Impact of service line replacement on lead, cadmium, and other drinking water quality parameters in Flint, Michigan

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Supplementary Information

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Standards preparation for phosphorus and metal analyses. Standards for metals and phosphorus analyses were prepared by serial dilution of a custom stock standard solution prepared by Ricca Chemical Company (Arlington, TX, USA) and acidification with a 35% nitric acid solution. The stock standard solution contained P at 300 mg/L, Zn at 200 mg/L, Fe at 100 mg/L, Al and Cu at 50.0 mg/L each, Pb and Mn at 20.0 mg/L each, and Cr, Ni, As, and Cd at 1.00 mg/L each. A lab fortified matrix sample was created by adding 263 µL of 1000-fold diluted stock solution to 5 mL of a randomly selected acidified sample.

Determination of minimum detection limit (MDL) and limit of quantification (LOQ) for metal analyses. The MDLs for metal analyses were determined according to the MDL procedure published by the US Environmental Protection Agency (US EPA).[1] Serial dilutions of the custom stock standard solution were prepared using 18.2 MΩ×cm Milli-Q water and acidified (final concentration of 7 µL 35% nitric acid per mL of sample). Diluted standard stock solutions were analyzed on the ICP-MS 16 times over the course of the study. The standard deviation for each metal at each dilution was calculated, and statistical outliers were determined using the method outlined in the Department of Natural Resources (DNR) Analytical Detection Limit Guidance document[2] and removed from the dataset for MDL determination. The MDL for each metal was determined by multiplying the standard deviation of the remaining instrument-measured concentrations by the appropriate Student's t-value at the 99% confidence interval (t-values at 99% confidence: 2.603 for Mn, Fe, and Cu; 2.625 for Zn; 2.651 for Al, P, Cr, Ni, As, and Cd; and 2.682 for Pb; t-values are based on the number of ICP-MS analyses of the stock solutions less the number of outliers identified for each metal). The LOQ was defined as 10 times the standard deviation according to the DNR Analytical Detection Limit Guidance.[2] The stock standard dilution level

used for MDL and LOQ determination was greater than the MDL but smaller than 10 times the MDL, as is required for the dilution level to be appropriate for MDL and LOQ calculations.

Sample processing, DNA extraction, and qPCR. Within 4 h of sample collection, each water sample was filtered through a 0.2 μm sterile membrane polycarbonate filter (EMD Millipore, Billerica, MA) on top of a 0.45 μm sterile cellulose ester backing membrane (Thermo Fisher Scientific). The filter was then transferred to a 2 ml nuclease free screw-cap tube and stored at -20°C. DNA was extracted from filters with a modified Maxwell® 16 LEV Blood DNA Kit (Promega, Madison, WI).[3] Briefly, each filter was dissolved in 500 μL of a 49:1 chloroform-isoamyl alcohol mixture (Sigma-Aldrich, St. Louis, MO). Three rounds of physical and chemical lysis were achieved by bead beating with 0.5 g of zirconium beads (BioSpec Products, Bartlesville, OK) and Maxwell lysis buffer. Extracted DNA was dissolved in 50 μl of molecular grade water and stored at -20°C until qPCR was performed. qPCR was conducted using a RealPlex 2 Mastercycler System (Eppendorf, Hauppauge, NY) to detect total bacteria, *Legionella pneumophila,* and *Mycobacterium* and *Legionella* genera. Targeted genes, primer sequences, amplicon sizes, limit of detection (LOD), LOQ, and standard sources for all qPCR assays are shown in SI Table S3. Each 10 μL reaction contained 1 μL DNA template, 1X Fast Evagreen qPCR Master Mix (Biotium, Fremont, CA), and 0.625 mg/ml bovine serum albumin (Life Technologies, Inc., Waltham, MA). For the quantification of total bacteria, *Mycobacterium* spp., and *L. pneumophila*, primers were added to achieve a final concentration of 500 nM, whereas a 400 nM final concentration was used in the *Legionella* genus assay. All assays had initial denaturation at 95°C for 5 min, except the total bacteria assay (95°C for 2 min). Cycling condition times, temperatures and cycle number varied (SI Table S3). All assays were followed by a melt curve analysis from 55 to 95°C for 20 min after post-cycling denaturation at 95°C for 5 min and

annealing at 55°C for 15 s. qPCR reactions were performed in triplicate and results were averaged for all samples and standards. No-template controls were carried out in duplicate for each qPCR run. Samples were quantified from a standard curve consisting of 10-fold serially diluted qPCR standards (10^1–10^6) gene copy/µL. qPCR standards consisted of purified PCR products prepared from either pure culture extracts or extracted environmental sample DNA (SI Table S3) and quantified using the Qubit double-stranded DNA high sensitivity kit (Invitrogen, Waltham, MA). A *Legionella pneumophila* strain Lp02 DNA extract was provided by Dr. Michele Swanson's laboratory as the *L. pneumophila* qPCR assay standard source.

Linear mixed-effects modeling. All linear mixed effects models were comprised of fixed effects (described below) and one random effect (i.e., home number). Prior to model generation, imputation of left-censored data was conducted on data points below the LOD by replacement with one-half the LOD, and censored data between the LOD and LOQ were replaced with the average of those two values.[4] Lognormal transformations of all metals, chlorine residuals, and total bacteria concentrations were performed prior to model selection to normalize data. Collinearity of explanatory variables was assessed using a correlation matrix (SI Table S4). Any variables in the matrix with a significant Kendall correlation were not included together in a model. Manual model selection was conducted using the log-likelihood of each model. Specifically, nested models were used to determine which parameters (i.e., pH, temperature, free chlorine residual, dissolved phosphorus level, percentage of a certain pipe material, and private service line (SL) type) increased the log-likelihood of the model significantly, and those parameters were left in the final model. All mixed-effect models contained only data of the same type of water sample (i.e., distribution system, premise plumbing, or hot water). Visit number (i.e., pre-SL replacement, two weeks and five weeks post-SL replacement) and sampling season were included as categorical

variables in all models regardless of log-likelihood values, unless otherwise specified. The lmer() function from the lme4 package was used to generate all models and determine the p-values associated with each explanatory variable.[5,6]

Results and Discussion

Temporal trends from Spring to Fall 2016 in total bacteria and select opportunistic pathogen abundance in water samples. Total bacteria concentrations ranged from 3.68×10^2 to 1.56×10^9 gene copies/L and *Mycobacterium* spp. and *Legionella* spp. concentrations were as high as 1.08×10^7 and 6.18×10^6 gene copies/L, respectively (SI Figure S8). Rhoads et al.[7] found 23% of samples collected in March 2016 from 17 Flint homes and small businesses to be positive for *Legionella* spp., while our results indicated 41% of samples contained detectable *Legionella* spp. during the same sampling time period (March–May 2016). Consistent with our results, Rhoads et al. also did not detect any *L. pneumophila* by qPCR.[7] Among the samples with quantifiable *Legionella* spp. concentrations in our study, levels were comparable with those reported by Schwake et al.[8] (i.e., 10^4 to 10^6 gene copies/L) for samples collected from homes and small business in Flint in 2015. Furthermore, Schwake et al.[8] did not detect *L. pneumophila* by qPCR. Although no *L. pneumophila* was detected by qPCR in these studies, Byrne et al.[9] reported culturing *L. pneumophila* strains from first flush samples collected from Flint homes in Fall 2016. Inability to detect genome copies by molecular methods at low environmental concentrations and differences in sampling locations could explain this variability.

Total bacterial levels in distribution system samples decreased significantly from Spring to Fall (Wilcoxon Rank Sum Test, p-value = 0.022) and the total number of *Legionella* spp. and *Mycobacterium* spp. positive samples followed the same trend. Specifically, *Legionella* spp.

positive samples decreased from 41% in the spring to 15% in the fall and *Mycobacterium* spp. were detected in 69% of samples in the spring and 53% of samples in the fall. While bacterial levels tend to increase with increasing temperature, our results signify that water quality changes other than temperature changes from Spring 2016 to Fall 2016 had a more profound impact on bacterial levels. Particularly, free chlorine levels inversely correlated with total bacterial concentrations in distribution system samples (SI Table S8), suggesting the addition of extra chlorine to the water in Flint starting in the summer of 2016[10] resulted in successful reduction of bacterial levels.

Total bacterial abundance in water samples before and after SL replacement. Total bacterial levels in each type of sample collected before, two weeks after, and five weeks after SL replacement did not change significantly (Wilcoxon Signed Rank Test, all p-value > 0.05). In addition, the total bacterial concentrations in distribution system samples were significantly lower than those in premise plumbing and hot water samples (Wilcoxon Signed Rank Test, all p-values < 0.05), consistent with previous studies.[11,12] Primary reasons for this trend include the ability of bacteria to grow in stagnant water that typically undergoes disinfectant residual decay and biofilm sloughing during flow changes.[13] Our modeling results for premise plumbing samples support these explanations, as chlorine residual was significantly inversely correlated with total bacterial levels (SI Table S9). Linear mixed-effects models established for total bacteria in premise plumbing samples indicate the positive correlation of increased total metal concentrations (e.g., total copper, total lead, total iron) with increased total bacterial abundance. For instance, a model developed with total lead levels shows a 0.6-log increase in total bacterial levels corresponding to a one-log increase in lead concentrations (SI Table S9). These results indicate the release of

particulate metals might be accompanied with biofilm sloughing from piping in premise plumbing samples.

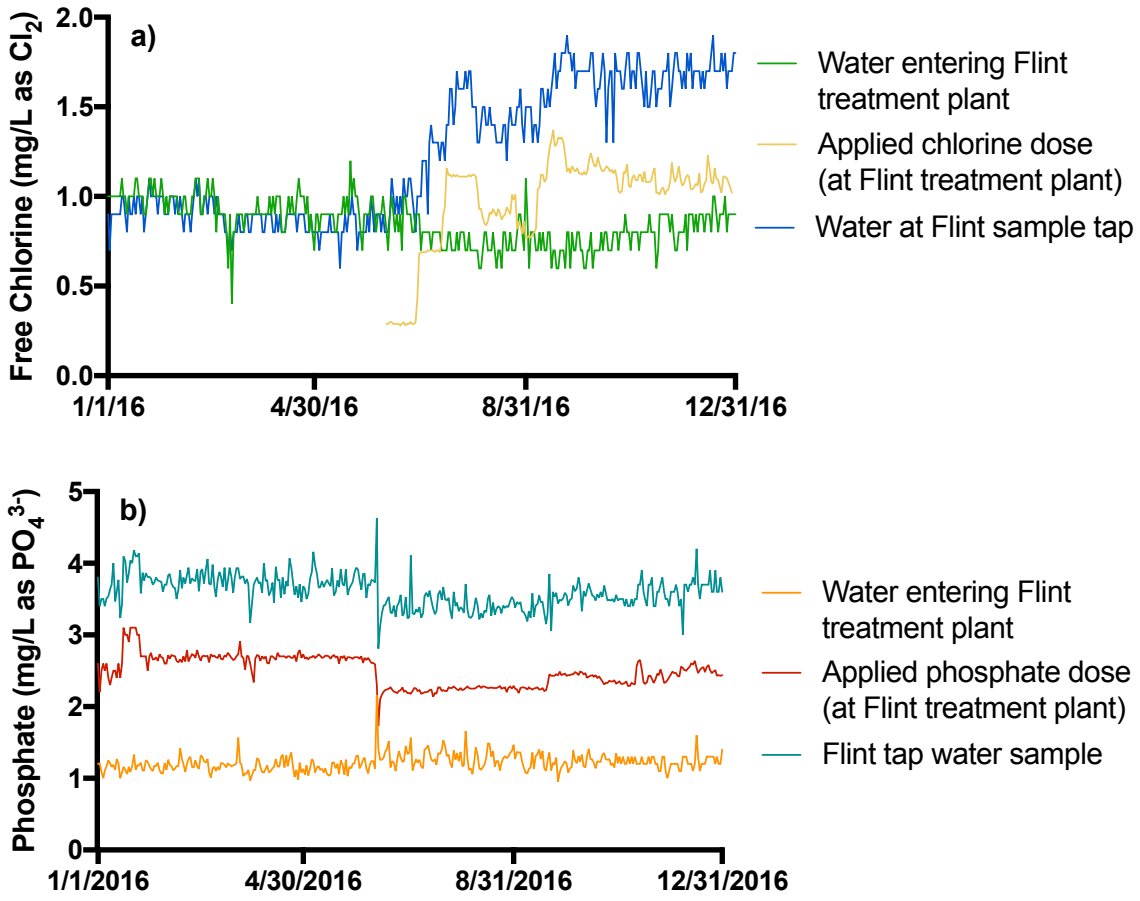

Figure S1. a) Free chlorine and b) orthophosphates levels in Detroit Water and Sewer Department water entering the Flint treatment plant, in water at a Flint tap, and applied to water prior to entering the Flint distribution system, and at a Flint tap throughout 2016. Data were obtained from 2016 City of Flint water treatment plant monthly operating reports.[10]

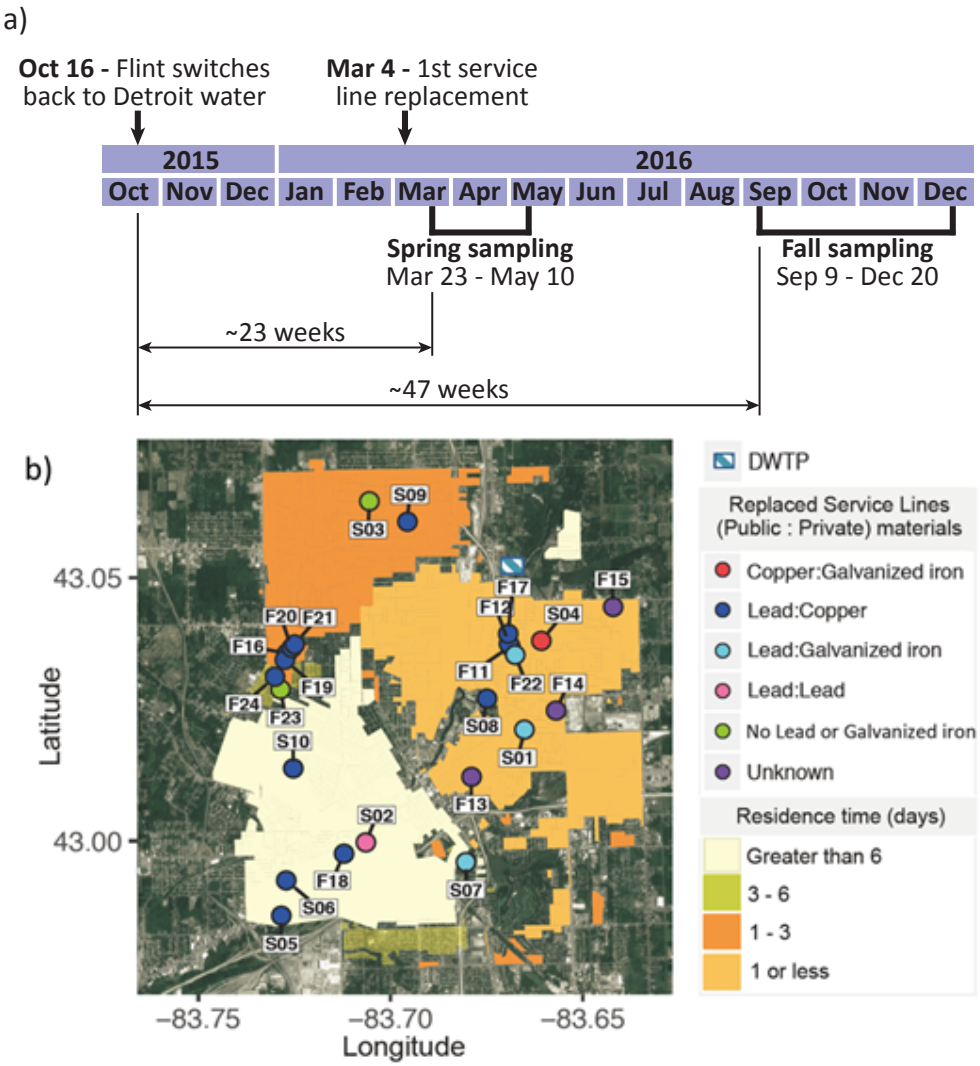

Figure S2. a) Sampling timeline and b) sampling locations in Flint, MI. Sampling locations S01-S10 and F11-F24 indicate homes sampled in Spring 2016 and Fall 2016, respectively.

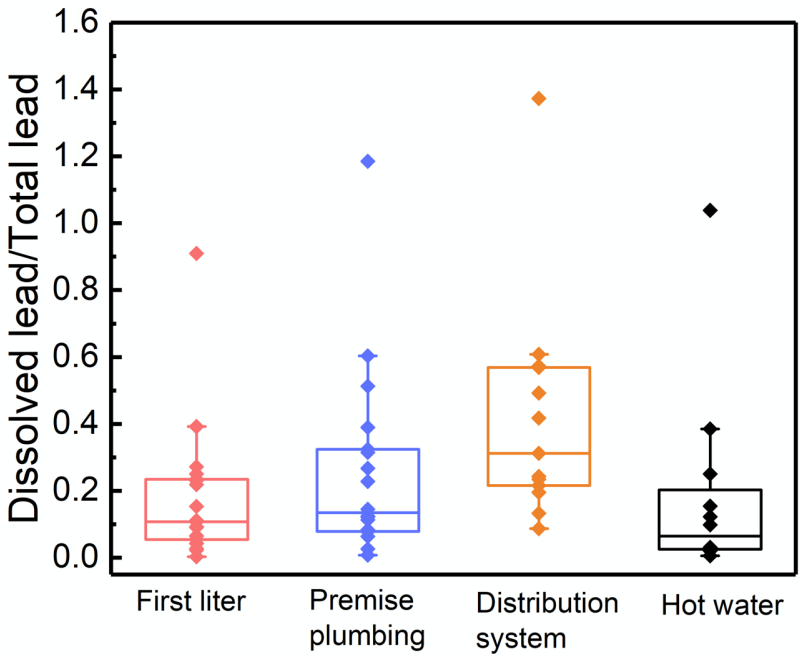

Figure S3. Median and interquartile range of ratios of dissolved lead to total lead concentrations. Note that the samples with lead levels lower than LOQ were not included.

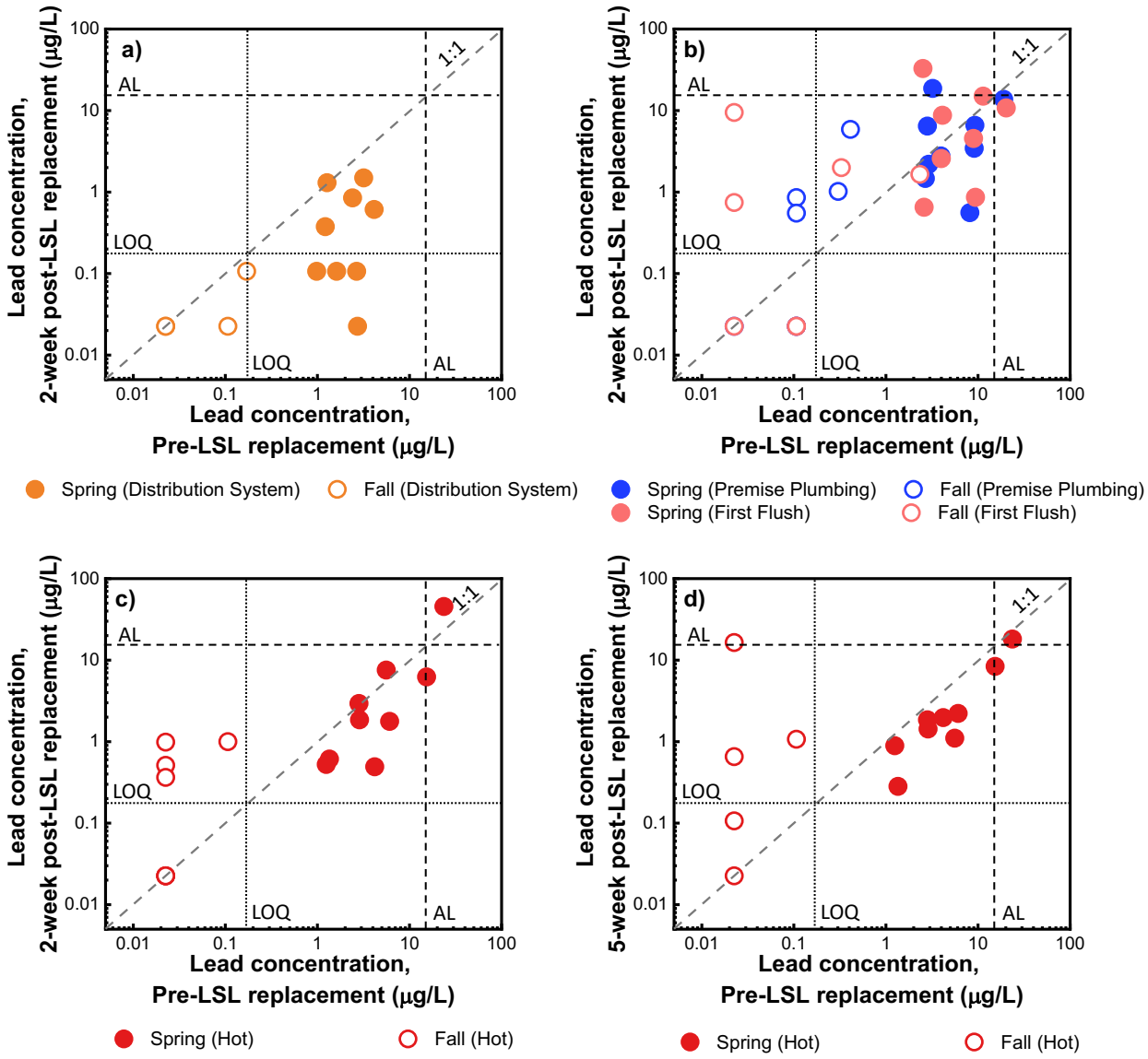

Figure S4. Lead concentration changes in 17 homes for a) distribution system and b) premise plumbing and first flush samples collected before service line replacement and five weeks after service line replacement in the Fall and Spring, and hot water samples collected c) before service line replacement and two weeks after service line replacement and d) before service line replacement and five weeks after SL replacement in the Fall and Spring. Dotted horizontal and vertical lines indicate the LOQ.

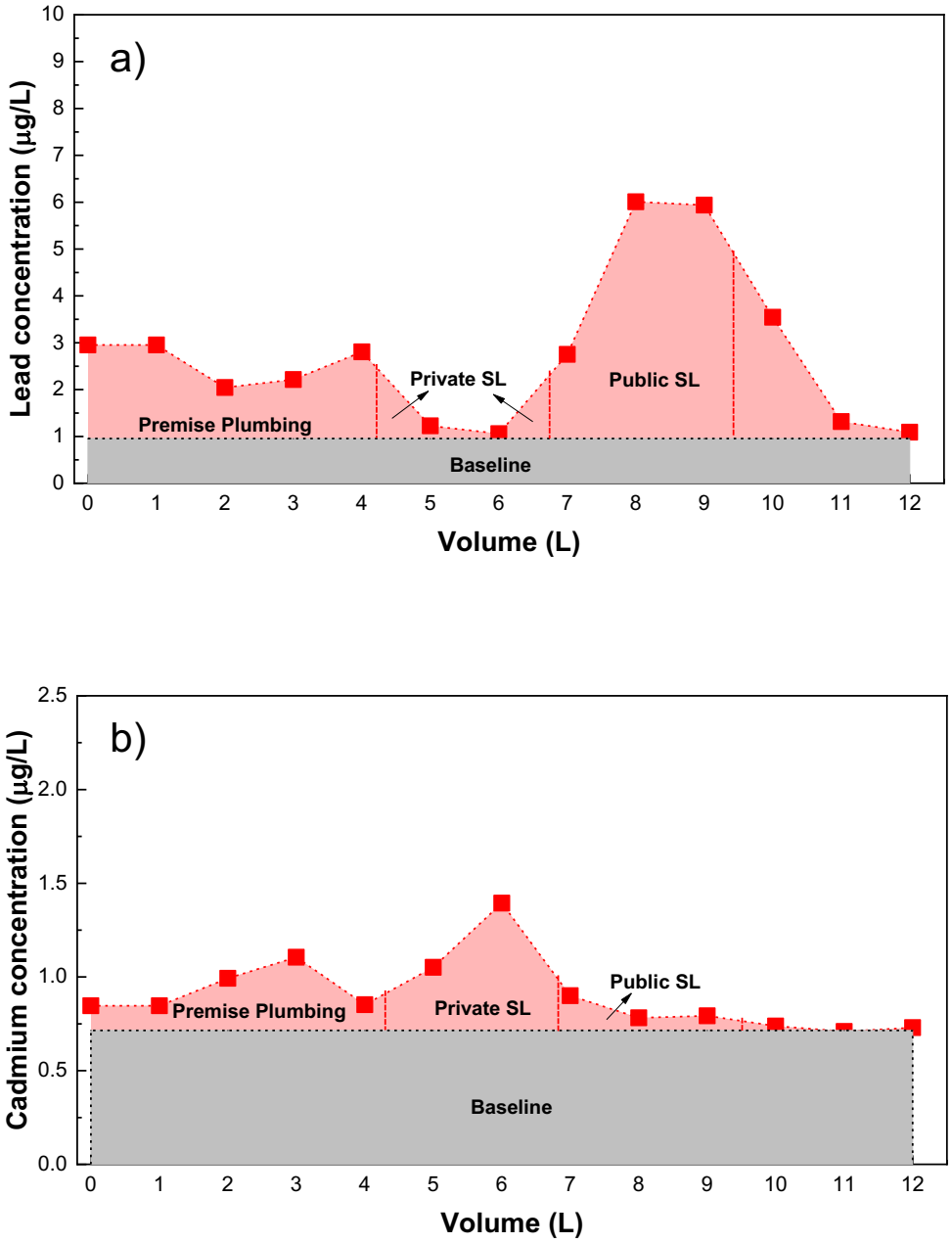

Figure S5. Profiles of the calculated percentages from each plumbing source (premise plumbing, private SL, and public SL) contributing to the (a) lead and (b) cadmium levels in water. The lead and cadmium concentrations represent the pre-LSL replacement conditions. Gray shading represents the baseline lead/cadmium levels, which indicate the contribution of metal concentrations from water in the distribution system being used at the tap. Red shading is considered to be the additional lead/cadmium contribution from each portion of pipe over the baseline metal level. The data points (red symbols) in each line were calculated by averaging the results of replicate pre-LSL replacement sampling events.

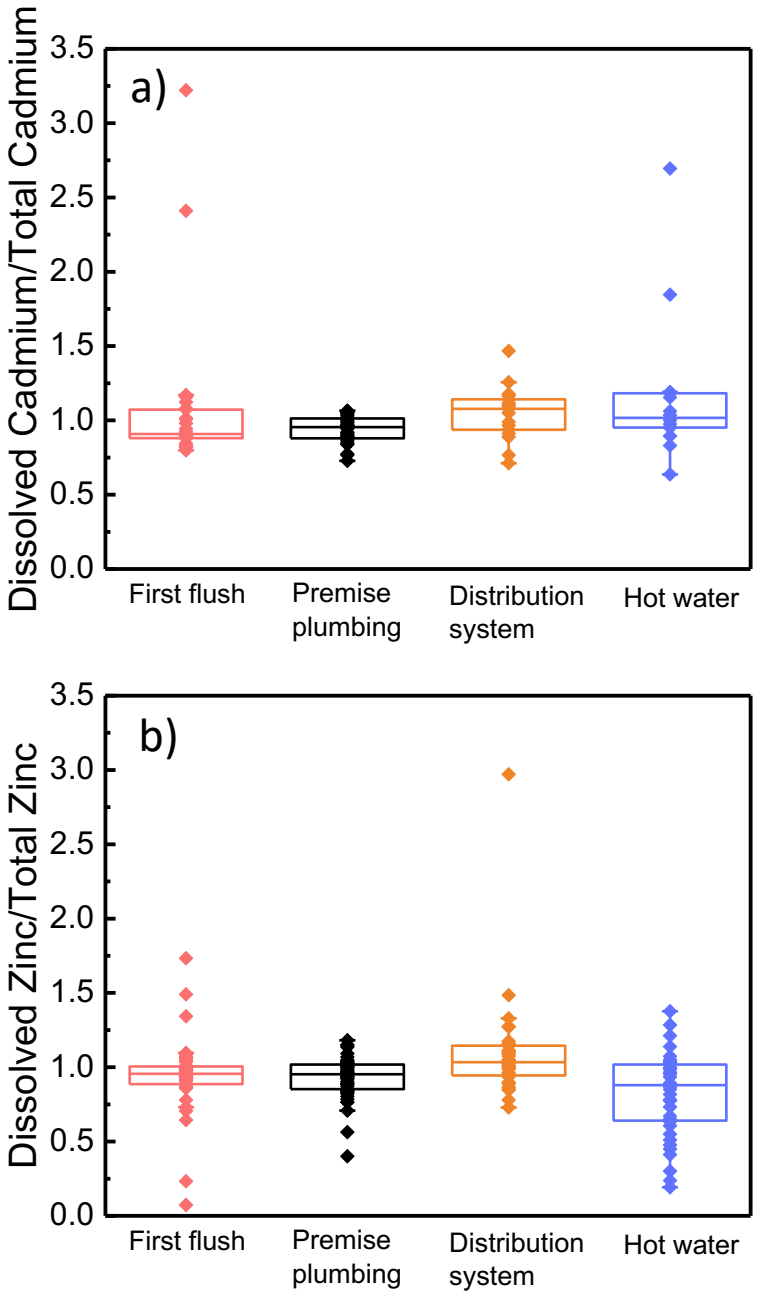

Figure S6. Median and interquartile range of ratios of a) dissolved cadmium to total cadmium concentrations and b) dissolved zinc to total zinc concentrations. Note that the samples with cadmium/zinc levels lower than LOQ were not included.

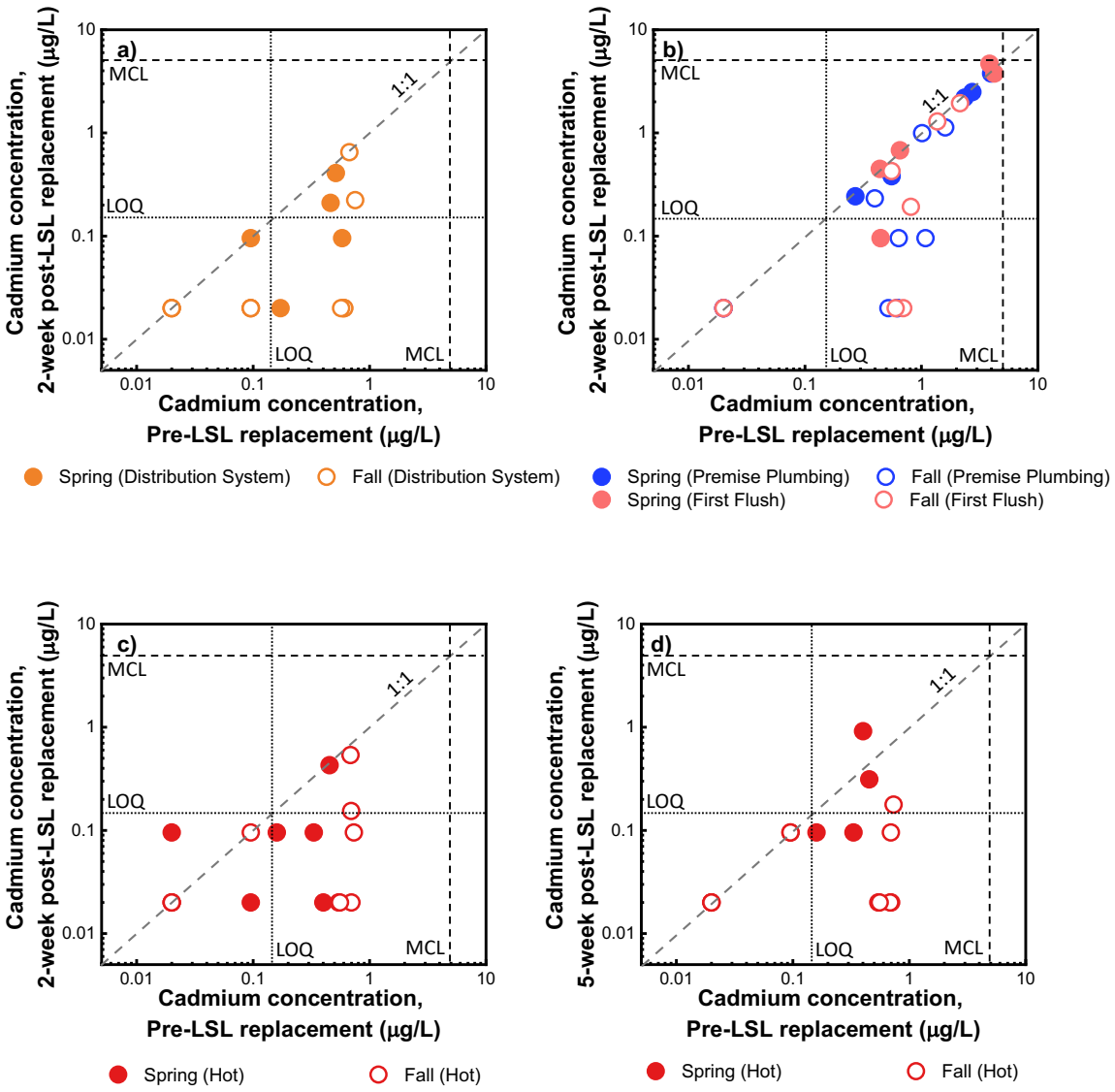

Figure S7. Cadmium concentration changes in 17 homes for a) distribution system and b) premise plumbing and first flush samples collected before service line replacement and five weeks after service line replacement in the Fall and Spring, and hot water samples collected c) before service line replacement and two weeks after service line replacement and d) before service line replacement and five weeks after SL replacement in the Fall and Spring. Dotted horizontal and vertical lines indicate the LOQ.

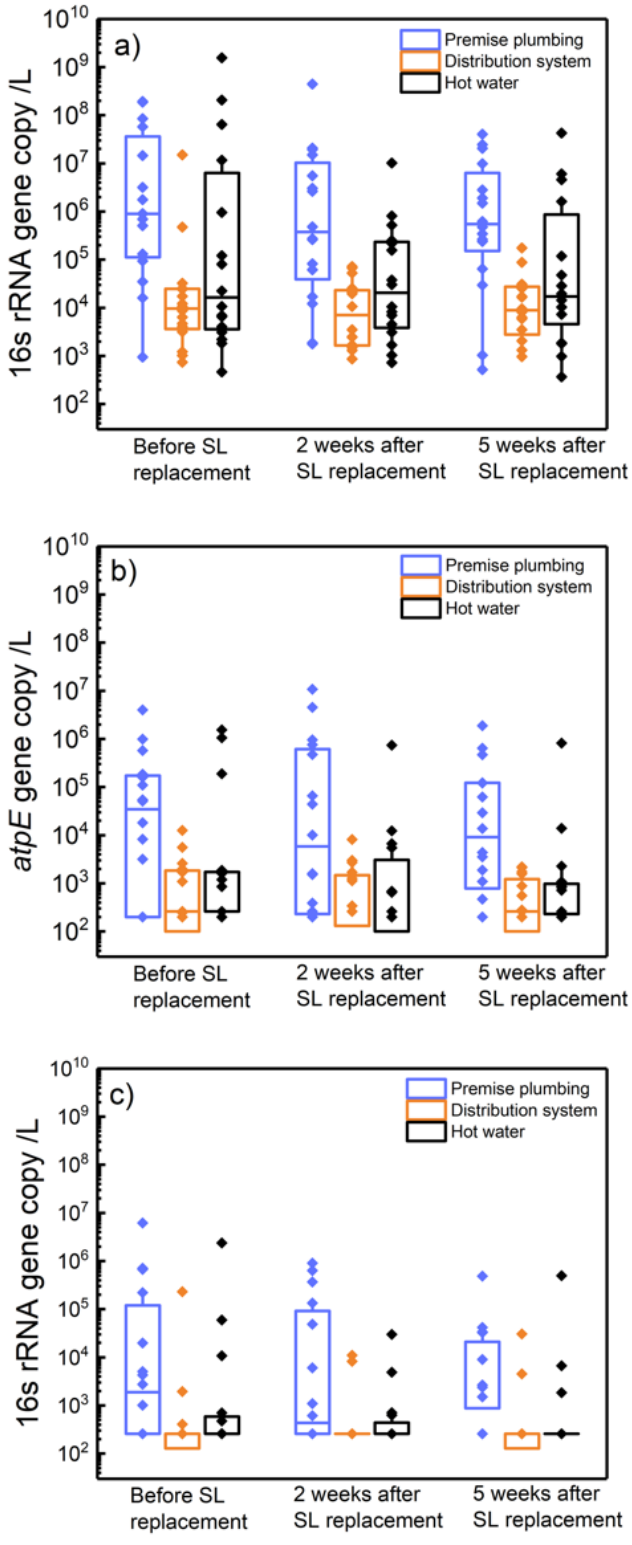

Figure S8. Gene copy concentrations of a) total bacteria, b) *Mycobacterium* spp., and c) *Legionella* spp. in premise plumbing, distribution system, and hot water samples.

Table S1. Composition of SL and premise plumbing pipe materials in all homes sampled in Flint prior to SL replacement.

House ID[a]	Public SL	Private SL	Premise plumbing material	Premise plumbing characterization		
				% Copper	% PVC	% Galvanized
S01	Lead	Galvanized	Copper, small amount of Galvanized	61.1	2	36.9
S02	Lead	Lead	Copper	96.5	0	3.5
S03[a]	Copper	Copper	PVC	100	0	0
S04	Copper	Galvanized	Copper, small amount of PVC	95	5	0
S05	Lead	Copper	Copper, small amount of PVC	94.3	5.7	0
S06	Lead	Copper	Copper, small amount of PVC	89.7	10.3	0
S07	Lead	Galvanized	Galvanized, small amount of PVC and copper	2.8	6.7	90.5
S08	Lead	Copper	Copper, small amount of PVC	95	4.7	0
S09	Lead	Copper	Copper + Galvanized, small amount of PVC	42.5	3.7	53.8
S10	Lead	Copper	Galvanized + copper, small amount PVC	11.5	2.8	83.1
F11	Lead	Galvanized	Galvanized + Copper	13.8	2.1	81.1
F12[a]	Lead	Copper	PVC	11.4	88.6	0
F13[a]	Unknown	Unknown	PVC	0	99.8	0.2
F14[a]	Unknown	Unknown	PVC + Copper	81.4	15.5	3.1
F15[a]	Unknown	Unknown	Copper	96.1	3.9	0
F16	Lead	Copper	Copper	95.6	4.4	0
F17	Lead	Copper	Copper	0	0	100
F18	Lead	Copper	PVC + Galvanized	0	10.1	89.9
F19	Lead	Copper	PVC + Copper	1.8	98.2	0
F20	Lead	Copper	Galvanized	4.4	1.7	93.9
F21	Lead	Copper	PVC + Copper	24.4	75.6	0

F22	Lead	Galvanized	Galvanized + Copper	52.6	0	44.8
F23[a]	Copper	Copper	Copper	70.9	29.1	0
F24[a]	Lead	Copper	PVC + Galvanized	2.6	34.9	62.5

[a]Initial water sample data taken at these homes are not included in this study because no post-replacement samples were taken.

Table S2. MDL and LOQ values for metals and phosphorus analyses.

Element	LOQ	MDL
Aluminum (µg/L)	14	3.8
Phosphorus (µg/L)	68	18
Chromium (µg/L)	0.16	0.042
Manganese (µg/L)	0.13	0.033
Iron (µg/L)	0.99	0.26
Nickel (µg/L)	0.69	0.18
Copper (µg/L)	0.29	0.074
Zinc (µg/L)	6.4	1.7
Arsenic (µg/L)	0.67	0.18
Cadmium (µg/L)	0.15	0.040
Lead (µg/L)	0.17	0.045

Table S3. qPCR targets, primers, and thermocycling conditions.

Target	Primer sequence (5'-3')	Primer reference	Amplicon size (bp)	LOQ[a] (gc/µL)	LOD[b] (gc/µL)	Cycling conditions		Standard Source
Total bacteria (16S rRNA gene)	Forward: ACTCCTACGGGAGGCAGCAG Reverse: ATTACCGCGGCTGCTGG	Fierer et al.[14]	189	214	157	35x	95°C, 5s 54°C, 5s 72°C, 25s	Environmental sample
Legionella spp. (16S rRNA gene)	Forward: AAGATTAGCCTSCGTMCGAT Reverse: GTCAACTTAYCGCGTTTGCT	Reverse primer from Miyamoto et al.[15] Modified from Lesnik et al.[16]	139	1035	271	40x	95°C, 20s 61°C, 20s 72°C, 20s	Environmental sample
Mycobacterium spp. (*atpE* gene)	Forward: CGGYGCCGGTATCGGYGA Reverse: CGAAGACGAACARSGCCAT	Radomski et al.[17]	164	276	189	35x	95°C, 20s 59.6°C, 30s 72°C, 30s	Environmental sample
L. pneumophila (*mip* gene)	Forward: CCGATGCCACATCATTAGC Reverse: CCAATTGAGCGCCACTCATAG	Wullings et al.[18]	150	5257	5257	40x	95°C, 20s 61°C, 20s 72°C, 20s	Pure culture extract (*L. pneumophila* Lp02)

[a]Limit of quantification (LOQ) values obtained by determining the lowest standard concentration quantified with 10 qPCR replicates that resulted in a relative standard deviation of concentrations less than 35%. The concentration in gene copies (gc)/L was then calculated based on an extraction volume of 50 µL and a filtration volume of 3.5 L.

[b]Limit of detection (LOD) values obtained by determining the lowest standard concentration quantified in which all 10 qPCR replicates were positive. The concentration in gc/L was then calculated based on an extraction volume of 50 µL and a filtration volume of 3.5 L. In addition, any samples in which one or more triplicates did not return a positive cycle value were considered below detection.

bp – base pairs

Table S4. Kendall's Tau correlation coefficient (above) and associated p-values[a] (below) obtained using Kendall correlation analysis between all water quality parameters with all sample types.

	Total bacteria	*Mycobacterium* spp.	*Legionella* spp.	Temperature	Total chlorine	Free Chlorine	pH	Total Al	Dissolved Al	Total P	Dissolved P	Total Cr	Dissolved Cr	Total Mn	Dissolved Mn	Total Fe	Dissolved Fe	Total Ni	Dissolved Ni	Total Cu	Dissolved Cu	Total Zn	Dissolved Zn	Total Cd	Dissolved Cd	Total Pb	Dissolved Pb
Total bacteria		0.61	0.52	0.08	-0.24	-0.25	0.08	0.01	-0.03	-0.18	-0.21	-0.06	0.01	0.32	0.30	0.22	0.08	0.25	0.31	0.29	0.21	0.26	0.22	0.08	0.07	0.26	0.17
		0	**9.67E-11**	0.49	**0.011**	**0.0080**	0.46	0.96	0.85	0.06	**0.030**	0.57	0.96	**3.8E-04**	**0.0010**	**0.017**	0.50	**0.0082**	**4.7E-04**	**0.0014**	**0.023**	**0.0054**	**0.020**	0.50	0.52	**0.0054**	0.09
***Mycobacterium* spp.**			0.41	-0.05	-0.07	-0.06	0.05	-0.05	-0.11	-0.07	-0.11	-0.04	0.01	0.25	0.20	0.23	0.13	0.16	0.16	0.18	0.19	0.21	0.19	0.04	0.04	0.24	0.19
			1.18E-06	0.69	0.50	0.57	0.71	0.71	0.30	0.56	0.28	0.75	0.95	**0.0074**	**0.040**	**0.015**	0.20	0.11	0.10	0.06	**0.046**	**0.029**	**0.046**	0.74	0.74	**0.0091**	**0.050**
***Legionella* spp.**				0.05	-0.31	-0.30	0.09	0.01	-0.05	-0.23	-0.33	-0.15	-0.09	0.40	0.39	0.29	0.10	0.27	0.33	0.22	0.16	0.23	0.19	0.13	0.12	0.29	0.20
				0.71	**4.49E-04**	**9.19E-04**	0.44	0.96	0.66	**0.013**	**2.4E-04**	0.12	0.42	**2.3E-06**	**4.1E-06**	**1.17E-03**	0.35	**0.0028**	**2.3E-04**	**0.017**	0.10	**0.012**	**0.043**	0.20	0.26	**0.0011**	**0.033**
Temperature					-0.49	-0.50	0.07	0.27	0.50	-0.29	-0.28	0.07	-0.13	0.11	0.10	-0.09	-0.27	0.28	0.25	0.04	-0.05	-0.06	-0.12	0.03	0.04	-0.01	-0.16
					1.52E-09	**8.23E-10**	0.55	**0.0030**	**1.1E-09**	**0.0013**	**0.0020**	0.51	0.22	0.28	0.34	0.39	**0.0030**	**0.0018**	**0.0065**	0.75	0.71	0.64	0.25	0.83	0.75	0.95	0.11
Total chlorine						0.84	0.04	-0.26	-0.32	0.33	0.40	0.03	0.06	-0.42	-0.41	-0.20	-0.03	-0.34	-0.37	-0.22	-0.15	-0.06	-0.01	-0.05	-0.07	-0.25	-0.10
						0	0.74	**0.0054**	**3.3E-04**	**2.3E-04**	**3.1E-06**	0.83	0.62	**6.4E-07**	**1.6E-06**	**0.040**	0.80	**1.1E-04**	**2.6E-05**	**0.022**	0.14	0.62	0.96	0.69	0.54	**0.0069**	0.33
Free Chlorine							0.06	-0.25	-0.30	0.32	0.39	0.00	0.02	-0.43	-0.40	-0.19	0.02	-0.35	-0.36	-0.23	-0.14	-0.04	0.00	-0.02	-0.05	-0.26	-0.12
							0.62	**0.0079**	**8.0E-04**	**3.0E-04**	**5.9E-06**	0.97	0.91	**4.7E-07**	**2.4E-06**	**0.042**	0.88	**8.6E-05**	**2.8E-05**	**0.016**	0.16	0.73	0.99	0.85	0.71	**0.0057**	0.26
pH								-0.11	0.04	-0.30	-0.25	0.06	0.01	0.01	0.12	-0.21	-0.23	0.07	0.04	-0.18	-0.20	-0.19	-0.23	-0.04	-0.07	-0.27	-0.23
								0.27	0.72	**0.0011**	**0.0082**	0.65	0.95	0.96	0.26	**0.030**	**0.013**	0.51	0.75	0.06	**0.039**	**0.048**	**0.013**	0.76	0.54	**0.0027**	**0.013**
Total Al									0.49	0.09	-0.02	0.09	0.03	0.22	0.07	0.29	0.11	0.11	0.13	0.18	0.09	0.02	-0.04	0.07	0.07	0.25	-0.01
									6.9E-12	0.33	0.86	0.36	0.80	**0.0065**	0.49	**2.47E-04**	0.24	0.22	0.15	**0.031**	0.36	0.83	0.74	0.43	0.49	**0.0015**	0.97
Dissolved Al										-0.03	-0.03	0.23	0.08	0.09	0.07	-0.01	-0.08	0.17	0.18	0.06	0.07	-0.13	-0.16	0.02	0.02	-0.01	-0.06
										0.82	0.76	**0.0047**	0.37	0.31	0.46	0.92	0.41	**0.046**	**0.029**	0.56	0.50	0.15	0.06	0.90	0.90	0.96	0.57
Total P											0.68	0.07	0.10	-0.28	-0.39	0.03	0.17	-0.29	-0.29	0.08	0.09	-0.08	-0.03	-0.18	-0.14	0.02	0.08
											0	0.44	0.27	**3.1E-04**	**2.4E-07**	0.77	**0.043**	**1.8E-04**	**1.9E-04**	0.42	0.33	0.37	0.79	**0.032**	0.11	0.83	0.41
Dissolved P												0.04	0.10	-0.42	-0.47	-0.12	0.08	-0.33	-0.30	0.01	0.06	-0.14	-0.07	-0.20	-0.15	-0.07	0.06
												0.74	0.30	**1.5E-08**	**7.7E-11**	0.19	0.38	**1.4E-05**	**8.2E-05**	0.96	0.56	0.12	0.46	**0.017**	0.07	0.45	0.57
Total Cr													0.38	0.02	-0.02	0.01	-0.04	0.15	0.09	0.03	0.04	-0.13	-0.11	-0.12	-0.11	0.00	0.00
													3.0E-07	0.88	0.85	0.93	0.71	0.07	0.31	0.78	0.68	0.15	0.21	0.16	0.24	0.99	1.00
Dissolved Cr														0.00	0.01	0.00	0.05	0.02	0.08	0.04	0.07	-0.13	-0.11	-0.14	-0.12	0.00	0.07
														0.97	0.93	0.97	0.63	0.85	0.39	0.70	0.46	0.14	0.24	0.10	0.18	0.97	0.50
Total Mn															0.75	0.51	0.23	0.36	0.38	0.24	0.19	0.21	0.16	0.15	0.11	0.41	0.14
															0	**2.4E-13**	**0.0050**	**1.8E-06**	**4.7E-07**	**0.0027**	**0.022**	**0.0090**	0.07	0.07	0.24	**1.7E-08**	0.10
Dissolved Mn																0.31	0.18	0.35	0.37	0.16	0.13	0.15	0.12	0.15	0.13	0.21	0.06
																6.5E-05	**0.030**	**2.4E-06**	**5.4E-07**	0.07	0.14	0.08	0.16	0.07	0.14	**0.0091**	0.51
Total Fe																	0.38	0.13	0.16	0.37	0.29	0.32	0.28	0.14	0.11	0.63	0.25
																	5.3E-07	0.14	0.05	**9.5E-07**	**1.8E-04**	**2.6E-05**	**4.1E-04**	0.10	0.21	**0**	**0.0015**
Dissolved Fe																		-0.06	-0.02	0.13	0.15	0.11	0.14	0.10	0.10	0.22	0.26
																		0.55	0.85	0.14	0.09	0.20	0.10	0.26	0.26	**0.0056**	**9.7E-04**
Total Ni																			0.82	0.23	0.19	0.11	0.08	-0.02	-0.03	0.18	0.12
																			0	**4.8E-03**	**0.018**	0.22	0.43	0.87	0.79	**0.035**	0.19
Dissolved Ni																				0.25	0.24	0.14	0.13	0.00	0.00	0.21	0.15
																				1.4E-03	**3.2E-03**	0.10	0.14	1.00	0.97	**0.0094**	0.07
Total Cu																					0.75	0.16	0.15	-0.19	-0.20	0.46	0.38
																					0	0.06	0.07	**0.018**	**0.017**	**9.8E-11**	**3.1E-07**
Dissolved Cu																						0.15	0.17	-0.20	-0.19	0.39	0.43
																						0.08	**0.036**	**0.013**	**0.022**	**2.2E-07**	**5.0E-09**
Total Zn																							0.88	0.51	0.49	0.31	0.10
																							0	**3.1E-13**	**6.4E-12**	**6.7E-05**	0.26
Dissolved Zn																								0.47	0.50	0.26	0.13
																								3.8E-11	**2.5E-12**	**9.6E-04**	0.13
Total Cd																									0.91	0.06	-0.09
																									0	0.54	0.36
Dissolved Cd																										0.04	-0.08
																										0.71	0.42
Total Pb																											0.49
																											0
Dissolved Pb																											

[a]p-values of significant correlations ($p < 0.05$) are indicated in bold and blue and were calculated using a Benjamini Hochberg correction.

Table S5. Results of total lead and dissolved cadmium linear mixed-effects models in distribution system samples.

	Response Variables[a]					
	log(total lead)			log(dissolved cadmium)		
	β	CI	p-value	β	CI	p-value
Fixed Effects						
(Intercept)	**-1.15**	**-1.41 to -0.88**	**3.28×10^{-11}**	**-1.2**	**-1.7 to -0.7**	**2×10^{-5}**
Percent galvanized premise plumbing[b]				**0.010**	**0.005 to 0.015**	**0.002**
Time period (spring)	**1.25**	**0.98 to 1.52**	**2.54×10^{-8}**	0.2	-0.2 to 0.7	0.3
Visit (two weeks post-SL replacement)	**-0.55**	**-0.86 to -0.23**	**1.34×10^{-3}**	**-0.6**	**-1.0 to -0.3**	**0.004**
Visit (five weeks post-SL replacement)	-0.29	-0.61 to 0.02	0.070	**-0.9**	**-1.3 to -0.5**	**1×10^{-4}**
Random Effects	Variance			Variance		
Home	0.002			0.09		
Observations (n)	51			51		

[a]The slope (β), confidence interval (CI), and p-value of any fixed effects with a significant impact on the response variable are indicated in bold.

[b]Percent galvanized premise plumbing refers to the fraction of premise plumbing materials in a home that is comprised of galvanized iron. Percentages were determined from premise plumbing surveys conducted in each home sampled.

Table S6. Lead masses associated with different portions of in-home and SL plumbing in Home 11 before and after SL replacement.[a]

	Volume (L)	Total lead mass before SL replacement (μg)		Total lead mass after SL replacement (μg)	
		Sampling event 1	Sampling event 2	Sampling event 3	Sampling event 4
Premise plumbing	0-4.2	13.20	7.32	1.12	1.43
Private SL	4.2-6.9	4.49	4.54	0.06	2.74
Public SL	6.9-9.3	11.48	15.08	0.05	0.42
Total in 12-L sample	**0-12**	**33.46**	**32.41**	**1.30**	**4.64**

[a]Lead masses were determined by integrating Home 11 total lead concentrations of the sequential sample profile obtained over the different portions of pipe in the home. Rectangular integration was used (average concentration of each sample was assumed to be the average concentration of that 1 L sample).
[b]Volumes of water in the premise plumbing, private SL, and public SL segments were determined through a home survey of pipe length, material, and diameter.
[c]Includes the total integrated lead concentration of 12 1 L samples.

Table S7. Cadmium masses associated with different portions of in-home and SL plumbing in Home 11 before and after SL replacement.[a]

	Volume (L)[b]	Total cadmium mass before SL replacement (μg)		Total cadmium mass after SL replacement (μg)	
		Sampling event 1	Sampling event 2	Sampling event 3	Sampling event 4
Premise plumbing	0-4.2	4.02	3.99	0.49	0.47
Private SL	4.2-6.9	3.06	3.02	0.11	0.05
Public SL	6.9-9.3	1.90	1,88	0.05	0.05
Total in 12 L sample[c]	**0-12**	**10.90**	**10.87**	**0.71**	**0.62**

[a]Cadmium masses were determined by integrating Home 11 total cadmium concentrations of the sequential sample profile obtained over the different portions of pipe in the home. Rectangular integration was used (average concentration of each sample was assumed to be the average concentration of that 1 L sample).
[b]Volumes of water in the premise plumbing, private SL, and public SL segments were determined through a home survey of pipe length, material, and diameter.
[c]Includes the total integrated cadmium concentration of 12 1 L samples.

Table S8. Kendall's Tau correlation coefficient (above) and associated p-values[a] (below) obtained using Kendall correlation analysis between all water quality parameters with distribution system sample type.

	Total bacteria	*Mycobacterium* spp.	*Legionella* spp.	Temperature	Total chlorine	Free Chlorine	pH	Total Al	Dissolved Al	Total P	Dissolved P	Total Cr	Dissolved Cr	Total Mn	Dissolved Mn	Total Fe	Dissolved Fe	Total Ni	Dissolved Ni	Total Cu	Dissolved Cu	Total Zn	Dissolved Zn	Total Cd	Dissolved Cd	Total Pb	Dissolved Pb
Total bacteria		0.54	0.47	-0.01	-0.42	-0.38	-0.04	-0.02	-0.09	-0.09	-0.27	-0.06	0.16	0.45	0.40	0.24	0.21	0.26	0.29	0.14	0.08	0.21	0.15	0.18	0.19	0.19	0.20
		0.0011	**0.0077**	0.97	**0.022**	**0.046**	0.89	0.94	0.76	0.78	0.19	0.87	0.52	**0.011**	**0.0310**	0.27	0.35	0.21	0.15	0.56	0.80	0.35	0.55	0.42	0.39	0.40	0.36
***Mycobacterium* spp.**			0.40	-0.07	-0.28	-0.26	-0.05	-0.07	-0.23	-0.10	-0.26	-0.07	0.16	0.33	0.36	0.21	0.14	0.18	0.12	0.04	0.05	0.08	0.05	0.05	0.06	0.16	0.13
			0.033	0.85	0.18	0.21	0.87	0.85	0.27	0.75	0.21	0.83	0.50	0.09	0.06	0.33	0.60	0.45	0.64	0.89	0.87	0.80	0.88	0.88	0.86	0.51	0.63
***Legionella* spp.**				-0.05	-0.40	-0.42	0.08	0.03	0.01	-0.14	-0.39	0.10	0.19	0.47	0.48	0.36	0.15	0.29	0.20	0.20	0.20	0.08	0.05	0.06	0.11	0.21	0.22
				0.87	**0.029**	**0.022**	0.79	0.92	0.96	0.58	**0.038**	0.75	0.38	**0.0077**	**0.0076**	**0.060**	0.53	0.16	0.36	0.36	0.36	0.81	0.88	0.86	0.71	0.33	0.31
Temperature					-0.02	-0.02	0.60	-0.28	0.21	-0.27	-0.25	0.17	-0.09	-0.13	0.02	-0.40	-0.45	-0.14	-0.18	-0.24	-0.28	-0.22	-0.30	0.12	0.08	-0.43	-0.29
					0.95	0.94	**1.2E-04**	0.17	0.33	0.19	0.23	0.46	0.78	0.61	0.93	**0.031**	**0.012**	0.57	0.45	0.27	0.17	0.31	0.15	0.64	0.80	**0.019**	0.15
Total chlorine						0.87	-0.03	0.15	0.03	0.30	0.47	-0.02	-0.28	-0.67	-0.66	-0.30	-0.24	-0.20	-0.26	-0.13	-0.14	0.07	0.07	-0.02	-0.03	-0.22	-0.26
						2.8E-14	0.92	0.56	0.91	0.14	**0.0080**	0.93	0.18	**4.2E-06**	**6.0E-06**	0.14	0.27	0.36	0.21	0.62	0.59	0.86	0.86	0.94	0.92	0.31	0.21
Free Chlorine							-0.06	0.17	0.05	0.31	0.51	-0.08	-0.30	-0.68	-0.68	-0.32	-0.22	-0.26	-0.27	-0.09	-0.09	0.10	0.10	0.00	-0.01	-0.24	-0.30
							0.87	0.47	0.87	0.12	**0.0034**	0.80	0.14	**2.5E-06**	**1.7E-06**	0.12	0.33	0.21	0.20	0.77	0.77	0.74	0.75	0.99	0.98	0.27	0.14
pH								-0.28	0.18	-0.35	-0.34	0.16	-0.04	-0.11	0.09	-0.32	-0.48	-0.16	-0.21	-0.19	-0.22	-0.33	-0.42	-0.04	-0.06	-0.44	-0.30
								0.17	0.45	0.08	0.09	0.51	0.89	0.70	0.78	0.11	**0.0075**	0.52	0.33	0.38	0.31	0.09	**0.022**	0.89	0.87	**0.0124**	0.14
Total Al									0.29	0.43	0.37	0.03	0.06	0.03	-0.15	0.34	0.26	0.22	0.20	0.33	0.32	0.27	0.25	0.05	0.06	0.28	0.12
									0.15	**0.017**	0.05	0.92	0.87	0.92	0.53	0.08	0.20	0.32	0.36	0.09	0.11	0.20	0.24	0.88	0.87	0.16	0.66
Dissolved Al										0.12	0.04	0.46	0.24	-0.09	-0.07	-0.07	-0.17	0.07	-0.03	0.20	0.27	-0.05	-0.05	0.06	0.02	-0.19	-0.13
										0.66	0.89	**0.010**	0.25	0.78	0.84	0.83	0.49	0.84	0.92	0.37	0.20	0.87	0.88	0.86	0.93	0.39	0.63
Total P											0.58	0.04	0.08	-0.13	-0.30	0.11	0.26	0.05	0.06	0.16	0.17	0.24	0.30	0.02	0.00	0.19	0.10
											2.2E-04	0.90	0.81	0.63	0.14	0.73	0.21	0.88	0.87	0.52	0.48	0.27	0.14	0.95	0.99	0.39	0.74
Dissolved P												-0.10	-0.06	-0.35	-0.52	-0.03	0.08	-0.12	-0.09	0.01	0.01	0.22	0.26	0.01	0.02	0.09	-0.07
												0.75	0.87	0.07	**0.0022**	0.92	0.80	0.66	0.78	0.96	0.96	0.31	0.21	0.98	0.93	0.78	0.86
Total Cr													0.41	0.03	0.09	0.03	-0.08	0.24	0.04	0.13	0.17	-0.12	-0.08	0.00	0.01	-0.09	-0.04
													0.026	0.92	0.78	0.93	0.79	0.25	0.89	0.63	0.46	0.66	0.81	0.99	0.96	0.78	0.89
Dissolved Cr														0.30	0.31	0.29	0.22	0.24	0.23	0.27	0.33	-0.04	0.00	-0.10	-0.06	0.16	0.28
														0.14	0.12	0.16	0.32	0.26	0.30	0.19	0.10	0.90	1.00	0.75	0.87	0.50	0.18
Total Mn															0.71	0.47	0.38	0.31	0.32	0.22	0.21	0.06	0.07	0.05	0.05	0.37	0.35
															3.5E-07	**0.0077**	**0.045**	0.13	0.12	0.31	0.35	0.86	0.85	0.87	0.87	**5.4E-02**	0.07
Dissolved Mn																0.24	0.19	0.26	0.21	0.13	0.12	-0.13	-0.15	-0.05	-0.04	0.15	0.21
																0.27	0.39	0.21	0.33	0.62	0.67	0.61	0.53	0.87	0.89	0.54	0.33
Total Fe																	0.47	0.36	0.39	0.38	0.38	0.22	0.25	0.04	0.05	0.58	0.42
																	0.0077	0.06	**0.038**	**0.046**	**0.046**	0.31	0.24	0.89	0.87	**0**	**0.023**
Dissolved Fe																		0.29	0.40	0.31	0.35	0.24	0.32	0.06	0.10	0.45	0.45
																		0.15	**0.029**	0.13	0.08	0.25	0.12	0.87	0.74	**0.012**	**0.012**
Total Ni																			0.78	0.26	0.20	0.16	0.13	0.10	0.13	0.36	0.35
																			1.3E-09	0.21	0.36	0.51	0.61	0.74	0.61	0.06	0.07
Dissolved Ni																				0.32	0.30	0.16	0.14	0.02	0.07	0.41	0.45
																				0.11	0.14	0.52	0.56	0.94	0.83	**0.023**	**0.011**
Total Cu																					0.78	0.04	0.06	-0.17	-0.22	0.16	0.21
																					1.3E-09	0.89	0.86	0.49	0.32	0.52	0.33
Dissolved Cu																						-0.01	0.07	-0.20	-0.21	0.20	0.27
																						0.98	0.86	0.36	0.34	0.36	0.20
Total Zn																							0.87	0.46	0.48	0.18	0.02
																							2.8E-14	**0.011**	**6.9E-03**	0.45	0.94
Dissolved Zn																								0.39	0.43	0.20	0.08
																								0.036	**0.019**	0.36	0.80
Total Cd																									0.89	0.05	-0.03
																									0	0.88	0.91
Dissolved Cd																										0.09	0.02
																										0.78	0.93
Total Pb																											0.61
																											6.8E-05
Dissolved Pb																											

[a]p-values of significant correlations ($p < 0.05$) are indicated in bold and blue and were calculated using a Benjamini Hochberg correction.

Table S9. Results of two linear mixed-effects models developed for total bacteria in premise plumbing using different explanatory variables.

	Response Variables[a]					
	log(total bacteria)			log(total bacteria)		
	β	CI	p-value	β	CI	p-value
Fixed Effects						
(Intercept)	**6.4**	**5.4 to 7.5**	**3×10^{-12}**	**5.2**	**4.1 to 6.2**	**9×10^{-11}**
log(total lead)	**0.5**	**0.2 to 1.0**	**0.01**			
log(free chlorine)				**-0.9**	**-1.5 to -0.3**	**0.005**
Time period (spring)	-0.5	-2.0 to 0.8	0.5	-0.3	-1.6 to 0.9	0.6
Visit (two weeks post-SL replacement)	-0.3	-0.8 to 0.2	0.2	-0.05	-0.5 to 0.4	0.8
Visit (five weeks post-SL replacement)	-0.5	-0.9 to 0.0	0.06	-0.1	-0.6 to 0.4	0.6
Random Effects	Variance			Variance		
Home	1.5			1.3		
Observations (n)	51			51		

[a]The slope (β), confidence interval (CI), and p-value of any fixed effects with a significant impact on the response variable are indicated in bold.

References

1 *Definition and Procedure for the Determination of the Method Detection Limit, Revision 2*, 2016.

2 Wisconsin Department of Natural Resources Laboratory Certification Program, *Analytical Detection Limit Guidance & Laboratory Guide for Determining Method Detection Limits*, 1996.

3 T. M. Webster, A. L. Smith, R. R. Reddy, A. J. Pinto, K. F. Hayes and L. Raskin, Anaerobic microbial community response to methanogenic inhibitors 2-bromoethanesulfonate and propynoic acid, *Microbiologyopen*, 2016, **5**, 537–550.

4 P. Hewett and G. H. Ganser, A Comparison of Several Methods for Analyzing Censored Data, *Ann. Occup. Hyg.*, 2007, **51**, 611–632.

5 D. Bates, M. Maechler, B. Bolker and S. Walker, Fitting Linear Mixed-Effects Models Using lme4, *J. Stat. Softw.*, 2015, **67**, 1–48.

6 A. Kuznetsova, P. B. Brockhoff and R. Haubo Christensen Bojesen, lmerTest: Tests in Linear Mixed Effects Models.

7 W. J. Rhoads, E. Garner, P. Ji, N. Zhu, J. Parks, D. O. Schwake, A. Pruden and M. A. Edwards, Distribution System Operational Deficiencies Coincide with Reported Legionnaires' Disease Clusters in Flint, Michigan, *Environ. Sci. Technol.*, , DOI:10.1021/acs.est.7b01589.

8 D. O. Schwake, E. Garner, O. R. Strom, A. Pruden and M. A. Edwards, Legionella DNA Markers in Tap Water Coincident with a Spike in Legionnaires' Disease in Flint, MI, *Environ. Sci. Technol. Lett.*, , DOI:10.1021/acs.estlett.6b00192.

9 B. G. Byrne, S. McColm, S. P. McElmurry, P. E. Kilgore, J. Sobeck, R. Sadler, N. G. Love and M. S. Swanson, Prevalence of Infection-Competent Serogroup 6 Legionella pneumophila within Premise Plumbing in Southeast Michigan, *MBio*.

10 City of Flint Water Treatment Plant, *City of Flint Water Treatment Plant Monthly*

Operating Report, Flint, 2016.

11 E. I. Prest, F. Hammes, S. Kötzsch, M. C. M. van Loosdrecht and J. S. Vrouwenvelder, Monitoring microbiological changes in drinking water systems using a fast and reproducible flow cytometric method, *Water Res.*, 2013, **47**, 7131–7142.

12 H. Wang, S. Masters, Y. Hong, J. Stallings, J. O. Falkinham, M. A. Edwards and A. Pruden, Effect of Disinfectant, Water Age, and Pipe Material on Occurrence and Persistence of Legionella, mycobacteria, Pseudomonas aeruginosa, and Two Amoebas, *Environ. Sci. Technol.*, 2012, **46**, 11566–11574.

13 H. Wang, M. A. Edwards, J. O. Falkinham and A. Pruden, Probiotic Approach to Pathogen Control in Premise Plumbing Systems? A Review, *Environ. Sci. Technol.*, 2013, **47**, 10117–10128.

14 N. Fierer, J. A. Jackson, R. Vilgalys and R. B. Jackson, Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays., *Appl. Environ. Microbiol.*, 2005, **71**, 4117–4120.

15 H. Miyamoto, H. Yamamoto, K. Arima, J. Fujii, K. Maruta, K. Izu, T. Shiomori and S. Yoshida, Development of a new seminested PCR method for detection of Legionella species and its application to surveillance of legionellae in hospital cooling tower water., *Appl. Environ. Microbiol.*, 1997, **63**, 2489–2494.

16 R. Lesnik, I. Brettar and M. G. Hofle, *ISME J*, 2016, 10, 1064–1080.

17 N. Radomski, A. Roguet, F. S. Lucas, F. J. Veyrier, E. Cambau, H. Accrombessi, R. Moilleron, M. A. Behr and L. Moulin, atpE gene as a new useful specific molecular target to quantify Mycobacteriumin environmental samples, *BMC Microbiol.*, 2013, **13**, 277.

18 B. A. Wullings, G. Bakker and D. van der Kooij, Concentration and Diversity of Uncultured Legionella spp. in Two Unchlorinated Drinking Water Supplies with Different Concentrations of Natural Organic Matter, *Appl. Environ. Microbiol.*, 2011, **77**, 634–641.