# EFFECTS OF TRACE METALS ON CHLORAMINATED DISINFECTION BYPRODUCT FORMATION OF DRINKING WATER BIOFILMS

By

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Bachelor of Science in Civil Engineering

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2019

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTERS OF SCIENCE
July, 2021

EFFECTS OF TRACE METALS ON

CHLORAMINATED DISINFECTION BYPRODUCT

FORMATION OF DRINKING WATER BIOFILMS

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ACKNOWLEDGEMENTS

I would like to acknowledge NSF for the funding provided in support of this research. I would like to thank Dr. Fu for his efforts in previous research leading to this study. I would like to thank Dr. Krzmarzick for providing me with the opportunity to work with him during my Masters, and overseeing my development as a researcher and an engineer. Thank you to Dr. Wilber and Dr. Lampert for agreeing to serve on my committee. A special thank you to all of the graduate and undergraduate research assistants with who I had the pleasure of working with on my research.

"Acknowledgements reflect the views of the author and are not endorsed by committee members or Oklahoma State University. "

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Date of Degree: JULY, 2021

Title of Study: EFFECTS OF TRACE METALS ON CHLORAMINATED DISINFECTION BYPRODUCT FORMATION OF DRINKING WATER BIOFILMS

Major Field: CIVIL ENGINEERING

Abstract:

The use of disinfecting chemicals on public water supplies is a practice conducted around the world today. Disinfectants such as chlorine, chloramine, and ozone can be added to a water supply to eliminate any bacteria and waterborne diseases in the water. Studies have found that these chemicals can also oxidize natural organic matter in the water, creating disinfection byproducts (DBPs). Presently, hundreds of DBPs have been identified, both volatile and nonvolatile. Due to the potential harms of these DBPs, countries have established regulations that limit their concentration in water and pushed for research to improve our understanding of their formation. Recent research has found that the presence of metal ions in treated water can influence the types and concentration of DBPs formed. The aim of this study was to determine the influence trace metals have on the production of DBP precursors from bacterial isolates. The samples tested in this study showed that metal's do have influence over DBP formation. When compared against a set of control samples, samples with the addition of metal ions had increase and decreased concentrations of the targeted DBPs. Some DBPs such as chloroform that were not present in the control samples, were present in high concentrations in samples with metal additives. Chloropicrin was present in the control sample, and with the addition of various metals, such as Mg, the formation was increased. The addition of metals such as Fe and Mn resulted in no formation of chloropicrin in solution.

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# CHAPTER I

# INTRODUCTION

## 1.0 INTRODUCTION

Disinfection byproducts (DBPs) can result from the treatment of public water due to the reactions between disinfectants and natural organic matter (NOM) in water. Presently, hundreds of DBPs have been identified. The most common of these results from the use of chlorine disinfection. Free or excess chlorine in the system oxidizes NOM present in the water, resulting in the formation of DBPs. These DBPs pose some potential health risks for humans, including cancer such as bladder cancer and other chronic and sub-chronic effects [7]. Regulation and research into how to limit the concentration of DBPs, has led many to consider alternative disinfectants. Chloramine is one alternative disinfectant that limits the formation of halogenated DBPs, but can also result in increased formation of nitrogenous DBPs. The identification and understanding of DBP precursors can also help limit DBP in a water system. The removal of these precursors before or during water treatment is key to controlling the formation of DBPs. However, it is still possible for precursors to be released by biofilms in the distribution pipe network and react with residual disinfectants to form DBPs. In oligotrophic systems

such as drinking water systems, trace metals and other major ions such as magnesium can influence bacterial metabolic processes and regulations. The quality of water, upstream treatment processes, and piping material used in the water system can influence the concentration of these elements in the water [20]. This in turn affects microbiological physiology [10], which can likely impact the release of DBP precursors from biofilms. Studies have shown that changes to selected trace metals shift DBP formations from precursors produced by bacterial isolates [16]. Further studies are needed to better understand and assess how this can impact public health.

The research outlined in this paper was focused on the influence metals have on the production of DBP precursors from bacterial isolates. The study aimed to determine the independent influence that each trace metal has on the formation of DBPs from individual bacterial isolates. This will be achieved by examining the effects metals have on DBP formation from bacteria-derived precursors using bacterial isolates. Bacterial isolates and biofilms will be exposed to various levels of metal ions, chloraminated, and then analyzed for halogenated DBPs. *Nitrosomonas europaea* is a bacteria species commonly found in pipeline biofilms for this study a strain of the species (ATCC 19718) was purchased and used for this study. The biofilm used will be collected from full-scale distribution systems that use chloramination. The production of DBP precursors will be monitored to determine the influence of trace metals in water distribution systems. The expectation of this study was that the addition of low concentrations of trace metals will increase the formation of DBPs significantly over base oligotrophic levels, as anabolic processes are stimulated. The addition of higher concentrations I expect may have toxicity responses and other responses, but minimal effects on the formation of DBPs.

# CHAPTER II

# LITERATURE REVIEW

## 2.0 INTRODUCTION

The formation of disinfection byproducts (DBPs) was first discovered by Rook in 1974, when he identified chloroform, a form of trihalomethane, in treated water [12,13]. Chemical disinfectants are used due to how susceptible natural water is to contamination by disease, bacteria, and other matter that can be extremely harmful to organisms. After disinfection of drinking water was introduced in the 1900s, deaths due to these diseases virtually ceased to occur. Common chemicals used include bromine, sodium hypochlorite, chlorine, and chloramine.

Further investigations found other types of DBPs including additional forms of trihalomethanes (THMs), haloacetic acids, chlorite, and bromate. Research conducted by Richardson identified more than 600 water disinfection byproducts in chlorinated tap water [12,13]. Presently hundreds of DBPs have been identified and reported. DBPs were deemed a public health issue after the National Cancer Institute published results linking chloroform to cancer in laboratory animals [3]. After this finding, public concern for

DBPs rose along with the need to better understand and regulate their presence in public water.

## 2.1 Water Treatment

Disinfection of public water is instrumental to maintaining public health. Before the use of disinfecting chemicals, contamination of public water by bacteria and waterborne diseases was the cause of death for millions around the world. Today, the most commonly used disinfectant in public water systems is chlorine [23]. Although chlorine was not discovered as a disinfectant until the year 1850, a chemist, Karl Scheele, was recognized for the identification of chlorine in 1774. In the process of disinfecting water, gaseous chlorine ($Cl_2$) or liquid sodium hypochlorite (i.e., bleach, NaOCl) is added to the water. Through either means, a reaction occurs forming hypochlorous acid (HOCl and $OCl^-$). This weak acid (pKa of 7.2) is a strong oxidizing agent in water and reacts with a wide variety of compounds [1]. In addition, as a disinfectant, chlorine is used to react with chemicals that impact the taste and smell of water systems [2]. Any reactions with the hypochlorous acid consume the chlorine and produces DPBs such as trihalomethanes, haloacetic acids, chlorite, and bromate [23].

Chloramination is one alternative method that can be used instead of chlorination. Chloramines are formed based on the reaction between ammonia and chlorine in the water system.

$$NH_3 + HOCl \leftrightarrow NH_2Cl + H_2O$$

This reaction can become more complicated when the chloramine used in the system reacts with more hypochlorous acid to tie up more "free" chlorine in the system. This results in the chloramine becoming further oxidized and can be seen in the following reactions:

$$NH_2Cl + HOCl \leftrightarrow NHCl_2 + H_2O$$

$$NHCl_2 + HOCl \leftrightarrow NCl_3 + H_2O$$

With both chloramination and chlorination, DBP reduction is important to meet regulatory standards. Minimizing the contact time with the disinfectant reduces the formation of DBPs. Water treatment plants also use chemicals to reduce disinfectants in the water distribution system. While both of these techniques minimize and eliminate the production of disinfection byproducts, biofilms present in distribution systems can still contribute to the production of the DBPs [12]. By understanding the reactivity of these biofilms with residual disinfectant, the impact and role they have in our DPB exposure can be determined.

By using regulation requirements that have been established, the reproduction of contamination will be minimized. When a municipal water treatment plant is subjected to bacterial regrowth, both the treatment plant and pipeline are exposed to deterioration of water quality. The control of the amount of biofilm formation and disinfection byproducts due to microbial restrain growth is seen by taking preliminary reactions such as preliminary disinfection and secondary disinfection. This will increase the relationship between natural organic matter (NOM) and the disinfectant [3].

In water systems, biofilms are often located on the interior of water distributing systems. A biofilm is known as a mixture of microbial cells surrounded by a matrix of exopolysaccharides that is secreted by those cells [23]. While possibly consisting of bacteria, yeast, fungi, and protozoa, biofilms are known for their variety of characteristics. Biofilms can form on solid and liquid surfaces, and the thick layer of exopolysaccharides makes them resistant to conventional methods of disinfection. Microorganisms attach to the sub-surfaces which will have an effect on the rate depending on the microbial water quality. It is through the release of biodegradable compounds from the materials which can enhance the suspended growth rate of the planktonic bacteria which leads to the formation of biofilms. Biofilms are seen to improve and be beneficial in industrial waste and the production of water. An analysis of chlorine concentration, temperature, and piping material was taken to see their effects on biofilm growth. Biofilm production is successful when placed in a favorable environment. Biofilm growth produced in observation is examined and documented in detail. By understanding the many factors of water techniques and identification properties, the purpose of each result is identified in the desired objective.

## 2.2 Disinfection Byproducts

### 2.2.1 DBP Formation

Most disinfectants used in water treatment are powerful oxidants, which allow them to kill harmful microorganisms in the water. This can also lead to reactions occurring between the disinfectant and the natural organic matter (NOM) in the water as it is

oxidized. These reactions can result in the formation of DBPs. Natural organic matter is a heterogeneous mixture composed of organic substances such as proteins, amino acids, humic acids, and methoxy-substituted aromatic units. Filtered NOM of 0.1 μm to 0.7μm is considered dissolved organic matter (DOM). DOM comes in a variety of structures including hydrophilic acids, hydrocarbons, and hydrophobic humic substances [16].

### 2.2.2 DBP Concerns and Regulations

Byproducts in water disinfection are linked to increased cancer, liver failure, kidney failure, anemia, and central nervous system problems [20]. The occurrence of DBPs in chlorinated drinking water has become an issue of interest to policymakers, engineers, epidemiologists, biologists, and risk assessors. The National Cancer Institute published findings linking chloroform to cancer in laboratory animals. Further studies found that exposure to DBPs can affect an organism's reproductive and developmental health. One study found that disinfection byproducts are seen to show a caution of health concerns in pregnant women, which can result in either miscarriage or birth defects [6]. With each of these findings, more concern and focus has been put on understanding and managing DBPs. The United States and Europe have each invested significant energy and time in investigating how DBPs can affect human health and how water treatment systems can best adapt to prevent the formation of these DBPs in the water. Regulations were quickly adopted that limited the disinfectants used to treat water and the number of DBPs allowed to exist in public water. In the United States, most of these regulations were created and enforced by the Environmental Protection Agency under The Safe Drinking Water Act and its subsequent amendments. This Act was established with the goal to educate on

health and enlighten on the problems that surrounded water systems. The Safe Drinking Water Act required the Environmental Protection Agency to regulate drinking water, establish health goals, and specify filtration and disinfection requirements. The EPA has established Microbial and Disinfection Byproducts Rules that provide a series of interrelated regulations that address risks from microbial pathogens and DBPs. These rules are broken into two stages. The Environmental Protection Agency developed the Stage I and Stage II DBPRs to control pathogens while minimizing the public health risk from disinfectants and DPBs This has been done by establishing a maximum contaminant level and a maximum residual disinfectant level. By limiting disinfectants such as chlorine and chloromine, which react with naturally occurring materials in water, one can reduce the number of DBPs produced in the water.

| TABLE 2-1<br>MRDLGs, MRDLs, MCLGs, and MCLs for Stage 1 Disinfectants and Disinfection Byproducts Rule | | | |
|---|---|---|---|
| **Disinfectant Residual** | **MRDLG (mg/L)** | **MRDL (mg/L)** | **Compliance Based On** |
| Chlorine | 4 (as $Cl_2$) | 4.0 (as $Cl_2$) | Annual Average |
| Chloramine | 4 (as $Cl_2$) | 4.0 (as $Cl_2$) | Annual Average |
| Chlorine Dioxide | 0.8 (as $ClO_2$) | 0.8 (as $ClO_2$) | Daily Samples |
| **Disinfection Byproducts** | **MCLG (mg/L)** | **MCL (mg/L)** | **Compliance Based On** |
| Total trihalomethanes (TTHM)[1]<br>- Chloroform<br>- Bromodichloromethane<br>- Dibromochloromethane<br>- Bromoform | N/A<br>N/A<br>ZERO<br>0.06<br>ZERO | 0.080 | Annual Average |
| Haloacetic acids (five)(HAA5)[2]<br>- Dichloroacetic acid<br>- Trichloroacetic acid | N/A | 0.060 | Annual Average |
| Chlorite | | 1.0 | Monthly Average |
| Bromate | | 0.010 | Annual Average |

N/A Not applicable because there are individual MCLGs for TTHMs or HAAs

1 Total trihalomethanes in the sum of the concentrations of chloroform, bromodichloromethane, dibromochloromethane, and bromoform

2 Haloacetic acids (five) is the sum of the concentrations of mono-, di-, and trichloroacetic acids and mono- and dibromoacetic acids.

Due to its popularity of use, chlorine disinfection has been recognized as the source chemical of most disinfection byproducts [21]. By switching to the alternative disinfectant, chloramines, the formation of halogenated DBPs is decreased, but the formation of nitrogenous DBPs is increased [22]. While this method decreases the formation of DBPs during water treatment, biofilms and DBPs can still form within the water system distribution network [26]. This occurs when residual disinfectants react with NOM and DBP precursors present in the distribution system resulting in the formation of Biofilms and DBPs. The effects of DBPs in distribution systems include the problems due to excessive growth and colonization of water distribution pipes by bacteria and other organisms. The ability for microbial growth to occur is determined by the

appropriate nutrients that are accessible. Indication of complications can result in taste and odor or corrosion problems found in water systems. This is a result of the drinking water network system being an oligotrophic system. Due to the low accumulation of dissolved salts in the drinking water distribution system, the bacterial metabolic process may be affected if trace metals are introduced to the system [10].

## 2.3 Effect of Metal Ions on DBP Formation

Recent studies have discovered the significance that metal ions play in the formation of DBPs. Metal ions can be introduced into a water supply at various points throughout the water system. The means by which these metal ions are introduced can also affect the concentration level of the metal ions in the water. Hard water taken from groundwater sources can have high concentrations of alkaline earth metal ions. Surface water can experience elevated concentration of ferrous ions ($Fe^{2+}$), ferric ions ($Fe^{3+}$), and cupric ions ($Cu^{2+}$) [16]. A study conducted by Navalon in 2009, investigated how the presence of $Ca^{2+}$, $Mg^{2+}$, and $Cu^{2+}$ impacted the formation of trihalomethanes during chlorination. The study used model compounds such as dicarboxylic acids and citric acids to represent NOM moieties. While no significant change was found in solutions containing dicarboxylic acid and histidine, solutions of citric acid and humic acid showed considerable change [8].

Another study investigated the effects of $Fe^{2+}$, $Fe^{3+}$, $Ca^{2+}$, and $Cu^{2+}$ ions on DPBs formed through the chlorination of NOM. The study found that the effect that these ions had on DBPs was strongly dependent on the model compound used and the nature of the metal

ions. The study also showed that metal ions such as $Ca^{2+}$ and $Cu^{2+}$ can have a greater influence on the formation of DBPs than some NOM present in water samples [8].

# CHAPTER III

# METHODOLOGY

## 3.1 Chemicals and Reagents

The experiments outlined in this paper used reagent grade or higher chemicals. A Pall Water purification system is used to deionize water. NaOCl stock solution (20 mg/L) was prepared by diluting 5% of sodium hypochlorite solution (Allied Signal). The following chemicals were purchased from Sigma Aldrich: sodium chloride, peptone, yeast extract, sodium thiosulfate, and metal salts. The metal salts were as follows: $MgCl_2$, $FeSO_4$, $MnSO_4$, $ZnSO_4$, $CuSO_4$, $Na_2MoO_4$, and $CoSO_4$.

## 3.2 Source Water and Biofilm

Water for this study was collected from after chlorination by the Oklahoma State University Water Treatment Plant (OSU WTP). The OSU WTP is located on the Oklahoma State University Stillwater Campus and provides potable water to the entire campus through 30 miles of underground pipe. The WTP sources water from the Lake Carl Blackwell reservoir located west of the WTP. The disinfection process of the WTP uses 25-100 pounds of liquefied chlorine gas each day [9].

The sample was collected from a hydrant fed by the water treatment plant. Hydrants act as dead-ends in water systems, which can allow water to stagnate at those locations. When water is allowed to stagnate the presence of microbial organisms and poor water quality. When collecting the sample, the initial water released by the hydrant was collected. Each sample was stored in wide-mouth containers at 4°C.

## 3.3 Direct Water Quality Factors

After collecting the source water sample, the quality of water was determined by measuring the pH, temperature, total organic carbon (TOC) content, particle concentration, and microbial concentration. The temperature and pH were determined immediately following the collection of each source sample. These factors play a significant role in biofilm formation in water. The pH of typical drinking water is often adjusted to meet the optimal condition to minimize the decay of disinfectants or prevent corrosion. The pH of the water also plays a key role in how active bacteria and biofilm growth in a system is. The fluctuation in the pH in the water can promote or inhibit nitrification. A pH of 7.0 is more favorable for the growth of nitrifying bacterial biofilms, while it may not be for other bacteria. The pH is a huge factor when determining the vulnerability of water and the impact it can have in an experiment. The pH of the water can also affect how the bacteria interact with the surface of the piping material. When the water source has a pH of 7, the presence of anionic groups on cell surfaces will cause some biofilm-forming bacteria to have a net negative surface charge. This can lead to electrostatic repulsion when the bacteria interact with negatively charged surfaces. If the

pH of the system were to drop, then the electrostatic repulsion between the bacteria and the surface would be reduced and increase the potential for bacterial attachment to occur.

The Hach method was employed to measure the total organic contamination level of the water. Each sample was then autoclaved and filtered through a 0.45 μm regenerated cellulose filter to ensure sterilization of the growth media. The particle concentration and microbial concentration were then measured to ensure the quality of the filtered sample. Once the water quality of each source water sample was determined; the samples were stored in wide-mouth containers at 4$^{o}$C.

## 3.4 Bacterial Selection and Growth

*Nitrosomonas europaea* was the bacteria isolate used for this study and was purchased from ATCC. *Nitrosomonas europaea* is a bacterial strain commonly found in pipeline biofilms of drinking water systems that utilize chlorination and chloramination. *Nitrosomonas europaea* is rod-shaped, gram-negative, and is seen to have aerobic metabolic function [3,6]. *Nitrosomonas europaea* is present in many biofilms, and is found in chloraminated systems [3]. *Nitrosomonas europaea* is ammonia -oxidizing bacteria and performs nitrification in the water distribution system [6]. Bacteria cells use this ammonia-oxidizing bacteria to produce energy. *Nitrosomonas europaea* was grown following the procedure outlined by the ATCC. This procedure is provided in Appendix 2.

The spread plate method was utilized to ensure bacterial growth in the source water samples. This method is used to separate microorganisms contained in a small volume of

water. This is achieved by spreading a small liquid sample containing bacteria over an agar plate. When formations of isolated bacterial colonized the distribution was evenly across the plate. These colonies were then be counted to determine the bacterial concentration of the sampled water.

## 3.5 Sample Preparation

Once the water quality was determined, the source water was used to create individual samples for this study. The samples were made in 50 mL Teflon centrifuge tubes. Biofilm formation occurs at the interface between the aqueous media and the surface of the rigid body. The formation and subsequent biomass rely on the adhesion between the tube wall and the aqueous solution. To account for this, the inner surface of each tube was scared to improve surface contact between the tube and the solution and disrupt the inner flow. Each sample was composed of 25 mL of the source water, 10 mL of organic matter, and 2 -mL of bacteria from the cultured plates. The organic matter consisted of filtered soil water that was made using soil from Lake Carl Blackwell, the WTP's water source. This was added to the solution to provide an additional humic substance. Once each sample was prepared, they were placed in a temperature-controlled room of 30.1°C, on an oscillating bed that continuously rolled each sample. This can be seen in IMAGE 3-1 below. Due to the scarring of the inner wall, each sample was able to experience random mixing and form a viscous sublayer of biofilm. Since the velocity distribution was not symmetrical, drag stress was placed on the surface wall and provided attachment sites for the microbial communities.


**IMAGE 3-1: Tubed reactors on oscillation table**

Each sample solution can be described as a batch culture. Being restricted to this vessel, the growth of the organism will not be indefinite, but instead will be subjected to a growth cycle. This cycle includes a lag phase, exponential phase, stationary phase, and ends with a death phase. The organism's growth cycle can be reflected by a growth curve that depicts the population of the organisms in the vessel over time [6]. To determine the optimal time for biofilm growth in the solution, a preliminary set of samples were created to establish a growth curve for the bacteria. This growth curve is provided in FIGURE 3-1 and shows that the stationary phase of the bacteria's growth cycle was reached at over 17 days. Based on this finding, the samples used in this study were allowed to roll for 14 days to maximize the number of bacterial colonies present in each solution.

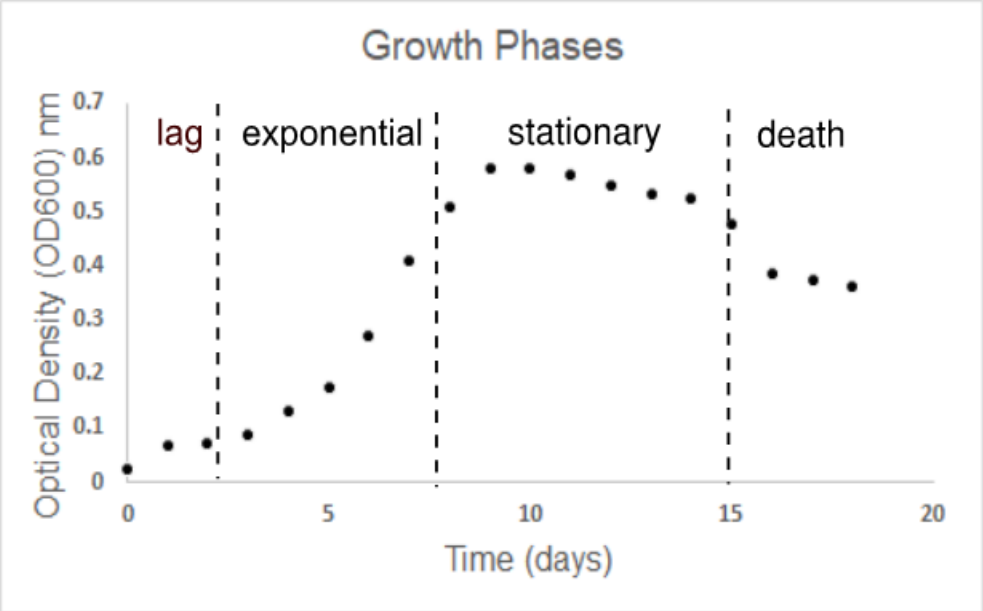


**FIGURE 3-1 : Growth Cycle Graph**

The bacterial growth rate of the sample was then quantified by using estimates provided by measuring the absorbance at 600 nm (OD600).

## 3.6 Addition of Metal Ions

Varying concentrations of metal ions were added to samples as solutions of their corresponding salts. For this study, $MgCl_2$, $FeSO_4$, $MnSO_4$, $ZnSO_4$, $CuSO_4$, $Na_2MoO_4$, and $CoSO_4$ were used and filtered through a sterile 0.2-mm cellulose filter. These concentrations are depicted in TABLE 3-1 and are based on typical ranges found in distribution networks [21]. These metal concentrations are as follows: for the major ion Mg, (0.1, 1, and 10 mM); for the minor elements and/or piping material metals Zn, Fe, and Mn, (0.1, 1, 10, and 100 mM); and for lower trace elements Mo and Co, (0.1,1, and

10 mM). For metals amended as salts with $SO_4^{2-}$, controls will be amended with equal molar amounts of $Na_2SO_4$ to control against effects of microbiologically relevant $SO_4^{-2}$.

After each sample was prepared with its concentration of one of the metal ions, they were returned to the oscillating bed and rolled for an additional 14- days.

**TABLE 3-1**

**Reported Range of Metals in Municipal Tap Water and Drinking Water Standards**

| Metal | Reported tap-water ranges (NRC) | | EPA Drinking Water Standards | Metal Concentrations to be Tested |
|---|---|---|---|---|
| | Low | High | | |
| Mg | 0.3 mg/L | 120 mg/L | None | 2.4, 24, 243 mg/L |
| Fe* | BQL | 1,300 μg/L | 300 μg/L (secondary) | 5.58, 55.8, 558, 5,580 μg/L |
| Mn | BQL | 2,500 μg/L | 50 μg/L (secondary) | 5.49, 54.9, 549, 5,490 μg/L |
| Zn* | BQL | 5,460 μg/L | 5,000 μg/L (secondary) | 6.54, 65.4, 654, 6,540 μg/L |
| Cu* | BQL | 1,400 μg/L | 1,300 μg/L (secondary) | 6.35, 63.5, 635, 6,350 μg/L |
| Mo | BQL | 1,024 μg/L | None | 9.59, 95.9, 959 μg/L |
| Co | BQL | 99 μg/L | None | 5.89, 58.9, 589 μg/L |

*Pipeline materials; BQL = Below quantification limits

## 3.7 Preliminary Biofilm Growth

The growth of biofilm in each sample solution was determined through the use of the OD600, a Bio-Rad Protein Assay, an FTIR scan, and a TOC analysis. The OD600 was used following the same procedure mentioned before. The Bio-Rad Protein Assay (Bio-Rad, USA) was used to determine the concentration of solubilized protein in each solution. Increased protein in water can increase the potential of biofilm formation. To conduct this test, dye reagent solution was made by diluting 1 part dye reagent concentration with 4 parts DDI water. The solution was then filtered through a Whatman #1 filter to remove particulates. Each test used 10 mL of each protein standard and sample solution piped into separate microtiter plate wells. These solutions were mixed with 200 mL of the diluted dye reagent. The samples were then incubated for ten minutes at room temperature and absorbance was measured.

The Fourier-Transform Infrared Spectroscopy (FTIR) method known as Attenuated Total Reflection (ATR) was then conducted to determine the carboxyl groups present in the biofilm solution. An FTIR ATR operates by directing an IR beam onto an optically dense crystal with a high refractive index at a certain angle. The sample solution is held in contact with the crystal. The internal reflectance creates an evanescent wave that extends beyond the surface of the crystal into the sample. An evanescent wave is created through the internal reflectance and extends past the surface of the crystal into the solution to determine the carboxyl functional group of the biofilm. Changes to the internally reflected IR beam are measured and used to generate an IR spectrum [19]. IMAGE 3-2 is a mosaic of the biofilm when exposed to ATR.

**IMAGE 3-2: Depicts Biofilm Growth in Sample**

## 3.8 EPS and IPS Homogenizing

The biofilm growth that is not on the tube wall will depend on the cell division and cell-to-cell communication to reproduce. This includes genes encoding proteins that synthesize intercellular signaling molecules and initiate matrix formation [6]. It is through the production of the cyclic dimeric guanosine monophosphate (c-di-GMP) that the bacteria are able to communicate. Secondary messengers are defined as regulatory

molecules that transmit signals to the first messenger which starts the process of biofilm formation. The initial attachment of biofilm to the surface is demonstrated by the phototrophic and chemolithotrophic processes. Glass beads were placed in the samples and vortexed for 2 minutes to homogenize the sample to ensure the biomass was relatively the same in each triplicate. A TOC analysis was also conducted to determine if any change had occurred in the total organic carbon concentration of each sample solution.

## 3.9 Chloramination Disinfection

Bacterial samples were chloraminated to quantify the levels of chloramine-reactive disinfection byproduct precursors. The samples were chloraminated following the Uniform Formation Condition (UFC) to mimic realistic chloramination conditions in typical U.S. distribution networks [18]. The disinfection process was achieved by adding 0.5 mL of 20mg/L NaOCl stock solution to 10 mL of sample solution. Samples were adjusted to pH of 8 with a phosphate buffer, spiked with 2.5 mg $Cl_2$/L of free chlorine, followed after 30 seconds with 0.53 mg N/L of ammonia chloride to achieve a 4.7:1 $Cl_2$:$NH_3$-N mass ratio [5,15,25]. Chloraminated sample solutions were stored in an incubator in 50 mL serum bottles at 22.7 +- C. After 72 hours, 1 mL of each sample was transferred to sterile 10 mL serum bottles and diluted with 4 mL HPLC grade water from Agilent, USA. Chloraminated samples were quenched with L-asorbic acid and analyzed for DBPs.

## 3.10 DBP Analysis

Calibration standards using Chlorinated Disinfection Byproducts Mix (Lot#TS150901003, SPEXCerti Prep, USA), which contains fifteen typical DBPs in 2000 mg/L were analyzed at 0.2 μg/L, 0.5 μg/L, 1 μg/L and 2 μg/L. Following chloramination, the samples were analyzed for halogenated DBPs following modified EPA Methods 551.1 and 552.3 [4,25,26].

Samples were allowed to equilibrate to room temperature before 10 mL of the solution was taken from each sample. The pH of each sample was then determined to ensure it was between the range of 4.5 and 5.5. The samples were then mixed by inverting each centrifuge tube twice, carefully as to not agitate the sample. Sample extraction was conducted by adding exactly 3.0 mL of MTBE to each solution followed by 10 g of NaCl. Each sample was then capped and consistently well shaken for four minutes. Each centrifuge was then inverted to allow the water and MTBE phases to separate. A portion of each solvent phase was transferred to autosampler vials using disposable Pasteur pipettes. Each autosampler vial was inspected to ensure no water was present after the transfer of solvent.

Analyses of DBPs were carried out with a gas chromatograph (Agilent 7890B) with an electron capture detector (ECD). Samples were extracted in MTBE and taken by a 10 μL silicon injector, and 2 μL of extracted samples injected through the ECD column.

## 3.11 GC ECD

Based on our method modified by USEPA method 551.1, the column used was a DB-1 fused silica capillary column (30 m x 0.32 mm I.D. with 1 μm film thickness). The GC temperature program consisted of an initial temperature of 35 °C for 9 min, ramping to 40 °Cat 5 °C/min and holding for 5 min, ramping to 120 °C at 20 °C/min and holding for 10 minutes, lastly ramping to 150 °C at 10 °C/min and holding for 5 min. Helium was used as the carrier gas for this method.

## 3.12 Metal Analysis

Metal concentration in drinking water was determined by inductive coupled plasma mass spectrometry (ICP-MS). The samples were filtered using a 0.45 μm pore-diam member filter. The filter blank was soaked in 0.5 N HCl or 1 N $HCO_3$ and rinse with deionized water. To reduce the interference from organics to convert metals into particulates. Nitric Acid Digestion was used for sample analysis.

The sample tubes and caps were soaked in 2 N $HNO_3$ for several days and rinsed with metal-free water. Next, 10 mL of acid samples were pipetted into sample tubes. Then the analyte was added for the following sample set. Next with a new pipette tip, 0.5 mL of concentrated HNO3 was added to all samples, blanks, and standards. The samples were digested for 2h at 105°C, then diluted back to the original 10 mL volume with metal-free water.

# CHAPTER IV

# RESULTS

## 4.1 Water Quality

Water sample testing was done to evaluate the amount of total organic carbons and to see the correlation between DBPs and TOC. The distribution of particulate versus dissolved TOC determined the amount of DBPs would increase as TOC increases. The TOC analysis can be seen in Appendix 1.

## 4.2 pH, Protein and OD600

Factors involved in the formation of DBPs, such as pH, can act as conditional indicators of bacteria growth. This resulting from the ammonia oxidizing bacteria within the system. The pH of each sample was tested at 0, 7, and 14 days and were found to decrease with time. This change is reflected in Figure 4-1 through Figure 4-7. These graphs show the change of pH of various concentrations of a metal ion against the change in pH of the control. For each batch culture conditions pH and nitrogen species were shown with the relative abundance to time. In parallel to the pH decrease over time, the total nitrite concentrations increased as seen in Table 4-1. Growth rate and protein increase rate as bacterial activated presenters were also detected in Table 4-1. The growth rate and

protein increase were measured at day zero and day three to determine the exponential growth.

## TABLE 4-1: pH, Protein, and Growth Change Table

| Sample ID | .1 Mg mM | 1 Mg mM | 10 Mg mM | .1 Zn µM | 1 Zn µM | 10 Zn µM | 100 Zn µM | .1 Fe µM | 1 Fe µM | 10 Fe µM | 100 Fe µM |
|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | 6.95 | 7.12 | 6.88 | 6.53 | 6.22 | 6.05 | 6.05 | 6.34 | 6.07 | 6.07 | 6.33 |
| Sample ID | .1 Mn µM | 1 Mn µM | 10 Mn µM | 100 Mn µM | .1 Mo µM | 1 Mo µM | 10 Mo µM | .1 Cu µM | 1 Cu µM | 10 Cu µM | Control |
| pH | 6.56 | 6.96 | 7.03 | 6.33 | 6.8 | 7.05 | 7.03 | 6.08 | 6 | 5.79 | 6.89 |
| Sample ID | .1 Mg mM | 1 Mg mM | 10 Mg mM | .1 Zn µM | 1 Zn µM | 10 Zn µM | 100 Zn µM | .1 Fe µM | 1 Fe µM | 10 Fe µM | 100 Fe µM |
| Protien day 0 | 0.073 | 0.0638 | 0.0438 | 0.0505 | 0.0672 | 0.0732 | 0.1847 | 0.1111 | 0.137 | 0.127 | 0.127 |
| Sample ID | .1 Mn µM | 1 Mn µM | 10 Mn µM | 100 Mn µM | .1 Mo µM | 1 Mo µM | 10 Mo µM | .1 Cu µM | 1 Cu µM | 10 Cu µM | Control |
| Protien day 0 | 0.0461 | 0 | 0 | 0.0308 | 0.1118 | 0.0623 | 0.0523 | 0 | 0.1115 | 0.1309 | 0.1358 |
| Sample ID | .1 Mg mM | 1 Mg mM | 10 Mg mM | .1 Zn µM | 1 Zn µM | 10 Zn µM | 100 Zn µM | .1 Fe µM | 1 Fe µM | 10 Fe µM | 100 Fe µM |
| Protien day 3 | 0.1075 | 0.0983 | 0.082 | 0.0865 | 0.1017 | 0.1077 | 0.2192 | 0.1456 | 0.1715 | 0.1615 | 0.1615 |
| Sample ID | .1 Mn µM | 1 Mn µM | 10 Mn µM | 100 Mn µM | .1 Mo µM | 1 Mo µM | 10 Mo µM | .1 Cu µM | 1 Cu µM | 10 Cu µM | Control |
| Protien day 3 | 0.3911 | 0.345 | 0.345 | 0.2788 | 0.4568 | 0.4073 | 0.3973 | 0.345 | 0.4565 | 0.4759 | 0.172 |
| Sample ID | .1 Mg mM | 1 Mg mM | 10 Mg mM | .1 Zn µM | 1 Zn µM | 10 Zn µM | 100 Zn µM | .1 Fe µM | 1 Fe µM | 10 Fe µM | 100 Fe µM |
| OD600 day0 | 6754463.53 | 624779.683 | 404196.383 | 662529.683 | 645613.05 | 650612.983 | 673113.05 | 651196.4 | 655529.7 | 651613 | 648363.05 |
| Sample ID | .1 Mn µM | 1 Mn µM | 10 Mn µM | 100 Mn µM | .1 Mo µM | 1 Mo µM | 10 Mo µM | .1 Cu µM | 1 Cu µM | 10 Cu µM | Control |
| OD600 day 0 | 665194.1 | 610108.45 | 588027.4 | 559077.7 | 686194.1 | 691944.1 | 582521.8 | 699694.1 | 637441.8 | 566356.2 | 593356.2 |
| Sample ID | .1 Mg mM | 1 Mg mM | 10 Mg mM | .1 Zn µM | 1 Zn µM | 10 Zn µM | 100 Zn µM | .1 Fe µM | 1 Fe µM | 10 Fe µM | 100 Fe µM |
| OD600 day 3 | 6775296.83 | 6435296.83 | 6125296.83 | 6825296.83 | 6651963.5 | 6695296.83 | 6911963.5 | 6695297 | 6738630 | 6695297 | 6681963.5 |
| Sample ID | .1 Mn µM | 1 Mn µM | 10 Mn µM | 100 Mn µM | .1 Mo µM | 1 Mo µM | 10 Mo µM | .1 Cu µM | 1 Cu µM | 10 Cu µM | Control |
| OD600 day 3 | 6860274 | 6288584.5 | 6080274 | 5786610 | 7060274 | 7100274 | 6016895 | 7180274 | 6545251 | 5683562 | 6116895 |

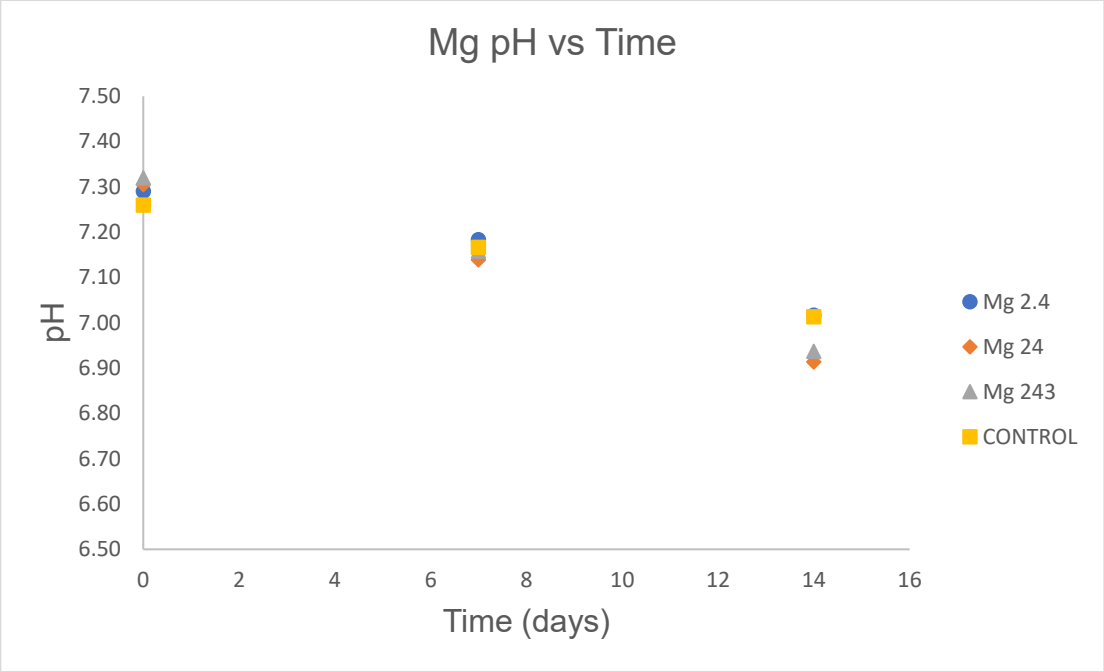


**FIGURE 4-1: pH of Mg vs Time Graph**

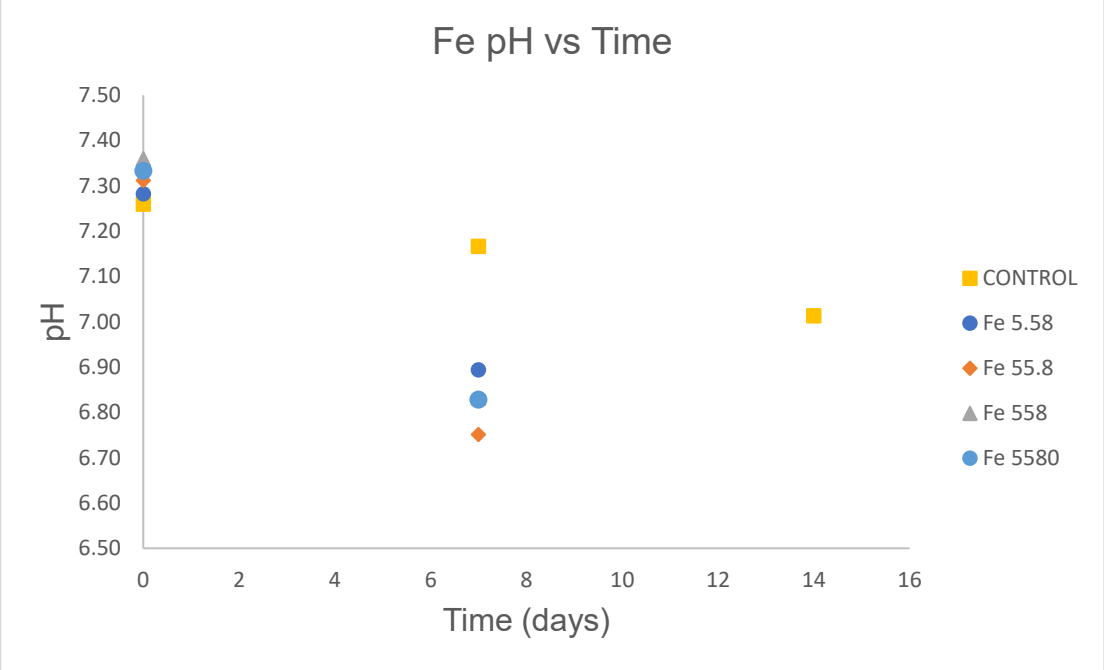


**FIGURE 4-2: pH of Fe vs Time Graph**

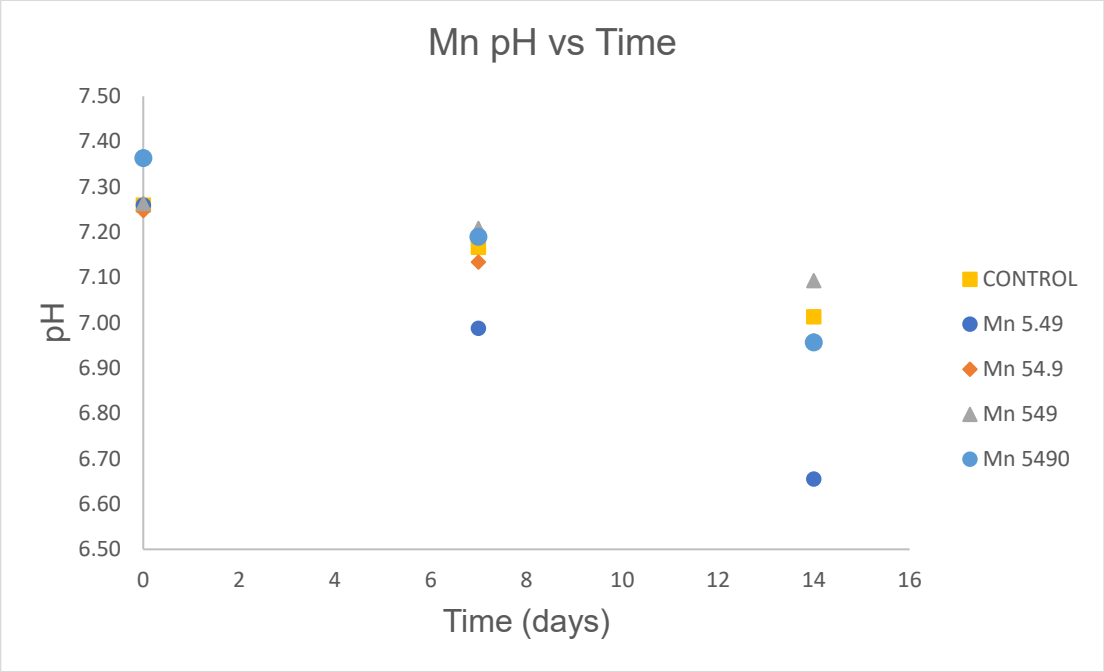


**FIGURE 4-3: pH of Mn vs Time Graph**

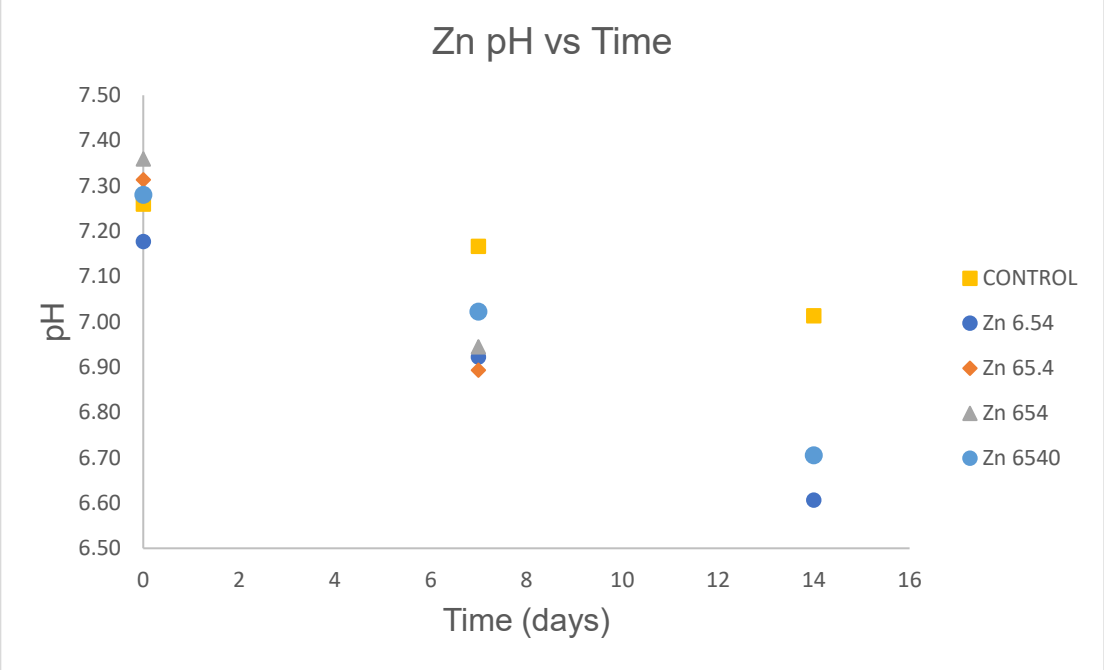


**FIGURE 4-4: pH of Zn vs Time Graph**

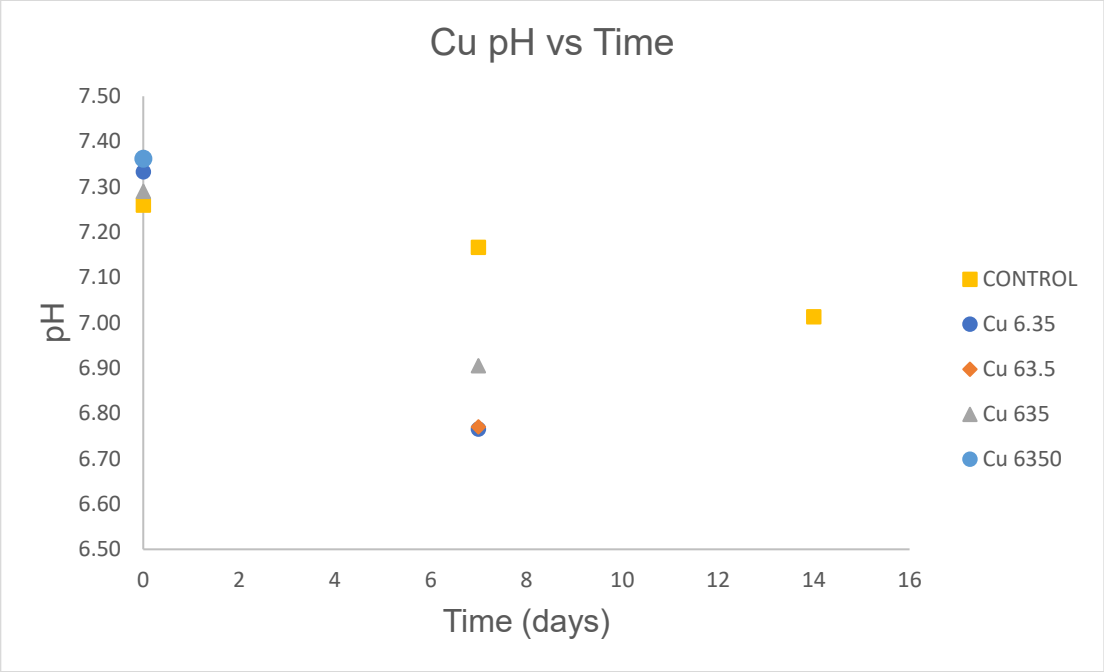


**FIGURE 4-5: pH of Cu vs Time Graph**

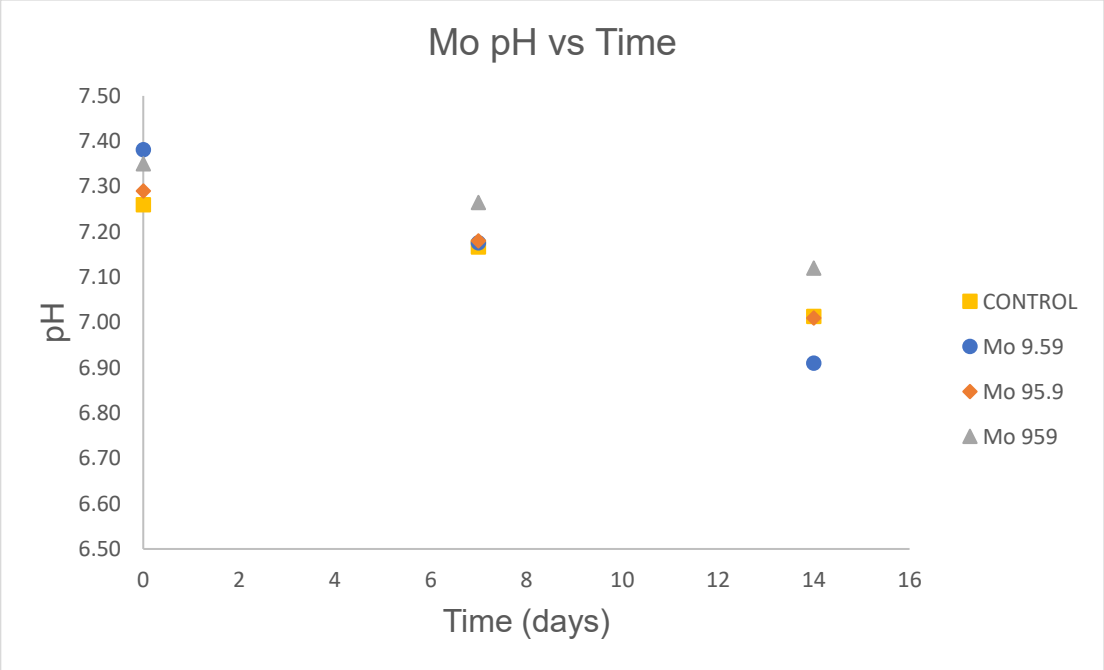


**FIGURE 4-6: pH of Mo vs Time Graph**

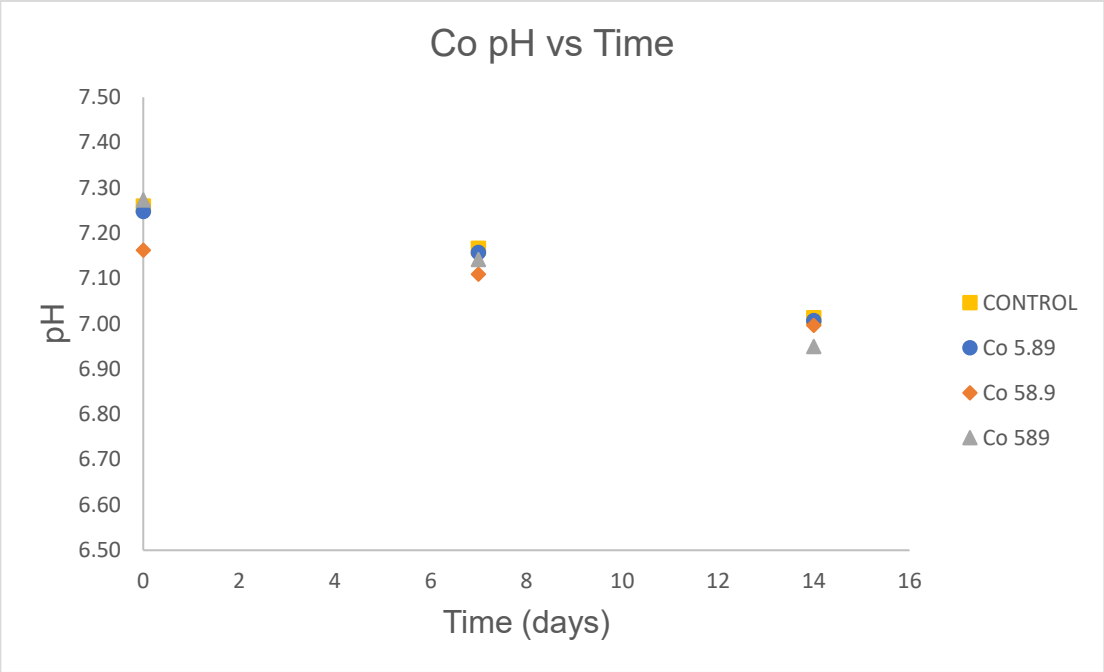


**FIGURE 4-7: pH of Co vs Time Graph**

## 4.3 FTIR ATR analysis

The detection of biofilm on specific markers were characterized using the FTIR-ATR. The FIGURE 4-8 represents the presence of protein and polysaccharide bands. The presence of the primary amide indicates the fouling layer of bacteria. This information correlates with the production of EPS on the attachment sites and changes as the biofilm grow and alter. For this study the biomass was observed with the ATR to indicate biofilm detachment from the surface over the 14-day period.

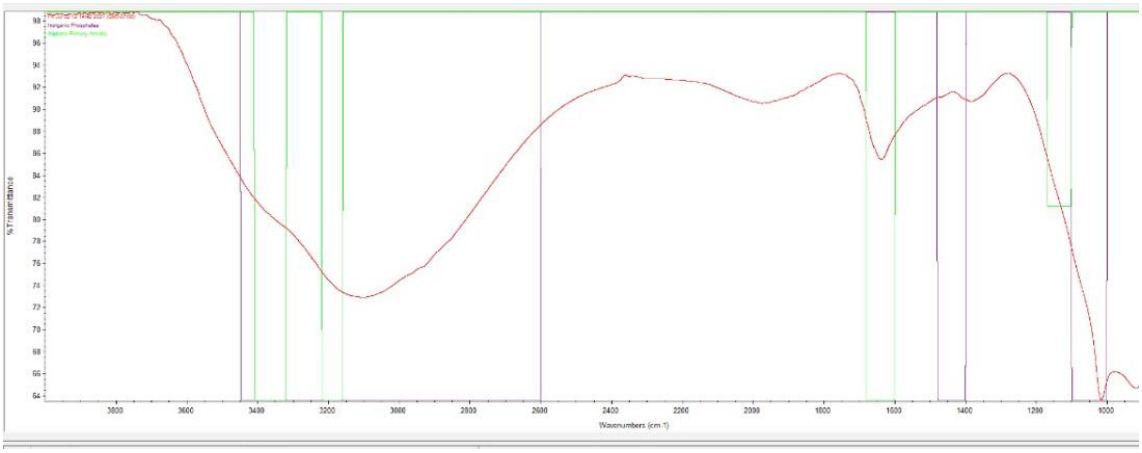

**FIGURE 4-8: FTIR ATR Analysis**

## 4.4 GC-ECD

The standard method was evaluated by plotting calibration curves of the relative peak area for each analyte versus the concentration. Standard calibrations were plotted for concentrations ranging from 0.1 to 2 µg/L. The curves obtained showed linearity for each analyte across the calibration range and can be seen in the following figures.

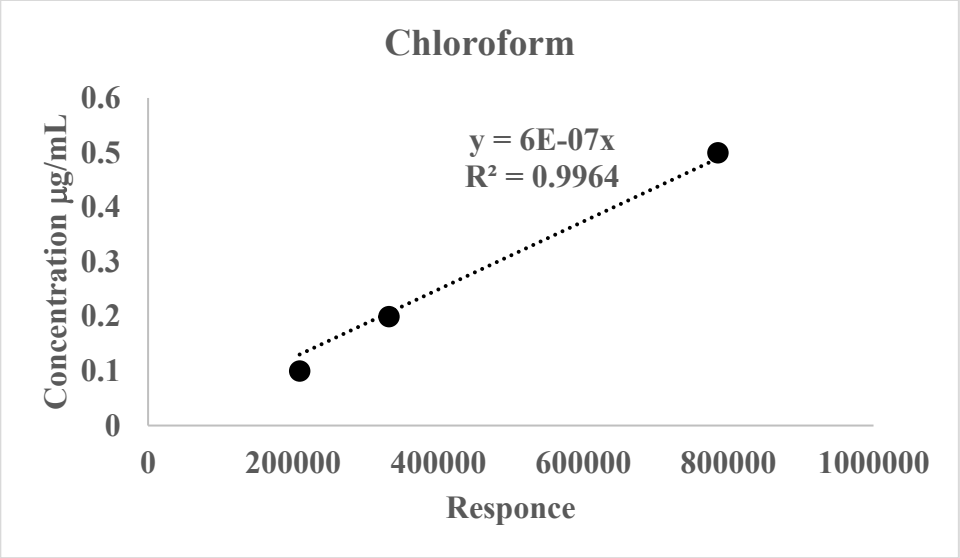


**FIGURE 4-9: Calibration Curve for Chloroform**

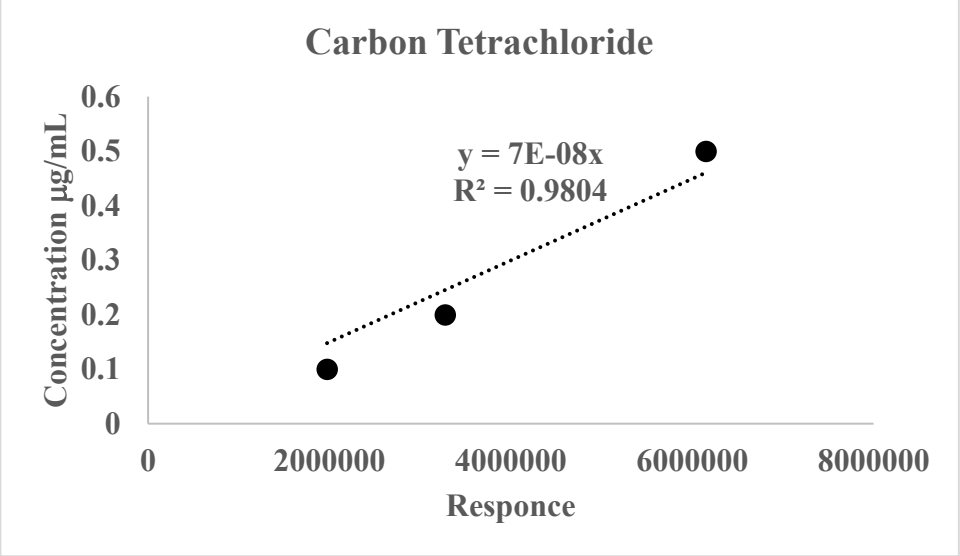


**FIGURE 4-10: Calibration Curve for Carbon Tetrachloride**

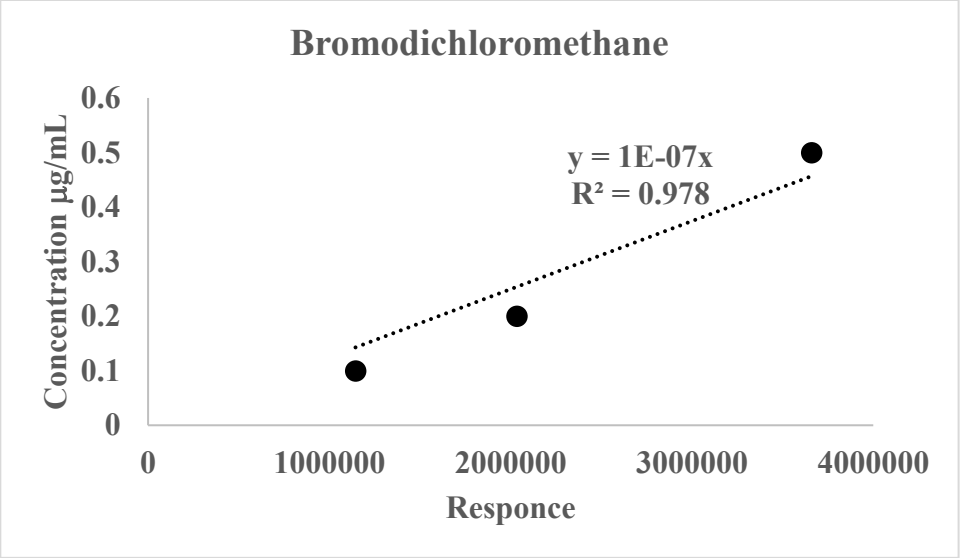


**FIGURE 4-11: Calibration Curve for Bromodichloromethane**

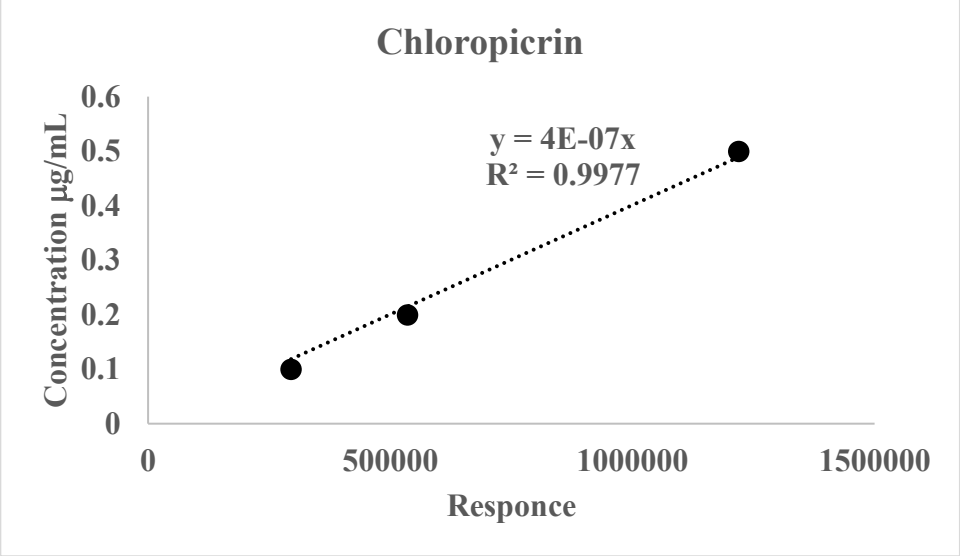


**FIGURE 4-12: Calibration Curve for Chloropicrin**

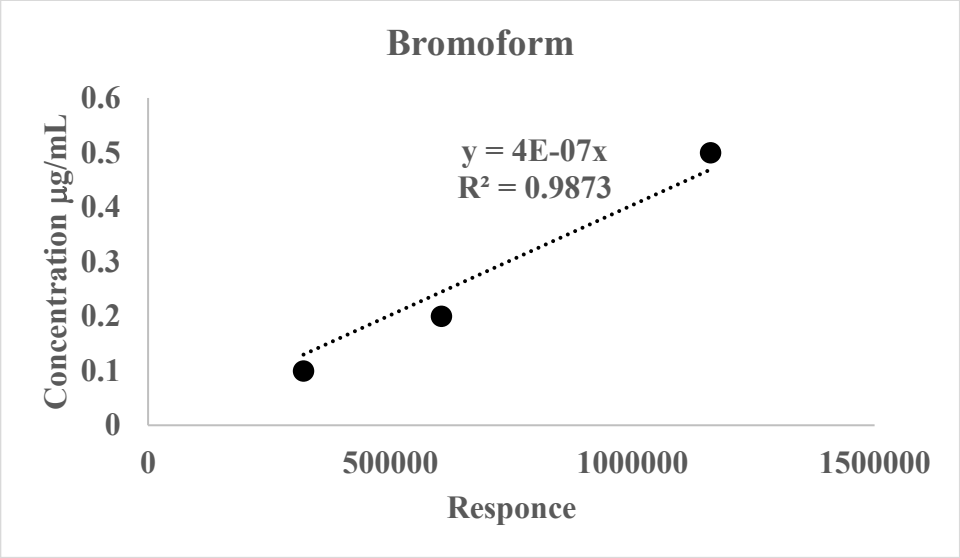


**FIGURE 4-13: Calibration Curve for Bromoform**

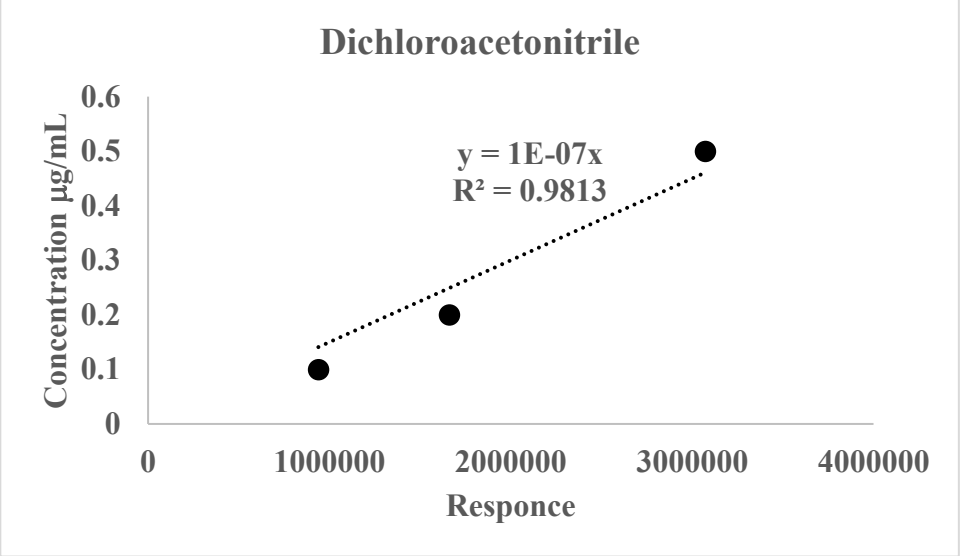


**FIGURE 4-14: Calibration Curve for Dichloroacetonitrile**

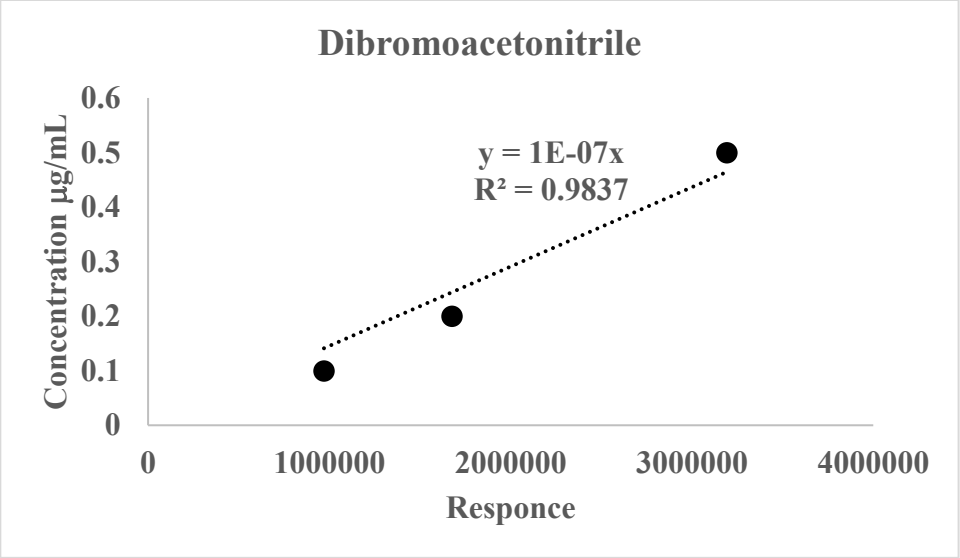


**FIGURE 4-15: Calibration Curve for Dibromoacetonitrile**

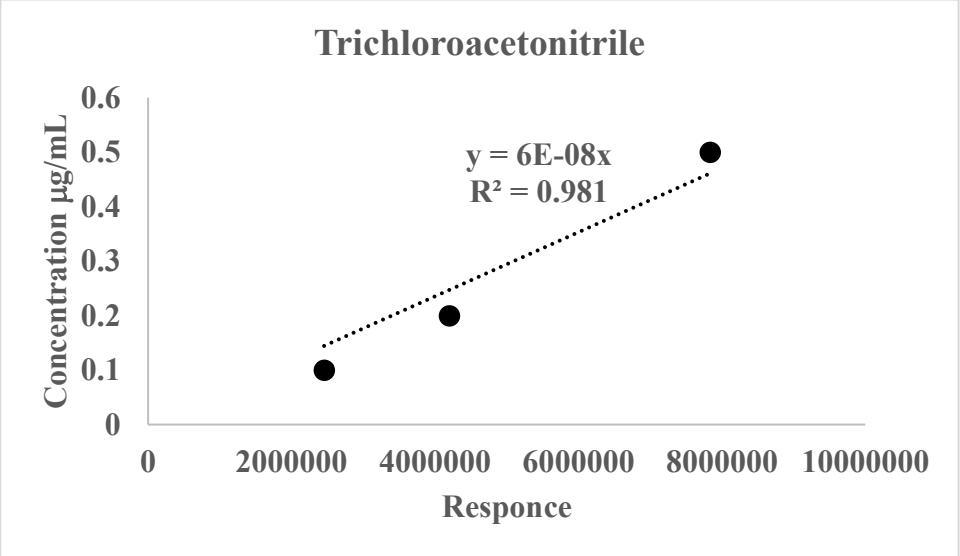


**FIGURE 4-16: Calibration Curve for Trichloroacetonitrile**

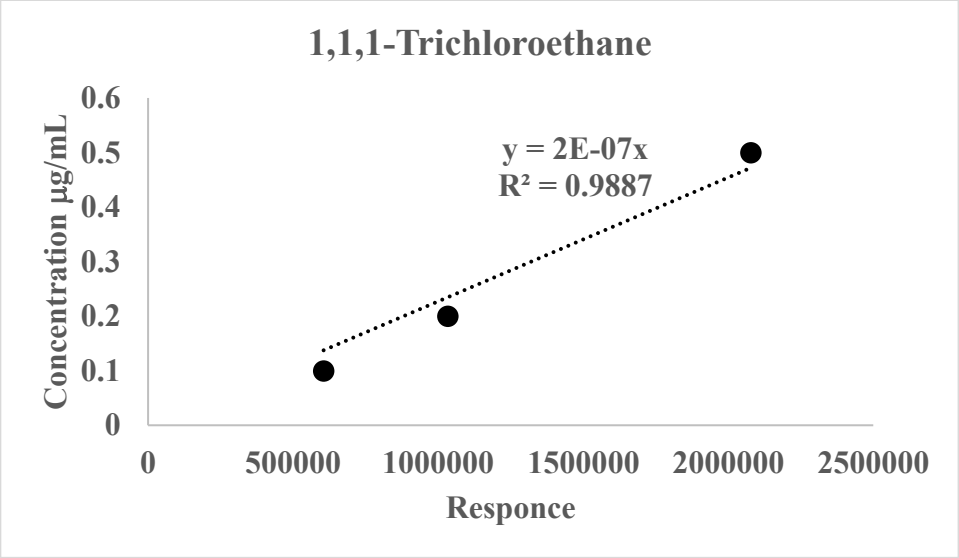


**FIGURE 4-17: Calibration Curve for 1,1,1-Trichloroethane**

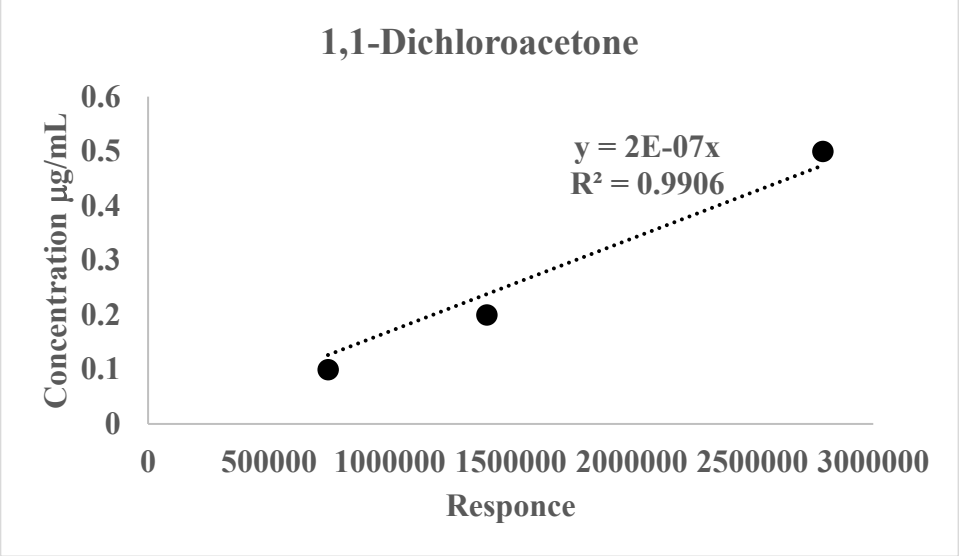


**FIGURE 4-18: Calibration Curve for 1,1-Dichloroacetone**

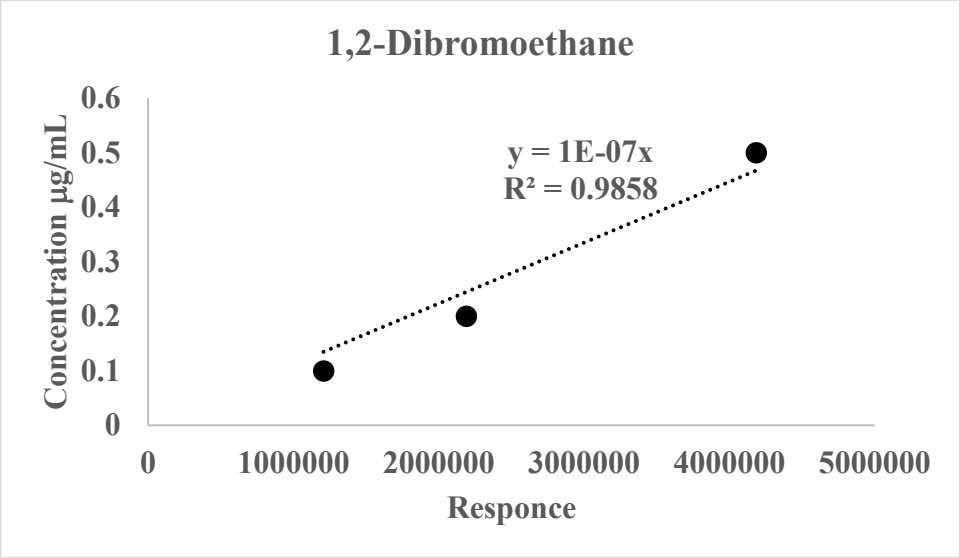


**FIGURE 4-19: Calibration Curve for 1,2-Dibomoethane**

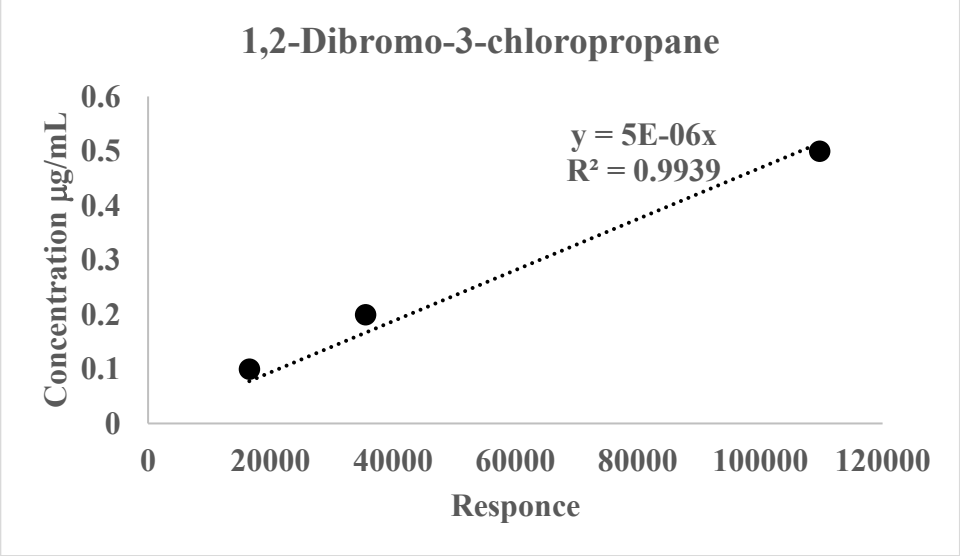


**FIGURE 4-20: Calibration Curve for 1,2-Dibromo-3-chloropropane**

The calculations were conducted using four standard samples at an estimated concentration. The concentrations were corrected by Standard Method 6232 B. using linear regression to determine the corrected concentration.

concentration of extract (ug/ml) = (peak area- intercept)/ gradient

For each compound, the average *RF* and standard deviation using the calibration standards were analyzed. Since the relative standard deviation was greater than 10%, a plot for each calibration curve was made to determine the compound present in each sample. Equation was utilized to calculate the individual response factors *(RFs)* for each standard analyzed. This equation can be utilized because the autosampler will hold the volume of injection constant.

$$RF = \frac{Nominal\ amount\ compond\ extracted, \mu g}{Response\ (peak\ area\ or\ height)}$$

The amount of compound for each standard was calculated using the following equation:

$$W_s = V_s \times C_s$$

Where:

$W_s$ = amount of compound, ug

$V_s$ = volume of standard extracted, L, and

$C_s$ = concentration of prepared standard, ug/L

The analytes from between the tested standard concentrations were observed, indicating that the method was performed properly. The analytes and the tested standards were corrected and observed in the following tables. From the table it can be conclude that the presence of low levels of trace metals increase DBPs.

| TABLE 4-2 : Chloroform Formation | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Concentration | CONTROL | Mg 2.4 | Mg 24 | Mg 243 | Fe 5.58 | Fe 55.8 | Fe 558 | Fe 5580 | Mn 5.49 |
| Sample A | 0.000 | 0.802 | 0.587 | 0.674 | 0.625 | 0.625 | 0.680 | 1.411 | 0.708 |
| Sample B | 0.000 | 0.505 | 0.555 | 0.530 | 0.559 | 0.659 | 1.077 | 1.453 | 0.615 |
| Sample C | 0.000 | 0.546 | 0.670 | 1.295 | --- | 0.708 | 1.411 | 1.111 | --- |
| Average | 0.000 ±0.000 | 0.618 ±0.148 | 0.604 ±0.016 | 0.833 ±0.072 | 0.592 ±0.033 | 0.664 ±0.017 | 1.056 ±0.198 | 1.325 ±0.021 | 0.661 ±0.046 |
| Concentration | Mn 54.9 | Mn 549 | Mn 5490 | Zn 6.54 | Zn 65.4 | Zn 654 | Zn 6540 | Cu 6.35 | Cu 63.5 |
| Sample A | 0.466 | 0.951 | 0.983 | 3.527 | 1.434 | 1.271 | 0.939 | 0.900 | 2.077 |
| Sample B | 1.050 | 0.808 | 0.822 | 2.208 | 2.175 | 1.016 | 0.939 | 2.985 | 2.186 |
| Sample C | --- | 0.783 | 0.580 | 2.093 | 1.512 | --- | --- | 3.745 | --- |
| Average | 0.758 ±0.292 | 0.847 ±0.071 | 0.795 ±0.080 | 2.609 ±0.659 | 1.707 ±0.371 | 1.144 ±0.128 | 0.939 ±0.000 | 2.543 ±1.042 | 2.131 ±0.055 |
| Concentration | Cu 635 | Cu 6350 | Mo 9.59 | Mo 95.9 | Mo 959 | Co 5.89 | Co 58.9 | Co 589 | |
| Sample A | 1.915 | 2.445 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | |
| Sample B | 4.005 | 0.608 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | |
| Sample C | --- | 1.721 | --- | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | |
| Average | 2.960 ±1.045 | 1.591 ±0.918 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | |

| TABLE 4-3 : Carbon Tetrachloride Formation | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Concentration | CONTROL | Mg 2.4 | Mg 24 | Mg 243 | Fe 5.58 | Fe 55.8 | Fe 558 | Fe 5580 | Mn 5.49 |
| Sample A | 0 | 0.01162 | 0.00585 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sample B | 0 | 0.00922 | 0.0175 | 0 | --- | 0 | 0 | 0 | 0 |
| Sample C | 0 | 0.00852 | 0.0102 | 0 | --- | 0 | 0 | 0 | --- |
| Average | 0.000 ±0.000 | 0.010 ±0.001 | 0.011 ±0.006 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 |
| Concentration | Mn 54.9 | Mn 549 | Mn 5490 | Zn 6.54 | Zn 65.4 | Zn 654 | Zn 6540 | Cu 6.35 | Cu 63.5 |
| Sample A | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sample B | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sample C | --- | 0 | 0 | 0 | 0 | --- | --- | 0 | --- |
| Average | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 |
| Concentration | Cu 635 | Cu 6350 | Mo 9.59 | Mo 95.9 | Mo 959 | Co 5.89 | Co 58.9 | Co 589 | |
| Sample A | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Sample B | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Sample C | --- | 0 | --- | 0 | 0 | 0 | 0 | 0 | |
| Average | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | |

| TABLE 4-4 : Bromodichloromethane Formation | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Concentration | CONTROL | Mg 2.4 | Mg 24 | Mg 243 | Fe 5.58 | Fe 55.8 | Fe 558 | Fe 5580 | Mn 5.49 |
| Sample A | 0.0115973 | 0.0687 | 0.05931 | 0.07602 | 0.104453 | 0.0627 | 0.0199 | 0.08555 | 0.100134 |
| Sample B | 0.0115973 | 0.0572 | 0.05088 | 0.08141 | --- | 0.0996 | 0.0159 | 0.0827 | 0.05319 |
| Sample C | 0.0115973 | 0.05421 | 0.06413 | 0.11471 | --- | 0.0581 | 0.06 | 0.06009 | --- |
| Average | 0.012 ±0.000 | 0.060 ±0.006 | 0.058 ±0.004 | 0.091 ±0.003 | 0.104 ±0.000 | 0.073 ±0.018 | 0.032 ±0.002 | 0.076 ±0.001 | 0.077 ±0.023 |
| Concentration | Mn 54.9 | Mn 549 | Mn 5490 | Zn 6.54 | Zn 65.4 | Zn 654 | Zn 6540 | Cu 6.35 | Cu 63.5 |
| Sample A | 0.058513 | 0.11402 | 0.07579 | 0.68395 | 0.283592 | 0.2412 | 0.2121 | 0.07931 | 0.082956 |
| Sample B | 0.0973336 | 0.10746 | 0.06577 | 0.40698 | 0.326684 | 0.1852 | 0.2121 | 0.10613 | 0.089311 |
| Sample C | --- | 0.09941 | 0.07732 | 0.38613 | 0.325193 | --- | --- | 0.15009 | --- |
| Average | 0.078 ±0.019 | 0.107 ±0.003 | 0.073 ±0.005 | 0.492 ±0.138 | 0.312 ±0.022 | 0.213 ±0.028 | 0.212 ±0.000 | 0.112 ±0.013 | 0.086 ±0.003 |
| Concentration | Cu 635 | Cu 6350 | Mo 9.59 | Mo 95.9 | Mo 959 | Co 5.89 | Co 58.9 | Co 589 | |
| Sample A | 0.0332159 | 0.08204 | 0.20235 | 0.09401 | 0.134203 | 0.4514 | 0.3367 | 0.19582 | |
| Sample B | 0.0622091 | 0.04823 | 0.13117 | 0.08151 | 0.134703 | 0.203 | 0.3242 | 0.242 | |
| Sample C | --- | 0.05153 | --- | 0.14284 | 0.141022 | 0.1806 | 0.2501 | 0.24765 | |
| Average | 0.048 ±0.014 | 0.061 ±0.017 | 0.167 ±0.036 | 0.106 ±0.006 | 0.137 ±0.000 | 0.278 ±0.124 | 0.304 ±0.006 | 0.228 ±0.023 | |

| TABLE 4-5 : Chloropicrin Formation | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Concentration | CONTROL | Mg 2.4 | Mg 24 | Mg 243 | Fe 5.58 | Fe 55.8 | Fe 558 | Fe 5580 | Mn 5.49 |
| Sample A | 0.0558047 | 0.88703 | 0.03479 | 0.73011 | 0.065794 | 0.0308 | 0 | 0 | 0 |
| Sample B | 0.0558047 | 0.65647 | 0.30132 | 0.41904 | --- | 0.0453 | 0 | 0 | 0 |
| Sample C | 0.0558047 | 0.64049 | 0.69344 | 2.19856 | --- | 0 | 0 | 0 | --- |
| Average | 0.056 ±0.000 | 0.728 ±0.115 | 0.343 ±0.133 | 1.116 ±0.156 | 0.066 ±0.000 | 0.025 ±0.007 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 |
| Concentration | Mn 54.9 | Mn 549 | Mn 5490 | Zn 6.54 | Zn 65.4 | Zn 654 | Zn 6540 | Cu 6.35 | Cu 63.5 |
| Sample A | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sample B | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sample C | --- | 0 | 0 | 0 | 0 | --- | --- | 0 | --- |
| Average | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 |
| Concentration | Cu 635 | Cu 6350 | Mo 9.59 | Mo 95.9 | Mo 959 | Co 5.89 | Co 58.9 | Co 589 | |
| Sample A | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Sample B | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Sample C | --- | 0 | --- | 0 | 0 | 0 | 0 | 0 | |
| Average | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | |

| TABLE 4-6 : Bromoform Formation | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Concentration | CONTROL | Mg 2.4 | Mg 24 | Mg 243 | Fe 5.58 | Fe 55.8 | Fe 558 | Fe 5580 | Mn 5.49 |
| Sample A | 0.0342677 | 0.04686 | 0.07614 | 0.14558 | 0.19775 | 0.1522 | 0.098 | 0.18884 | 0 |
| Sample B | 0.0342677 | 0.04216 | 0.06666 | 0.06998 | --- | 0.1545 | 0.1544 | 0.09961 | 0.207624 |
| Sample C | 0.0342677 | 0.04532 | 0.05802 | 0.12128 | --- | 0.1136 | 0.2158 | 0.09385 | --- |
| Average | 0.034 ±0.000 | 0.045 ±0.002 | 0.067 ±0.005 | 0.112 ±0.038 | 0.198 ±0.000 | 0.140 ±0.001 | 0.156 ±0.028 | 0.127 ±0.045 | 0.104 ±0.104 |
| Concentration | Mn 54.9 | Mn 549 | Mn 5490 | Zn 6.54 | Zn 65.4 | Zn 654 | Zn 6540 | Cu 6.35 | Cu 63.5 |
| Sample A | 0.1480458 | 0.25254 | 0.23211 | 0.23328 | 0.130809 | 0.1804 | 0.0392 | 0 | 0.1259 |
| Sample B | 0.3721964 | 0.124 | 0.19314 | 0 | 0.1079 | 0.1581 | 0.0392 | 0 | 0.099536 |
| Sample C | --- | 0 | 0.1358 | 0.07436 | 0.101305 | --- | --- | 0 | --- |
| Average | 0.260 ±0.112 | 0.126 ±0.064 | 0.187 ±0.019 | 0.103 ±0.117 | 0.113 ±0.011 | 0.169 ±0.011 | 0.039 ±0.000 | 0.000 ±0.000 | 0.113 ±0.013 |
| Concentration | Cu 635 | Cu 6350 | Mo 9.59 | Mo 95.9 | Mo 959 | Co 5.89 | Co 58.9 | Co 589 | |
| Sample A | 0.0539396 | 0.08905 | 0.1199 | 0.07562 | 0.061347 | 0.0591 | 0.0838 | 0.0078 | |
| Sample B | 0.0643843 | 0.07174 | 0.08724 | 0.05419 | 0.023546 | 0.0668 | 0.0702 | 0 | |
| Sample C | --- | 0.07967 | --- | 0.02858 | 0.077852 | 0.0349 | 0.0476 | 0.04966 | |
| Average | 0.059 ±0.005 | 0.080 ±0.009 | 0.104 ±0.016 | 0.053 ±0.011 | 0.054 ±0.019 | 0.054 ±0.004 | 0.067 ±0.007 | 0.019 ±0.004 | |

| TABLE 4-7 : Dichloroacetonitrile Formation | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Concentration | CONTROL | Mg 2.4 | Mg 24 | Mg 243 | Fe 5.58 | Fe 55.8 | Fe 558 | Fe 5580 | Mn 5.49 |
| Sample A | 0.0106507 | 0 | 0.03039 | 0.05202 | 0.027921 | 0.009 | 0 | 0 | 0 |
| Sample B | 0.0106507 | 0.01997 | 0 | 0 | --- | 0.0446 | 0 | 0 | 0.027508 |
| Sample C | 0.0106507 | 0.02065 | 0.03177 | 0.04091 | --- | 0 | 0.0154 | 0 | --- |
| Average | 0.011 ±0.000 | 0.014 ±0.010 | 0.021 ±0.015 | 0.031 ±0.026 | 0.028 ±0.000 | 0.018 ±0.018 | 0.005 ±0.000 | 0.000 ±0.000 | 0.014 ±0.014 |
| Concentration | Mn 54.9 | Mn 549 | Mn 5490 | Zn 6.54 | Zn 65.4 | Zn 654 | Zn 6540 | Cu 6.35 | Cu 63.5 |
| Sample A | 0 | 0.03668 | 0.03537 | 0.17078 | 0.10797 | 1.4616 | 0.0399 | 0.03053 | 0.031047 |
| Sample B | 0.0588036 | 0.04721 | 0.03151 | 0.13546 | 0.125035 | 0.0941 | 0.0399 | 0.04996 | 0.022937 |
| Sample C | --- | 0.02858 | 0.01503 | 0.14183 | 0.093549 | --- | --- | 0.05871 | --- |
| Average | 0.029 ±0.029 | 0.037 ±0.005 | 0.027 ±0.002 | 0.149 ±0.018 | 0.109 ±0.009 | 0.778 ±0.684 | 0.040 ±0.000 | 0.046 ±0.010 | 0.027 ±0.004 |
| Concentration | Cu 635 | Cu 6350 | Mo 9.59 | Mo 95.9 | Mo 959 | Co 5.89 | Co 58.9 | Co 589 | |
| Sample A | 0.0299381 | 0.0667 | 0.11431 | 0.0313 | 0.042834 | 0.1195 | 0.0725 | 0.0498 | |
| Sample B | 0.0565968 | 0.05758 | 0.04521 | 0.03359 | 0.049913 | 0.0591 | 0.0573 | 0.07498 | |
| Sample C | --- | 0.0351 | --- | 0.02464 | 0.039897 | 0.0511 | 0.0643 | 0.07283 | |
| Average | 0.043 ±0.013 | 0.053 ±0.005 | 0.080 ±0.035 | 0.030 ±0.001 | 0.044 ±0.004 | 0.077 ±0.030 | 0.065 ±0.008 | 0.066 ±0.013 | |

| TABLE 4-8 : Dibromoacetonitrile Formation | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Concentration | CONTROL | Mg 2.4 | Mg 24 | Mg 243 | Fe 5.58 | Fe 55.8 | Fe 558 | Fe 5580 | Mn 5.49 |
| Sample A | 0.0268054 | 0.1653 | 0.13977 | 0.19802 | 0.123209 | 0.0198 | 0.1049 | 0.08033 | 0.348466 |
| Sample B | 0.0268054 | 0.16648 | 0.16969 | 0.11317 | --- | 0.0173 | 0.0984 | 0.07801 | 0.456443 |
| Sample C | 0.0268054 | 0.15641 | 0.22342 | 0.3478 | --- | 0.0221 | 0.1077 | 0.07225 | --- |
| Average | 0.0268054 | 0.16273 | 0.17763 | 0.21966 | 0.123209 | 0.0197 | 0.1037 | 0.07686 | 0.402454 |
| | ±0.000 | ±0.004 | ±0.035 | ±0.097 | ±0.000 | ±0.002 | ±0.004 | ±0.003 | ±0.054 |
| Concentration | Mn 54.9 | Mn 549 | Mn 5490 | Zn 6.54 | Zn 65.4 | Zn 654 | Zn 6540 | Cu 6.35 | Cu 63.5 |
| Sample A | 0.3430045 | 0.07798 | 0.27862 | 0.6745 | 0.217587 | 0.1676 | 0.0717 | 0.01018 | 0 |
| Sample B | 0.1153473 | 0.11074 | 0.27042 | 0.15415 | 0.091396 | 0.0785 | 0.0717 | 0.03067 | 0.020097 |
| Sample C | --- | 0.03952 | 0.25817 | 0.14648 | 0.074323 | --- | --- | 0.0388 | --- |
| Average | 0.2291759 | 0.07608 | 0.26907 | 0.32504 | 0.127769 | 0.123 | 0.0717 | 0.02655 | 0.010049 |
| | ±0.114 | ±0.029 | ±0.008 | ±0.247 | ±0.064 | ±0.045 | ±0.000 | ±0.012 | ±0.010 |
| Concentration | Cu 635 | Cu 6350 | Mo 9.59 | Mo 95.9 | Mo 959 | Co 5.89 | Co 58.9 | Co 589 | |
| Sample A | 0.0103919 | 0.00837 | 0.05582 | 0.03519 | 0.006058 | 0.0521 | 0.0567 | 0.04611 | |
| Sample B | 0.0038305 | 0.01405 | 0.00677 | 0.02373 | 0.030936 | 0.0867 | 0.0653 | 0.02073 | |
| Sample C | --- | 0.01725 | --- | 0.02217 | 0.008048 | 0.0106 | 0.0674 | 0.03423 | |
| Average | 0.007 | 0.013 | 0.031 | 0.027 | 0.015 | 0.050 | 0.063 | 0.034 | |
| | ±0.003 | ±0.003 | ±0.025 | ±0.006 | ±0.012 | ±0.017 | ±0.004 | ±0.013 | |

| TABLE 4-9 : Trichloroacetonitrile Formation | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Concentration | CONTROL | Mg 2.4 | Mg 24 | Mg 243 | Fe 5.58 | Fe 55.8 | Fe 558 | Fe 5580 | Mn 5.49 |
| Sample A | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sample B | 0 | 0 | 0 | 0 | --- | 0 | 0 | 0 | 0 |
| Sample C | 0 | 0 | 0 | 0 | --- | 0 | 0 | 0 | --- |
| Average | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| | ±0.000 | ±0.000 | ±0.000 | ±0.000 | ±0.000 | ±0.000 | ±0.000 | ±0.000 | ±0.000 |
| Concentration | Mn 54.9 | Mn 549 | Mn 5490 | Zn 6.54 | Zn 65.4 | Zn 654 | Zn 6540 | Cu 6.35 | Cu 63.5 |
| Sample A | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sample B | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sample C | --- | 0 | 0 | 0 | 0 | --- | --- | 0 | --- |
| Average | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| | ±0.000 | ±0.000 | ±0.000 | ±0.000 | ±0.000 | ±0.000 | ±0.000 | ±0.000 | ±0.000 |
| Concentration | Cu 635 | Cu 6350 | Mo 9.59 | Mo 95.9 | Mo 959 | Co 5.89 | Co 58.9 | Co 589 | |
| Sample A | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Sample B | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Sample C | --- | 0 | --- | 0 | 0 | 0 | 0 | 0 | |
| Average | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | |
| | ±0.000 | ±0.000 | ±0.000 | ±0.000 | ±0.000 | ±0.000 | ±0.000 | ±0.000 | |

| TABLE 4-10 : 1,1,1-Trichloroethane Formation | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Concentration | CONTROL | Mg 2.4 | Mg 24 | Mg 243 | Fe 5.58 | Fe 55.8 | Fe 558 | Fe 5580 | Mn 5.49 |
| Sample A | 0.000 | 0.426 | 0.457 | 0.351 | 0.067 | 0.429 | 0.837 | 0.818 | 0.503 |
| Sample B | 0.000 | 0.365 | 0.470 | 0.394 | --- | 0.529 | 0.373 | 0.194 | 0.408 |
| Sample C | 0.000 | 0.427 | 0.474 | 0.698 | --- | 0.732 | 0.907 | 0.039 | --- |
| Average | 0.000 ±0.000 | 0.406 ±0.030 | 0.467 ±0.007 | 0.481 ±0.021 | 0.067 ±0.000 | 0.563 ±0.050 | 0.706 ±0.232 | 0.350 ±0.312 | 0.455 ±0.047 |
| Concentration | Mn 54.9 | Mn 549 | Mn 5490 | Zn 6.54 | Zn 65.4 | Zn 654 | Zn 6540 | Cu 6.35 | Cu 63.5 |
| Sample A | 0.315 | 0.537 | 0.394 | 1.851 | 0.640 | 0.424 | 0.369 | 0.000 | 0.000 |
| Sample B | 0.523 | 0.517 | 0.400 | 1.290 | 0.819 | 0.330 | 0.369 | 0.000 | 0.000 |
| Sample C | --- | 0.468 | 0.387 | 1.093 | 0.666 | --- | --- | 0.000 | --- |
| Average | 0.419 ±0.104 | 0.507 ±0.010 | 0.394 ±0.003 | 1.412 ±0.280 | 0.708 ±0.090 | 0.377 ±0.047 | 0.369 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 |
| Concentration | Cu 635 | Cu 6350 | Mo 9.59 | Mo 95.9 | Mo 959 | Co 5.89 | Co 58.9 | Co 589 | |
| Sample A | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | |
| Sample B | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | |
| Sample C | --- | 0.000 | --- | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | |
| Average | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | |

| TABLE 4-11 : 1,1-Dichloroacetone Formation | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Concentration | CONTROL | Mg 2.4 | Mg 24 | Mg 243 | Fe 5.58 | Fe 55.8 | Fe 558 | Fe 5580 | Mn 5.49 |
| Sample A | 0.0354367 | 0.20638 | 0.11219 | 0.13216 | 0.125401 | 0.2863 | 0.7547 | 0.71435 | 0.281371 |
| Sample B | 0.0354367 | 0.10116 | 0.48141 | 0.19797 | --- | 0.4396 | 0.7915 | 0.58347 | 0.149351 |
| Sample C | 0.0354367 | 0.15124 | 0.17316 | 0.30893 | --- | 0.6639 | 1.2973 | 0.43096 | --- |
| Average | 0.035 ±0.000 | 0.153 ±0.053 | 0.256 ±0.185 | 0.213 ±0.033 | 0.125 ±0.000 | 0.463 ±0.077 | 0.948 ±0.018 | 0.576 ±0.065 | 0.215 ±0.066 |
| Concentration | Mn 54.9 | Mn 549 | Mn 5490 | Zn 6.54 | Zn 65.4 | Zn 654 | Zn 6540 | Cu 6.35 | Cu 63.5 |
| Sample A | 0.145289 | 0.20098 | 0.19167 | 0.5795 | 0.326961 | 0.2353 | 0.1828 | 0.35738 | 0.537266 |
| Sample B | 0.2121964 | 0.14661 | 0.1815 | 0.34166 | 0.248469 | 0.1965 | 0.1828 | 0.45492 | 0.43752 |
| Sample C | --- | 0.13351 | 0.11919 | 0.33644 | 0.245226 | --- | --- | 0.9008 | --- |
| Average | 0.179 ±0.033 | 0.160 ±0.027 | 0.164 ±0.005 | 0.419 ±0.119 | 0.274 ±0.039 | 0.216 ±0.019 | 0.183 ±0.000 | 0.571 ±0.049 | 0.487 ±0.050 |
| Concentration | Cu 635 | Cu 6350 | Mo 9.59 | Mo 95.9 | Mo 959 | Co 5.89 | Co 58.9 | Co 589 | |
| Sample A | 0.2851337 | 0.94891 | 0.08272 | 0.06978 | 0.062311 | 0.1761 | 0.2103 | 0.11786 | |
| Sample B | 0.7042462 | 0.38175 | 0.05908 | 0.05198 | 0.087467 | 0.1093 | 0.2062 | 0.15755 | |
| Sample C | --- | 0.61446 | --- | 0.22951 | 0.821517 | 0.1108 | 0.1299 | 0.11884 | |
| Average | 0.495 ±0.210 | 0.648 ±0.284 | 0.071 ±0.012 | 0.117 ±0.009 | 0.324 ±0.013 | 0.132 ±0.033 | 0.182 ±0.002 | 0.131 ±0.020 | |

| TABLE 4-12 : 1,2-Dibromoethane Formation | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Concentration | CONTROL | Mg 2.4 | Mg 24 | Mg 243 | Fe 5.58 | Fe 55.8 | Fe 558 | Fe 5580 | Mn 5.49 |
| Sample A | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sample B | 0 | 0 | 0 | 0 | --- | 0 | 0 | 0 | 0 |
| Sample C | 0 | 0 | 0 | 0 | --- | 0 | 0 | 0 | --- |
| Average | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 |
| Concentration | Mn 54.9 | Mn 549 | Mn 5490 | Zn 6.54 | Zn 65.4 | Zn 654 | Zn 6540 | Cu 6.35 | Cu 63.5 |
| Sample A | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sample B | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sample C | --- | 0 | 0 | 0 | 0 | --- | --- | 0 | --- |
| Average | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 |
| Concentration | Cu 635 | Cu 6350 | Mo 9.59 | Mo 95.9 | Mo 959 | Co 5.89 | Co 58.9 | Co 589 | |
| Sample A | 0 | 0 | 0 | 0 | 0 | 0.1328 | 0.1251 | 0.10258 | |
| Sample B | 0 | 0 | 0 | 0 | 0 | 0.0179 | 0.0692 | 0.08626 | |
| Sample C | --- | 0 | --- | 0 | 0 | 0.067 | 0.0829 | 0.14844 | |
| Average | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.000 ±0.000 | 0.073 ±0.057 | 0.092 ±0.028 | 0.112 ±0.008 | |

| TABLE 4-13 : 1,2-Dibromo-3-chloropropane Formation | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Concentration | CONTROL | Mg 2.4 | Mg 24 | Mg 243 | Fe 5.58 | Fe 55.8 | Fe 558 | Fe 5580 | Mn 5.49 |
| Sample A | 0.358125 | 0.91234 | 0.85926 | 0.86869 | 2.460567 | 0.4867 | 0 | 1.07877 | 0.716582 |
| Sample B | 0.358125 | 0.91233 | 1.06126 | 0.50104 | --- | 1.6517 | 0.4396 | 1.11586 | 1.739159 |
| Sample C | 0.358125 | 0.89168 | 1.19211 | 1.66446 | --- | 0.405 | 1.4031 | 0.69069 | --- |
| Average | 0.358125 ±0.000 | 0.90545 ±0.010 | 1.03754 ±0.137 | 1.0114 ±0.486 | 2.460567 ±0.000 | 0.8478 ±0.569 | 0.6142 ±0.586 | 0.96177 ±0.192 | 1.227871 ±0.511 |
| Concentration | Mn 54.9 | Mn 549 | Mn 5490 | Zn 6.54 | Zn 65.4 | Zn 654 | Zn 6540 | Cu 6.35 | Cu 63.5 |
| Sample A | 1.262115 | 2.21353 | 1.95855 | 1.27922 | 0.570672 | 1.7091 | 0.3999 | 0.49142 | 0.790596 |
| Sample B | 3.6915818 | 1.6375 | 1.76889 | 0.97428 | 0.57048 | 1.3197 | 0.3999 | 0.57594 | 0.676775 |
| Sample C | --- | 1.81753 | 1.61599 | 0.70084 | 34.42691 | --- | --- | 0.38092 | --- |
| Average | 2.4768484 ±1.215 | 1.88952 ±0.241 | 1.78114 ±0.140 | 0.98478 ±0.236 | 11.85602 ±15.960 | 1.5144 ±0.195 | 0.3999 ±0.000 | 0.48276 ±0.080 | 0.733686 ±0.057 |
| Concentration | Cu 635 | Cu 6350 | Mo 9.59 | Mo 95.9 | Mo 959 | Co 5.89 | Co 58.9 | Co 589 | |
| Sample A | 0.4465079 | 0.64168 | 0.59128 | 0.85046 | 0.143069 | 0.3519 | 0.2185 | 0.26159 | |
| Sample B | 0.4795962 | 0.52494 | 0.77932 | 0.705 | 0.340197 | 0.3977 | 0.0754 | 1.21371 | |
| Sample C | --- | 0.39854 | --- | 0.71107 | 1.637445 | 0.6371 | 0.367 | 0.90782 | |
| Average | 0.463 ±0.017 | 0.522 ±0.058 | 0.685 ±0.094 | 0.756 ±0.073 | 0.707 ±0.099 | 0.462 ±0.023 | 0.220 ±0.072 | 0.794 ±0.476 | |

The analysis targeted 12 analytes consisting of DBPs and chlorinated solvents, each found commonly in water systems. Of these, only 8 were found present in the control samples. These included dichloroacetonitrile, bromodichloromethane, trichloroethene,

1,1-dichloroacetone, chloropicrin, bromoform, dibromoacetonitrile, and 1,2-dibromo-3-chloropropane. These concentrations were used to establish a baseline for comparison to determine how the addition of metal ions would affect DBP formation. Chloroform while not found present in the control samples, was found in samples containing Mg, Fe, Mn, Zn, and Cu. While Zn showed the greatest concentration of chlorine in the sample, Mg and Fe reflected a more consistent correlation between the increase in metal and the increase in chlorine present. 1,1,1-Trichloroethane was found present in samples containing Mg, Fe, Mn, and Zn, with the presence of Zn resulting in the greatest concentration of it. Carbon tetrachloride was only found present in samples containing 2.4ug of Mg and 24ug of Mg. When the presence of Mg was increased to 243ug, carbon tetrachloride no longer formed within the sample water. Trichloroacetonitrile was not found present in any sample. Dichloroacetonitrile was found present in every sample, but at concentrations greater and weaker than that of the control average. Samples containing Zn, Cu, Mo, and Co almost all resulted in concentrations greater than that of the control samples. Samples containing Cu at 63.5ug resulted in a decrease in the formation of dichloroacetonitrile. The presence of Mg, Fe, and Mn in samples decreased the formation of dichloroacetonitrile, with samples containing Fe at 5580 ug preventing its formation. Bromodichloromethane and 1,1-dichloroacetone were both found present in each sample, with almost all samples showing an increased concentration when compared to the control. While chloropicrin was found present in the control, it was only found in samples containing Mg and low levels of Fe. Only samples containing Co showed the presence of 1,2-dibromethane. Bromoform was found in samples containing each metal, typically at higher concentrations than that of the control. Dibromoacetonitrile and 1,2-dibromo-3-

chloropropane were found in each sample. While their concentrations fluctuated, they were found to have higher concentrations when Mg, Fe, Mn, and Zn were present in the solution. When Cu, Mo, and Co were present, the concentration of dibromoacetonitrile and 1,2-dibromo-3-chloropropane were found lower than the average concentration of the controls.

From these results, one can clearly see that the inclusion of metal in water has a direct impact on the formation of biofilms. When compared against the control, the addition of each metal ion resulted in clear change to the DBP yielding. Samples showed either increased or decreased production levels when compared against the control. Aside from Mo, the addition of each metal to the sample solution resulted in the formation of DBPs not found in the control sample. These changes were also reflected by the DBP spread ratios that showed variations based on changes to the metal ion concentration added to each sample. These results indicate that metals have the potential to influence DBP formation. The ICP-MS was used to provided quality control on the additional of metal ion in the drinking water samples. Samples were analyzed periodically on the ICP-MS to verify change in concentrations. This is because the molecular weight of the disinfection byproduct precursors impact how dissolved the organic material in the samples may be.

# CHAPTER V

# CONCLUSION

## 5.1 Summary

The goal of this thesis was to determine the independent influence that trace metals have on the formation of disinfection byproducts from individual bacterial isolates. The study used 72 samples to measure how the change between metal ions or in their concentration would affect the DBP formation. These samples were produced using water collected from a fire hydrant fed by the Oklahoma State University Water Treatment Plant. The metals used in the study included the following: $MgCl_2$, $FeSO_4$, $MnSO_4$, $ZnSO_4$, $CuSO_4$, $Na_2MoO_4$, and $CoSO_4$. The tests and methods described in Chapter 3 were used to determine the effects of each metal at given concentrations.

The results of this study showed that the addition of trace metals in water can both increase and decrease the formation of DBPs. Based on the data outlined in Chapter 4 of this paper, the following conclusions can be made:

- The increase of protein and EPS will alter the characteristic of biofilm over the first 14-days.

- The addition of metals to water solution will directly impact the potential for DBP growth in the solution
- Metals such as Mg, Fe, Mn, Zn, Cu, and Co resulted in the formation of DBPs that were not found present in the control solution. These DBPs included Chloroform, 1,1,1-Trichloroethane, Carbon Tetrachloride, and 1,2-dibromoethane.
- The addition of metals in the sample solution caused some DBPs found in the control samples not to form.
- The formation of Chloropicrin was found in the control samples after 14 days of being rolled. Aside from Mg, this formation of Chloropicrin was mitigated by every tested metal.
- Trichloroacetonitrile was unable to form in any sample tested.

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# APPENDIX

## A.1 ADDITIONAL FIGURES AND TABLES

### A.1.1 pH Data

#### A.1.1.1 pH Table

| Bottle ID | Sample Description | pH[1] | pH[2] | pH[3] | pH[4] |
|---|---|---|---|---|---|
| 1 | Mg 2.4 A | 7.29 | 7.17 | 6.98 | 7.98 |
| 2 | Mg 2.4 B | 7.28 | 7.15 | 6.95 | 7.98 |
| 3 | Mg 2.4 C | 7.30 | 7.24 | 7.12 | 8.12 |
| | Average: | 7.29 | 7.18 | 7.02 | 8.03 |
| 4 | Mg 24 A | 7.30 | 7.15 | 6.94 | 7.94 |
| 5 | Mg 24 B | 7.31 | 7.12 | 6.88 | 7.88 |
| 6 | Mg 24 C | 7.31 | 7.15 | 6.92 | 7.92 |
| | Average: | 7.31 | 7.14 | 6.91 | 7.91 |
| 7 | Mg 243 A | 7.32 | 7.14 | 6.91 | 7.91 |
| 8 | Mg 243 B | 7.32 | 7.22 | 7.05 | 8.05 |
| 9 | Mg 243 C | 7.33 | 7.12 | 6.85 | 7.85 |
| | Average: | 7.32 | 7.16 | 6.94 | 7.94 |
| 10 | Fe 5.58 A | 7.23 | 6.82 | 6.34 | 7.34 |
| 11 | Fe 5.58 B | 7.34 | 6.97 | 6.55 | 7.55 |
| | Average: | 7.28 | 6.89 | 6.45 | 7.45 |
| 12 | Fe 55.8 A | 7.24 | 6.69 | 6.07 | 7.07 |
| 13 | Fe 55.8 B | 7.35 | 6.78 | 6.16 | 7.16 |
| 14 | Fe 55.8 C | 7.35 | 6.79 | 6.16 | 7.16 |
| | Average: | 7.31 | 6.75 | 6.13 | 7.13 |
| 15 | Fe 558 A | 7.36 | 6.74 | 6.07 | 7.07 |
| 16 | Fe 558 B | 7.36 | 6.93 | 6.43 | 7 |
| 17 | Fe 558 C | 7.37 | 6.83 | 6.23 | 7 |

| | | | | | |
|---|---|---|---|---|---|
| | Average: | 7.36 | 6.83 | 6.24 | 7.02 |
| 18 | Fe 5580 A | 7.37 | 6.74 | 6.05 | 7.05 |
| 19 | Fe 5580 B | 7.37 | 6.92 | 6.41 | 7 |
| 20 | Fe 5580 C | 7.26 | 6.83 | 6.33 | 7 |
| | Average: | 7.33 | 6.83 | 6.26 | 7.02 |
| 21 | Mn 5.49 A | 7.29 | 6.96 | 6.56 | 7.56 |
| 22 | Mn 5.49 B | 7.23 | 7.02 | 6.75 | 7.75 |
| | Average: | 7.26 | 6.99 | 6.66 | 7.66 |
| 23 | Mn 54.9 A | 7.26 | 7.14 | 6.96 | 7.96 |
| 24 | Mn 54.9 B | 7.25 | 7.13 | 6.96 | 7.96 |
| 25 | Mn 54.9 C | 7.24 | 7.13 | 6.96 | 7.96 |
| | Average: | 7.25 | 7.13 | 6.96 | 7.96 |
| 26 | Mn 549 A | 7.23 | 7.16 | 7.03 | 8.03 |
| 27 | Mn 549 B | 7.23 | 7.19 | 7.09 | 8.09 |
| 28 | Mn 549 C | 7.33 | 7.28 | 7.16 | 8.16 |
| | Average: | 7.26 | 7.21 | 7.09 | 8.09 |
| 29 | Mn 5490 A | 7.23 | 7.15 | 7 | 8 |
| 30 | Mn 5490 B | 7.43 | 7.22 | 6.94 | 7.94 |
| 31 | Mn 5490 C | 7.43 | 7.21 | 6.93 | 7.93 |
| | Average: | 7.36 | 7.19 | 6.96 | 7.96 |
| 32 | Zn 6.54 A | 7.21 | 6.95 | 6.63 | 7.63 |
| 33 | Zn 6.54 B | 7.00 | 6.86 | 6.66 | 7.66 |
| 34 | Zn 6.54 C | 7.32 | 6.96 | 6.53 | 7.53 |
| | Average: | 7.18 | 6.92 | 6.61 | 7.61 |
| 35 | Zn 65.4 A | 7.28 | 6.78 | 6.22 | 7.22 |
| 36 | Zn 65.4 B | 7.34 | 7.03 | 6.66 | 7.66 |
| 37 | Zn 65.4 C | 7.32 | 6.87 | 6.36 | 7.36 |
| | Average: | 7.31 | 6.89 | 6.41 | 7.41 |
| 38 | Zn 654 A | 7.35 | 6.73 | 6.05 | 7.05 |
| 39 | Zn 654 B | 7.37 | 7.16 | 6.89 | 7.89 |
| | Average: | 7.36 | 6.94 | 6.47 | 7.47 |
| 40 | Zn 6540 A | 7.32 | 7.11 | 6.83 | 7.83 |
| 41 | Zn 6540 B | 7.24 | 6.94 | 6.58 | 7.58 |
| | Average: | 7.28 | 7.02 | 6.71 | 7.71 |
| 42 | Cu 6.35 A | 7.31 | 6.73 | 6.08 | 7.08 |
| 43 | Cu 6.35 B | 7.38 | 6.82 | 6.2 | 7 |
| 44 | Cu 6.35 C | 7.31 | 6.75 | 6.13 | 7 |
| | Average: | 7.33 | 6.77 | 6.14 | 7.03 |

| | | | | | |
|---|---|---|---|---|---|
| 45 | Cu 63.5 A | 7.32 | 6.81 | 6.23 | 7 |
| 46 | Cu 63.5 B | 7.41 | 6.74 | 6 | 7 |
| | Average: | 7.37 | 6.77 | 6.12 | 7.00 |
| 47 | Cu 635 A | 7.32 | 7.15 | 6.92 | 7.92 |
| 48 | Cu 635 B | 7.26 | 6.66 | 6 | 7 |
| | Average: | 7.29 | 6.91 | 6.46 | 7.46 |
| 49 | Cu 6350 A | 7.36 | 6.29 | 5.16 | 6.23 |
| 50 | Cu 6350 B | 7.35 | 6.21 | 5 | 7.02 |
| 51 | Cu 6350 C | 7.37 | 6.61 | 5.79 | 6.88 |
| | Average: | 7.36 | 6.37 | 5.32 | 6.71 |
| 52 | Mo 9.59 A | 7.39 | 7.13 | 6.8 | 7.8 |
| 53 | Mo 9.59 B | 7.37 | 7.23 | 7.02 | 8.02 |
| | Average: | 7.38 | 7.18 | 6.91 | 7.91 |
| 54 | Mo 95.9 A | 7.31 | 7.13 | 6.88 | 7.96 |
| 55 | Mo 95.9 B | 7.22 | 7.17 | 7.05 | 8.05 |
| 56 | Mo 95.9 C | 7.34 | 7.25 | 7.1 | 8.1 |
| | Average: | 7.29 | 7.18 | 7.01 | 8.04 |
| 57 | Mo 959 A | 7.41 | 7.30 | 7.13 | 8.13 |
| 58 | Mo 959 B | 7.43 | 7.35 | 7.2 | 8.034 |
| 59 | Mo 959 C | 7.21 | 7.15 | 7.03 | 8.03 |
| | Average: | 7.35 | 7.27 | 7.12 | 8.06 |
| 60 | Co 5.89 A | 7.33 | 7.22 | 7.04 | 8.04 |
| 61 | Co 5.89 B | 7.23 | 7.12 | 6.95 | 7.95 |
| 62 | Co 5.89 C | 7.18 | 7.14 | 7.03 | 8.03 |
| | Average: | 7.25 | 7.16 | 7.01 | 8.01 |
| 63 | Co 58.9 A | 7.14 | 7.09 | 6.99 | 7.99 |
| 64 | Co 58.9 B | 7.14 | 7.11 | 7.01 | 8.01 |
| 65 | Co 58.9 C | 7.21 | 7.13 | 6.99 | 7.99 |
| | Average: | 7.16 | 7.11 | 7.00 | 8.00 |
| 66 | Co 589 A | 7.23 | 7.09 | 6.88 | 7.92 |
| 67 | Co 589 B | 7.26 | 7.19 | 7.05 | 8.05 |
| 68 | Co 589 C | 7.33 | 7.16 | 6.92 | 7.92 |
| | Average: | 7.27 | 7.14 | 6.95 | 7.96 |
| 69 | Control | 7.26 | 7.105 | 6.89 | 7.89 |
| 70 | Control | 7.26 | 7.105 | 6.89 | 7.68 |
| 71 | Control | 7.26 | 7.290 | 7.26 | 8.01 |
| | Average: | 7.26 | 7.17 | 7.01 | 7.86 |

Notes:

$pH^1$ -pH of water source with humic substance at day zero (0).

$pH^2$- pH of water source with humic substance and AOB at day seven (7).

$pH^3$- pH of water source with humic substance, AOB and metal ions at day fourteen (14)

$pH^4$- pH of water source after addition of phosphate buffer

### A1.1.2 pH Graphs

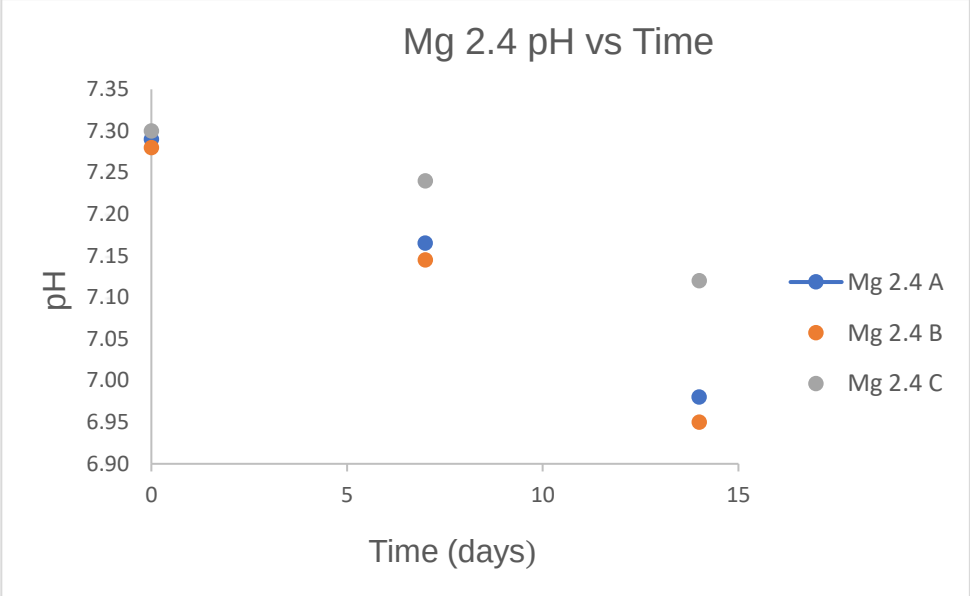


**FIGURE A1-1**

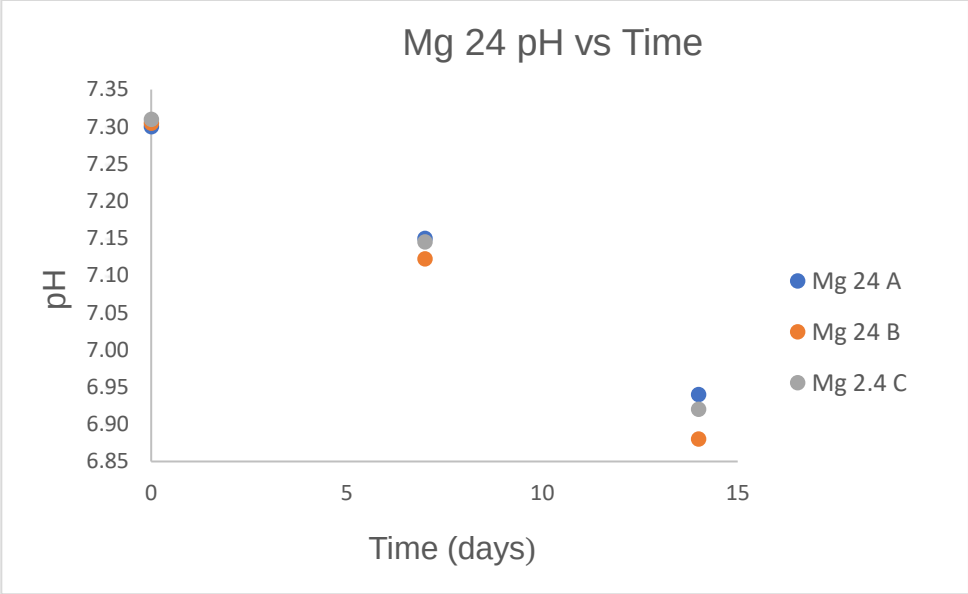


**FIGURE A1-2**

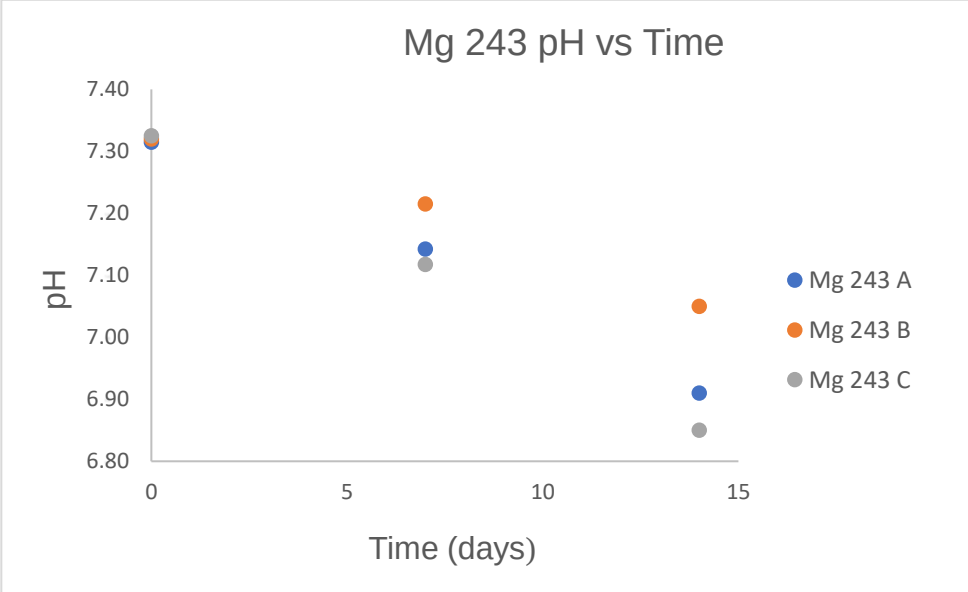


**FIGURE A1-3**

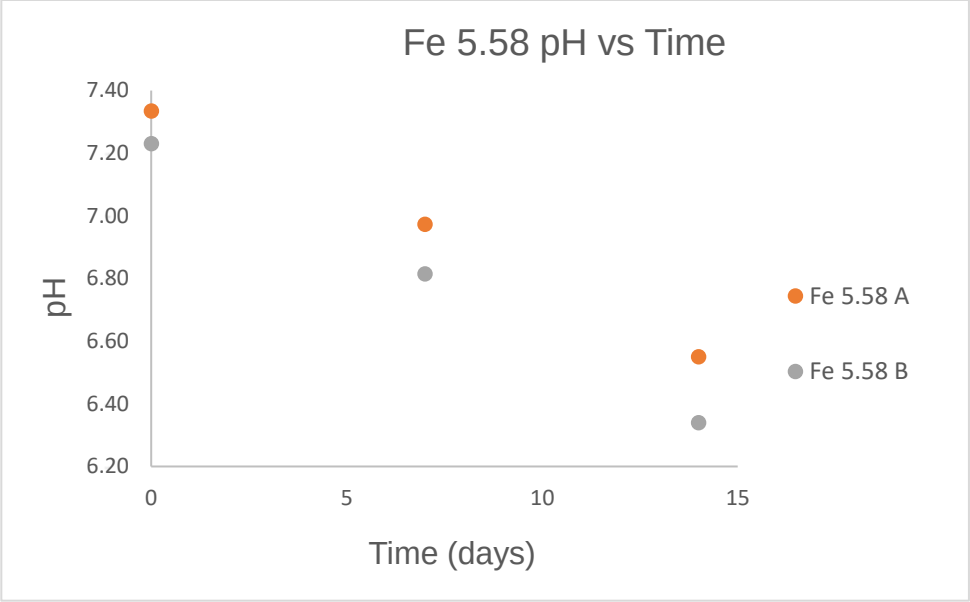


**FIGURE A1-4**

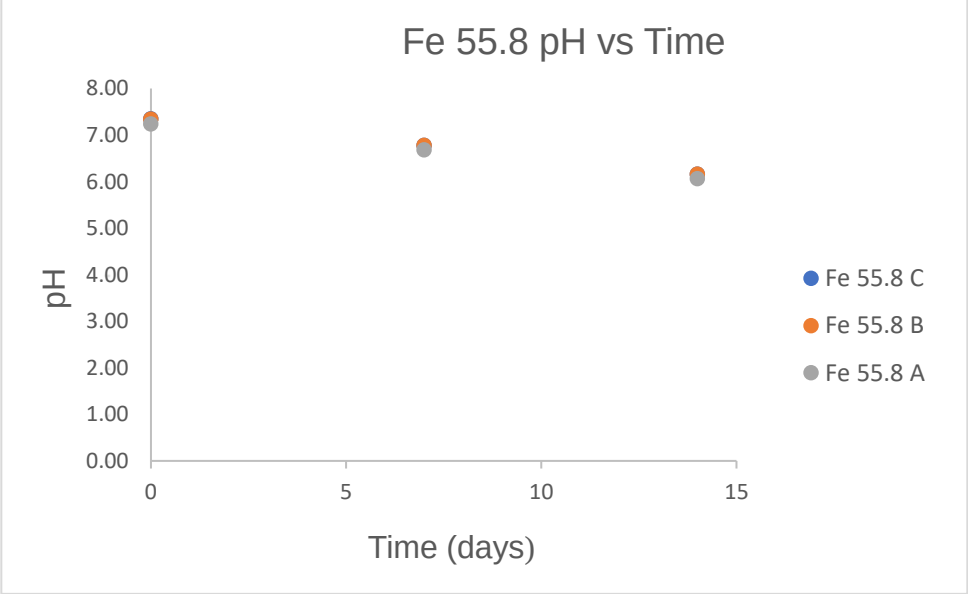


**FIGURE A1-5**

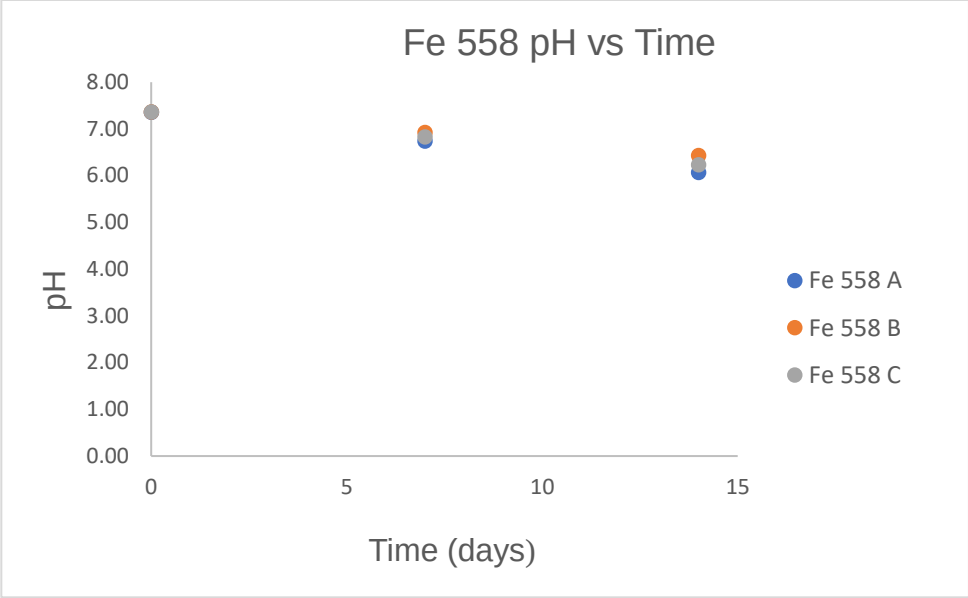


**FIGURE A1-6**

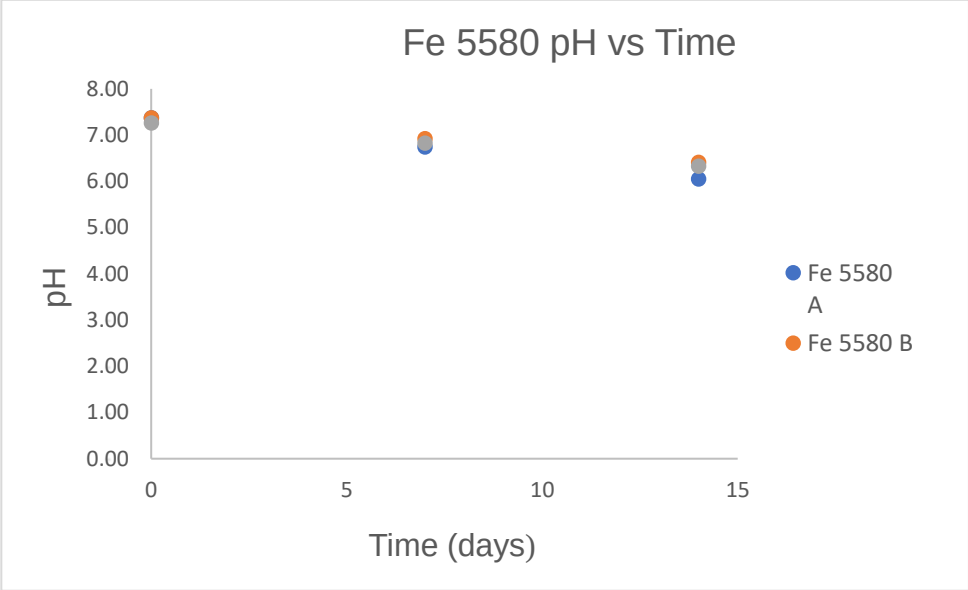


**FIGURE A1-7**

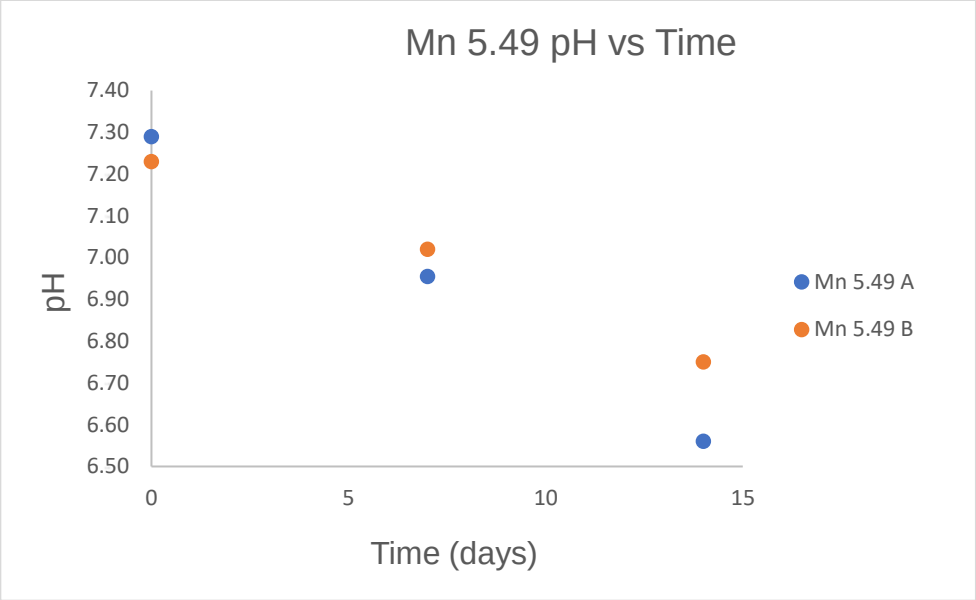


**FIGURE A1-8**

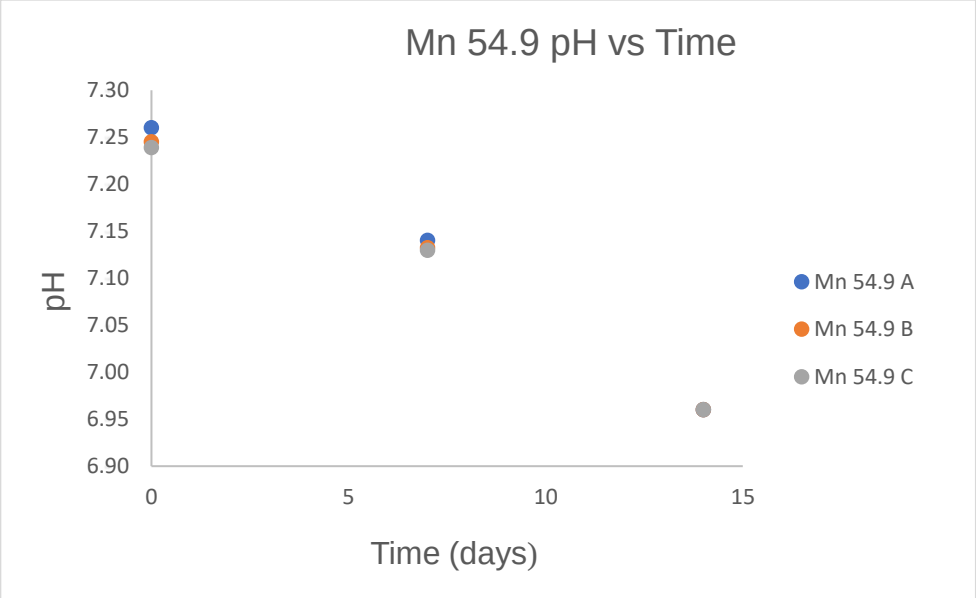


**FIGURE A1-9**

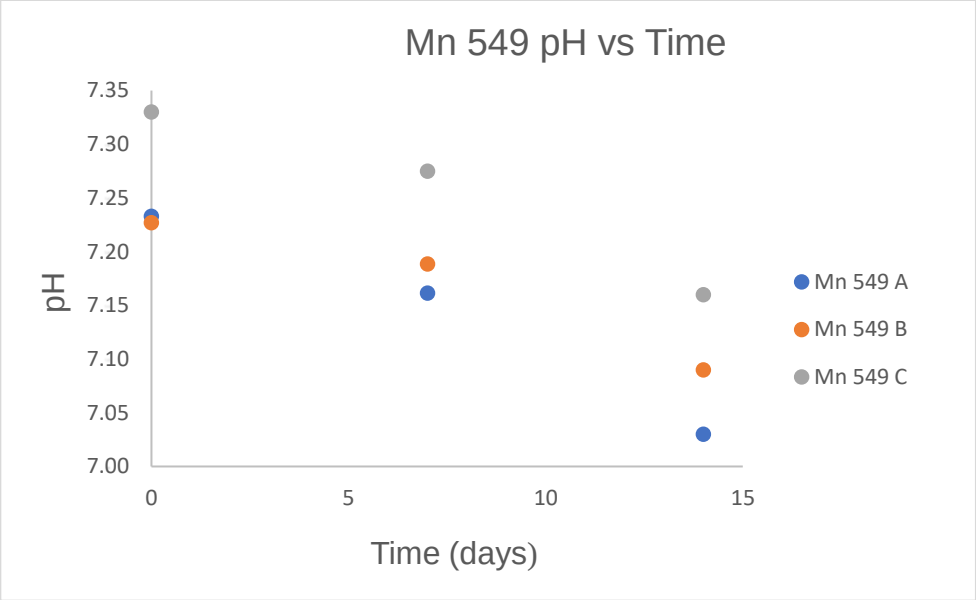


**FIGURE A1-10**

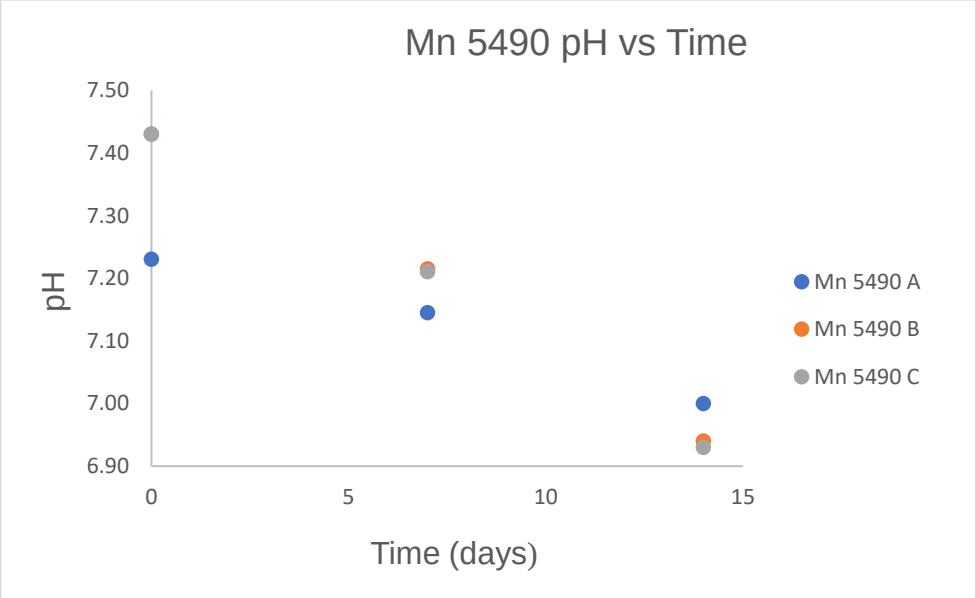


**FIGURE A1-11**

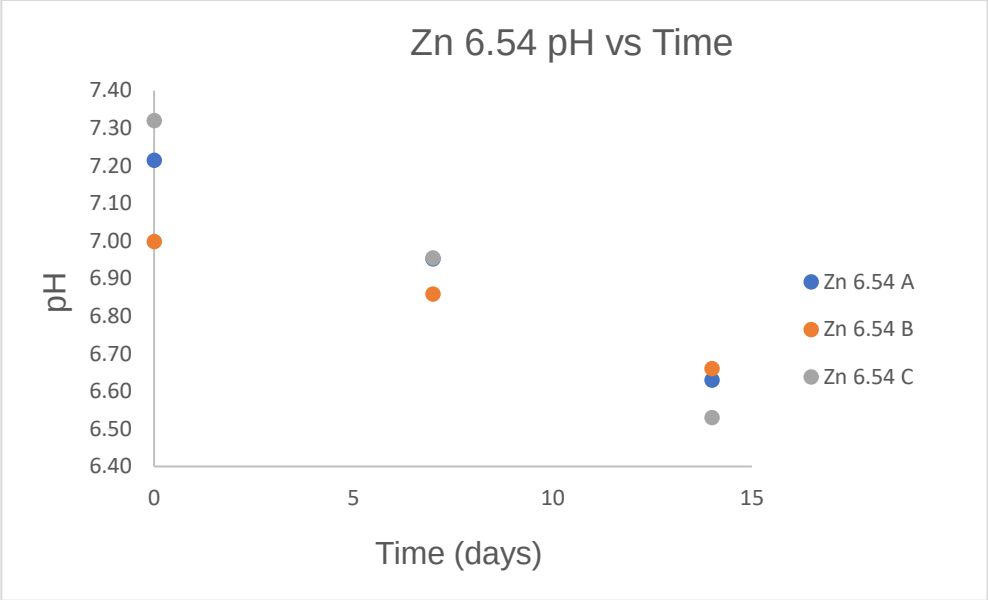


**FIGURE A1-12**

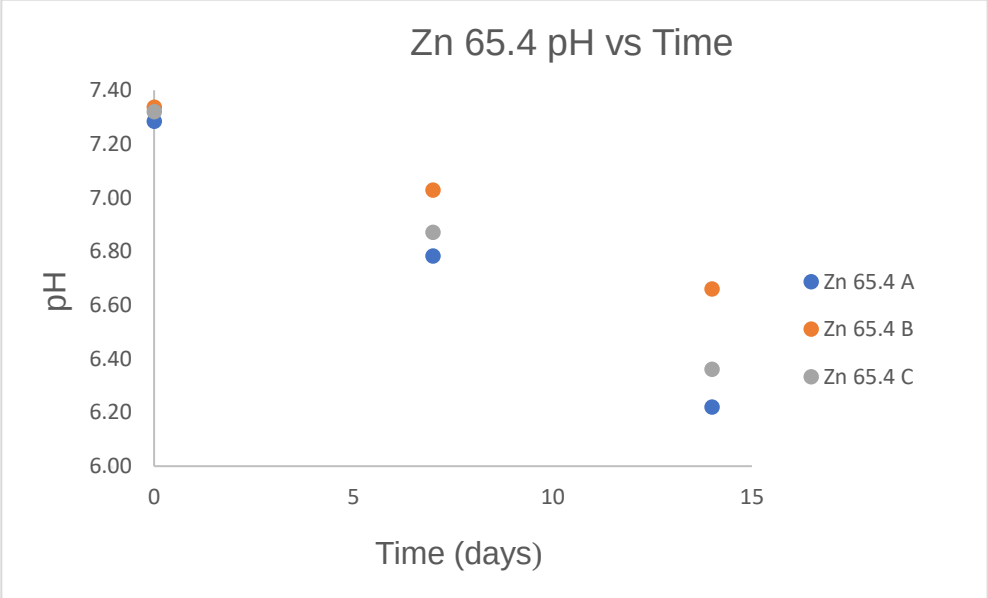


**FIGURE A1-13**

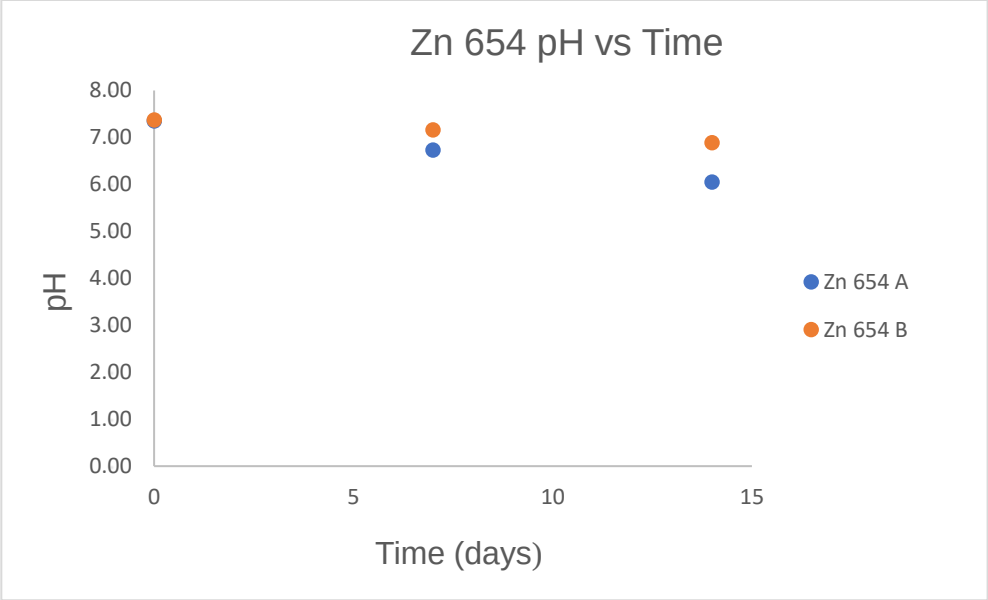


**FIGURE A1-14**

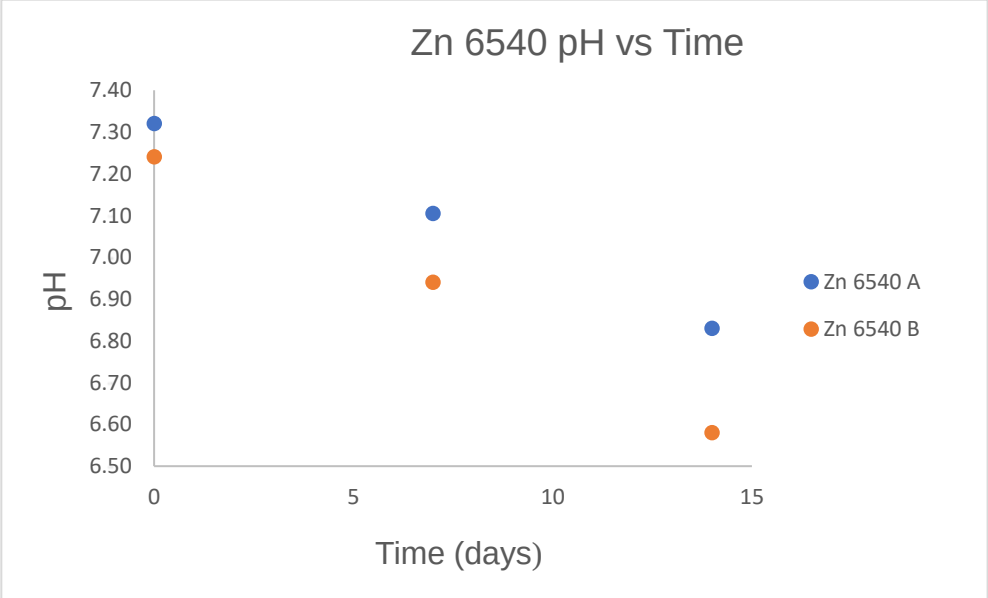


**FIGURE A1-15**

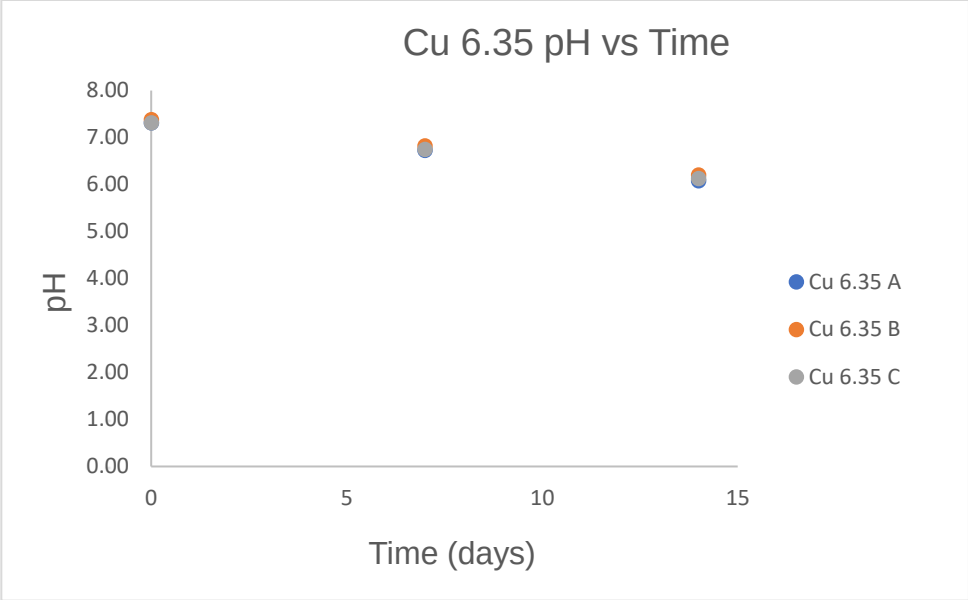


**FIGURE A1-16**

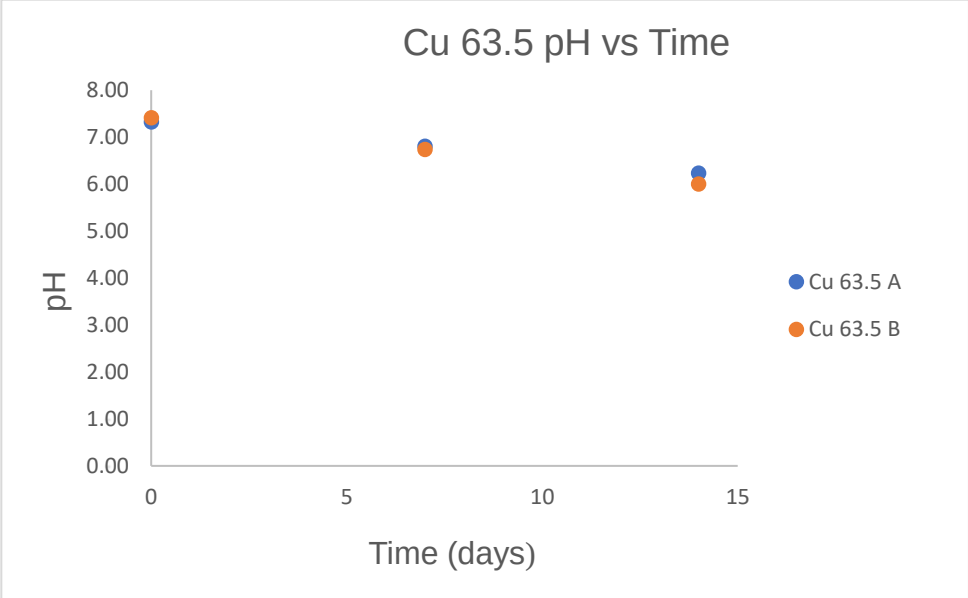


**FIGURE A-17**

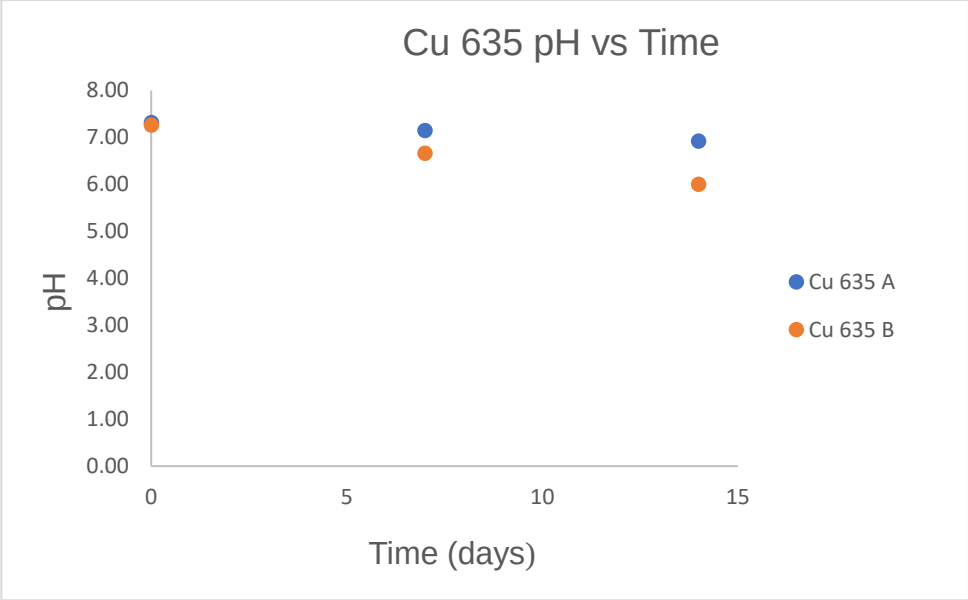


**FIGURE A-18**

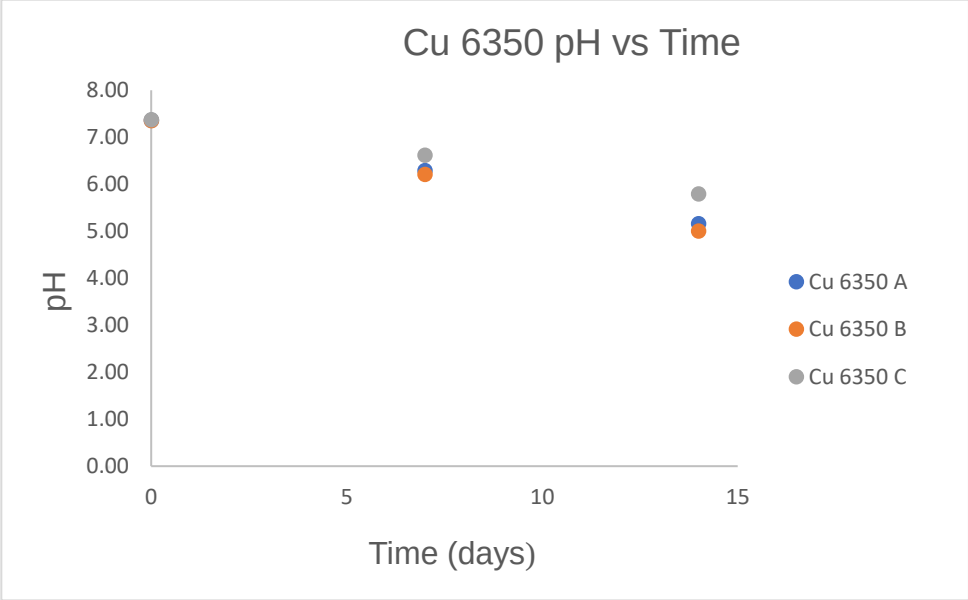


**FIGURE A-19**

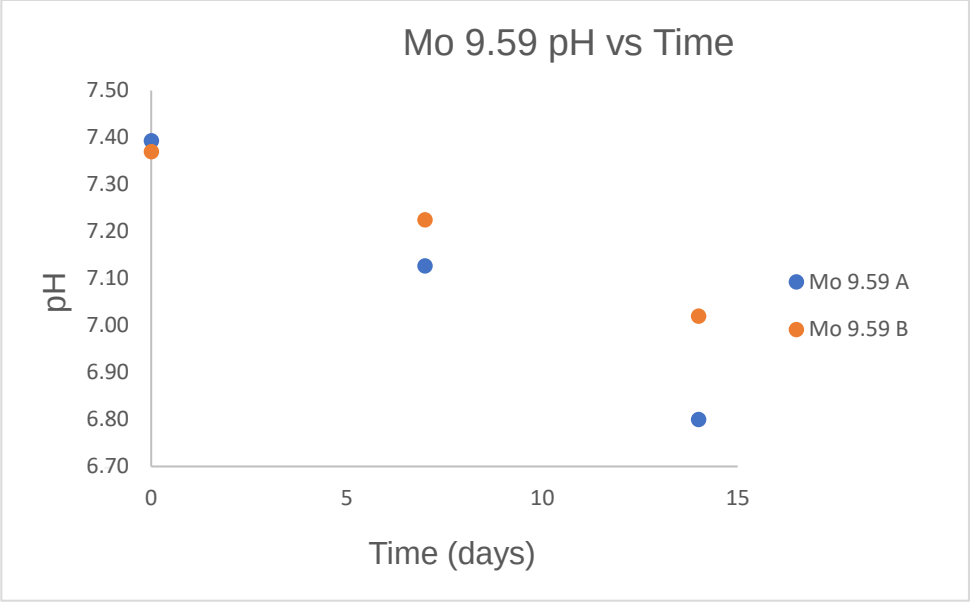


**FIGURE A1-20**

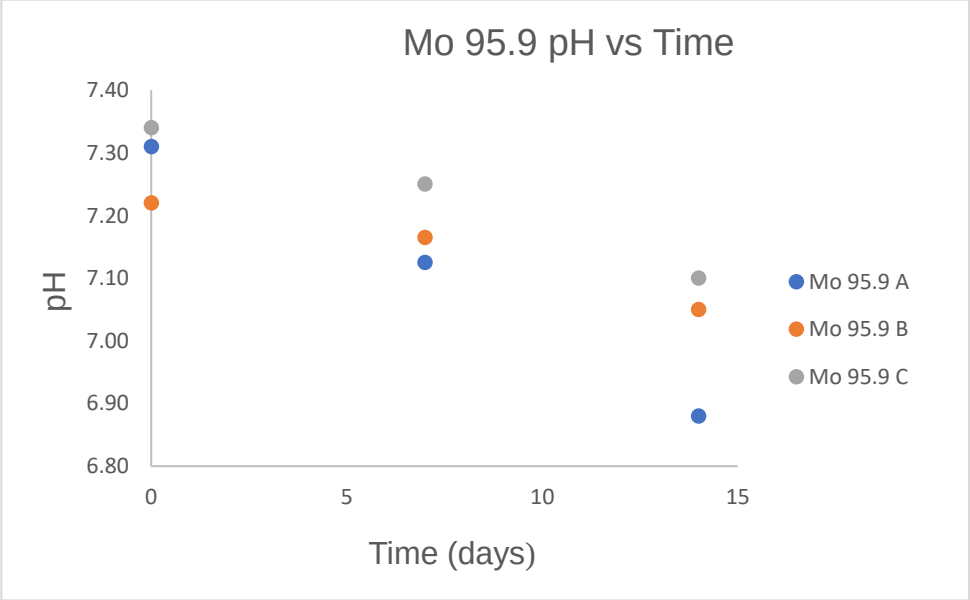


**FIGURE A1-21**

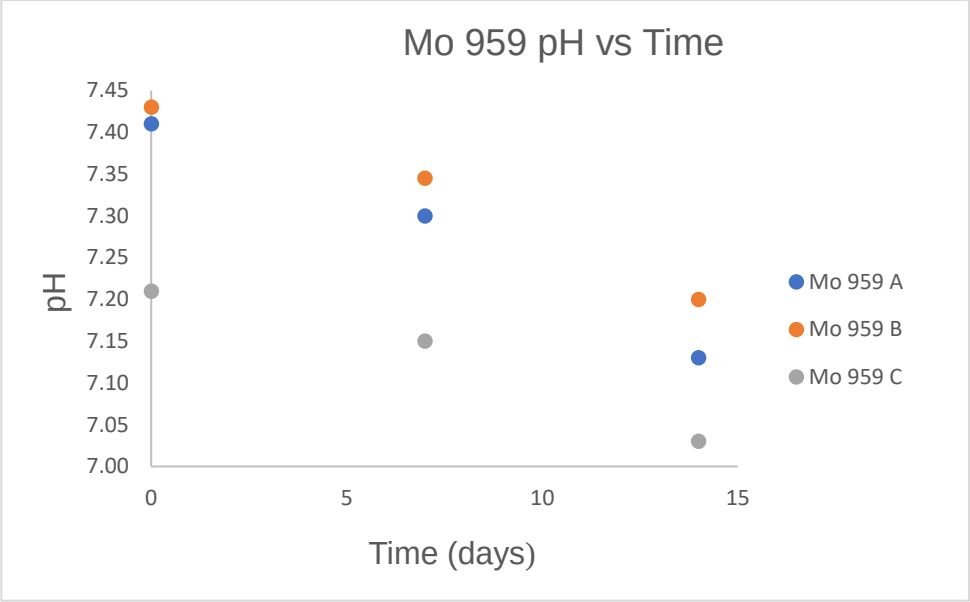


**FIGURE A1-22**

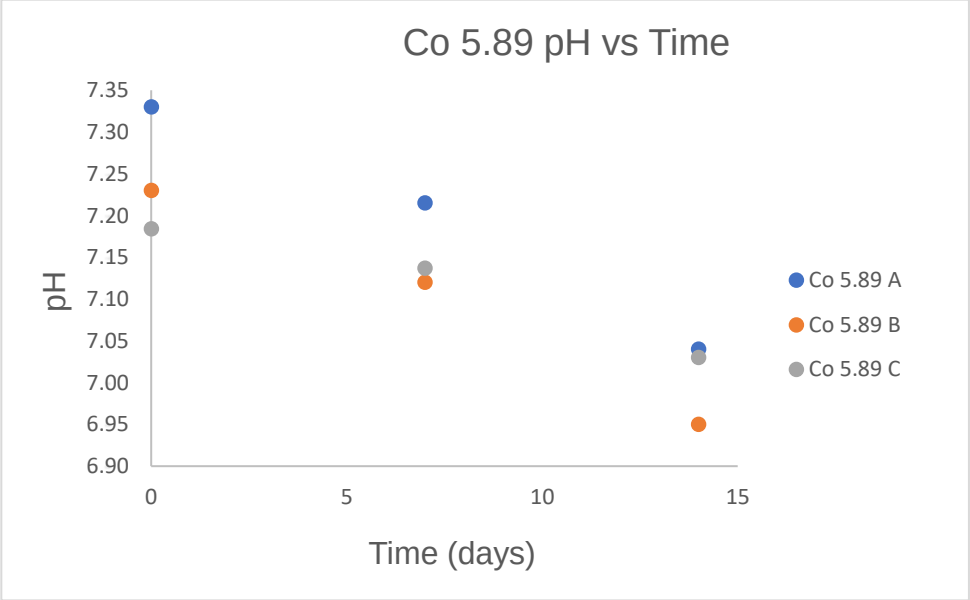


**FIGURE A1-23**

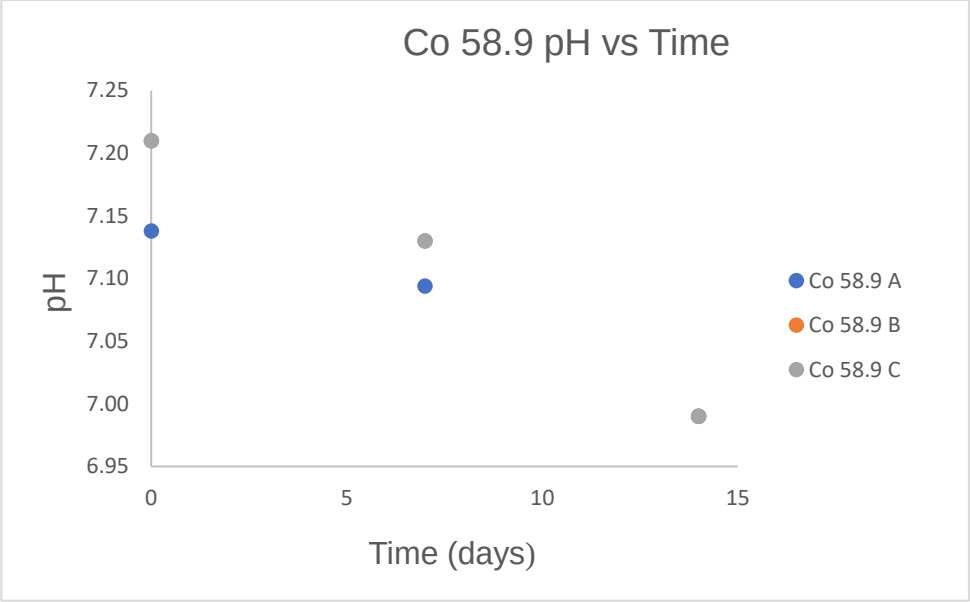


**FIGURE A1-24**

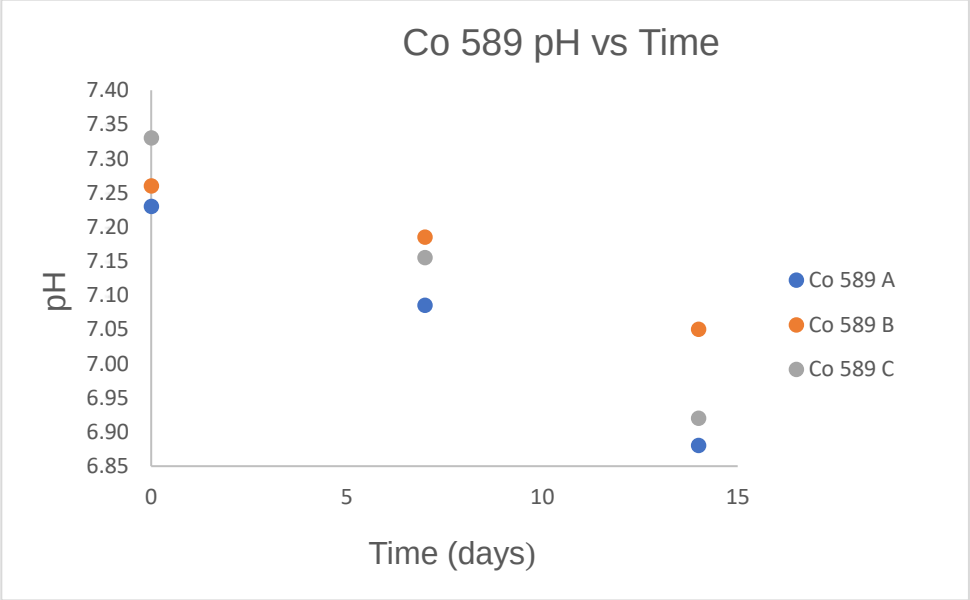


**FIGURE A1-25**

## A.1.2 TOC DATA

| TABLE A1-2 | | | | | |
|---|---|---|---|---|---|
| SAMPLE | | | | | |
| ID | METAL | CONC. | NUMBER | TOC mg/L | TOC g |
| 1 | Mg | 2.4 | A | 1.8 | 0.0000054 |
| 2 | Mg | 2.4 | B | 1.9 | 0.0000057 |
| 3 | Mg | 2.4 | C | 2.1 | 0.0000063 |
| 4 | Mg | 24 | A | 2.3 | 0.0000069 |
| 5 | Mg | 24 | B | 2.1 | 0.0000063 |
| 6 | Mg | 24 | C | 1.8 | 0.0000054 |
| 7 | Mg | 243 | A | 1.8 | 0.0000054 |
| 8 | Mg | 243 | B | 2.3 | 0.0000069 |
| 9 | Mg | 243 | C | 1 | 0.000003 |
| 10 | Mg | 243 | D | 1.4 | 0.0000042 |
| 11 | Fe | 5.58 | A | 1.5 | 0.0000045 |
| 12 | Fe | 5.58 | B | 1.9 | 0.0000057 |
| 13 | Fe | 55.8 | A | 2.1 | 0.0000063 |
| 14 | Fe | 55.8 | B | 1.7 | 0.0000051 |
| 15 | Fe | 55.8 | C | 1.9 | 0.0000057 |
| 17 | Fe | 558 | A | 1.5 | 0.0000045 |
| 18 | Fe | 558 | B | 1.6 | 0.0000048 |
| 19 | Fe | 558 | C | 1.6 | 0.0000048 |
| 20 | Fe | 5580 | A | 1.3 | 0.0000039 |
| 21 | Fe | 5580 | B | 1.3 | 0.0000039 |
| 22 | Fe | 5580 | C | 1.7 | 0.0000051 |
| 23 | Mn | 5.49 | A | 1.7 | 0.0000051 |
| 24 | Mn | 5.49 | B | 1.7 | 0.0000051 |
| 25 | Mn | 54.9 | A | 2 | 0.000006 |
| 26 | Mn | 54.9 | B | 1.6 | 0.0000048 |
| 27 | Mn | 54.9 | C | 1.6 | 0.0000048 |
| 28 | Mn | 549 | A | 1.3 | 0.0000039 |
| 29 | Mn | 549 | B | 1.4 | 0.0000042 |
| 30 | Mn | 549 | C | 1.5 | 0.0000045 |
| 31 | Mn | 5490 | A | 1.4 | 0.0000042 |
| 32 | Mn | 5490 | B | 1.5 | 0.0000045 |
| 33 | Mn | 5490 | C | 1.6 | 0.0000048 |
| 34 | Zn | 6.54 | A | 1.2 | 0.0000036 |
| 35 | Zn | 6.54 | B | 1.9 | 0.0000057 |
| 36 | Zn | 6.54 | C | 2.1 | 0.0000063 |
| 37 | Zn | 65.4 | A | 2.3 | 0.0000069 |

| | | | | | |
|---|---|---|---|---|---|
| 38 | Zn | 65.4 | B | 2.2 | 0.0000066 |
| 39 | Zn | 65.4 | C | 1.8 | 0.0000054 |
| 40 | Zn | 654 | A | 1.9 | 0.0000057 |
| 41 | Zn | 654 | B | 2.1 | 0.0000063 |
| 42 | Zn | 6540 | A | 1.9 | 0.0000057 |
| 43 | Zn | 6540 | B | 1.9 | 0.0000057 |
| 44 | Cu | 6.35 | A | 1.7 | 0.0000051 |
| 45 | Cu | 6.35 | B | 1.7 | 0.0000051 |
| 46 | Cu | 6.35 | C | 1.2 | 0.0000036 |
| 47 | Cu | 63.5 | A | 1.4 | 0.0000042 |
| 48 | Cu | 63.5 | B | 1.6 | 0.0000048 |
| 49 | Cu | 635 | A | 1.9 | 0.0000057 |
| 50 | Cu | 635 | B | 1.3 | 0.0000039 |
| 51 | Cu | 6350 | A | 1.2 | 0.0000036 |
| 52 | Cu | 6350 | B | 1.8 | 0.0000054 |
| 53 | Cu | 6350 | C | 1.7 | 0.0000051 |
| 54 | Mo | 9.59 | A | 1.2 | 0.0000036 |
| 55 | Mo | 9.59 | B | 1.9 | 0.0000057 |
| 56 | Mo | 9.59 | C | 2.1 | 0.0000063 |
| 57 | Mo | 95.9 | A | 2.3 | 0.0000069 |
| 58 | Mo | 95.9 | B | 2.2 | 0.0000066 |
| 59 | Mo | 959 | A | 1.8 | 0.0000054 |
| 60 | Mo | 959 | B | 1.9 | 0.0000057 |
| 61 | Mo | 959 | C | 2.1 | 0.0000063 |
| 62 | Co | 5.89 | A | 1.9 | 0.0000057 |
| 63 | Co | 5.89 | B | 1.9 | 0.0000057 |
| 64 | Co | 5.89 | C | 2.2 | 0.0000066 |
| 65 | Co | 58.9 | A | 1.8 | 0.0000054 |
| 66 | Co | 58.9 | B | 1.7 | 0.0000051 |
| 67 | Co | 58.9 | C | 2.1 | 0.0000063 |
| 68 | Co | 589 | A | 2.2 | 0.0000066 |
| 69 | Co | 589 | B | 2.1 | 0.0000063 |
| 70 | Co | 589 | C | 1.9 | 0.0000057 |
| 71 | CONTROL | | A | 1.4 | 0.0000042 |
| 72 | CONTROL | | B | 1.3 | 0.0000039 |
| 73 | CONTROL | | C | 1.5 | 0.0000045 |

## A.2 Standard Operating Protocols

### A.2.1 ATC Method

**ATCC medium: 2265 Nitrosomonas europaea medium**

| | |
|---|---|
| **Solution 1:** | |
| $(NH_4)_2SO_4$ (for 50 mM $NH_4^+$) ..... | 4.95 g |
| $KH_2PO_4$..... | 0.62 g |
| $MgSO_4$. $7H_2O$ ..... | 0.27 g |
| $CaCl_2$. $2H_2O$ ..... | 0.04 g |
| $FeSO_4$ (30 mM in 50 mM EDTA at pH 7.0) ..... | 0.50 g |
| $CuSO_4$. $5H_2O$ ..... | 0.20 g |
| Distilled Water ..... | 1.20 L |
| *Filter Sterilize* | |
| **Solution 2:** | |
| $KH_2PO_4$..... | 8.2 g |
| $NaH_2PO_4$ ..... | 0.7 g |
| Distilled Water ..... | 300.0 ml |
| *Bring to pH 8.0 with 10N NaOH. Filter Sterilize.* | |
| **Solution 3 (buffer):** | |
| $Na_2CO_3$ anhydrous ..... | 0.6 g |
| Distilled Water ..... | 12.0 ml |
| *Filter Sterilize.* | |
| **Complete medium:** | |
| Combine Solution 1, 2, and 3. Dispense aseptically into desired aliquots | |

## A.3 Equipment and Materials

### A.3.1 Chemicals

### A.3.2 Source Water Locations

#### A.3.2.1 Water Treatment Plant

Oklahoma State Energy Services operates OSU's Water Treatment Plant. This Plant sources raw water from Lake Carl Blackwell, which is located 8 miles west of Stillwater.

The water treated at the WTP is made potable and the distributed to every building on Campus as drinking water and for processing waste. Image A3-1 is of the aerial view of the plant.

Address: 398 W Hall of Fame Ave, Stillwater, OK 74075

**Image A3-1**

### A.3.2.2 Fire Hydrant

Hydrant Location: 36°07'42.6"N 97°04'40.6"W

Hydrant Address: 398 W Hall of Fame Ave, Stillwater, OK 74075

### A.3.3 Equipment

#### A.3.3.1 Oscillation Table

A Clever Commercial Hot Dog Machine-11 Roller was used as an oscillation table to roll each sample continuously during this study. While the machine provides a heating element, it was not used in this study to maintain sample temperature. Parameters of the machine are provided in Table 3.3.1 below:

**TABLE 3.3.1**

**PARAMETERS OF TECHNOLOGY**

| Type | Volt(s) | Power (KW) | Temperature scope | Rollers | Size (mm) |
|---|---|---|---|---|---|
| CRS201710 | 110 | 1.4 | 0°C - 250°C | 11 | 580X480X170 |

VITA

Alissa Hall

Candidate for the Degree of

Master of Science

Thesis: EFFECTS OF TRACE METALS ON CHLORAMINATED DISINFECTION BYPRODUCT FORMATION OF DRINKING WATER BIOFILMS

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