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Water Quality During Extended Stagnation and Flushing in a College Residential Hall

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Abstract

Water quality can change drastically within a building during periods of little to no water use: residual disinfectant can decay, leading to microbial growth, and metals can leach into the water. This study aimed to understand the change in water quality within a 4-story, 10,000 ft^2 residential building over five months of building closures driven by the coronavirus (COVID-19) pandemic. Sampling events occurred seven times between April and September 2020. Complete flushing of cold and hot water was performed; samples were collected immediately after flushing, then one and four weeks after flushing, with building re-occupancy occurring before four weeks. A total of 90 full samples were collected. Chlorine residual was not detectable (>0.2 mg/L) for any sample collected during the five months before flushing. Flushing refreshed chlorine concentrations, and a minimum of 0.3 mg/L total chlorine was brought to all cold water outlets. Flushing reduced the average lead concentration in cold water from 2.4 ppb to 1.0 ppb, however, the concentration rebounded to an average of 2.6 ppb just one week after flushing. Additionally, during flushing lead was measured at a maximum of 150 ppb as discolored water slugs moved through the system. Flushing reduced *Legionella* spp. concentrations in cold and hot water, and these concentrations remained reduced one week later. Levels of *Legionella* spp. were further reduced when normal use was

resumed. Results suggest that flushing can refresh water quality in the short term, but its efficacy over a longer period is uncertain.

Water impact statement

Water quality in a large residential building was studied for up to five months of stagnation followed by flushing. Flushing reduced *Legionella* spp. concentrations, and at sampling events one and subsequently four weeks post-flushing there remained a reduction. But, average lead and copper concentrations rebounded to higher than pre-flushing levels just one week after flushing. This work helps elucidate the tradeoffs associated with flushing to refresh building water quality.

1. Introduction

The closure of schools, businesses, and other buildings across the United States due to the coronavirus (COVID-19) pandemic resulted in little to no occupancy in many buildings (1–10). This was concerning because drinking water quality in buildings can deteriorate during periods of stagnation, with the accumulation of heavy metals and bacterial growth causing potential health risks (11–13). The primary focus of this study was to better understand changes to cold and hot water chemistry and microbiology in a large residential building after extensive periods of stagnation and flushing. At the time this study was initiated, few evidence-based national or industry guidelines existed for how to reopen shutdown buildings.

Exposure to water with high pathogenic concentrations can pose acute health risks. Drinking water-associated pathogens that can cause infections in immunocompromised or otherwise susceptible individuals (DWPI) like *Legionella pneumophila* can cause fatal disease (14). *L. pneumophila* grow in building plumbing without temperature control (15). Ideal *L. pneumophila* growth conditions are found in plumbing allowing amplification even when not detected in influent water (16), or when disinfectant is

present (17,18). Stagnation may provide particularly ideal conditions, with disinfectant decay, nutrient availability, and warm water temperatures. A study analyzing water quality in student dormitories discovered that the microbial community composition changed and cell counts increased by a factor of 10,000 cells/mL following six days of stagnation (19). Biofilms may also have an impact on the water microbiome during stagnation (6,20). Many pre-existing studies only examined stagnation up to a few days, but buildings like dormitories, summer homes, and homes for sale may go unused for weeks or months at a time.

Building plumbing often contains metal components that can corrode and leach metals, including lead and copper, into the water. Copper is widely used in indoor plumbing and is a gastrointestinal tract irritant at certain levels in drinking water (21). Lead can cause damage to the kidneys, nervous system, and reproductive system, and is of particular risk to children. In building plumbing, lead can leach from lead, copper, and galvanized steel pipes (22). Lead has also been shown to leach from brass valves considered “lead-free” (23) (“lead free” is defined as a maximum weighted average of 0.25% lead across the wetted surface (24)), and regulations have changed over time. Increased lead and copper concentrations have been observed with stagnation periods of just 30 minutes to 8 hours (25). Lead and copper levels increase after periods of stagnation (22,26). In a study performed in schools and large buildings in Canada, researchers compared several stagnation and flushing times, and found that the highest copper and lead concentrations were recorded in the first 250 mL after 8-hour stagnation (25). In addition to corrosion, stagnant water can contribute to issues such as taste and odor throughout building plumbing (27).

To address water quality degradation, several shock remediation strategies have been suggested by experts, including hyperchlorination, thermal shock, and flushing (25). To fully flush plumbing, all water within the building and entering the building must be replaced with fresh water. Flushing has been recommended (28,29) as flushing can replenish disinfectant residual (30), increase the temperature in hot water lines (29), and decrease the temperature in cold water lines outside the range of pathogen

growth (31,32). Still, flushing is imperfect in practice, requires significant time, and may only provide temporary improvement to water quality. For example, lead concentrations that decreased with flushing following 8 hours of stagnation returned to >45% of the first draw concentration after just 30 minutes of subsequent stagnation (25). Flushing can also be considered a waste of water if it does not improve water quality.

The goal of this study was to better understand the chemical and microbiological changes in hot and cold water quality over months of stagnation with subsequent flushing strategies employed. First draw hot and cold samples were collected and analyzed during five months of stagnation. A flushing plan was developed and implemented to replenish disinfectant residual. Additional sampling one week after flushing prior to reoccupation, and four weeks after flushing after occupancy in the building resumed allowed investigation into the long-term effects of flushing.

2. Methods

2.1 Water source and residential plumbing

The 10,000 ft^2 [929 m^2] residential building in this study was located in Indiana and constructed in 1930. The history of plumbing rennovcations was unknown to current residents and management. Typically, 30 to 35 students lived there during the academic year. There were three half bathrooms on the basement, first, and third floors respectively. On the second floor, there was one large bathroom comprised of four showers, three toilets, three bathroom sinks, and one utility sink (Figure 1). There was an industrial style kitchen with a dish sprayer in the basement. Irrigation and washing machines were also located off basement supply lines. There was no central air conditioning in the building. While a large room on the third floor contained multiple air conditioning units, no water outlets were in this room and air conditioning was turned off during most of the study period, resulting in high in-building temperatures.

A public water system supplied treated groundwater to the building. The groundwater source was oxidized, aerated, filtered, chlorinated for secondary disinfection with free chlorine, and a corrosion

inhibitor was added before distribution. The highest monthly average of chlorine residual measured throughout the distribution system in 2020 was reported to be 1.05 mg/L, with a range of 0.94 to 1.48 mg/L(33)]. Iron and polyvinylchloride water distribution pipes conveyed water to the building water service line. The domestic cold and hot water plumbing was made of galvanized steel. Upstream of the utility room, the pipe diameter was 1.6 in [4.0 cm]. It then decreased to 1.25 in [3.175 cm] before entering the utility room and splitting into hot and cold lines. The hot water line was connected to the softener before the water heater, while cold water was not softened. The softener was comprised of two 18 gallon [68 L] tanks, with one for primary use and the other for use during backwash. The water heater was 100 gallons [379 L] with a maximum temperature setting of 82°C (Ruud, Atlanta, GA, USA). From the water heater, hot water flowed into a 115 gallon [435 L] insulated holding tank that did not have an independent heating supply.

2.2 Water sampling, flushing, and onsite analysis

Routine sampling trips occurred monthly between April and August 2020, representing one (April) to five (August) months of stagnation after the building was vacated on approximately March 16, 2020. Immediately following the routine sampling in August, flushing was performed for the building plumbing. One week and four weeks following the flushing activity, first-flush samples were collected at the ten locations. The last sampling trip, four weeks after flushing, represented normal use conditions for the building. At this time, the typical 30 to 35 residents had been living in the home for approximately 3 weeks.

Ten full samples were collected per trip, with a total of 50 full samples collected before the building water system was flushed. Samples were collected in the following order: basement bathroom sink hot, basement kitchen cold, a 2nd floor bathroom sink cold then hot, another 2nd floor bathroom sink cold then hot, 2nd floor shower “A” set to hot, 2nd floor shower “C” set to hot, 3rd floor bathroom sink cold then hot (Figure 1). The sampling order was designed to collect samples closest to the building entry point first, and then move to the most distal point in the building to limit sampling interference. Samples were collected in the same order at each sampling event for consistency. The 3rd floor bathroom and 2nd floor

showers had single handle faucets while other outlets had separate hot and cold handles. With single handle faucets, the angle was adjusted fully to either side before initiating flow.

The flushing strategy was designed as follows. First, the kitchen sink was flushed for cold water for 15 minutes, until a consistent chlorine residual was detected in the water. Full samples were collected at 2, 5, 10, and 15 minutes, with temperature and chlorine analyzed more frequently. Then, aerators were removed at all sinks possible and each tap was flushed for cold water for at least two minutes, starting nearest the point of entry and moving away. After cold water was flushed at each tap, the kitchen sink was flushed for hot water for 60 minutes. Water temperature remained stable after 60 minutes of flushing, and the water had theoreticaly moved through the hot water tank based on calculated water volumes. Full samples were collected at 0, 20, 30, 40, 50, and 60 minutes, with chlorine and temperature measured more frequently. Similarly, each tap was then flushed for hot water for at least 2 minutes, moving away from the point of entry. Additional samples were collected at multiple taps for metals analysis, capturing slugs of discolored water. Flushing was sometimes extended to allow discolored slugs to clear the system. Full samples were collected immediately after flushing for the ten previously described sampling locations.

For each trip, approximately 2 L of first draw samples was collected per location, for a total of about 20 L per trip [5.28 gal]. Water samples were collected sequentially: 150 mL for onsite water quality analysis (described below), two 125 mL HDPE bottles for metal (Al, As, Be, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Zn) and *L. pnemophila* analysis. Then, one 15 mL centrifuge tube for total cell count (TCC) and intact cell count (ICC) analysis, one 250 mL amber glass bottle filled for total organic carbon (TOC) analysis, and lastly one 1 L HDPE bottle filled for quantitative polymerase chain reaction (qPCR) analysis. Alkalinity and ion samples were also collected at the first two sampling locations in a 250 mL amber bottle and 125 mL HDPE bottle, respectively following collection of other samples. Field (Type 1 water filled on-site) and trip (closed bottles of Type 1 water brought to the site) blanks were analyzed as controls during each trip.

Onsite water quality analysis included temperature, pH, total chlorine, and dissolved oxygen (DO) concentration. Temperature and pH were measured with an Oakton 450™ pH probe. Total chlorine was measured with HACH® Pocket Colorimeter chlorine test kits by adding *N,N*-diethyl-phenylenediamine (DPD) reagent to 10 mL samples. DO was measured using a Hach SL1000 Portable Parallel Analyzer.

2.3 Chemical laboratory analysis

Metal concentrations were quantified by inductively coupled plasma-optical emission spectrometry (iCAP 7400 Duo ICP-OES, Thermo Scientific) and an autosampler (ASX-280, CETAC Teledyne). Ion concentrations were quantified by ion chromatography (Metrohm 940 Professional IC Vario). TOC was measured using a Shimadzu TOC-L CPH/CPN, following USEPA method 415.1 (34). Alkalinity concentration as $CaCO_3$ was determined by titrating 50 mL sample with 0.025 N H_2SO_4 to a pH of 4.6. Before titration, 250 µL of bromocresol green indicator solution was added to each sample.

2.4 Microbiological analysis

Total cell counts. Flow cytometry (FCM) was used to determine total cell count (TCC) following Swiss Research method 366.1. (35). Briefly, each water sample was stained 1:100 with SYBR Green I nucleic acid gel stain diluted in filtered dimethyl sulfoxide (DMSO). Live (intact cells) and dead cells were differentiated by using propidium iodide, which selectively binds to intact cells. The samples were incubated in the dark at 37° C for 13 minutes. Samples from each fixture were analyzed using a Cytoflex flow cytometer (Beckman-Coulter Inc., Brea, CA, USA). A constant gating strategy was applied to all samples.

Legiolert. Starting with the flushing event in August and onward (Trips 5, 6, and 7), Legiolert (IDEXX, Westbrook, ME, USA) was used to identify and quantify *L. pneumophila* presence in samples collected at all ten sampling locations as described in Section 2.2. An additional Legiolert sample was collected at a bathroom sink on the first floor during Trips 5, 6, and 7 that had been previously unsampled. A total of 16 cold water samples and 18 hot water samples were collected for Legiolert analysis during these

sampling trips. Manufacturer instructions were followed. Positive wells were counted after seven days as any wells that have brown color and/or turbidity greater than a negative control.

qPCR. Approximately 1L of water was filtered onto 47 mm polycarbonate filters with pore size 0.4 μm (Millipore #HTTP04700) for future molecular biology analysis. DNA was extracted from the filters using the Qiagen DNeasy PowerWater DNA extraction kit (Qiagen, Germantown, MD, USA). DNA extracts were kept frozen in a -80°C freezer until qPCR analysis was conducted. The following targets were quantified using qPCR: *Legionella* spp. (targeting 23S rRNA), *L. pneumophila* (macrophage infectivity potentiator: *mip* gene) (36–38). For every assay performed, a standard curve of at least seven points (10-10^7 gc/rxn), a non-template control, and samples were tested in triplicate on an Applied Biosystems QuantStudio 5 (Life Technologies, Thermo-Fisher, Foster City, CA). Standard curves were generated using gBlock gene fragments (Integrated DNA Technologies, Coralville, IA). Amplification reaction mixtures (final total volume of 10 μL) contained 1 μL template (10x diluted DNA), 5 μL of 2x iTaq Universal Probes Supermix (BioRad, CA, USA), 250 nM of each primer and 93.75 nM of probe. The thermal cycling protocol included 2 min at 95° C for initial denaturation, followed by 40 cycles of two steps consisting of 15 s at 95° C and 30 s at 60° C ((12). A minimum R^2 value of 0.97 was used. Efficiency ranged 85% - 100% for *Legionella* spp and 70-100% for *L. pneumophila* mip. The limit of detection (LOD) was defined as the lowest concentration at which at least 95% of the standard replicates were detected (39), resulting in an LOD of 10,000 gc/L for both 23S rRNA and *mip.*

2.5 Statistical analysis

Statistical analysis for water quality data was performed using Python (version 3.10.6). qPCR results less than the LOD were replaced with one half the LOD for plotting and statistical analyses. Metals concentrations below the LOD were replaced with one half the LOD, and concentrations below the LOQ were replaced with half the LOQ (Table S1). Wilcoxon signed-rank test was used to compare concentrations of metals, TOC, total cell counts, and *Legionella* spp. before and after flushing (40). A type I error at the significance level of 0.05 was selected for all analyses.

3 Results and Discussion

3.1 In-building stagnation and chlorine decay

Building water use according to available water bills steadily decreased from March to June (Figure 2). The total water use during the billing before the building was vacated (period from 02/06/2020 to 03/04/2020) was 30,000 gallons. Then, from 4/04/2020 to 5/05/2020, the first full billing period without building occupants, the total water use dropped to 1,000 gallons. While sampling did occur during this month, only about 3 to 4 gallons were collected. The water use during this period can likely be attributed to residents using toilets and faucets while they collected belongings. The billing unit for this building was 100 gallons, but the sensitivity of the water meter was in 1,000 gallon increments. Thus, the 1,000 gallon use may have been substantially less, but resulted in passing a threshold. The following billing period (5/03/2020 to 6/03/2020) had a billed usage of zero gallons, showing that sampling did not bring the water use above a billable threshold. The final sampling trip occurred four weeks after flushing, and reflected "normal" use conditions (9/10/2020), as all residents had returned to the building approximately three weeks prior to the sampling date. The billed usage for the billing period from 09/04/2020 to 10/05/2020 was 35,000 gallons, which is comparable to the water use in the month before the building was vacated (the billed water use from 02/06/2020 to 03/04/2020 was 30,000 gallons).

Disinfectant residual is added to water to prevent pathogens from growing after disinfection (40), and its absence can allow for microbial regrowth (41). For the 70 first-draw samples collected throughout the study, only 2 of 70 contained chlorine at the state regulatory minimum detected limit (> 0.2 mg/L as Cl_2) (Table S2), despite addition of free chlorine at the distribution system level. These two samples were collected during the final sampling trip, when the residents had returned, and water use returned to "normal" conditions. The limit of detection for the HACH® Pocket Colorimeter was reported to be 0.02 mg/L as Cl_2, and 52% of samples (n=47) never reached this threshold. The absence of disinfectant residual may be due to its decay during water stagnation in the plumbing or service lines (19,27). It is also possible that the water delivered to the building did not contain a detectable residual (42), as previously observed at a building several properties away in the same water distribution system (12).

Ambient temperature may also be responsible for the low concentrations, as chlorine residual detection has been reported to be lower in warmer months (42,43).

3.2 Water and outdoor temperatures

The temperature of the sampled first-draw water increased during the study, especially comparing April and May to June and July (Table S3). Reported average outdoor temperatures correlated with average hot (R^2 = 0.863) and cold water temperatures (R^2 = 0.847) on sampling dates (Table S3). Because air conditioning was limited to a room without water fixtures and was often turned off, the outdoor temperature had a strong effect on indoor temperatures and thus water temperatures. Samples taken from the basement were notably cooler, as was ambient temperature. Average hot and average cold water temperatures from each sampling date were also very similar (Table S3). Failure to control indoor temperatures likely has ramifications for building water quality.

3.3 Copper, zinc, and lead concentrations

Copper concentrations were typically higher in hot water than in cold water (Figure 3a), except for location 2, the kitchen sink. At this location, copper concentrations increased from 226 ppb in April to 603 ppb in July. Flushing in August reduced the copper concentrationat the kitchen sink tap to 92.9 ppb, and the concentration returned to 594 ppb just one week later (Figure 4a). While the health-based copper action level of 1,300 ppb (44) was not exceeded, the rebound is notable and observable across the building (Figure S1). Zinc concentrations showed a similar pattern at location 2 (Figure S2) and was well below the 5,000 ug/L secondary aesthetic based MCL. The metal concentrations suggest that a different pipe material connected the kitchen sink (Location 2) to the main galvanized steel plumbing system. While copper pipe was not visible on inspection, the kichen sink apparatus may have had copper, or particulate may have accumulated on aerators. Additionally, some locations had higher copper concentrations immediately after flushing than with stagnation, potentially due to disruption of sediments.

Similarly, lead concentrations were typically higher in hot water than in cold water (Figure 3b), though lead was detected at all locations during at least one sampling event. Lead concentrations have been reported to be higher in hot water tanks due to sediment and precipitant accumulation within the tank (45). Lead concentrations have also been shown to be higher in the summer than winter in a study conducted at a housing complex in Saudi Arabia, possibly because warmer temperatures encourage leaching into water (45). It is likely that the higher concentrations of lead in hot water can be attributed to deposits in the water heater or scale behind shower heads for locations 7 and 8.

The American Association of Pediatrics (AAP) and Advisory Committee on Childhood Lead Poisoning Prevention of the Centers for Disease Control and Prevention (CDC) state there is no safe level of lead in blood (46,47). Still, the AAP recommends that water fountains in schools should not exceed water lead concentrations of 1 ppb (46), and the U.S. Food and Drug Administration (FDA) limit for lead concentration in bottled water is 5 ppb (48). All ten sampling locations had lead concentrations greater than 1 ppb, and two of those sampling locations had lead concentrations that also exceeded 5 ppb. The highest lead concentrations were often observed at Location 7 (a shower). It is unclear why this shower had higher lead concentrations, though shower head materials (all shower heads were unique), use patterns, and collected particulates may have contributed.

Flushing seemed to be more effective for reducing lead than copper concentrations (Figure S1). Copper concentrations measured immediately before and immediately after flushing were not significantly different ($p=0.922$, $r=0.022$), nor were copper concentrations measured immediately after and one week after flushing ($p=0.846$, $r=0.044$). Other studies have similarly shown that flushing does not consistently reduce cold water copper concentrations (13,25,49,50). There was a significant difference ($p=0.044$, $r=0.271$) in lead concentrations between stagnant samples and flushed samples. One week after flushing, however, lead concentrations had no significant change ($p=0.225$, $r=0.271$) from the concentrations prior to flushing (Figure 4b), and even rebounded to higher levels at a shower. These results are consistent with a study of a large building that reported a rebound in lead concentrations when

a stagnation period follows flushing (25). The immediate effect of flushing on ions concentrations was limited (Table S4). As many studies observe an immediate impact on metals concentrations flushing, even after building closures related to COVID-19 (51), examining this longer-term rebound is critical.

During flushing, slugs of discolored water were flushed through the system (Figure S3). This occurred when multiple faucets were open, likely maximizing flow rates through upstream plumbing and service lines, potentially mobilizing sediments. Samples of discolored water slugs were collected and analyzed for metals (Table S5). Notably, the maximum concentrations of aluminum (341.9 ppb) and iron (22,945 ppb) exceeded secondary maximum contaminant levels in hot water. In cold water, the maximum lead concentration was 150.5 ppb from a dish sprayer, where faucet fixtures (i.e., aerator) could not be removed. Flushing was strategically continued until water ran clear, and concentrations on return trips were notably lower. For residents undertaking their own flushing procedures, this discolored water would likely be cause for concern.

3.4 Microbial growth and organic carbon

Total cell counts were higher on average in hot water ($\bar{x}$ = 5.87 log cells/mL, n=42) than cold water ($\bar{x}$ = 5.32 log cells/mL, n=28) (Figure 5a). Intact cell counts had a similar trend (Figure S4). There was a significant change in the total cell count concentration immediately before and one week after flushing (p=0.006, r=0.616). TOC concentration, a commonly used indicator of the concentration of particulate and dissolved organic material present in water, followed a similar trend and was consistently higher in hot water ($\bar{x}$ = 3.36 mg/L, n=36) than cold water ($\bar{x}$ = 0.81 mg/L, n=24) (Figure S4). Flushing also resulted in a significant change in TOC concentration, as measured immediately before and one week after flushing (p=0.002, r=0.693).

A study of a residential green building in the same service area reported similar results, with higher bacteria and TOC concentrations in hot water than cold water (52). That hot water system included multiple tanks that may have been held at low temperatures. The higher cell counts and TOC

concentration in hot water in the present study may also be attributed to unique hot water system design (Figure 1). After moving through the water softener and water heater, hot water was directed to an insulated holding tank with a capacity of about 115 gallons [435 L]. This holding tank likely remains warm during building occupancy, with many users requiring large amounts of hot water. On inspection in June, the pipes leaving the tank were at room temperature (while the pipes leaving the heater were noticeably hot). Thus, stagnation allowed this holding tank to cool to ambient temperatures, which likely fell into suitable growth ranges for many bacteria.

The lack of change in general microbial parameters over the five months of low water use is notable. Although increases in total cell and *L. pneumophila* concentrations have sometimes been observed (53) this “growth curve” may reach an equilibrium at a certain point. Without new nutrients entering the system (e.g., nitrogen, oxygen, phosphorous from fresh water), a carrying capacity may be reached within the pipe (54). For example, a previous study indicated a carrying capacity effect with higher total growth in pipes that were flushed more frequently (15). Rhoads and Hammes (2021) have also highlighted the lack of clarity regarding stagnation and *Legionella* spp. in building plumbing; although it has been widely accepted that stagnation supports *Legionella* spp. growth, the authors summarize studies showing a lack of *Legionella* spp. growth during stagnation and assert that there is no consensus on stagnation water quality and *Legionella* spp. growth and occurrence (6).

Total cell count and TOC concentrations decreased for the samples collected during trip 7, representing normal use conditions. The average total cell count concentration measured during trip 7 was 3.70 log cells/mL and 4.70 log cells/mL for cold and hot water, respectively. There was a significant difference in the total cell count concentration one week after flushing compared to during normal use ($p=0.002$, $r=0.693$)., The average TOC concentration measured during trip 7 was 0.93 mg/L and 1.15 mg/L for cold and hot water, respectively. There was no significant difference observed in TOC concentrations one week after flushing and during normal use ($p=1.0$, $r=0.0$), although the concentrations were substantially lower for samples collected at trip 6 and trip 7 as compared to the stagnant samples collected throughout

the study. The decrease in concentrations of these microbial parameters was expected, due to chlorine residual detection and increased water use during the final sampling trip. Microbiological measurements indicate growth was occurring in the building plumbing during the stagnant periods, but flushing and the return to normal building occupancy helped reduce the total cell counts at all fixtures tested.

3.5 Legionella detection by qPCR and Legiolert during stagnation and flushing

Legionella spp. *w*ere quantified using qPCR for all sampling events. It is important to note that presence of *Legionella* spp. does not necessarily indicate the presence of a pathogen. *Legionella* spp. gene copy numbers were consistently higher in hot water fixtures than cold (Figure 5b); the mean hot water *Legionella* spp. concentration for each sampling trip ranged from 6.39 x 10^4 gc/L to 1.22 x 10^5 gc/L, while the mean cold hot water *Legionella* spp. concentration for each sampling trip ranged from below the detection limit to 6.79 x 10^4 gc/L. Flushing generally reduced *Legionella* spp. gene copy numbers in cold water: from an average of 2.73 x 10^4 gc/L in trip 5 to 9.27 x 10^3 gc/L one week after flushing and 8.97 x 10^3 gc/L after the building returned to full occupancy. Similarly, flushing reduced *Legionella* spp. gene copy numbers in the hot water fixtures from 9.82 x 10^4 gc/L in trip 5 to 5.72 x 10^4 gc/L 1 week after flushing and 6.39 x 10^3 gc/L after the building was returned to its normal operating conditions. The change in *Legionella* spp. was significantly different between the samples collected immediately before and one week after flushing ($p=0.020$, $r=0.522$). Another significant change in *Legionella* spp. occurred between one week after flushing and during normal use ($p=0.020$, $r=0.529$). Still, *Legionella* spp. reductions were not consistent at all fixtures: at sampling point 10, *Legionella* spp. concentrations remained elevated in hot water before the building was fully occupied.

Samples were collected for analysis with the Legiolert method from all sampling location during Trip 5, 6, and 7, as well as a bathroom sink on the first floor which had been previously unsampled. While only an incidental observation, at the first floor bathroom sink [cold], concentrations of *L. pneumophilia* measured by Legiolert increased during flushing from 2.0 MPN/100 mL (stagnant first flush) to 176.7 MPN/100 mL after 2 minutes of flushing. Levels remained low one week after flushing (2.0 MPN/100mL), and during

normal operation (2.3 MPN/100 mL). Cold water from a different sink on the second floor presented a *L. pneumophila* concentration of 3.0 MPN/100 mL during normal use as well. This is consistent with other studies that have reported decreased *L. pneumophila* presence following flushing (55). During flushing, the concentration exceeded suggested acceptable levels by several institutions. The National Academies of Science, Engineering, and Medicine have suggested a single sample concentration less than 106 CFU/100mL as the “acceptable” level for faucets (56). The American Industrial Hygiene Association (AIHA) states that all water systems should be below 100 CFU/100mL (57).

The increase in *L. pneumophila* concentration, in cold water, during flushing and at low levels at sporadic taps may be attributable to several phenomena. While not often studied in cold water, *Legionella* spp. presence has been noted in cold water at point of use taps in houses throughout the United States (58) *Legionella* spp. has been detected in samples from distribution systems during the summer and late fall where the water temperature was greater than 18 °C (59). With the increase in *L. pneumophila* during flushing, localized biofilms within the building may also be a cause, as flushing can destabilize biofilms. While low level water use for sampling occurred throughout the house, the location with highest *L. pneumophila* was not disturbed for four months prior to the flushing study.

L. pneumophila was detected in cold water only in this study. However, as discussed, there was little difference between the temperatures of the cold and hot water in the building plumbing. The optimal temperature range for *Legionella* spp. growth is 25°C to 43°C (60), and 64% of first-draw hot samples (n=27) and 57% of first-draw cold samples (n=16) fell within that range. The lack of temperature control is a possible explanation for the *L. pneumophila* presence within the cold building plumbing.

Although both cold and hot water samples were collected for Legiolert and qPCR analysis, *L. pneumophila* presence was detected with the Legiolert test only and was never confirmed via qPCR targeting the *mip* gene. *L. pneumophila* was similarly not detected by qPCR in a study conducted in a home located in the same distribution system (12). The collection order for each of the analyses may

have contributed: the qPCR sample was the last water sample collected at each tap. However, false positive results from the Legiolert test have been described. The specificity of Legiolert has been reported as 96.4% for potable samples (61). Other Gram-negative bacteria, including *P. aeruginosa*, *P. mirabilis*, and *S. marcescens,* can produce false positive results with Legiolert (62). Disagreements between Legiolert and DNA-based methods have been reported, particularly for samples with low concentrations of *L. pneumophila* as measured by Legiolert(8).The lower limit of detection for Legiolert as compared to our *mip* qPCR assay might explain the discrepancy in the results. The Legiolert method can detect *L. pneumophila* concentrations as low as 1.0 MPN/100mL (10 MPN/L), while our *L. pneumophila mip* qPCR LOD was determined to be 10 gc/mL [10,000 gc/L], accounting for multiple concentration and dilution steps. Additionally, our sequential sampling procedure may cause discrepancy, with water for analysis by Legiolert and DNA extraction collected in separate bottles (i.e., not using a split sample strategy).

Analysis of *Legionella* spp. and *L. pneumophila* data in this study potentially call into question the efficacy and purpose of flushing efforts. Flushing and sampling for this study required significant effort and time. While flushing had some effect on microbial quality of water, return to full occupancy likely had the strongest effect on *Legionella* spp. in hot water. At the same time, the highest concentrations of *L. pneumophila* were anecdotally detected during flushing. Several studies have examined DWPI over the course of pandemic-related building closures and subsequent flushing activities with inconsistent results. While some recommissioning studies observed no significant decrease to *Legionella* spp. with flushing (51), others have observed only short term benefits from flushing that may fade between a few days and a month after flushing(63,64). Other studies have also pointed to the limited benefits of flushing (65,66). The recovery of the entire microbial community may take longer (67), and variations in initial concentrations of DWPI, and thus relative risks are likely (8). Flushing for re-occupancy may serve to strategically limit building occupants' exposure to easily dislodged biofilms and scale. However, if undertaken, it likely needs to occur closely to building occupation to maintain water quality. Still, a flushing operation may contain exposure to only affect trained professionals and could be useful when building occupants are particularly sensitive or vulnerable.

3.6 Building volumes and water age

Understanding the plumbing layout is critical to understanding the real water age and origin location of each sample. As each sampling location was sampled monthly, the water likely stood stagnant at the absolute distal end for only one month in each location. Each sampling event should have moved water such that total water age increased throughout the study, but this is difficult to prove without flow data at each tap and a detailed plumbing drawing. In addition to the authors' sampling activities, other low amounts of water use likely occurred (e.g., by maintenance staff). The volume of water in the building pipes was calculated to be about 35 gallons (14 gallons hot, 22 gallons cold). Approximately 20 L [5.28 gal] (8 L cold, 12 L hot) of water was collected per trip for a total 100 L [26.42 gal] prior to flushing. This represents about 75% of the building water volume, meaning the building plumbing was not completely overturned by sampling activities prior to flushing. According to calculations, flushing - for 15 minutes (cold) and 60 minutes (hot) - was able to overturn the entire water volume in pipes as well as upstream tanks, even with known inefficiencies in overturning tanks.

This water age of specific slugs sampled is critical to take into account, as is the location at which stagnation occurs (i.e., which material it takes place in) as it may affect all water quality parameters. For example, if the service line had a copper component, it is possible that the spike in copper at location 2 in July was because a "slug" of water that stagnated in the service line finally reached that point. While the in-premise water age was close to 5-months before flushing, the full water age (from the point of treatment) was not calculated for the distribution system in this study. Occupancy of many buildings surrounding the study building was impacted by the COVID-19 pandemic, impacting the accuracy of any models or estimates.

4 Conclusions

Metal concentrations were typically higher in hot water than cold water, possibly due to particulate matter in the water heater or destabilization of scale. An important exception to this is the copper concentration in the cold water from the kitchen sink, likely attributed to different pipe material at this location. Total bacterial and *Legionella* spp. concentrations tended to be higher in hot water than cold water samples. A return to normal use reduced overall levels, and an increase during the five months of stagnation was not consistently observed. Likely, a carrying capacity was reached prior to the first sampling trip, and growth was limited without a steady influx of nutrients.

A flushing plan was developed based on knowledge of the plumbing system and empirical measurements (chlorine, temperature) during flushing. While flushing improved water quality in the short term by introducing a chlorine residual and reducing heavy metal concentrations, one week later the quality had returned to similar conditions as some earlier stagnation samples, especially for metals. One week after flushing, *Legionella* spp. remained elevated at some taps, though it was generally lower. A return to normal operation resulted in a significiant decrease in both bacterial and *Legionella* spp. concentrations, even from levels 1 week after flushing. These results suggest that flushing alone is not guaranteed to be a completely effective strategy for refreshing building water quality over an extended period of stagnation if the building will remain unused.

5. Conflict of interest

The authors declare no conflict of interest.

6. Acknowledgment

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7. Figures and Tables

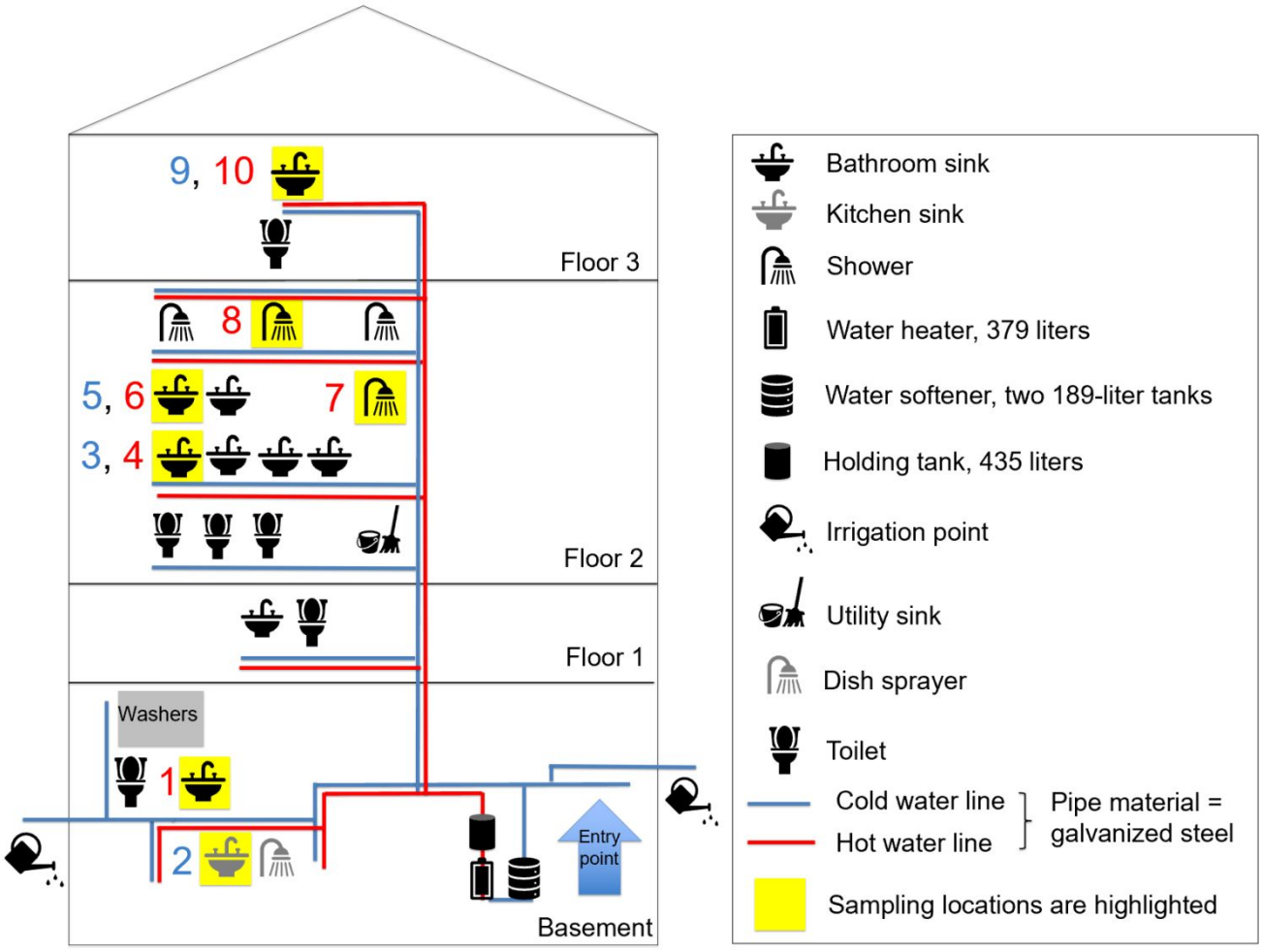

Figure 1. Building and plumbing layout. Sampling locations are highlighted and labeled with sampling order. Blue labels indicate cold water samples; red labels indicate hot water samples.

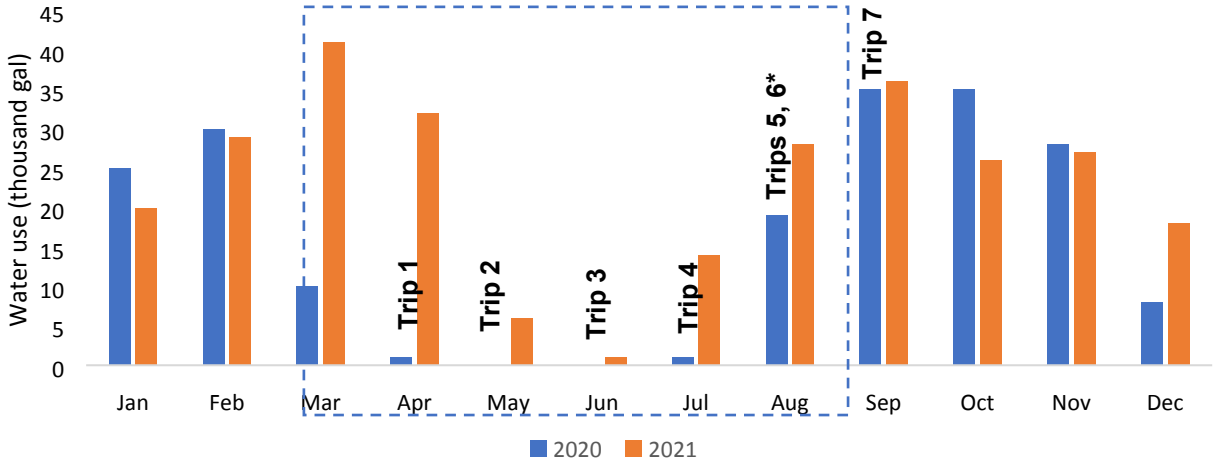

Figure 2. Water use in the building in 2020 (the dashed box indicates the building closure March 2020-August 2020) and 2021 (summer reduced occupancy May 2021 – August 2021). Each bar represents a single data point for the billed water use from each month. The building was officially re-occupied in late August 2020. Water use appears high in August 2020, in part due to flushing of the plumbing, as well as all residents reoccupying the building by August 26, 2020.

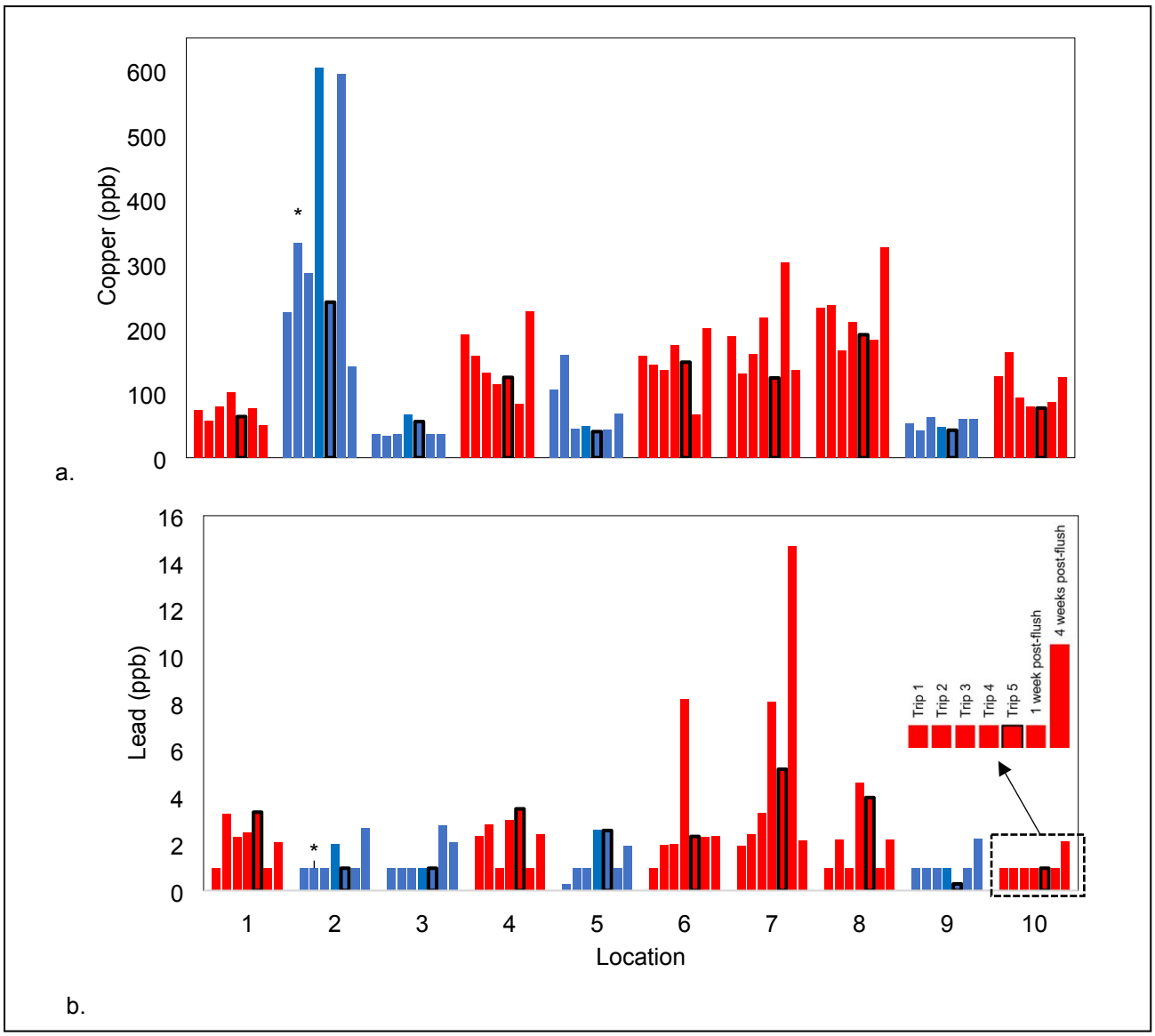

Figure 3. First draw (a) copper and (b) lead concentrations at 10 locations that drew either hot (red) or cold (blue) water. Each bar represents a single data point from each sampling trip (Trip 1 - April, Trip 2 - May, Trip 3 - June, Trip 4 - July, Trip 5 - August, Trip 6 - 1 week post-flush, and Trip 7 - 4 weeks-post flush, in order), and the location numbers are as defined in Figure 1. Bars outlined in black are the final stagnant sampling event prior to flushing (Trip 5 - August). For lead, concentrations below the Limit of Detection (LOD=0.57 ppb) are shown as half the LOD. Concentrations below the Limit of Quantification (LOQ=1.91 ppb) are shown as half the LOQ. *On this trip, a toilet near Location 2 was flushed immediately before sampling, potentially interfering with this result.

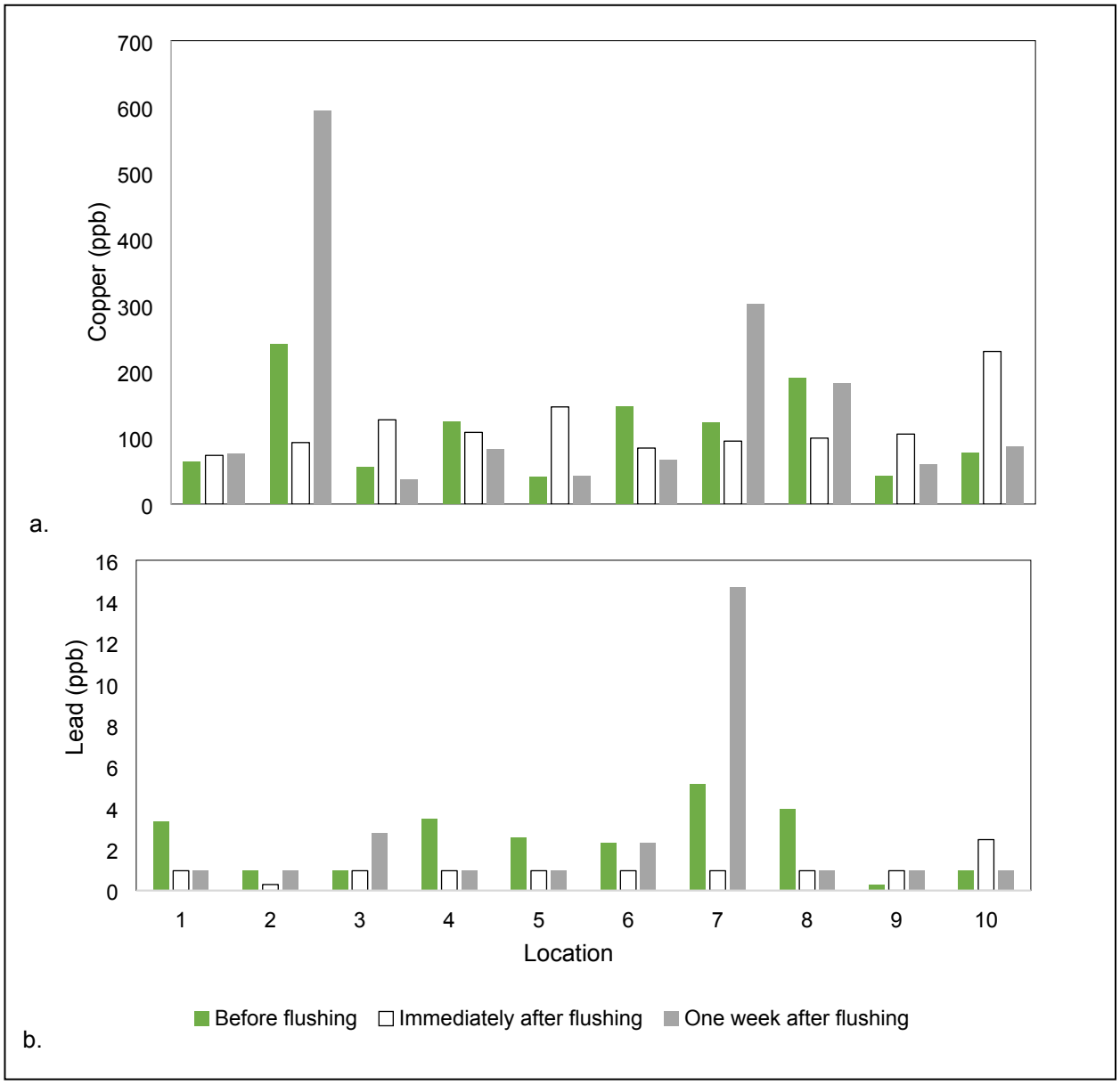

Figure 4. (a) Copper and (b) Lead concentrations at 10 locations in the building before (green), during (white) and after (gray) flushing. Green bars represent single data points from stagnant samples before flushing (Trip 5 – August, in Figure 3), white bars represent single data points from samples taken immediately after flushing (not in Figure 3), and gray bars are single data points from stagnant samples taken one week later (1 week post-flush, in Figure 3).

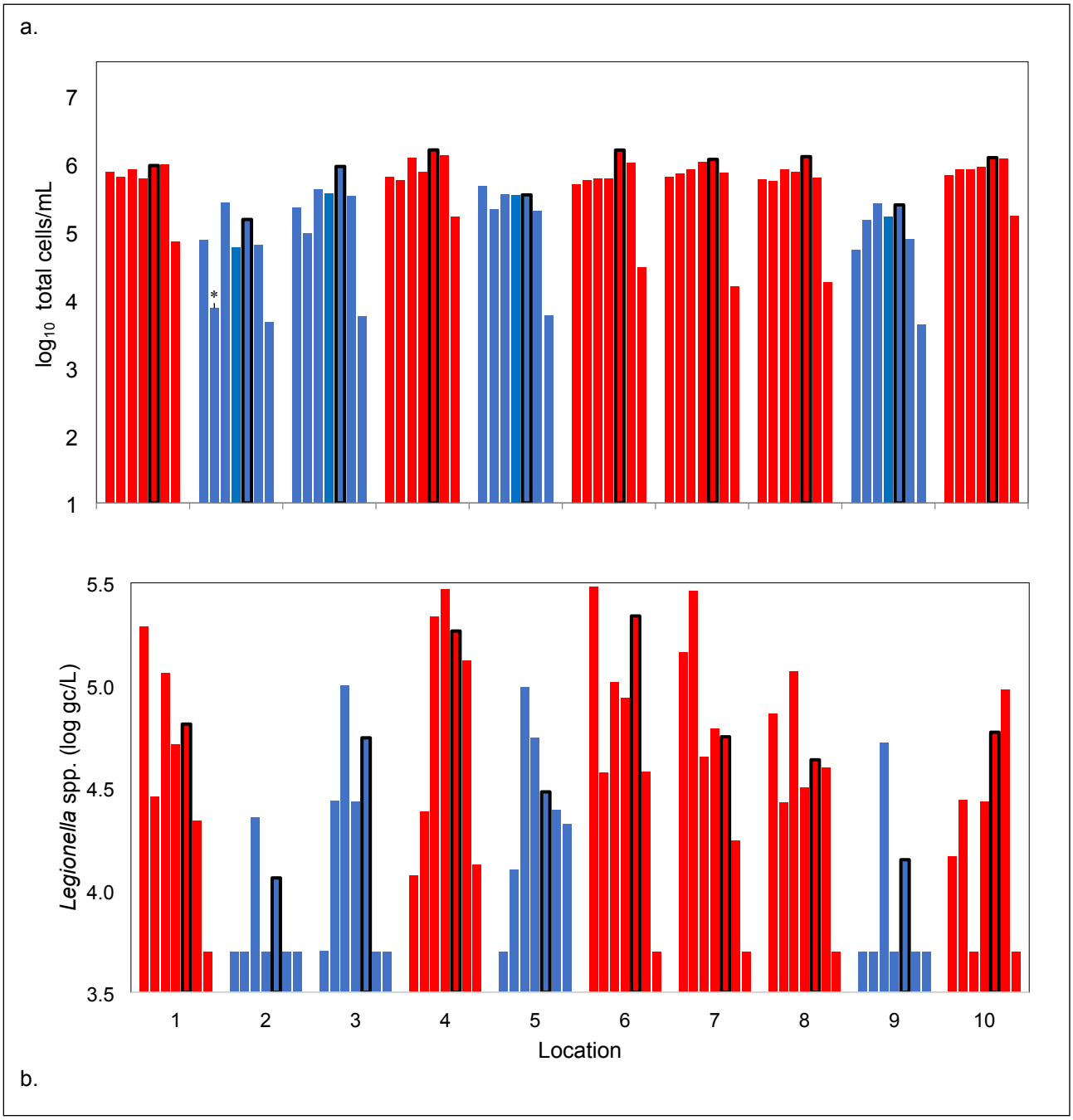

Figure 5. First draw (a) total cell counts and (b) *Legionella* spp. (log_{10} gc/L water) detection at 10 locations that drew either hot (red) or cold (blue) water. Each bar represents a single data point from each sampling trip (Trip 1 - April, Trip 2 - May, Trip 3 - June, Trip 4 - July, Trip 5 - August, Trip 6 - 1 week post-flush, and Trip 7 - 4 weeks-post flush, in order), and the location numbers are as defined in Figure 1. Bars outlined in black are the final sampling event prior to flushing. For *Legionella* spp., concentrations below the Limit of Detection (LOD=4 log_{10} gc/L = 10 gc/mL) are shown as half the LOD

(5 gc/mL = 3.7 $\log_{10}$ gc/mL). *On this trip, a toilet near Location 2 was flushed immediately before sampling, potentially interfering with this result.

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