2012). Smith et al. (2001) also demonstrated through logistic regression that watersheds with large proportions of urban land cover or agriculture had a chance of being contaminated by pathogens. Due to the warmer temperatures, precipitation will be falling more frequently as rain instead of snow and causing more runoff events. The excess of nutrients in a watercourse can lead to algal blooms and eutrophication which produce harmful toxins that impact ecosystems and human health.

Water resources are not only stressed by climatic events but also by a growing population. During the 20th century, water policies changed the geography of water resources with the common philosophy at the time of build centralized, large-scale infrastructure to easily transport water and anticipate future demand. In the Southwest, this philosophy led to the building of very large dams and aqueduct systems (Gleick 2010). The aim of these projects was to support and encourage population and economic growth in the region. The project thus changed the spatial distribution of people from 1920 to 2000 as the population growth of the seven states in the Southwest and the Great Plains that share the Colorado River grew 762% (Gleick 2010). These seven states included New Mexico, Arizona, California, Nevada, Utah, Wyoming, and Colorado. The population in the Southwest is still among the fastest growing in the United States, and development in the region has often occurred in locations where water is not easily accessible. While water scarcity is expected to cause a spatial shift in population, that shift usually entails people moving to other regions in the United States away from areas of scarcity. In this case however, people are moving to areas of scarcity, the Southwest. The population of the Southwest is expected to increase by 68% by 2050 to approximately 94 million. (Garfin et al. 2014). This significant increase in population will inevitably put large amounts of pressure on already stressed water resources.

There are few major rivers in the Southwest to supply the demand for surface water to the current population. Complex water distribution networks and interstate agreements have spatially altered the resource to try and distribute the valuable resource fairly (Konieczki and Heilman 2004). One such agreement, and arguably the most important for the Southwest, is the Colorado River Compact of 1922. The Colorado River is approximately 1500 kilometers long and courses through Colorado, Utah, Arizona, Nevada, and California. Its watershed also extends into Wyoming and New Mexico. Overall, the Colorado River serves approximately 30 million people (Arthur 2016). The Compact was crucial in fairly and legally allocating the water rights of the Colorado to the seven different states and was negotiated between those states and the federal government (Arthur 2016). Although the Compact has been amended several times, the primary purpose of it was to ensure that Upper Basin States (New Mexico, Utah, Wyoming and Colorado) would not be negatively impacted by the Low Basins States' (Arizona, Nevada, and California) future claims to water from the river as their populations increased rapidly (Arthur 2016). In New Mexico, the allocation of water is further complicated because historic water rights are also managed and distributed through an extensive network of irrigation channels locally known as acequias. Acequias with deep history are independently governed by local communities and water resource managers, which can impede water resource management plans at broader scales.

Unfortunately, while agreements like the Colorado River Compact do help allocate resources, they also cause many problems between states. For example, the Colorado River Compact over-allocates the river because the compact was negotiated during an anomalously wet period (Arthur 2016). The long-term mean discharge of the river is around 15 million acre-feet but 16.5 million acre-feet are actually allocated (Arthur et al 2016). Main waterways in the Southwest, including the Colorado River, but also the Sacramento-San Joaquin River system, the Rio Grande River, and rivers in the Great Basin, had a 5-37 percent decrease in flow in the twentieth century (Union of Concerned Scientists n.d.). Anticipated increases in population and decreasing water supply due to a changing climate will only increase the disparity between the two and cause city water divisions to seek alternatives in order to provide enough water for their communities.

With surface water resources growing scarcer, many cities and farmers are turning to ground water. However, groundwater can take thousands of years to be recharged naturally and will not be replaced at the same rate as it is currently being depleted (DuMars and Minier 2004). Groundwater is also susceptible to many of the same contaminants as surface water and therefore may not be an option if contamination causes watershed managers to seek alternative sources of water. Many southwestern states, including New Mexico, are using groundwater for meeting the demands of water-intensive agriculture and increasing population centers, but this will eventually prove to be unsustainable. One of the problems facing groundwater use is the lack of regulation. Regulation and monitoring of groundwater extraction is rare especially compared to the highly-monitored use of surface water (Lambert 1981). In most states, it is the local right of the landowner to drill a well and extract as they please (Hand 2014). This leaves water authorities with no way of predicting future levels of the resource. In the 1980s, the growing importance and depletion of groundwater caused Arizona to pass the Groundwater Management Act, which created five highly regulated groundwater basins and severely limited ground water pumping (Hand 2014). The law was fairly progressive for the time and several states have chosen to follow suit by adopting similar policies. New Mexico's groundwater is regulated by the Office of the State Engineer (OSE) (DuMars and Minier 2004). The Office of the State Engineer in New Mexico is allowed to declare ground water basins if they impact surface water or if their levels are drastically dropping (New Mexico Office of the State Engineer). Once a basin is declared, a permit is required for any new uses of the basin (New Mexico Office of the State Engineer). The government is able to regulate the supply of groundwater through permitting; however, existing users are grandfathered in. There are 33 declared groundwater basins in New Mexico and they cover approximately 90 percent of New Mexican land (Barroll 2003).

New Mexico provides a prime case study to examine climate's impact on watershed management because the state experiences many of the challenges explained thus far. The state experiences extreme temperature variation throughout the year. Temperatures can range from around 100 degrees Fahrenheit in the summer to around 20 or 30 degrees Fahrenheit in some areas during the winter (Western Regional Climate Center n.d.). The more arid portions of the state have a mean of 10 inches of rain annually while higher elevations could experience around 20 inches (Western Regional Climate Center n.d.). New Mexico is currently the sixth fastest warming state in the United States and has seen an increase in temperature of one degree Fahrenheit in the last century (Environmental Protection Agency and Union of Concerned Scientists n.d.). Since the 1950's its mean annual snowpack has been gradually decreasing, affecting the headwaters of the Rio Grande, San Juan, Colorado and Navajo rivers and jeopardizing local water supply (Environmental Protection Agency n.d.).

Cities in New Mexico are at risk of facing urban water shortages just like El Paso did in the 1980s and 1990s (Earl 1996). Due to its arid climate, limited groundwater and a rapidly growing population, El Paso was severely limited by a shortage of water. With a changing climate it is unclear if cities in New Mexico will run into a similar paucity of water augmented by changing precipitation patterns. Moreover, there is uncertainty about how water scarcity in the state might affect the production, contamination and supply of water in New Mexican cities. For states like New Mexico that rely on the predictable delivery of water, too little or too much water at the wrong time can have significant negative impacts on water quality, agriculture, power, transport, contamination and access (Falkenmark 2001, O'Lear et al. 2013 and Rippey 2012). Recent conservation efforts and water restrictions have reduced water use but have not helped water quality and will not be sufficient if current water supply and demand trends continue. The literature regarding the overall effects of a changing climate in New Mexico are well documented. However, how rising temperatures and precipitation variation may change watershed management practices and thus influence water quality still needs to be monitored and further researched.

3.4 Water Contaminants and Testing

3.4.1 Introduction

While water quantity is a constant concern for those in the Southwest, water quality is of equal importance to water managers. More than 40 years after the Clean Water Act was implemented, however, a significant fraction of United States rivers and lakes fail to meet the standards set by the Clean Water Act due to high levels of fecal bacteria (Gross and Stelcen 2012). Stormwater is the leading contributor of these contaminants because it easily transports the bacteria that have accumulated throughout the environment to rivers and streams. Protection from these contaminants is an important and challenging problem facing environmental scientists and regulators. Water quality testing for these bacteria depend upon both the needs of water resource managers and the available technologies but ultimately the testing is necessary to enhance the environmental security of the region.

3.4.2 Stormwater

In undeveloped areas, precipitation tends to infiltrate into the ground. When urban areas are created that include infrastructure such as parking lots, buildings and roads, the opportunity for infiltration is greatly limited. Instead, stormwater runoff is directed into drains that ultimately debouch into rivers or lakes. Along the way, runoff collects contaminants such as animal waste or fertilizer from human and animal activities. The primary river contaminants in the United States are listed below (Table 1).

Table 1. Known primary river contaminates common in river systems. **(**Source: https://www.nrdc.org/issues/water-pollution.)

Pollutant	Sources	Health/Environmental Impacts
Total Coliform	Soil, intestines of animals	- Gastrointestinal illnesses, jaundice, fatigue
Nitrates	Fertilizer, soils, sewage, septic tanks, industrial pollution	- Interferes with the ability of your red blood cells to transport oxygen - Eutrophication - Especially harmful to infants
Lead	Pipes, chemical waste, sludge	- Neurological problems, paralysis, infertility etc. - Negatively impact plant growth
Copper	Mining, agriculture, pesticides, sludge, rock weathering	- Altered brain function, enzyme activity and blood chemistry in aquatic life - Human tissue injury and disease (genetic disorders), nausea, diarrhea
Cadmium	Fertilizer, mining, smelting, sewage, industrial waste, weathering of rock	- Damage to the immune system, central nervous system, bone fractures, reproductive failture etc. - High uptake by plants, impacts animals through nerve or brain damage
Mercury	Forest fires (air), mining, fossil fuels, coal, landfills,	- Neurotoxin (for human and animals) - Muscle weakness, lack of coordination, speech/hearing impairment

In New Mexico, especially during monsoon season, short, intense periods of rain provide plenty of water to wash away containments that have accumulated on urban surfaces. The stormwater that enters drainage systems not only influences the physicochemical variables of the receiving water, but also the organisms living in the receiving water and the rivers hydrology (Baralkiewicz et al. 2014). Potential sources of contamination typically are classified into two groups: point sources and non-point sources (Rivera and Rock 2011). Point sources are easily identifiable such as raw sewage draining from a pipe. Non-point sources are more challenging because they diffuse or widely disperse in the environment, such as wildlife or unfocused urban runoff. This thesis aims primarily to examine non-point sources because the samples were taken during a storm event, and not placed intentionally immediately upstream and downstream of a given point source. Traditional stormwater management approaches focus on peak flow storage and not targeted pollutant reduction.

The Environmental Protection Agency (EPA) upholds the Clean Water Act (CWA) through various programs and permits including the National Pollutant Discharge Elimination System (NPDES) permit (Environmental Protection Agency 2017). Through the CWA certain cities are required to maintain NPDES permits and develop stormwater management programs (SWMPs) (Environmental Protection Agency 2017). The NPDES stormwater program regulates discharge from three sources: municipal separate storm sewer systems (MS4s), construction activities and industrial activities (Brown and Olson 2016). For the purposes of this study only two of the potential three sources are relevant to the study area, MS4s and to a lesser extent industrial activities. The NPDES permits have requirements to minimize discharge of pollutants and stormwater runoff falls under this permit

(Environmental Protection Agency 2017). There have been two MS4 phases in the United States. In 1990 Phase I required medium to large cities (population of100,000 or more) to obtain NPDES permit coverage for their stormwater discharge (Brown and Olson 2016, Environmental Protection Agency 2017). The City of Santa Fe was less than 100,000 people and thus did not have to comply with the permit. However, in 1999 the EPA expanded the program and Phase II permits included urbanized areas with 50,000 to 100,000 people (Environmental Protection Agency 2017). Santa Fe became covered under the Phase II permit at that time but the Phase II permit was not issued until 2007. The SWMPs the stormwater division of Santa Fe must maintain requires six components that are considered minimum control measures. These components include, public education and outreach, public involvement and participation, illicit discharge detection and elimination, construction site storm water runoff control, post-construction storm water management, and pollution prevention (City of Santa Fe 2017). The City of Santa Fe believes they need to do more to comply with the NPDES permit requirements and think microbial source tracking will be the best way to determine what is polluting the watercourses so they are better informed to prevent this discharge. They are one of a very few city water divisions to use microbial source tracking and they will be better prepared than most to meet EPA requirements.

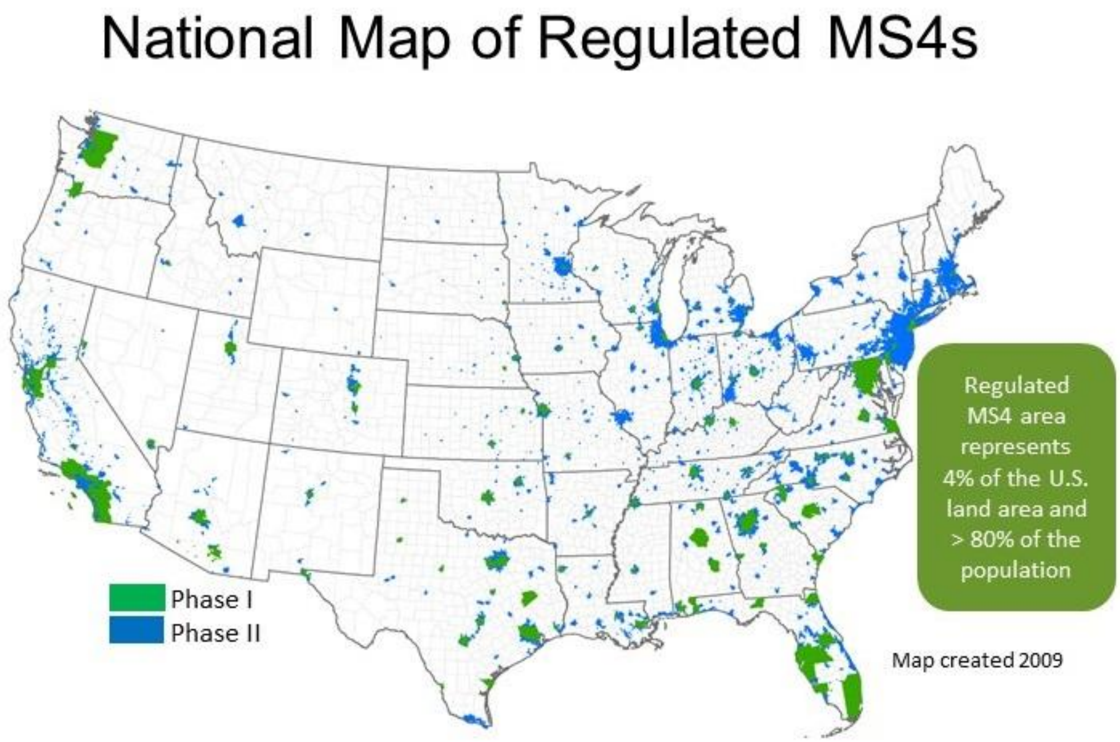

Figure 2. Municipal separate storm sewer systems (MS4s) map of phase I (population 100,000 or more) and phase II (population between 50,000 – 100,000) Environmental Protection Agency NPDES permits. (Source: Environmental Protection Agency https://www.epa.gov/npdes/stormwater-discharges-municipal-sources.)

3.4.3 Nitrates

The nitrogen cycle has been altered strikingly by human activity. Since 1950 global human nitrogen production has increased and is currently 30% greater than nitrogen produced by natural sources (Fields 2003). Nitrate levels in water vary across the United States due to natural and human processes. The Midwest has, in general, the highest nitrate ion concentrations varying from approximately 1.2 to >1.8 mg/L while New Mexico is in the moderate range 0.90 – 1.2 mg/L (United States Geological Survey 2015). In 1997, the United States Environmental Protection Agency enacted the Clean Water Action Plan after determining nutrients—

especially nitrate—contribute significantly to water pollution (United States Environmental Protection Agency 1997).

The primary anthropogenic source of nitrogen is fertilizer (Ward et al. 2005, Fields 2004, Ghaly and Ramakrishnan 2015). Nitrogen in the form of nitrate, nitrite and ammonium is essential for plant growth and is often added by farmers to increase crop yield (United States Geological Survey 2015). Other influential sources include nitrogen oxides from cars, utilities and animal and human waste (Ward et al. 2005). Nitrate is able to directly enter the water systems like most contaminants through runoff (United States Geological Survey 2015).

Nitrates are nutrients for plants and excess concentrations can cause eutrophication (Chislock 2013). Eutrophication is the overstimulation of aquatic plants and algae due to excess nutrients (United States Geological Survey 2015, Chislock 2013). The consequences of overstimulation are potential clogged water intakes, used up dissolved oxygen and overgrowth, which can block light from penetrating the water (United States Geological Survey 2015). Excess nitrates can also have adverse health effects for humans. Excessive nitrate can cause restriction of oxygen transport in the bloodstream and is particularly harmful to young infants (Majumdar 2003, Richard et al. 2014).

In New Mexico, nitrates are the most common contaminant found in water, and the public water supply is routinely tested for nitrates (New Mexico Environment Department 2017). The City of Santa Fe complies with the federal drinking water standard limit of 10ppm for nitrates. However, rivers and private wells in the city are rarely, if ever, tested for nitrates. They do not occur naturally in the area but levels can rise quickly because of rainfall and agricultural activity. The City is interested in learning about nitrate levels in the Santa Fe River because of its proximity in some locations to agricultural fields and septic tanks. The Santa Fe River is a source for the acequias in the city and a source of irrigation for many residents and therefore the quality of the water is important and needs to be investigated further.

3.4.5 Pet Waste

Pet waste is one of many pollutants that enters rivers, streams and lakes from stormwater runoff. Once the pet waste enters a water system it decays and in the process uses dissolved oxygen and releases ammonia. This change in the ecosystem can lead to the die off of fish and other organisms. Pet waste is a challenge to control in watercourses because it is a nonpoint source. The waste also contains micronutrients that cause excessive weed and algal growth. Negative consequences from this algal growth include zones of hypoxia, blockage of the sun, bad odor and die off of other plants and microorganisms (Hobbie et al. 2017). Controlling excess nutrients is a crucial step in improving urban waters and ecosystems and a ban on phosphorus containing detergents has greatly reduced phosphorus inputs. Nitrogen and phosphorus are both naturally occuring in the soil and water but humans are exponentially adding to that concentration. Approximately 40 percent of United States rivers and streams have elevated phosphorus levels and 28 percent have elevated nitrogen levels (Environmental

Health Perspectives 2014). Pet waste also contains disease carrying bacteria that may be unsafe for human contact. Diseases associated with pet waste include *Salmonellosis, Giardiasis and Campylobacteriosis* (World Health Organization n.d.). Studies have shown that pet waste is significant in urban watersheds with high housing density because they are likely to have high per area rates of pet ownership (Hobbie et al. 2017). Thus, any urban locations will likely have some type of pet waste issue.

3.4.6 Total Coliform: *Escherichia coli* and *Bacteroidaceae*

Clean water is essential for safe drinking water and is a goal of every city's water division. When the water supply is from natural bodies of water such as rivers, reservoirs or groundwater, however, it is susceptible to potentially dangerous contaminants such as *Escherichia coli*. These sources contain nutrients that microorganisms such as bacteria or viruses can use to sustain life (World Health Organization 2005). Most of these microorganism are common in the environment and generally harmless. Nevertheless, runoff from soil in the area, discharge from sewage, and leaking septic tanks into water bodies does have the potential to cause diseases in humans and pose a significant threat (World Health Organization 2005).

E. coli is part of a group of organisms called coliforms, which are common bacteria in the digestive track of animals and humans (Rogers and Peterson 2011). Total coliform is a collection of bacteria. Fecal coliform is a subset of total coliform that exist in feces (Rogers and Peterson 2011). *E. coli* is a subgroup of fecal coliform

(Rogers and Peterson 2011). *E. coli* is present in large quantities in the intestines of warm blooded animals (World Health Organization 2005, Rogers and Peterson 2011). Once in the water supply, it will survive for only a couple days and is a sign for public health and water professionals of contamination from either human or animal waste (Percival et al. 2013, Scheffe 2007). Some *E. coli* strains are pathogenic and can cause illnesses usually associated with diarrhea and stomach pain (Centers for Disease Control and Prevention 2015). The presence or absence of *E. coli* is used by water authorities as one of several conservative indicators of sanitary drinking water conditions. It has been a widely used indicator in the past because its cultivation and detection methods are inexpensive, little training is needed to collect and preform the test and their presence indicates the potential existence of pathogens (Rivera and Rock 2011).

Fecal *Bacteroidacetes* are an alternative to more traditional indicators such as *E. coli.* They are different because they are all anaerobes and thus indicate recent fecal contamination (Converse et al. 2009). An additional advantage to using *Bacteroidacetes* as indicators is that they are more abundant in feces of warm-blooded animals than *E. coli* with certain strains associated with humans (Converse et al. 2009). A high degree of host specificity allows the identification of the digestive system of the host animal (Converse et al. 2009). These bacteria through MST could solve the problem of identification, tracking and monitoring sources of contamination by specific host.

3.4.7 Water Quality and Human Health

Fecal pollution is a primary water quality concern because of the potential infectious microorganisms it can contain. Pathogens, which include viruses, bacteria, protozoa and parasites, are sometimes found in water. Most microorganisms are non-pathogenic but the mixture that are pathogenic come from a variety of sources. The severity of human health effects from waterborne pathogens can vary from mild gastroenteritis to severe potentially fatal diarrhea, hepatitis, dysentery and typhoid fever (World Health Organization n.d.). The species of pathogen varies geographically due to the pathogens ideal habitat and temperature but routes of transmission include ingestion, inhalation and contact.

The United States enjoys relatively disease free water due to their stringent control and implementation methods. However, microbes evolve constantly to overcome defense mechanisms put into place by our water treatment systems and outbreaks do happen. Water quality during storm events is crucial because in the United States more than 50 percent of waterborne illnesses are associated with extreme rain events. The most frequent adverse health outcome in the United States from water quality contamination is intestinal (enteric) illness that causes gastroenteritis (World Health Organization n.d.). The most common waterborne disease in the United States is giardia (Table 2). It's so prevalent because it can be found in many locations throughout the United States regardless of climate. Giardia parasites are usually found in animal droppings which can then enter the water supply. Waterborne diseases in the United States can easily be avoided with cleaner water infrastructure and more advanced and specific water quality testing methods.

Table 2. Waterborne diseases in the United States including their symptoms and sources. (Source: http://www.healthguidance.org/entry/15740/1/Waterborne-Diseases-in-the-USA.html).

Pathogen	Symptoms and Sources
Cryptosporidium	- Intestinal disorders, diarrhea - Cryptosporidium cysts are difficult to detect - Resistant to common disinfection methods
Shigella bacteria **(Dysentery)**	- Destroy intestinal wall cells, diarrhea - An amoeba and a bacterium - Resistant to common water treatment methods
Giardiasis	- Nausea, diarrhea, dehydration - Usually from water taken from streams, ponds and lakes - Animal droppings
Legionella pneumophila **(Legionnaires' Disease)**	- Fever, decreased liver/renal function, loss of coordination - Poorly maintained water towers and potable water systems
Hepatitis A Virus	- Vomiting, decreased liver function, jaudice - Fecal matter, drinking water, swimming pools

3.4.8 Microbial Source Tracking

Microbial source tracking is a new water quality technique that aims to identify sources of fecal pollution. The concept uses microbiological, genotypic, phenotypic and chemical methods to identify pollutants (Scott et al. 2002). Abiding by the Clean Water Act can be challenging because of the inability of traditional methods, such as those previously used by the Santa Fe Water Division, to identify the contamination sources. The benefit of MST is the ability to determine the source of contaminates so that effective control measures can be implemented to protect waterways and human health. Understanding the origin of pollution in waterways is paramount in assessing associated health risks and the only way to determine the proper remedies.

MST utilizes indicator microorganisms to predict the presence of pathogenic microbes. Indicator microorganisms are ideally nonpathogenic, have survival characteristics similar to potential pathogens of concern and are known to coexist with potential pathogenic microorganisms (Hagedorn et al. 2011). Total and fecal coliform testing are used extensively with many water divisions including the City of Santa Fe, as indicators for water quality. However, in recent years scientists have learned that the coliforms' ecology, prevalence and stress differ from those of many pathogenic microorganism of concern for which coliforms are used as a proxy (Duris et al. 2015, Harwood et al. 2014).

Microbial source tracking is a growing field and thus has developed a wide variety of methods. MST methods are generally broken into three categories: chemical, microbiological and genotype (Scott et al. 2002). Examples of these include F-specific RNA coliphage, MAR analysis, fulsed-field gel electrophoresis, coprostanol, repetitive element PCR and ribotyping (Mauffret et al. 2012, Furukawa and Suzuki 2013, Staley et al. 2012). To simplify the differences between methods, two other categories can be used: library- dependent and library-independent.

Library-dependent MST uses isolate by isolate identification of bacteria cultured from samples that are then compared to a "library" of bacterial strains from suspected fecal sources (Rivera and Rock 2011). This method requires the development of phenotypic or genotypic fingerprints for bacterial strains of suspected contaminant sources (Rivera and Rock 2011). Library-dependent methods require time to develop a library, highly trained personnel and are usually temporally and geographically specific (Rivera and Rock 2011). Library-independent MST identifies a specific genetic marker or gene target in the water sample and thus no "library" is needed (Rivera and Rock 2011). The analysis for this project used library-independent MST through real-time quantitative polymerase chain reaction (qPCR). PCR is a common library-independent approach that amplifies a target gene in a short amount of time after it has been isolated from a water sample. Real-time quantitative polymerase chain reaction (qPCR) measures the amount of microbial DNA present instead of simply detecting a presence or absence of the DNA (Wilks 2012). Most of the new development in the field of MST has been geared towards quantitative methods and adapting qPCR methods (Harwood 2014). With this MST method and the several others in existence, scientists and municipal authorities have the ability to determine the scale of response required when indicator bacteria are detected.

3.5 Research Statement

Recently, Santa Fe River water has tested positive for *E. coli* at some regularly tested sites (Appendix A), and thus microbiological pollutants are present along the river, raising the concern of the City of Santa Fe Water Division. Previous research suggests this type of contamination is especially associated with storm events when significant concentrations may enter the river with runoff. The city is concerned that bacteria may enter the water via runoff from agriculture fields and facilities, urban landscapes, and septic tanks. The quality of the water must be managed to prevent impacts to the local population, the riparian ecosystem, and groundwater. Watershed managers can mitigate pollution if they know the sources by educating the public, regulating water quantity and implementing stormwater capture architecture. To prevent further water and ecosystem degradation, the city must determine the primary sources of contamination because of the frequent recreational and agricultural use of the Santa Fe River. This thesis aims to answer the research question:

What are the sources of fecal contamination found in the Santa Fe River?

CHAPTER FOUR
RESEARCH DESIGN

The Rio Grande is the principle drainage system for the Sangre de Cristos mountains which extend from Santa Fe into southern Colorado. The Santa Fe River is a small river that lies in the Santa Fe Watershed (City of Santa Fe 2016). The watershed's headwaters are at 12,408 feet, right below Lake Peak, but the Santa Fe River begins at Santa Fe Lake in the upper reaches of the Santa Fe Watershed in the Sangre de Cristo Mountains. The river runs 46 miles through the City of Santa Fe before it joins the Rio Grande River south of Cochiti Reservoir. Its eastern portion is channelized through most of downtown and threads under roads and bridges while the western residential and industrial areas have more native riparian vegetation surrounding the river (New Mexico Environment Department 2012)

The river is fed by snowmelt and rain but the Santa Fe Living River Ordinance additionally allows 1,000 acre-feet to be released from McClure and Nichols reservoirs into the river each year (New Mexico Environment Department 2017). Before reaching Santa Fe, the river runs into two reservoirs, McClure Reservoir and Nichols Reservoir. All sample sites for this thesis were taken downstream of the two reservoirs (Figure 5).

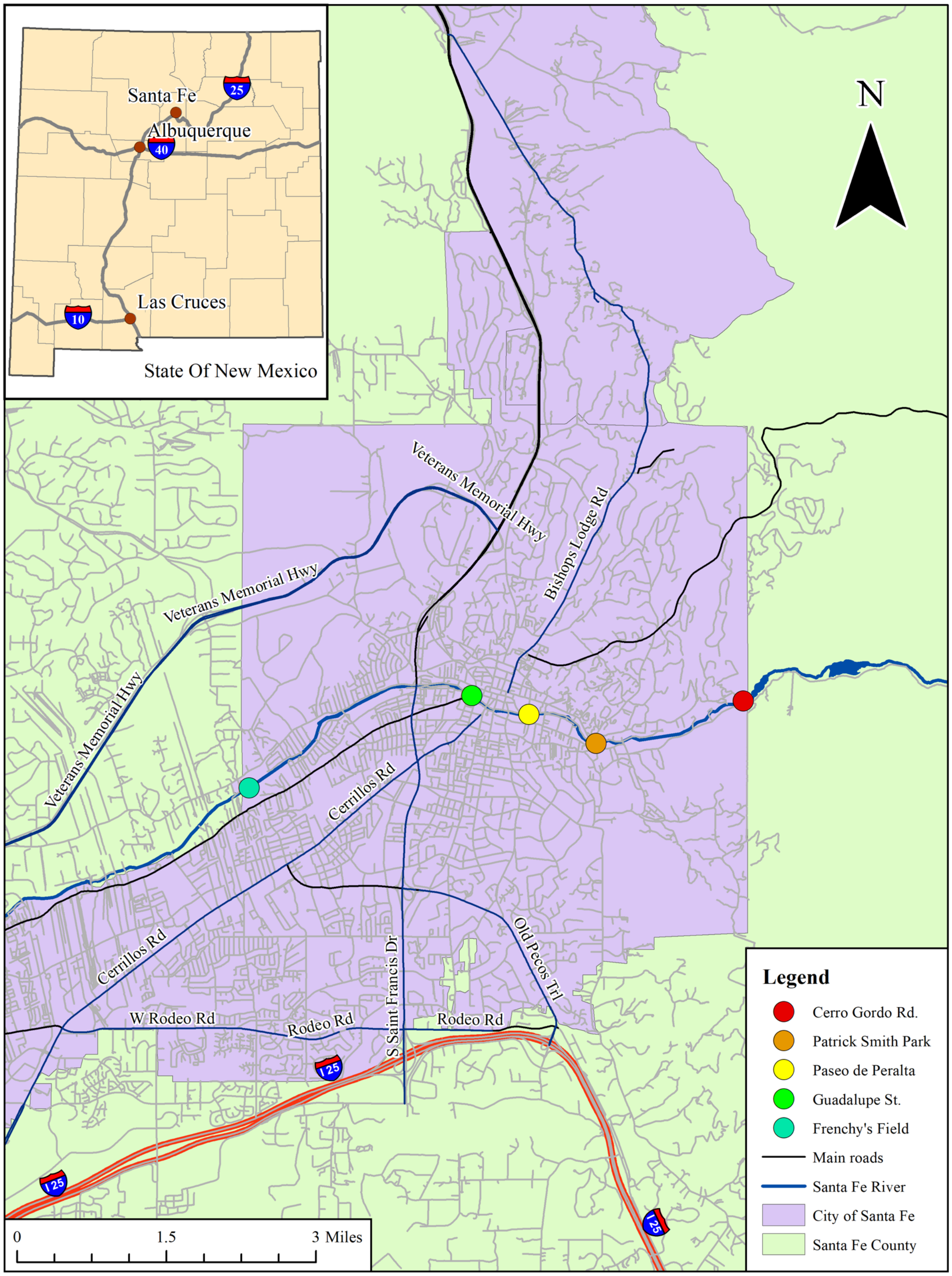

Figure 3. The five MST sample sites along the Santa Fe River. Map by author in 2018.

4.1 Sample Sites

Sample sites were chosen based on previous state and city water division testing locations as well as high traffic areas of interest to the city. The water division already performs water quality tests at three of the locations chosen, Cerro Gordo Rd., Guadalupe St and Frenchy's Field (Appendix B). These sites were chosen because Cerro Gordo Rd. had recently tested positive for *E. coli* from New Mexico Environment Department (NMED) river assessment sampling and the water division wanted to identify the source of contamination in the area. Guadalupe St. was also chosen because it is in the heart of town and would provide a prime example of the water quality near downtown Santa Fe. Frenchy's Field, the farthest downstream sample site, was chosen due to existing data on the location and because it was much farther downstream near farms and horses where the water quality could potentially change drastically. The Patrick Smith Park location was chosen because it is a very popular and large dog park where humans and dogs often play in the river. Finally, the Paseo de Peralta location was chosen because much like Guadalupe St., it is a central road leading to downtown. The genetic biomarkers, similar to the river sample sites, were selected based on the cultural surroundings of each sample site such as proximity to farms, streets, walking pathways, beaver dams and tree cover.

Cerro Gordo Road.

The location of this sample site is below both Nichols and McClure reservoirs and just below a small lake that beavers now occupy. This is the furthest upstream of the five sampling sites. The sample was collected about three meters downstream of the Cerro Gordo Road crossing. The area has a heavy canopy and though houses are in the vicinity, none are nearby. There is a hiking trail farther upstream near the small beaver pond and deer have been seen in the area. The river channel in this section is narrow and characterized by sand and granular gravel with some pebble and cobble gravel (Figures 6 and 7).

Figure 4. The Santa Fe River below Cerro Gordo Rd. Photographed by author in 2018.

Figure 5. The Santa Fe River above Cerro Gordo Rd. Photographed by author in 2018.

Patrick Smith Park

At this location, the sample site is downstream (west) of the park. A drainage line that goes under the park and spills into the Santa Fe River downstream of the park is just upstream of the sampling site. The park has a playground and a large grass field that is frequented by humans and dogs (Figure 8). There is no barrier between the river and the park and therefore dogs and humans can easily walk along and in the river (Figure 9). On the opposite side of the river, a walking path lies between the river and E. Alameda St., with several houses on the opposite side of the road. The river in this section is mostly made up of sand and granular gravel with some cobble gravel. There is a dense canopy of trees above the sample site as well as upstream of the site.

Figure 6. Patrick Smith Park, looking west. Note the dog. The Santa Fe River is to the right of the photo, among the trees. Photographed by author in 2018.

Figure 7. The Santa Fe River between Patrick Smith Park and East Alameda Street. A runner is jogging along an unofficial path. Photographed by author in 2018.

Paseo de Peralta

The sample at this location was taken just downstream (west) of the intersection of East Alameda St. and Paseo de Peralta. A drainage system joins the river immediately west of the Paseo de Peralta bridge, and the river is confined by stone walls (Figures 10 and 11). It is a high-traffic location for both vehicles and people. With distance north of the river, there is a walking path, East Alameda, and businesses. On the opposite side of the river are more businesses with a grassed buffer. Human activity along the river is evidenced by the presence of trash along the river banks. The grain size at the location varied from sand to cobble to boulder gravel.

Figure 8. The Santa Fe River upstream of the East Alameda St. and Paseo de Peralta street crossing facing West. Photographed by author in 2018.

Figure 9. The Santa Fe River under Paseo de Peralta facing West. Photographed by author in 2018.

Guadalupe St.

At this location the sample was collected just downstream of the intersection of West Alameda St. and Guadalupe St. The location is very similar to the Paseo de Peralta sample site with a drainage discharge point just west of the intersection (Figures 12 and 13). It is a busy intersection and has businesses located on either side of the river with a road on the north side of the river. The location is challenging for dogs and people to access because of the steep embankment on either side of the river. The walking path is not next to the river but above it in the form of a sidewalk. Vegetation is very dense but there is evidence of human activity in the form of broken bottles around the site. The river bed is primarily sandy pebble and cobble gravel with occasional boulders (Figure 12 and 13).

Figure 10. The Santa Fe River at the Guadalupe St. and East Alameda Rd. Photographed by author in 2018.

Figure 11. The Santa Fe River at the Guadalupe St. and East Alameda Rd. Photographed by author in 2018.

Frenchy's Field Park

Frenchy's Field Park is off Agua Fria St. and has a large grass area for dogs to play. A playground is situated between the grass area and the Santa Fe River (Figure 14). On the other side of the river are houses. Though there are trees, the canopy is not as dense in this location as the upstream locations. In this location, the El Camino Real hiking trail is within the channel, and several other unofficial trails cross the river in the area as well (Figure 15). Dog feces were found at multiple locations along the river. No trash was observed. Some shrubs exist along the thalweg margin and the channel banks. The grain size at the location is primarily sandy pebble gravel.

Figure 12. The Santa Fe River below Frenchy's Park off of Agua Fria Rd. Photographed by author in 2018.

4.2 Data Collection

Water samples were collected from these five sites on September 28th 2017, during a storm event. Sample collection followed the Quick Guide to Water Sample Collection (2016). At each site 500ml of river water was collected with 500ml sample bottles. Gloves were worn at each location to avoid any contamination. Once each sample was collected, the bottle was placed into a cooler to keep samples cold and preserve any bacterial DNA. Once sampling was complete, samples were packed with ice in a cooler and sent overnight to Source Molecular laboratory in Miami Florida.

At Source Molecular, the samples were tested using microbial source tracking looking for five different species groups (humans, dogs, beaver, ruminants, and avian). The type of genetic biomarkers tested per sample depended on the surroundings of each site (Table 3). All biomarkers were not tested at each site because of budget limitations. Instead, probable biomarkers were selected based on the unique geographic setting of each sample site.

Table 3. Microbial source tracking sample locations and respective biomarkers along the Santa Fe River.

Location	Biomarkers Tested
Cerro Gordo Rd.	Human, dog, avian, beaver
Patrick Smith Park	Human, dog, avian
Paseo de Peralta (crossing E. Alameda)	Human, dog, avian
Guadalupe St. (crossing W. Alameda)	Human, dog, avian
Frenchy's Field	Ruminant, human, dog

4.3 Genetic Biomarkers: How biomarkers were chosen for this project

There is a large range of potential genetic biomarkers that MST studies can utilize and those chosen must suit the water body in question (Harwood 2014). Source Molecular has created a bank of hundreds of fecal samples that have been collected throughout the United States from a variety of sources including human, animal, septage, and sewage (Source Molecular 2017). Through this extensive library of fecal sources, the laboratory has determined which bacteria are predominantly in specific hosts. From this information, water samples can be tested for predetermined genetic biomarkers.

4.3.1 *Bacteroidetes*

Bacteroidetes were one biomarker selected to test for human, dog, ruminant and beaver contamination.

Bacteroidetes are the primary alternative to traditional indicator organisms like *E. coli*. Bacteroidetes are anaerobes and thus indicate recent fecal contamination. Some members of the phylum can be pathogenic (Thomas et al. 2011). The majority of microbes in the gastrointestinal track belong to the *baceroidetes* phyla and they are also more abundant in warm-blooded animal than *E. coli* (Thomas et al. 2011). The genus *Bacteroides* is a gram-negative anaerobic bacteria under the phylum *Bacteroidetes.* Thus, these bacteria are favored for MST because the bacteria are primarily found in intestinal tracts and mucous membranes of warm-blooded animals and humans. Among this phyla the genus *bacteroides* are the most abundantly represented (Thomas et al. 2011). Certain strains of *bacteroides* are used to identify specific hosts. The genetic biomarker *B. dorie* targets the anaerobe species *Bacteroides dorie,* often shed from human gastrointestinal tracts and ending up in human feces. The bacteria are a perfect genetic biomarker because they are found worldwide. The DNA sequence with the human-associated marker of *B. dorie* is located on the 16S ribosomal ribonucleic acid (rRNA) gene. This gene is used for most host fecal pollution identification because of its specificity and sensitivity (Source Molecular 2017). While other *bacteroides* can be used, such as *B. stercoris* and *B. fragilis*, *B. dorei* qPCR assay is the highest preforming human-associated assay amongst the variety of human biomarkers tested and thus was chosen as the best indicator of human contamination. Terminal restriction length polymorphism of the 16S rRNA gene can be used to determine differences in populations of certain animals and thus allowed testing for *bacteroides* as the target gene for four (human, dog, beaver and ruminant) of the five hosts in question for this project (Bernhard and Field 2000 and Fogarty and Voytek 2005).

4.3.2 Helicobacter

Heliobacter was the biomarker used to identify avian contamination.

The genus *Heliobacter* is a group of gram-negative, microaerophilic bacteria that colonize the gastrointestinal tract of mammals as well as birds (Li et al. 2015). There are a total of 20 strains of *Helicobacter* and some of them such as *Helicobacter pylori* are pathogenic to humans (Ahmed et al. 2016). Certain DNA sequences within strains of the *Heliobacter* genus are specific to wild birds and the bird-associated gene biomarker 16S rRNA in *Helicobacter pametensis* was used as the targeted gene for this project (Ahmed et al. 2016).

4.4 Data Processing: Real-time Quantitative PCR

At Source Molecular, each water sample was filtered through 0.45 micron membrane filters to concentrate the bacteria. Each filter was placed in a separate sterile 2ml tube containing a mix of beads and lysis buffer. Each tube was shaken to cause physical and chemical cell disruption. Three separate samples were taken from the lysis buffer and bead mixture and centrifuged for one minute. The DNA was then extracted from each centrifuged sample using the Generite DNA-EZ ST1 extraction kit.

Once the DNA was extracted, it was subject to real-time quantitative polymerase chain reaction (qPCR). In this process, the 16S rRNA gene was amplified and run on an Applied Biosystems StepOnePlus real-time thermal cycler in a reaction mixture of oligonucleotides in the form of forward primers and reverse primers (complementary and specific to the unique bacteria 16S rRNA sequence in question), a fluorescent reporter molecule known as a probe, and an optimized buffer. During the reaction, the temperature of the mixture was raised to 95 C to allow the double stranded DNA to separate. It was then lowered to 55 C to allow the primers and probe to bind to the single stranded DNA target. The reaction was plotted on an amplification curve of fluorescence intensity vs. cycle number.

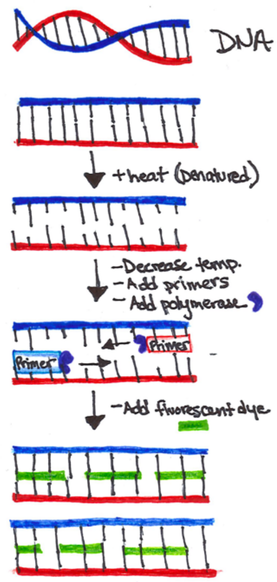

Figure 13. An example of each step in the PCR reaction after one cycle.

Quantification began at the threshold cycle (Ct), the number of cycles required for a fluorescent signal to exceed background level. The Ct value was then compared to the standard curve generated from serial dilutions of known concentrations for each host in question. The target gene copy numbers from the reaction were extrapolated from the standard curve to provide quantification. This process was repeated twice per sample for each genetic biomarker and the number of copies between the two qPCR tests were averaged to provide the final quantification. To ensure accuracy and avoid bias (false positives or negatives), a positive control and a negative control were run alongside each sample to ensure a properly functioning reaction. The positive control, containing the organism's genomic DNA that is known to give a signal, and the negative control that had no DNA in the sample to guard against any contamination that may have been in the sample.

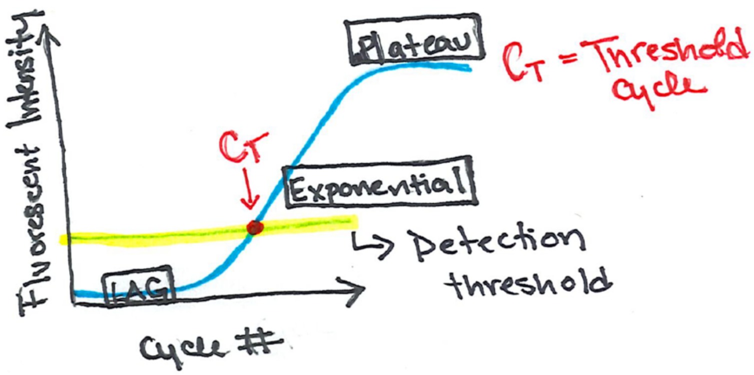

Figure 14. An example of an amplification curve generated from a PCR reaction demonstrating the three phases of PCR (lag, exponential and plateau).

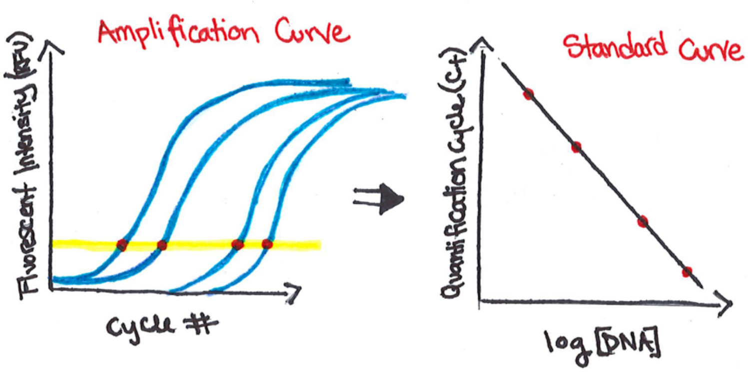

Figure 15. An example of four known concentrations run through PCR and plotted on the amplification curve. The Ct values of each sample are plotted on the standard curve along with the known DNA concentrations.

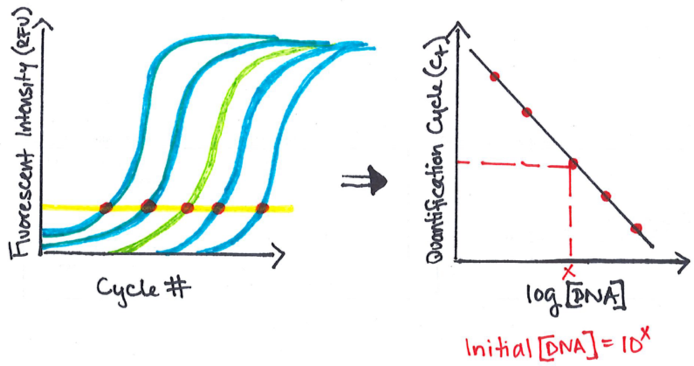

Figure 16. An example of an unknown concentration of DNA run through PCR and plotted on the amplification curve (green). The Ct value is plotted on the standard curve to determine intial DNA concentration.

Three types of DNA analytical results are possible with the qPCR procedure:

- *Non-detect results* indicate the host associated fecal gene biomarker was either not detected in the test replicates, or detected in one replicate but not the other.
- *Detected results* indicate the host associated fecal gene biomarker was detected at a quantifiable level in both replicates. The copy number measurements in Table 3 are relative to 500ml of water, not absolute measurements of copies in the river.
- *Detected not quantified (DNQ) results* indicate the host-associated fecal biomarker was detected in both replicates but the quantities were below the limit of quantification. The limit of quantification is determined by the standard curve created with serial dilutions. If the signal was not quantifiable but detected it means the signal was lower than the lowest standard curve signal.

CHAPTER FIVE

RESULTS

No samples yielded non-detect results (Table 4). Nine samples yielded quantifiable detected results. All five locations yielded detectable results for dog whereas three of the five locations yielded detectable results for human. Only the farthest upstream location in the sampled reach of the river, Cerro Gordo Rd. location, yielded detectable results for bird.

For five of the samples, the host-associated fecal biomarker was detected, but results were below the limits of quantification and yielded DNQ results. DNQ results include those for beaver and ruminant where tested, three-quarters of the tested locations for bird, and two of the five locations tested for human.

Table 4. Detection and quantification of the fecal gene biomarker by real-time quantitative polymerase chain reaction (qPCR) for each sample site on the Santa Fe River. Abbreviation: DNQ, biomarker was detected, but not quantified.

Gene Biomarker	Sample Location	Marker Quantified (copies/100ml)
Human	Cerro Gordo Rd.	**$2.67x10^2$**
	Patrick Smith Park	**DNQ**
	Paseo de Peralta (crossing E. Alameda)	**DNQ**
	Guadalupe St. (crossing W. Alameda)	**$4.72x10^2$**
	Frenchy's Field	**$2.88x10^3$**
Dog	Cerro Gordo Rd.	**$4.54x10^3$**
	Patrick Smith Park	**$3.16x10^4$**
	Paseo de Peralta (crossing E. Alameda)	**$1.13x10^5$**

	Guadalupe St. (crossing W. Alameda)	**2.26x10⁴**
	Frenchy's Field	**2.14x10⁴**
Bird	Cerro Gordo Rd.	**4.13x10³**
	Patrick Smith Park	**DNQ**
	Paseo de Peralta (crossing E. Alameda)	**DNQ**
	Guadalupe St. (crossing W. Alameda)	**DNQ**
Beaver	Cerro Gordo Rd.	**DNQ**
Ruminant	Frenchy's Field	**DNQ**

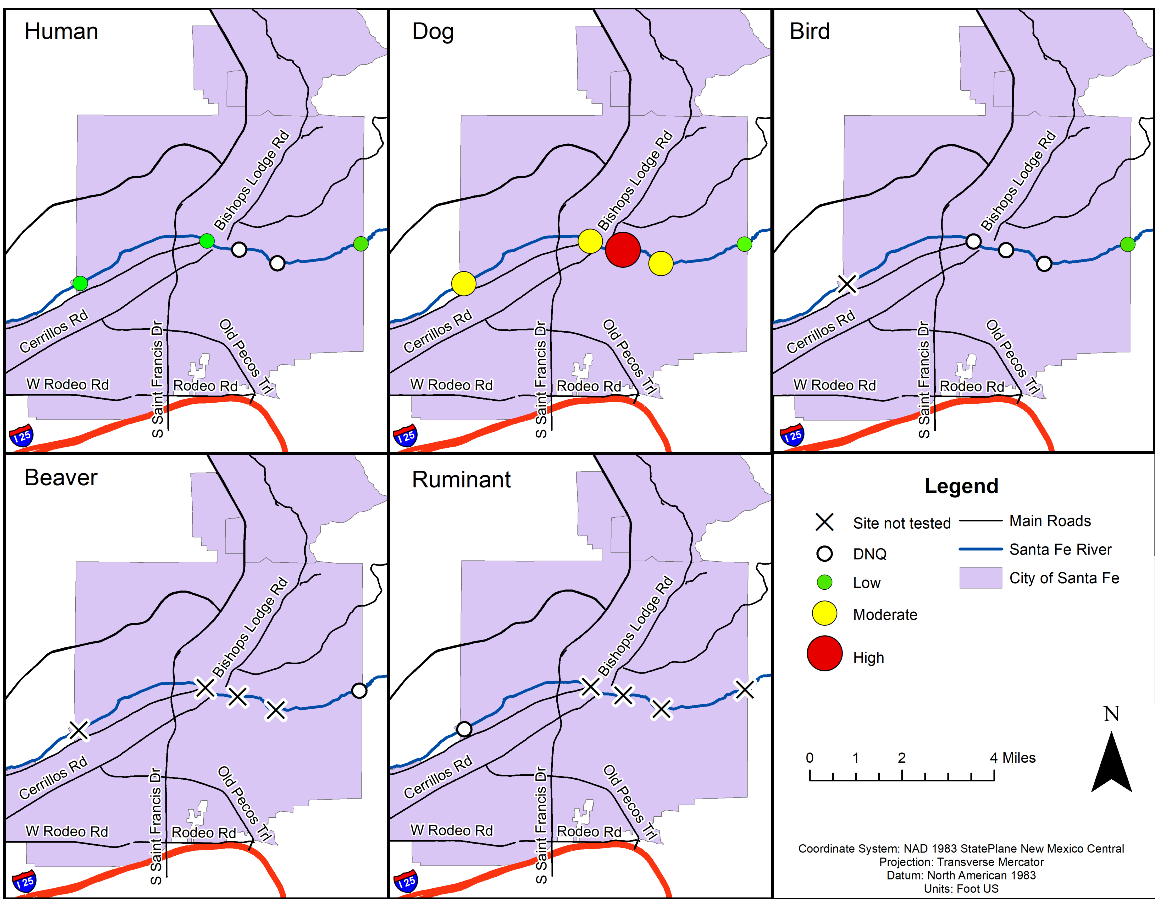

Figure 17. Biomarkers tested for at each samples site. Low concentrations are 10,000 copies or less per 100 ml of water. Moderate concentrations are 10,000 to 100,000 copies. High concentrations are over 100,000 copies.

The assignment of qualitative concentrations of fecal pollution in each sample are based on Cao et al 2013 (Appendix C). Low concentrations are considered less than 10,000 copies per 100 ml. Medium concentrations are between 10,000 and 100,000 copies per 100 ml and high concentrations are higher than 100,000 copies per 100 ml.

Within this classification system, the concentration of fecal pollution in all but four of the tests is “low” (Appendix C). Concentrations greater than “low” are recorded only for dog. Three of the four locations tested for dog are classified as “moderate” whereas one location is classified as “high”. The only “low” classification for dog is recorded at the Cerro Gordo Rd. location at the upstream end of the sampled river reach.

CHAPTER SIX

DISCUSSION

6.1 Analysis

The results provided valuable insight into the primary sources of contamination in the Santa Fe River and invalidated several assumptions formed by myself and professionals at the City of Santa Fe Water Division about the likely conclusions of the testing. Based on the local geography surrounding the sample sites, water division officials and I anticipated contamination at each site with each biomarker tested. While contamination of each biomarker tested was present at every sample site, several were at lower concentrations than expected. Additionally, the largest biomarker concentration at a site was often different than the biomarker anticipated.

Human Biomarker

The presence of human fecal contamination from either the homeless or septic tanks was the primary concern of the City of Santa Fe because of the potential pathogens that human waste contains. At all sites in which the human genetic biomarker was tested, the concentration was low. For two sites, Patrick Smith Park and Paso de Peralta, the concentration was too low to quantify. Based on proximity to high population areas and the evidence of human presence, I anticipated the two most likely locations for human contamination would be Paseo de Peralta and Guadalupe St. Both locations had trash and clothing on the river banks and are near downtown. The water at these locations was also noticeably darker, an additional indication of contamination, than the other locations tested (University of Florida 2004). However, there was not enough copies present at Paseo de Peralta to even quantify. Unexpectedly, the largest concentration per 100ml of water ($2.88x10^3$) was at Frenchy's Park. Frenchy's Park is the furthest location downstream tested and though it is surrounded by a neighborhood. Patrick Smith Park also did not have enough genetic material to quantify and has a similar layout to Frenchy's Park. Both have playgrounds and large fields and are frequented by people and their dogs. The only major difference between the two parks is the two-to-three-foot drop down to the river at Patrick Smith Park while Frenchy's Park has a gradual slope leading to the river. The difference in water quality could be because of where the parks are located. Patrick Smith Park is in the middle of several wealthy neighborhoods in Santa Fe while Frenchy's Park is closer to lower income housing and is a more likely location for homeless encampments. Alternatively, the higher concentration of human biomarkers could be because Frency's Park is further downstream than Patrick Smith Park and the river may have collected contaminants further upstream that became more concentrated in this location.

Dog Biomarker

The dog genetic material was the most prevalent biomarker at all sites. The highest concentration was at Paseo de Peralta and moderate concentrations were found at Patrick Smith Park and Guadalupe St. There were low concentrations at Cerro Gordo Rd. and Frenchy's Field, although these concentrations were still higher than those of the other biomarkers found at any of the other locations. The lowest concentration of dog genetic material was at Cerro Gordo Rd. This result was expected because there are no walking paths near the sample site and there is a very steep incline down to the river at this location. However, the highest biomarker concentration of those tested at the Cerro Gordo Rd. location was the dog biomarker ($4.54x10^3$). Recent positive *E. coli* tests at the site prompted the City of Santa Fe Water Division to assume the cause of contamination to be the beavers in Two Mile Reservoir directly upstream of the sample site (Appendix A). However, the MST testing suggests the source is instead dogs ($4.54x10^3$) or birds ($4.14x10^3$). The contamination is unlikely to be human ($2.67x10^2$) and it is not from beavers (DNQ). The data suggests the *E. coli* contamination is likely from dogs but because access to the sample site is difficult, the contamination may come from the popular hiking trails directly upstream of the sample site that are frequented by dogs and their owners.

The greatest concentration of any of the biomarkers tested was at the Paseo de Peralta sample site for the dog biomarker ($1.13x10^5$). This concentration is considered to be high for 100ml of water (Cao et al 2013). The Paseo de Peralta sample site is much easier to access than the Guadalupe St. site and thus may explain the difference in concentrations between the two sites that are so close to one another. The Paseo de Peralta site is along a popular walking path and the last place the river is easily accessible before entering downtown. It was unexpected that this site would yield a higher concentration than both of the dog parks. This may be because people that frequent the parks bring bags for fecal waste or use the bags and trashcans provided for them at the park while this service does not exist along the river.

Bird/Beaver/Ruminant Biomarker

The presence of the avian genetic biomarker registered low concentrations at the four sites for which it was tested. Birds frequent most of the testing locations but I anticipated that the presence of a dense canopy would prevent some of the bird's feces from reaching the river. It is also possible that the birds prefer other locations for roosting. The presence of the beaver genetic biomarker was only tested at one site, Cerro Gordo Rd., because of its proximity to Two Mile Reservoir which contains active beaver dams. While present, unexpectedly there was not enough to yield quantifiable results. Apparently, beavers are not as significant a polluter here as anticipated, particularly when compared to concentrations of the dog biomarker. Finally, the presence of the ruminant biomarker was only tested for at one location, Frenchy's Park, because there are some small farms in the area, and it is already an established sample site for the City of Santa Fe. Ruminants are mammals that only eat plants such as horses or cows. Source Molecular offers specific biomarkers for horse and cow however, due to the budget constraints and the assumption that cows and horses will likely be at the same location, a broader biomarker was chosen for this project to increase the probability of finding contamination. Somewhat unsurprisingly the result for the ruminant biomarker was DNQ. Frenchy's Park is still in town with many neighborhoods surrounding it. It is likely the ruminant biomarker would be more prevalent further downstream and future tests should include downstream sample sites where more farms are located.

It is challenging to discern any trends along the Santa Fe River from this testing because samples from different storm events were not obtained. Similarly, the range and number of sample sites is limited and thus more sampling should occur in the future to validate water quality claims in the tested areas. The human biomarker is the only one that shows a trend as concentrations increase the further downstream the sample was taken. This conclusion is not unexpected since the river must cross downtown and several other populated areas where it can pick up additional pollutants. It is clear that much of the contamination comes from dogs regardless of which section of the river is analyzed. The results demonstrate the value of MST because what were assumed by the City of Santa Fe as potentially significant or primary pollutants at the various sites did not always appear to be significant pollutants based on the data. If the city based its remediation efforts solely on assumptions, it would not be addressing the root of the problem (Table 5). City officials brainstormed many possible pollutant sources at the beginning of this project. They predicted any contamination found in the river would likely be due to humans from septic tanks, inappropriate waste disposal or human waste. Instead, waste from pets is the highest pollutant and while it was on their list of potential pollutant sources no remediation efforts were being put into place because it was thought to still be an unlikely source.

Table 5. City of Santa Fe assumptions of Santa Fe River contamination. (Source: https://www.env.nm.gov/swqb/TMDL/Santa%20Fe%20River/FINALDRAFTSFRTMDL_WQCCapproved_041117.pdf)

TMDL Watershed	Probable Pollutant Sources
Santa Fe River (Santa Fe WWTP to Guadalupe Street)	Flow Alteration, Drought-Related Impacts, Inappropriate Waste Disposal, Irrigation Return Flow, On-Site Treatment Systems (Septic), Urban Runoff/Storm Sewers, Wastes from Pets, Wildlife other than Waterfowl
Santa Fe River (Guadalupe Street to Nichols Reservoir)	Flow Alteration, Dams/Diversion, Drought-Related Impacts, Inappropriate Waste Disposal, On-Site Treatment Systems (Septic), Urban Runoff/Storm Sewers, Wastes from Pets, Wildlife other than Waterfowl

6.2 Environmental Protection Agency Requirements (MS4's and Pilot Project)

At the end of 2016 the United States Environmental Protection Agency released its final changes to the regulations governing how small MS4s, such as the City of Santa Fe, obtain NPDES general permits (United States Environmental Protection Agency 2016). The final MS4 General Permit Remand Rule clearly establishes what is necessary for the MS4 permit to be granted by establishing what is necessary to "reduce the discharge of pollutants from the MS4 to the maximum extent practicable, to protect water quality, and to satisfy the appropriate water quality requirements of the Clean Water Act" (United States Environmental Protection Agency 2016). The revision of the Phase II stormwater rule was required due to petitions filed by environmental groups, industry groups and municipal organizations (United States Environmental Protection Agency 2016). It led to the remand of the rule because of the lack of procedures for permitting authority review, failure to require public notice and the lack of opportunity to request a hearing for authorization to discharge on Notice of Intent (NOIs), Environmental Defense Center v. U.S. Environmental Protection Agency, 344 F. 3d. 832 (9th Circuit) (United States Environmental Protection Agency 2016).

The older version of the rule also did not require any permitting authority to review the BMPs. Due to these issues the court found it did not comply with the standards articulated by the Clean Water Act because there was no way to ensure compliance was achieved. Clarifications of the requirements for small MS4 permits are in the rules and clearly states that it is the permitting authority's responsibility and not that of the small MS4 permittee to establish the terms and conditions of the permit that must meet the MS4 regulatory standard (United States Environmental Protection Agency 2016). The rule also emphasizes requirements must be "clear, specific and measurable" and include "narrative, numeric, or other types of requirements" (United States Environmental Protection Agency 2016). The City of Santa Fe is planning on using these MST results to specifically fulfill the "measurable" and "numeric" requirements laid out in the new MS4 permit process. The goal is to expand their testing sites and test over multiple storm events. Once a definite baseline is formed and sources of contamination are identified, the water divisions BMP's will be adjusted to reduce or block the sources of contamination. MST testing occurring after the change in best management practices (BMP's) will quantitatively demonstrate improvement in stormwater management and the health of the Santa Fe River.

In 2017, the City of Santa Fe also was awarded an EPA pilot project grant of $150,000 to create a toolkit for managing stormwater pollution (The National Association of Clean Water Agencies 2017). Five other communities were chosen to participate in the EPA program. The goals of the program include developing an asset management plan and creating an economically vibrant stormwater system while also creating a far-reaching best management practices system (Hubbard and Durant 2017). The data collected for this project will contribute to the latter of these goals. Information from MST can dictate BMP's that may serve as examples for other communities. Once a pollutant is identified plans must be made and implemented for reducing the loading of the target pollutant. The BMP treatment method will depend highly on the type and nature of the pollutant and the characteristics of the watershed. Currently, the data from this project suggests the City of Santa Fe should create initiatives to control dog pollution. They can do this through television and radio ads, public education and awareness, increased availability of trashcans and dog bags, or promote volunteer clean up days.

6.3 Watershed Implications

The recognition of water pollutants in the future is important because the literature on climate change demonstrates its impact on surface water quality will be negative. Watershed management currently has measures in place for preserving water quantity as the climate changes but fewer measures are in place for preserving water quality with a changing climate because less is known about how climate change will impact water quality. Watershed managers are replenishing ground water and generating multiple water sources for cities in the Southwest in anticipation of warmer temperatures and potentially less rain and snowfall. Proper planning and water reduction education are key components to water quantity management in anticipation of climate change.

The City of Santa Fe is also currently expanding efforts to improve and protect the watershed by practicing adaptive water management and anticipating how climate change may impact the area. Increasing temperatures and drought are projected for the southwest and therefore the city is working to protect forested slopes above the reservoirs against wildfire. After seeing the devastating effects of the 2000 Cerro Grande fire in Los Alamos County, the City of Santa Fe partnered with the Santa Fe National Forest, the Nature Conservancy and the Santa Fe Watershed Association to perform frequent thinning and prescribed burns in the Sangre de Cristo Mountains (FEMA 2018). To sustain watershed protection, thinning and prescribed burns are carried out at five to seven-year intervals (City of Santa Fe n.d.). The U.S. Forest Service conducted the most recent thinning project. In this project, 5,500 acres of forest surrounding the two reservoirs were hand-thinned (Miller 2015). However, recent water quality tests on the Santa Fe River reveal that fire protection is actually adding to the contamination of the river. Thinning and prescribed burns in the watershed have increased sediment erosion and runoff, leading to increased turbidity levels. None of these levels have yet exceeded drinking water standards but the sediment levels have impacted water treatment plant operations by clogging pipes.

Climate change will affect the quantity of water available but measures to safeguard the watershed against a changing climate, such as prescribed burns, will likely also affect the water's quality. Therefore, water quality research should include not just the Santa Fe River but also other sources the city relies upon including groundwater. As described in the literature review, a decrease in water quantity will negatively impact raw water quality because contamination will not be diluted. Higher temperatures and more variable rain will decrease the water quantity but increase the demand per unit of irrigation area. Plants easily take up contaminants and the Santa Fe River is a primary provider of irrigation water. Warmer water temperatures will also boost the abundance of microorganisms while heavy rain will expand sediment, pollutant and nutrient loading. The latter is what this thesis aims to monitor. With more testing, a baseline can be created to determine if climate change or changes in watershed management are contributing to the pollutant load in the river.

Due to the uncertainty surrounding water quality and climate change, watershed managers are focusing on frequent and updated testing to monitor any slight changes in water quality that could be byproducts of climate change. Dissolved organic matter, pathogens and micropollutants, such as the pet waste found in the Santa Fe River, are susceptible to a rise in concentration due to heavy rainfalls and temperature increases whether that be in the soil, water or air (Delpla et al. 2009). A rise in water temperature in the Santa Fe River may enable new pathogens that enter the water via pet waste to thrive. Additionally, fluctuations in the dissolved oxygen and pH of the river can negatively impact aquatic ecosystem health and thus overall quality. As temperature increases the solubility of oxygen decreases (NASA n.d.). Appendix B lists the most recent measurements of Santa Fe River dissolved oxygen, pH and temperature for three of the sample sites tested in this thesis (Cerro Gorde Rd., Frenchy's Field and Guadalupe St.). In general, freshwater fish require a minimum DO level of 4mg/L but their eggs can require as much as 11mg/L (Fodriest Environmental 2013). The DO levels for the three sites tested from 2012- 2016 was 2.83-9.25 mg/L. This range could be a problem if fish were regularly inhabiting the Santa Fe River. Microbes need much less DO (1-2mg/L) and if the oxygen in a water system is used up then the bacteria can start reducing nitrate and sulfate to survive (NASA n.d.) Therefore, while fish and other plants in the ecosystem will be negatively impacted by the change in DO microbes, including pathogens, will continue to thrive. The full impact of climate change on surface water is challenging to predict because there is a lack of information on micropollutant occurrence and fate. Studies such as this thesis can add data to the occurrence and type of micropollutants and future similar longer term studies can provide more information about the impact climate change is having on mircopollutant occurrence and fate.

The fecal contaminants of concern for this thesis, except for the bird and beaver, are all human related. Restricting recreational access to the river might be a necessary policy to prevent further damage to the watershed. Future restoration projects should include planting more willows and cottonwoods around the river's edge along with shrubs. These will serve as natural barriers to erosion and pollution as well as slow down runoff allowing water to infiltrate and replenish low groundwater supply. Contaminants entering the river from runoff are more challenging to prevent but creating campaigns to remind owners to clean up after their dogs and provide trashcans can improve the water quality and thus advance watershed resilience. Continuing to monitor the human contamination in the river, such as septic tank leakage, should also be a top priority because those are pollutant problems that can easily be solved while controlling contamination from animals like birds and ruminants is more challenging. There are also a variety of methods to catch stormwater runoff and use it to support a river environment. The City of Santa Fe is working with the Surrounding Studio design studio to limit polluted stormwater from entering the Santa Fe River through landscape architecture. The goal of the partnership is to redirect stormwater through “oxbow” infiltration structures and create “stormwater acequias” which will redirect road runoff into linear canals with water absorbing wicks (Figure 21). Once this project is fully implemented, continuing MST tests will provide data on the effectiveness of these river restoration projects.

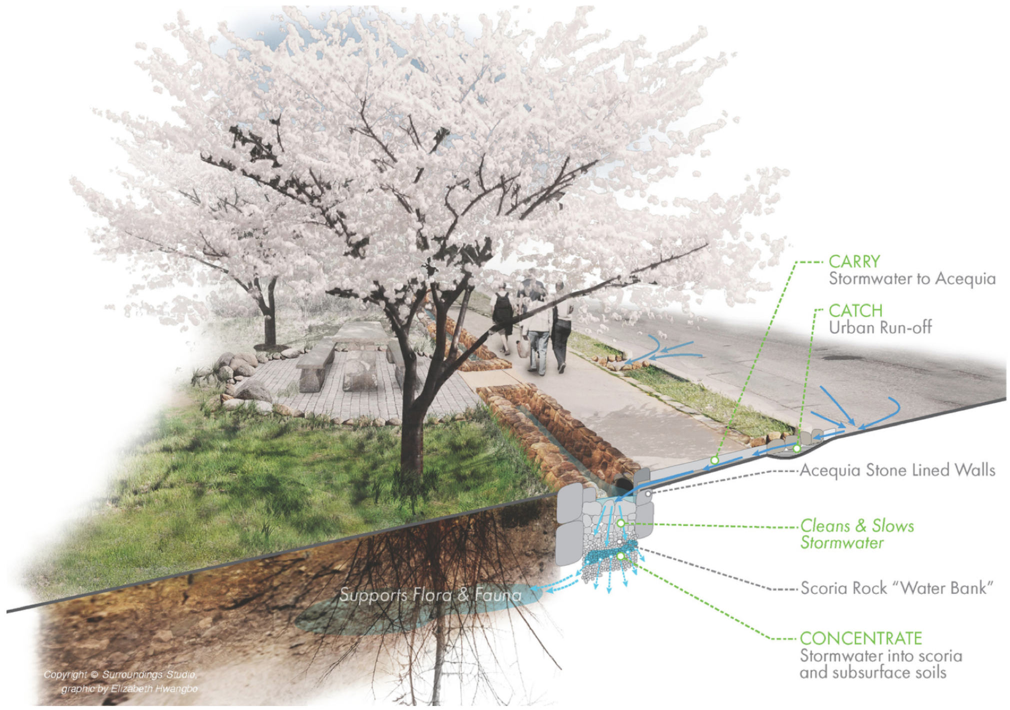

Figure 18. Proposed stormwater runoff capture design for the City of Santa Fe create by Surroundings Studio. Source: https://surroundings.studio/epdr.

In addition to remediation efforts, there must be public education and awareness about the water quality in Santa Fe. Currently, the City of Santa Fe Water Conservation Division invests heavily in water quantity awareness but not in water quality awareness. The conservation division provides strategies for residents to be more water efficient through finding and fixing leak programs, rebates and indoor and outdoor water saving tips. They also organize Project WET (Water Education for Teachers) which teaches educators hands-on learning activities related to water to teach their students in elementary and middle schools. Along with the WET program, the conservation division holds the annual children's poster contest, calendar contest and water fiesta. There is however, no public awareness campaign to keep the Santa Fe River clean, only to conserve water as much as possible. This is likely a trend in most southwestern cities. But it is important to also inform and educate citizens of a watercourse's quality before they use it for recreation or irrigation. Public awareness campaigns can also highlight the primary contaminants in a water system and ways to minimize water exposure to those pollutants. In the case of the Santa Fe River, dog pollution was the primary pollutant and thus reminding the public to pick up after their dogs by providing more trashcans and bags for people along river paths or creating volunteer clean up days or even erecting fences in areas with the highest pollution are all steps the city can take to minimize this type contamination.

6.4 Limitations

There were several limitations for this project that can be addressed in the future. The most significant issues are the small number of sample sites and the relatively narrow range of source biomarkers for which a presence could be tested. Microbial source tracking is very expensive as it is a new procedure and each biomarker tested for is an additional cost. There was approximately $5,000 available to spend on testing for this project. Therefore, only five sites could be sampled for four or fewer biomarkers. While an effort was made to spread out sample sites to piece together a complete picture of the water quality in the Santa Fe River, the sample size was too small to completely achieve that goal. Temperature and chemical changes associated with seasonal variation can also significantly affect the survival of microorganisms targeted adding to the necessity of replicate testing during different storm events in the future.

With a greater fiscal commitment, additional strategically located samples sites could produce a more precise assessment of contaminants and their source. For example, in addition to sampling immediately downstream of a drainage pipe, coupling those results with a sample from a site immediately upstream of the same drainage pipe would allow an assessment of the contributions of that particular pipe. This approach to individual drainage pipes could be refined further by collecting samples during runoff events as water enters sewer grates, more precisely focusing on contamination sources. At a broader scale, additional sites along the urban part of the river can test additional point sources and other potential areas of concern (Figure 22).

Secondly, the seasonality and paucity of precipitation events in Santa Fe's southwest climate, coupled with the City of Santa Fe Water Division procedures, timetable and constraints, left no choice in precipitation events to sample. The precipitation event that generated runoff for sampling occurred well after the monsoon season ended, starting the night of September 27th 2017. It rained in Santa Fe only once after the sampling storm event and did not rain for several weeks after that last event. The late sampling date occurred because of delays associated with the City of Santa Fe Water Division's approval process. The sampling occurred after a night of steady rain. It was not possible to sample during the night and although the sampling commenced first thing in the morning, it is likely results were impacted by the rain beginning the night before. Ideally, sampling should occur soon after a storm event begins. The exact time will depend on when the stormwater reaches the river that is being sampled. Once noticeable stormwater discharge has entered the river, samples should be taken to get the most realistic concentrations of contaminants. It rained continuously throughout the sampling day and therefore runoff was still entering the Santa Fe River. However, it is likely that the concentrations would be higher if sampling was able to occur near the beginning of the storm event because the initial runoff would hold more pollutants.

6.5 Future Work

To continue to comply with the NPDES permit and utilize the EPA pilot program grant, the City of Santa Fe would like to do additional microbial source tracking at the same sample sites along the Santa Fe River this spring. Their goal is to create a baseline of the level of contamination throughout the river to determine if remediation efforts in the future are effective or not. They plan to build this testing into their budget so that additional sites along the river can be tested along with additional markers to create a comprehensive picture of the water quality. Ideally, the water division wants to sample at the beginning of storm events in the spring and likely also during monsoon season, both of which were not possible for this project. The city was most concerned about potential human contamination because of the health risks associated with it. Though concentrations were found to be low at all sites, additional testing could provide insight into whether this human contamination is coming from the homeless, sewer leaks, or septic system failures.

The city is interested in learning about nitrate levels in the Santa Fe River because of its proximity in some locations to farming and septic tanks. The Santa Fe River is also the primary source for acequias in the city, which are in turn a source of irrigation for many residents. Therefore, the quality of the water is important and needs to be investigated further.

The city plans to take samples from the five sample sites used for this project as well as a larger range of sample sites to create a holistic picture of water quality in the river and determine any discernable trends. For this project, the northeast portion of the river was well sampled but it is still unclear what pollutants might be effecting the southwest portion of the river. This area is more rural, with few houses and some farms. For future testing, I recommend sampling the river where it crosses Old Santa Fe Trail, St. Francis Dr. Alto Park and Siler Rd because they are all high trafficked areas (Figure 21). Alto Park is also a very large park between Patrick Smith Park and Frenchy's Park and could clarify why different quantities and types of contaminates are at the other two parks. To provide data on the southwestern portion of the river I recommend sampling from major roads crossing the river in the area including San Ysidro crossing, South Meadows and 599 (Figure 21). There are also small parks along the river throughout the city; sampling at these locations could clarify the land-use patterns associated with dog fecal contamination.

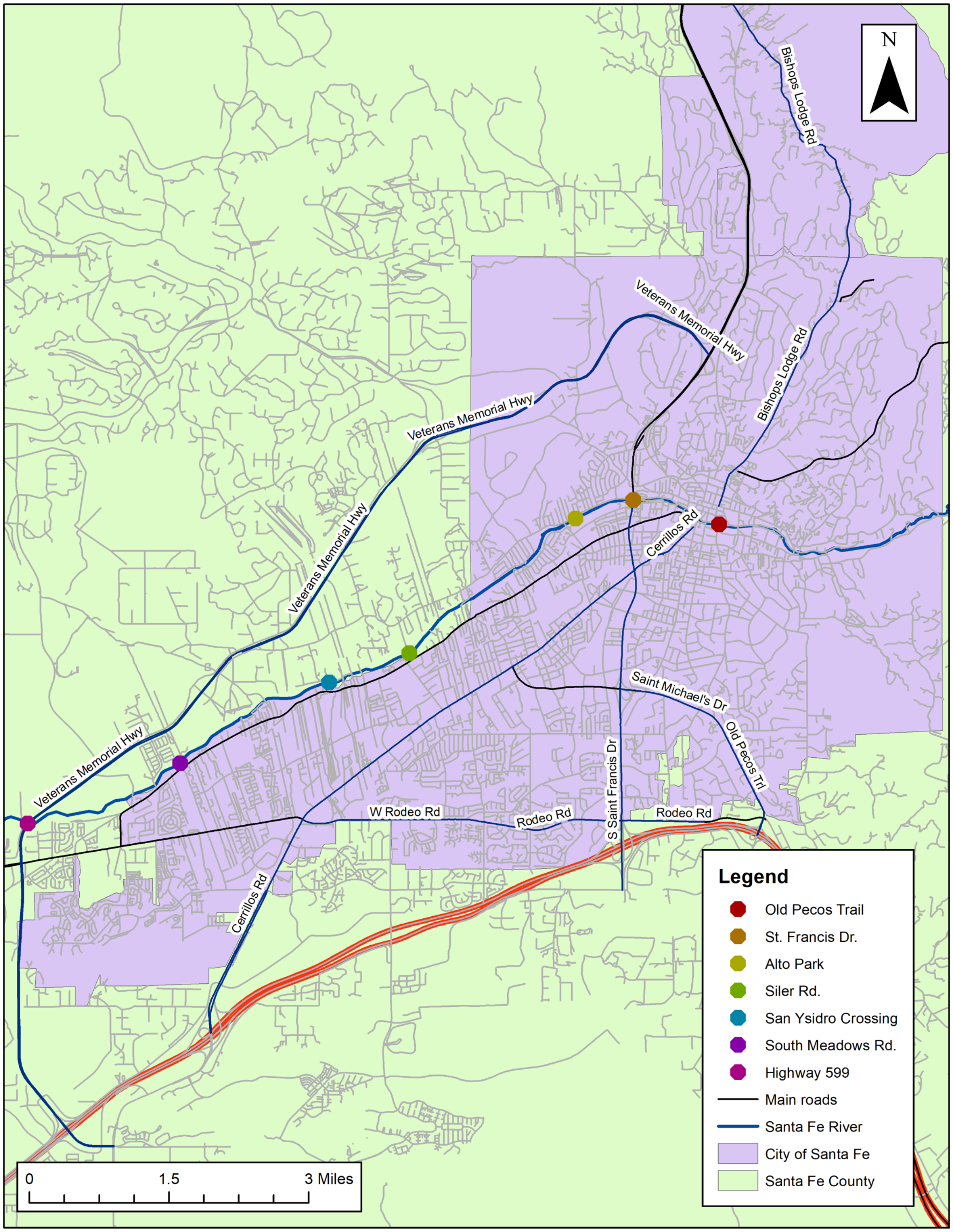

Figure 19. Proposed, future MST sample sites along the Santa Fe River for the City of Santa Water Division. Map by author in 2018.

MST testing is still expensive and therefore may not be a viable annual test for some water divisions. MST testing however, is still valuable even without the creation of a water quality baseline. Testing only when contamination is detected may be the most cost effective measure. Additionally, water divisions that monitor large watercourses will likely only want to preform MST testing when they cannot determine the source of contamination. Monitoring a watercourse of a large size would require sampling dozens of sites along the river which would become expensive quickly. Frequent MST testing thus is likely only feasible for smaller watercourses until the testing becomes less expensive.

Finally, the water division could test for additional viruses and bacteria based on the information provided by the MST. Water-borne diseases caused by various bacteria, viruses, and protozoa remain a public-health problem. Most these cases are caused by *Shigella* spp*., Giardia, Cryptosporidium,* and *Salmonella,* all of which have been found in municipal watersheds in the United States. If there was an outbreak of a waterborne illness in Santa Fe, MST results could be used to identify pathogens and contaminated locations along the river.

6.6 Conclusion

Maintaining water quality is a continuous battle for many municipal water divisions in the United States. Minimizing microbial contamination is crucial for not only the health of that ecosystem but also human health. This project has identified sources of contamination along a portion of the Santa Fe River using microbial source tracking to aid the City of Santa Fe Water Division in complying with EPA water quality requirements. The research demonstrates the need to address pollution caused by dog feces along the Santa Fe River. This study provides a baseline for the city to gauge future contamination, and to assess potential remediation efforts. The data generated from this project will also help the city create BMP's for their EPA pilot project that has the potential to influence water management throughout the United States.

CHAPTER NINE

APPENDIX A: Santa Fe River *E. coli* Data

Table 6. Table of *E. coli* results for the Santa Fe River Nichols Reservoir to the Waste Water Treatment Plant from 2012 to 2016.

Sample Date/ Time	Station Name	*E. coli* Concentration (cfu/100ml)
2012-06-04 11:15:00.0	Santa Fe River below Cerro Gordo RD	14.5
2012-07-18 14:30:00.0	Santa Fe River below Cerro Gordo RD	119.8
2013-05-07 14:40:00.0	Santa Fe River below Cerro Gordo RD	167
2013-05-07 15:40:00.0	Santa Fe River ~75m u/s of Sandoval St	178.9
2013-05-14 13:45:00.0	Santa Fe River ~75m u/s of Sandoval St	88.2
2013-09-17 12:40:00.0	Santa Fe River ~75m u/s of Sandoval St	387.3
2013-10-08 14:40:00.0	Santa Fe River ~75m u/s of Sandoval St	66.3
2013-10-08 15:50:00.0	Santa Fe River below Cerro Gordo RD	15.6
2014-03-27 08:30:00.0	Santa Fe River ~75m u/s of Sandoval St	6.3
2014-04-22 14:00:00.0	Santa Fe River below Cerro Gordo RD	1
2014-04-22 14:15:00.0	Santa Fe River ~75m u/s of Sandoval St	1
2014-05-27 10:15:00.0	Santa Fe River below Cerro Gordo RD	61.3
2014-05-29 09:15:00.0	Santa Fe River ~75m u/s of Sandoval St	98.7
2014-06-25 08:40:00.0	Santa Fe River ~75m u/s of Sandoval St	69.7
2014-07-23 08:50:00.0	Santa Fe River ~75m u/s of Sandoval St	727
2014-07-23 11:35:00.0	Santa Fe River below Cerro Gordo RD	344.8
2014-08-20 11:30:00.0	Santa Fe River ~75m u/s of Sandoval St	101.9

2014-10-01 10:30:00.0	Santa Fe River ~75m u/s of Sandoval St	579.4
2014-10-15 11:20:00.0	Santa Fe River ~75m u/s of Sandoval St	547.5
2014-11-14 13:00:00.0	Santa Fe River below Cerro Gordo RD	3
2016-06-02 11:00:00.0	Santa Fe River 5 meters u/s of Guadalupe St	32.37
2016-06-13 10:00:00.0	Santa Fe River 5 meters u/s of Guadalupe St	135.4
2016-06-29 09:45:00.0	Santa Fe River 5 meters u/s of Guadalupe St	307
2016-08-05 16:30:00.0	Santa Fe River 5 meters u/s of Guadalupe St	>2419.6
2013-10-08 10:00:00.0	Santa Fe River above CRd 56 d/s of river preserve	123.6
2014-03-27 11:00:00.0	Santa Fe River above CRd 56 d/s of river preserve	56.5
2014-04-22 17:45:00.0	Santa Fe River above CRd 56 d/s of river preserve	139.6
2014-05-28 16:40:00.0	Santa Fe River above CRd 56 d/s of river preserve	88
2014-06-25 12:15:00.0	Santa Fe River above CRd 56 d/s of river preserve	686.7
2014-07-23 15:25:00.0	Santa Fe River above CRd 56 d/s of river preserve	501.2
2014-08-20 13:55:00.0	Santa Fe River above CRd 56 d/s of river preserve	>2419.6
2014-10-01 12:10:00.0	Santa Fe River above CRd 56 d/s of river preserve	130.8
2014-10-15 12:35:00.0	Santa Fe River above CRd 56 d/s of river preserve	195.6

APPENDIX B: Santa Fe Water Division previous testing of Cerro Gordo Rd., Frenchy's Field and Guadalupe St. for other water quality indicators.

Table 7. Table of the most recent dissolved oxygen, pH and temperature results for the Santa Fe River. Testing for the indicators occurred in 2014 and 2016.

Sample Site	Dissolved Oxygen (mg/L)	PH	Temperature
Cerro Gordo Rd.	9.25	8.1	13.82
Cerro Gordo Rd.	7.25	8.03	17.62
Cerro Gordo Rd.	8.8	8.21	15.7
Cerro Gordo Rd.	8.08	7.87	11.88
Cerro Gordo Rd.	8.40	7.72	11.92
Cerro Gordo Rd.	7.71	8.27	13.85
Cerro Gordo Rd.	7.75	7.64	17.91
Frenchy's Field	8.99	7.93	8.33
Frenchy's Field	8.5	7.6	16.63
Frenchy's Field	7.03	8.51	29.47
Frenchy's Field	7.55	8.12	24.84
Frenchy's Field	7.64	8.23	16.07
Frenchy's Field	5.93	8.31	29.56
Guadalupe St.	5.86	8.78	14.6
Guadalupe St.	6.68	8.22	15.73
Guadalupe St.	6.43	8.36	15.43
Guadalupe St.	2.83	8.44	21.95

APPENDIX C: New Mexico Environment Department (NMED) Sample Sites

Table 8. Storm Water Quality Bureau 2012-2016 Santa Fe River *E. coli* sampling sites.

Santa Fe River below Cerro Gordo RD
Santa Fe River ~75m upstream of Sandoval St
Santa Fe River 5 meters upstream of Guadalupe St
Santa Fe River below St Francis Dr.
Santa Fe River below Frenchy's Field
Santa Fe River at County Road 68A (San Isidro Crossing)
Santa Fe River above Hwy 599
Santa Fe River immediately upstream of WWTP effluent channel
Santa Fe River above County Road 56 downstream of river preserve

APPENDIX D: Biomarker Concentration Interpretations

Table 9. Concentration interpretation for the fecal gene biomarker by real-time quantitative polymerase chain reaction (qPCR) for each sample site on the Santa Fe River.

Gene Biomarker	Sample Location	Concentration of Fecal Pollution in Sample
Human	Cerro Gordo Rd.	Low concentration
	Patrick Smith Park	Low concentration
	Paseo de Peralta (crossing E. Alameda)	Low concentration
	Guadalupe St. (crossing W. Alameda)	Low concentration
	Agua Fria St.	Low concentration
Dog	Cerro Gordo Rd.	Low concentration
	Patrick Smith Park	Moderate concentration
	Paseo de Peralta (crossing E. Alameda)	High concentration
	Guadalupe St. (crossing W. Alameda)	Moderate concentration
	Agua Fria St.	Moderate concentration
Bird	Cerro Gordo Rd.	Low concentration
	Patrick Smith Park	Low concentration
	Paseo de Peralta (crossing E. Alameda)	Low concentration
	Guadalupe St. (crossing W. Alameda)	Low concentration
Beaver	Cerro Gordo Rd.	Low concentration
Ruminant	Agua Fria St.	Low concentration

APPENDIX E: How Data will be Shared with the City of Santa Fe

This research was presented in December 2017 at the Santa Fe River Commission meeting to County Commissioner Anna Hansen and her staff. She was intrigued by the results and wants to work on allocating funds for additional MST testing. In addition to the City of Santa Fe Water Division, the Santa Fe Watershed and the Nature Conservancy were also present at the meeting. The Nature Conservancy runs trails and restoration around Two Mile Reservoir, right above the Cerro Gordo Rd. sample site, and requested the data from the sample site as well as a copy of this thesis to further their restoration and public awareness efforts. At the end of this project all data and the final paper will be given to the City of Santa Fe Water Division.

CHAPTER TEN

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