Image caption: Biofilms accumulate inside drinking water distribution pipes.

Accumulation of Lead by Biofilms in Water Distribution Systems

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Abstract: Lead accumulation in humans is detrimental at very low doses, especially in developing children. With millions of lead pipes and lead solder used in American homes before the 1980s, it is important to understand the interactions between lead pipes, their respective distribution systems, and the water flowing through them. This study examines the interaction between lead sources and biofilm, using a pipe loop system to determine how biofilms behave in the presence and subsequent absence of lead source. It also provides insight regarding lead activity in premise plumbing systems that have lead segments and how much of a threat these segments pose. A pipe loop with different pipe materials including lead was constructed to simulate water flows and stagnation periods of a typical household. Biofilms from the pipe loop were removed and analyzed for growth, lead concentration, and microbial community structure. In the presence of lead source, biofilms were shown to adsorb lead at concentrations as high as 48.39 μg/cm^2. This demonstrates that biofilms have the capability of accumulating lead in drinking water distribution systems. Lead levels in the biofilm ultimately decreased after the lead source was removed. No dissolved lead was observed releasing from the biofilm. The decrease of lead concentration within biofilm was likely due to detachment of the biofilm from the pipe. Biofilms can be a previously unrecognized source of lead following lead pipe removal. As the lead-laden biofilm detaches over time, a flushing regime and temporary avoidance of drinking tap water is recommended following pipe removal. This will ensure the safety of drinking water regarding lead concentration.

Key Points:

- Biofilm growth is ubiquitous in lead-containing water distribution systems.
- Biofilm grown within the water pipes accumulated lead at concentrations as high as 48.39 μg/cm^2 as well as other elements.
- No dissolved lead release was observed from biofilm after lead pipe was removed within the pipe loop system.

Introduction

Recently, lead (Pb) in the water supply has become a hot button issue following the early 2014 discovery of lead-contaminated drinking water in Flint, Michigan. Many scientists, government workers, and citizens nationwide now have serious concerns that other American communities may be at risk for potential lead contamination in drinking water. While the issue in Flint is believed to have been caused by a failure to use necessary corrosion control in the pipes, lead in distribution systems is a problem ranging across the United States.

Before the 1980's, many pipes used lead solder in order to connect lead pipes to copper pipes, and a number of lead pipes are still in use in distribution systems around the nation. This is a serious issue, as research has found that even small amounts of lead can be very hazardous to human health, especially young children in important developmental phases. Due to the severity of the effects of lead, the EPA has set a Maximum Contamination Level Goal (MCLG) at zero. Achieving this goal would essentially require removing all lead and lead containing parts in the entirety of a drinking water distribution system (DWDS). However, to perform such a removal would be a massive undertaking in economic terms as well as physical labor required. Thus, it is important to learn the consequences of slowly removing lead from DWDSs.

Disappointingly, a recent study found that replacing pipes in the system might actually exacerbate the problem due to the fact that in DWDSs, perceptible amounts of lead can be found within soft deposits and solids (St. Clair et al., 2016). We hypothesize another possible source of lead contamination is biofilm that develops throughout the DWDS. Biofilms are a group of cells that aggregate together and often adhere to an external surface by extracellular polymeric substances. In DWDSs biofilms have been shown to be ubiquitous (Berry et al., 2006). The goal of the present project is to discover the role biofilms play concerning lead contamination in DWDSs. It is very important not only to the state of Arkansas, but to society as a whole, to determine if trace amounts of lead are being accumulated and released into the water by biofilm in DWDSs.

Methods

Replaced Pipe Sampling

A 1-ft lead pipe was collected from 1023 Haskell St., Tulsa, OK 74106 on November 15, 2016. The pipe sample was preserved on ice and delivered to the University of Arkansas lab the next day. To access the biofilm and scale within the pipe, the pipe was cut open and into three equal pieces. Two of the pieces were used for lead analysis in scale and biofilm using ICP-MS. Pipe A was cut longitudinally to allow easy access to scraping the biofilm and scale with a metal spatula. Pipe B was left intact and the biofilm and scale was removed with a sponge that was pushed through the pipe and then sonicated. Then, metal analysis using ICP-MS was performed. The remaining piece was used for DNA analysis following the method below.

Pipe Loop Construction and Operation

Five types of pipe materials are included in the pipe loop: lead pipes (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 loop, 12 pieces of 6" long removable pipe sections were installed in the overall pipe loop. The total pipe length per train is 30-ft. The pipe loop configuration is shown in Figure 1, and the actual pipe loop is shown in Figures 2 and 3. After pipe loop construction, the entire system was flushed at high velocity for 30 min to ensure that there were no leaks in the system. During the initial operation, the pipe loop was placed in the A.B. Jewell plant, and water had a chloramine residual of 2.75 mg/L. Water in the pipe loop flowed in an intermittent mode at a flow rate of 1.0 gpm during the hours of 6:00am-9:00am, 11am–1:30pm, 4:00pm–6:30pm, and 9:30pm–10:30pm. The flow was designed to simulate a typical residential water usage pattern. There was no flow in other time periods and water was allowed to stagnate in the pipes during these times. The pipe loop was operated in two different stages. In Stage one, 2 ft of lead pipe in each train served as the initial source of lead contamination. This

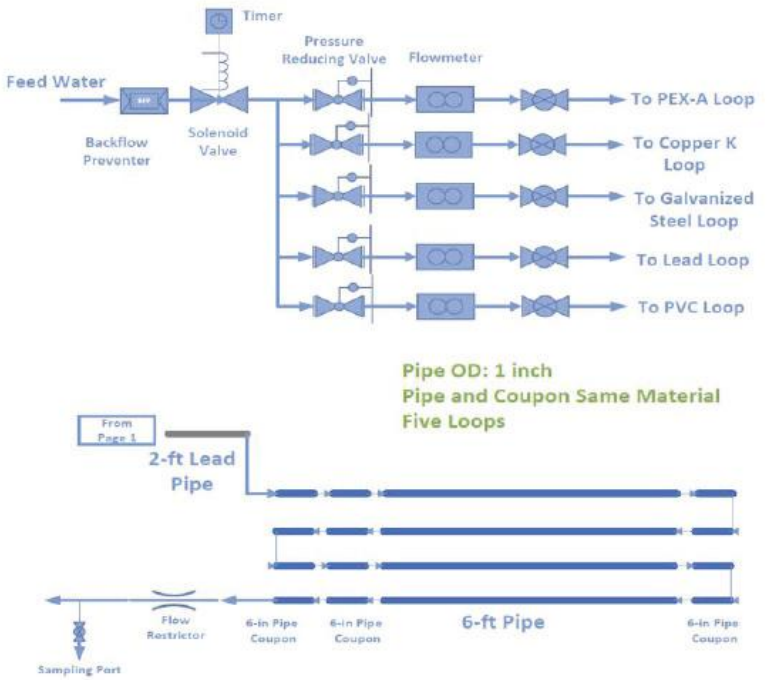

Figure 1: Pipe loop construction configuration.

Figure 2 (left) and 3 (right). On the left, a pipe loop displaying PEX-A train (on top of pipe loop), Galvanized Steel train (top of loop wall) and Copper-K train (bottom of loop wall). On the right, a pipe loop displaying Lead train (top of pipe wall) and PVC train (bottom of pipe wall).

Table 1: Elemental concentrations within deposits collected from the two pieces of removed pipe.

		Lead
Pipe Sample A	Conc. (μg/cm²)*	22.26
	Distribution (%)	38.71
Pipe Sample B	Conc. (μg/cm²)*	472.44
	Distribution (%)	70.27

*Surface area for pipe sample A and B is 49.98, and 24.47 cm^2, respectively.

stage lasted from January 23, 2017, to September 5, 2017. In Stage two, the 2 ft of lead pipes were removed from all trains and the system continued to operate until October 26, 2017.

Pipe Loop Sampling

Pipe loop samples were collected on February 17, March 22, April 21, July 11, October 6, and October 26, 2017. On each sampling day, two 6-in pipe coupons (duplicates) were collected from each train composed of different pipe materials. Each pipe sample was placed in a one gal zip-loc bag with approximately 80 mL of water from its respective pipe train. The samples were then preserved on ice and transported to the University of Arkansas lab on the same day for processing. Each pipe coupon was sonicated using a Branson Sonifier 3800 (Emerson, Ferguson, MO) for 30 min within the collection bag to dislodge the biofilm from the pipe interior. Following the sonication step, the water from each gal Ziplock bag was filtered through separate 0.22 μm filters (Pall Corporation, Port Washington, NY). Each filter was then dried completely in the oven at 98°C. The filters were preserved in -20°C until subsequent processing.

Metal Analysis

Dried filters from the previous step were placed in 20 mL centrifuge tubes for storage and digestion. Five mL of deionized distilled (DDI) water from a Barnstead Gen pure Pro UV/UF 501311950 (Thermo Fisher Scientific, Waltham, Massachusetts) was added into the centrifuge tube and then sonicated for 30 min in a VWR Model 751 Sonicator (Radnor, PA). A solution of 1 mL of H_2O_2, 0.42 mL of HCl and 0.2 mL of HNO_3 was then added to each of the centrifuge tubes. That mixture was digested for 24-h in a Blue M model M01440A oven (Thermo Fisher Scientific, Waltham, Massachusetts) set at 50 °C. After 24-h, the mixture was diluted to 10 mL using DDI water. One mL was then removed from the solution and 9 mL of 2% HNO_3 was added to that 1 mL for a final dilution of 10x. Elemental levels were calculated on the 10x dilution using a Thermo Sci. Icap Q (Bremen, Germany) Inductively Coupled Plasma Mass Spectrometer (ICP-MS).

DNA Analysis

DNA was extracted for subsequent analyses from the filter containing the biofilm using a soil DNA extraction kit (Power Soil DNA Isolation Kit, Mo-Bio, Carlsbad, CA). The protocol recommended by the manufacturer was followed. DNA extracts were preserved in -20°C until subsequent processing. To quantify bacteria concentration, 16S rRNA was first amplified using PCR. PCR reactions were completed following the procedure used by Walden, Carbonero and Zhang, 2017. The presence of 16S rRNA genes was confirmed by gel electrophoresis. For bacteria community analysis, DNA extracts were submitted to the sequencing facility in Food Science at the University of Arkansas for next generation sequencing. Sequencing and data analysis was performed according to the procedure used by Walden, Carbonero and Zhang, 2017.

Results and Discussion

Replaced Pipe Scale Analysis

Lead concentrations were normalized by surface area (μg/cm^2) as well as the percentage of lead compared to the overall total solids recovered. Results are shown in Table 1. For both pipe samples, lead was abundant in the deposit collected with concentrations going as high as 472.44 μg/cm^2. Notice that pipe A has a much lower lead concentration than B. We believe this was caused by the rinsing procedure after pipe A was cut open to remove the metal shavings.

Replaced Pipe Biofilm Growth

Figure 4 is the gel image showing the presence of universal bacteria genes (16S rRNA). It confirmed the biofilm

presence within pipelines from the DWDS in Tulsa, OK.

Biofilm Growth

PCR and Gel Electrophoresis showed positive bacterial genes from the pipe coupons, one example is shown from March 22, 2017, in Figure 5. This shows the biofilm growth within the pipe loops.

Biofilm Lead Adsorption

Results from ICP-MS showed each type of pipe in the pipe loop had biofilm that adsorbed lead. The metal concentrations are normalized in two ways – by surface area (μg/cm^2) and by dry weight (μg/mg). These are shown in Tables 2 and 3. The surface areas for the five pipe materials are 98.00 cm^2, 91.20 cm^2, 86.23 cm^2, 79.67 cm^2, and 112.70 cm^2 for PVC, galvanized steel, lead, PEX-A, and Copper Type K, respectively. The largest adsorption of lead for all materials occurred on October 6, 2017. We speculate this is due to the lead source that was removed in September which dislodged particles of lead or lead scale were then able to attach to the biofilm. The highest reported adsorption of lead was in a lead pipe coupon at 40.18 μg/cm^2 and 738.10 μg/mg. The largest adsorption recorded for a non-lead pipe coupon was in galvanized steel at 42.77 μg/cm^2 and 98.76 μg/mg. However, the lead concentration found in the galvanized steel pipe biofilm may have been inflated. A recent study found that the zinc coating in galvanized steel pipes contained up to 2% of lead (Martin et al., 2015). In other pipe materials, the PEX coupon was shown to have adsorbed 11.75 μg/cm^2 and the Copper Type K coupon had adsorbed 70.02 μg/mg.

Lead Release

The lead concentration in biofilms initially increased after the lead source was removed. This data is shown in Tables 2 and 3. The largest change occurred in the Copper Type K with an increase of 21.44 μg/cm^2. We speculate that the removal of the lead source dislodged particulate lead or lead scale, which then attached to the biofilm. During the next sampling period the lead levels in each train decreased. However, dissolved lead levels in water did not increase during this time. This indicates that the lead may not have released from the biofilm into the water after the lead source pipes were removed; instead, particulate lead was released from biofilm and pipe deposits as biofilm detachment hap-

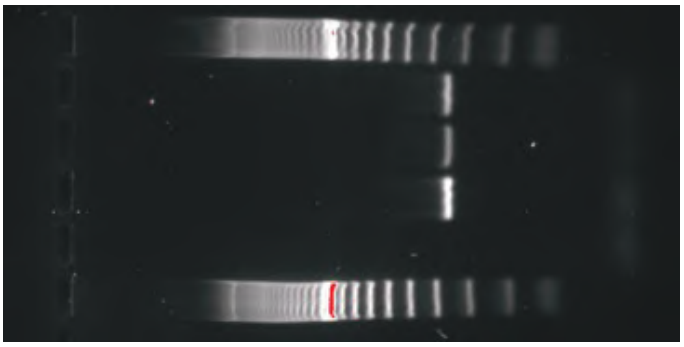

Figure 4: Gel electrophoresis image showing the successful amplification of DNA extracted from lead pipe deposits in the City of Tulsa. The wells contain: ladder, triplicate DNA samples, negative control, and ladder (in vertical order).

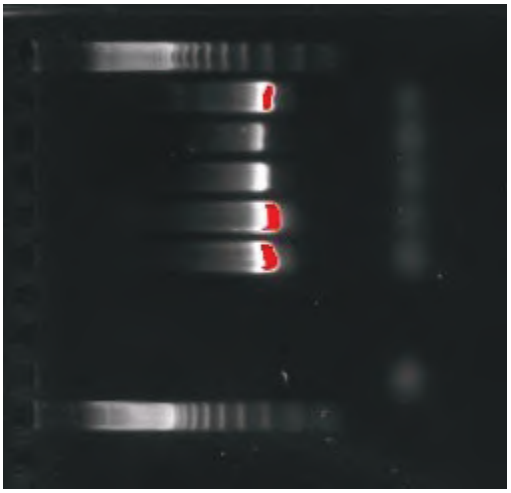

Figure 5: Gel electrophoresis image showing the successful amplification of DNA extracted from biofilms in the pipe samples from the pipe loop on March 22, 2017. The wells contain: ladder, 5 DNA extracts from Galvanized Steel, Copper Type K, Lead, PEX-A and PVC pipes, negative control, and ladder (in vertical order).

Table 2: Lead adsorbed by biofilms measured in μg/cm^2.

Date Collected	Pipe Material				
	Lead	PVC	PEX-A	Steel	Copper-K
17-Feb-17	3.01	0.07	0.04	0.05	0.04
22-Mar-17	5.25	0	0.02	0	0
21-Apr-17	9.16	0.05	0.05	0.02	0.32
11-Jul-17	7.26	0.08	0.03	0.04	0.02
6-Oct-17	23.05	7.57	11.75	10.87	21.44
26-Oct-17	23.5	1.3	0.07	1.41	0.45
26-Oct-17-Long	18.49	0.4	0.34	0.76	1.7

Table 3: Lead adsorbed by biofilms measure in μg/mg.

Date Collected	Pipe Material				
	Lead	PVC	PEX-A	Steel	Copper-K
17-Feb-17	117.94	0.31	0.16	0.7	0.3
22-Mar-17	1565.99	0.65	0.37	0	0.68
21-Apr-17	738.1	9.37	15.23	3.33	36.3
11-Jul-17	29.53	0.4	0.12	0.18	0.09
6-Oct-17	83.52	38.82	57.79	70.02	98.76
26-Oct-17	104.98	8.18	0.33	9.44	1.7
26-Oct-17-Long	54.52	2.08	0.63	2.52	3.24

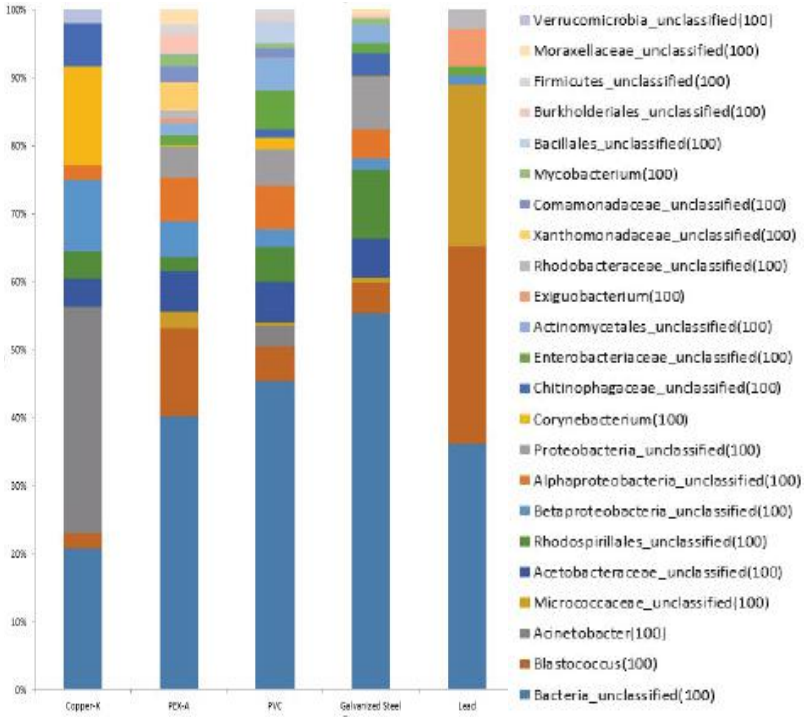

Figure 6: Stacked bar chart of the most abundant species of bacteria present in each pipe coupon from March 22, 2017.

pened. Ultimately, if this were a real system the particulate lead or dislodged biofilm would be consumed by human use or enter the sanitary sewer.

DNA Sequencing

DNA sequencing was performed on all pipe samples. Microbial communities were determined for each pipe loop material over time. An example of one microbial community is shown above in Figure 6. It shows different pipe material accumulated distinct microbial communities within the biofilm.

Conclusions

Scale pipe deposits in the replaced lead pipe from DWDS at the City had lead deposits with concentrations as high as 472.44 $\mu g/cm^2$. It also showed positive biofilm growth within the replaced pipe.

Biofilm formed within the pipe loop adsorbed lead at varying levels with concentrations as high as 48.39 $\mu g/cm^2$. Adsorption of lead occurred in all five pipe materials when there was a lead source pipe present. After the removal of the lead source, lead concentration in the biofilms rose on average by 13.45 $\mu g/cm^2$. Lead levels in biofilm then decreased in the next sampling period, however, no dissolved lead was observed releasing from the biofilm. We recommend continuing this research by conducting further pipe loop tests using other variables such as disinfectant, source water, and treatment processes.

Lead is an ongoing problem at both regional and national level. The present research indicates that lead can be adsorbed into biofilms but no dissolved lead was released back into the water above detection limit. Additionally, a major finding is that when our lead source was removed in all five pipe trains, the lead concentration in the biofilm rose briefly. This indicates that when lead pipe is replaced in premise plumbing that a certain amount of lead released can be stored for a brief period by the biofilm. Our recommendation is that a flushing regime occurs following lead pipe removal to ensure that all stored lead is removed before continuing usage.

Acknowledgements

This material is based upon work supported by the U.S. Geological Survey under grant agreement No. G16AP00040 and administered by the Arkansas Water Resources Center. The views and conclusions contained in this document are those of the authors and should not be interpreted as representing the opinions or policies of the U.S. Geological Survey.

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