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Accumulation of Lead by Biofilms in Drinking Water Distribution Systems

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Accumulation of Lead by Biofilms in Drinking Water Distribution Systems

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Civil Engineering

by

Kaleb Belcher
University of Arkansas
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University of Arkansas

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ABSTRACT

Recent crises, such as the one in Flint, MI, indicate that lead exposure from drinking water is a major health concern in the United States. Over six million lead service lines are still in use in the United States, and a universal protocol to safely remove these lead service lines from drinking water distribution systems has not yet been established. This paper calls to attention the potential hazard that biofilms pose as a source of lead in distribution systems, even after the removal of lead pipes. This study used a simulated water distribution system containing a lead source pipe and various pipe materials with periodic flow and stagnation conditions of a typical household to create, characterize, and determine lead accumulation capabilities of biofilms within the water pipes. Biofilms developed in all pipe materials with an overall range of 1.44×10^3 to 5.90×10^5 gene copies per cm^2 of pipe surface. Pipe material affected the biofilm growth with plastic pipes supporting higher quantities of biofilms. Biofilms accumulated lead in all pipe materials with a maximum accumulation of 25.22 $\mu g/cm^2$. In addition, all pipe trains experienced an increase in lead accumulation immediately following the removal of the lead source with a maximum increase of 21.42 $\mu g/cm^2$ in the galvanized steel pipe and then a gradual decrease during a period of one month afterward. The lead source also had an effect on the microbiome of the biofilms collected during the project. One genus specifically, *Sphingobium,* increased in all pipe materials following the removal of lead. This research provides valuable information regarding the timing and process of safe lead service line removal from drinking water distribution systems and helps minimize the human exposure to lead contamination within drinking water.

ACKNOWLEDGEMENTS

Special thanks to my advisor, Dr. Wen Zhang for her expertise and guidance, especially in writing. Additionally, I want to thank Dr. Franck Carbonero for assistance with DNA sequencing. I'd also like extend gratitude to Mr. Roy Foster, Dr. Hua Jiang and other City of Tulsa Staff for both funding the project and assisting with gathering data during this study.

DEDICATION

This thesis is dedicated to my wife and parents, for the extensive help that was given to me in everything from advice and review to a reassuring word when things were looking down. I couldn’t have done it without you.

TABLE OF CONTENTS

I. INTRODUCTION

Before the passing of the 1986 Safe Drinking Water Act Amendment, the use of lead solder in drinking water distribution systems (DWDSs) was common practice. As of 2016, there are approximately 6.1 million lead service lines currently in use in the United States (Cornwell et al., 2016). In adults, consumption of lead can lead to renal problems and high blood pressure. Previous research indicates that in children below the age of 5, blood lead levels as low as 5 µg/dL correspond with an impedance of cognitive and academic growth (Canfield et al., 2003). Because of the health consequences of lead consumption, the EPA has set a Maximum Contamination Level Goal (MCLG) for lead at zero. To reach such a goal would entail the removal of all lead sources in DWDSs. While efforts have been put toward the replacement of lead pipes in the U.S., lead contamination in drinking water remains a problem in multiple regions. Several events in the past twenty years have highlighted this risk of lead contamination. The city of Washington D.C. experienced high lead levels in its drinking water from 2001 to 2004 as a result of switching from free chlorine to chloramine as the disinfectant used in its distribution system (Edwards & Dudi, 2004). More recently in 2014, the lead crisis in Flint, Michigan occurred as a result of a system-wide change of water chemistry due to a switch in water sources. The corrosive nature of the new water source and the discontinued use of a corrosion inhibitor such as orthophosphate resulted in lead release to the treated water (Pieper et al., 2018). While these two examples were perhaps the most well-known instances of lead contamination in recent years, increased lead levels in drinking water can happen in a variety of circumstances and can go unnoticed by the general public.

One condition that can increase lead levels in distribution systems is galvanic corrosion (Wang et al., 2012). Galvanic corrosion occurs when two metals of different nobility (in this case Copper and Lead) are near each other in a distribution system. The difference in nobility causes one metal to act as an anode while the other acts as a cathode, ultimately accelerating corrosion in lead pipes. Furthermore, other commonly-utilized pipes in distribution systems have been found to potentially contain previously unrecognized sources of lead. These include lead leaching from PVC pipes which use heavy metals as a stabilizer (Zhang & Lin, 2014) as well as Galvanized Steel pipes in which the zinc coating can have up to 5% lead (Clark, Masters & Edwards, 2015). Unfortunately, research indicates that even the process of partial removal of lead service lines may aggravate the problem instead of solving it, as disturbing the accumulated scale on pipe interiors can release lead (St. Clair et al., 2016).

Although drinking water is treated with disinfectant, planktonic or suspended cells still exist within drinking water systems. These cells stick to the surfaces of pipe walls and subsequently are embedded in a slimy extracellular matrix which is composed of polymeric substances. These collections of cells are known as biofilms. Of the total microbial cells in a DWDS, nearly 95% are present in biofilms on pipe surfaces (Moritz et al., 2010). Biofilm's existence within DWDSs is ubiquitous, as biofilms have the ability to use a variety of carbon sources and are able to resist disinfection (Camper, 2004; Emtiazi et al., 2004). The presence of biofilms inevitably lead to degradation of water quality within the distribution system. Types of degradation include growth of taste and odor forming bacteria (Zhou et al., 2017), accelerated corrosion of pipes (Teng et al., 2008), as well as hosting bacteria that promotes nitrification (Zhang & Edwards, 2009).

In addition to causing water quality degradation, biofilms have also been shown capable of sorbing metals in a variety of situations. Metals sorbed by biofilms include lead and chromium in a biofilter (Vilchez et al., 2011), copper, lead, and iron in shower hoses (Proctor et al., 2017), and zinc, copper, and lead in a flow chamber (Ancion et al., 2010). Although biofilms have been shown to adsorb metals, they have yet to be considered as a source of lead contamination in DWDSs. This study focused on the interactions between lead, various pipe materials and biofilms within the DWDS. Simulated distribution system experiments were conducted at water treatment plants in Tulsa (Oklahoma) to mimic DWDSs with different pipe materials. Biofilms were analyzed for lead accumulation, bacteria concentration and bacteria community profile.

II. MATERIALS AND METHODS

Construction and Operation of Simulated Distribution Systems. A simulated distribution system mimicking a DWDS was constructed at the water treatment plants in Tulsa, Oklahoma (see Figure 1).

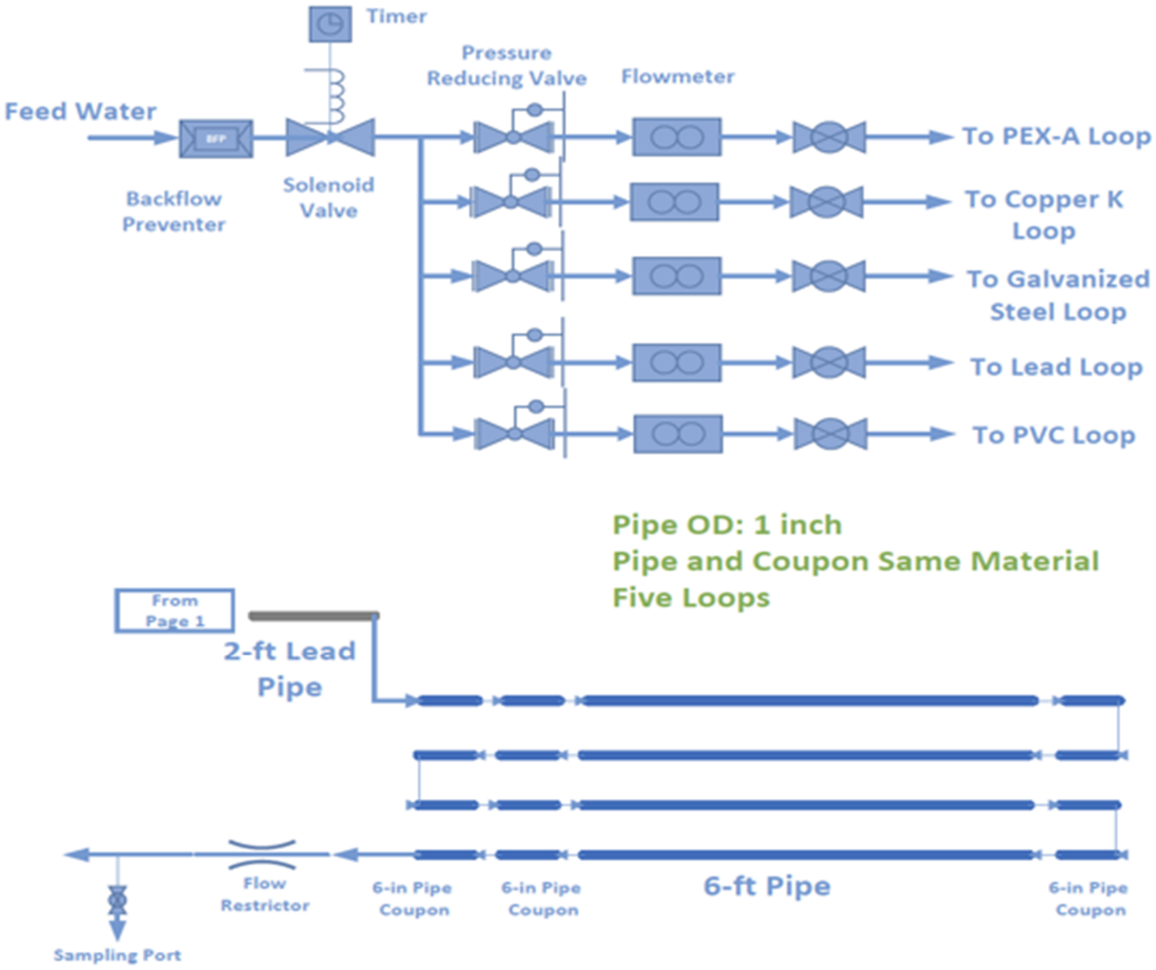

Figure 1: Simulated distribution system design configuration.

Five trains within the simulated distribution system, each comprised of a different pipe material, were operated in parallel in each experiment. The pipe materials include lead (¾” ID × 1” OD), PEX-A (¾”), Copper Type K (¾” ID × 7/8” OD), galvanized steel (¾” ID × 1” OD), and PVC

(¾” Schedule 40). Within each train, 12 pieces of 6” long pipe coupons were installed strategically for biofilm sampling purposes. Additionally, four 6-foot segments in designated pipe material were placed in the middle of each pipe train so the overall length was 30-ft per train. An additional 2-foot section of lead pipe was installed at the beginning of each pipe train to serve as the lead source. After simulated distribution system construction, the entire system was flushed at a high velocity of approximately 6 gallons per minute (gpm) for 30 minutes to ensure that there were no leaks in the system. During simulated distribution system operation, water flowed in an intermittent mode to simulate a typical residential water usage pattern. One gpm of water flowed within the system during the time intervals of 6:00am - 9:00am, 11:00am – 1:30pm, 4:00pm – 6:30pm, and 9:30pm – 10:30pm; water stayed stagnant within the system at all other times. The first simulated distribution system was constructed and operated at the A.B Jewell plant for 11 months, where influent water had a chloramine residual of 2.80 mg/L and an ambient pH of 8.0. The second simulated distribution system was constructed and operated at the Mohawk plant for 5 months, where influent water had a chloramine residual of 2.88 mg/L and a pH of 8.2. Each round of operation included two different stages. All trains in stage one contained a lead pipe (2-ft) which served as the initial source of lead contamination. This stage lasted from January 23, 2017 to September 5, 2017 for A.B Jewell plant and November 13, 2017 to April 24, 2018 for the Mohawk plant. In stage 2, the initial 2-ft of lead pipe was removed from all trains and the system continued to operate without the lead source. The second stage was operated until October 26, 2017 and May 25, 2018 for the A.B. Jewell plant and the Mohawk plant, respectively.

Water Sampling and Analysis. Water quality parameters such as water temperature, alkalinity, hardness, pH, total dissolved solids (TDS), disinfectant type and residual level, and influent flow rate were recorded at both water treatment plants daily using the Standard Methods for the

Examination of Water and Wastewater (Eaton et al., 1998).Using the water quality parameters above the Langelier Saturation Index (LSI) was calculated. LSI is used by many water treatment facilities to measure the corrosivity of treated water. The index indicates whether the calcium carbonate in distribution systems will precipitate and form a scale on the pipe wall (LSI>0.5), dissolve from the existing scale (LSI<-0.5), or be in equilibrium. The equation to calculate LSI is shown below.

$$LSI = (pH) + (Temperature) + (Hardness) + (Total\ Alkalinity) - (TDS\ Factor)$$

During the simulated distribution system operation, 500 mL of effluent samples were collected daily for month one, twice a week for month two and weekly for the remaining time of the experiment. These samples were used for recoverable and dissolved lead quantifications via inductively coupled plasma mass spectrometer (ICP-MS) using EPA Method 200.8 Revision 5.4.

Pipe Coupon Sampling. Figure 2 shows the sampling events for the A.B Jewell plant and Figure 3 shows the sampling events for the Mohawk plant.

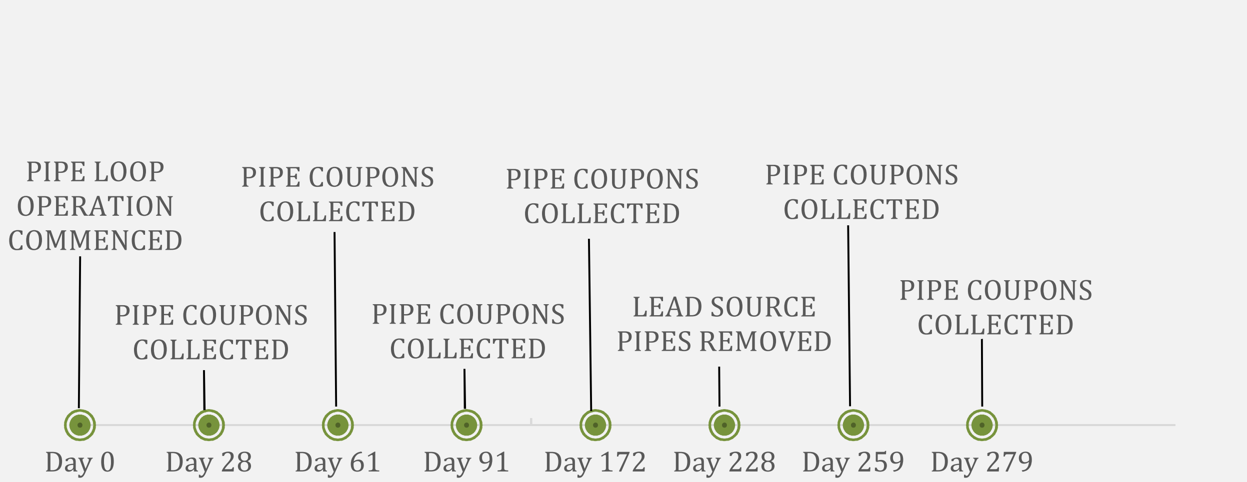

Figure 2: Timeline of simulated distribution system operation at A.B. Jewell Water Treatment Plant.

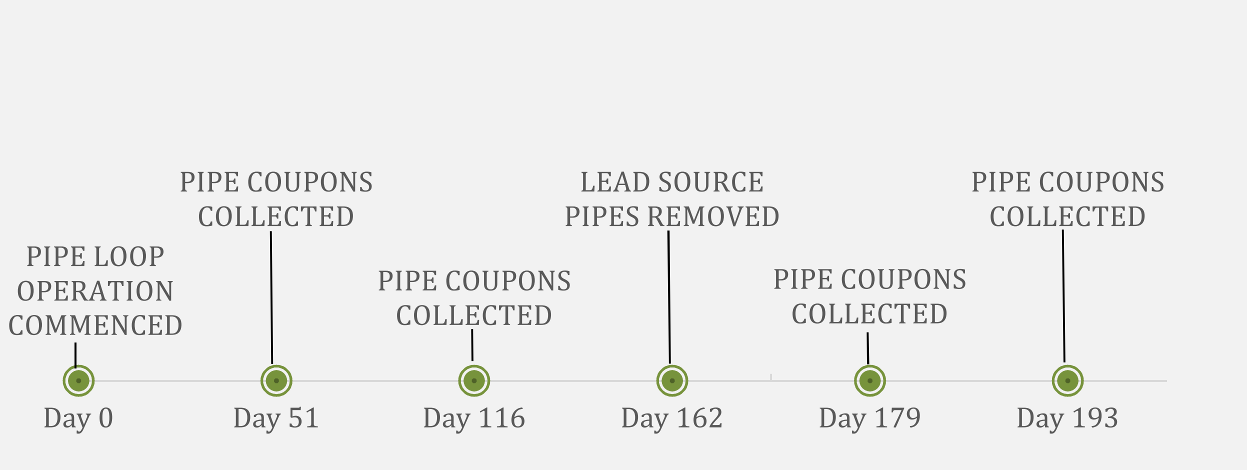

Figure 3: Timeline of simulated distribution system operation at Mohawk Water Treatment Plant.

On each sampling day, two 6-inch pipe coupons (duplicates) were collected from every train composed of different pipe materials. Each pipe sample was placed in a one-gallon Ziploc bag with approximately 80 mL of water from its respective pipe train. The samples were then preserved on ice during transportation from Tulsa, OK to the University of Arkansas lab in Fayetteville, AR on the same day. Each pipe coupon was sonicated using a Branson Sonifier 3800 (Emerson, Ferguson, MO) for 30 minutes within the collection bag to dislodge the biofilm from the pipe interior. One of the duplicate coupons was used for metal and total solids analysis, while the other was used for microbial analysis.

Metal Analysis. Following dislodging of biofilms via sonication, half of the water volume from one pipe coupon was filtered through a 0.22-µm filter (Pall Corporation, Port Washington, NY) for metal analysis. To prevent interferences from pipe sonication, two control lead pipes were sonicated to determine the amount of lead released during sonication. This value of lead released from the control pipes were subtracted from the lead measured from the pipe coupons. The filter was then dried completely in the oven at 98°C. Dried filters were placed in 20-mL centrifuge tubes for storage and digestion. 5 mL of DI millipore water (Thermo Sci, Gen pure Pro UV/UF

501311950, Waltham, MA) was added into the centrifuge tube and then sonicated for 30 minutes (VWR model 751, Radnor, PA). The filter was digested using a solution comprised of 1 mL of H_2O_2, 0.42 mL of HCl, and 0.2 mL of HNO_3. The mixture was then allowed to digest for 24 hours in an oven (Blue M model M01440A, Waltham, MA) set at 50ºC. After 24 hours, the mixture was diluted to a total volume of 10 mL using DI millipore water. Samples were analyzed using a Thermo Sci. Icap Q (Bremen, Germany) ICP-MS at the Arkansas statewide mass spectrometry facility. The ICP-MS was operated in KED mode to remove polyatomic interferences. Elemental levels were calculated using a multi-element calibration standard run in sequences with the samples. The detection limit for lead was at 0.07 parts per billion.

Total Solids Analysis. The remaining water from the metal analysis was used for total solids analysis. Briefly, the water samples were evaporated in the oven at 98°C overnight in beakers, which were weighed before and afterwards. The weight of solids was then divided by the water volume to calculate the total solids concentration.

Microbial Analysis. Following sonication of pipes to dislodge biofilm, the water was filtered through a 0.22 μm filter (Pall Corporation, Port Washington, NY). DNA was extracted from the filter with a soil DNA extraction kit (Power Soil DNA Isolation Kit, Mo-Bio, Carlsbad, CA). The protocol recommended by the manufacturer was followed.

16S rRNA qPCR. Bacteria concentration was quantified using quantitative polymerase chain reaction (qPCR). The 16S ribosomal RNA universal bacterial primer set 1392R, 5'-ACGGGCGGTGTGTAC-3' and 1055F, 5'-ATGGCTGTCGTCAGCT-3 was used to target universal bacteria genes. Each 20 μL qPCR reaction mixture contained SYBR Green Supermix (SsoAdvanced Universal SYBR Green Supermix10, Bio-Rad Laboratories, Hercules, CA), 10 μM forward primer, 10 µM reverse primer, up to 10 ng DNA template, and nuclease free water as needed. G-block gene fragments with a size of 353 bp were purchased from Integrated DNA Technologies (Skokie, Illinois) as gene standards. Duplicate standard curves were included in every qPCR plate with gene concentrations ranging from 2.58 to 2.58×10^6 gene copies per microliter. Thermal cycling (CFX Connect Real Time System, Bio-Rad Laboratories, Hercules, CA) consisted of initial denaturing at 95ºC for 5 minutes and 35 cycles of the following: denaturing at 95ºC for 30 s, annealing at 60ºC for 60 s, and extension at 72ºC for 60 s. Each qPCR reaction had one negative control containing nuclease-free water in lieu of DNA template.

DNA Sequencing. Following extraction, DNA was submitted for next generation sequencing. Sequencing and data analysis was performed according to the procedure used by Walden, Carbonero & Zhang, 2017. Briefly samples were processed with Illumina MiSeq and subsequently analyzed in Mothur following the standard operating procedure for 16S rRNA gene amplicons (Kozich et al., 2013). Subsequent output files were graphed using Microsoft Excel 2013 (Microsoft Corporation, Redmond, WA). Chao1 and Shannon index were both calculated using PAST software v 3.20 (Hammer et al., 2001). A non-metric multidimensional scaling (MDS) plot was also generated in PAST.

III. RESULTS & DISCUSSION

Water Quality Parameters

Various water parameters were measured during the course of the experiments at the A.B. Jewel Plant and the Mohawk Plant. Quarterly averages of each parameter for the A.B. Jewel Plant are shown below in Table 1. Over the course of the experiment conducted at the A.B. Jewel Plant, pH trended downward from 8.4 to 7.8. Alkalinity, hardness, and conductivity all increased over time. Turbidity, chlorine residual and Langelier Index were both consistent during the experiment.

Table 1: Average water quality parameters at A.B. Jewel Plant from Day 1 through Day 279 of the experiment.

	pH	Water Temperature (C°)	Alkalinity (mg/L of $CaCO_3$)	Hardness (mg/L of $CaCO_3$)	Turbidity (NTU)	Conductivity (ms/cm)	Total Cl_2 Residual (mg/L)
Day 1-Day 90	8.22	11.19	99.34	108.82	0.06	234.39	2.80
Day 91-Day 180	7.89	21.33	108.32	128.40	0.09	308.70	2.70
Day 181-Day 270	7.72	27.93	117.28	136.72	0.08	326.48	2.74
Day 270-279	7.79	22.07	124.23	144.35	0.06	342.92	2.71

Effluent water samples were collected at various frequencies during the course of experiment. Recoverable and dissolved lead were measured in each pipe train over the course of the experiment. Recoverable lead includes both particulate and dissolved lead. Dissolved lead and pH measurements over time are shown below in Figure 4.

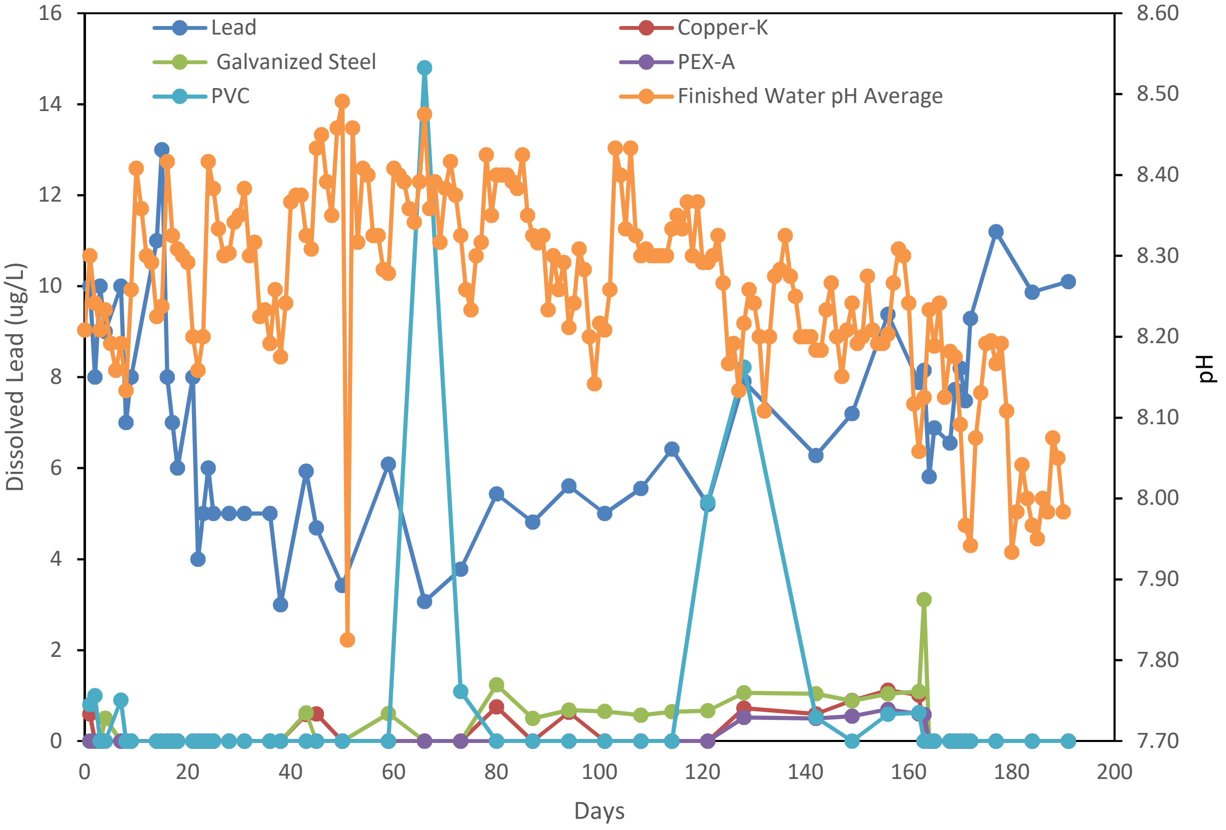

Figure 4: Dissolved lead in each pipe train at the A.B. Jewel Plant overlaid with pH in simulated distribution system.

During the experiment, effluent from the lead pipe train had the greatest amount of dissolved lead with a maximum of 324 µg/L and an average of 26.94 µg/L. Effluent from the PEX-A train had the lowest amount of dissolved lead with a maximum of 29.6 µg/L and an overall average of 2.04 µg/L. The order of dissolved lead for the A.B Jewel treatment plant from greatest to least amount of dissolved lead was as follows: Lead>Galvanized Steel>PVC >Copper-K>PEX-A.

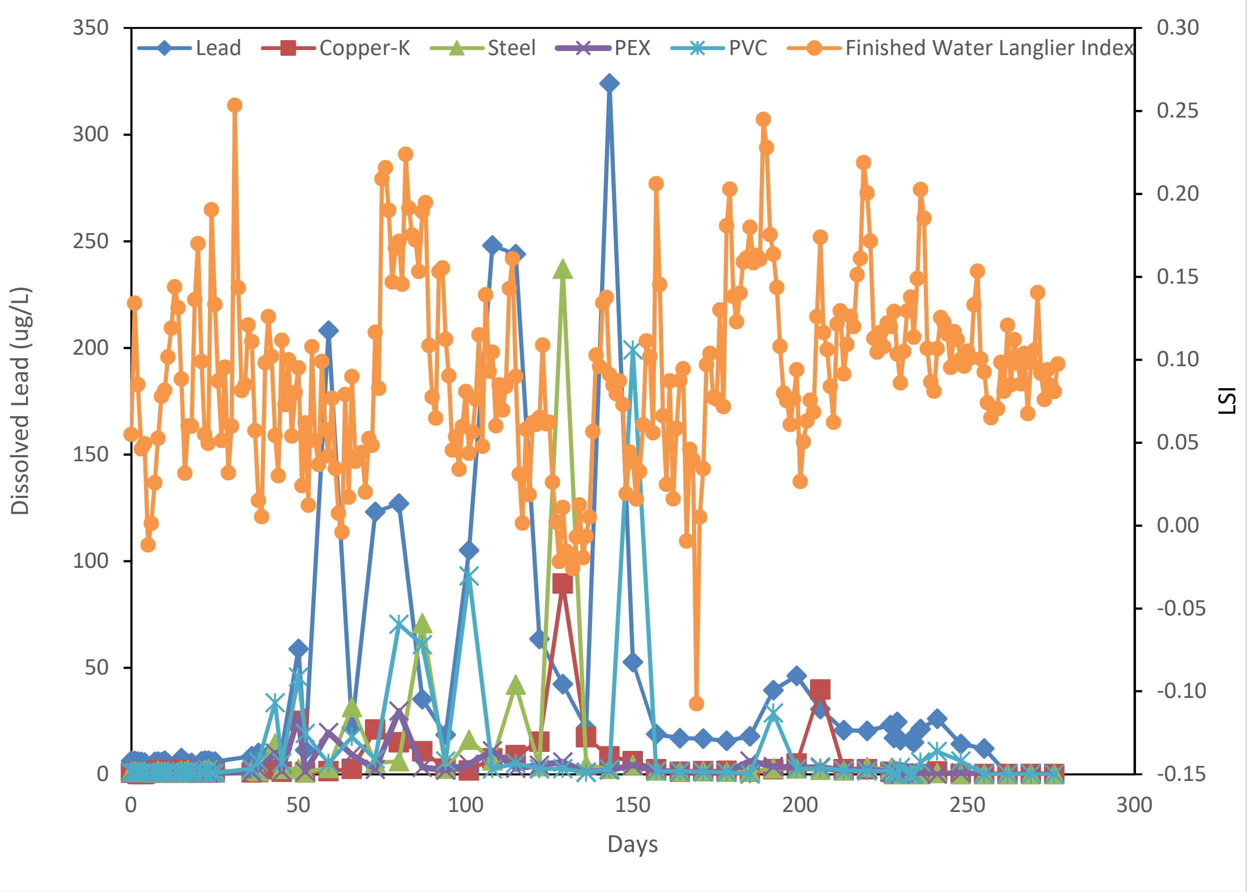

Figure 5: Dissolved lead in each pipe train at the A.B. Jewel Plant overlaid with Langelier Saturation Index in a simulated distribution system at A.B. Jewel Plant.

Over the course of the experiment, the LSI was calculated using water quality parameters from above. As can be seen from Figure 5, the treated water at the A.B. Jewel plant was consistently non-corrosive. Additionally, it does not appear as if there is a relationship between LSI and dissolved lead in this experiment.

In the Mohawk Plant's simulated distribution system, water quality parameters were also recorded for the full duration of the experiment. Quarterly averages of each parameter are shown below in Table 2. During the course of the Mohawk Plant simulated distribution system experiment, pH had a slight downward trend from 8.4 to 8.0. Conductivity and temperature increased slightly, while alkalinity, hardness, turbidity and chlorine residual were relatively stable.

Table 2: Average water quality parameters at the Mohawk Plant from Day 1 through Day 193 of the experiment.

	pH	Water Temperature (C°)	Alkalinity (mg/L)	Hardness (mg/L)	Turbidity (NTU)	Conductivity (ms/cm)	Total Cl_2 Residual (mg/L)
Day 1-Day 90	8.31	9.39	90.56	89.49	0.04	217.30	2.86
Day 91-Day 180	8.26	11.81	93.96	96.47	0.04	227.89	2.90
Day 181-Day 193	8.05	23.18	90.00	94.64	0.04	231.59	2.84

Effluent water samples were collected at varying frequencies from each pipe train and measured for recoverable and dissolved lead from day 0 through day 193 at the Mohawk Plant. The corresponding graph of the dissolved lead and pH is shown in Figure 5. In the Mohawk Plant experiment, water from the lead pipe train had the highest average amount of dissolved lead at 6.96 µg/L. The highest maximum amount of dissolved lead was measured in water from the PVC pipe train at 14.8 µg/L. The water from the PEX-A pipe train had the lowest amount of dissolved lead with an average of 0.07 µg/L. The order of dissolved lead for the Mohawk treatment plant from greatest to least amount of dissolved lead was as follows: Lead>PVC>Galvanized Steel>Copper-K>PEX-A, which varied slightly from the AB Jewel results.

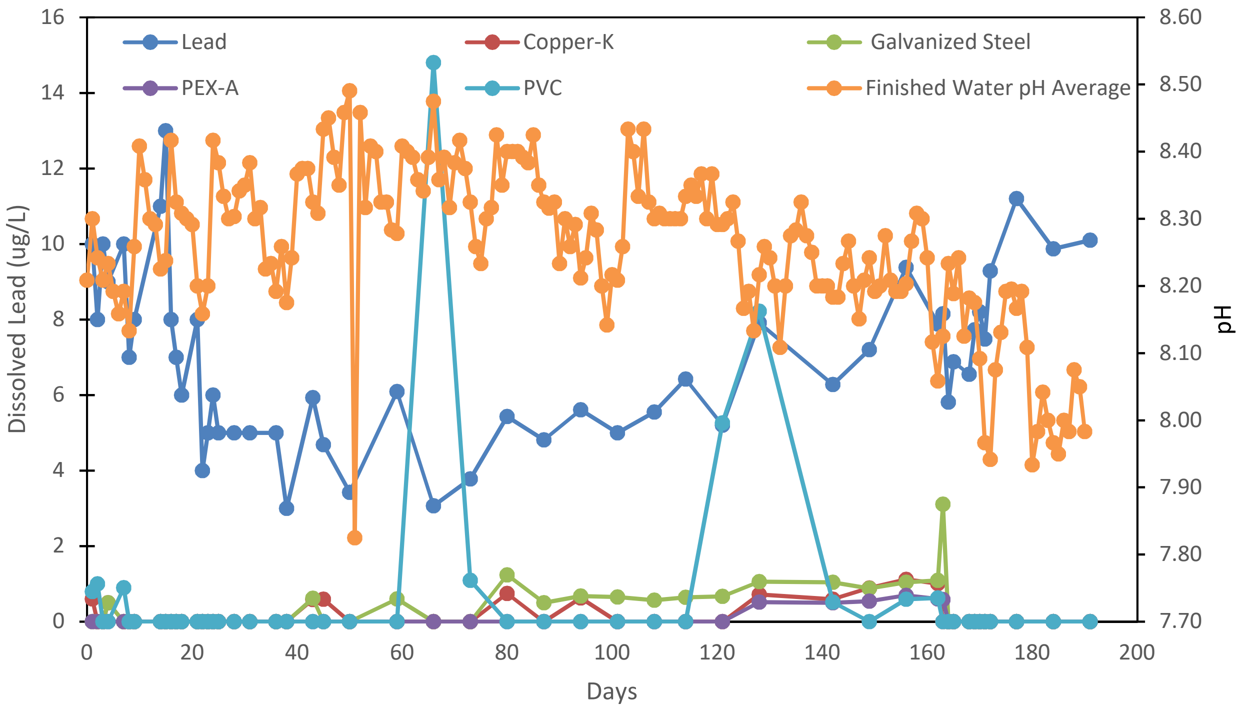

Figure 6: Dissolved lead in each pipe train at the Mohawk Plant overlaid with pH in simulated distribution system at Mohawk plant.

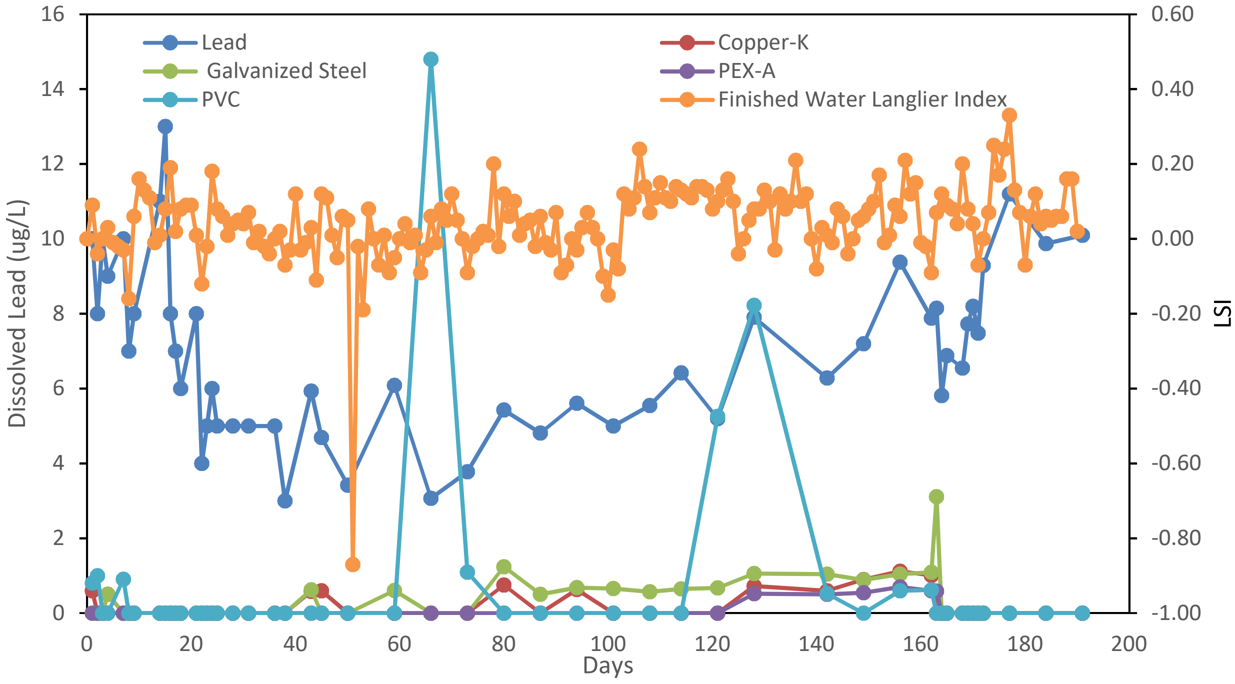

Figure 7: Dissolved lead in each pipe train at the Mohawk Plant overlaid with Langelier Saturation Index in a simulated distribution system at Mohawk plant.

The LSI was also calculated during the experiment at the Mohawk Plant. Over the course of the experiment, the treated water, with the exception of a single day, was relatively non-corrosive. As mentioned, there was a single point on day 107 where the water was below the -0.5 threshold and therefore deemed corrosive. Similar to the results from the experiment at the A.B. Jewel plant, the LSI does not appear to play a major role in the amount of dissolved lead being released into the simulated distribution system.

The differences in water quality at the two Tulsa water treatment plants affected the simulated distribution system experiments, especially the water temperature. The A.B. Jewel experiment was run primarily in the spring and summer and the Mohawk experiment was done mainly in the winter and spring. The A.B Jewel project had an average temperature of 20.98º C whereas the Mohawk project had a much lower average temperature of 12.10 ºC. The low temperature slowed down the biofilm formation within the simulated distribution system, which will be discussed in detail later. In addition, other water quality parameters also affected the experiments. For example, the water at the A.B. Jewel Plant had higher hardness, turbidity, and conductivity, but lower pH, resulting in the higher amount of dissolved lead. Furthermore, the type of pipe material appears to have played an impact in the amount of dissolved lead. The ranking of pipe materials regarding dissolved lead concentration within pipe effluent was similar comparing the two simulated distribution system experiments. The only difference was that in the A.B. Jewel Plant, the galvanized steel pipe train had a higher average dissolved lead than the PVC pipe train. High lead levels in galvanized steel and PVC coincides with other research indicating that both pipe materials can release dissolved lead (Lasheen et al., 2008; Clark et al., 2015). In PVC manufacturing, the process of stabilization introduces salts and heavy metals, including lead, which creates a point source. Galvanized steel has a zinc coating that can contain up to 1% of lead. The Copper-K pipe train’s average dissolved lead came in at second lowest overall. This result was surprising since in previous experiments galvanic corrosion accelerated lead release in copper-lead service lines (Wang, Jing et al., 2012). This unexpected result may have been due to the fact that the two dissimilar metals were not directly touching, as they were connected by XX. However, other research has shown that even with lead and copper not directly touching, galvanic corrosion still occurs even if at a reduced rate (Clark et al., 2013).

Metal Accumulation in Biofilms.

Accumulation of lead by biofilms occurred in all pipe materials at the A.B. Jewel Plant (Figure 8). Biofilms in the Lead pipe train adsorbed the greatest quantity of lead of all the pipe trains, with a maximum amount of 19.05 µg/cm^2 and an average accumulation of lead at 8.57 µg/cm^2. Biofilms within the PVC pipe train had the lowest amount of lead accumulation, with an overall average of 1.35 µg/cm^2. Prior to lead source pipe removal, no visible trend was observed in all trains regarding lead accumulation within biofilms. However, following the removal of the lead source pipe on day 228, all pipe trains saw an increase of accumulated lead. The biggest increase occurred in the Galvanized Steel pipe train, in which the lead accumulation increased over 1000x fold.

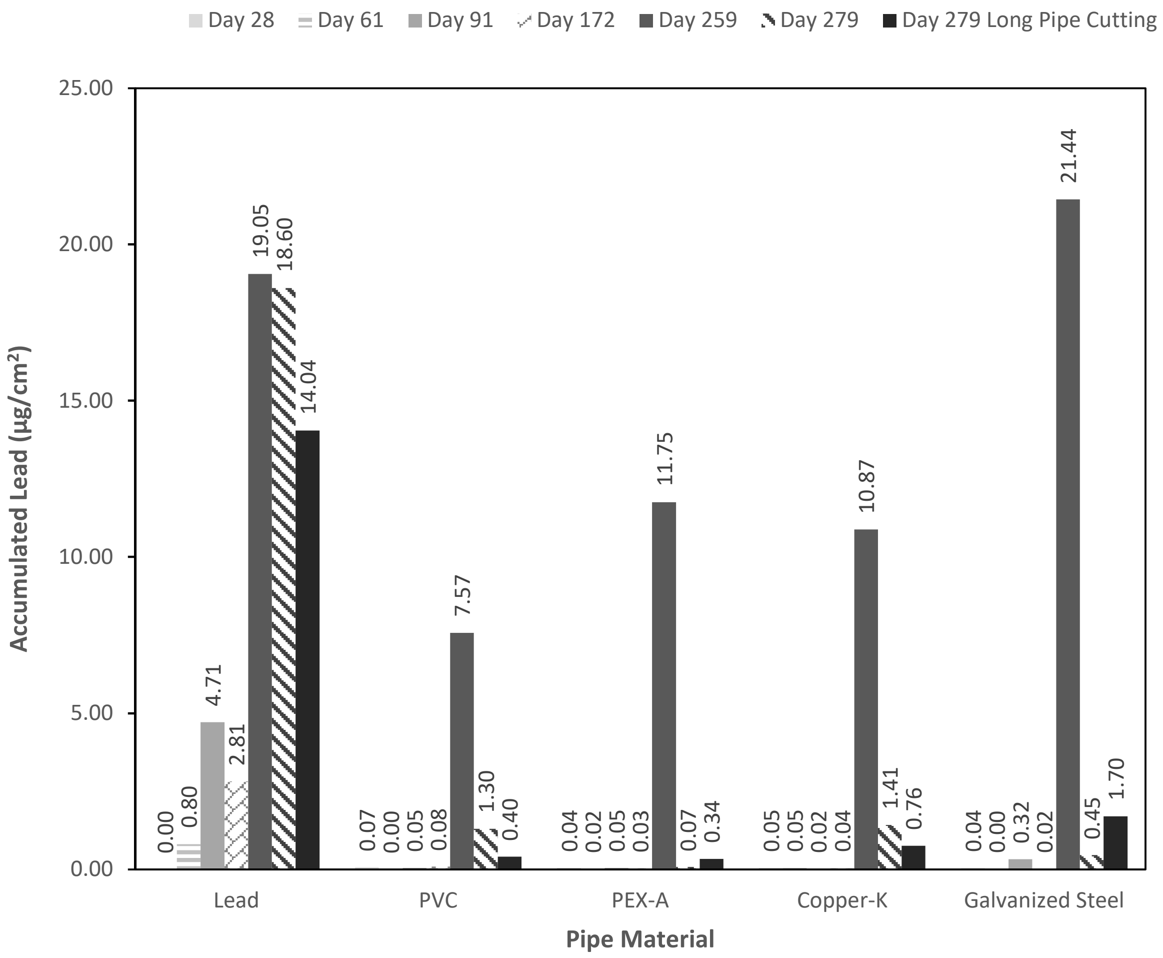

Figure 8: Lead accumulation in biofilms in different pipe materials in the simulated distribution system experiment at A.B. Jewel Plant.

In the Mohawk Water Treatment Plant's simulated distribution system, lead also accumulated in biofilms from all trains. Quantity of lead accumulation in biofilms from each train is shown in Figure 9). Biofilms within the lead pipe train had the greatest accumulation of lead with a maximum amount of 25.22 µg/cm^2 and an average at 8.07 µg/cm^2, which is comparable with the result from A.B. Jewel Plant. The pipe train with the lowest biofilm accumulation of lead was the Copper Type K train, with an average value of 0.06 µg/cm^2. Following the removal of the lead source pipe, the lead accumulation varied in different materials. Biofilms from the Copper Type K and Galvanized Steel trains both saw an increase in lead, both of which exceeded a doubling of previously accumulated lead. Lead level decreased within biofilms from the PEX-A pipe train following the removal of the source pipe. Lead concentration in biofilms from the PVC pipe train fluctuated over time.

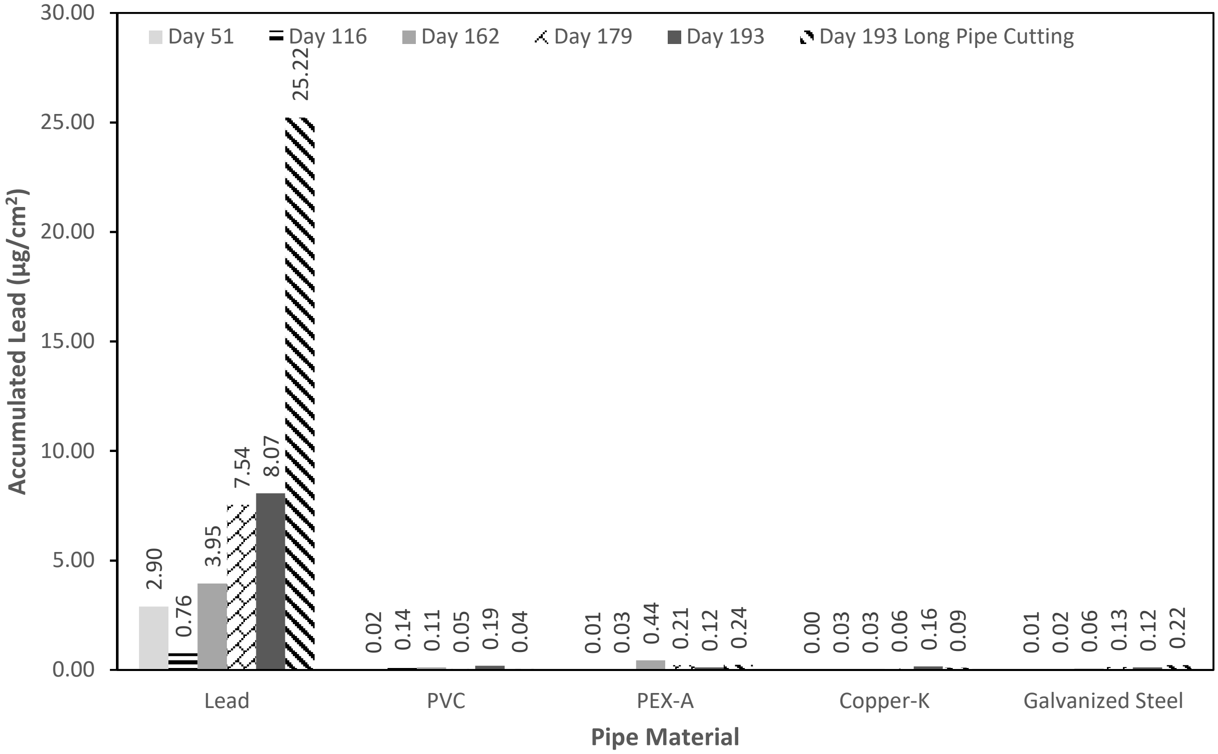

Figure 9: Lead accumulation in biofilms in different pipe materials from the simulated distribution system experiment at Mohawk Water Treatment Plant.

In simulated distribution system experiments at both the A.B. Jewel Water Treatment Plant and the Mohawk Water Treatment Plant, biofilm grew on all five of the tested pipe materials and adsorbed lead. After the removal of the lead source pipe, lead was still found in all biofilms. Accumulated lead levels immediately increased following the removal of the source pipes, but then generally decreased. In the non-lead pipe trains, six of the eight pipe trains saw an increase in lead adsorption by biofilm following the removal of the lead source pipe. Spikes in lead levels have been previously reported following the replacement of lead service lines (Trueman et al., 2016; Brown & Cornwell, 2015). This was believed to be a result of pipe disturbances in the process of lead pipe removal. While both experiments saw some increase in the accumulated lead, the A.B. Jewel simulated distribution system saw a much more drastic increase after the lead source pipe was removed. We hypothesize that due to the higher dissolved lead levels during the A.B. Jewel experiment, more scale was deposited onto the pipe walls. The scale was then disturbed during the removal of the lead source pipes which caused the drastic spikes. In addition, the largest pH drop of the experiment occurred at the A.B. Jewel plant around the time of source pipe removal. Prior research has observed that lowering pH correlates with the dissolution of lead scale, which is what most likely occurred during this experiment (Xie et al., 2010). The subsequent gradual decrease in accumulated lead after the source pipe removal indicates that lead was being removed from the biofilm by either desorption or biofilm degradation. Overall, the accumulated lead in the biofilm represents a previously unrecognized lead source in drinking water, posing a potential threat to the public health. Even considering a small amount of accumulated lead such as 0.1 $\mu g/cm^2$ would represent over 450 µg of stored lead in a very short water service line (25 feet, 3/4" ID). To the best of the authors' knowledge, this study is the first to provide evidence that biofilm can be a source of lead contamination in DWDSs.

Bacteria Quantification in Biofilms

The biofilms accumulated on all pipe coupons were analyzed for the concentration of universal bacterial genes (16S rRNA) and normalized by the surface area of the pipe coupon. In the simulated distribution system study at the A.B. Jewell Plant, bacteria concentration ranged from 1.0×10^3 to 3.0×10^5 gene copies per cm^2 (Figure 10). Pipe coupons used to determine bacteria concentration were collected on days 28, 61, 91, 172, 259, 279 of the experiment. Following the last pipe coupon removal of the final coupon from the system two cuttings were taken from the simulated distribution system and are labeled as Long Pipe Cutting 1 & 2.

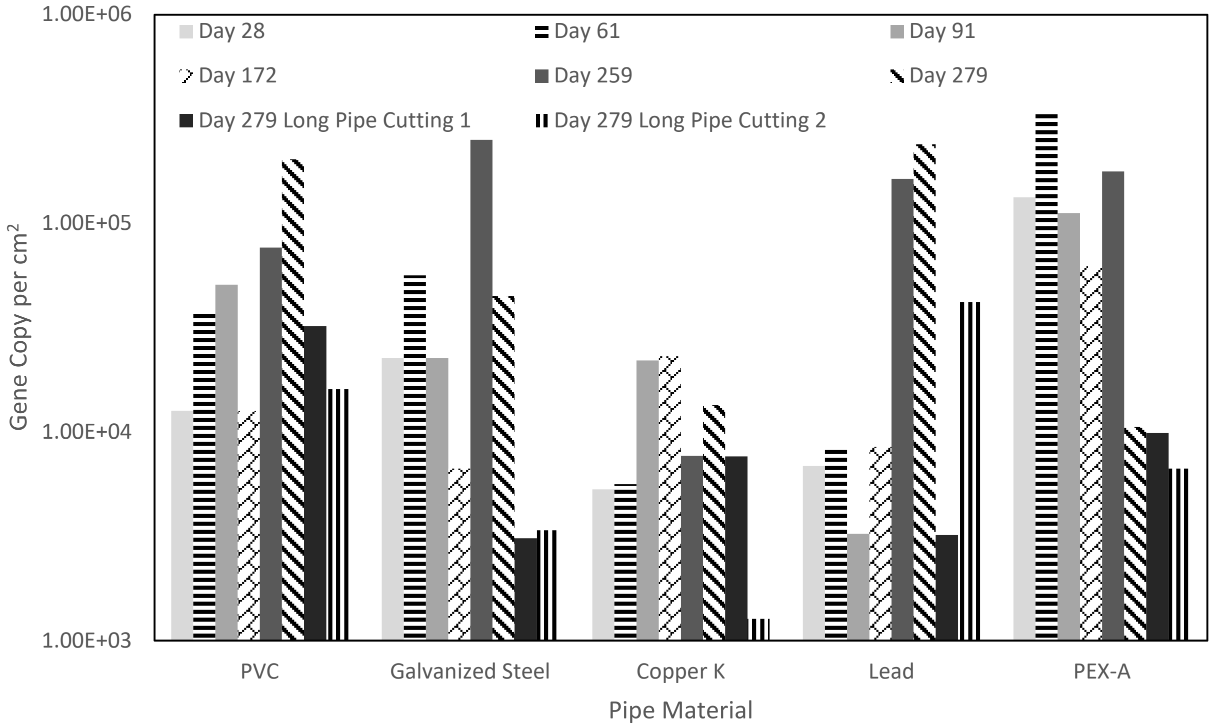

Figure 10: Bacteria concentration in biofilms accumulated on pipe coupons from different pipe materials at A.B. Jewell plant, including two long pipe cuttings from each material on day 279.

In the simulated distribution system at A.B. Jewell Plant, biofilms grown in the Copper Type K pipe train accumulated the lowest quantity of bacteria, with amounts ranging from 5.3×10^3 to 2.7×10^4 gene copies per cm^2 and an average of 1.1×10^4 gene copies per cm^2 over the course of the experiment. The PEX-A pipe train had the highest quantity of biofilm with bacteria concentration ranging from $6.2x10^4$ to $3.4x10^5$ gene copies per cm^2 and an average of $1.1x10^5$ gene copies per cm^2 over the course of the study.

Bacteria concentration from pipe coupons was analyzed in the same fashion for the simulated distribution system experiment at the Mohawk Plant, ranging from 2.48×10^3 to 5.90×10^5 gene copies per cm^2 (Figure 11). Pipe coupons used to determine bacteria concentration were collected on days 51, 116, 162, 179, and 179 of the experiment. Following the last pipe coupon removal of the final coupon from the system a single cutting was taken from the simulated distribution system and are labeled as Long Pipe Cutting 1.

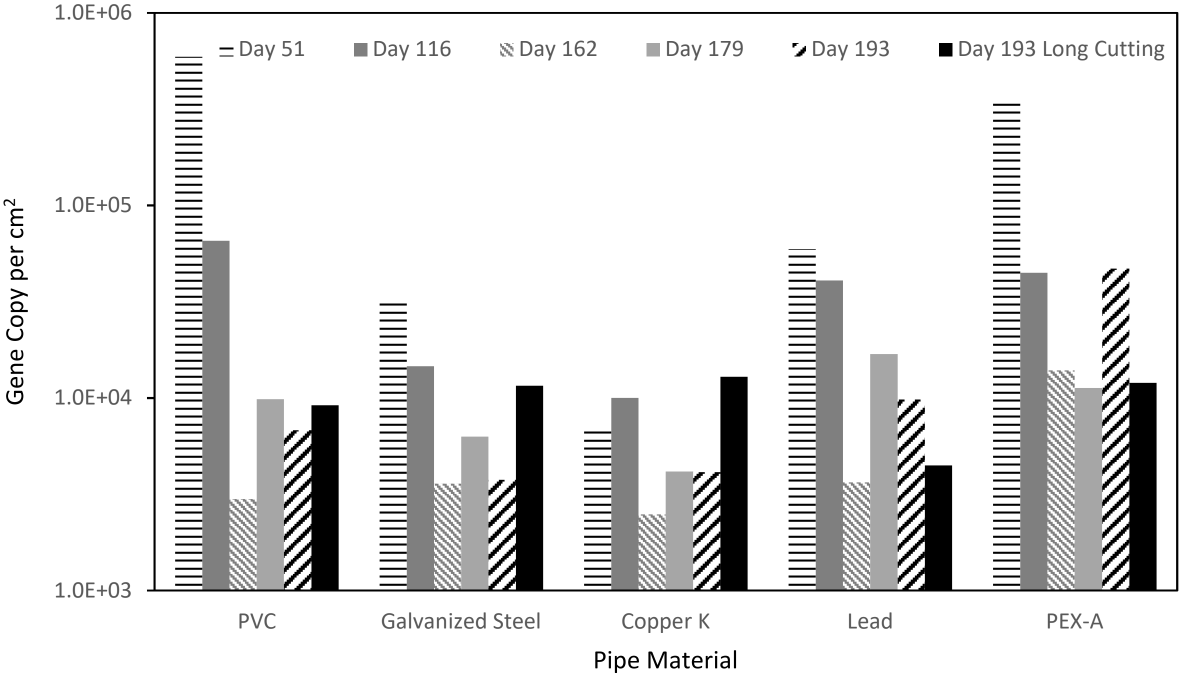

Figure 11: Bacteria concentration in pipe coupons from different pipe materials at the Mohawk Plant.

The Copper Type K pipe train also had the lowest bacteria concentration in the Mohawk plant ranging from 2.48×10^3 to 9.98×10^3 gene copies per cm^2, and an average of 5.93×10^3 gene copies per cm^2. The PVC pipe train had the highest bacteria concentration ranging from $2.98x10^3$ to $5.90x10^5$ gene copies per cm^2, and an average amount of $1.14x10^5$ gene copies per cm^2.

The measured values of gene copies per cm^2 were lower than previous studies (Liu et al., 2016, Ren et al., 2015). It is hypothesized that the lower gene copies are a result of the young biofilm age (several months in this study versus 10 to 11 years from real DWDSs in previous research). In addition, the simulated distribution system experiments used freshly treated drinking water, with the highest disinfectant residuals possible in their respective distribution systems. Biofilm reduction by monochloramine has been confirmed in all previous research (LeChevallier et al., 1988; Chang & Craik, 2012). In this study, the bacteria concentrations within biofilms not only differed with pipe materials, they also varied from the same pipe material within the two simulated distribution system experiments. This difference was likely due to a variety of different water quality parameters. For example, the average temperature in the Mohawk plant was 12.10ºC, whereas in the A.B Jewel plant the average temperature was 21.12 ºC. The findings of these studies agree with Hallam et al. (2001) in that lower temperature decreased biofilm activity. Other than temperature, pipe material appears to play a major role in biofilm formation, which was well established in water distribution pipes (Niquette et al., 2000; Yu et al., 2010; Jang et al., 2011). Results from this research showed no drastic differences in the bacteria concentration within biofilms from different pipe materials. This both agreed (Proctor et al., 2017) and disagreed that differing pipe materials have different bacterial concentrations(Ren et al., 2015). There were trends that were consistent in the two studies in terms of biofilm quantification. The plastic pipes (PEX and PVC) had the highest average amount of biofilm over the course of the study. Similar to our results, some studies indicate PEX has more biofilm growth than PVC (Rożej et al., 2014; Gamri et al., 2015), while others indicate the opposite (Yu et al., 2010). Previous studies indicate that various components, such as volatile organic compounds, can migrate from the plastic materials (Skjevrak et al., 2003; Kowalska et al., 2011 Ryssel et al., 2015), which may promote microbial growth in DWDSs (Schwarts et al., 1998; Van der Kooij et al., 2005). It has been found previously that copper pipes have less microbial growth than other pipe materials (Lehtola et al., 2004; Inkinen et al., 2014). This was supported in our study where the Copper-K pipes had consistently less biofilm than other pipe materials.

Microbial Community Analysis

A total of 700,210 high quality sequences were obtained from forty-five biofilm samples during the A.B. Jewel Plant simulated distribution system experiment. Overall sample coverage was 96.0%, which indicates that the sequencing captured the majority of unique operational taxonomic units (OTUs). Some samples did not have good coverage or had low sequences (<500) and as a result were not included in the results.

In the A.B. Jewel Plant simulated distribution system, there were a total of 23 phyla detected in biofilms collected from the pipe coupons. *Actinobacteria, Proteobacteria, Firmicutes* and Unclassified Bacteria comprised the majority of the bacterial communities (62.6-99.6%) in all samples.

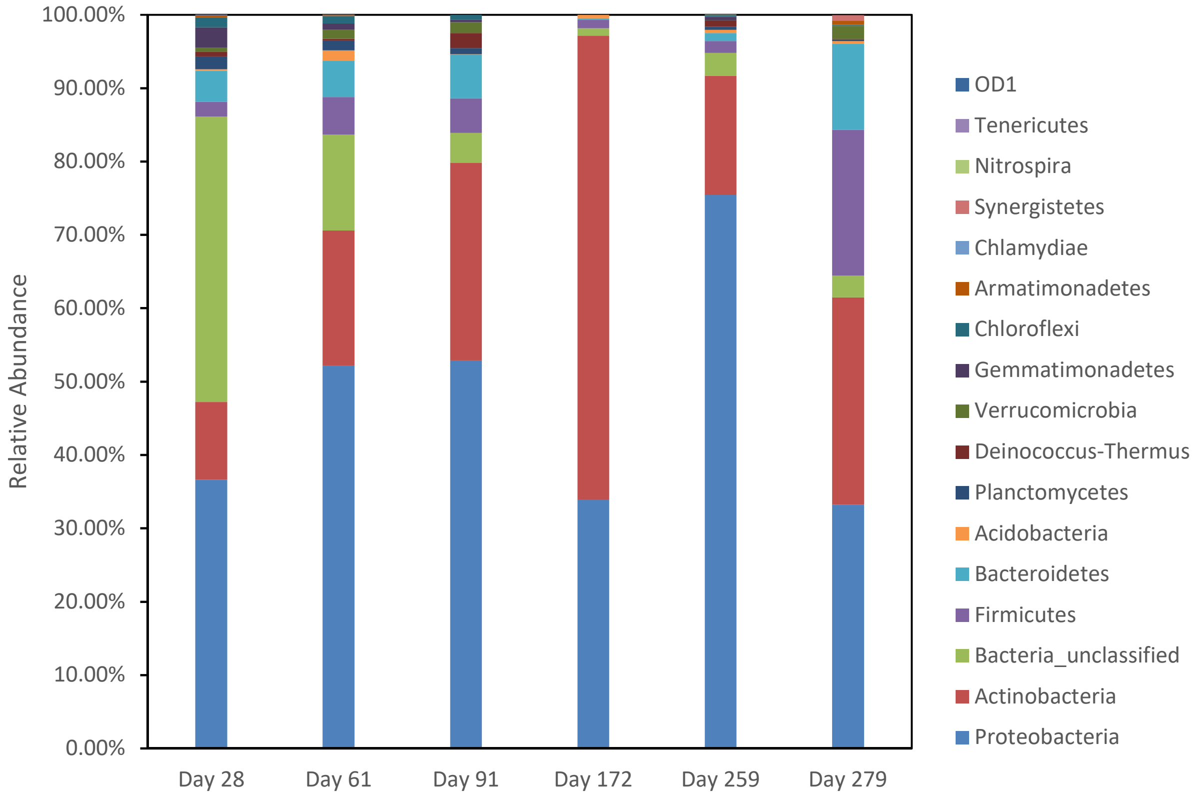

Figure 12: Composition of biofilm phyla in PEX-A pipe train at the A.B. Jewel Plant.

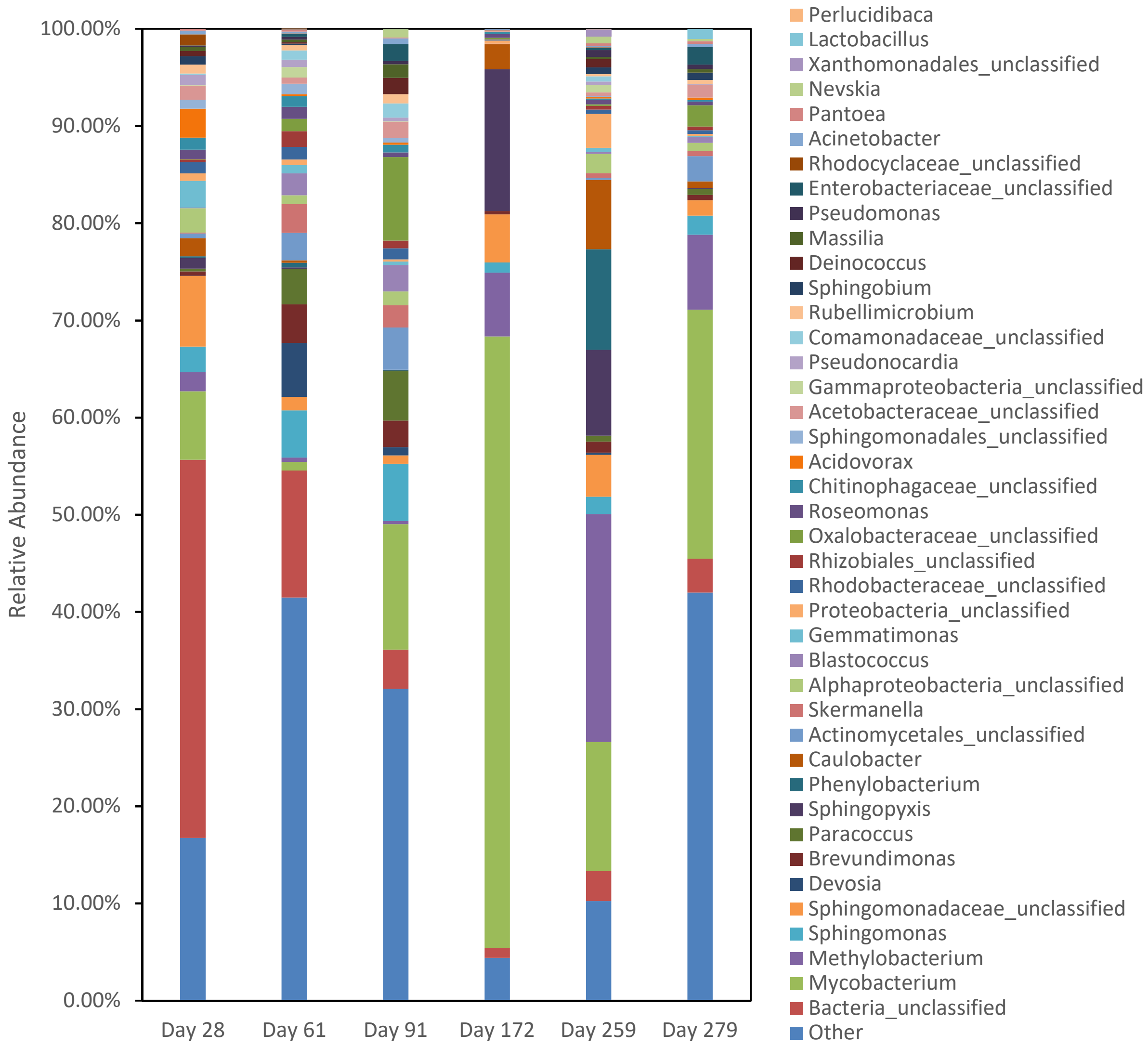

Figure 13: Composition of biofilm genera in PEX-A pipe train at the A.B. Jewel Plant.

During the course of the A.B. Jewel experiment, up to 22 phyla were detected in the PEX-A pipe train (Figure 12). The phyla present were consistent throughout the course of the experiment, with *Actinobacteria, Proteobacteria, Firmicutes* and Unclassified Bacteria representing the majority of the phyla. However, the number and relative abundance of phyla fluctuated over the course of the experiment. For the first two months, the total number of phyla was equal to 14. The total then steadily decreased until it reached its minimum in July of 2017 (after 172 days from the beginning of the experiment), with only 6 phyla detected. Following the lead source removal in September 2017 (228 days from the beginning of the experiment), the number of phyla increased to 12. *Firmicutes* increased by nearly 20% a month after the lead source removal.

The number of genera found in the biofilms at the A.B. Jewel experiment was high. Such high quantities of genera in this case results in low relative abundance from each genera. Therefore, any genera that represented less than 0.5% of the genera across all pipe materials was summed together and labeled "other". One notable point from the genera in the PEX-A pipe train include the fact that *Mycobacterium* drastically increased around day 172 before decreasing again following the lead removal on day 228.

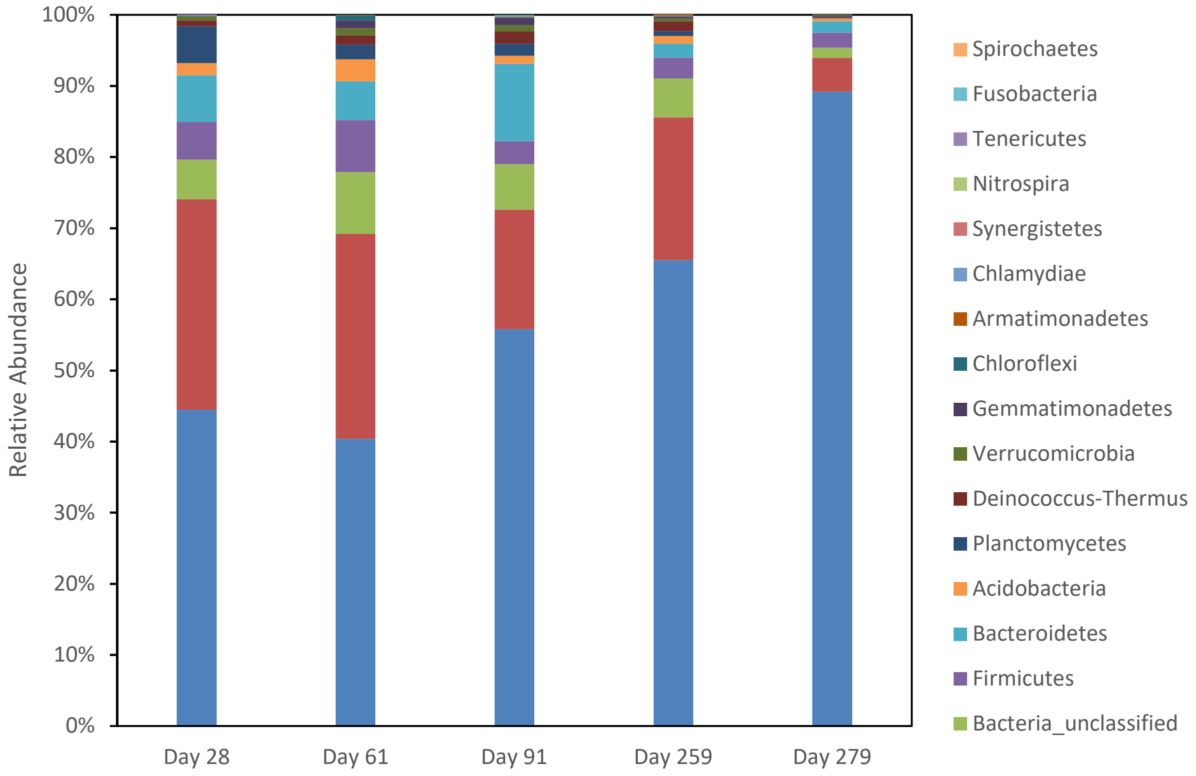

Figure 14: Composition of biofilm phyla in PVC pipe train at the A.B. Jewel Plant.

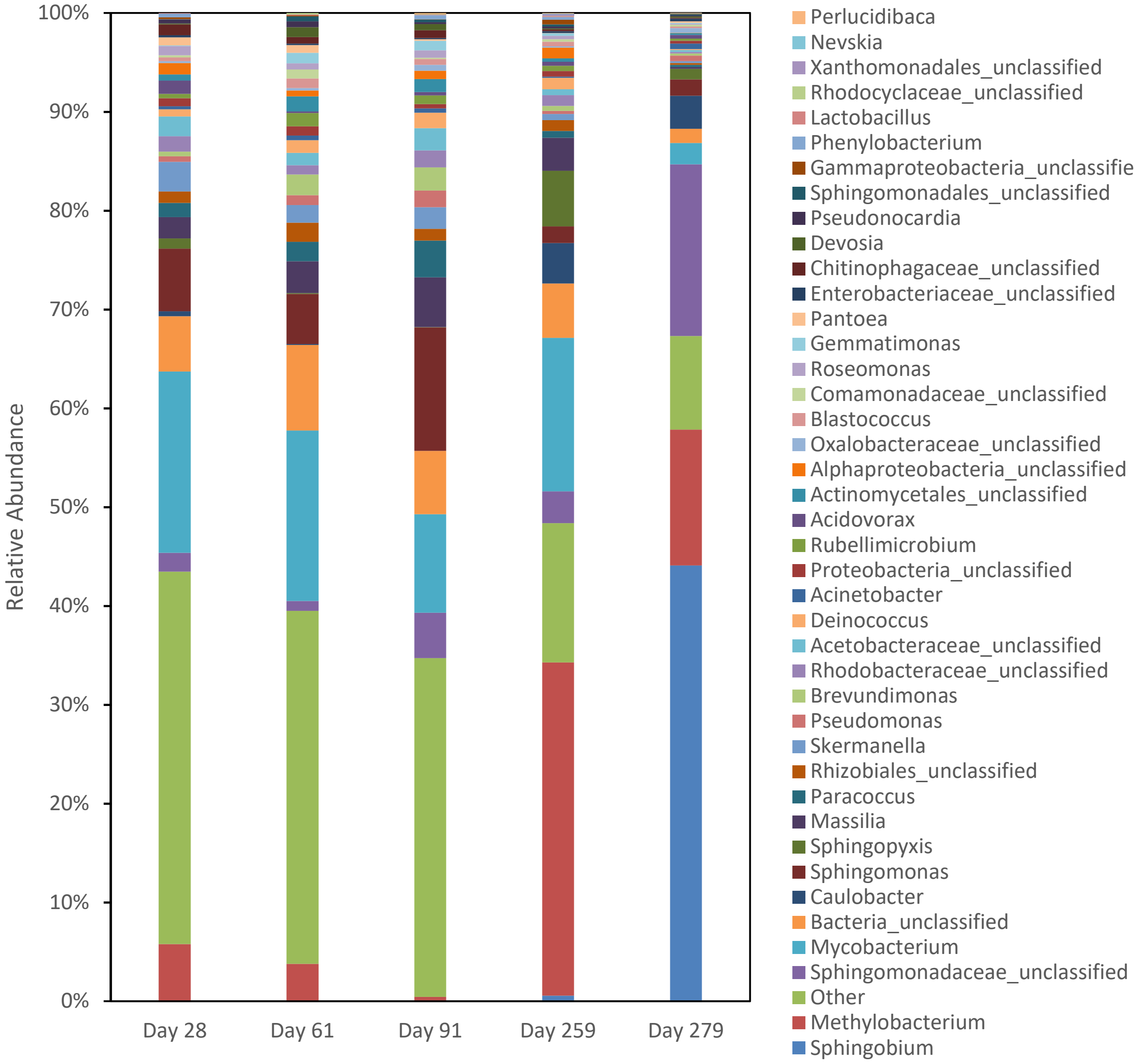

Figure 15: Composition of biofilm genera in PVC pipe train at the A.B. Jewel Plant.

In the PVC pipe train, 18 phyla were detected within biofilms throughout the experiment (Figure 14). Consistent with other pipe materials, the PVC pipe train was dominated by *Actinobacteria, Proteobacteria, Firmicutes* and Unclassified Bacteria. During the first three months of the simulated distribution system operating, the number of phyla increased from 13 to 14. The number of phyla briefly jumped to 16 following the removal of the lead source. The diversity of phyla then declined to an average of 12 phyla on the last sampling period. *Fusobacteria* and *Tenericutes* were both present in the pipe train before the removal of the lead source and were no longer present after its removal, whereas both *Spirochaetes* and *Synergistetes* were not present before lead source removal and appeared afterward.

Following the removal of lead on day 228there was a major shift in genera of the PVC pipe train (Figure 15). Both *Sphingobium* and *Methylobacterium* increased. *Sphingobium* represented a greater abundance in the PVC at the end of the project than any other pipe train.

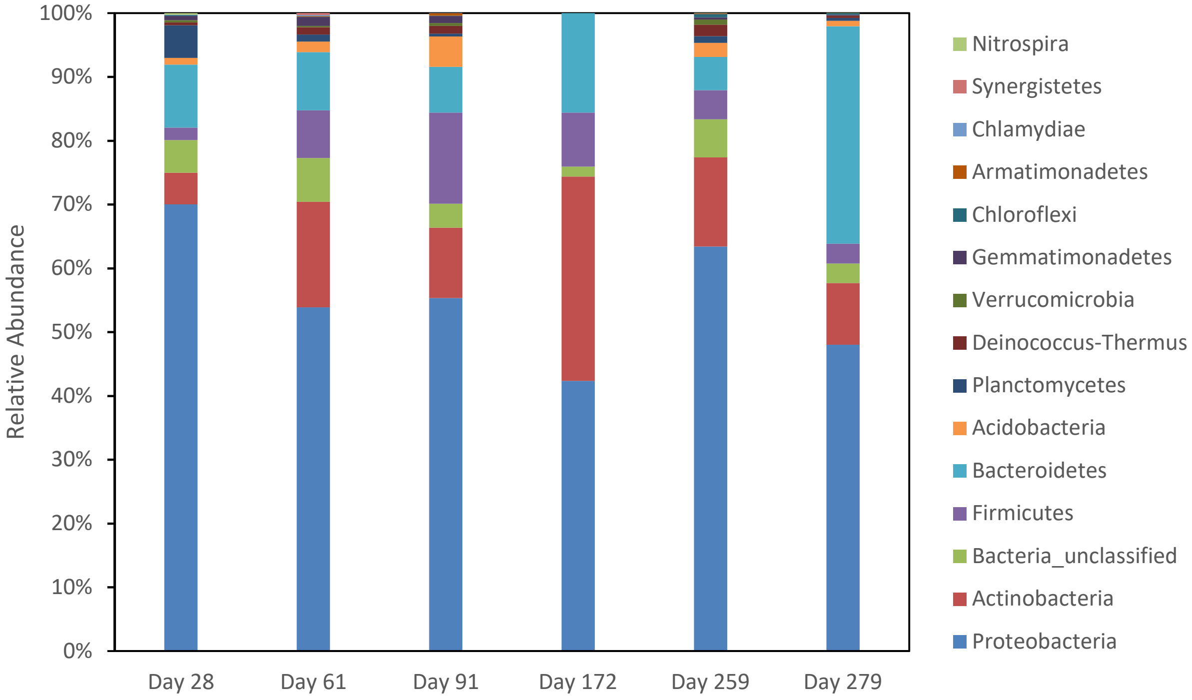

Figure 16: Composition of biofilm phyla in Galvanized Steel pipe train at the A.B. Jewel Plant.

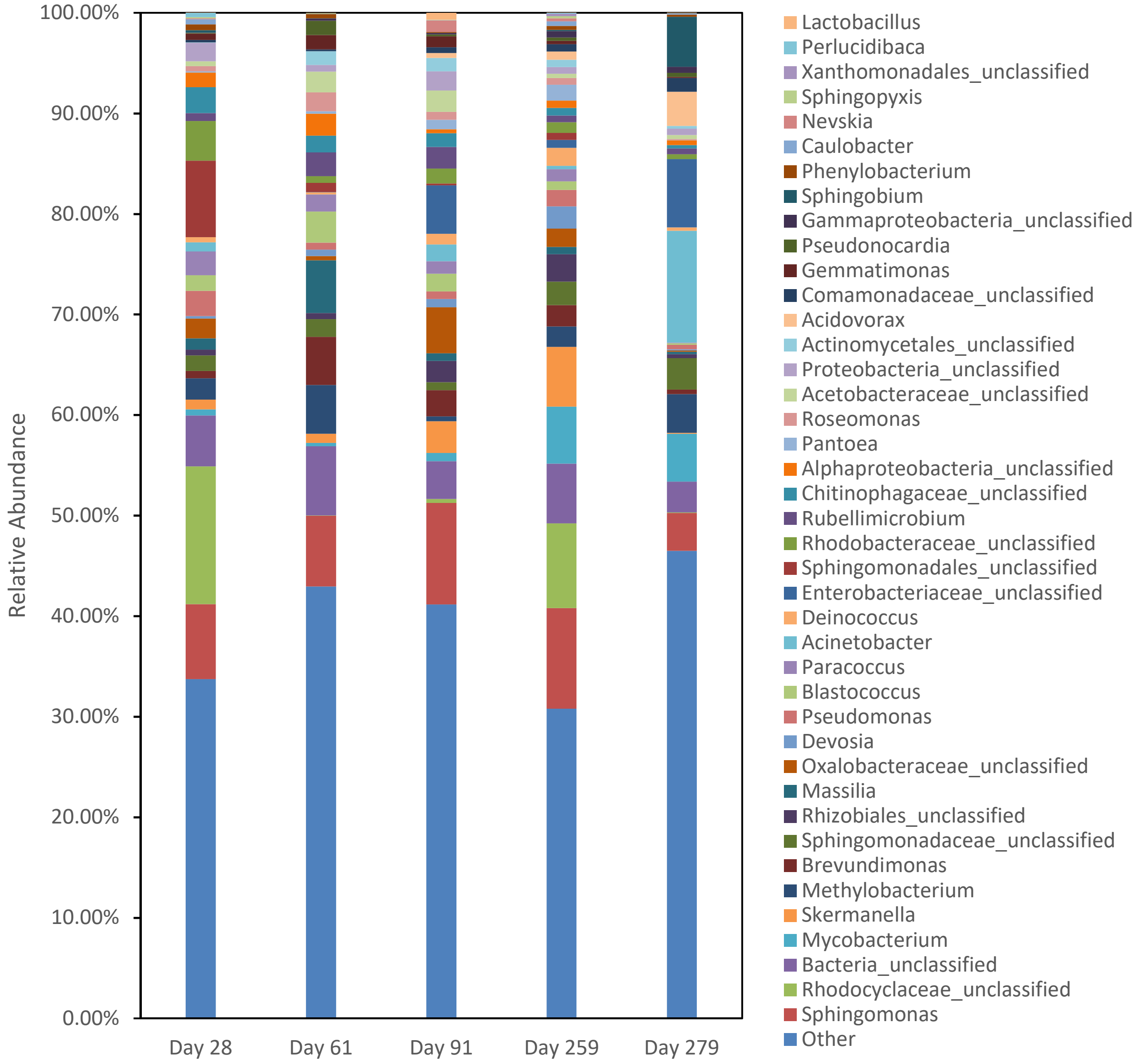

Figure 17: Composition of biofilm genera in Galvanized Steel pipe train at the A.B. Jewel Plant.

In the Galvanized Steel pipe train, the microbial community was comprised of 15 phyla (Figure 16). Galvanized Steel had the lowest total number of phyla represented in its pipe train as compared to other pipe materials. The community was dominated by a few phyla, namely, *Actinobacteria, Proteobacteria, Firmicutes* and Unclassified Bacteria. The relative abundance of several phyla shifted during the experiment. *Bacteriodetes'* average relative abundance rose 29% after the lead source pipe was removed. In addition, the average relative abundance of *Chloroflexi* more than doubled after removing the lead source pipe.

Among the genera in the galvanized steel, the *Mycobacterium* increased around 100-fold following the removal of the lead source pipes to 5.66% of the community (Figure 17).

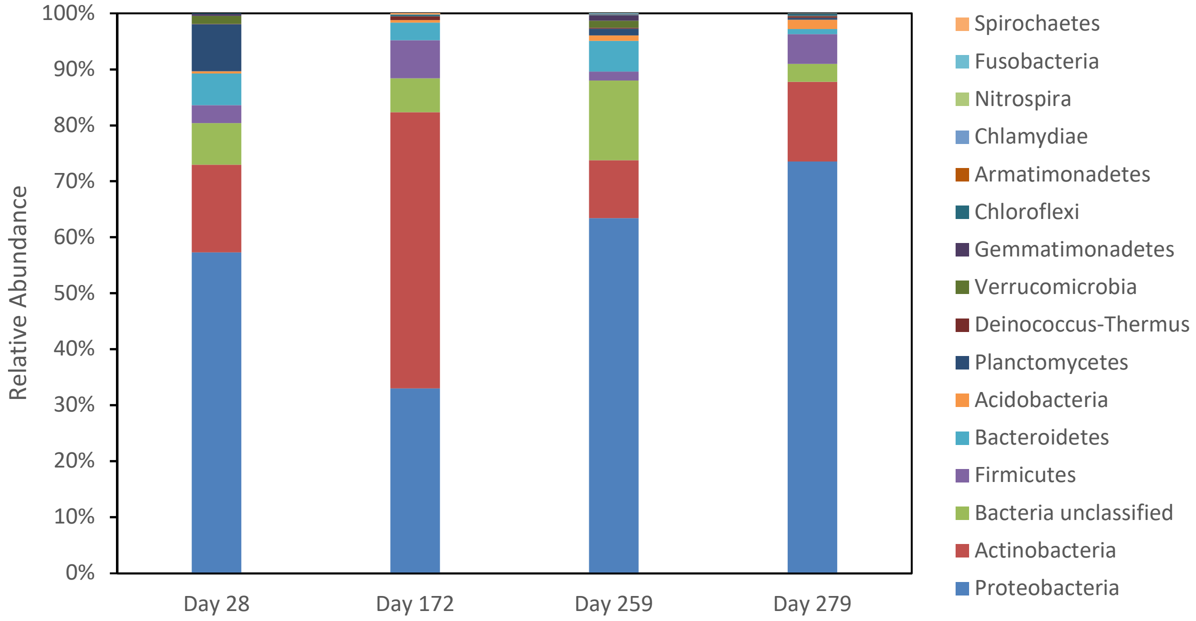

Figure 18: Composition of biofilm phyla in the Lead pipe train at the A.B. Jewel Plant.

The total number of phyla in the Lead pipe train over the course of the study was 17 (Figure 18). *Actinobacteria, Proteobacteria, Firmicutes* and Unclassified Bacteria were also the dominant phyla. Although the lead source pipe removal did not impact the presence of lead within this train, shifts in the community still occurred. *Acidobacteria* was low in relative abundance for the first three months. However, by the last month of the experiment, it represented the fifth most abundant phylum in the lead train.

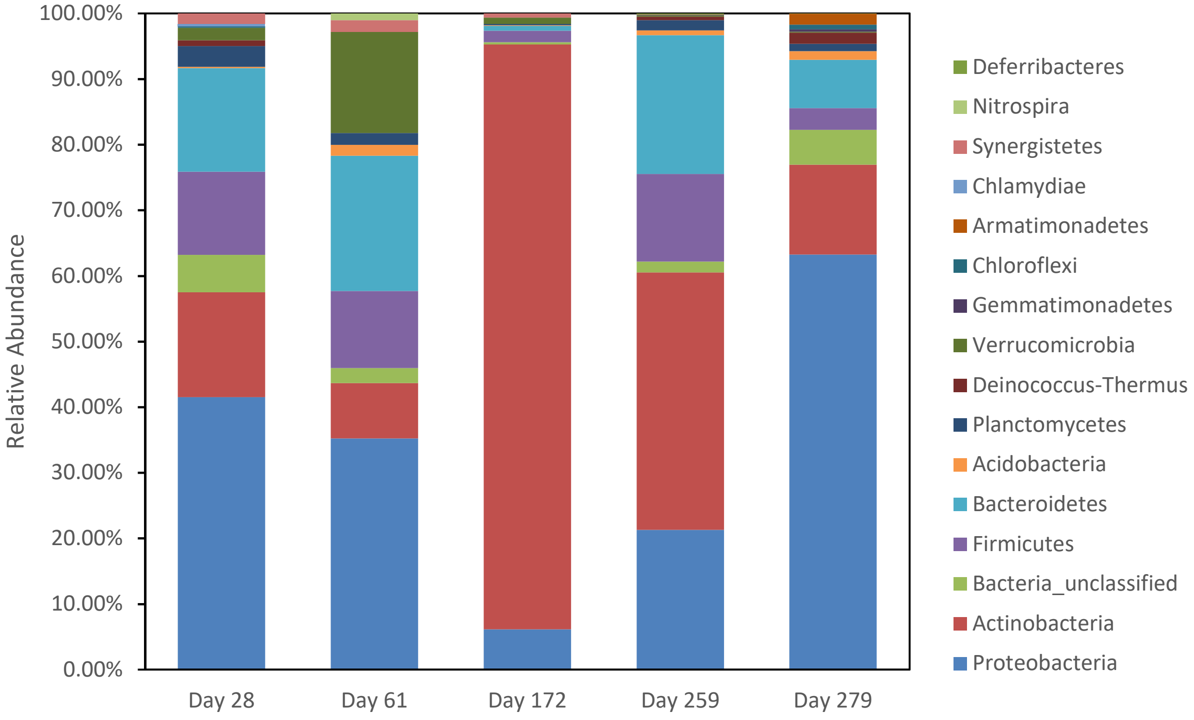

Figure 19: Composition of biofilm phyla in the Copper Type K pipe train at the A.B. Jewel Plant.

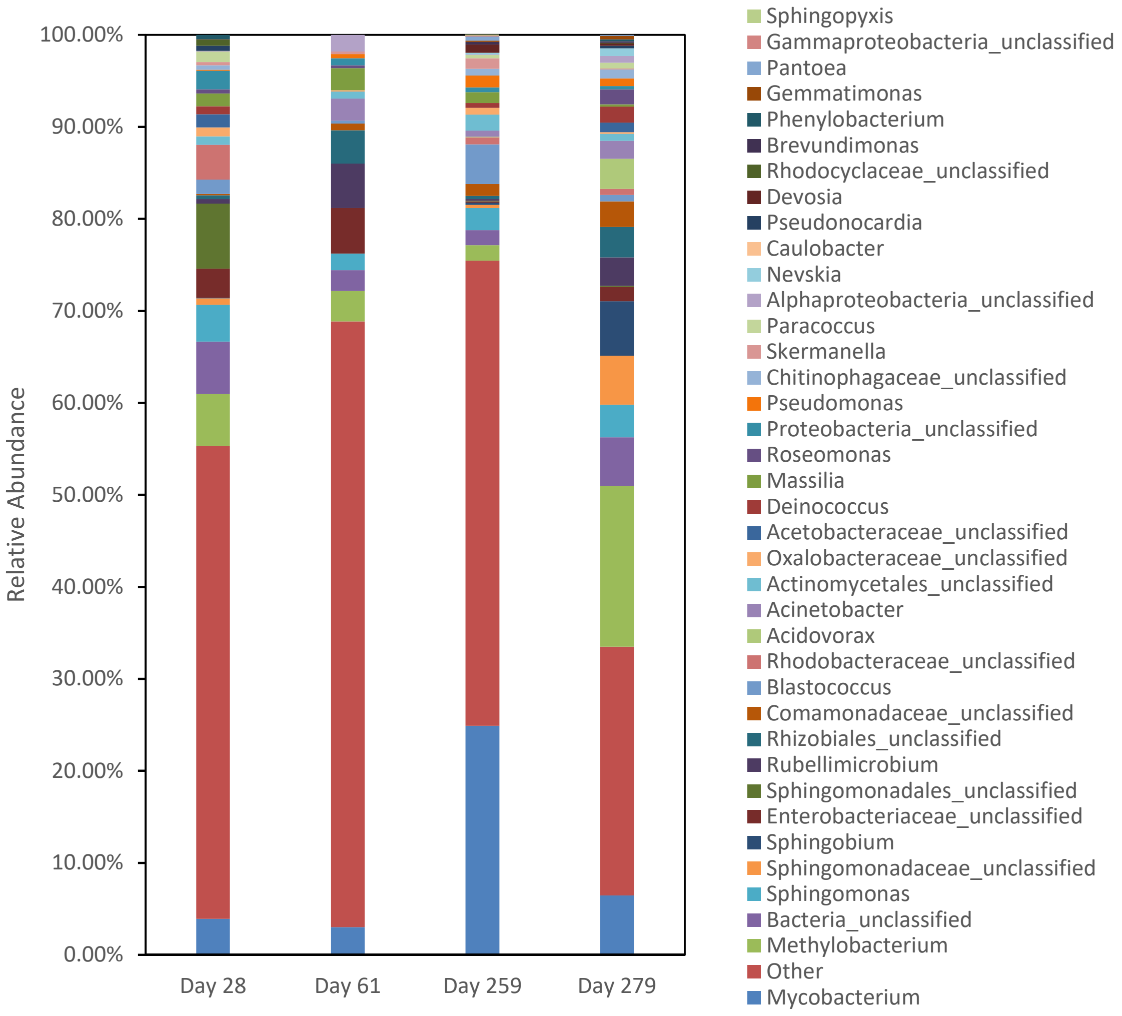

Figure 20: Composition of biofilm genera in the Copper Type K pipe train at the A.B. Jewel Plant.

In the Copper Type K pipe train, a total of 17 phyla were identified during the course of the study (Figure 17). Similar to other pipe trains, *Actinobacteria, Proteobacteria, Firmicutes* and Unclassified Bacteria were the four most abundant phyla. While *Proteobacteria* was the most abundant phyla in the Copper Type K pipe train, it was lower than other pipe materials, especially in the presence of the lead source pipe. The other pipe trains had a range of *Proteobacteria* average abundance from 42.4 to 59.7% in the presence of lead, while the Copper Type K only had an average abundance of 20.72%. Following the removal of the lead source, the Copper Type K pipe train's abundance of *Proteobacteria* saw a 15.2% increase. *Actinobacteria* decreased by 50% after the removal of the lead source. Several phyla appeared in the Copper Type K pipe train only after the lead source removal, namely, *Armatimonadetes* and *Deferribacteres. Mycobacterium* appears to have increased over fivefold after the removal of the lead source pipe, which is consistent with Galvanized Steel pipe train. Additionally, several genera appeared after the removal of lead that were not previously present, including *Acidovorax, Nevskia* and *Devosia.*

Overall, the dominant phyla in the study (*Actinobacteria, Proteobacteria* and *Firmicutes*) match numerous other studies on biofilms in DWDSs (Liu et al., 2012; Sun et al., 2014; Wu et al., 2014). No single phylum or genus appeared to dominate in different pipe materials. There were several genera that appeared to respond to the presence of lead. *Sphingobium* increased following the removal of lead in all non-lead pipe materials; and *Sphingomonadaceae Unclassified* increased in PEX-A, Galvanized Steel and Copper-K following the lead source removal. *Methylobacterium* increased in the two plastic materials. *Mycobacterium* increased in Copper-K and Galvanized Steel pipe trains.

Different diversities were also observed in different pipe trains temporally. Both the Shannon and Chao index were calculated and are shown below in Figure 21 and Figure 22. The Shannon index is used as a measure of diversity which takes into account both abundance and evenness. The Chao1 index is a which estimates the diversity by extrapolating based on the number of rare OTUs. The highest biofilm diversity occurred in the PEX-A pipe train on day 61 of the experiment with a Chao1 index of 356 and a Shannon index of 4.40. The lowest diversity occurred in the PEX-A pipe train on day 172 with a Chao1 index 51 and a Shannon index of 1.47. Some studies indicate higher levels of richness and diversity (Inkinen et al., 2017) whereas others are similar to our results (Lu et al., 2014) in simulated distribution systems.

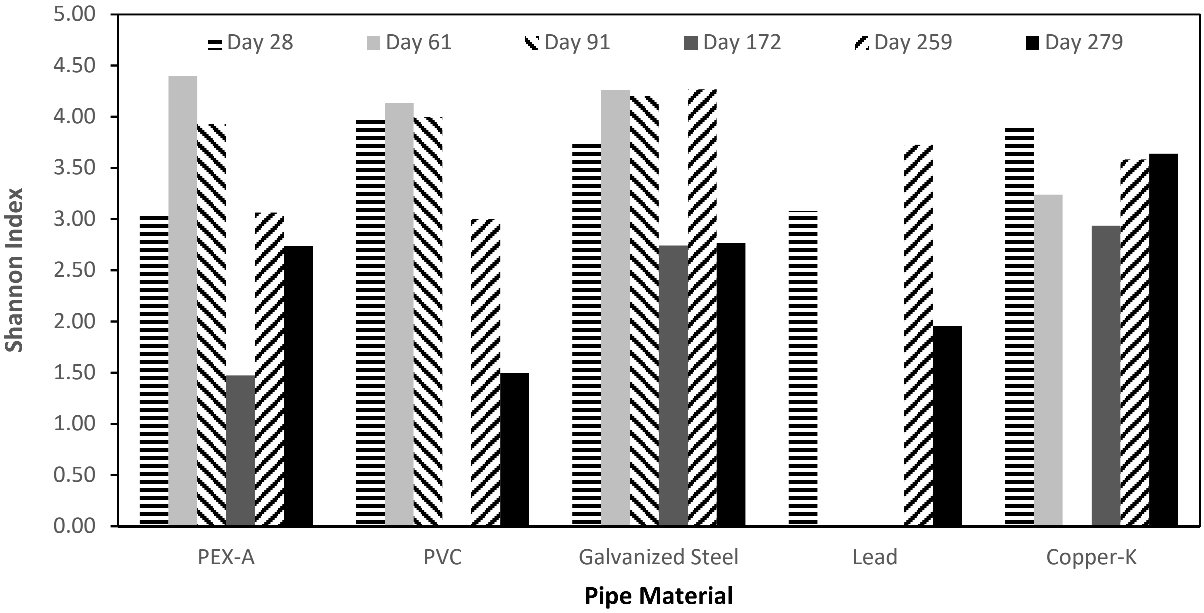

Figure 21: Shannon diversity index of biofilms from the A.B. Jewel plant.

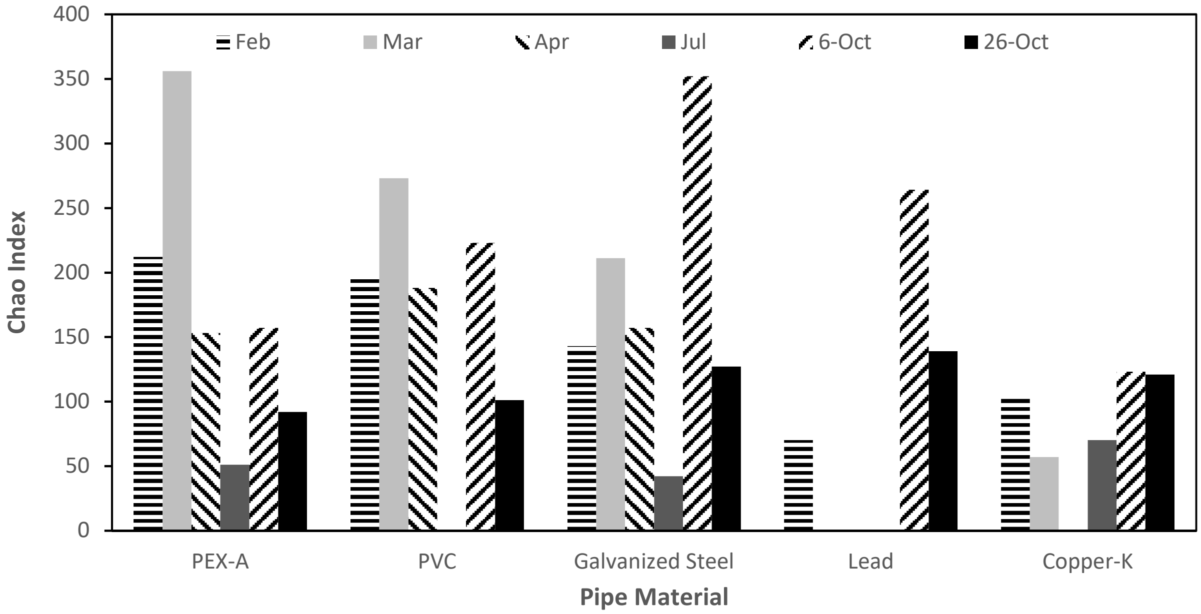

Figure 22: Chao1 richness index of biofilms at the A.B. Jewel plant.

Furthermore, a non-metric multidimensional scaling plot shows the similarity of the microbial communities in different pipe materials over time (Figure 23). The biofilms clustered in two distinct ways. In general, they either cluster according to pipe materials – metal (Lead, Copper-K, Galvanized Steel) or plastic (PVC, PEX-A), or according to seasons.

Figure 23: Non-metric MDS plot of pipe coupons in A.B. Jewel experiment with material and date labels.

Possible Pathogens

The sequences from biofilms in all the pipe trains were compared to identify pathogens. Sequences assigned to the genera *Escherichia, Legionella* and *Mycobacteria* were identified in the biofilm samples. At least one pathogenic sequence was found in all of the forty five samples collected. Of the potential pathogens identified, *Mycobacteria* was the most common and was identified in 97.8% of the samples. *Legionella* followed, being identified in 48.8% of samples. *Escherichia* was identified in 4.4% of samples. Within the specific pipe materials, *Legionella* was most prevalent in Lead, where it was positively identified in 61.5% of the coupons. Following Lead, *Legionella* was identified in 50% of the coupons in PEX-A, PVC and Galvanized Steel and 25% of the Copper Type K coupons. These results are similar to those of Proctor et al (2017) in which PEX type pipes supported a larger population of *Legionella* than Copper. The highest relative abundance of *Legionella* was measured in July (day 172). This agrees with other studies that indicate higher temperatures in water support more *Legionella* growth (Yee and Wadowsky, 1982, Ohno et al., 2003). *Escherichia* was only identified in the Lead pipe coupons. *Mycobacteria* was identified in all pipe materials. Copper pipe coupons had the highest average relative abundance of *Mycobacteria*. Exposure to the above pathogens, especially in facilities with immunocompromised persons, is a health risk that is increasingly becoming recognized (Karakousis et al., 2004, Aumeran et al., 2007; Bartram, 2007, Falkinham et al., 2008). The relative abundance of pathogens measured in this study were low within the biofilm, which limited the threat to human health. However, the fact that pathogens were present indicates the importance of maintaining a safe DWDS, as the pathogens in biofilms could be the seed for suspended growth at locations with low disinfectant residual in the system (Edagawa et al., 2008; Wang, Masters et al., 2012).

IV. CONCLUSION

In both simulated distribution system experiments, which used different source waters, biofilm developed and accumulated lead in all five pipe materials. Pipe materials play an important role in lead accumulation within biofilms. Results showed that plastic pipes supported more biofilm growth than metal pipes. In the A.B. Jewel simulated distribution system, PEX-A had the most growth with an average of 3.0×10^5 gene copies per cm^2. PVC was the most abundant in the Mohawk plant, with an average of 1.14×10^5 gene copies per cm^2. Copper-K had the least biofilm growth in both A.B. Jewel and Mohawk simulated distribution systems, with averages of 1.1×10^4 and 5.93×10^3 gene copies per cm^2 respectively. Biofilms on metal pipes accumulated higher quantity of lead, with the lead pipes adsorbing up to 25.22 $\mu g/cm^2$ at the Mohawk plant. In addition, the microbiome of biofilms develop in distribution systems was also impacted by the presence of lead. Unique genera, such as *Sphingobium*, appeared after the removal of lead in all the different pipe materials. For DWDS maintenance, the removal of lead pipes is a high priority to minimize the lead exposure in drinking water. However, biofilm detachment and lead release were observed after the removal of lead source pipe in both experiments, indicating that strategies must be developed to recognize biofilms as a potential lead source.

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