COPPER AND ZINC MODIFIED CANADIAN NATURAL ZEOLITE AS A MEANS FOR REDUCING THE NUMBER OF *E. COLI* COLONY FORMING UNITS IN CONTAMINATED DRINKING WATER

by

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Abstract

Clean drinking water is essential for all life on Earth. Over 400, 000 people die annually from lack of clean drinking water, and the demand on water is predicted to increase in all sectors. The demand for safe drinking water presents an ongoing challenge for new water purification techniques. Since the 1950s, natural zeolite has been investigated as a means of water purification due to its stable, crystalline, porous structure and cation exchange capacities. Recent publications have indicated that natural zeolite modified with metal cations provides a stable treatment media for the elimination of *E. coli* bacteria in drinking water. In this series of analysis, Canadian natural zeolite from the Bromley River Valley, Kamloops BC, was modified with zinc and copper sulphates to create three novel water treatment options. Treatment of *E. coli* contaminated water was most effective with zinc modified zeolite, which also significantly lowered the pH in comparison to the copper and copper/zinc modified zeolite. Of the three zeolites, all released too much copper and zinc into solution that may be linked to the low cation exchange capacity (CEC) of the Canadian zeolite. *E. coli* colony forming units were reduced; however, they were not sufficient to meet drinking water standards. Future studies will focus on optimizing the ratio of modified zeolite needed to treat a given amount of pathogen in solution.

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Glossary

CanNZ: Canadian Natural Zeolite from the Bromley River Valley

CanZZ: Canadian zinc modified zeolite

CanCZ: Canadian copper and zinc modified zeolite

CanCU: Canadian copper modified zeolite

CEC: Cation Exchange Capacity

CFU: Colony Forming Units

CubNZ: Cuban Natural Zeolite

CubZZ: Cuban zinc modified zeolite

ICP-OES: Inductively coupled plasma optical emission spectroscopy

WHO: World Health Organization

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Chapter 1 - Introduction

1.1 Water

Even in countries blessed with substantial water and land resources (e.g. Canada), water is not always available when and where needed (Bereski et al. 2017). Sustainable development requires protecting the resources for future generations, while meeting the needs of present society. Taking into account climate change, social influences, and increasing demands on water resources, the country is experiencing new challenges and competition for water. As demands on water increase, effective management of water resources, including reuse of contaminated surface and underground water streams, will be essential for a healthy environment. Thousands of crises have dramatically affected water management and governance criteria. Waterborne disease, such as *Escherichia coli* (*E. coli)* outbreaks in Walkerton, ON, Canada killed seven, and induced illness in 2,300 people between 2000 and 2005 (Bradford et al. 2015). In this specific case, studies showed that the source of the pollution was livestock manure that had been applied on farmland as fertilizer.

Further, access to clean water within a country is not always consistent. In 2015, over 90 First Nations reserves had boil water advisories. Waterborne illness was 26 times higher on reserves than the national average (Bradford et al. 2015) with 30% of reserve treatment facilities actively posing a risk to community health (Bradford et al. 2015). Despite several million dollars dedicated by the Government of Canada in 2020, there are still 58 communities without access to reliable drinking water (Environment of Canada 2022). Some treatment options require constant maintenance and chemical additives, such as chlorine, that have been shown to worsen common skin conditions among reserve children (Warrick and Patrick 2019). Additionally, it seems that the water in remote communities is often negatively impacted by local industry, such as mining and manufacturing. In 2011, Robert J Patrick outlined that the issue with water treatment extended beyond policy and funding to include a lack of appropriate water treatment technology (Patrick 2011). To meet the water needs of small communities, a purification technology needs to be affordable, require minimal maintenance and skill to operate, be reliable, and be able to treat fecal matter pathogens and industrial by-products such as volatile organic compounds and heavy metals (Warrick and Patrick 2019, Ravishnakar and Jamuna 2011, Bereskie et al. 2017, Government of Canada 2017).

1.2 Previous techniques for water purification

Reactive chemicals (e.g. chlorine) and/or ultraviolet (UV) radiation are usually used to destroy water pathogens (KDF 2020). Several technical reports studied silver (Ag) and zinc (Zn) oxide nanoparticles for treating microbiological contamination (Xiaolei et al. 2013, Aarestrup and Hasman 2004, Ravishnakar and Jamuna 2011, Kallo 2001, Fuentes et al. 2014, Hrenovic et al. 2012). Kinetic Degradation Fluxion (KDF) filters release small quantities of Cu and Zn (K. Inc. 2020). Some articles have related the use of Ag/Zn zeolite for the same purpose (Orha et al. 2011, Aarestrep and Hasman 2004, Prabir et Wang 2019). The use of natural and modified porous zeolitic minerals as multifunctional media is one of the efficient approaches for decontamination of polluted water resources (Wang and Peng 2010). Zeolites are composed of a durable alumino-silicate structure that forms a porous charged material. These unique chemical and physical characteristics make zeolite appropriate for a multitude of environmental applications where effective, low-cost materials are needed to bind, absorb, adsorb, fill, and filter (Ravinshankar and Jamuna 2011, Boles et al. 1977, Orha et al. 2011, Fuentes et al. 2014). Molecular sieve and ion exchange properties, as well as availability and relatively low cost, are the major factors that make natural zeolites commercially attractive for environmental remediation and industrial applications (Flanigen and Mumpton 1981). The discovery of zeolite deposits with relatively high purity in Canada, United States, and other countries in the 1950s marked the era of commerce for natural zeolite-based water filtration (Mumpton 1978).

1.3 Zeolites

Natural zeolites are ubiquitous silicate formations found in cavities of basalt and trap rock formations; however, commercially available zeolites are most often found in sedimentary deposits (Hay and Sheppard 2001). Zeolites are crystalline hydrated alumino-silicates of alkaline earth cations, capable of exchange with cations in solution (Mumpton 1978). Since the 1950s, natural zeolites have been highly valued for their ability to remove toxic cations and rivals the adsorption of synthetic sieves (Fuentes et al. 2007). Along with quartz and feldspar, zeolites are tetra silicates that form a three-dimensional SiO_4^{-4} tetrahedral, where all tetrahedral corner oxygens are shared (Kitsopoulos 1999). The sharing of oxygen ions provides zeolites with infinite (repeating units) three dimensional structures. The alumino-silica structures are negatively charged and very strong in nature. This combination of properties allows cations to be shared freely between the internal zeolite structure and its surrounding environment. For example, two sodium ions with a positive charge each may be exchanged with a single calcium ion with a two plus charge. It is apparent in zeolite studies that the ion affinity changes based on the pore size and charge of the zeolite backbone (Boles et al. 1977, Ming and Dixon 1987).

Filtration and purification characteristics of natural zeolites can be applied to removing impurities existing in water in the form of insoluble, colloidal, and dissolved physical states, which are of mineral, organic or biological origin (Kallo 2001). Zeolites were used to reduce the levels of ammonium and other impurities in water treatment plants in Budapest (Hungary), Colorado (USA), Tbilisi (Georgia) and Ukraine (Kallo 2001, Mumpton 1999). In both pilot- and full-scale applications, the ion exchange and filtering properties of clinoptilolite-rich tuffs were utilized with subsequent treatment systems. Practical applications of phillipsite-rich tuff from Tenerife, Canary Islands, were also shown to favorably remove indicator bacteria and dissolved organic matter from water in a packed percolator reactor (Harleman et al. 2009). A patented micro-filtration system (i.e. Jossab Aqualite), functions by integrating clinoptilolite into an appropriate technical set-up for purifying drinking water in emergencies where the requirement of safe drinking water has been critical for public health (Hrleman et al. 2009). The technique can filter out particles down to the size of 1–2 μm without any chemical additives. The subsequent UV filter ensures complete removal of bacteria and parasites. For emergency situations, the mobile units have a capacity ranging between 7-15 m^3/h which corresponds to fresh water for 5000–12000 persons per 8–10 hours of use or up to 20000–25000 persons for 20 hours of use, in compliance with the World Health Organization's (WHO) standards (Government of Canada 2017) (W.H.O. 2019). Mobile water purification units (Fig. 1) were utilized between 1999- 2006 in Rosersberg, Sweden; Grozny, Chechneya; Belgrade, Kosovo for rapid transportation to the point-of-need and immediate performance at the emergency site (Figure1-1) (Harleman et al. 2009). This system is further proposed for the elimination of radioactive fallout and for the removal of arsenic or geogenic pollutants from groundwater (Harleman et al. 2009).

Due to the stability of zeolite and its cation exchange properties, researchers have been attempting to produce zeolite-based water treatment apparatus (Mumpton 1999, Flanigan and

Mumpton 1977, Faghihian et al. 1999, Madji et al. 2015, Fuentes et al. 2014, Hrenovic et al. 2012). By incorporating zeolites into filtration units, it was possible to remove unwanted cations and contaminants from solution. In other studies, the modification of zeolites with positively charged molecules allowed for the delayed release of antimicrobial agents to treat environmentally sourced water (Alsammarraie et al. 2018, Filali et al. 200, Fuentes et al. 2006, Lalley et al. 2014).

(Figure 1-1) A mobile water purification unit based on natural zeolite, capacity: 4–7 m^3*/h, weight: 1800 kg.*

Some natural zeolites are well known as physical adsorbents of pathogens, such as Giardia, cryptosporidium, bacteria and their spores (Fuentes et al. 2014). Most of these organisms and their spores are in the size range of 0.5-10 microns; therefore, the zeolite powder can adsorb a high percentage of these microorganisms while the water passes through the zeolite

(Hughes 2003). In 1990, a team of researchers at the University of Havana developed a zinc-modified zeolite that had microbicidal effects against bacteria, yeast, and protozoans (Fuentes 2014). According to their report, they purified natural zeolite and loaded Zn cations using zinc sulphate. The product released zinc cations in a slow-release fashion, meeting the drinking water standard of less than 5.0 mg/L recommended for levels of zinc (Government of Canada 2017).

The applications for zeolite use in water treatment are abundant. The natural properties of zeolite as cation exchangers and sieves are well documented for many zeolites in many countries; however, the natural zeolites of the Bromley River Valley in Kamloops BC have never been evaluated or tested in application for drinking water remediation (Kondo et al. 2019). By establishing the natural properties of the zeolitic tuff and evaluating subsequent cation modification, it may be possible to develop a system for the purification of contaminated water (Djordie et al. 2011).

1.4 Mechanism of action on prokaryotes

The cellular functions of bacterial pathogens are affected by at least three mechanisms linked to metal modified zeolite. The first mechanism of action affects the optimum pH range for bacterial reproduction (Aarestrup and Hasman 2004). By releasing metal ions into solution, the resulting pH can impacted bacterial reproduction and prevented further propagation (Shameli et al. 2011). A second mechanism (Sharma et al. 2009) indicated that free cations such as Ag and Zn pass through the cell membrane and interact with the negatively charged DNA/RNA molecules, preventing translation and transcriptional activities. The third potential mechanism was damage done to the pathogen’s cell wall as it passed over or through the zeolite pore (Hrenovic et al. 2012).

Transition metals in their ionic forms are known to bind with DNA, and zinc, specifically, was utilized in zinc fingers as a regulatory element (Anupama et al 2014, Pierce 2020). In some studies, the binding for covalent ions, such as zinc, with DNA showed a strong correlation and was likely interfering with the DNA transcription and regulation of the pathogens being studied (Anupama et al. 2014, Khedr et al. 2011).

1.5 Metal cations and consumption

Between 20 and 40mg of zinc is required daily for the activity of approximately 100 enzymes in the human body and plays a role in the immune system, protein synthesis, wound healing, DNA synthesis, and cell division (Anupama et al 2014, Plum et al. 2010). Zinc also supports normal growth and development during pregnancy, childhood, and adolescence, and is required for a proper sense of taste and smell (Plum et al. 2010). A daily intake of zinc is required to maintain a steady state because the body has no specialized zinc storage system (Leda et al. 2019). Various studies have shown that zinc is effective against a wide variety of microorganisms, especially those pathogens living in water that cause the gastrointestinal, pulmonary, and skin infections that most commonly affect humans (Gomes et al. 2020). According to the Guidelines for Canadian Drinking Water Quality, the removal of microbiological contaminants, such as bacteria, protozoa and viruses is a high priority (Government of Canada 2017). As a result of challenges with routine analysis of harmful microorganisms that could be present in inadequately treated drinking water, the microbiological guidelines focus on indicators (e.g. *E. coli* and total *coliforms*) and treatment goals. In addition to microbiological guidelines, there are chemical and physical parameters for drinking water (Government of Canada 2017, W.H.O. 2019):

1. health-based and listed as maximum acceptable concentrations (MAC);

2. based on aesthetic considerations and listed as aesthetic objectives (AO); or
3. established based on operational considerations and listed as operational guidance values (OG).

Zinc has AO: ≤ 5.0 mg/L or 5 ppm (AO is based on taste), which is slightly higher than the number from WHO guidelines (W.H.O. 2019). Water with zinc levels above the AO tends to be opalescent and develops a greasy film when boiled. Therefore, it is important to control the release of zinc from modified zeolite to water. Water with copper above AO will be blue in appearance and will be explored as both an antimicrobial agent and indicator.

Copper is the third most abundant transition metal in the human body (Osredkar et al. 2011). Copper cations are involved in many biological processes including immune response, nervous system maintenance (myelin sheaths) and defense against oxidative stress. Too much copper in a diet is also linked to many diseases, including increased rates of diabetes, and neurological impairments that vary depending on the age of the population (Leda et al. 2019). Some sources recommend roughly 1mg/ day whereas others recommend avoiding copper supplementations due to uncertain side effects of oversaturation. The B.C. safe water drinking guidelines have a minimum acceptable concentration of less than 1 mg/L (1ppm) designated to protect bottle feeding babies (Government of Canada 2017). Keeping the concentration below 2 ppm is required to avoid damage to kidneys (Government of Canada); however, other sources report no side effects until water reaches a concentration of 6ppm Cu (Osredkar et al. 2011). Some zeolite researchers have demonstrated that copper modified zeolite could reduce colony forming units by almost 100% in drinking water and synthetic effluent but also indicate that minimal amounts of copper were desorbed into solution (Hrenovic et al. 2012).

Copper ions have several proposed mechanisms for action against prokaryotic organisms. One method involves the formation of reactive oxygen species (ROS) (Osredkar et al. 2011, Angelova et al. 2011, Pavelkova et al. 2018) through the Haber–Weiss/Fenton reaction to form OH^- radicals (Pavelkova et al. 2018).

Fenton reaction $Cu^{1+} + H_2\,O_2 \rightarrow Cu^{2+} + OH^- + \cdot OH$

Haber ─ Weiss $Cu^{2+} + \cdot O_2 \rightarrow Cu^+ + O_2$

These free radicals have been shown to cause damage by attaching and inhibiting DNA transcription and regulation (Osredkar et al. 2011). In certain illnesses, the human host and the bacterial infection will battle over the limited reserves of copper in the body, indicating that copper is essential for a successful infection (Pavelokova et al. 2018). Evidently prokaryotes and eukaryotes have developed systems for incorporating limited trace metal resources, and for combating the risks associated with oversaturation (Leda et al 2019).

Due to the rarity of silver in natural environments, there are no guidelines addressing silver in drinking standards; however, the BC guidelines for aquatic systems indicate that there should be less than 2ug/L (2ppb) (Government of Canada 2017). Researchers have compared the effect of silver, copper and zinc ions against *E. coli* and have found that silver is a substantially superior antimicrobial (Prabir and Wang 2019, Janicijevic et al. 2020). Historically, silver has been used for treating injuries to prevent infection (Rosabal et al. 2005, McEnvoy and Zhang 2014), and to treat contaminated water; however, in 2022, silver is 230 times the price of zinc, and 75 times the price of copper, making silver-based treatment options extremely expensive. Theoretically, the silver treated nanoparticles will outperform modified materials with copper and zinc (Ravishankar and Jamuna 2011). It is still worth investigating copper and zinc, however, as they are much more cost efficient. A tool or system designed with significantly cheaper materials that can still eliminate the contaminants will be more accessible for smaller communities (Warrick and Patrick 2019).

1.6 pH of Water

The pH of water plays a significant role in its properties. Commercially sold water ranges from pH 6 - pH 10 (Khan and Chohan 2010. For drinking water purposes, some researchers propose that drinking more basic water will prevent certain illnesses such as cancer (Renal et al. 2011) while lower pH beverages might be the result of flavour additives such as lemons and limes. In terms of water quality, pH lower than 6.5 and above 8 will taste different and may be unappealing. Few studies exist; however, sheep have been observed preferring tap water over neutralized AMD indicating a taste difference (Horvath 1985).

In natural water tables, the pH greatly affects which organisms thrive and are present in the ecosystem. For example, a shift towards a higher pH will result in more cyanobacteria, which create bad odors, tastes and can be toxic to humans and animals (Government of Canada 2017). Low pH bodies of water are a risk to wildlife, as many species of plants and animals are not able to survive sudden or prolonged exposure to highly acidic water. Microorganisms are also challenged by changes in pH. Extremely basic and acidic solutions are beneficial as antimicrobials (Lee et al. 1997). Cleaners such as vinegar, with a pH of 2-3, and bleach, with a pH of 11-13, are common household cleaners valued for their ability to kill germs and eliminate bad odors.

A final consideration is that the free cations and anions in solution will affect the final pH (Zhang et al. 2018). Acidic solutions are known to increase the solubility of transition metals ((Król at al. 2020). There seems to be no significant indication that pH influences the speed of dissociation; however, the total amount of cations desorbed will change (Zhang et al. 2018). As the zeolite and the cation treatment dissociate, the competition between H^+ and the dissolved metals for ligands (e.g., OH^-,CO_3^{2-}, SO_4^{2-}, Cl^-, $S2^-$, and phosphates) becomes more and more significant (Lee et al. 1997, Zhang et al. 2019). This system may not be comparable as the zeolite framework will not completely dissolve in solution. The ideal pH range for the resulting water will be between 6 and 8 (Government of Canada 2017) and will be monitored by pH probe.

1.7 Pathogen in water

There are many water borne pathogens that exist across the world (Fuentes et al. 2014) Among the most common is *E. coli,* a member of the *Enterobacteriaceae* family. *E. coli* is a gram-negative bacterium that is typically found in the guts of mammals and wastewater effluent. As an environmental pathogen, the concentration of *E. coli* is notably less in lakes and rivers than in ponds, swamps, and municipal wastewater (Hrenovic et al. 2012). The dangers associated with *E. coli* are specifically present in bodies of water contaminated with fecal material. Rain and snow melt may drive fecal matter into water streams and wells. Natural disasters, such as hurricanes and flooding, can compromise entire wastewater treatment facilities, creating potential for potation to spread within cities and water treatment systems. Most water treatment facilities are effective at removing *E. coli* from drinking water; however, it is during times of natural disaster or system overload when the risks to human health are the highest.

This research elected to use a less infectious strain of *E.* coli then is typically found in contaminated water. The use of a less infectious strain comes at the risk that this organism might not be a good representative organism for other coliforms, or pathogenic micro-organisms. A benefit to using this organism means that the lab equipment and procedures needed for handling the BLS1 organism translate into cost savings for the project and safety for the community.

1.8 Summary

Water resources continue to be fundamental to human existence, and water security is important for healthy communities. Despite a multitude of techniques for water treatment being developed to purify contaminated drinking water, there is no singular solution for all situations. This research will test the feasibility of a low cost, easily operated filtration system using a metal modified Canadian zeolite. Zinc and copper modifications are a logical option to pursue, as they have demonstrated some anti-microbial properties and are significantly cheaper than silver. Therefore, silver nano-particles will only be used in certain comparison studies. *E. coli* is not the only water borne pathogen of concern; however, due to its simplicity to cultivate and the existence of a BSL1 strain, it is a good organism to start with. *E. coli* is also a ubiquitous organism; therefore, it will provide the largest scope of the possible infectious organisms to target.

A group of Cuban researchers have patented a water purification system using zinc modified zeolite (Fuentes et al. 2014). This research team from the Zeolites Engineering Laboratory of Havana have shared some of their zeolite sample, and modified zeolite for comparison during this study. The Cuban natural zeolite (CubanNZ) and Cuban zinc modified zeolite (CubanZZ) will be used as benchmarks for comparisons to the Canadian natural zeolite (CanNZ) and Canadian zinc-modified zeolite (CanZZ). In addition to zinc modification CanNZ will be modified into a copper (CanCu), and zinc/copper hybrid form (CanCZ).

1.9 Research Question

1) Do Canadian natural zeolites from the Bromley River Valley leach unwanted heavy metal contaminants (arsenic, cadmium, lead, mercury, etc.) into water?
2) Will Canadian natural zeolite modified with copper and zinc be able to maintain a steady concentration of treatment ions in solution?
3) Will the metal modified Canadian zeolites be able to eliminate the growth of *E. coli* over a short period of time (1hour-24hours)?

1.10 Objectives

1) Evaluate the Canadian natural zeolite for undesirable impurities via acid digestion and elemental analysis by ICP-OES
2) Modify the Canadian natural zeolite using pre-established methodologies for metal loading and determine their effects on the elemental composition of the metal (zinc and copper) modified Canadian zeolite.
3) Determine if the metal modified Canadian zeolites have the capacity to reduce the colony forming units of *E. coli.*

1.11 Hypothesis

Water treated with Canadian natural zeolites that have been modified with copper and zinc will be able to effect the *E. coli* colony forming units in solutions over a 24-hour period, while maintaining a concentration of metal ions that meets drinking water safety guidelines.

Chapter 2 - Materials and Methods

2.1 Collection of Zeolite Samples

Zeolite samples were collected from the Bromley River deposit in Kamloops, BC, and analysed in the Northern Analytical Lab (NALS) at the University of Northern British Columbia. Bromley River zeolite deposits are open pit; therefore, representative samples were collected using surface sampling and hand-dug excavation methods. Prior to modification, particles were between powdered and 4mm (-10+18 mesh). Samples were sieved into 0.5-1mm, 1-3mm and 3-5mm diameters. At UNBC, characterization tests were performed using X-ray diffraction (XRD), scanning electron microscopy with electron dispersive spectroscopy (SEM EDS) and inductively coupled plasma optical emission spectroscopy (ICP-OES).

X-ray fluorescence (XRF) was used to determine the classification of zeolite (Alver et Sakizci 2010). X-ray diffraction (XRD) was performed using a Rigaku Miniflex 300 to identify the crystalline structures of the zeolite sample. The surface morphology of the Canadian zeolite was determined using scanning electron microscopy (SEM) operating at 10 keV of acceleration voltage and coupled with energy dispersive X-ray analysis (EDAX).

2.2 Elemental Analysis

Zeolites are characteristically durable and, as such, require strong acids to fully dissolve the structure (Peru and Collins 1993). The use of hydrofluoric acid (HF) is required to destroy a zeolite; however, HF requires special considerations for laboratory safety. Hydrochloric acid and nitric acid are used to digest most soil samples and is well established in EPA Method 3050 (EPA 1996). Powdered Bromley sample was digested along with coarse Bromley sample (1mm-3mm) and a commercial comparator, ZeoDigest. Zeolite samples were digested in solutions of $4NO_3$:1HCl, $1NO_3$:4HCl, $2.5NO_3$:2.5HCL and $3NO_3$:1.5HCl. The tubes were then topped to 15 mL using deionized water (DI). The resulting solutions were prepared for analysis using ICP-OES to compare elemental values. The 3.5 NO3: 1.5HCl mixture was selected for the subsequent digestions. Ground zeolite samples were dried at 100°C for 24 hours, digested using the modified EPA method 3050b (EPA 1996) with 2.5ml hydrochloric acid and 2.5mL nitric acid (based on results of the above digestion investigation) and analyzed for elemental composition using ICP-OES (ECS 4010 CHNS-O Analyzer, Costech Analytical Technologies Inc.) with a modified EPA method 200.2 (EPA 1994).

2.3 Cation Exchange Capacity (CEC)

Cation exchange capacity (CEC) was determined using established ammonium acetate procedures (Kitsopoulos 1999). Powdered zeolite was dried in a 100°C oven for 24 hours. Then, 10 mL of 1 molar, pH 7 solution of ammonium acetate was added to 1 g of each zeolite sample. The ammonium acetate solution was changed once every 24 hours for three days. At each exchange, the supernatant was discarded. After 72 hours, 10 mL isopropanol was used to wash the samples to remove excess ammonium acetate. The zeolite samples were then dried at room temperature for 24 hours. Ammonium ions were replaced within the zeolite structure by washing six times with 10 mL of 10% NaCl. The wash was saved for ICP- OES analysis of sodium ion concentration.

Zeolites possess a natural positive charge due to the structure of the aluminum, oxygen and silica tetrahedron structure. This charge allows the material to readily exchange ions with the surrounding medium. Analyzing the zeolite's ability to exchange ions was part of the initial characterization and allows for an estimation of potential loading and desorbing. The cation exchange method was based off previous work from Greek zeolites (Kitsopoulos 1999) and employed a variation of the Berthelot method adapted for the AA3 auto analyzer (Garcia and Baez 2012) (Kanda 1995) (Ming and Dixon 1987) (Hendershot and Laland 1993).

Zeolite samples were ground to a powder less than 125 um in diameter. Between 100 and 150mg of dried sample was transferred to 10mL of 2M NH_4OAc pH7. The zeolite was placed on a shaker and the solution was changed every 24 hours, three times, to ensure each ion exchange site was filled with ammonium. The NH_4OAc solutions were saved for later analysis by ICP-MS for cations Na, K, Ca and Mg. The resulting rinse solutions were diluted in HCl to match the matrix solution for the ICP and compared for the cations mentioned above. Each rinse solution was centrifuged at 10, 000 rpm for 5 minutes to separate the supernatant and zeolite components. After the ammonium loading, the zeolites were rinsed five times with warm DI- and five times with isopropanol. Each wash was shaken by hand and centrifuged at 5000 rpm for 10 minutes before decanting. The final samples were left to dry in the oven at 30^{O}C for 24 hours before continuing.

The dried zeolite samples were weighed and transferred into new 15 mL falcon tubes. The zeolite samples were exposed to six rinse solutions of 10 mL 10% NaCl acidified to 0.005M HCl. The rinse solutions were saved in 100mL volumetric flasks. The final CEC analysis was performed using ICP-OES following EPA method 9081 (EPA 1986). Commercially available natural zeolite products from United States (e.g. KMI from Nevada) and from Cuba were used as benchmark samples to be compared with the Canadian natural zeolite and modified zeolites (Table 3-2).

2.4 Modification of zeolite

The selected sample was purified using a sodium chloride rinse (0.5M NaCl) in order to remove water-soluble impurities. The zeolites were washed in a 1:10 mass to mass (m/m) ratio for 24 hours at room temperature. The resulting zeolites were rinsed with 10 mL of DI on repeat until the solution was clear of sodium (roughly 30-50 mL) (Yeasmin, et al. 2016). Sodium was detected through the use of silver nitrate drops, which formed white precipitate in solution with sodium cations.

Canadian natural zeolites, converted into sodium form following the above method, were used to create zinc-modified Canadian natural zeolites following the methods outlined by the Cuban ZZ project (Fuentes et al. 2014). The reaction was allowed to take place at 100ºC for 24 hours. The resulting supernatant was decanted and the modified zeolites were washed several times with DI until the rinse was clear. The newly formed zeolites were then transferred to a metal tray and dried at 100°C for 24 hours.

A 0.5M $ZnSO_4 \bullet 7H_2O$ solution was prepared for zeolite modification. The mass-to-mass ratio of solution and sodium form zeolite was 10:1. Six Erlenmeyer flasks were used to soak 10 g of zeolite in 100 mL of the zinc sulfate solution. The flasks were then placed in a temperature-controlled shaker for 24 hours at 30°C. Three different zinc sulfate solutions were created in 250 mL volumetric flasks. 50 mL of solution was saved from each flask for elemental analysis to determine the efficiency of the loading.

After the zinc sulfate and zeolite exposure had occurred, the Erlenmeyer flasks were decanted into falcon tubes for further elemental analysis. The zeolite was then rinsed three times with 50 mL of DI water and dried at 100°C. 1.00 g of sample was saved from each reactor flask for future analysis, and the other zeolites were mixed into one homogeneous sample. A 0.5M $CuSO_4 \bullet 5HO$ solution was prepared and used in the same manner as the 0.5 M zinc solution for modification. A mixture of 0.5M CuSO and 0.5M ZnSO was prepared following the same procedure as above.

Sample Calculations (Skoog 2014):

(287.56g/mol ZnSO*7HO) x (0.5mol/1000mL)x (250mL/1 solution)=35.95gZnSO*7HO

(249.68g/mol CuSO*5HO) x (0.5mol/1000mL)x (250mL/1 solution)=31.21g CuSO*5H0

2.5 Ion Chromatography

Ion chromatography was utilized to determine the amount of sulphate present in solution after modified zeolite had been combined with *E. coli* treated water. Nitrates, carbonates and nitrites were also investigated using ion chromatography. CanNZ, CanZZ, CubanNZ, CubanZZ and SSZ were place in a 1:100 m/m ratio with water collected from Tabor Lake, Prince George, BC. Samples were placed on a shaker for 24 hours, then filtered for particulate matter and transferred into ion chromatography tubes.

The analysis was performed with a pump speed of 1.5 mL/min running 23mmol KOH eluent with suppression ASRS of 4 millimeters at 86 milliamps.

2.6 *E. coli* Acquisition and Storage

E. coli is often used as an indicator organism for safe water drinking (Hrenovic et al. 2012) (Fuentes et al. 2014). *E. coli* strain ATCC8793 was ordered from The American Type Culture Collection (ATCC) in an 8-mini pack (Product number ATCC-8739-mini-PACK). The bacteria were stored as per supplier instructions at -20°C. One of the eight packs was propagated into nutrient broth and incubated for 18 hours at 37°C. Glycerol was added to the nutrient broth (Difco BD 234000) to form a 10% volume to volume (v/v) mixture. 200 uL of *E. coli* were transferred to sterile Eppendorf tubes and re-frozen at -20C for use in the anti-bacterial trials.

2.7 Minimum Inhibitory concentration (MIC):

The effect of zinc on the growth rate of *E. coli*, had to be verified. A small test was performed using an Optical Emission Spectrometer (OES) and *E. coli* grown in increasing concentrations of zinc solution. A sample of frozen zeolite was thawed and transferred into 10 mL of sterile nutrient broth. The nutrient broth was then inoculated with *E. coli* and spiked with zinc sulphate. The range of zinc was 0, 0.2, 0.5, 1, 2, 3, 4 and 5ppm in each test tube. The test tubes were incubated at 37°C for 24 hours, then analyzed for optical density at 600nm wavelength.

2.8 Timed desorption of cations

The rate and profiles of the zeolite desorption had to be established prior to application on the target organism. 1 g of CanNZ, CanZZ, Cuban ZZ and SS zeolites were placed in 100 mL of sterile Tabor Lake water. The flasks were shaken for 24 hours and sampled at 10, 30, 60 180 300 720 and 1440 minutes. Triplicates were performed for mean and standard deviation.Antimicrobial assay in Tabor Lake water with metal modified zeolites

To compare the effectiveness of modified zeolites on *E. coli* growth, 0.1 g of CanZZ, CanNZ, synthetic silver zeolite or CubanZZ were added to 100 mL of autoclaved Tabor lake water. The lake water was then spiked with approximately 2000 cfu/mL of *E. coli*. The resulting solution was incubated at 37°C. One set of samples was analysed after one hour of exposure and another set of samples was analysed at three hours. The procedure was performed in triplicate to compare mean and standard deviation (n=10). Samples were analysed for *E. coli* and coliforms with the IDexx quant-tray 2000 technique (Rice, et al. 2012).

2.9 Colony counting

Bactericidal effects of the developed media on micro-organisms present in drinking water will be studied using reference strain ATCC 8793 for *E. coli.* The contact time was varied (30min, 1 hour, 2 hours), and the amount of zeolite in solution was held constant (1g/100mL). The most efficient media was then used to test actual water samples. The Colilert and membrane filtering techniques were used for bacteriological studies (Rice et al. 2012). The Colilert method simultaneously detects and quantifies both total coliforms and *E. coli* 24 hours after sampling. The known amount of zeolite was in contact with un-modified and modified zeolite at a given temperature and pH. At a given time, the zeolites were removed from the treated water. The post copper, zinc and copper/zinc modified Canadian zeolites were dried at 100°C for 24 hours, and digested using the modified EPA method 3050b for elemental analysis (EPA 1996).

2.10 Cu and Zn Modified Canadian Zeolites on *E. coli* in Nutrient Broth

The effects of CanCU, CanZZ, and CanCZ, on *E. coli* in nutrient broth was analysed using the IDEXX Quanti-Tray 20, 000 (Rice et al. 2012). A sample of *E. coli* was inoculated into 100 mL of nutrient broth for 18 hours then 1 mL of grown culture was transferred into sterile 100 mL portions of nutrient broth. Four replicates for 0.5 g, 1.0 g, 2.0 g, and 2.5 g of CanCU, CanZZ, and CanCZ were mixed into individual flasks of inoculated broth and incubated at $37^{O}C$ for 24 hours. All cultures of *E. coli* were analysed using the IDEXX Quanti-Tray 20, 000 method and compared using mean, standard deviation and data analysis described below.

2.11 Final pH of solution

The initial and final pH of solutions treated with metal modified Canadian natural zeolite was assessed using an Orion pH probe. The pH probe was calibrated three times with pH 3, pH7 and pH 10 solution. A solution of DI was used to rinse the probe between samples (Orion 2011).

2.12 Data Analysis

The final pH of solution was analyzed for normality using a Shapiro-Wilk. Due to non-normal data, a Kendall- Theil regression was applied. Following, a Levene's F-test a Krustal Wallis was applied to determine the effects of final pH on *E. coli* cfu.

The effects of metal modified Canadian zeolite were analysed for normality using a Shapiro-Wilk. Following the normal data analysis, a linear regression (R^2) was determined along with a Cook's distance less than 1. A Levene's F test was then applied, followed by a 1-way ANOVA.

The effect of the metal modified zeolites on the colony forming units of *E. coli* in inoculated growth media was analysed statistically using SPSS software. The entire dataset for post treatment *E. coli* colony forming units was normalized (log base 10). The data was then analyzed for normality using both a Shapiro-Wilk test (a=0.05). Once the data was determined to be normal, a regression analysis was performed with an investigation for collinearity between the mass of zeolite and the type of modification used. Probability plots were formed as well to investigate the distribution of values along with the Standard Residual. Cook's Distance was investigated with a limit of 1.0. This data set (n=48) was then analyzed using a Pearson-Correlation and Linear Regression. To determine if there were differences between the effects of the metal modified Canadian zeolites, a Levene's F Test was applied to determine homogeneity of variance. This data set did not posses equal variances; therefore, a Krustal-Wallis analysis was applied.

Chapter 3 - Results and Discussion

3.1 Leaching of Toxic Elements

3.1.1 Digestion

Zeolites are characteristically stable structures and therefore typical digestion methods use hydrofluoric acid (HF) (Boles et al 1977). Barring access to HF, several combinations of nitric acid and hydrochloric acid were attempted (Table 3-1). The implication of incomplete digestion is that small amounts of cations locked within the silicate structure of the zeolite might not be represented during analysis. These protected ions would impair the accuracy of the elemental analysis. EPA method 200.2 is typically used to digest soils and sludge, while only employing (NO_3) and (HCl)(EPA 1994). Two replicates were digested per mixture of acid (n=8). The mean was calculated, and all variations were compared to the EPA method 200.2(EPA 1994). Visually, there were still intact masses of zeolite in the base of the acid bath; however, the elemental analysis in (Table 3-1) allows for some comparison of the digestion methods. Table 3-1 indicates that toxic elements; Pb, As, Cr and Ti are recovered in comparable quantities despite the mixture of HCl and NO_3. Given the consistent measurements (Table 3-1) the applied digestion method of $1.5HCl:3.5HNO_3$ was acceptable (Figure 3-1).

Table 3-1:The difference between the mean of Canadian natural zeolite digested with HCl and NO_3 and EPA method 3050b(EPA 1996.

	4HNO3 :1HCL	1HNO3 :4HCL	2.5HNO3 :2.5HCL	4HNO3 :1HCL	1HNO3 :4HCL	2.5HNO3 :2.5HCL
	2mm	2mm	2mm	Ground	Ground	Ground
As	0.00	-1.14	-0.65	-0.71	0.37	0.71
B	0.97	-0.54	0.71	0.80	-1.91	-0.80
Ba	86.59	26.05	62.38	76.38	-28.44	-76.38
Be	0.32	0.19	0.28	0.17	-0.02	-0.17
Ca	1480.45	348.93	1098.04	1190.59	-371.5	-1190.59
Cd	0.14	0.05	0.07	0.11	-0.15	-0.11
Ce	10.14	9.80	6.32	-20.92	46.34	20.92
Co	0.54	0.56	0.36	0.14	-0.58	-0.14
Cr	0.86	0.09	-0.30	2.36	-49.33	-2.36
Cu	1.67	0.56	1.51	0.67	-3.20	-0.67
Fe	859.95	-659.43	45.93	998.29	-1703.80	-998.29
K	4486.47	2925.22	3176.02	2109.09	378.30	-2109.09
Mg	320.16	35.11	90.22	328.04	-173.24	-328.04
Mn	20.00	10.98	12.35	9.21	-5.56	-9.21
Na	1501.56	1024.78	1199.66	530.57	-2.80	-530.57
Ni	0.96	0.24	0.45	0.12	-1.55	-0.12
Pb	2.69	2.92	5.16	1.79	-1.13	-1.79
Ti	15.97	-66.36	-13.85	39.47	-105.26	-39.47
Zn	15.69	2.50	7.61	12.00	-14.31	-12.00

3.1.2 Post Treatment Elemental Analysis.

The Canadian metal modified zeolites were analysed pre- and post-bacterial treatment for comparison to the natural zeolite. Table 3-2 shows the copper and zinc modification greatly increased the amount of copper and zinc from the natural Canadian zeolite, and that only a small portion of the additional cations are used in this treatment.

Table 3-2 Analysis of modified zeolites and post bacterial trial zeolites for the elements listed column (mg element/kg of sample).

Metals (mg/kg)	Zn Zeolite	Zn Zeolite Bio	Cu Zeolite	Cu Zeolite Bio	Cu/Zn Zeolite	Cu/Zn Zeolite Bio
As	<9.00	<9.00	<9.00	15.00	<9.00	17.00
B	2.00	3.00	2.00	2	2.00	2.00
Ba	373.53	331.23	417.08	378.67	331.89	411.07
Ca	3342.00	3308.00	2998.00	2844.00	2963.00	3355
Cr	0.97	1.24	1.43	1.29	1.23	0.98
Cu	8.00	11.00	11945.00	6088.00	7195.00	2942.00
Fe	1410.00	1539.00	1476.00	1739.00	1884.00	1658
Hg	<3.75	<3.75	<3.75	<3.75	<3.75	<3.75
K	13728.00	18666.00	14234.00	22601.00	12665.00	20011.00
Mg	1058.00	939.00	1060.00	984.00	903.00	1105.00
Mn	8.00	7.00	7.00	9.00	11.00	5.00
Na	8167.00	6321.00	7571.00	7162.00	7054.00	5929.00
Ni	1.00	1.00	1.00	2.00	2.00	2.00
P	47.50	268.50	272.60	<14.00	<14.00	260.30
Pb	10.40	10.10	25.80	29.60	23.10	24.00
S	111.00	89.00	577.00	231.00	2001.00	98.00
Zn	11526.00	10103.00	55.00	49.00	7083.00	4203.00

Comparing initial readings with the post modified zeolite and the post application zeolite (Table 3-1 with Table 3-2) demontrated, toxic ions did not leach into solution. The elements As, Cd, Cr and Ti were extracted during the conversion of Canadian natural zeolite into metal modified Canadian zeolite (CanZZ, CanCu and CanCZ). The element Pb, however, did not reduce in quantity from initial analysis (Table 3-1). Instead, the concentration of Pb in solution increased when treated with metal modified Canadian zeolite (Table 3-5). This suggests that the CanNZ has a high affinity for Pb, and that initial digestion may not have revealed the full extent of toxic cations present within the zeolite structure. Table 3-1 showed that there is a low amount of lead available in the zeolite structure; however, Table 3-2 showed that metal modified zeolite possessed more of this toxic ion (2-5ppm vs 10-25ppm). It was clear from this comparison, that the digestion method used to analyze the natural zeolite did not fully represent the composition of the material. The increase in total cations indicated that HF digestion methods were more appropriate for zeolite analysis (EPA 1994). Additionally, it was important to recognize the competition between cations within the zeolite structure (Orha et al. 2011) because the copper and zinc cations might have out competed the lead cations for binding sites. The increase in lead detection was likely due to the zeolite’s preference for copper and zinc cations over lead cations. This preference allowed a more favourable set of conditions for lead cations to release into the acid bath (Orha et al. 2011). Consequently, the digestion of silicate-based material using hydrochloric and nitric acid was insufficient (EPA 1994).

One limitation of this thesis is the lack of replicates and statistical application on early experiments. More replicates would have provided a stronger empirical data set from which to draw conclusions. Including a known silicate-based standard in digestion would have informed the completion of a digestion method, which would address the issues encountered using hydrochloric and nitric acid. The use of a known standard would have allowed for some form of correction value to be generated in the case of discrepancy (Ming and Dixon 1987). Further replication would improve the empirical nature of the digestions by bringing the mean calculations closer to the true value of the zeolite material, and could be applied in predictive models.

3.1.3 Physical characterization

The undigested material was analyzed using SEM-EDS and XRF (Figure 3-1,Table 3-3). Chemical composition of the clinoptilolite phase of Canadian natural zeolite tuff was obtained by EDS analysis. The aluminum silica ratio of 5.8 was determined by SEM-EDS (Table 3-3) and supported the identification of Bromley River Valley zeolite as clinoptilolite (Boles, et al. 1977). Additionally, the XRF (Figure 3-1) matched database comparisons (Alver and Sakizci 2010). The energy dispersive X-ray spectroscopy (EDS) of the Canadian natural zeolite sample provided the chemical composition of the Canadian natural zeolite. In comparison with known literature, this sample matched the chemical composition of clinoptilolite, shown in Table 3-3 (Boles et al. 1977, Hrenovic et al. 2012). The scanning electron micrograph, when applied to the Canadian natural zeolite, revealed its crystalline structure and the X-ray fluorescence read out matched the layout of clinoptilolite, as shown in Figure 3-1 (Alver and Sakizci 2010).

Table 3-3: Chemical composition of the clinoptilolite phase of Canadian natural zeolite tuff obtained by EDS analysis.

SiO_2	**TiO_2**	**Al_2O_3**	**Fe_2O_3**	**MnO**	**MgO**	**CaO**	**K_2O**	**Na_2O**	**P_2O_5**	**BaO**	**SrO**	**L.O.I.**	**Total(%)**
70.18	0.15	12.09	1.40	0.00	0.56	1.70	3.90	0.86	0.01	0.05	0.02	8.83	99.75

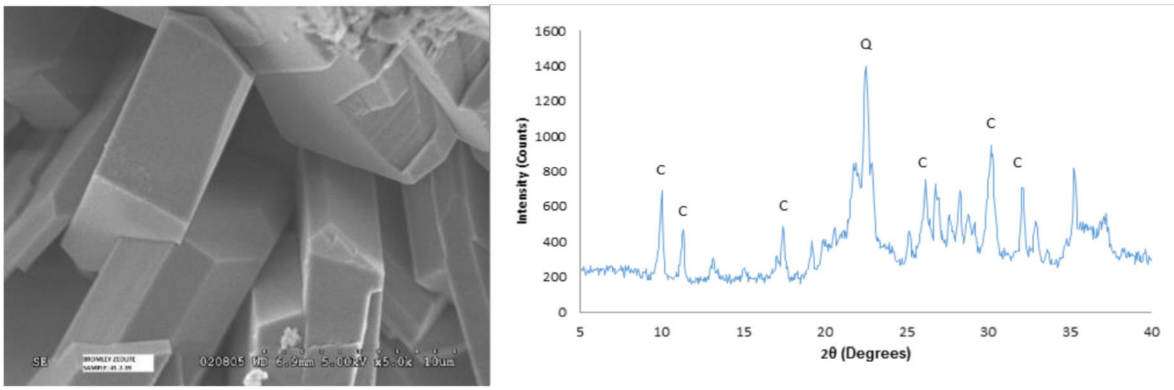

Figure 3-1: Scanning electron micrograph and XRF readings for the Canadian natural zeolite indicating clinoptilolite sample

3.1.4 Cation Exchange Capacity (CEC)

The cation exchange capacity of the Canadian natural and Canadian modified zeolites was low in comparison with the comparator zeolites (Table 3-4). The zeo-digest and the KMI zeolites possessed a CEC more than twice as high as the Canadian zeolites. The Cuban zeolites measured at a closer CEC to the Canadian materials; however, the CubanNZ was still 1.5x that of the CanNZ. The difference in CEC may have contributed to the differences in desorption profiles. The Cuban zeolite displayed a slow release of zinc cations (Figure 3-3). The Canadian zinc modified zeolite released more zinc rapidly, despite being modified using the same material and methodology.

Ion preference may have also played a significant role in the desorption of treatment ions. Table 3-4 indicated that the CanZZ adsorbed more of the Mg in solution than the CubanZZ. If the Canadian zeolite significantly prefers the ion mixture of Tabor Lake water, then it will readily exchange more cations than the CubanZZ. Further investigation into the Canadian zeolite's ion preferences could lead to discovering preferred environmental conditions that facilitate a lower total zinc desorption and a slower desorption profile (Kistsopoulos 1999).

The Cation Exchange Capacity (CEC) of Canadian zeolite and its zinc modified forms were compared with (a) a zeolite food additive, ZeoDigest, (b) the Cuban natural zeolite, (c) Cuban zinc modified zeolite and (d) KMI Nevada deposit. The CEC values obtained for the comparators were much higher than the CEC values for the Canadian zeolites. The Canadian Natural Zeolite had a lower CEC value than any of the comparators. Finer granules of zeolite also registed a smaller CEC value (Table 3-4)

Table 3-4 The mean cation exchange capacity (n=12) cation exchange capacity for natural and metal modified zeolites.

Sample Name	Zeodigest	CubanNZ	CubanZZ	KMI	CanNZ powder	CanNZ 2mm
meq/100g	293.25	148.66	113.58	220.44	94.47	103.57

3.2 Controlled Desorption of Treatment Ions

3.2.1 Cation Exchange with Environments

The elemental desorption for CanNZ, CanZZ, CubanZZ and Synthetic Silver zeolites were compared over a 24-hour period (Figure 3-2). Figure 3.2 displays the natural Cuban and Canadian zeolites in comparison with their zinc modified formats against the background elemental composition of Tabor Lake and a synthetic silver zeolite. There was no detectible silver analyzed in any other sample than the ones containing the synthetic silver zeolite, used as a baseline comparator.

The Synthetic Silver zeolite performed as expected by eliminating all *E. coli* CFU in solution (Table 3-5). There was no Ag in solution unless SS zeolite was present, and quickly after contact, the concentration of Ag increased rapidly (Figure 3-2). As the zeolite performed ion exchange with the solution, the Ag concentration plateaued due to complete desorption or a limit on exchanged had been reached (Alsammarraie et al. 2018) (Ravishankar and Jamuna 2011) (Orha et al. 2011). In Figure 3-3, the concentration of Ag plateaued as the concentration of magnesium approached 0ppm, indicating that the synthetic silver zeolite is reached an ion exchange limit with the Tabor Lake water which prevented further Ag cations from being released. It is possible that there was more silver that could be desorbed but didn't have a counter ion for exchange (Orha et al. 2011) (Filali et al. 2000).

Magnesium was one of the best exchange ions for clinoptilolites, as demonstrated in Figure 3-2. The silver zeolite had the most exchange occurring with the magnesium, followed by the CanNZ. The zinc modified zeolites demonstrated the least amount of exchange. Sodium was another readily available cation for exchange and appeared to increase in solution for every zeolite except for the CanZZ, which indicated a release of sodium by the zeolitic material.

The CubanZZ released zinc into solution; however, the CubanZZ maintained a lower concentration of zinc in solution over time. The Cuban ZZ maintained roughly a 5ppm concentration of zinc which is consistent with their documentation (Fuentes et al. 2014). This concentration is acceptable for drinking water standards (The Government of Canada 2017). The release of zinc into solution by the CanZZ was three times greater than standards and continued to increase over time (Figure 3-2). The minimum zinc concentration was above the drinking water standard and possibly indicates insufficient washing during the modification process (Figure 3-2). The CubanZZ also did not consume all of the available cations for exchange such as Mg. This zeolite seemed to maintain a 5ppm equilibrium with solution that is desirable for drinking and long-term storage.

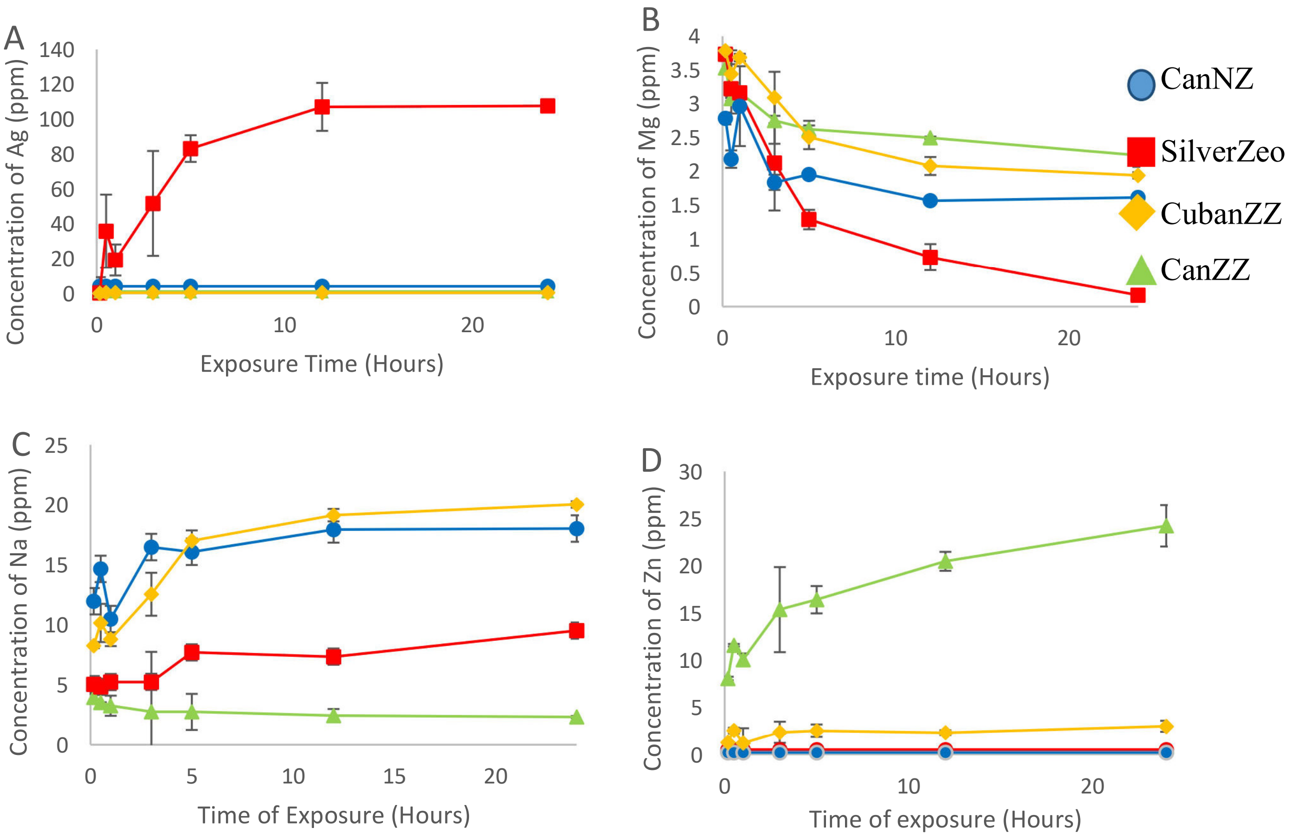

Figure 3-2:The mean and standard deviation for metal ion desorption of metal modified Cuban Zeolite (CubanZZ), zinc modified Canadian zeolite(CanZZ), natural Canadian zeolite (CanNZ), natural Cuban zeolite (Cuban NZ) and synthetic silver zeolite (n=10).

The Cuban zinc modified and natural zeolite released a large amount of sodium into solution (Figure 3-2). The expectation was that there would be limited sodium desorption from the zinc modified zeolite; however, this sodium may have been left over from the modification process. In this concentration of 20-25ppm, sodium is closer to oceanic salt water than desired drinking water conditions. 20ppm NaCl is above the tolerance of many prokaryotic species including *E. coli*. It is possible that the CubanZZ includes NaCl in its mechanism of action as a disruption to cell membranes by lysis (Pierce 2020).

The CanZZ had a very sudden release of zinc into solution, and it provided more zinc as time passed; however, it did not plateau as rapidly and may have taken longer than 24 hours to reach equilibrium with solution. The CanZZ also did not fully exchange with Mg in solution, indicating that there were still viable cations for exchange. With this information, it would not be considered safe to drink water treated with the CanZZ in a volume ratio of 1g per 100mL, instead, it would be appropriate to perform further tests with a larger volume of water to see if the CanZZ could maintain a concentration of Zn in solution that meets drinking water guidelines (The Government of Canada 2017).

3.2.2 Ion chromatography Sulphates

Ion chromatography was utilized to determine the amount of sulphate present in solution after modified zeolite had been combined with *E. coli* treated water. Figure 3-3 displays the change in sulphate concentration in Tabor Lake water after the addition of natural and metal modified zeolite. The Cuban zeolites leeched sulphate in solution up to 15ppm for the natural zeolite, and over 25ppm for the Cuban zinc modified zeolite. There was no change in sulphate quantities detected in assays with synthetic silver zeolite, CanNZ or CanZZ. Nitrates, carbonates, and nitrites were also investigated using ion chromatography; however, no changes were observed.

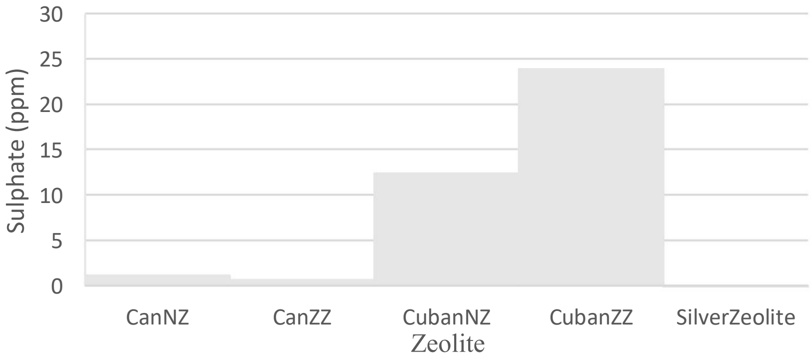

Figure 3-3: The mean sulphate concentration(ppm)) for duplicate treatments in Tabor Lake water (n=10).

Sulphate concentration of solutions can have an effect on the microbial community in solution (Aarestrup and Hasman 2004). The modification for the treated zeolites was performed using zinc and copper sulphates and, therefore, it was pertinent to determine if the Canadian modified zeolites were releasing sulphate into solution. Figure 3-3 shows that the CanNZ, CanZZ and SS zeolites released trace amounts of sulphate into solution. In contrast, the CubanNZ and the CubanZZ released more sulphate into solution.

The Canadian and Cuban zeolites were clinoptilolites and were modified with zinc following very similar reaction conditions (Fuentes et al. 2014). Sulphates attached to CubanZZ and not the CanZZ indicated potential disparities in the washing and loading procedures performed by the two different research groups. Further collaboration between the two research groups may identify the origin of the sulphates in the CubanZZ, or a method for maintaining the sulphate within the CanZZ for use as an anti-microbial.

3.2.3 Final pH of Treated Solutions

The final pH of nutrient broth was analysed for normality using the Shapiro-Wilk and for equal variances using Levene's F-test. The data collected from the final pH was neither normal (Shapiro-Wilk p=0.02<a=0.05) nor did it possess equal variance (F-Test p<0.01, < a=0.05). A Kruskal-Wallis non-parametric analysis was applied and found a significant difference between final pH (KW p=0.01<A=0.05). A Mann-Whitney U test was applied between groups with a Bonferonni Correction of 0.015. It was determined that the pH of the CanCu zeolite was significantly different from the pH of CanCZ and CanZZ. (MWU p<0.001, <a=0.015 for both groups). The mean and standard deviation final pH is displayed in figure 3-4.

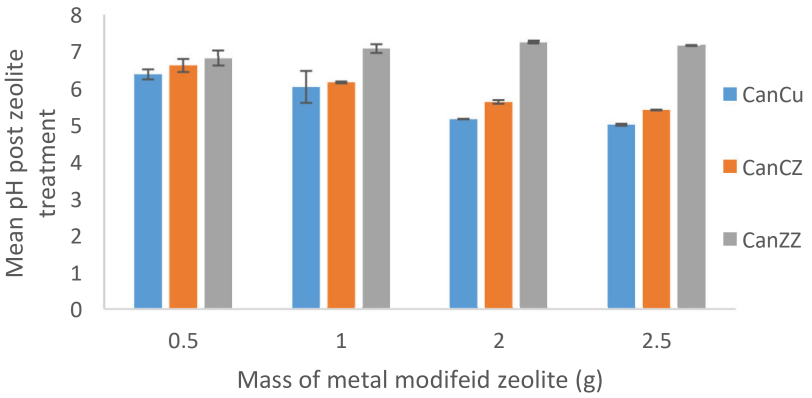

Figure 3-4: Mean and Standard deviation of final pH in nutrient broth after treatment with metal modified Canadian zeolite CanCu, CanCZ and CanZZ (n=16).

The treatment on *E. coli* that involved copper ions was effective at reducing the pH of solution (Figure 3-4). The decrease in pH may be due to the Haber-Weiss reaction which lowers the pH of solution while forming reactive oxygen species (Zhang et al 2018, Angelova et al. 2011, Osredkar et al. 2011). It may be this combination of decreasing solution pH and reactive oxygen species that leads to the anti-bacterial properties of copper treatment options (Hrenovic et al. 2011). The pH limit of *E. coli* is roughly 4.7 (Breidt et al. 2004), which is below the lower limits of this sample; however, the acidity may still be pressuring the organism.

3.3 Effects of Metal Modified Zeolite on *E. coli*

3.3.1 Minimum Inhibitory Concentration (MIC)

E. coli were grown in 10ml of nutrient broth with increasing zinc sulphate concentrations. The inoculated broths were incubated at 37^{O}C for 24 hours and analyzed with a spectrophotometer set to 600nm. Figure 3-5 indicates that there was a decrease in *E. coli* growth at 2ppm with only a slight recovery at 3ppm.

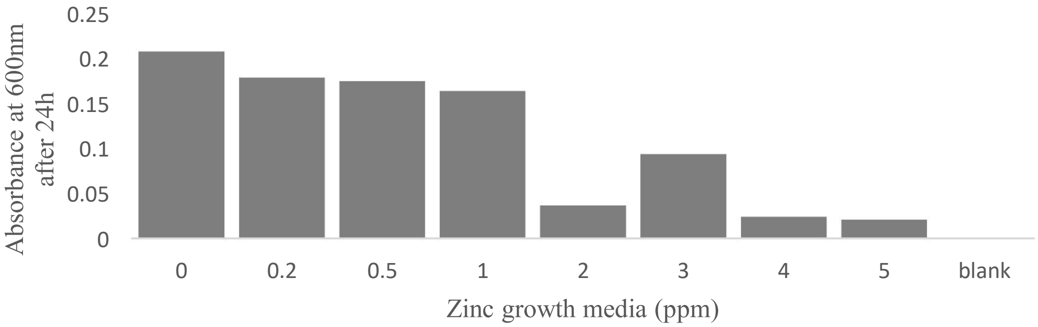

Figure 3-5: The absorbance of 600nm wavelength light by growth media at 37^{O}C for increasing zinc sulphate concentration (n=8).

The *E. coli* bacteria were introduced to favorable nutrient and environmental conditions; however, the bacteria did not reproduce to the same population density due to the increase in zinc sulphate concentration. The sample with 2ppm zinc sulphate registered a lower OD600 nm rating than the *E. coli* grown in conditions without treatment. At 5ppm there was still growth of the organism counter to certain literature (Rodrigues-Fuentes et al. 2014). While zinc is demonstrated to inhibit the growth of *E. coli* in treated solutions, the trial does not indicate if the treated solution is killing the bacteria or simply inhibiting growth.

One could determine if the *E. coli* were dead or simply inhibited by plating the broth and looking for colony forming units, as done in other studies (Hrenovic et al. 2012). Performing this experiment with more replicates would provide the opportunity for statistical predictions of future treatments (Aziz et al. 2019). An additional consideration is that both zinc and sulphate are present in solution. Considering that both zinc and sulphate have anti-bacterial properties in isolation, this study may be overestimating the effectiveness of either treatment; however, it is unclear that any published documents address this consideration.

This phase of the experiment benefited from low costs and ease of use. Using spectrophotometers minimized training requirements compared to other methods such as the SEM or ICP. The low volume (1-3mL) requirements per sample allowed for quicker analysis and would allow for simpler management of larger sample sizes. A set of descriptive statistics predicting the strength of the effect of zinc on *E. coli*, would improve the experiment design (Hrenovic, et al. 2012, Aarestrup and Hasman 2004).

3.3.2 Comparing Canadian and Cuban Zeolites on *E. coli*

The main goal for producing metal modified zeolites was to eliminate *E. coli* colony forming units from drinking water. The patented Cuban ZZ has demonstrated success in antimicrobial activity (Fuentes et al. 2014), as well as other research groups (Hrenovic et al. 2012, Prabir and Wang 2019, Filali et al. 2000, Khedr et al. 2011, Shameli et al. 2011). CanNZ had no quantifiable effect on the *E. coli* numbers; whereas, the zeolites containing metal had some effect. The Cuban ZZ had no quantifiable effect in the short-term exposure of 1 hour, but there was a measurable decrease in 3 hours (Table 3-5).

Table 3-5 The effect of zeolite treatment on Tabor Lake water spiked with 2000 CFU E. coli after one hour and three hours of exposure. (n=10).

Zeolite	1 hour exposure		3 hour exposure	
	Mean E.coli cfu/100mL	Mean coliform cfu/100mL	*Mean E.coli* cfu/100mL	Mean coliform cfu/100mL
CanZZ	<1	<1	<1	<1
CanNZ	>2,431	>2,431	>2,431	>2,431
SS	<1	<1	<1	<1
CubanZZ	>2,431	>2,431	488	461
Blank (no zeolite no *E. coli*)	<1	<1	<1	<1
Positive control (*E. coli)*	>2,431	>2,431	>2,431	>2,431

In contrast, the CanZZ was more successful at killing the *E. coli* within the first hour of contact. This might be explained by the different release profiles of the zeolites (Figure 3-3). The CanZZ had a much more immediate release of zinc into solution and maintained at a higher concentration than the CubanZZ (Figure 3-3).

3.3.3 The Effect of Zn and Cu modified Zeolites on *E. coli* in Nutrient Broth

The effect of metal modified zeolite on the mean Log10CFU of *E.coli*, was strong, as demonstrated by the R^2 values in (Table 3-6). Based on the Krustal-Wallis analysis there was no significant difference between the effects of metal modified zeolite on the target organism. Despite the strong R^2 values, the *E. coli* numbers never reached values acceptable for human consumption (Government of Canada 2017). In addition to the concerns with *E. coli* cells, the treated water did not maintain a safe drinking standard for either zinc or copper. The copper concentration reached over ten times the safe drinking standards, and the zinc concentration was more than double. The CanZZ zeolite displayed the highest R^2 value; however, the CanCZ treatment desorbed double the amount of zinc. If zinc were the main active ingredient in *E. coli* reduction, then the CanCZ should have performed much better than the CanZZ treatment. Additionally, some researchers have published on the success of copper modified filtration units using substantially lower copper concentrations (Ayben and Ulku 2004).

E. coli typically thrives in the bodies of mammals and enters the environment through biological secretion, where it is found in high concentrations (Hunter 2003). Testing metal modified zeolite on the elimination of *E. coli* required an environment that was more favorable than the autoclaved Tabor Lake water. Copper and zinc modified zeolites were tested on *E. coli* in nutrient broth and strong antimicrobial effects were observed (Figure 5-6); however, the metal modified zeolite desorbed more zinc into solution than is acceptable by drinking water guidelines (Government of Canada 2017). In all three trials with metal modified Canadian zeolite, the concentrations of copper and zinc were far more elevated than is acceptable by drinking water standards. Based on the regression analysis performed, the CanCu zeolite had the strongest effect on the *E. coli* (Table 3.5 R2=0.771>0.495>0.446); however, the Kruskal-Wallis analysis revealed that there was no significant difference between groups (p=0.054>a=0.05).

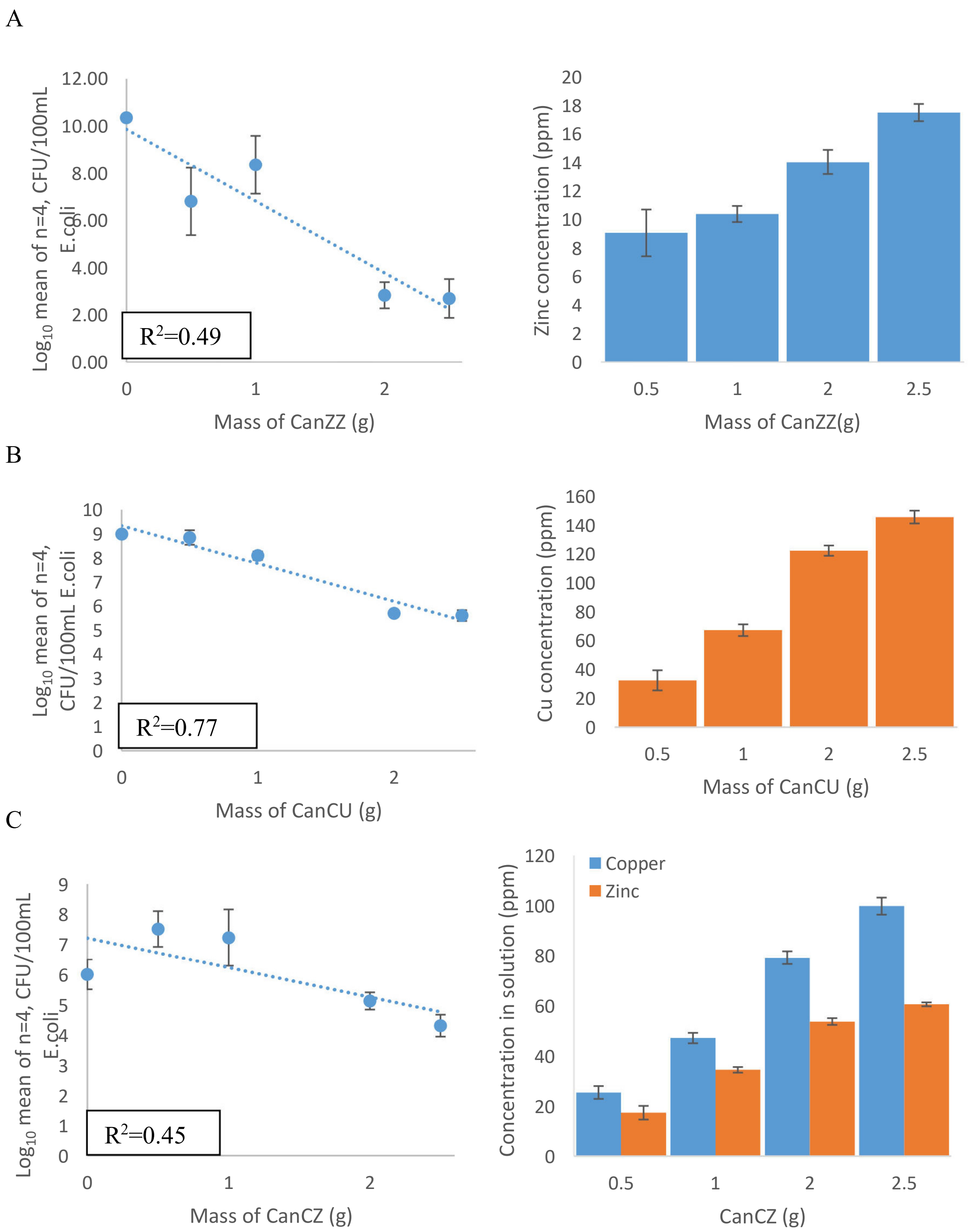

Figure 3-6The relationship between the mass of metal modified Canadian zeolite treatment and the loss of colony forming units by E. coli after 24 hours of exposure.

The success of the CanZZ on solutions containing 2000cfu of *E. coli* in Tabor Lake water, as shown in Table 3-3, justified testing solutions more favourable to *E. coli.* The line graph in Figure 3-6 displays the mean and standard deviations (n=16) *E. coli* cfu after 24 hours of exposure with metal modified Canadian zeolite. The associated bar graphs demonstrate the mean and standard deviations (n=16) of the active cations in solution after 24-hours of treatment. The copper and zinc concentrations achieved in solution were far above the safe drinking standards and the *E. coli* was not reduced to zero colony forming units in any of the trials (Breskie et al. 2017, W. H. O. 2019, Environment Canada 2022).

The zinc leached in nutrient broth (Figure 3-6) was comparable with the quantity of zinc leached into Tabor Lake water by CanZZ (Figure 3-2). After 24 hours, 1 gram of CanZZ released 12ppm zinc into nutrient broth (Figure 3-6) while 1 gram of CanZZ released 17ppm zinc in Tabor Lake water. This variation may be due to a difference in counter ions for which zinc could exchange or it may be due to natural variation within the zeolite sample (Orha et al. 2011, Kallo 2001, Filali et al. 2000). The *E. coli* bacteria was spiked at a higher concentration than in Tabor Lake trials (10^8 to 10^{10} cfu) which may have played a factor in the survival rate. A comparable paper testing zinc modified Croatian zeolite (Hrenovic et al. 2012) commenced their trials with 10^6-10^8 cfu *E. coli.* They reported that their zeolites were able to eliminate up to 100% of the *E. coli* over the period of 24 hours. They also indicate that minimal changes in pH were observed; however, they do not report their final cation concentrations in solution (Hrenovic et al. 2012).

The antibacterial activity recorded during the Tabor Lake and MIC experiments follows expectations outlined by previous researchers (Hrenovic et al. 2012, Fuentes et al. 2014). Table 3-5 shows that the metal modified, and silver synthetic zeolites can reduce the quantity of *E. coli* to zero in a very short time; whereas, the Canadian natural zeolite has next to no effect.

3.4 Avenues for Future Research or Improvement

Smaller inoculations of *E. coli* are recommended for analysis in future studies. Large quantities of organism required extended periods for growth in stable conditions and lead to large differences in organism counts. During the metal modified Canadian zeolite trials on *E. coli,* it was challenging to maintain a consistent amount of starting organism. The CanCu, CanZZ and CanCZ zeolites were tested against different starting concentrations of *E. coli,* which might have had an impact on the effectiveness of three treatments. Additionally, working with elevated colony counts required multiple dilutions, inoculations and related transfers between glassware and media, which may have compounded errors, expressing themselves in large standard deviations such as those in Figure 5-5. The volume of solution per sample was also challenging. Large volumes of nutrient broth required long periods of autoclaving and substantial volume to develop a sample size from which to draw statistical significance.

Colony counting techniques are widely used and considered a good technique for quantifying the number of coliforms in environmental solutions, however; the process required substantial large frames for analysis to ensure that the counts would fall within detection (Burlage et al. 1998). The method applied during the MIC determination addressed concerns of timing and volume. The spectrophotometer detected the density of cells and can be related to CFU/mL and OD600nm, prepared in advance. The costs were low, requiring only disposable or washable cuvettes. Using smaller solution sizes of 10mL in test tubes, instead of 100mL in Erlenmeyer flasks, would allow the experimenter to perform ten times the analyses for a similar amount of broth prepared.

In a colony counting design, timing is important as the organisms will continue to grow or be eliminated as time progresses. If a sample falls outside of the range covered by the plates, then it is challenging to redo a sample. However, if a tube of broth were too thick with target organism, a dilution could be performed immediately and analysed immediately after. The rapidity of this correction would protect the researcher from errors due to growth and time discrepancies. Relating the OD600nm to a

CFU/mL would only require one round of plating to create a relationship curve between cells and OD600nm in a certain solution. It is recommended that future studies apply the use of a spectrophotometry either in conjunction with colony counting, or in lieu of, to simplify the research.

Chapter 4 - Conclusions

Canadian Natural zeolite could play a role in water filtration as it does not leach any toxic metal or sulphates into solution in water treatment studies. The treated zeolite revealed more lead during the digestion procedure than the natural zeolite, indicating that digestion methods with hydrochloric acid and nitric acid were insufficient at complete digestion. The low CEC suggested that the zeolite may not have been effective at removing environmental contaminants, but further investigation will be needed for certainty. Areas of future inquiry include cation preference and determining an optimal ratio of zeolite used per volume of solution treated. The metal modified Canadian zeolite from Bromley deposit displayed a bactericidal effect on *E. coli* in both lake water and in nutrient broth; however, the amount of metal cations desorbed into solution was too high for safe consumption. The pH of solution may have played a significant role along with the metal cations in solution; however, the pH data set and the cation treatment data set did not share enough qualities for simple comparison. Analysis of comparator zeolites revealed that international zeolite samples released large volumes of sulphate and sodium into solution, bringing into question the main mechanism of action. The sulphate concentration reached by Cuban zeolite was well above *E.* coli tolerance and the amount of sodium in solution was far too high for human consumption, despite the zinc concentration being considered safe. In future studies, larger sample sizes and simpler experimental designs may provide a better understanding of the role that zeolites can play in water treatment.

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