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Water impact statement

Water management interventions designed to improve microbial water quality in buildings may have unintended consequences for other contaminants. A comprehensive water quality evaluation of three interventions (flushing, water heater set point change, and a combination) demonstrated the presence of contaminant tradeoffs as well as incomplete *L. pneumophila* mitigation in a highly colonized green building.

Water Quality Trade-offs for Risk Management Interventions in a Green Building

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Abstract

Premise plumbing water quality degradation has led to negative health impacts from pathogen outbreaks (e.g., *Legionella pneumophila* and non-tuberculous mycobacteria), as well as chronic effects from exposure to heavy metals or disinfection by-products (DBP). Common water quality management interventions include flushing, heat shock (thermal disinfection), supplemental disinfection (shock or super chlorination), and water heater temperature setpoint change. In this study, a *Legionella pneumophila*- colonized Leadership in Energy and Environmental Design (LEED) certified building was monitored to study health-relevant water quality changes before and after three controlled management interventions: (1) flushing at several points throughout the building; (2) changing the water heater set point; and (3) a combination of interventions (1) and (2) by flushing during a period of elevated water heater set point (incompletely performed due to operational issues). Microbial (culturable *L. pneumophila,* the *L. pneumophila mip* gene, and cATP) and physico-chemical (pH, temperature, conductivity, disinfectant residual, disinfection by-products (DBPs; total trihalomethanes, TTHM), and heavy metals) water quality were monitored alongside building occupancy as approximated using Wi-Fi logins. Flushing alone resulted in a significant decrease in cATP and *L. pneumophila* concentrations (p = 0.018 and 0.019, respectively) and a significant increase in chlorine concentrations (p = 0.002) as well as iron and DBP levels (p = 0.002). Copper concentrations increased during the water heater temperature setpoint increase alone to 140°F during December 2022 (p = 0.01). During the flushing and elevated temperature in parts of the building in February 2023, there was a significant increase in chlorine concentrations (p = 0.002) and iron (p = 0.002) but no significant decrease in *L. pneumophila* concentrations in the drinking water

samples (p = 0.27). This study demonstrated the potential impacts of short term or incompletely implemented interventions which in this case were not sufficient to holistically improve water quality. As implementing interventions is logistically- and time-intensive, more effective and holistic approaches are needed for informing preventative and corrective actions that are beneficial for multiple water quality and sustainability goals.

1. Introduction

The development of water- and energy- saving approaches to building construction is an important approach to increasing sustainability in the built environment. Green buildings (e.g., Leadership in Energy and Environmental Design or "LEED" buildings) provide an opportunity to reduce energy and water demands as well as carbon footprint, providing an economic and environmental benefit(1). Both green and conventional buildings can present water quality challenges. However, water quality in green buildings may have higher concentrations of opportunistic pathogens compared to their conventionally constructed (non-LEED) counterparts, due to factors such as increased water age or varying temperatures (2–4) and stagnant and low water use from using low flow, water-efficient fixtures.

Building plumbing is a portion of the drinking water distribution system between the water main and final point of use. Building plumbing has an essential role to play in determining the final quality of drinking water consumed by customers. Exposure to microbial contamination through drinking water is a concern for public health. Opportunistic pathogens including *Legionella pneumophila*, *Pseudomonas aeruginosa*, and *Mycobacterium avium* complex (MAC) are well suited to persist in drinking water distribution systems (DWDS) and building plumbing (5–7). *Legionella pneumophila* has been identified as the most common cause of drinking water-associated waterborne disease outbreaks in the US (8,9). Identifying proper methods to limit the growth and persistence of opportunistic pathogens, especially *L. pneumophila*, presents an opportunity to reduce the disease burden associated with the proliferation of these pathogens in building plumbing. *L. pneumophila* has been identified in green buildings as a high concern due to its occurrence in high concentrations (10–12).

While microbial risks often drive water safety considerations due to their acute effects, chemical constituents are also important health concerns. Small diameter pipes and low flow fixtures potentially accelerate the accumulation of both chemical and microbial contaminants and promote biofilm growth in the premise plumbing (13,14). Additionally, water quality trade-offs exist (e.g., tradeoffs between niches for pathogens such as inverse relationships between *L. pneumophila* and *M. avium*; remediating disinfection by-products (DBPs) but creating conditions conducive to corrosion leading to metal leaching or microbial growth). Additionally, water quality trade-offs exist (e.g., free chlorine is more effective for reducing *Mycobacterium* spp. but are less effective for reducing *L. pneumophila* compared to chloramine disinfectants) and as such, maintaining quality is a delicate balance that requires holistic strategies (15).

Water quality entering a building distribution system may differ substantially from the quality at the point of exposure to the consumer due to water quality deterioration as it travels through the complex plumbing. Water management plans are recommended by multiple organizations (16–18), however implementation of interventions (e.g., flushing or water heater set point change) can be logistically challenging and resource-intensive for facilities managers, especially under low-occupancy conditions (4,10,19,20). Building water use or demand, occupant water use activities, and overall occupancy are likely to influence water quality but are typically not directly observable or controllable over a relevant time scale for water quality maintenance in buildings.

This research will result in an understanding of how interventions designed to improve water quality in buildings affect multiple water quality variables. Interventions can be described as any approach or method performed by building facilities that can be used to improve microbial water quality. Typically, strategies to improve water quality in premise plumbing have primarily focused on *L. pneumophila* and have relied on raising water temperatures within pipes and/or flushing water from the pipes (21–23). Thermal inactivation studies report that a 1 h heat exposure was associated with a 4-log reduction of culturable *L. pneumophila* at 70 °C and 2.5-log reduction at 55 °C and 60 °C in simulated drinking water, with no culturable *L. pneumophila* detected after 1 h exposure at 60 °C and 70 °C (24). The times required to obtain 1-log inactivation (90% reduction) of *L. pneumophila* were 2500 min (45°C), 380 min (50°C), <5 min (60°C), and <1 min (70°C) (25–28). Furthermore, *L. pneumophila* could return in water systems subjected to heat treatment (28,29). Although flushing of taps could result in the reduction of *Legionella* spp. concentrations by up to 2-logs (30,31), *Legionella* spp. has been shown to repopulate or colonize premise plumbing within two to seven days after flushing (Hozalski et al., 2020). Therefore, more research is needed to improve the techniques employed to remove pathogens from premise plumbing while simultaneously improving overall water quality.

Previous studies assessed the influence of different intervention strategies (e.g., flushing, heat shock, water heater set point change, and supplemental disinfection), on the presence of contaminants or pathogens in premise plumbing and water distribution systems in an effort to guide control strategies (33–41). However, there is a gap in the literature for studies systematically evaluating the simultaneous impact of multiple water quality interventions on a suite of human-health relevant contaminants. Given that typical water quality interventions in buildings may have drawbacks (e.g., water use, heat/energy use, lower sustainability, and water quality trade-offs), there is a need to understand how building water quality interventions as well as human behavior patterns affect chemical and microbial contaminants in real world settings.

An integrated proactive approach to improve water quality in premise plumbing has not yet been developed, in part due to the complexities and logistical challenges involved in long-term grab sampling campaigns. The overall goal of this research is to provide an improved understanding of management interventions on building water quality. Consequently, this study aims to: (1) evaluate the water quality impacts of three management interventions in a data-rich, operational green building; and (2) quantify trade-offs and synergistic benefits associated with individual and combined controlled water quality interventions, with the goal of informing management practices in an increasingly automated building environment.

2. Materials and methods

2.1 Building description

We studied water quality in a building on the Arizona State University (ASU), Tempe campus without a water softener (a different building than previously studied for *Legionella* spp. colonization that contained a whole building water softener) (10). The building receives municipal treated drinking water as domestic cold water (DCW) from the City of Tempe which uses a free chlorine residual disinfectant. The City of Tempe uses 0.8% of sodium hypochlorite utilizing an on-site Hypo Generation (OSHG) system by passing an electrical charge through a brine mix as well as UV for disinfection.

The facility is a 7-floor institutional research building that opened in May 2012 with an area of 327,256 ft^2. The building includes laboratory spaces, clean rooms, administrative and office spaces, high bay spaces, and a 250-seat auditorium. The building earned a gold rating on the U.S. Green Building Council's (USGBC) Leadership in Energy and Environmental Design (LEED) rating system. It has a high-performance façade with vertical sunshades to reduce heat gain and incorporate passive cooling strategies. The design optimized the building envelope and integrated extremely efficient mechanical systems to reduce energy use by 40.7% below a typical laboratory building. ASU allocated energy produced by the photovoltaic array on the parking structure adjacent to the building, supplying an additional 11.6% of its energy use beyond the savings achieved by the building design. Renewable energy reduced the building's energy costs by over 16% because the peak energy load is also reduced.

The plumbing pipes are copper, but the water main is constructed from insulated 6 in. ductile iron, at the basement with one riser to feed the breakrooms and bathrooms. The copper riser starts at 2 in. and becomes 1.25 in. by the seventh floor. About half the space is dedicated to offices for professors and graduate students. The first and second floors house the lobby, K-12 educational spaces, and laboratory spaces. Excluding the first floor, each floor has a small breakroom containing a manual sink with separate hot and cold water. The floors have one breakroom and one alcove with three bathrooms and two drinking water fountains. Bathrooms are located vertically on top of each other for all floors, and breakrooms start on the second floor and are also vertically collocated. In total excluding the lab space, this large building has 4 showers, 7 manual faucets in the breakroom, 14 drinking water fountains, 58 automatic faucets in bathrooms, 72 toilets, and 25 urinals. There are two water heaters in the building which were originally set to 120° F from the time of installation. However, this is not the temperature at the point of use taps or fixtures because each heater has a mixing valve installed to avoid scalding risk. One valve is a digital magnetic type and the other is a thermostatic type. One heater serves hot water for basement to the 2nd floor which has thermostatic valve while the other serves 3rd to 7th floor of the building which has a digital magnetic valve. Domestic cold water (DCW) gets split between industrial cold water that goes through the reverse osmosis (RO) and a water softener system, then to the storage tank that goes to labs. The other DCW stream is not softened and serves the remainder of applications in the building (note that no industrial water was sampled). There is a flat plate heat exchanger for meeting the needs of process chilled water. No water softening is performed for the whole building.

2.2 Building occupancy

Wi-Fi logins per floor were recorded and used as a surrogate for approximate building occupancy. Direct counts of building occupants are not observed over this study duration. However, hourly Wi-Fi logins (point connection of a Wi-Fi-enabled device) were used to approximate occupancy trends on each floor in the building. Wi-Fi signals were obtained as previously described from the Arizona State University Information Technology (ASUIT) with counts of the number of connections of wireless devices (phones, tablets, or personal computers) by floor (4,10,19). We analyzed occupancy for basement, 2nd, 3rd, and 7th floor in the building for this study as these were the floors where the water quality sensors were present for constant monitoring of water quality parameters.

2.3 Municipal water quality data

Grab sampling water quality parameters from the LEED building values were also compared with the City of Tempe Water treatment plant daily analysis report for September 2022 to March 2023. The treated drinking water for the City of Tempe comes from two conventional surface water treatment plants, the Johnny G. Martinez water treatment plant (JGMWTP) or the South Tempe water treatment plant (STWTP) depending on the demand or plant function and time of year. The source of drinking water influent for Tempe is mostly surface water from Salt River Project (SRP) reservoirs which is diverted to SRP canals (Salt and Verde River Watersheds) and some groundwater wells. There is fluctuation in the water chemistry depending on the blending of source water that goes to the water treatment plant.

Seasonal changes such as precipitation, snowmelt from mountains, and stormwater runoffs also affect the water quality. JGMWTP and STWTP reported daily monitoring values for turbidity, pH, total hardness as $CaCO_3$ along with calcium and magnesium hardness, chloride ions, total alkalinity, temperature, and total organic carbon (TOC) concentrations in its daily chemical report which is published monthly (42,43). During several months of the year, only one drinking water treatment and distribution plant is in operation and the other is offline unless the demand is high.

2.4 Sample collection

Baseline building water quality testing was performed from April 2022 to July 2022. Several pilot sampling events were performed during this time to analyze overall building water quality and evaluate a single flushing event on 11 July 2022. The impacts of water quality management interventions were further assessed from September 2022 to February 2023. Water was collected between 7am-8 am Arizona time from the point-of-entry (POE), hot and cold-water side of faucet taps with each handle opened to maximum in the breakrooms, automatic faucets receiving mixed hot and cold water for floors 2, 3, and 7, hot and cold water from janitor's closet in the basement, and hot water from a basement shower set to maximum hot water. The early morning timeframe was chosen to maximize the likelihood of sampling before building water demand increased in the building. Sampling locations and type of fixtures in the sampled areascan be found in **Table 1** and **Supplemental Table SI.1.**

The first draw samples were collected in sterile 2 L Nalgene polypropylene (PP) bottles and in 0.5 L glass bottles for disinfection by-product analysis. From the first draw volume, 1.1 L was separated for microbial analysis in PP bottles, pre-dosed with 1 mL of 2.4 % of sodium thiosulfate solution as a chlorine quencher. The remaining sample was tested for physico-chemical water quality parameters. Controls were used for each sampling event including a field blank and trip blank. The blank samples were 1.1 L of autoclaved deionized (DI) water in sterile sampling bottles. The trip blank was kept on the sampling cart during sampling trips. The field blank was brought to the sampling site and opened to expose it to the site environment.

2.5 Interventions and communication with building facilities managers

2.5.1 Flushing. After obtaining the results from preliminary testing in April 2022, facilities were contacted, and flushing was reported to be performed by facilities' staff at the basement breakroom hot water faucet and all the showers in basement for ~ 30 minutes from Monday to Thursday in the early morning at ~7 am. These actions did not overlap with later controlled water quality intervention testing (September 2022 and later). During the controlled flushing events starting in September 2022, the research team performed controlled flushing for 20 minutes at the showers, janitors' closet, and breakroom manual faucets at ~ 1pm. T0 represents samples before flushing and T1 represents samples after 20 minutes of flushing intervention. The 20 minutes of flush timing was chosen based on the pipe lengths and volume of waterlines per EPA flushing recommendations for premise plumbing (44). This duration was calculated from floor plans, building drawings and maps, along with considering the fixture flowrates and corresponding to the volume of water necessary to completely turn over the water within the building. From the building drawings, the length of riser pipe was 174.65 m with an internal diameter of 0.0381 m. At a flow rate of ~14.5 gpm, the time to flush and turn over water on each floor was calculated and ranged up to 10 min. To ensure complete turnover, this estimate was doubled for the flush time used for the intervention.

2.5.2 Temperature/ water heater set-point changes. For the water heater setpoint increase, sampling was performed according to updates from facilities managers. Sampling was done before and after incremental water heater setpoint changes during November through December 2022.

After a request was made to change the setpoint, facilities' staff started working with the water heater valve serving the 3rd to 7th floor in December 2022. The temperature setpoint was increased stepwise to 140° F. The temperature settings were increased gradually by the building facilities from 120° F to 133° F and then finally to 140° F. In December 2022, the valve serving the 3rd to 7th floors was repaired, and in January 2023, the facilities team started working with the other valve serving the basement to the 2nd floor, completing this process in February 2023. In February, once the team was informed that both the valves were set to 140°F, we performed a combination sampling event by flushing while the water heater was set at an elevated temperature. **Figure 1** shows a detailed sampling and intervention timeline.

To check the peak hot water temperature after set point changes, temperature readings every 10 seconds were observed and time to stabilize and reach the highest temperature was also noted. An observation of the peak temperature and consultation with facilities confirmed that the valves were not functioning in April 2023, and the additional sampling did not proceed per communications from facilities' staff regarding outstanding parts for fixing remaining valve issues.

2.6 Physico-chemical water quality analysis

Water quality parameters such as pH, temperature, conductivity, and free and total chlorine (disinfection concentration) for the water samples were tested onsite after each grab sample was collected at each location. The pH was measured using a pH30 pH tester probe (Oakton Instruments). The temperature of the water samples was measured using a IR002 Infrared Thermometer (Ryobi Tools). Temperature profiles for manual hot water faucets were also measured by recording temperature readings every 10 seconds and noting the highest temperature reached and time needed to stabilize the water stream at the highest temperature. A DR 900 colorimeter (HACH) was used for total and free chlorine concentration (method DPD 8167 and DPD 8021 respectively, range 0.02 – 2 mg/L Cl_2). Conductivity of water samples (µS/cm) was measured using an Orion Versa Star Pro pH/ISE/Conductivity/Dissolved Oxygen Multiparameter Benchtop Meter (Thermo Fisher Scientific).

2.7 Disinfection by-product (DBP) analysis

Trihalomethanes (THM) analyses were conducted using a THM-100 analyzer from Aqua Metrology Systems (AMS). The first draw water samples were collected in headspace-free 500 mL glass bottles with PTFE-sealed lids. Each sample was dechlorinated with 1mL of 2.4%

sodium thiosulfate solution prior to collection and kept at 4°C until analyzed. All samples were analyzed within one week of collection. The THM-100 used a method characterized by a purge, trap, and desorption process, where the THM species were dissolved in a solution with a chemical reagent. The reagents consisted of a diluter (60-90% acetonitrile and 10-40% picolines), a retainer, a developer, a dechlorinator, and THM 3 standards for calibration. All of the reagents were prepared and provided by the manufacturer (Aqua Metrology Systems). The dissolved species and the reagent underwent a modified Fujiwara reaction, and the solution was subsequently analyzed spectrophotometrically. Change in absorbance was related to relative abundance of THM4 species, as well as total THM. Accuracy was reported by the manufacturer as ±10%, or ±5% standard deviation at THM = 50 μg/L. The quantitation range was reported as 5-200 μg/L THM. Instrument self-calibration was conducted every 2-3 weeks using supplied reagents. The instrument was continually monitored remotely by AMS to ensure the instrument was operating correctly and calibration was accurate.

2.8 Metals analysis

For metals analysis, 10 mL of the water samples were acidified with 2% by volume HNO_3 (Nitric acid 67 - 70%, ARISTAR® ULTRA, ultrapure for trace metal analysis, VWR Chemicals BDH ®). Metal concentrations in the drinking water samples were analyzed for iron, copper, calcium, and magnesium using inductively coupled plasma mass spectrometry (ICP-MS, Perkin Elmer NexION 1000). The lower detection limit for the ICP-MS is 1 ppb for all metals except for copper (5 ppb). Calcium and magnesium concentrations commonly contribute to the water hardness levels whereas the plumbing consists mostly of copper pipes along with a copper main.

2.9 Cellular Adenosine Triphosphate (cATP)

cATP was used as a surrogate for live microbial activity in the collected drinking water samples (Pistelok et al., 2016; Richard et al., 2021). cATP was measured by using a portable, commercial light absorbance ATP test kit with a wide detection range (LuminUltra: PhotonMaster/PBM Equipment Set EQP PBM-PAC; Quench Gone Aqueous QGA-100C). All grab samples were processed using this kit where 50 mL water sample was filtered using a syringe filter (sterile 25 mm, 0.7-um pore size, inline glass fiber), that traps the bacterial biomass/cells on a porous filter membrane. The cells were lysed with 1 mL of lysing reagent (UltraLyse7, LuminUltra Technologies), and cATP was washed from the filter membrane into 9 mL of buffer solution (UltraLute, LuminUltra Technologies). A mixture of two enzymes, luciferin and luciferase (Luminase, LuminUltra Technologies) were added to the extracted sample in a 1:1 ratio (volume/volume) and the mixture was rapidly introduced into the luminometer (PhotonMaster, LuminUltra Technologies). The light produced by the enzymatic reaction was measured and reported in relative light units (RLU). Then a 1 ng/mL concentration of ATP solution (standard/calibrant) (UltraCheck™1, LuminUltra Technologies) was used to confirm the accuracy of the luminometer in measuring within the confidence limit of this test kit. Finally, the cATP concentration of the analyzed sample was converted from RLU to picograms of cATP per ml of the grab water sample. When the cATP value is <1 (pg cATP/ml) the water fixture is considered to have good control over microbial activity in the drinking water and when the cATP value is >10 (pg cATP/ml) the manufacturer suggests that corrective actions should be taken (45,46).

2.10 Culturable *L. pneumophila* analysis

100 mL of dechlorinated water sample was used for *L. pneumophila* culture-based testing, and the remaining 1 L water sample was filtered for further analysis with quantitative polymerase chain reaction (qPCR) for the *mip* gene. The Legiolert™ (IDEXX Laboratories, Westbrook, Maine, USA) test was performed according to the manufacturer's instructions for potable water. Legiolert (IDEXX Laboratories, Inc.) is a liquid culture assay, enzyme-substrate reaction-based method (47). Quantification with 95% confidence limit of viable *L. pneumophila* is based on the most probable number (MPN) technique (range 1 - 2272.6 MPN/100 mL). This culture method is specific for *L. pneumophila* detection. Aquadur colorimetric hardness test strips (Macherey–Nagel, Germany) were used to determine the hardness of the drinking water related sample before it was processed using the Legiolert method. Depending on the water hardness range, a volume of either 0.33 mL (for low hardness water) or 1 mL (for high hardness water) of hardness supplement was added to the 100 mL of the water sample as high hardness could have a negative impact on *L. pneumophila* growth. A Legiolert blister pack of powdered reagents and nutrients was added to the sample in a sterile glass bottle and shaken gently until completely dissolved. The sample solution was poured into a Quanti-tray Legiolert tray (96 well plate consisting of 6 big wells and 90 small wells) that was immediately sealed using the Quanti-

Tray™ Sealer PLUS using a Quanti-Tray/Legiolert 96 well rubber insert. Plates were incubated at 39 ± 0.5 °C in an incubator in a humid environment for 7 days, after which the Legiolert plates were read. The number of positive wells (corresponding to wells that are turbid and/or with brown coloration) was counted and the MPN was determined using the IDEXX MPN generator 1.4.4. According to IDEXX recommendation, quality assurance and quality control protocols (QA-QC) were performed by culturing positive and negative control (*L. pneumophila* serogroup 1 and *Enterococcus faecalis*, respectively). The lower and upper limit of viable *L. pneumophila* detection for the IDEXX Legiolert was <1 and >2,272.6 MPN/100 mL respectively (48). Serotyping was performed on a subset of samples, as previously described (10).

The 1 L water sample remaining after processing this 100 mL grab samples for IDEXX was filtered using an Isopore 47 mm diameter 0.2 µm pore polycarbonate membrane filter (EDM Millipore, GTTP04700) and vacuum pump filtration apparatus. The filters were placed aseptically in 2 mL sterile tubes and stored in the -80 °C freezer prior to further processing. A filtration control (autoclaved DI water) was included.

2.11 DNA extraction

Filters with the captured biomass were aseptically transferred using sterile tweezers to 2 mL Qiagen power bead tubes with 1.4 mm ceramic beads (Qiagen, Hilden, Germany). A Precellys Evolution bead-beating homogenizer (Bertin Technologies) was used to grind the filters and lyse the microorganism's cell walls. The Precellys was set to 10,000 rpm, 3 cycles for 15 seconds with 10 seconds pause program to maximize the DNA yield. A Qiagen Dneasy PowerSoil kit (with inhibition removal technology) was used to extract DNA from the filters used for all water samples and trip and field blanks. DNA concentrations and quality of extracted nucleic acids were measured with a spectrophotometer (Thermo Scientific NanoDrop 2000). The elution buffer used for eluting the DNA in the last of Power soil DNA extraction protocol was used for blanking the Nanodrop. All extracted DNA samples were stored at −80°C until further molecular analysis.

2.12 qPCR assays

Primers and probe sequences specific to the macrophage infectivity potentiator *mip* gene target from *L. pneumophila* species were used for gc/μL quantification in the water samples (49). The *mip* gene is a single-copy gene so it can be assumed that one gene copy (gc) was equivalent to one microorganism (acknowledging that the presence of gene copies does not denote viability of the microbe). Quantitative real-time polymerase chain reaction (qPCR) was performed on the Biorad CFX 96 (Hercules, CA) thermocycler. Each water sample was tested in triplicate. The optimized assay details and cycling conditions are shown in **Section A** of the **Supplemental Information Tables SI.1 to SI.3**. A 25 μL PCR reaction was used with 12.5 μL universal probe super mix (Biorad), 1.250 μL of 10 μM concentration forward and reverse primer stocks, 0.625 μL of 10 μM probe, 6.375 μL PCR grade nuclease-free water, and 3 μL of extracted DNA template. All PCR reagents (nucleotides) used in this assay were synthesized by IDT (Coralville, IA). The recovery efficiency of the culture-based and molecular methods was evaluated as described in **Supplemental Information Section B**.

2.13 Data analysis

For the grab water samples, the normality of the data was tested using the Shapiro-Wilk test. As the water quality data were not normally distributed, a non-parametric Wilcoxon signed ranked test was performed to determine statistical differences between parameters pre- and post-management interventions in the building. When the *p*-value was less than 0.05, the differences were considered statistically significant. A non-parametric Kendall's Tau correlation coefficient was computed in order to consider different limits of detection for the compared variables, where +1 is perfect positive association and -1 represents perfect negative association (50). The cutoffs for interpreting the correlation coefficient (Tau) showing if association is negative or positive and if it is strong, moderate are weak were used from (51). The cutoffs for the correlation coefficient were negligible (0 - 0.1), weak (0.1 - 0.39), moderate (0.4 - 0.69), strong (0.7 - 0.89), or very strong (>0.9).

Water quality data were pooled across all the sampling events to study the overall association between different parameters monitored during this study. Due to single grab samples being the focus of the study, statistical pooling tests were not conducted for correlation analysis. All statistical tests were performed in R studio (R software version 2023.03.1). Arithmetic means were computed for positive samples when reporting compared values (excluding non-detects).

Results3.1 Summary of significant differences in water quality before and after interventions

Significant differences from interventions are summarized in **Section D** of the **Supplemental Information Table SI.5** and relative changes from the previous sampling point by building location are summarized in **Figure 9.** Flushing significantly decreased *L. pneumophila* in September 2022 (p = 0.019), but not during February 2023 (p = 0.270). The *L. pneumophila* counts decreased for the 3rd and 7th floor in the building when the water heater temperature was set to the highest available setting of 140 °F (p = 0.002).

Iron concentrations significantly increased during both flushing events (p = 0.002) and resulted in brown-colored at the outlet of the faucet after flushing for 20 minutes. Copper concentrations did not show any significant changes with respect to flushing interventions (p = 0.600). No significant changes in calcium and magnesium concentrations were observed.

Total and free chlorine significantly increased post-flushing (p = 0.002), which is to be expected as flushing allows for additional incoming fresh city water with higher disinfectant residuals. There was a significant increase in THM concentrations after the flushing event in September (p = 0.002). This may have occurred due to flushing replenishing the free chlorine residual that can react with the organic matter present in the system to form more DBPs (52).

During November 2022 through February 2023, the building facilities worked to increase the water heater setpoint to 140°F for both the mixing valves. Facilities started with the valve that serves 3rd to 7th floor, using a stepwise increase from 120°F to 133 °F and finally to a 140°F setpoint. In January, facilities increased the setpoint of the other valve serving the basement through 2nd floor. Even after setting the valves at a high temperature setpoint, there was no significant increase in temperature observed at the outlet water of the fixtures for the overall building. This could be due to incomplete implementation of the intervention actions. Water quality parameters for the pilot and intervention study are described below.

Tradeoffs were observed in health-related parameters (culturable *L. pneumophila,* TTHMs, and copper) (**Figure 9**). The original flush-only event reduced culturable *L. pneumophila* concentrations at all points in the building. However, *L. pneumophila* regrew before the first temperature change, and worsened after the initial incomplete temperature rise. Once the final heat and flush event was performed, *L. pneumophila* concentrations decreased on most floors. In contrast, the first flush-only event increased copper concentrations on the third floor of the building and above. Copper concentrations increased consistently on higher floors throughout the interventions, with lower floors also showing an increase during the heating interventions. TTHMs decreased during the first heat intervention but subsequently increased or had mixed impacts during the second and third temperature increases on floors 7 and 3 and finally decreased during the final combined flush and temperature increase event in February 2023. These results indicated that there were mixed effects of the individual and combined interventions with respect to overall water quality for health-relevant variables of interest.

3.2 Pilot study

We observed widespread colonization of *L. pneumophila* in this green building during the initial pilot sampling campaign (**see Supplemental data file**). Concentrations were above the upper limit of quantification of the assay (>2,273 MPN/ 100 mL) for 36/65 pilot samples including the flushing test (multiple IDEXX dilutions, qPCR, metals, and DBP (TTHM) analyses were not performed for pilot samples).

The pilot flushing study conducted on 11 July 2022 demonstrated decreases in culturable *L. pneumophila* in two locations (automatic faucet on 3rd floor as well as the manual faucet (both hot and cold water) on the 7th floor (see descriptions in **Table 1**). ATP concentrations decreased at the POE, shower, manual faucet and restroom on the 2nd floor, and all 3rd and 7th floor locations, but increased in the janitor closet and manual faucet on the 2nd floor. Free chlorine increased at all locations except the basement janitor's closet (cold water), manual hot water on the 2nd floor, and automatic faucet on the 3rd floor. Conductivity increased at all building locations (excluding the POE). Temperatures decreased for cold water samples from manual faucets (floor 2, 3, and 7) but increased at other locations. pH remained consistent from 7.48-7.89 during the flushing test.

3.3 Physico-chemical water quality measurements

Several physico-chemical water quality parameters such as water temperature, pH, conductivity, total and free chlorine, DBPs, and metals were tested during this sampling campaign. First draw water temperatures ranged from 48°F to 125°F from September 2022 to February 2023. The mean temperature of the city water inlet to the building was 65°F, which ranged from 48°F to 92°F. The mean shower temperature in the basement for hot water was 79°F, and it reached its highest of 98°F post-flushing. The mean cold- and hot- water tap temperature from the janitor's closet and breakrooms was 75°F and 92°F, respectively. The automatic sensor faucets with mixed cold and hot water in the restrooms have an intermediate mean temperature of 87°F. The 3rd floor breakroom hot water temperature was higher compared to other floors, and this could potentially be from the higher water demand on the 3rd floor in this building. The city water inlet had a lower temperature than locations within the building. We also examined the temperature profile for the hot water faucets to observe how quickly hot water reached its highest temperature in April 2023. The highest temperatures were reached after running the hot water faucet in the breakrooms for one minute. As there are two water heater valves, one serving basement to 2nd floor and other for 3rd to 7th floors, we tested the highest temperature the water reached at these four locations (hot water taps in basement, 2nd floor, 3rd floor, and 7th floor). After running the water for a minute, the temperature reached its peak and it stabilized. As the outlet water temperature depends on the mixing valve settings for the water heater, we observed lower temperatures for the basement and 2nd floor of 76°F and 73°F, respectively. Higher temperatures were observed for the 3rd and 7th floor of 88°F and 87°F, respectively, after running the water for one minute. During December 2022, the 3rd floor hot water temperature reached a maximum of 125°F and the 7th floor reached 110°F as the valve was set to 140°F. After the February 2023 sampling event, the valves had operational issues and lower outlet temperatures were observed as a result.

The mean pH of the grab water samples was 7.9 with a range of 7.6 to 8.4, which falls within the recommended 6.5 to 8.5 for drinking water by the USEPA. The water pH from locations inside the building was observed to be slightly higher than the city drinking water pH for most of the instances.

The conductivity of the grab water samples ranged from 888 to 1612 μS/cm. The conductivity was also observed to be slightly higher inside the building's plumbing system compared to the city water entering the building. We observed a decrease in conductivity during December 2022 (**Figure 2**). This could have been due to a decrease in salts (e.g., sodium chloride) in the source water as confirmed by the City of Tempe operators. The observed decrease in conductivity thus corresponded to seasonal, operational, and maintenance changes at the drinking water treatment facility (53). In December 2022, the JGMWTP was operational, and the STWTP was offline. The average turbidity of influent water was 3.2 NTU consistent with seasonal trends indicating lower turbidity in winter months.

The mean concentration of total chlorine values for city water entering the building was 0.78 ppm with a range of 0.63 to 0.91 ppm (**Figure 3**). After the water entered the building, there was a loss of disinfectant residual. During September 2022, in the pre-flushing samples, the mean chlorine concentration of the water inside the building was 0.15 ppm (less than 0.20 ppm recommended value), ranging from 0.0 to 0.77 ppm. The mean total chlorine concentration increased post-flushing and was observed to be 0.40 ppm. During February 2023, the mean total chlorine concentration was 0.13 ppm and increased to 0.29 ppm after flushing. Overall, the hot and mixed water samples tended to have lower chlorine concentrations compared to the cold-water samples on the same floors (p = 0.0001). The city water always had chlorine concentrations above the recommended 0.2 ppm minimum disinfectant residual concentration for the incoming water (54).

Similar trends were observed for free chlorine concentrations. The mean concentration of free chlorine values for city water entering the building was 0.65 ppm with a range of 0.57 to 0.72 ppm. The mean concentration decreased to 0.09 ppm when water entered the building. This concentration increased to 0.28 ppm after flushing the fixtures for 20 minutes in September 2022. In February 2023, the mean free chlorine concentration was 0.08 ppm which was observed to increase to 0.25 ppm after the flushing. The 2nd and the 3rd floor had higher chlorine concentrations compared to the basement and the 7th floor due to higher occupancy and high-water demands. The changes in water heater setpoint did not significantly impact the overall chlorine concentrations, chlorine values from September 2022 to November 2022 were compared with chlorine values from December 2022 to February 2023 time period (p =0.56). During the September 2022 flushing event, 10 out of 12 locations had an increase in chlorine concentrations and during February 2023 flushing event, 11 out of 12 locations had increases in chlorine concentrations.

3.4 Metal concentrations

Metals concentrations observed throughout the study duration are shown in **Figure 4**. The calcium and the magnesium concentrations ranged from 3,200 to 6,100 ppb and 13,600 to 28,300 ppb, respectively. The building does not have a full building softener system installed to soften the city water, which typically has hardness values ranging from 130 to 280 total hardness as ppm of $CaCO_3$. Flushing in September 2022 and February 2023 and increasing the temperature setpoint in December 2022 did not significantly impact calcium and magnesium concentrations (p = 0.68). The pH of the water was observed to have a moderate positive correlation with calcium concentrations (Kendall's Tau= 0.43) which is expected given that hard water tends to be alkaline.

Copper concentrations ranged widely from 4 to 1,265 ppb. Cold water samples had lower concentrations of copper compared to hot water samples (p = 0.0005). Showers had the highest copper concentrations (ranging from 162 to 1,265 ppb). The cold-water faucet samples had a mean of 123 ppb with a range of 4 to 686 ppb, whereas the hot water faucet samples had a mean of 292 ppb with a range of 58 to 872 ppb. The MCL for copper in drinking water is 1,300 ppb (55). Although this value was not exceeded in this study, concentrations close to this value (e.g., 1,265 ppb at the shower) were observed in some instances. Concentrations of copper did not show any significant changes with the flushing intervention taking place in the building but did increase significantly (p = 0.01) during the period when mixing valves were set to the higher temperature setpoint. In December 2022, when the water heater mixing valve setpoint was being increased in a stepwise manner, the copper concentrations started to increase with the water temperature increase.

Iron is an essential nutrient for *L. pneumophila* and stimulates its growth (56). Iron concentrations also ranged widely from 1 to 1027 ppb in the building. The secondary MCL for iron in drinking water is 300 ppb based on taste and odor concerns (57). Iron values were significantly different (p = 0.002) after both flushing events in September 2022 and February 2023. In September, the mean iron concentration was 3.9 ppb which increased to 295 ppb after flushing. Similarly in February 2023, the mean iron concentration increased from 1.4 ppb to 186 ppb after flushing. All 12 locations had an increase in iron concentrations after both flushing events. The iron concentrations increased significantly after flushing and were observed to exceed the MCL (300 ppb) (57) post flushing at various fixtures on 10 February 2023 and 12 September 2023, with values ranging from 104.8 ppb to 1,027.4 ppb. No significant change in iron concentrations was observed from increasing the water heater setpoint during December 2022 to January 2023.

3.5 Disinfection byproduct (DBP) concentrations

There was a gradual decline in concentrations of TTHMs over the course of the study from September 2022 through February 2023 (**Figure 5**). In cooler seasons, it was expected to see an overall gradual decrease in TTHM concentrations due to lower temperature profiles (58,59). In September, we observed lower TTHMs in hot water lines, higher TTHMs in cold water lines, and the highest TTHMs at the POE sample during September 2022. TTHM concentrations decreased starting in November 2022. Additionally, a significant increase in TTHM concentrations (p = 0.002) was observed after flushing in September 2022. Before the flush, the mean TTHM concentration was 73 ppb and it increased to 88 ppb post flushing (**Figure 5**). The trends from February 2023 sampling event were more in line with expected trends; the highest concentration of TTHM was observed in hot water, with lower concentrations in cold water, and the lowest concentrations at the POE (58). There was a decrease in THMs after flushing the faucets for 20 minutes (not significant, p = 0.06). The mean TTHM concentration before flushing was 52 ppm, which decreased to 37 ppb post flushing.

The USEPA's maximum allowable annual mean level for TTHM is 80 ppb (ug/L) (60). From September 2022 to November 2022, the TTHM values exceeded the MCL (80 ppb) on 6 instances for the 3rd and 7th floor. There was a significant increase (p =0.002) in TTHM concentrations post-flushing in September 2022. The mean TTHM concentration during the month of September 2022 was 83 ppb, which decreased to 49 ppb in the winters during November 2022 to February 2023. We also observed a weak positive correlation (Kendall's Tau = 0.21) between free chlorine concentration and TTHM concentrations.

3.6 cATP concentrations

Cellular adenosine triphosphate (cATP) in drinking water samples was tested to monitor the overall microbial activity. cATP ranged from 0.1 to 83 pg/mL (**Figure 6a**). Values exceeding 10 pg/mL for drinking water distribution systems and premise plumbing indicate potential microbial water quality deterioration according to the cATP test manufacturer (45,46). The city water entering the building had the lowest cATP concentration (1.51 – 0.06 pg/mL), and thus lower microbial activity. Hot and mixed water samples had higher ATP values (0.21 – 83.1 pg/ml) compared to cold water samples (0.15 – 21.3 pg/mL) (p = 0.002). We observed a significant decrease in mean ATP values post-flushing in September 2022 (mean concentration of 20.8 pg/mL compared to 2.14 pg/ml; p =0.018) (**Figure 6a**). 10 out 12 locations had a decrease in cATP concentrations after the flushing event in September 2022. This indicated that flushing was overall useful in reducing microbial loads in the system.

3.7 Culturable *L. pneumophila* concentrations

L. pneumophila was not detected at the POE during the pilot or intervention testing study. The mean recovery efficiency of the IDEXX method was 67.1±7.3% (**Section B** of the **Supplemental Information Table SI.4**). A wide range of *L. pneumophila* concentrations were detected in positive samples from 1 to 227,300 MPN/ 100mL (**Figure 6b**). Water samples from hot and mixed faucets had higher concentrations of *L. pneumophila* compared to cold water samples (p =0.0001). The hot and mixed water concentrations ranged from 1 to 227,300 MPN/ 100 mL with a mean of 26,393 MPN/ 100mL. The cold-water concentrations ranged from 1 to 5,390 MPN/ 100 mL with a mean of 316 MPN/ 100 mL. Overall, the 3rd floor had the lowest *L. pneumophila* concentrations, (mean = 686 MPN/ 100 mL) potentially due to higher occupancy and higher hot water line temperatures. A significant decrease in *L. pneumophila* was observed post-flushing in September 2022, with a mean concentration decrease from 863 MPN/100 mL to 43 MPN/100 mL (p = 0.019) (**Figure 6b**). There was a slight decrease in *L. pneumophila* concentrations during the second flushing intervention in February 2023, with the mean concentration decreasing from 23,065 MPN/mL to 12,113 MPN/ mL (not statistically significant, p = 0.27). During the first flushing event in September 2022, there was a decrease in *L. pneumophila* concentrations for every floor and all sampling locations (n = 12). The same trend was not observed for the other two intervention events. This could be due to potential ad-hoc flushing by the facilities' staff outside of investigator interventions during April 2022 to September 2022 after the pilot sampling was conducted. No significant changes in overall *L. pneumophila* concentrations were observed throughout the building during the period of increasing the water heater setpoint during December 2022 to February 2023 (p =0.21). *L.*

pneumophila concentrations were compared based on floors, noting that the valve that served the 3rd to 7th floor was set to a high temperature setpoint of 140°F during December 2022. We observed the *L. pneumophila* concentrations were significantly lower (p = 0.002) compared to basement and 2nd floor counts. *L. pneumophila* in water samples were identified as both serotype 1 and serogroup 2–14.

3.8 *L. pneumophila mip* gene concentrations

Quality assurance/quality control for the qPCR assays was previously performed in a prior study (10). All quantification assays followed the MIQE guidelines (61), and the no template controls (NTC) did not show any amplification throughout the study. No PCR inhibition was observed in the tested samples as evidenced by consistent Cq differences between undiluted and ten-fold diluted samples. The mean recovery efficiency of the qPCR method was 61.3±6.7% (**Section C** of the **Supplemental Information Table SI.4**).

The city drinking water entering the building had low *mip* gc values which were below the quantifiable limit. After the water entered the building, a wide range from 10 to 1.14×10^9 gc/L was observed (**Figure 6c**). The hot water faucet on the 7th floor had the highest concentration of the *mip* gene (mean concentration of 1.32×10^8 gc/L) followed by the shower and hot water faucet in the janitor's closet located in the basement (mean concentration of 4.28×10^7 gc/L). There were no significant changes in *mip* gene concentration observed during flushing interventions in September 2022 and February (p = 0.169 and 0.307 respectively) or during the process of increasing water heater temperature setpoint (p = 0.43).

3.9 Building occupancy

Wi-Fi logins in the building were used as a proxy of occupancy, which also served as an indicator ofas water usage and demand on each floor in the building with respect to time and day of the week as direct water demand information was not available. Occupancy was observed to be the highest on 2nd and 3rd floors with an mean of 42 logins per hour compared to the 7th floor and least in the basement (mean of 9 logins per hour) (**Figure 7**). Occupancy increased gradually until mid-workday (1-2 pm) compared to early morning (before 8am) during which time sampling was performed ($p = 0.002$). Occupancy was lower on the 7th floor (mean of 24 logins per hour) during the study. Variation in occupancy (hourly Wi-Fi logins) throughout the day (12 AM to 11:59 PM) when sampling events were performed for each floor is represented in **Section C** of the **Supplemental Information Figure SI.1.** High variability is observed over the course of the day.

3.10 Correlations between water quality variables and building factors

Culturable *L. pneumophila* counts showed a weak negative correlation with free chlorine concentration (Kendall's Tau = -0.31), a moderate positive correlation with cATP (Kendall's Tau = 0.45) and a weak positive correlation with the *mip* gene concentration (Kendall's Tau = 0.15) (**Section D** of the **Supplemental Information Table SI.6**). Culturable *L. pneumophila* had a weak negative correlation with building occupancy (Kendall's Tau = -0.19). Free chlorine concentrations were positively correlated with occupancy (Kendall's Tau = 0.25). The free chlorine concentration was negatively correlated with outlet water temperature (Kendall's Tau =

-0.39), consistent with an expectation that chlorine decays faster at warmer temperatures (62). The TTHM concentrations were positively correlated with the free chlorine concentrations (Kendall's Tau = 0.21). The water pH was positively correlated with calcium concentration (Kendall's Tau = 0.43). Temperature was positively correlated with occupancy (Kendall's Tau = 0.19) (**Figure 8**).

3. Discussion

4.1 Temperature

Temperature and disinfection residuals in the water are the most frequently recommended physicochemical parameters to monitor overall water quality. Due to the warm ambient temperatures, seasonal variations, and temperature setpoint adjustments, water temperatures typically ranged from 48°F to 125°F (9°C to 52°C) in the first draw of grab samples. *Legionella* spp. are known to grow best in the range of 25°C to 45°C, making the building plumbing a favorable place to colonize and grow in higher concentrations (63). When there is low activity in the building, warming of cold water and cooling of hot water can result in temperatures that provide ideal growth conditions for microbes. However, high temperatures can also be a concern for scalding (64).

In this study, the increase in temperature setpoint for both the valves was incomplete due to several logistic issues such as finding an appropriate replacement for old valves, contractor availability, and other valve assembly repairing complications. The set point was increased from 120°F to 133°F and then finally set it to 140°F. While sampling was performed in coordination with the building manager, no major increase in temperature at the fixture outlet occurred in many building locations in the first flush and time to peak temperature was > 1min. For the basement to 2nd floor, there was a minimal temperature rise, whereas for the 3rd to 7th floor, we observed a temperature increase and the hot water faucets reached a mean temperature of 115°F at the outlet during December 2022. Fluctuating warm water temperatures favor microbial growth in the waterlines and influence the biofilm dynamics in these pipes, and thus could contribute to release of pathogens in the bulk drinking water (65). Material accumulation forming deposits in the waterlines may also accelerate with temperature along with metal releases in water (65).

4.2 Chlorine Concentrations

The disinfectant residuals in the building were generally low (<1 ppm) and could have contributed to higher microbial growth within the system. Low water demands in the building could potentially cause stagnation and increase the water age. Increased water age can deteriorate the water quality of a building (66,67). The flushing intervention was designed to increase the chlorine concentration by bringing in fresh city water which has a higher disinfectant residual. Flushing resulted in higher chlorine concentrations as well as ATP values in some cases but resulted in tradeoffs for DBP formation. Additionally, as the building is LEED-certificated, water flushing can go against water saving goals and result in a burden on facilities' management resources. Direct flushing at the hot and cold mixed water lines was not performed as these locations had automatic activation via sensors in comparison to manual lines with separate hot and cold-water handles. Therefore, additional water stored in the final sections of pipe/fixtures could have contributed to higher contaminant observations at these fixtures.

4.3 Conductivity

Seasonal changes in building samples were compared with monthly city water quality reports (53). Turbidity was observed to decrease as TOC decreased in the City of Tempe finished water, with an overall decrease in turbidity and TOC in winter months. The drop in conductivity that was observed in the building in December 2022 could therefore be due to a decrease in sodium chloride, consistent with water quality variation observed for the City of Tempe (53). Water with fluctuating high conductivity can have an unpleasant taste and odor, cause mineral deposits on plumbing fixtures, and/or indicate water quality issues (68,69). Turbidity in tap water should be very low, however TOC may exert some chlorine demand.

4.4 Copper Concentrations

Heavy metals such as copper from copper piping can corrode during stagnation and flow cycles, releasing them into the bulk water (70). Copper tends to dissolve to a greater degree in hot water compared to cold water (71). Copper can be released both in dissolved and particulate form and corrosion rates differ and depend on the electrochemistry of water (72). There could also be microbially-induced corrosion when copper-tolerant bacteria colonize the pipes and prevent the formation of a brown protective insoluble oxide layer that plays a role in controlling free copper release (73,74). When the protective layer is absent, there is a higher chance of pitting and faster release of copper. In our study, we observed that copper concentrations were highest in the shower water and in samples from the 3rd to 7th floor when the digital magnetic mixing valve was set to the highest temperature setpoint of 140°F.

4.5 Iron Concentrations

The water collected after 20 minutes of flushing intervention was cloudy and brown/ orange in color which is potentially from the high iron concentrations in the water. This could be due to flow disturbances in the pipes resulting in mobilization of mineral sediments or deposits (30,71). Iron in bulk water may affect disinfectant demand and tends to form scales with high surface area suitable for biofilm formation (75). Scale formation on the inner surfaces of pipes favor microbial growth in the drinking water system by providing potentially large surface areas for biofilm growth and protection from disinfectants (76,77).

4.6 DBPs Concentrations

DBPs are carcinogenic and pose a risk to human health through multiple exposure routes (60). They are formed when disinfectants like chlorine interact with natural organic matter (NOM) in water (78). The USEPA Disinfectants and Disinfection Byproducts Rule (DBPR) requirements address Trihalomethanes (TTHM) and Haloacetic acids (HAA5). In our study, we observed that TTHM values were higher from September 2022 to November 2022 and then gradually decreased starting December 2022 to February 2023. Cooler seasons are expected to lower the rate of reaction between the natural organic matter and chlorines which ends up in lower DBP formation (79,80). As flushing brings in more fresh disinfectant residual from city water, the organic matter that has been accumulated in the system from high water age reacts with the chlorine to form more DBPs which could explain this trend of increasing DBP concentration (81,82). Higher water temperature, higher water age from stagnation, and corrosion products creating sediments are all known to potentially increase DBP concentrations in the drinking water system (80,81).

There were seasonal trends observed in the TTHM concentrations. In cooler months, it was expected to see an overall gradual decrease in their concentration in the grab water samples. In September, lower concentrations of TTHMs were observed in hot water lines, with higher TTHMs in cold water lines, and the highest TTHMs at the POE sample. Additionally, an increase in TTHMs was observed after flushing in September. These trends were not expected due to typically higher TTHMs reported in hot water lines compared to cold water or POE samples (58). The trends from the February sampling event were more consistent with expected trends; the highest concentration of TTHMs were observed in hot water, with lower concentrations in cold water, and the lowest concentrations at the POE. There was a decrease in TTHMs after flushing the faucets for 20 minutes.

The potential causes for the September trends could be related to changes in building water usage, or other factors related to changes in the plumbing system beyond the control of the investigators such as unreported repairs, changes in infrastructure (e.g., replaced parts), or other water quality changes. JGMWTP and STWTP were both running during September 2022, with low turbidity ranging from 0.05- 0.09 NTU in treated effluent, and TOC from 1.5-4.5 mg/L. The TTHM instrument and all probes were calibrated prior to use. Sampling error due to the timing of sample collection (samples taken at different times) could have also played a role in observed contaminant differences. Floor sampling was randomized to attempt to control for the effect of sampling order. Building water usage changes were concomitant with other factors and could have had an impact on contaminant levels, and therefore it is challenging to isolate the impacts of specific building water management interventions. Building water usage was monitored via Wi-FI logins as a proxy, and the time of day was standardized (early morning to target the time of day prior to building occupancy) to target a stagnation time of at least 24h. Occupancy data were obtained for the floors on which a water quality sensor was present (2,3, and 7) but could be expanded to collection from other building floors to more comprehensively evaluate overall building occupancy. There could also have been flushing or other actions performed in tandem by facilities that were not captured during communications with the research team. The trends observed could also be related to biofilm formation and sloughing, temperature fluctuations affecting the volatility of DBPs, changes in source water quality, or building practices (58,81,83).

4.7 Microbial Water Quality Parameters

The cATP values exceeded 10 pg/mL at several locations in the building during sampling period indicating high active biomass in the waterlines (45). The cATP values were lower in cold water samples compared to hot and mixed water samples. This could be because the hot water lines were not maintained at high enough temperature and the intermediate temperature favors more microbial growth. The cATP values decreased after flushing events but did not show any significant decrease during the period of increasing water heater setpoint.

L. pneumophila colonization was widespread and persistent throughout the building. Many factors such as warm temperatures, low or no disinfectant residuals, low flow fixtures, and low water demand can potentially contribute to this *Legionella* problem in commercial and institutional buildings. Although the first flushing event in September resulted in a significant decrease in *Legionella count*, later, high concentrations were observed consistently irrespective of management interventions. High concentrations were observed in hot and mixed water lines, especially on floors with less occupancy than the cold-water lines in the building. Recovery efficiencies of the *L. pneumophila* methods were consistent with previously reported studies (84,85), however recoveries throughout various locations of the plumbing system could be impacted by extraction method, stagnation, and changes in chemistry throughout the system (86,87).

4.8 Impact of Sample Timing and Locations

Samples were collected on weekdays in the morning 7 to 8 am and post flushing samples were collected in the afternoon 1 to 2 pm, and therefore occupancy changes are a confounding factor in this analysis. Previous work has indicated that increases in microbial cell count are commonly observed after overnight stagnation (88). The doubling time of *Legionella* is approximately 2 h at mid-log phase within a host cell, however this is likely to be temperature-dependent with first order growth rates in drinking water ranging from 0.83 to 0.25 h^{-1} at temperatures of 25-42 °C (89). The *Legionella* concentrations were slightly higher on floors with less occupancy, in shower water lines, and in hot or mixed water lines compared to cold waterlines. The sampling study period also covered different seasons that tend to affect physicochemical water quality variables such as temperature, conductivity, dissolved solids, amount of natural organic matter, and DBP concentration (90).

4.9 Impact of Management Interventions

Three interventions (flushing, increasing the water heater temperature setpoint, and a combined water heater set point change and flush) were evaluated for their impacts on water quality during this study. Building interventions such as flushing require less resources than changing a water heater set point, which can be logistically challenging due to labor, equipment, and parts costs in large commercial and institutional buildings. A significant decrease was observed in cATP and viable *L. pneumophila* concentrations for some locations but not others throughout the building during the first flushing event. cATP concentrations were observed to significantly decrease from flushing in September 2022 for 10 out of 12 locations. *L. pneumophila* counts significantly decreased from flushing in September 2022 for 12 out of 12 locations. More mixed trends were observed for the later flushing event as set point temperatures were being changed.

Furthermore, the incompletely implemented temperature intervention did not have a significant decrease on microbial water quality variables (*L. pneumophila,* ATP) which it was intended to address. This suggests that one-time interventions such as flushing and water heater set point changes may not completely resolve water quality issues and a routine flushing plan is typically recommended as a preventative action (66). Other authors have also suggested that hot water lines should be flushed longer (for ~75 minutes) compared to cold water lines (~20-30) minutes (41).

4.10 Building water quality tradeoffs

Combined interventions such as flushing and increasing the water heater setpoint have previously resulted in a greater decrease of *L. pneumophila* counts in building plumbing compared to a single intervention action. Combined interventions such as flushing and increasing the water heater setpoint have previously resulted in a greater decrease of *L. pneumophila* counts in building plumbing compared to a single intervention action (12,33,91). However, when observing combined interventions, it is challenging to parse out the effects of a particular action.

It is also challenging to manage both microbial and chemical risks in building plumbing and tradeoffs between microbial, chemical, and DBP aspects of water quality were observed in this study. This suggests that no single intervention was universally effective in improving the overall water quality (**Figure 10**). Comparisons of microbial and DBP risks have highlighted a dependency of such calculations on site-specific water quality monitoring data, and in the future could be applied in the context of building water management plans (92).

The LEED certification by the USGBC addresses the design and construction phase of the building but is not revisited during regular building operation and maintenance. LEED certification is awarded based on a building's overall sustainability, with a greater number of points earned associated with a higher certification level. It is thus important to ensure that LEED principles such as energy efficiency and water savings are followed. By implementing management interventions there could be tradeoffs such as additional energy requirements from set point changes, and increased water usage from flushing. Thus, it is challenging to design and operate buildings that simultaneously maximize benefits for both sustainability and health.

4.11 Generalizability and limitations. The results of this study are generalizable to similar building types such as buildings served by chlorinated city water, copper plumbing, similar size (e.g., number of floors and square footage), and moderate occupancy. The study building has office and lab spaces rather than high-occupancy areas like classrooms and cafeterias and therefore may be more generalizable to other commercial and institutional buildings compared to other types of academic buildings. Certain aspects of specific microbial communities and dynamics governing contaminant-tradeoffs are likely to be partially building-specific, however microbial washout and metals (re)mobilization generally have been reported as a result of in-building interventions elsewhere (6,93).

The presented analysis is exploratory and could be expanded to include additional multivariate analysis of associations. While this dataset provides a holistic view of contaminant occurrence under various in-building interventions, the use of single grab samples and many potential explanatory variables (e.g., occupancy, floor, sampling location, fixture type, hot vs. cold water, pre vs. post flush, pH, temperature, conductivity, total chlorine, free chlorine, DBP, ATP, culturable LP, qPCR LP, metals) with 12 samples per each pre- or post-flush sampling event limits the extent of multivariate statistical analysis possible.

5. Conclusions

Through this study, a lack of disinfectant residual (low free chlorine concentrations in the building), low water heater set point, and temperature fluctuations in the hot and cold waterlines may have contributed to *L. pneumophila* proliferation. Incomplete implementation of management intervention actions did not mitigate *L. pneumophila* colonization and additional management is needed to control these issues. If water heater temperature setpoints can be increased to 140°F, it can discourage *L. pneumophila* growth in the hot water lines, however the plan should integrate system-specific knowledge of other components such as mixing valves and their associated performance. In this study, flushing interventions had a significant effect on replenishing disinfection residual, and reducing cATP and *L. pneumophila* concentrations, however other considerations (metals, DBPs) did not improve consistently across the interventions performed. A statistically significant and consistent contaminant decrease was not observed across the single or combined interventions, and it was common to observe mixed trends for different locations in the building during interventions. These observations highlight the challenges of balancing risks for drinking water, and the need to monitor multiple water quality aspects to avoid replacing one risk with another. Finally, water quality management in the building can likely be improved through longer-term routine and proactive actions rather than single interventions. An improved understanding of the building's plumbing and fixture performance can help to inform building water management plans.

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Author contributions

KAH: Project funding, concept origination, project management, manuscript writing; **SJ**: Sampling lead, sample processing, microbial analysis, PCR, manuscript writing, data analysis; **RR:** Pilot sampling lead, manuscript review, data analysis; **DH:** TTHM analysis, manuscript writing, data analysis; **JB, MC, VK, KC, EJ, NB:** Sampling assistance, sample processing, physico-chemical measurements, microbial sample processing; **MM-H:** Metals analysis, manuscript review; **JA, LV-G:** manuscript review, data analysis; **TB:** Project funding, manuscript review, project management.

Figures

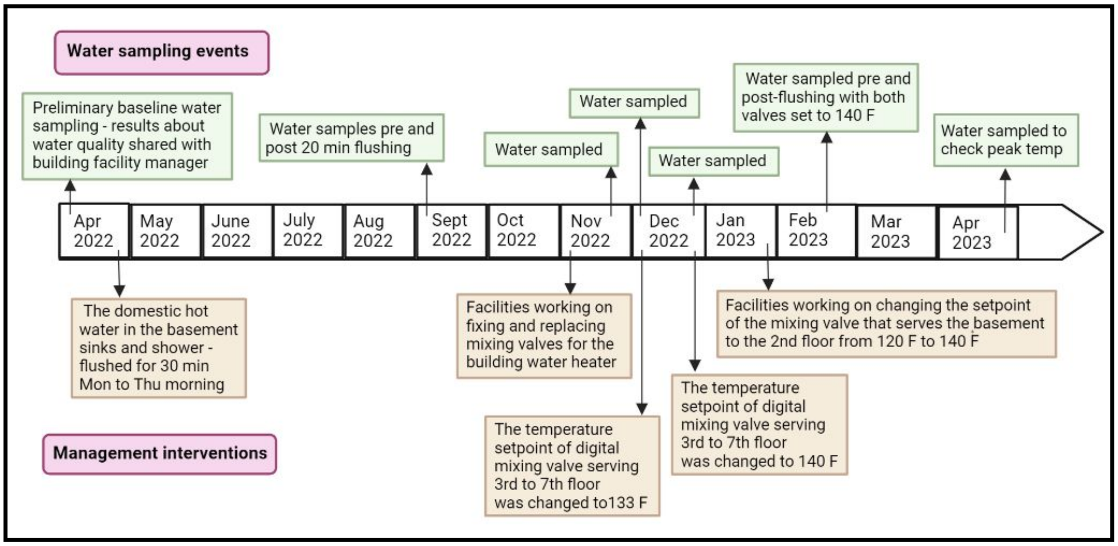


Figure 1. Timeline of water sampling events and building management interventions throughout the study period April 2022 to February 2023.

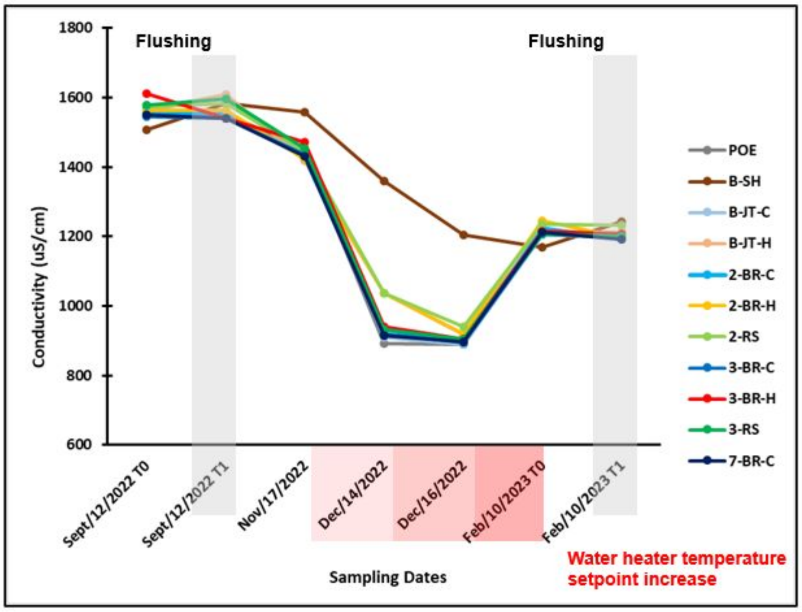


Figure 2. Conductivity profiles of grab water samples collected during September 2022 to February 2023. The grey shaded areas in the background of the plots represent in flushing events during September 2022 and February 2023. T0 time points were collected before the flushing interventions and T1 time points were collected after 20 minutes of flushing. The red gradient background represents the time during which temperature setpoints were being increased for the water heater. The X axis points are not evenly spaced in terms of time; the axis only shows the sampling events during this study along with interventions. Sampling locations focused are POE= Point of entry; B-SH= bathroom shower, hot water; B-JT-C=Basement Janitor's closet, cold water; B-JT-H= Basement Janitor's closet, hot water; BR-C= Breakroom floors 2,3, or 7

cold water; BR-H= Breakroom floors 2,3, or 7 hot water; RS= restroom floors 2,3, or 7 mixed hot and cold water.

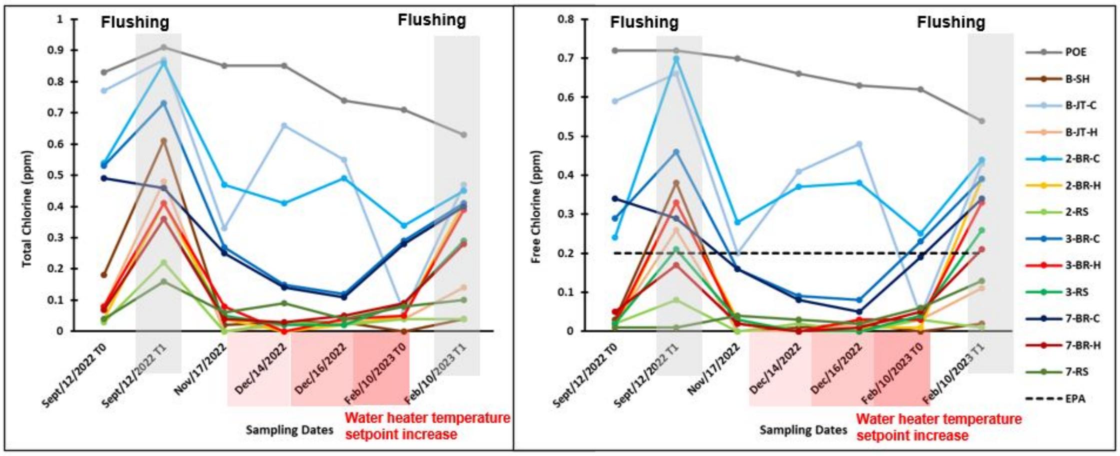


Figure 3. Total (left) and free (right) chlorine concentration in grab water samples collected during September 2022 to February 2023. The grey shaded areas in the background of the plots represent in flushing events during September 2022 and February 2023. T0 time points were collected before the flushing interventions and T1 time points were collected after 20 minutes of flushing. The red gradient background represents the time during which temperature setpoints were being increased for the water heater. The X axis points are not evenly spaced in terms of time; the axis only shows the sampling events during this study along with interventions. Sampling locations focused are POE= Point of entry; B-SH= bathroom shower, hot water; B-JT-C=Basement Janitor's closet, cold water; B-JT-H= Basement Janitor's closet, hot water; BR-C= Breakroom floors 2,3, or 7 cold water; BR-H= Breakroom floors 2,3, or 7 hot water; RS= restroom floors 2,3, or 7 mixed hot and cold water.

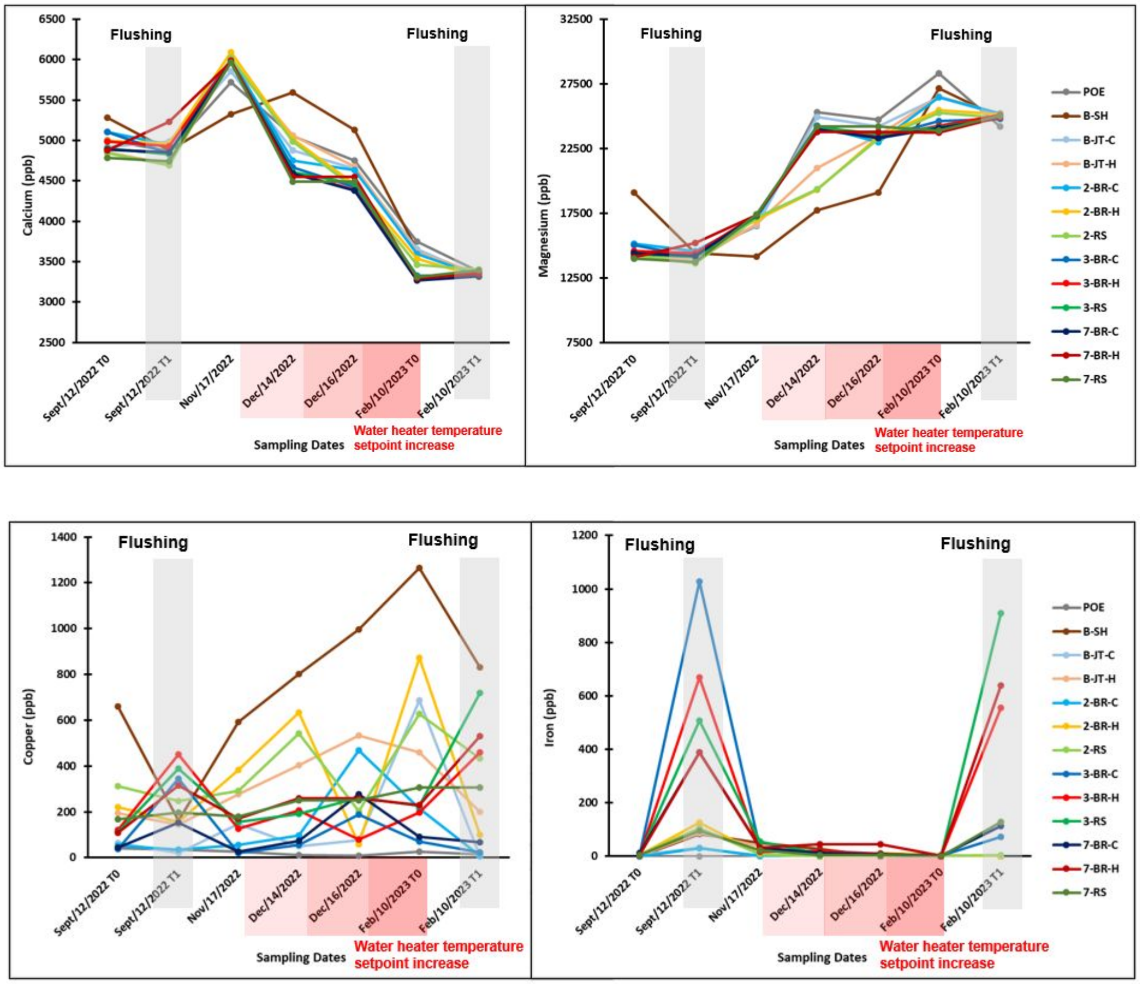


Figure 4. Metals concentrations in grab water samples collected during September 2022 to February 2023. The grey shaded areas in the background of the plots represent in flushing events during September 2022 and February 2023. T0 time points were collected before the flushing interventions and T1 time points were collected after 20 minutes of flushing. The red gradient background represents the time during which temperature setpoints were being increased for the water heater. The X axis points are not evenly spaced in terms of time; the axis only shows the sampling events during this study along with interventions.

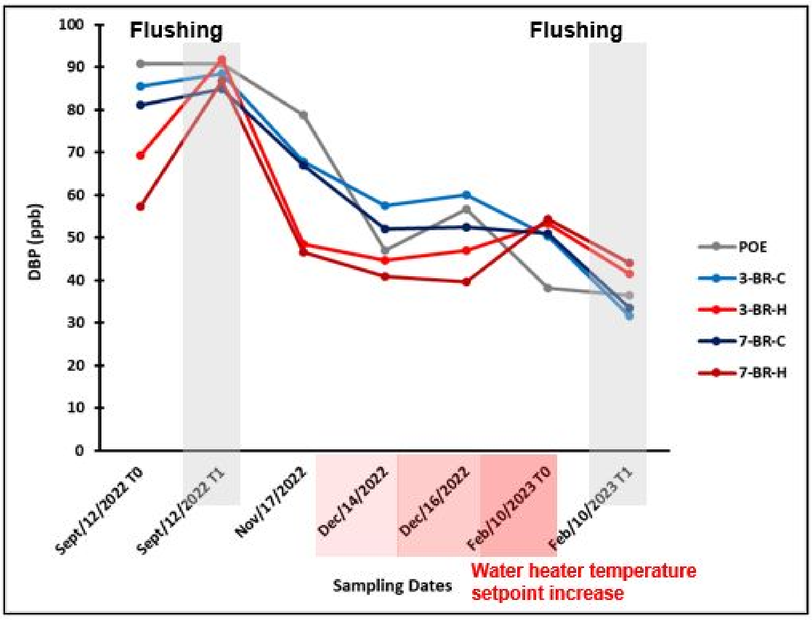
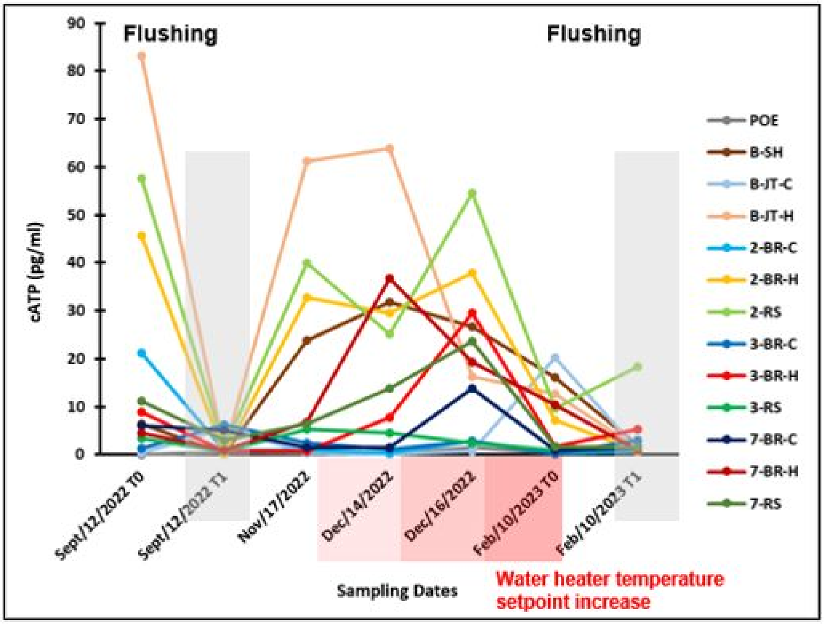
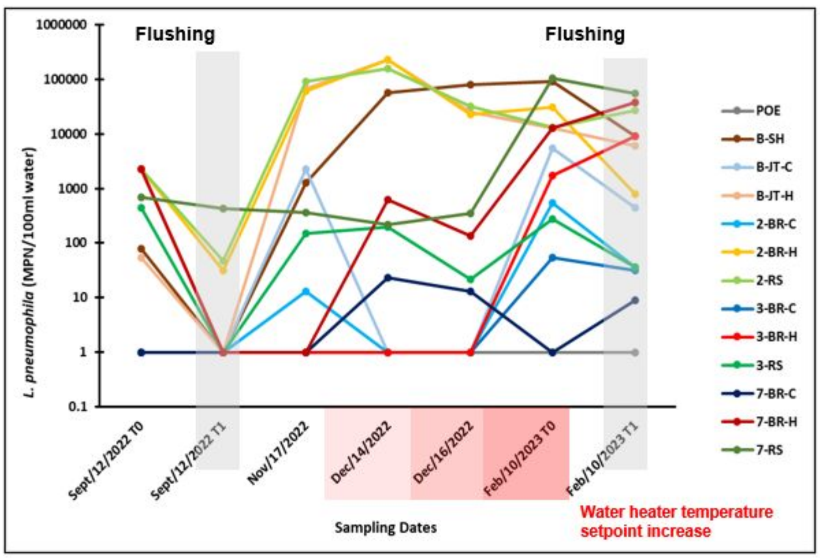


Figure 5. TTHMs concentration in grab water samples collected during September 2022 to February 2023. The grey shaded areas in the background of the plots represent in flushing events during September 2022 and February 2023. T0 time points were collected before the flushing interventions and T1 time points were collected after 20 minutes of flushing. The red gradient background represents the time during which temperature setpoints were being increased for the water heater. The X axis points are not evenly spaced in terms of time; the axis only shows the sampling events during this study along with interventions.



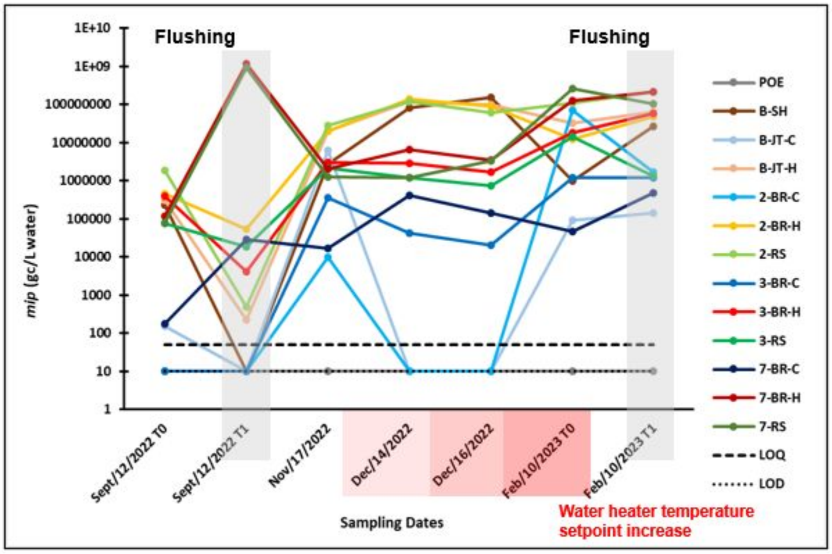
Flushing
Flushing
cATP (pg/ml)
POE
B-SH
B-JT-C
B-JT-H
2-BR-C
2-BR-H
2-RS
3-BR-C
3-BR-H
3-RS
7-BR-C
7-BR-H
7-RS
Sept/12/2022 T0
Sept/12/2022 T1
Nov/17/2022
Dec/14/2022
Dec/16/2022
Feb/10/2023 T0
Feb/10/2023 T1
Sampling Dates
Water heater temperature setpoint increase

a)



Flushing
Flushing
L. pneumophila (MPN/100ml water)
POE
B-SH
B-JT-C
B-JT-H
2-BR-C
2-BR-H
2-RS
3-BR-C
3-BR-H
3-RS
7-BR-C
7-BR-H
7-RS
Sept/12/2022 T0
Sept/12/2022 T1
Nov/17/2022
Dec/14/2022
Dec/16/2022
Feb/10/2023 T0
Feb/10/2023 T1
Sampling Dates
Water heater temperature setpoint increase

b)



c)

Figure 6. Microbial measurements in grab water samples collected during September 2022 to February 2023 for (a) cATP; (b) culturable *L. pneumophila*; and (c) *L. pneumophila* quantified via qPCR. The grey shaded areas in the background of the plots represent in flushing events during September 2022 and February 2023. T0 time points were collected before the flushing interventions and T1 time points were collected after 20 minutes of flushing. The red gradient background represents the time during which temperature setpoints were being increased for the water heater. The X axis points are not evenly spaced in terms of time; the axis only shows the sampling events during this study along with interventions.

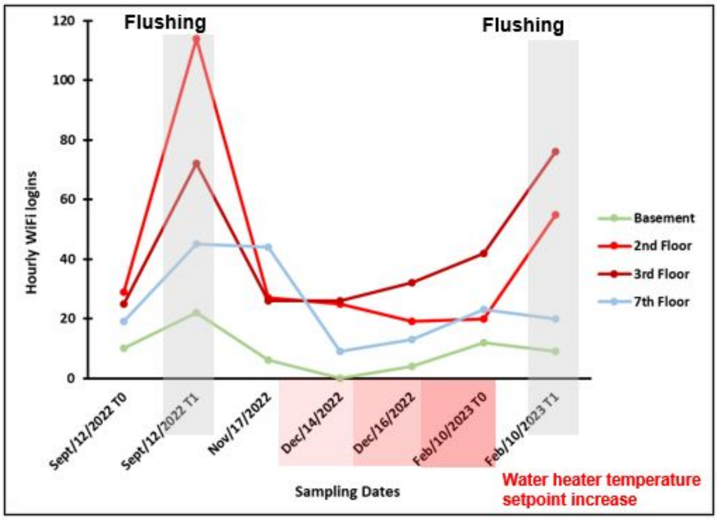


Figure 7. Occupancy trends in the building during the study for floors basement, 2, 3, and 7 respectively. The grey shaded areas in the background of the plots represent in flushing events during September 2022 and February 2023. T0 time points were collected before the flushing interventions and T1 time points were collected after 20 minutes of flushing. The red gradient background represents the time during which temperature setpoints were being increased for the water heater. The X axis points are not evenly spaced in terms of time; the axis only shows the sampling events during this study along with interventions.

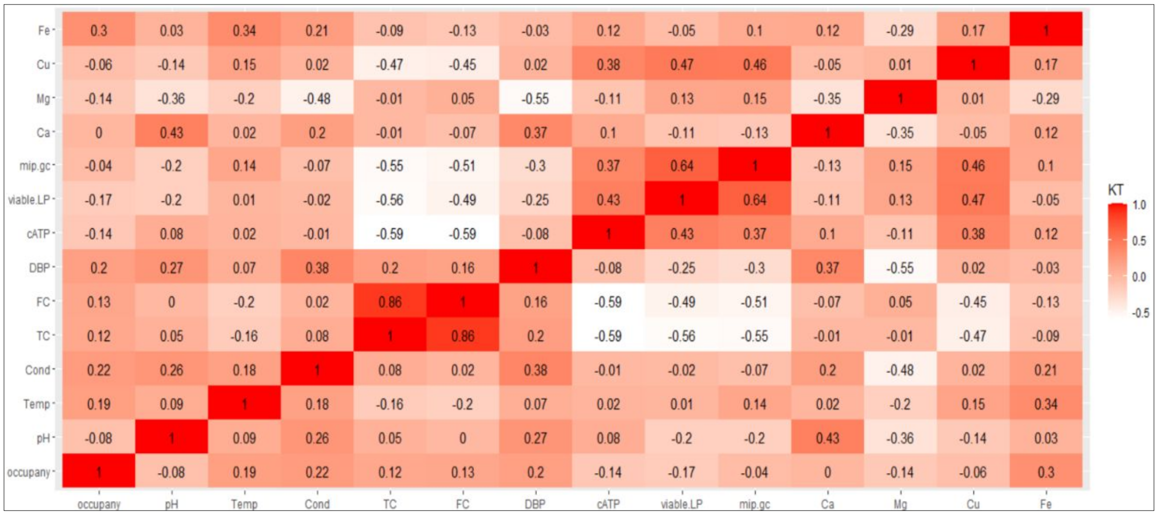


Figure 8. Kendall's Tau heat map summarizing all correlation coefficient between building water quality parameters considered in this study.

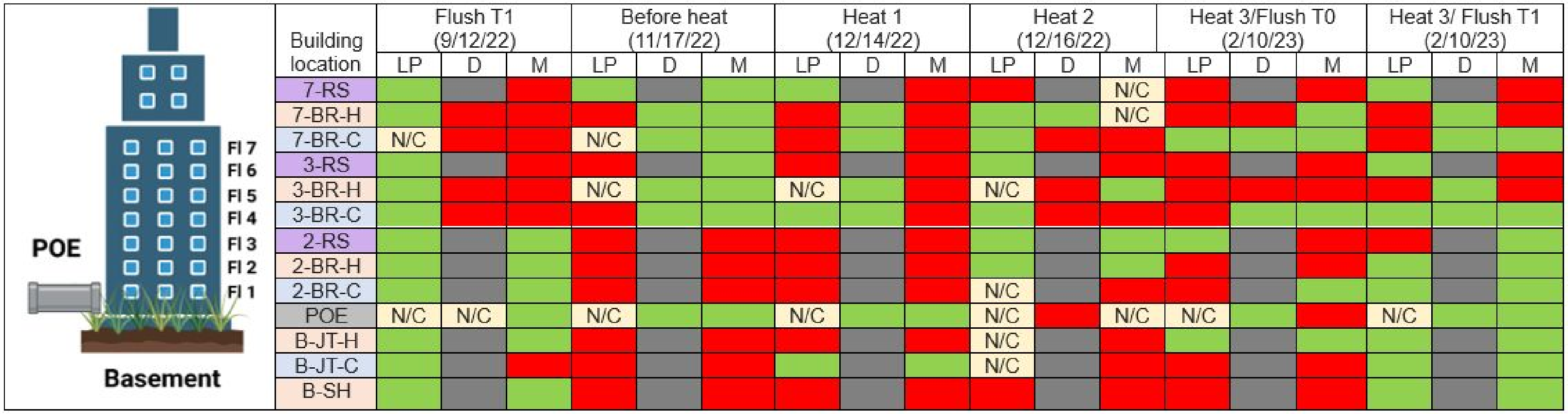


Figure 9. Map of building water changes over the course of the study. LP= culturable *L. pneumophila;* D= Disinfection by-products (TTHM); M= heavy metals (copper only). Red=increase from prior sampling event; yellow=no change; green=decrease; N/C= no change; grey= not measured at that location. Building locations: blue= cold water; pink=hot water; purple=mixed hot and cold water. See Table 1 for building location key.

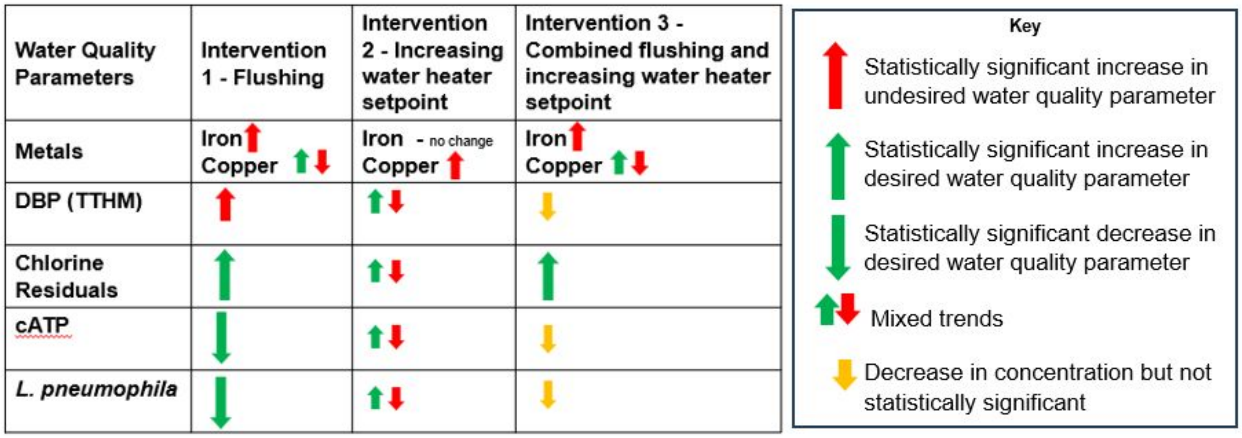


Figure 10. Summary of impacts of various water quality intervention in the building performed during the study period.

List of Tables

Table1. Water sampling locations, fixture types, and water type (hot, cold, or mixed)

Location in the multi-story building	Fixture type	Water type	Sample code/ID
City distribution system (Tempe)	City water influent—point of entry outside the building	Cold water	POE
Shower in basement restrooms	Manual fixture with handle	Hot water	B-SH
Janitor's closet in basement	Manual faucet with handle	Cold water	B-JT-C
Janitor's closet in basement	Manual faucet with handle	Hot water	B-JT-H
Breakrooms floors – 2,3,7	Manual faucet with handle	Cold water	BR-C
Breakrooms floors – 2,3,7	Manual faucet with handle	Hot water	BR-H
Restrooms floors – 2,3,7	Automatic faucet with sensor	Mixed water	RS

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