

What happens in your water system? Impact of materials, temperature, stagnation and chlorination on water quality and biofilm formation

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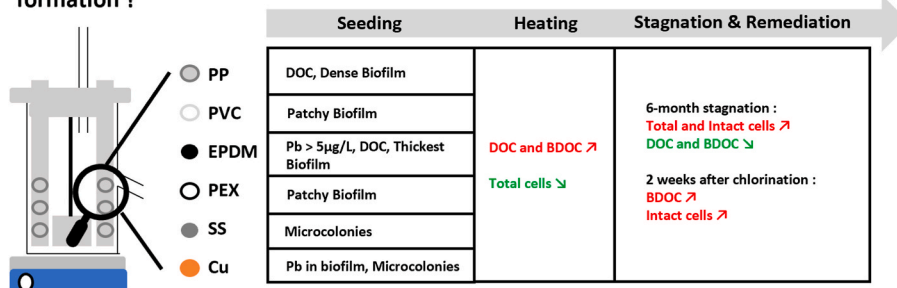
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HIGHLIGHTS

- EPDM leaches Pb in drinking water (>5 µg/L) while copper biofilms accumulate Pb.
- Materials show diverse biofilm densities: EPDM > PP > PVC > PEX > Cu – SS.
- Heating at 40, 55 and 60 °C leads to striking leaching of DOC/BDOC by all materials.
- Limited cells recovery after 6-month stagnation in copper previously at 55–60 °C.
- High BDOC release followed shock chlorination, highlighting the need of flushing.

GRAPHICAL ABSTRACT

How do materials, temperature, and stagnation affect water quality and biofilm formation ?



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ABSTRACT

Building water distribution system materials are diverse and their impact on water quality depends on nutrients, stagnation, and temperature. The main objective was to investigate the interplay between material type, temperature and stagnation on water quality and biofilm formation potential. CDC biofilm reactors were used to compare concentrations of metals, total (DOC) and biodegradable (BDOC) dissolved organic carbon, and total and viable cells densities in water and biofilms in contact with coupons of 6 materials: polypropylene (PP), polyvinyl chloride (PVC), ethylene propylene diene monomer (EPDM), cross-linked polyethylene (PEX), stainless steel (SS) and copper (Cu). All materials were colonized by bacteria, with denser biofilm observed on EPDM > PP > PVC > PEX > Cu – SS. Copper and EPDM reactors showed different dynamics of accumulation and release of Cu and Pb, with bulk Pb concentrations exceeding 5 µg/L. Increasing temperatures from 25 °C to 40, 55 and 60 °C resulted in an increase by at least a factor 2 of DOC and BDOC, a temporary decrease in cell viability (%) and contrasting trends for suspended total bacteria (up to 1.4-log). Biofilm densities remained lower for all reactors after two months of constant heating at 40 °C, even further for copper reactors heated to 55 °C (2.5-log) and 60 °C (2.7-log). A 4-week stagnation at room temperature promoted total bacteria recovery in water and biofilm for all materials except copper. Extending stagnation to 6 months (COVID-19 shutdown) further increased total bacteria and the viable fraction in water, except in copper reactors.

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1. Introduction

Water distribution systems are complex systems composed of a large diversity of materials, including metals and plastics, in service lines and fixtures (Waines et al., 2011). Copper (Cu) pipes were often used in water distribution systems because of their potential antimicrobial properties (Moritz et al., 2010; Waines et al., 2011). Stainless steel (SS) represents a good alternative to copper pipes because of its long period of resistance to corrosion (Waines et al., 2011; Proctor et al., 2020). Plastics like polypropylene (PP) and polyvinyl chloride (PVC) are mostly found in drinking water distribution systems (DWDS), while ethylene propylene diene monomer (EPDM) rubber and cross-linked polyethylene (PEX) are found in flexible plumbing hoses and sealing gaskets (Waines et al., 2011; Proctor et al., 2020). While the choice of the materials relies mostly on their cost and ease of installation, materials also greatly impact the water quality in the DWDS as their surface structure and leaching potential (metals, nutrients) influence the growth of biofilms on their surfaces (Lehtola et al., 2004; Waines et al., 2011). Materials release carbon that can be used as nutrient by microorganisms and favour the formation of biofilms (Mao et al., 2018). Also, some plastic materials like PEX leach inorganic and organic compounds that can be hazardous for the consumers (Stern and Lagos, 2008). Concentrations of some metals as lead (Pb) or copper (Cu) are regulated in drinking water because of their impact on human health, especially children (Health Canada, 2018, 2019).

Temperature influences the solubility of chemicals in water, and Hametner et al. showed that the increase of water temperature from 23 °C to 60 °C in a polypropylene system led to an increase of total organic carbon (Hametner, 1999). Microbial quality of water is also dependent on temperature, with 1-log higher concentrations of culturable cells being monitored in hot water distribution systems compared to cold water in some buildings (Bédard et al., 2018). In the Canadian province of Quebec, temperature in hot water distribution systems is regulated as follow: (1) at least 60 °C at the water heater outlet, (2) at least 55 °C in the recirculation loop, (3) less than 43 °C at the tap in healthcare and long term care facilities to prevent risk of scalding (Régie du bâtiment du Québec (RBQ), 2014).

Stagnation alters water quality, especially in building water distribution systems. However, factors controlling both microbial and chemical water quality under prolonged stagnation periods and the effectiveness of the remediation strategies proposed are still not well understood (Proctor et al., 2020). Among the multiple recommendations issued for the safe reopening of buildings after the COVID-19 pandemic shutdown, flushing and shock disinfection were the most advised corrective actions (Canadian Standard Association (CSA), 2020; Proctor et al., 2020; Régie du bâtiment du Québec (RBQ), 2020; Grimard-Conea et al., 2022). Depending on the duration of the building closure, the age and state of the plumbing system, and the water quality parameters considered (chemicals like copper or lead, or microbial), recommissioning flushing can be sufficient (American Water Works Association (AWWA), 2020; Canadian Standard Association (CSA), 2020; Proctor et al., 2020; Régie du bâtiment du Québec (RBQ), 2020). Yet, the efficiency of flushing remains site-dependent and there is still a lack of data concerning the benefits of flushing on the short- and long-term (Grimard-Conea et al., 2022). For shock chlorination, both low free chlorine concentrations (10 ppm) with prolonged contact time (up to 24 h) and high free chlorine concentrations (200 ppm) with short contact times (3 h) are recommended, but the latter can damage plumbing systems (Canadian Standard Association (CSA), 2016; Proctor et al., 2020; International Association of Plumbing and Mechanical Officials (IAPMO), 2021).

The main objective of this study was to investigate the interplay between material type, temperature and stagnation on water quality and the potential for biofilm formation, and to provide insight on the evolution of water quality and biofilm densities under various operating conditions common in building water systems. This study specifically

aimed to: (1) assess the variation of water quality and biofilm formation potential among reactors containing coupons of 6 different materials in contact with dechlorinated tap water, (2) evaluate the impact of an increase in temperature on the water quality and biofilm, and (3) monitor the evolution of water quality and biofilm formation after an extended period of stagnation (6 months) and as a result of the application of remediation measures.

2. Materials and methods

2.1. Pilot set up and operation

A pilot composed of 12 CDC Biofilm Reactors (BioSurface Technologies Corporation, USA) was operated from May 2019 to October 2020 (17 months) as described in Cazals et al. (2023). Reactors contained 600 mL of tap water dechlorinated overnight and were operated at 80 rpm in semi-batch with semi-weekly change of 75% of the water volume. In each reactor, 24 coupons (two sides with a surface of 1,27 cm² each) of a specific material were inserted in 8 polypropylene rods immersed in water (Figure S.1). The reactors associated with 4 plastics (polypropylene (PP), polyvinyl chloride (PVC), ethylene propylene diene monomer (EPDM) rubber, and cross-linked polyethylene (PEX)) and 2 metals (stainless steel (SS) and copper (Cu)) are summarised in Table 1.

After 3 months of operation (wk 14), the amoeba *Vermamoeba vermiformis* (ATCC 50237TM) was added at a final concentration of 1.5 x 10³ cells/mL. Two environmental starved strains of the bacteria *Legionella pneumophila* (Lp) (serogroup 1 (ID Q067501-02) and serogroup 5 (ID Q076826-03)) were added 6 weeks later (wk 20) in 11 out of 12 reactors (Bédard et al., 2021). Final concentrations in reactors were 1.5 x 10⁷ cells/mL (serogroup 5 strain) and 2 x 10⁷ cells/mL (serogroup 1 strain). PP_A was inoculated with only one Lp strain at a final concentration of 1.5 x 10⁷ cells/mL. It was initially planned to not have any Lp in this reactor, but we took the opportunity to evaluate the competition effect between the 2 Lp species. Both strains were selected as they were both extracted from biofilms formed in water distribution systems of two hospitals, and because of their health relevance as *L. pneumophila* serogroup 1 causes most of *Legionella* pneumonia (Yu et al., 2002). All microorganisms were cultured as described in Cazals et al. (2022) before addition into the reactors.

Two weeks after the Lp inoculation (wk 22), reactors temperature was increased except for reactor PP_A which was left at 25 °C for the entire experiment (Table 1). The temperatures were chosen to reproduce typical temperatures found in hot water distribution systems.

Two months after the temperature increase (wk 33), stagnation occurring in hot water pipes during holidays was simulated by bringing reactors to ambient temperature and stopping mixing for 4 weeks. Mixing and weekly change of water were then resumed (wk 37) but were halted again because of the COVID-19 shutdown. Thereafter, the pilot was kept stagnant at room temperature for 6 months. During stagnation periods, reactors were kept closed and the only oxygen exchanges were through small rigid tubing on reactor tops. In September 2020, the

Table 1
Characteristics of the 12 CDC Biofilm reactors.

Reactor ID	Material	<i>L. pneumophila</i>	T° after increase of the set point
PP_A	Polypropylene	1 strain	25 °C
PP_B	Polypropylene	2 strains	40 °C
PVC	Polyvinyl chloride	2 strains	40 °C
EPDM	EPDM rubber	2 strains	40 °C
SS	Stainless steel	2 strains	40 °C
PEX	Cross-linked Polyethylene	2 strains	40 °C
Cu_A	Copper	2 strains	40 °C
Cu_B	Copper	2 strains	55 °C
Cu_C	Copper	2 strains	60 °C

reactors were sampled again, 72 weeks from initial pilot start up. Then, reactors were rinsed twice with dechlorinated tap water, with a 5 min agitation at 80 rpm between each rinsing to simulate a building water distribution system flushing event, as recommended by the Canadian Standard Association after a stagnation of at least 2 weeks (Canadian Standard Association (CSA), 2016). The reactors were left at 80 rpm mixing without water change for two weeks and were sampled again. Shock chlorination was simulated by filling the reactors with a 20-ppm chlorine solution for 24 h without mixing. The reactors were then emptied, refilled with dechlorinated tap water at 80 rpm and left without water changes. The last samples were collected 2 weeks after the shock chlorination. A schematic of the timeline of the pilot's operation is given in Figure S.2.

Water samples were collected in sterile polypropylene bottles. For each biofilm sampling, 3 coupons were removed from a single rod, and each coupon was immersed in 2 mL of sterile 0.2 μm -filtered ultrapure water. The coupons were then manually scraped on both sides with sterile inoculation loops for 1 min, then vortexed for 1 min, and finally sonicated for 1 min in a sonication bath. The volumes recovered from the 3 coupons were pooled into a single sample (final volume of 6 mL), equivalent to a 7,62 cm^2 surface.

2.2. Analytical methods

General physicochemical parameters - Once a week, pH, conductivity, turbidity, and dissolved oxygen concentrations were measured at the time of water change. pH was measured using the Hach 186 Multi-parameter HQ40d and the PHC301 probe (Hach, USA), conductivity was assessed with a ECTestr Low conductivity meter (ThermoScientific, USA), turbidity was measured using Hach TL2300 turbidimeter (Hach, USA), and dissolved oxygen concentration was measured with the Hach 186 Multi-parameter HQ40d and the 187LDO101 probe (Hach, USA). Temperature in each reactor was monitored with a resistance temperature detector (RTD) probe provided with the reactors.

Dissolved organic carbon (DOC) was determined following the method of the American Public Health Association (American Public Health Association (APHA) et al., 2012) and its biodegradable fraction (BDOC) was measured using a previously published method (Servais et al., 1989). Concentrations of calcium (Ca), chromium (Cr), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), lead (Pb) and zinc (Zn) were measured using a PerkinElmer NexION 300x mass spectrometer (PerkinElmer, USA).

Scanning Electron Microscope (SEM) pictures - A JSM-7600F Schottky Field Emission Scanning Electron Microscope (JEOL, Japan) was used to characterize the surface of coupons from each material before and 3 months after the launch of the pilot. Specimens were sputter coated with gold and double-sided conductive tape was used to maintain them on a standard specimen holder. Their elementary composition was estimated using energy-dispersive X-ray spectroscopy.

Flow cytometry - Total and viable bacterial densities were estimated by flow cytometry with a BD Accuri™ C6 flow cytometer until September 2020, and a BD Accuri™ C6 Plus flow cytometer after (BD Biosciences, USA). Staining with SYBRGreen I (SG, 100x concentrated) or a mix of SYBRGreen I and Propidium Iodide (SGPI, final concentration of 6 μM of propidium iodide) was used, and samples were analysed according to the protocol published by Gatza et al. (2013).

2.3. Statistical analysis

Using RStudio version 4.2.2, a Mann-Whitney *U* test was conducted in pairs on the copper concentrations measured in copper reactors before the increase of temperature to assess if they were significantly different.

All data from water samples and concentrations of microorganisms in biofilm samples were standardized and principal component analysis

(PCA) was applied to identify the main factors responsible for variations in water quality and biofilm microbial composition using FactoMineR and factoextra R packages.

3. Results

3.1. General water quality parameters

3.1.1. Metal concentrations

Concentrations of copper and lead were measured in water and biofilm samples (Fig. 1).

Reactors with plastic or stainless-steel coupons showed an average bulk copper concentration ($492 \pm 191 \mu\text{g/L}$) almost twice as low as in the feed water ($735 \pm 208 \mu\text{g/L}$) (Fig. 1). Contrarily, average copper concentrations were 30% higher than feed water before temperature increase in copper reactors ($905 \pm 135 \mu\text{g/L}$). During operation at room temperature, bulk copper concentrations in copper reactors were not significantly different ($p > 0.05$). Fig. 2 shows the evolution of the dynamics on partitioning of Cu between the bulk and solid phases in copper reactors.

Heating reactors with copper coupons to 40 °C, 55 °C and 60 °C decreased copper concentrations in water by factors of 1.6 X to 5 X but resulted in major increases (between 1.8 X and 3.8 X) of accumulated Cu onto the biofilm coupons (Fig. 2a). Moreover, visible precipitation was noted on the glass reactor outer vessels at 55 °C and 60 °C (Fig. 2b) and the surface of the coupons changed to blackish colour (Fig. 2c), typical of tenorite formed at the pH, alkalinity and temperatures found in this experiment. Bulk copper concentrations in copper reactors doubled after the 4-week stagnation at room temperature while shock chlorination led to a 50% reduction of biofilms copper concentrations.

While the lowest copper concentrations in water were measured in the EPDM reactor, it harboured the highest average biofilm copper concentration ($145 \pm 73 \mu\text{g/L}$) amongst the non-copper reactors (Fig. 1). EPDM was also a significant source of lead in water, with an average concentration of $5.12 \pm 4.22 \mu\text{g/L}$ (Fig. 1). For instance, the highest average concentration of Pb in biofilm from plastic reactors was measured in EPDM biofilms ($1.49 \pm 1.08 \mu\text{g/L}$ compared to an average of $0.67 \pm 0.16 \mu\text{g/L}$ in other plastic biofilms). The lowest Pb concentrations in water were measured in copper reactors, with an average concentration of $0.94 \pm 0.19 \mu\text{g/L}$ compared to $2.07 \pm 1.70 \mu\text{g/L}$ in plastic reactors (Fig. 1). Reactors with the lowest concentrations of Pb in water (the copper ones) were the ones with the highest concentrations in the biofilms (Fig. 1). Pb concentrations in copper biofilms increased by 2-fold when heated compared to when reactors were set at room temperature.

3.1.2. Dissolved organic carbon (DOC) and biodegradable organic carbon (BDOC)

DOC and BDOC concentrations were measured in all the reactors and the feed water (Fig. 3).

DOC and BDOC concentrations in feed water were stable, with average values of $1.89 \pm 0.23 \text{ mg/L}$ and $0.12 \pm 0.05 \text{ mg/L}$. In general, addition of microorganisms in reactors was linked to the decrease of DOC and BDOC. Maximal DOC and BDOC concentrations were measured in all reactors after the increase of temperature. During the heating phase, DOC and BDOC concentrations higher than those of most of plastic reactors were assessed in copper reactors, especially at 55 °C and 60 °C. BDOC concentrations increased by at least 3-fold in copper reactors after heating to 55 °C and 60 °C. EPDM, Cu_B and Cu_C reactors displayed a reduction of DOC after the 4-week stagnation. After the 6-month stagnation, the BDOC concentrations measured were at least 2.7-fold lower in all the reactors except PP_A, which remained stable throughout the study. Finally, shock chlorination led to an increase of BDOC concentrations in all reactors, especially in copper reactors where they were at least multiplied by 3.

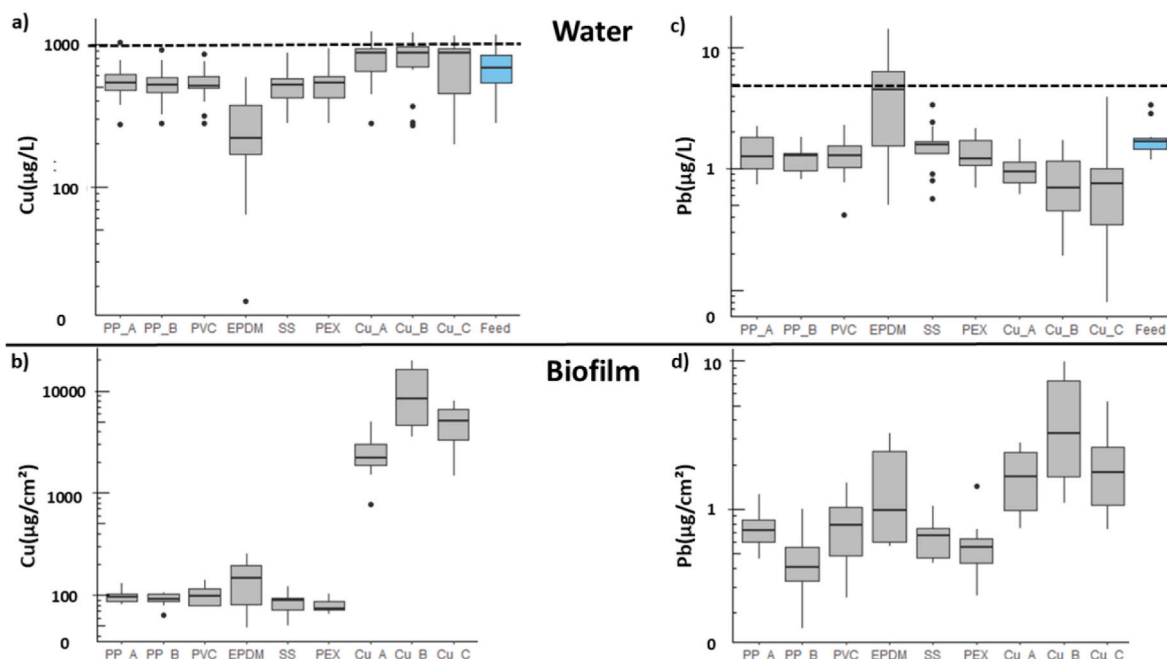


Fig. 1. Concentrations of copper and lead in water (a and c respectively) and biofilm samples (b and d respectively). The dotted lines represent the limits of 1 mg/L for copper (a) and 5 µg/L for lead (c) as recommended by Health Canada (Health Canada, 2018, 2019). Mean result is presented for duplicates of copper reactors at the same temperature (detailed reactors description in Table 1). The feed water is presented in blue color.

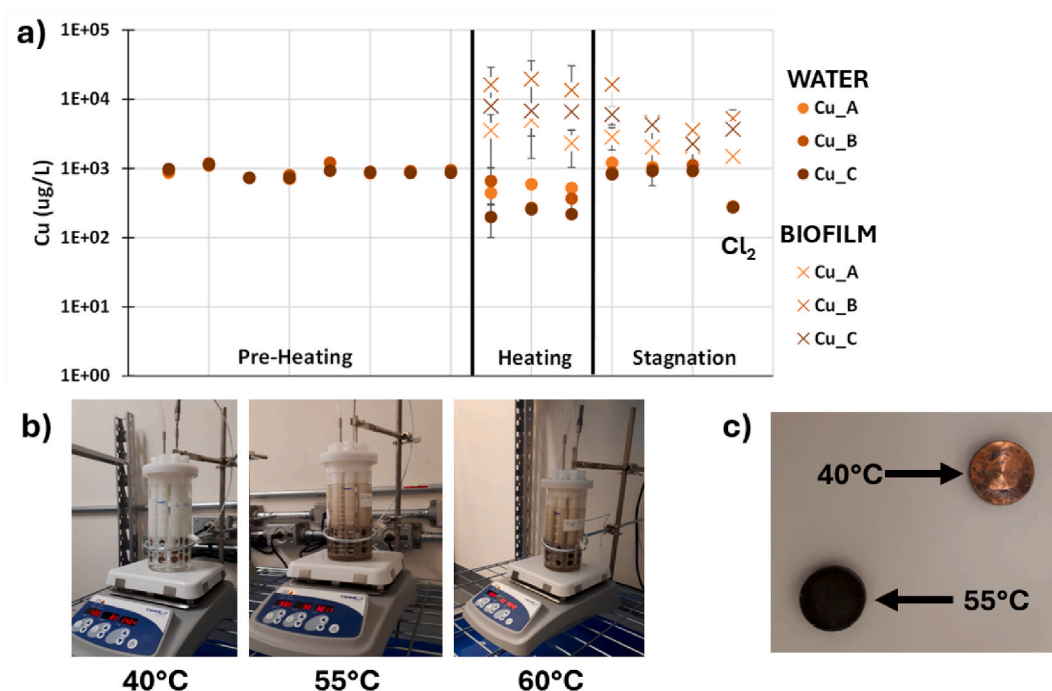


Fig. 2. Temporal evolution of copper concentrations in water and biofilms of copper reactors (a) and pictures of the reactors (b) and coupons (c) after heating. Cu_A: mean value of the 2 reactors heated at 40 °C, Cu_B: mean value of the 2 reactors heated at 55 °C, Cu_C: mean value of the 2 reactors heated at 60 °C.

3.2. Biological parameters

3.2.1. Biofilm structures

SEM pictures of the coupons were taken before the pilot start up and after 3 months of inoculation with feed water (Fig. 4).

New EPDM coupons showed a preferential structure for biofilm development, with many pores available for attachment and development of bacteria. New PVC coupons also presented crevices where

bacteria could adhere, while new PEX coupons presented a grainy structure. Interestingly, new PP coupons seemed to already have some biomass or a smooth substance on them, while regular surfaces were observed on metallic coupons. After 3 months of biofilm development, differences in biofilm's coverage and structure were noticed among the materials. Scattered biofilms with a thin layer of extracellular polymeric substances (EPS) were observed on PVC and PEX while more complex and dense biofilms were observed on EPDM and PP, the thickest biofilm

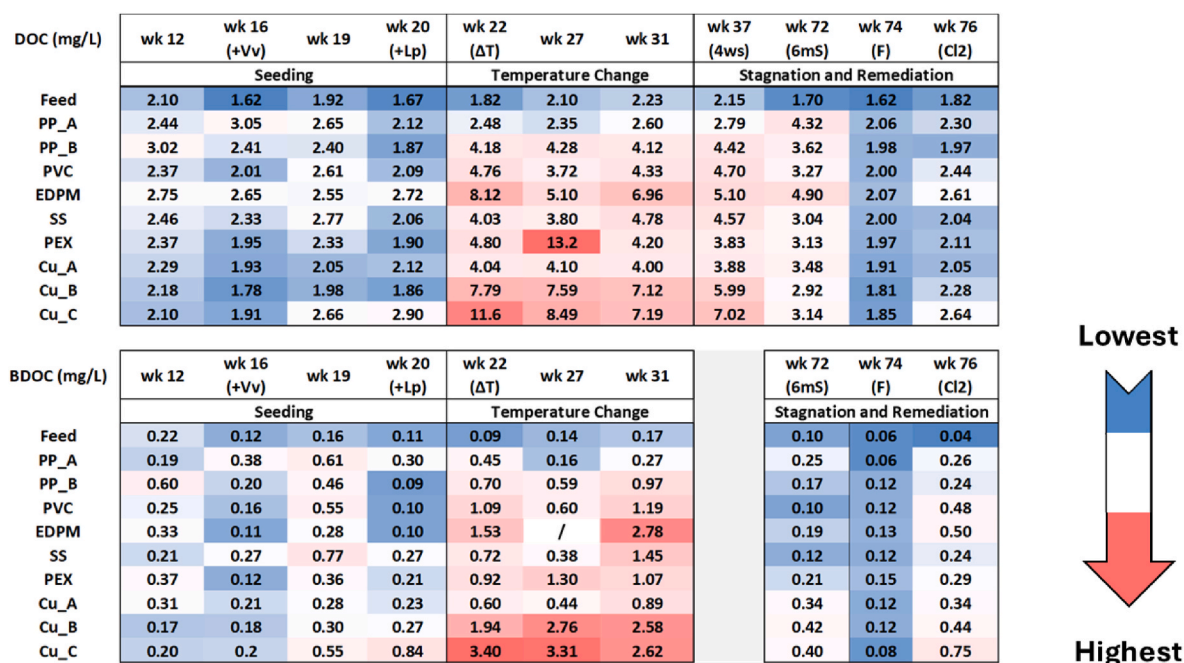


Fig. 3. Heatmaps of the concentrations of dissolved organic carbon (upper) and biodegradable dissolved organic carbon (lower) in water of all reactors and feed water. Lowest values are in blue while higher ones are in red. Samples were collected three days after interventions (+Lp, +Vv and ΔT) and 2 weeks after flushing (F) and chlorination (Cl₂).

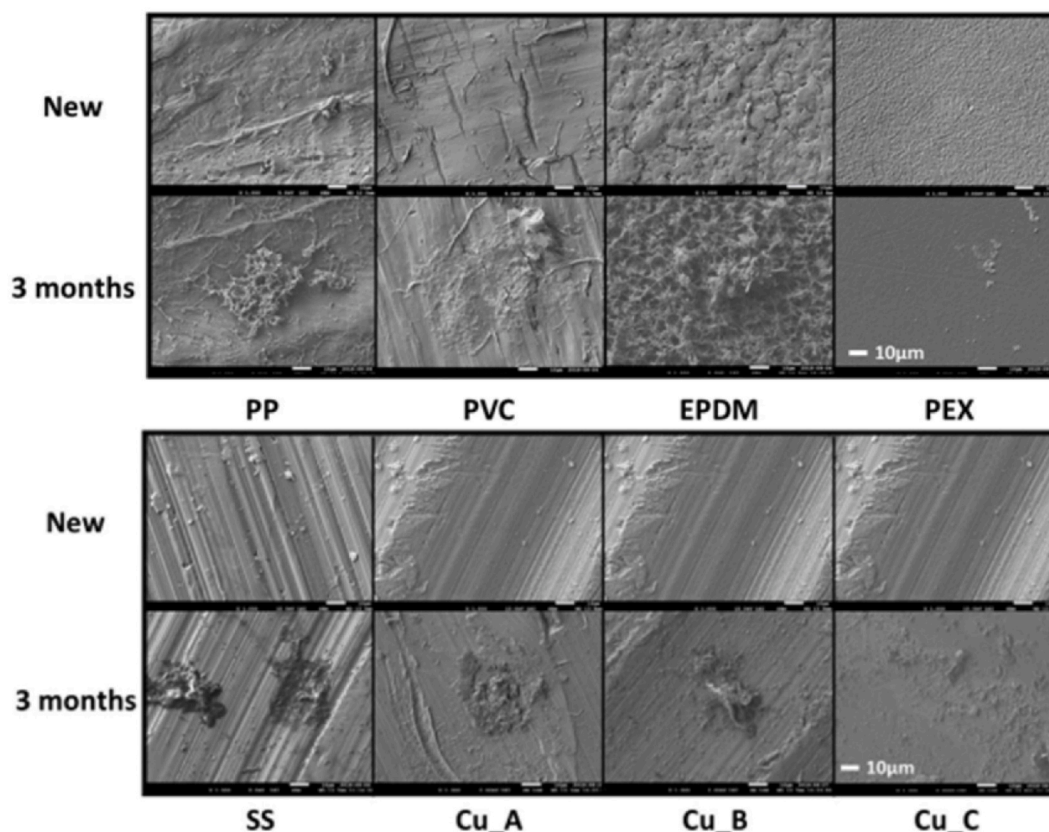


Fig. 4. Pictures of coupons used in the pilot-scale study before (upper lines) and 3 months after the launch of the pilot (lower lines) taken with a scanning electron microscope (SEM), 1,000X.

being observed on EPDM coupons. Dispersed microcolonies were observed with some scattered biofilms on SS and copper coupons.

3.2.2. Bacterial densities

Total and viable bacterial populations in water and biofilm samples were estimated using flow cytometry (Fig. 5).

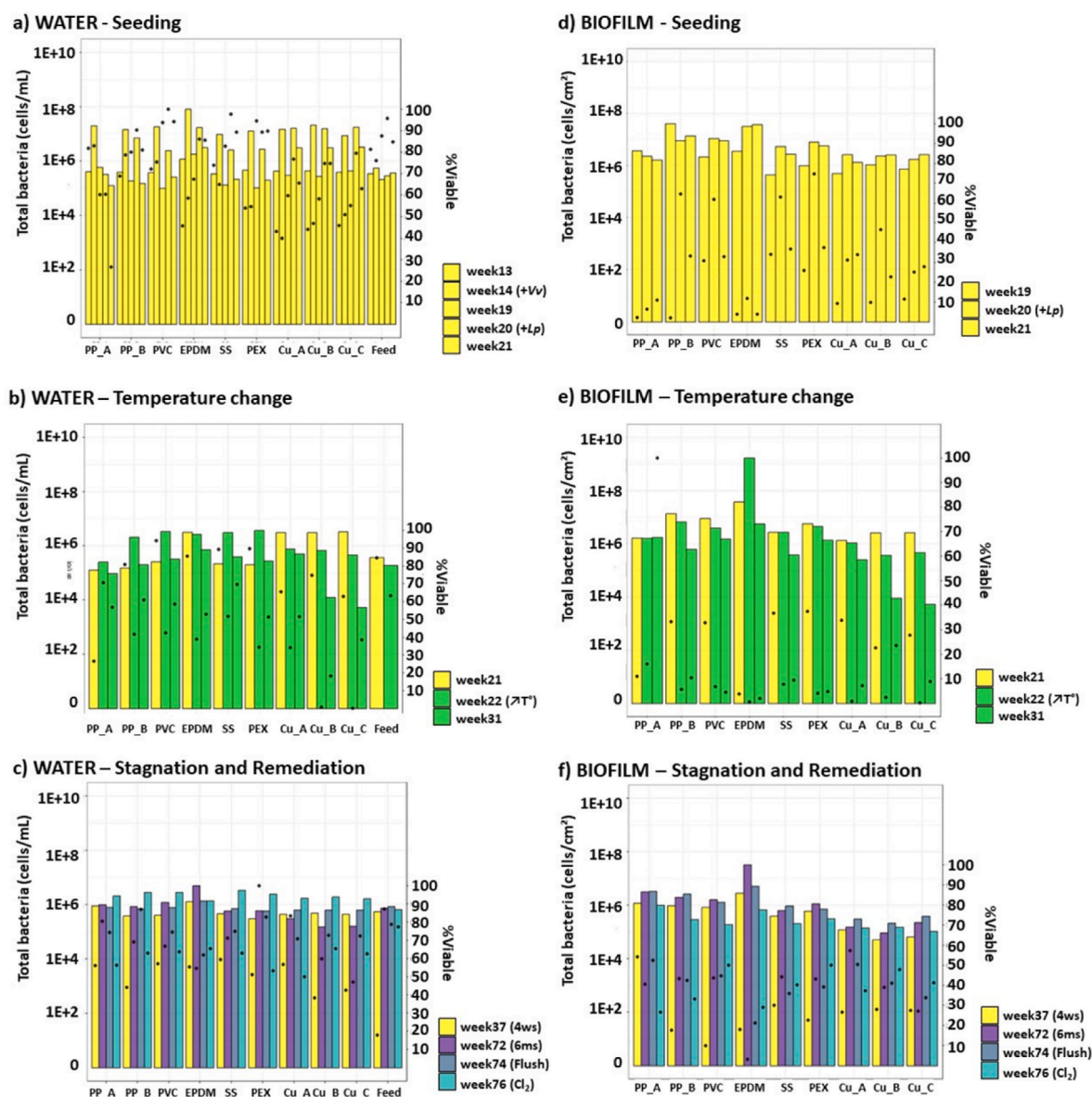


Fig. 5. Temporal evolutions of the concentrations of total cells (bars) and the percentage of viable cells (black dots) measured by flow cytometry in water (a, b and c) and biofilms (d, e and f) of all the reactors. Yellow for water at room temperature, green (b and e) for when water temperature is increased, purple for after the 6-month stagnation (c and f). Blue and cyan are for samples taken 2 weeks after flushing and 2 weeks after chlorination (c and f). Cu_A reactors were heated at 40 °C, Cu_B at 55 °C and Cu_C at 60 °C during the heating phase.

Overall, total cell concentrations were similar in both water and biofilm samples, but the percentage of intact cells was lower in biofilms than in water. Total bacterial populations in biofilms increased by at least 0.5-log over the seeding period, except in the PP_A and PP_B reactors. The studied materials were ranked according to their biofilm density at the week 21 (Fig. 5d) and considering SEM observations (Fig. 4): EPDM > PP > PVC > PEX > Cu - SS.

Interestingly, during the first week of heating, a 1-log increase in total bacterial densities was noted in water of all reactors except the EPDM and Cu ones (Fig. 5b). On the opposite, a drop of the proportion of intact cells was noticed in water and biofilm samples of all reactors on the first week of heating, especially in copper reactors. After 2 months of heating, a 1-log decrease of total bacterial populations was observed in all reactors except copper reactors set at 55 °C and above, where a larger decrease (2-log) was noted (Fig. 5b). In biofilms, total bacteria decreased by at least 0.5-log after 2 months of heating (Fig. 5e). The greatest decrease (2.5-log) was noticed in copper reactors set at 55 °C and 60 °C. Again, an opposite trend was observed for the percentage of

intact cells, increasing after several weeks of heating (Fig. 5b and e).

After the 6-month stagnation, the total cell densities decreased by 50% in water from copper reactors while they increased by 2-fold in average in the other reactors (Fig. 5c). A slight increase of the total cells was also noted in biofilms after the 6-month stagnation (Fig. 5f). The 4-week and 6-month stagnation periods led to an increase of at least 30% of the percentage of intact cells in water of all the reactors except EPDM (Fig. 5c). The same trend was noticed in biofilms except on the PP_A and EPDM coupons where the percentage decreased by 15% (Fig. 5f).

A 0.5-log increase of the total bacterial population in water was noted 2 weeks after shock chlorination and was related to a leaching of bacteria from biofilms as the total cells densities slightly decreased in biofilms (Fig. 5c and f). After the shock chlorination, a decrease of the percentage of intact cells was noticed in all the water samples except in the reactor with EPDM coupons. Interestingly, an increase of the fraction of intact cells was observed in all the biofilms during that same time, except the ones on PP coupons and on copper coupons set at 40 °C.

3.3. PCA analyses

The PCA Biplot in Fig. 6 shows that water samples are clustered by condition/week, suggesting that the environmental conditions affecting the water system impact more the water quality than the materials themselves. It appears that 5 weeks after inoculation of *V. vermiformis* in the reactors, water quality was close to its initial state, as well as after the 6-month stagnation and the simulated flushing (Fig. 6). DOC and BDOC are closely correlated with *L. pneumophila* concentrations measured by both culture and qPCR, while total and intact cells are more sensitive to metals concentrations (Fe, Al, Zn and Ca). Finally, Cu and Pb concentrations appear to be inversely correlated.

PCA biplot in Fig. 6 shows that characteristics of biofilms are less clustered by condition/week than parameters of bulk water quality as compared to Fig. 6, but total and intact cells densities measured by flow cytometry are still closely correlated.

4. General discussion

4.1. Biofilm formation potential

DWDS are complex systems with a large diversity of materials which directly impact water quality. Both plastic and metallic materials were colonized, with a biofilm density and extent dependent on the material type, as previously reported (Waines et al., 2011). The observed dense biofilm on EPDM agrees with the rapid full colonization (indicated by adenosine triphosphate (ATP)) and complex biofilm structure of EPDM plumbing previously observed after 28 and 84 days (Waines et al., 2011). SEM observations (Fig. 4) highlight differences of biofilm structure on colonized materials. For PVC, the presence of crevices (Fig. 4) suggests a surface favourable for biofilm adherence compared to PEX and SS surfaces. The colonization observed corresponds to the 10^7 cells/cm² reported by Neu et al. (2019) for 12-month old biofilms on PVC-P shower hose fed by tap water. Although a similar potential was observed for PVC and PEX in this study, major differences have been reported for a same material depending on the manufacturer, especially for PVC (Proctor et al., 2016), which could lead to a change in ranking order between those two materials. As observed on our coupons, the development of a thin EPS layer was described for 84-day old biofilms on PEX (Waines et al., 2011). For metallic materials, our observations of scattered biofilms on SS and copper coupons (Fig. 4) concur with previous findings (Waines et al., 2011). Observations made in the present study are typical of young biofilms composed of individual cells and empty areas, except on EPDM and PP (Martiny et al., 2003). Formation of different biofilm structures depending on materials can then be linked to preferential adherence of opportunistic pathogens in these biofilms and their release in water systems (Lee et al., 2021; Cazals et al., 2023). Biofilms can also cause undesirable changes in water colour, odour and taste (Prest et al., 2016).

Because nature and availability of nutrients influence bacterial growth, understanding their source is crucial to evaluate the risk of bacterial amplification in the water system according to the nature and the sequence of materials used (Prest et al., 2016). Here, concentrations of DOC and BDOC in bulk water were closely correlated to *L. pneumophila* concentrations (Fig. 6). When considering total and biodegradable dissolved organic carbon present in water, two sources should be considered: 1) initial and sustained release from the material itself and 2) release from accumulated organic matter and biofilm. Materials used in drinking water systems can leach organic and inorganic components in water, especially new plastic pipes, mainly because of the stabilizers, plasticizers and antioxidants they contain (Stern and Lagos, 2008). This release, either short term (14 days (Neu et al., 2020)), or long term (8 months (Proctor et al., 2016)), leads to higher concentrations of biodegradable carbon and increased bulk and biofilm cell densities. PEX pipes are particularly prone to leaching total (TOC), dissolved (DOC), and assimilable (AOC) organic carbon in the early days

of utilization (Lund et al., 2011; Connell et al., 2016).

In this study, similar DOC and BDOC concentrations were measured in all reactors after 12 weeks compared to feed water (Fig. 3). The minimal leaching observed is attributed to the low surface to volume ratio (0.1 cm^{-1}) per coupon material in the CDC reactors and the progressive elimination of the initial migration of DOC/BDOC as observed by Neu et al. (2020) with the weekly water changes. The release from accumulated organic matter and biofilm from the surface is believed to be the primary source of the major increases in DOC and BDOC concentrations observed for all materials, especially metals, after heating. This hypothesis is confirmed by the highest concentrations released in copper reactors, where the bulk of DOC and BDOC does not originate from the metallic material. Interestingly, the largest DOC migration corresponds to the largest biofilm loss (Fig. 5). The decrease in DOC and BDOC observed after the addition of high concentrations of microorganisms on weeks 14 and 20 (Fig. 3) is likely due to the carbon consumption for microorganisms' cellular multiplication, as previously described in water distribution systems in China (Liu et al., 2002).

4.2. Concentrations of metals

Varying dynamics in lead and copper concentrations between water and biofilm were observed reflecting the sequence of precipitation/accumulation and release driven by temperature and stagnation (Fig. 1).

An important release of Pb was noticed in the EPDM reactor, exceeding Canadian drinking water guidance level of 5 µg/L (Health Canada, 2019). This suggests the presence of Pb in this brand of EPDM, possibly a stabilizer similar to those used in old unplasticized polyvinyl chloride (uPVC) pipes (Al-Malack, 2001). As EPDM exact composition can vary from one manufacturer to another, not all EPDM rubbers may release lead in water, but our results highlight a potential risk. This also concurs with previously reported lead accumulation on the surface of plastic pipes (Salehi et al., 2017).

Contrastingly, lower Pb concentrations were measured in water with copper reactors, while significant accumulation of Pb concentrations were measured on copper coupon biofilms (Fig. 1). Increasing temperature above 55 °C decreased bulk water concentrations (55 °C (−43%) and 60 °C (−80%)) and increased the concentration on the copper coupons. We hypothesize that precipitation, co-precipitation and biofilm entrapment of Pb occur on copper coupons (Belcher and Zhang, 2018). It raises the possible issues of increases of Pb in water resulting from: (1) detachment from the biofilm and surface into the water during transient events, such as a flowrate increase (flushing) causing a resuspension of the biofilm or particulate; (2) desorption from the surface/biofilm resulting from reduced concentrations in water. Indeed, flushing resulted in small increases in Pb concentrations in almost all reactors after two weeks, for example the SS reactor exceeded incoming water concentrations by a factor 2.8 X. Pb concentrations remained stable despite prolonged stagnation, in contrast with other studies where stagnation increased Pb leaching (Inkinen et al., 2014).

A decrease of copper concentrations was noticed in all reactors water after the 6-month stagnation, except for copper reactors previously heated to 55 °C and 60 °C (Cu_B and Cu_C). This concurs with the previously reported high copper leaching during the first 10 h of stagnation and the subsequent decrease of Cu concentrations over time (Merkel et al., 2002). Among all the non-copper reactors, the highest average copper concentrations were measured in EPDM biofilms, possibly due to the accumulation of copper on the plastic (Salehi et al., 2017) and the binding and sequestering of copper ions by extracellular polymeric substances (Beech and Sunner, 2004). Overall, copper concentrations in water remained under the maximum acceptable value of 2 mg/L for all reactors, but sometimes exceeded the aesthetic objective of 1 mg/L as defined by Health Canada (Health Canada, 2018).

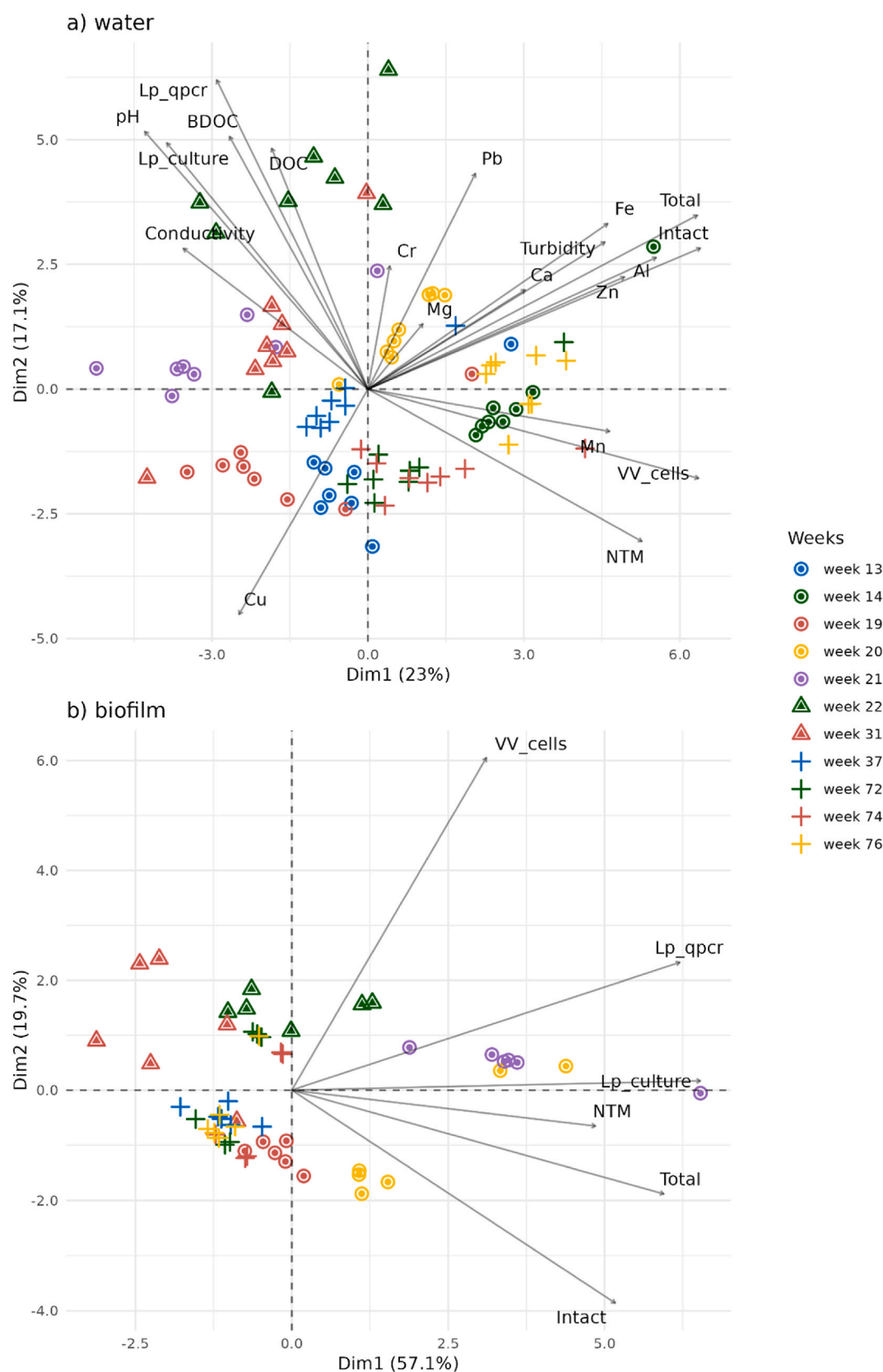


Fig. 6. PCA Biplot with data from all water samples except Cu reactors heated at 55 °C and 60 °C (a) and with data from all biofilm samples from Cu reactors (b). Lp_qpcr represents the concentrations of *L. pneumophila* estimated by qPCR, Lp_culture the concentration of *L. pneumophila* measured by enzymatic culture test, and VV_cells the concentrations of the amoeba *Vermamoeba vermiformis* in cell equivalents, and NTM the concentrations of non-tuberculous mycobacteria measured by qPCR (data presented in Cazals et al. (2023)).

4.3. Impact of stagnation on microbial densities

Stagnation impacted the release and use of DOC/BDOC, affecting intact and total cells in bulk and solid phases. Substrate utilization can be estimated by considering the changes of DOC in reference to the initial BDOC concentrations. Utilization of substrate will depend on both the lability of the available carbon and the ability of the intact bacteria to use it. As an example, the EPDM, Cu_B and Cu_C reactors displayed a consumption of DOC after the 4-week stagnation, suggesting high carbon lability. This is supported by the high concentration of BDOC measured in those reactors, above 2.5 mg/L at the onset of stagnation. Partial utilization of the available carbon resulted in the highest increases in intact cells in those reactors (Figs. 3 and 5). Fixed and suspended biomass were exposed to significant levels of organic carbon during the following 6-month stagnation. Indeed, concentrations of DOC and BDOC measured in all reactors except PP_A after 6 months of stagnation suggest depletion of the available carbon substrates during the extended stagnation (Fig. 3). Kinetics differences of carbon utilization (Figs. 3 and 5) may reflect differences in the nature of the labile carbon sources: in copper reactors, the main source is most likely the dead biomass, while in the EPDM, the material is a source of carbon of unknown lability after heating. As a result, viable percentages remained relatively constant in water from the EPDM reactor despite an observed increase in total cells. The peak of carbon may have occurred early on during stagnation, leading to depletion of nutrients before the end of stagnation and limited growth (Proctor et al., 2020). This is not observed for short-term stagnation, as total bacteria increase was detected in the water after the 4-week stagnation at room temperature.

Carbon usage also led to an increase in total bacterial populations and percentage of intact cells in the water of other plastic reactors. BDOC concentrations elevated by heating provided an initial BDOC stock sufficient for bacteria to remain viable during a prolonged 6-month stagnation. These findings acknowledge a deterioration of the microbial water quality after extended periods of stagnation (4 weeks and 6 months), agreeing with studies correlating stagnation times with increased total cells densities and intact cells proportions (Lipphaus et al., 2014).

4.4. Impact of flushing and chlorination on microbial densities

Differences were noted between reactors two weeks after the simulated flushing. In general, DOC and BDOC concentrations decreased, directly reflecting feed water levels (Fig. 3). Concentrations of total cells increased or decreased slightly depending on the material (Fig. 5c). Flushing sometimes promotes the washout of high concentrations of chemicals or microbes that were present in the stagnant water (Lehtola et al., 2006). Renewal in nutrients and dissolved oxygen could explain the slight increase in intact cells percentage that was measured in almost all the reactors (water and biofilm) two weeks after flushing. Flushing can also lead to a short-term reduction in microbial densities, but renewed nutrients and oxygen brought by flushing may promote cell recovery after a few days of stagnation (Grimard-Conea et al., 2022). In addition, small changes in total bacterial densities were also noted in biofilms 2 weeks after flushing for all reactors except PEX and EPDM (Fig. 5f). These results suggest that flushing is not efficient to eliminate biofilm as previously mentioned (Proctor et al., 2020), and should not be followed by further stagnation.

During shock chlorination, chlorine decay measured after 24 h in the EPDM reactor was much faster than in other reactors, suggesting that EPDM is reactive to chlorine or that the denser biofilm formed on these coupons consumed chlorine. Copper coupons had the next highest chlorine consumption, faster than plastic materials other than EPDM, which concurs with previous studies (Raetz, 2010). Copper concentrations in copper biofilms were reduced by 50% after the shock chlorination. This is expected as chlorine will react with copper scales and copper accumulated in the extracellular polymers of the biofilm. Most

importantly, a drastic BDOC concentration increase by 2- to 10-fold was measured in all reactors 2 weeks after the shock chlorination, despite rinsing the reactors prior to sampling. The largest releases of DOC were observed for the PVC, EPDM and Cu_C reactors, which aligns with a previous study reporting significantly higher TOC migrations in chlorinated EPDM and PEX compared to non-chlorinated ones (Mao et al., 2018). The large increase of BDOC concentrations after chlorination in all reactors may be linked to the release of dead oxidized cells from the surface biofilms. This highlights the need to rinse thoroughly a system after chlorination to evacuate all the dead cells that can serve as nutrients and could promote microbial growth. After chlorination, total cell concentrations clearly increased in bulk water of all reactors (except EPDM) while they decreased in all biofilms, showing a transfer of the bacteria from biofilms to bulk water. The decrease of intact cell percentages in water after chlorination also agrees with the previous report of a rapid decrease of intact cells after a shock chlorination (Besmer et al., 2017). In contrary, intact cell percentages increased in all biofilms except on EPDM coupons, suggesting that viable cells preferably remain in the biofilm after injured and dead cells are released and evacuated in bulk water.

4.5. Impact of temperature on microbial densities

Temperature greatly influences bacteria growth potential in water systems (Prest et al., 2016). After a week of heating at 40 °C, densities of total cells increased in water of all non-copper and EPDM reactors while the percentage of intact cells decreased, suggesting the release of dead cells in the water. In the copper and EPDM reactors, bulk densities of total cells decreased. In biofilm, all cell densities and viability decreased, again except for the EPDM reactor. This concurs with a previous study reporting a decrease in culturability and ATP viability in the biofilms of a hot water system compared to a cold water one (Inkinen et al., 2014). A most striking observation is the marked and sustained DOC and BDOC increases in all the reactors set at 40 °C by a factor of up to 2.6 X and 27.8 X respectively during the 10 weeks of heating, and even more in copper reactors at 55 °C and 60 °C (min 2.5 X and max 9.5 X). The sustained increases were observed despite the regular semi-weekly water changes. These results agree with previous studies showing an increase of assimilable organic carbon (AOC) and total organic carbon (TOC) concentrations with higher temperatures of 55–60 °C (Hametner, 1999; Inkinen et al., 2014). DOC and BDOC concentrations intensified with increasing temperatures from 40 °C to 60 °C with fold changes of up to 7.5 X in BDOC and 2.7 X in DOC in heated copper reactors. The high release of DOC from copper pipes and coupons in hot water suggest that heating periodically and at suboptimal temperature can generate substantial amounts of nutrients that supply surviving microorganisms or downstream microbiomes. Indeed, minerals and microbes released from copper strongly impacted the development of biofilm and the presence of *L. pneumophila* on downstream non-copper materials (Lu et al., 2014). The nutrients produced during heating can also be consumed by microorganisms if temperature decreases or during stagnation periods.

Regardless of elevated BDOC in Cu reactors heated to 55–60 °C, maintaining the temperature over time prevents bacterial increases and depresses viability (Figs. 3 and 5b and S.3), in agreement with the demonstrated efficacy of thermal control in large buildings (Bédard et al., 2015, 2016). If heating is not applied continuously (thermal shocks vs. thermal regime), it is critical to thoroughly rinse the system after the thermal shock, to flush all the nutrients released by the materials and biofilms during heating. Stagnation of the water at lower temperature after a heat treatment without efficient flushing may lead to rapid microbial regrowth, eliminating the benefits of shock thermal treatment, as these conditions promote the necrotrophic growth of opportunistic pathogens (Temmerman et al., 2006).

4.6. Recommendations

Our results underline the importance of not only considering the cost of materials for building water distribution systems but also their potential impact on water quality. For instance, the release of metals and other internal components of materials at the start of use or after changes of environmental conditions must be considered. As every material has its own advantages and disadvantages, and different parts of the same system can be made of different materials, a register listing all materials must be kept up to date. This will help building managers to identify hazards and hazardous events and define adequate control measures in water safety plans.

Intermittent heating and shock chlorination have been linked to important release of nutrients by materials and biofilms in our study, highlighting the importance of a thorough rinse and the prevention of stagnation in water systems after such events to prevent microbial regrowth.

5. Conclusions

- All materials were colonized by bacteria after 14 weeks at 25 °C, but density and structure of biofilms were impacted by material type: EPDM > PP > PVC > PEX > Cu – SS
- EPDM leached up to 14 µg/L of Pb in water while EPDM biofilms accumulated up to 256 µg/cm² of Cu. Although EPDM is a plastic material, it should be used with caution in water systems harbouring high concentrations of copper.
- Precipitation, co-precipitation, and biofilm entrapment of Pb occurred on copper coupons with Pb concentrations as high as 9.8 µg/cm². Such dynamics can result in release of Pb in water either from leaching back into lower concentration water or through biofilm sloughing.
- All materials leached substantial concentrations of DOC and BDOC when heated at 40 °C; increasing temperature to 55 °C and 60 °C increased this release by up to 4.2 X and 7.2 X-fold respectively. It highlights the importance to thoroughly flush a system after periodic curative heating to prevent microbial regrowth once the temperature is lowered.
- High availability of nutrients due to heating which occurred prior to a 6-month stagnation at room temperature led to an increase of total bacterial densities and viability.
- Shock chlorination led to a transfer of dead and injured cells from biofilms to the liquid phase and BDOC concentrations increased by up to 10 X-fold. Again, thorough flushing must be carried out to evacuate nutrients and cells to prevent microbial growth.

CRedit authorship contribution statement

Margot Cazals: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Emilie Bédard:** Writing – review & editing, Supervision, Project administration, Methodology, Conceptualization. **Juan Francisco Guerra Maldonado:** Visualization, Data curation. **Michèle Prévost:** Writing – review & editing, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.chemosphere.2025.144248>.

Data availability

Data will be made available on request.

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