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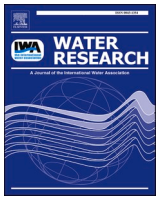

Assessing the health risks associated with the usage of water-atomization shower systems in buildings

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ABSTRACT

In the context of climate change policies, buildings must implement solutions to reduce energy and water consumption. One such solution is showering with water atomization showerheads, which can significantly reduce water and energy usage. However, the lack of risk assessment for users' health has hindered the widespread adoption of this technology. To address this gap, we assess the risk of spreading bacteria, in particular the pathogenic bacterium *Legionella pneumophila*, from shower hose biofilms of different ages grown under controlled or uncontrolled conditions considering different levels of water hardness, during showering using water atomization showerheads (ECO) or continuous flow showerheads (STA). We compared the aerosol and bioaerosol emission – total, viable and cultivable – during a 10 min shower event between the two shower systems. We showed that the water-atomization showerhead emitted slightly more nanoparticles smaller than 0.45 μm and slightly fewer particles larger than 0.5 μm than the continuous flow showerhead. Additionally, ECO showerheads emitted fewer cultivable bacteria than STA, regardless of the biofilm's age or growth conditions. When *Legionella pneumophila* was detected in biofilms, ECO showerheads released slightly less cultivable *Legionella* in the first flush of shower water compared to the STA, ranging from 6.0×10^2 to 1.6×10^4 CFU·L^{-1}. However, cultivable *L. pneumophila* was not detected in the aerosols emitted during showering with either showerhead. These findings suggest that emerging water-drop emission technologies might affect human exposure to aerosols differently than traditional systems, emphasizing the importance of assessing the health risks associated with any new shower system. Additionally, these findings provide valuable insights for achieving a balance between water and energy conservation.

1. Introduction

To meet basic human needs, an adequate supply of water is required not only for drinking and cooking, but also for personal and domestic hygiene, including handwashing, bathing, and laundry, in order to protect public health. In low- and middle-income countries, significant efforts have been made in the past two decades to provide access to sufficient quantities of safe drinking water. However, these efforts need to continue to ensure universal access to an adequate water supply for personal hygiene. In contrast, most high-income countries still use excessive amounts of water for personal and domestic hygiene, and they must reduce household water consumption levels in anticipation of decreasing water availability. Although water-efficient household appliances have been implemented for washing machines and toilets (Rhoads et al., 2015), further efforts can be made to reduce water usage per capita during showering. Indeed, in most high-income countries, it is common for individuals to shower daily, despite the fact that the frequency of showering should be based on local climate, weather conditions, and physical activity levels (Chenoweth et al., 2016). To tackle the challenge of reducing water consumption in high-income countries while preserving user habits, and to offer solutions for low – and middle-income countries, several alternative shower technologies have been brought to the market in recent years. One such technology involves water atomization, which generates a fine mist of small water droplets (Panao and Delgado, 2014). This technology has allowed for a drastic decrease in water flow rate, from 9 L·min^{-1} for traditional showerheads based on continuous flow, to 5 L·min^{-1} or even 3 L·min^{-1} for those using water atomization (Niculita-Hirzel et al., 2021). However, generating smaller water droplets through water atomization may increase the risk of exposure to pathogenic bioaerosols, such as *Legionella pneumophila*, which can be associated with water contamination.

Inhalation of *L. pneumophila*, a frequent contaminant of water pipes,

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can cause serious, life-threatening pneumonia, known as "Legionnaires' disease", which often requires hospitalization (ECDC, 2020; Barskey et al., 2020). Despite antibiotic treatment, the disease can be fatal for 5% to 10% of patients. The incidence of Legionnaires' disease has been continuously increasing since 1996 (ECDC, 2020; OFSP, 2019; Santé Publique France, 2018), and a change in customer washing habits – preferring showers over baths – has been implicated (O'Brien et al., 2000). The importance of this mode of transmission is supported by the prevalence of *L. pneumophila* in water systems and its regular isolation from domestic showers (Hayes-Phillips et al., 2019; Stout and Yu, 2003). *L. pneumophila* has been shown to incorporate pre-established biofilms as a secondary colonizer at a temperature between 20 °C and 45 °C in sanitary water pipes, taps, showerheads, hot tubs, air handling systems, etc., where it can reproduce and significantly increase in number chronically, making it more hazardous than the classic waterborne pathogens.

In a previous study, we monitored the number of inhalable particles emitted by showerheads that use either water-atomization or continuous-flow technology to assess the risk of inhaling water droplets during showering. We showed a slight increase in inhalable particles emitted by water-atomizing showerheads compared to those using continuous-flow technology. It's important to note that this observation was made in shower systems where biofilm formation was absent (Niculita-Hirzel et al., 2021). To further investigate potential differences in *L. pneumophila* emission, we conducted a comparative glovebox experiment using one showerhead representative of water atomization technology and one of continuous-flow technology with a calibrated solution of planktonic *L. pneumophila* (Niculita-Hirzel et al., 2022). The results of this last study indicate that there was no significant difference between the two showerhead models regarding the release of *L. pneumophila* in the first flush of water or its emission in aerosols during showering with an equivalent volume of water (Niculita-Hirzel et al., 2022). However, we did not investigate how water atomization technology, particularly the low water flow rate in the associated hose, may affect the development and colonization of biofilm by *L. pneumophila*, or the subsequent release of bacteria and inhalable particles from the biofilm. This is relevant given that the rate of biofilm colonization by *L. pneumophila* and its detachment from biofilms are influenced by water flow and streamflow patterns. It has been demonstrated that *Legionella* spp. have a significantly higher biofilm colonization rate under turbulent flow conditions (Reynolds number (Re) $>$ 1000 compared to laminar conditions (Re $<$ 1000), and the lower biofilm-associated *Legionella* spp. counts are detected under stagnant flow conditions (Re = 0) (Liu et al., 2006). Moreover, continuous release of noninfectious doses of *L. pneumophila* from biofilms (erosion detachment) have been observed at low water flow rates (5 to 50 $\mu L \cdot min^{-1}$) in flow chambers (Mampel et al., 2006) or under laminar flow conditions (Liu et al., 2006). Punctual release of high concentration of bacteria can been released (sloughing detachment) for a few days before the total removal of the biofilm from the surface at a water flow rate as high as 50 $\mu L \cdot min^{-1}$ (Mampel et al., 2006) or under turbulent conditions (Liu et al., 2006). Therefore, sloughing of *L. pneumophila* from the biofilm represents a risk of infection. Evaluating the impact of this new shower technology on bacterial attachment and detachment is of fundamental importance in assessing the risk of spreading infection and contamination in clinical and public health settings by using water atomization showerheads.

The objective of this research was to assess the potential risk associated with the utilization of water atomization technology in shower systems where mature biofilms were intentionally allowed to develop, specifically its impact on the attachment and detachment of bacteria in biofilms, particularly of *L. pneumophila*, as well as on the release of bioaerosols from these biofilms during showering, in comparison to shower systems using continuous flow technology.

2. Materials and methods

2.1. Shower model

The showers were run in an experimental shower cab measuring 1.5 m × 1.5 m × 2.5 m which was equipped with a mechanical extraction ventilation system and connected to a drinking water supply system, and to the building's water-heating system (Niculita-Hirzel et al., 2021). The shower system is composed of a Polyvinyl Chloride (PVC) flexible shower hose (180 cm long and of a diameter of 9 mm), and a showerhead STA (continuous flow) or ECO (water-atomizing). The STA showerhead has a spray angle of 5° and 51 nozzles of a diameter of 0.8 mm while the ECO showerhead has a spray angle of 36° and six nozzles of 1.1 mm of diameter. The STA and ECO showerheads imposed a Reynolds number (Re) of 250 and 135 respectively, and an average water speed of 2.7 $m \cdot s^{-1}$ and 1.4 $m \cdot s^{-1}$.

2.2. Controlled biofilm growth under control-lab conditions

The system installed in the shower cab allowed for experimental replication by running six independent shower systems simultaneously – three with an ECO showerhead and three with a STA showerhead – with the same water tap. Over the span of six months, a shower event of five minutes, at a water flow rate of 5.2 or 10.2 $L \cdot min^{-1}$ respectively, was run during working days (five days a week) with a water temperature of 38 °C. The system allows not to drain water in the shower system. Six months after shower system installation, 24 h after the last showering event, three types of samples from each shower system were collected during the showering event: the first flush of water (1 L), the aerosols emitted during a shower event of 10 min with warm water at 38° ± 1 °C, and the hose biofilm.

2.3. Uncontrolled biofilm growth under real-use conditions

Thirteen shower hoses were collected from domestic shower systems in August 2020, and an additional three hoses were obtained in August 2022 (Table 1, Fig. 1). The shower hoses had been in daily use with a water temperature of 37° ± 2 °C for 6 months to 10 years with continuous flow showerheads for the 2020 hoses, and for 1 to 2 years with water atomization showerheads for the 2022. The dwellings were supplied with a representative diversity of water hardness (from 1.50 to 3.80 $mmol \cdot L^{-1}$). All hoses were transferred to the lab in individual plastic bags within one day. To compare the number of bacteria released in water to that emitted in aerosols during a standard shower event, hoses were installed in the shower cabin one after another, either with a STA or an ECO showerhead. A standard shower event consisting of 10 min shower with warm water at 38° ± 1 °C was run with each shower head. Two consecutive series of experiments were conducted on the hoses collected in 2020, one with STA and one with ECO showerheads. To mitigate the impact of the local water supply on the biofilm in these hoses, we scheduled the showers 24 h apart and randomized the order of the showerheads. Biofilm extraction was performed after testing STA and ECO successively.

2.4. Sampling collection

During each shower event, the first liter of water was collected in a glass bottle. Simultaneously, the bioaerosols emitted were collected in a Coriolis collection tube containing 15 mL of PBS (VWR Chemicals, USA) with the Coriolis®μ air sampler (Bertin Instruments, Montigny-le-Bretonneux, France) at a flow rate of 300 $L \cdot min^{-1}$. To determine the number of inhalable particles generated during showering, we used a GRIMM 1.109 real-time particle monitor (GRIMM Aerosol Technik GmbH & Co. KG, Germany). To ensure compatibility with the instrument's functioning, cab air dilution by half with outdoor air was conducted, resulting in an air relative humidity of 50%. This method of

Table 1
Characteristics of the different hoses collected.

House ID	City	Zip code of the city	Water hardness (mmol/l)	Nb of hoses with STA	Biofilm age (years)	Nb of hoses with ECO	Biofilm age (years)
1	Lausanne	1005	1.87	2	0.6	3	0.6
2	Prêles	2515	3.02	1	2	2	2
3	Nods	2518	3.8	1	10	1	2
4	Evilard	2533	1.84	1	0.5		
5	Zofingen	4800	3.12	1	0.7		
6	Berne	3008	2.2	1	1		
7	St.Légier-La Chiésaz	1806	2.6	1	2		
8	Champoz	2735	1.5	1	2.5		
9	Neuchâtel	2000	1.96	1	3		
10	Neuchâtel	2000	1.96	1	4		
11	Le-Mont-sur-Lausanne	1052	2	2	5		

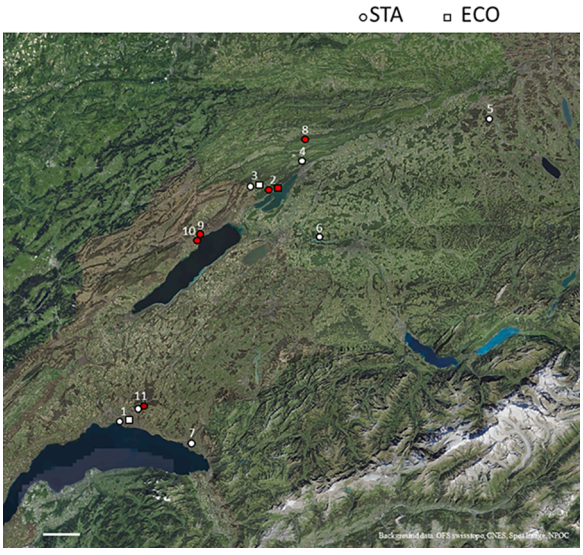

Fig. 1. Location of dwellings from which the shower hoses were collected. The shower hoses that were used with an STA showerhead are indicated by circles while the ones used with a ECO showerhead are indicated by squares. Biofilms in which *Legionella pneumophila* was diagnosed are shown in red, while those in which L. *pneumophila* was not detected are shown in white. The white bar indicates the map scale: 10 km. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

aerosol monitoring has been previously validated (Niculita-Hirzel et al., 2021). The Coriolis®μ air sampler and the GRIMM 1.109 real-time particle monitor were placed at a distance previously shown to be representative of aerosols emission for each showerhead, respectively, 45 cm from the water-atomization showerhead and 65 cm from the continuous-flow showerhead (Niculita-Hirzel et al., 2021). To establish the baseline bacterial levels in the warm water and ambient air, we collected one liter of water from the outlet and one aerosol sample each day before operating the shower systems. The biofilm was extracted from overall hoses by sonication and an aliquot of the obtained suspension was directly analyzed as described here after (detailed in the Biofilm extraction from hoses). One hundred milliliters of water and the total volume of aerosols were concentrated by centrifugation at 4000 g for 5 min at 4 °C, and by resuspension in 1 mL of PBS. Each sample was analyzed by flow cytometry to determine the total bacterial cell concentration and the proportion of viable bacteria, and cultured on R2A agar to determine the concentration of cultivable bacteria. The presence of *Legionella pneumophila* in samples was screened by culture on *Legionella* specific media (detailed in the Cultivability section).

2.5. Biofilm extraction from hoses

The overall length of hoses of 180 cm long and of a diameter of 9 mm was used for biofilm removal via repeated sonication as previously described (Proctor et al., 2016). Each hose was cut into two sections and, if necessary, the outer casing removed. Briefly, the method consisted in plug an adapted stopper at one end of each hose section, fill the hose section with sterile glass beads (3 mm diameter) and 0.2 μm filtered mineral water (Evian, France), seal the other extremity of the hose section with a sterile stopper, and invert ten times. Then, to detach the biofilm, the hose sections were sonicated by submersion in a sonication bath (Branson 5210, Branson, USA) for 5 min. The biofilm suspension was collected in 50 mL tubes (VWR International, USA) by removing the stopper from one end gently inverting the hose section, taking care not to lose glass beads during the process. and the hose sections refilled with freshly filtered mineral water. Subsequently, the hose sections were refilled with freshly filtered mineral water to repeat the steps of sonication. The sonication and collection steps were performed a total of five times. Finally, the total biofilm suspension (overall volume ranging between 80 and 100 mL) was sonicated for 1 min before further analyses.

2.6. Flow cytometry assay

The total bacterial cell concentration (TCC) and viability were determined by flow cytometry assay (FCA). Two hundred microliters of each sample were labeled with 1 μl of propidium iodide (PI) at 1 mg·mL^{-1} (Invitrogen P3566, Life Technologies Europe BV, Zug, Switzerland) and with 2 μl of SYBR™ Green I nucleic acid gel stain (SYBR) diluted 100x (Invitrogen S7563, Life Technologies Europe BV, Zug, Switzerland) and analyzed using a CYTOFLEX S2 (Beckman Coulter, US). The FCA profiles of the samples were obtained by using a combination of SYBR fluorescence for all cells (with damaged and non-damaged membranes) and PI red fluorescence for cells with damaged membranes. The SYBR signal was detected in the FITC—H channel (488 nm excitation and 525 nm acquisition), and the PI signal was detected in the APC channel (640 nm excitation and 660 nm acquisition). A volume of 40 μL was measure at a medium sheath flow velocity of 30 μL·min^{-1}. When needed, biofilms suspensions were diluted with filtered PBS (VWR Chemicals, USA) to do not exceed 10^5 cells·ml^{-1}. Data analysis was performed using the FlowJo™ software (version 10.7, Ashland, USA). Manually set gates for intact and damaged cells were applied for each sample type (aerosols, water, and biofilm) based on SYBR and PI fluorescence alone, as well as on the intrinsic fluorescence of unstained sampled (Fig. 2). Intact cells were considered viable, and damaged cells were considered dead in the present manuscript.

2.7. Cultivability

To proportion of cultivable bacteria in biofilm, water and bioaerosols

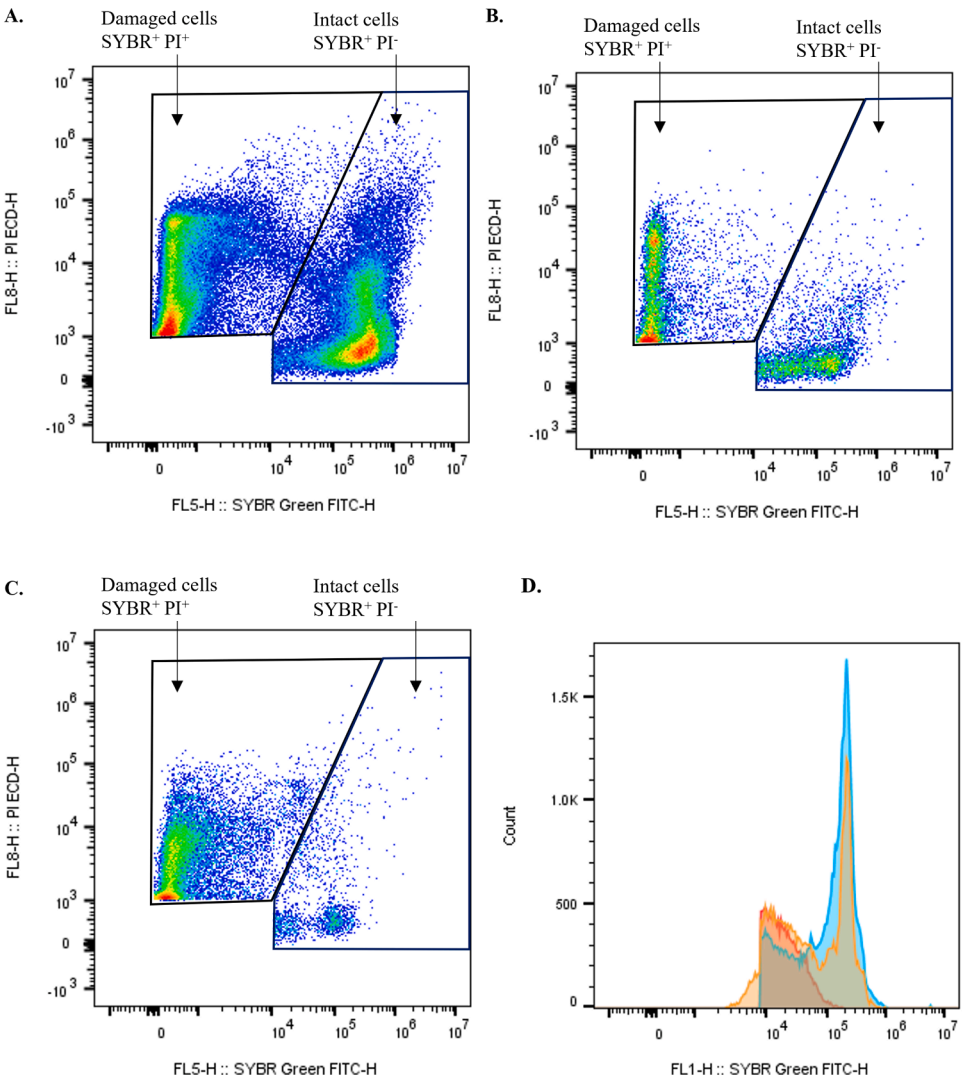

Fig. 2. Gating for counting intact and damage cells in biofilm, water, and aerosol samples. Each scattergram represents flow cytometric dot plots of green (FL5) vs. red (FL8) fluorescence of (**A.**) biofilm, (**B.**) water and (**C.**) aerosol samples stained concurrently with SYBR Green I and PI. Manual gates were set for each sample type based on SYBR Green I and PI fluorescence signals, as well as on the intrinsic fluorescence of unstained sampled, and are indicated by black boxes. Only events with positive SYBR Green I fluorescence were considered as cells. (**D.**) An exemplary superposed histogram was generated to visualize the fluorescence signals detected under the Green channel in water samples, comparing unstained (in red), SYBR Green I-stained (in blue), and SYBR Green I and PI dual-stained (in orange) conditions. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

samples was estimated by serial dilution (1:10) and plating on Reasoner's 2A (R2A) agar (Oxoid PO5149A, Thermo Fisher Diagnostics AG, Pratteln, Switzerland). After 5 days of incubation at 30 ± 1 °C, the colony-forming unit (CFU) were enumerated, overall and per phenotype, and one representative colony for each phenotype and each sample was subcultured on R2A for further identification.

To detect *L. pneumophila* in samples, 100 µL of each biofilm or water sample and 500 µL of each bioaerosol sample were plated onto Glycine Vancomycin Polymyxin Cycloheximide (GVPC) agar (Oxoid PO5074A, Thermo Fisher Diagnostics AG, Pratteln, Switzerland). The plates were incubated at 36 ± 1 °C in a humid atmosphere with 2.5% CO_2 for 10 days and read from day 5. Suspected colonies with a mottled surface or an iridescent and faceted cut-glass appearance (Figure S1), were counted and subcultured on Buffered Charcoal Yeast Extract (BCYE) agar with L-cysteine (Oxoid PO5072A, Thermo Fisher Diagnostics AG, Pratteln, Switzerland) and BCYE without L-cysteine (Oxoid PO5028A, Thermo Fisher Diagnostics AG, Pratteln, Switzerland) for 7 days at 37 ± 1 °C. The ones growing on BCYE with Cysteine, but not on BCYE without, were identified as described in the paragraph 2.5.

2.8. Bacteria identification

One hundred twenty colony forming units (CFUs) with distinct phenotypes on R2A or GVPC agar plates were isolated and subcultured on the respective media to obtain pure cultures (see examples Figure S2). The DNA of each isolate was extracted following the FastDNA spin kit for soil protocol (MP Biomedicals, LLC, France). The V6-V8 region of the bacterial 16S rRNA was amplified with the forward primer 967F and the reverse primer 1391R. The PCR reactions were performed in 25 µl reaction mixture using 1 µL of DNA, 0.3 µM of each primer, 0.2 mM dNTP mix, 1x KAPA HiFi fidelity buffer and 0.5 U of Kapa HiFi (HotStart) DNA polymerase (Kapa Biosystems KR0369, USA). The DNA samples were amplified using the Biometra T1 thermocycler (Biolabo Scientific Instruments, Châtel-Saint-Denis, Switzerland) under the following conditions: an initial denaturation at 95 °C for 2 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 54 °C for 40 s and extension at 72 °C for 1 min, with a final extension performed at 72 °C for 10 min. The amplicon products were visualized using gel electrophoresis and fragments of about 400–500 bp purified using the NucleoSpin® Gel and PCR Clean-up kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany). Finally, each purified fragment was sequenced with the 967F primer in a Sanger sequencing lab (Microsynth AG, Balgach, Switzerland). Taxonomic identification was done by blasting the obtained quality trimmed sequences against the non-redundant database with blastn (https://blast.ncbi.nlm.nih.gov/).

2.9. Statistical analysis

The GRIMM 1.109 gives data in particles·L^{-1} for 31 size channels (between 0.25 and 32.00 µm). To determine the number of particles·L^{-1}

inhaled, we pooled the data provided by the instrument for the channels between 0.25 and 10.00 μm and called this PM_{10}. The data provided by the instrument for the channels between 0.50 and 10.00 μm were pooled, taking in account the statistical significance of each channel and called this range PM_{10}-$PM_{0.50}$. The values obtained in CTT·mL^{-1} and CFU·mL^{-1} from biofilm samples were converted per unit surface (CTT·cm^{-2} and CFU·cm^{-2} respectively) as following:

$$\frac{Cb \times D \times Vb}{2 \times \pi \times r \times h} \quad (1)$$

where *Cb* is the concentration of bacteria per mL, *D* the dilution factor (if applied), *Vb* the volume of biofilm solution recovered after the different steps of sonication, *r* the rayon of the hose and *h* the length of hose section.

The values obtained in CTT·mL^{-1} and CFU·mL^{-1} from bioaerosol samples were converted per air volume (CTT·m^{-3} and CFU·m^{-3} respectively) as following:

$$\frac{Ca \times Va}{De \times t} \quad (2)$$

where *Ca* is the concentration of bacteria in the aerosol sample, *Va* the volume of solution recovered after aerosols sampling, *De* is the Coriolis air sampling debit and *t* the duration of the shower.

Descriptive statistics (means with standard deviations (SD)) were used to describe the inhalable fractions, the total bacterial cell concentration, the viability proportion and the cultivable bacteria concentration in samples.

For further statistical analysis, total bacterial cell concentration, cultivable bacteria concentration, PM_{10}, PM_{10}-$PM_{0.35}$ and PM_{10}-$PM_{0.50}$ were log transformed. Multivariate analysis of variance was conducted to explore correlations between these different variables, as well as with water hardness gradient and biofilm age. Kruskal–Wallis nonparametric tests were performed to identify differences between groups. Paired *t*-tests were conducted to determine whether there was a statistically significant difference between the biofilm samples collected from the two sections of the hose, or between the water or aerosols samples collected with ECO and STA showerheads. Two-sample test of proportions were conducted to determine whether bacterial species are more frequently released from biofilm with an ECO or STA showerheads. A *p* value of less than 0.05 was considered statistically significant. All analyses and graphs were carried out using STATA 14 software (StataCorp LLC., College Station, TX, USA).

3. Results

3.1. Biofilm data

To determine whether showerhead technology affects biofilm growth by modulating access to available nutrients or oxygen, the bacterial density and physiological state in biofilms grown with ECO or STA showerhead systems were compared in two sections of the same hose. No significant differences were found in bacterial density or physiological state, including the proportion of dead cells and concentration of cultivable bacteria, between the two sections for each type of showerhead (ECO: $p = 1.0$, $p = 0.87$, $p = 0.34$; STA: $p = 0.77$, $p = 0.67$, $p = 0.67$; Table S1, Fig. 3A). Furthermore, no significant differences were observed between the biofilms grown under controlled conditions with ECO and STA in terms of the density of total cell count (TCC·cm^{-2}) ($p = 0.0.23$), proportion of dead cells ($p = 0.15$) or density of cultivable bacteria (CFU·cm^{-2}) ($p = 0.15$) (Table 2). Similar results were observed when all biofilms, grown under controlled and uncontrolled conditions, were considered (Fig. 3A, Table S1).

In the overall biofilms grown under controlled and uncontrolled conditions, the density of total cell counts showed a significant correlation with that of cultivable bacteria (regression coefficient (Coef.) = 0.59 $p = 0.00$) and with the proportion of dead cells in the biofilm (% DCC; Coef. = 0.01 $p = 0.01$). No correlation was observed between TCC·cm^{-2} or CFU·cm^{-2} and the age of the biofilm (Coef. 0.07 $p = 0.09$,

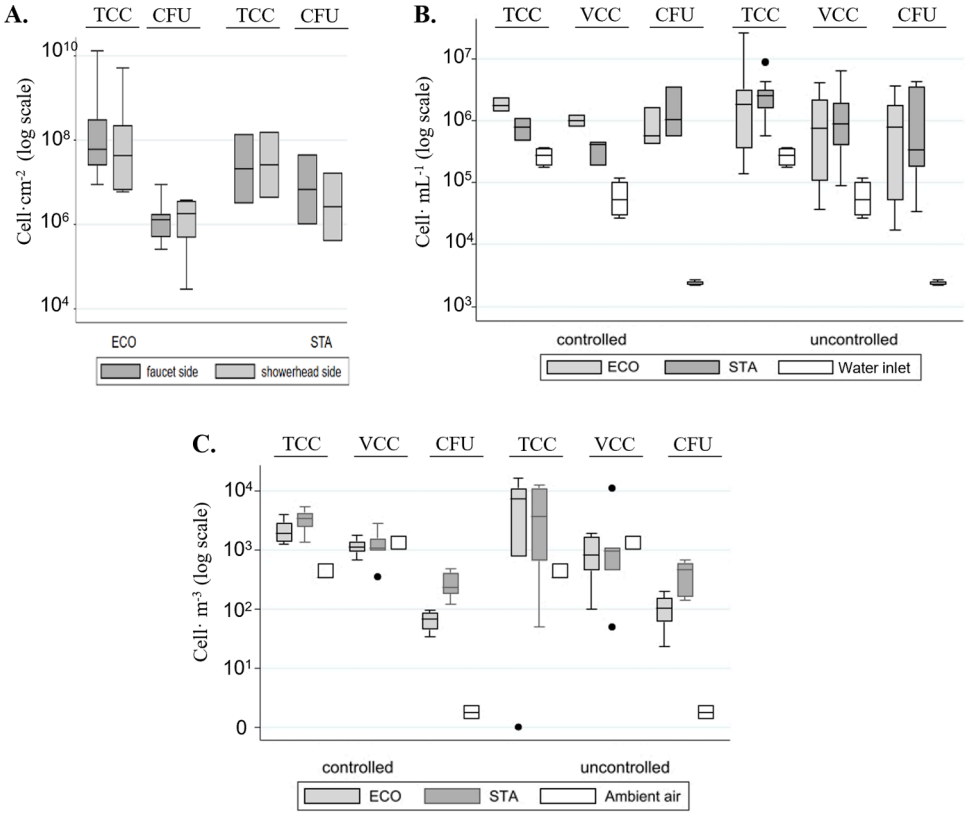

Fig. 3. Variation in bacterial concentration (total cell count (TCC), viable cell count (VCC) and cultivable bacteria (CFU)) in (**A.**) biofilm samples obtained from section closed to the faucet and section closed to the showerhead coupled with showerhead ECO or STA; (**B.**) water samples obtained with ECO or STA showerheads, or directly from the water inlet; (**C.**) bioaerosols samples obtained during showering with ECO or STA showerheads, or present in the ambient air. The y axis is given in logarithmic scale. The box covers the values between the first (Q1) and third quartile (Q3), and the line in the box indicates the median value. The whiskers cover all the data values between Q1 and the minimum value that is, the lowest 25% of data values, and between Q3 and the maximum value that is, the highest 25% of data value. The outliers are indicated by black dots.

Table 2
Mean number and standard deviation of the total (TCC) and cultivable (CFU) bacterial populations, along with the respective proportion of dead bacteria (% DCC) obtained from the biofilm grown under controlled conditions, water and aerosols.

	TCC[a]	% DCC	CFU[a]
Biofilm			
ECO	$2.2 \times 10^7 \pm 3.1 \times 10^7$	45.3 ± 8.3	$7.9 \times 10^5 \pm 8.3 \times 10^3$
STA	$9.3 \times 10^6 \pm 2.3 \times 10^6$	57.9 ± 7.8	$3.1 \times 10^5 \pm 1.9 \times 10^5$
Water			
ECO	$1.8 \times 10^6 \pm 4.7 \times 10^5$	44.9 ± 2.1	$8.8 \times 10^5 \pm 6.7 \times 10^5$
STA	$7.9 \times 10^5 \pm 3.2 \times 10^5$	54.6 ± 12.1	$1.7 \times 10^6 \pm 1.6 \times 10^6$
water inlet	$2.9 \times 10^5 \pm 1.0 \times 10^5$	37.4 ± 24.7	$2.4 \times 10^3 \pm 2.2 \times 10^2$
Aerosols			
ECO	$2.3 \times 10^3 \pm 1.1 \times 10^3$	65.9 ± 39	67 ± 27
STA	$3.4 \times 10^3 \pm 1.6 \times 10^3$	44.3 ± 25.9	95 ± 51
Ambient air	$4.2 \times 10^2 \pm 1.4 \times 10^2$	62.4 ± 31.7	3.6 ± 1.4

[a] expressed per cm^2 for biofilm samples, per mL for water samples and per m^3 for aerosol samples.

Coef. 0.08 $p = 0.08$), but the proportion of dead bacteria was positively associated with age (Coef. 3.34 $p = 0.01$). The water hardness used during biofilm growth had a significant impact, leading to higher densities of bacteria, both total and cultivable (Coef. = 0.07 $p = 0.00$, Coef. = 0.06 $p = 0.00$, respectively). However, no significant association was observed between water hardness and the proportion of dead bacteria in the biofilm (Coef. = 0.85 $p = 0.21$).

Legionella pneumophila was detected in a wide range of concentration ($6.0{\cdot}10^2$ to $2.8{\cdot}10^6$ CFU·cm^{-2}) within the hose biofilm of five out of the eleven houses included in the study. A significant correlation was found between the density of cultivable *L. pneumophila* in the biofilm and the density of overall cells ($p = 0.01$), as well as total cultivable bacteria in the biofilm ($p = 0.04$). The comparison of *L. pneumophila* concentration in biofilms grown for two years with ECO and STA showerheads in one house, reveled similar concentrations for the two systems (ECO: $3.1{\cdot}10^3$ CFU·cm^{-2}; STA: $3.7{\cdot}10^4$ CFU· cm^{-2}).

3.2. Bacteria released in water from biofilm

The bacteria present in the first water flush were compared between samples collected during showering with ECO or STA showerheads where biofilms were grown under controlled condition for 6 months. A significant difference between samples collected with the ECO and STA showerheads was observed in the concentration of total cells released ($p = 0.03$, Table 2), as well as of the viable cells ($p = 0.02$, $VCC_{ECO} = 1.0 \times 10^6 \pm 2.3 \times 10^5$ $VCC_{STA} = 3.5 \times 10^5 \pm 1.4 \times 10^5$). However, no significant difference in the proportion of damaged cells or in the concentration of cultivable bacteria was observed in the water released by either showerhead ($p = 0.24$, $p = 0.46$ respectively; Table 2)). When the experiment was conducted with shower systems where biofilms were grown under uncontrolled conditions, no significant difference in the concentration of total cells, viable cells or cultivable bacteria was observed in the water released by either showerhead, regardless of the age or growth conditions of the biofilm ($p = 0.45$, $p = 0.47$, $p = 0.74$ respectively). The concentration of bacteria in water samples collected using ECO or STA was significantly higher than that in the water inlet ($p = 0.01$, $p = 0.01$ and $p = 0.01$, respectively), confirming the release of bacteria from the hose biofilm (Fig. 3B).

To track the release of bacteria from biofilms, bacterial species isolated from biofilms, such as *Brevundimonas, Caulobacter*, and *Sphingomonas* were researched in overall water samples obtained with ECO or STA showerheads. *Brevundimonas* was detected in 50% of water samples released with an ECO showerhead and 67% of water samples released with a STA showerhead from biofilms containing this taxon (two-sample test of proportions $p = 0.56$). *Caulobacter* was detected in 67% of both types of samples (two-sample test of proportions $p = 1.00$). *Sphingomonas* was detected in 100% of water samples released with an ECO showerhead and 50% of water samples released with a STA showerhead (two-sample test of proportions $p = 0.046$). No significant difference was observed in the amount of bacteria released in the initial flush of water between the two types of showerheads (*Brevundimonas* $Median_{ECO} \pm SD$: $4.5{\cdot}10^4 \pm 8.2{\cdot}10^4$ $Median_{STA} \pm SD$: $1.0{\cdot}10^5 \pm 8.5{\cdot}10^6$, paired *t*-test $p = 0.09$; *Caulobacter* $Median_{ECO} \pm SD$: $2.5{\cdot}10^4 \pm 1.2{\cdot}10^5$ $Median_{STA} \pm SD$: $3.0{\cdot}10^5 \pm 4.4{\cdot}10^5$, paired *t*-test $p = 0.87$; *Sphingomonas* $Median_{ECO} \pm SD$: $1.5{\cdot}10^5 \pm 2.6{\cdot}10^5$ $Median_{STA} \pm SD$: $1.9{\cdot}10^5 \pm 4.8{\cdot}10^5$, paired *t*-test $p = 0.056$). The concentration of cultivable *L. pneumophila* in the first flush of showering ranged from 6.0×10^2 to 1.6×10^4 CFU·L^{-1} depending on the level of *Legionella* contamination in the shower hose biofilm. However, the concentration was comparable between the two showerhead systems (ECO and STA), indicating that the showerhead type did not significantly influence the number of *L. pneumophila* release in water.

3.3. Aerosols emitted during showering

Aerosols and bioaerosols emission during showering were compared systematically between the ECO and STA shower systems showerheads where biofilms were grown under controlled condition for 6 months. The particle size profile of inhalable water droplets emitted during showering showed a significant difference between the two systems. The ECO showerhead emitted slightly more water droplets than STA in the nano size range (0.25, 0.28, 0.30, 0.35 and 0.40 μm). However, when considering only water droplets larger than 0.50 μm ($PM_{10} - PM_{0.5}$), ECO emitted fewer such water droplets than STA ($p = 0.025$, Fig. 4). Notice the high contribution of particles smaller than 0.35 μm from ambient air.

Regarding the emission of bioaerosols, ECO showerheads emitted significantly fewer cultivable bacteria than STA showerheads irrespective of whether the biofilms were grown under controlled or uncontrolled conditions ($p = 0.006$, $p = 0.004$; respectively; Table 2, Fig. 3C). However, no significant difference in the concentration of total or viable bacteria emitted during showering with ECO or with STA was observed under both controlled ($p = 0.58$, $p = 0.67$; respectively), and uncontrolled conditions ($p = 0.32$, $p = 0.99$; respectively; Table 2, Fig. 3C). Notice that only the cultivable bacteria were detected in significantly higher concentrations in the bioaerosols collected during showering compared to the ambient air ($p = 0.04$).

During showering with both showerhead types, bacteria from the biofilm were detected in the aerosols emitted, albeit sporadically (15% during showering with ECO and 18% with STA showerheads). However, no significant difference in the concentration of these taxa in the bioaerosols was observed between the two showerhead systems ($z = -0.26$, $p = 0.80$). *L. pneumophila* was not detected in any of the bioaerosols screened.

4. Discussion

In this research, we compared the impact of two showerhead systems with distinct water flow and streamflow patterns on the long-term attachment of bacteria, including *Legionella*, to hose surfaces, on their release from biofilm into water and aerosols during showering in a full-size shower system.

Hydrodynamics play a crucial role in the formation and development of biofilms, and affect biofilm thickness and density (Tsagkari and Sloan, 2018). Previous studies have shown that different flow conditions can cause substantial changes in biofilm morphology and growth in pipes, specifically turbulent flow can accelerate biofilm growth in drinking water (Tsagkari and Sloan, 2018; Tsvetanova, 2020). The water flow and streamflow pattern have been also shown to have crucial effect on the initial attachment to the pipe surfaces (Tsvetanova, 2020), in particular of *Legionella* (Liu et al., 2006; Mampel et al., 2006). Our results complete the existing data with the impact of water flow and streamflow pattern on biofilms that reached the quasi-stationary state. We showed that after more than 6 months, biofilms submitted to

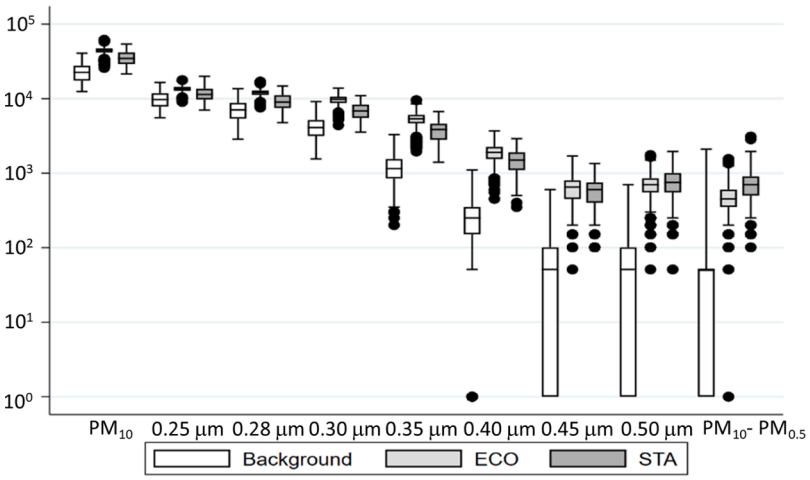

Fig. 4. Variation in the concentration of inhalable water droplets emitted during a 10 min showering with ECO or STA compared to the natural concentration of particles in ambient air. The seven size channels that contribute the most to the PM_{10} were individually represented. The y axis is given in logarithmic scale. The box cover the values between the first (Q1) and third quartile (Q3), and the line in the box marks the median value. The whiskers cover all the data values between Q1 and the minimum value that is, the lowest 25% of data values, and between Q3 and the maximum value that is, the highest 25% of data value. The outliers are indicated by black dots.

different water flow and streamflow pattern during showering using two distinct commercial showerhead systems, shared similar characteristics. Specifically, we observed no significant difference in the rate of biofilm colonization by bacteria or in the bacterial physiological status. However, there was a tendency for *Legionella pneumophila* to have a higher biofilm colonization rate under the ECO flow condition ($Re = 135$) compared to the STA flow condition ($Re = 250$). This suggests that even a slight difference in *Re* water flow value may matters for *Legionella* proliferation in the biofilm, as previously suggested for larger variations in water flow behavior (Liu et al., 2006; Tsagkari and Sloan, 2018). To confirm this tendency, further experiments with a larger number of samples, naturally colonized by *Legionella*, are needed. However, the fact that we observed no significant difference in the rate of biofilm colonization by bacteria or in the bacterial physiological status does not support the results of previous studies conducted on biofilms grown in quasi-stationary status which has indicated that water velocity has an impact on biofilm cell density, although the magnitude of this effect was reported to be minor (Tsvetanova, 2020). One possible explanation for this finding is that the biofilm within the shower hose plays a significant role in the release of bacteria during showering, with the hose biofilm being the primary contributor. In contrast, the biofilm in showerheads, which are exposed to different water velocities, contribute less to the release of bacteria. Nevertheless, the mean level of colonization by cultivable bacteria in the biofilms was in the same order of magnitude as that observed in previous studies involving biofilms growing on PVC material (Tsvetanova, 2020).

Our study also yielded interesting results regarding the aerosols emitted by the two showerhead systems. We found that while the water atomization showerhead emitted slightly more particles in the nano size range smaller than 0.45 µm than the continuous flow showerhead, it emitted less particles commonly considered to be large enough to carry a bacterium. The difference in the emission of particles in the nano size range is consistent with the results of previous studies (Cowen and Ollison, 2006; Niculita-Hirzel et al., 2021; Zhou et al., 2007). Furthermore, the observed difference in the concentration of particles, commonly considered large enough to carry a bacterium, between the two shower systems aligns with the predictions of QMRA models, which indicated a lower risk of infection during showering with a water-efficient showerhead compared to a conventional showerhead (Wilson et al., 2022). However, a recent study suggests that *Legionella pneumophila* particles with a smaller aerodynamic diameter as small as 0.26 µm can carry these pathogenic bacteria deep into the lungs (Allegra et al., 2016). This raises questions about the advantage of water atomization technology in reducing exposure to bacterial pathogen. Nevertheless, the results obtained in the present study on the cultivable fraction of emitted bacteria in aerosol support a decreased in the risk of user exposure to bioaerosols emitted by ECO compared to STA. Previous experiments conducted with calibrated and homogenous solutions of planktonic *L. pneumophila* have also shown that the atomizing showerhead emits fewer bacteria – cultivable, viable, and total – than the conventional showerhead for the same shower duration due to reduced water usage. However, to our surprise, this difference was less significant than expected, no significant difference being observed in the total cell counts or viable fraction of bioaerosols emitted during a 10 min showering session with the two showerheads and hoses that had 6-month-old biofilms. One potential explanation for the lack of difference in bioaerosol concentration between the two showerheads could be the method of collection. Bioaerosols were collected as a cumulative dose, while particles large enough to carry a bacterium were monitored in real-time, which is more sensitive to fluctuations in their concentration during showering. Such variation in bacteria emission is supported by the higher concentration of bacteria released in the first flush of water – total and viable – with ECO than STA. Another explanation could be that the contribution of bacteria emitted in aerosols in shower cabs during showering to the overall concentration of bioaerosols is too small to be distinguish from the background level of ambient air bacteria, except for the cultivable fraction.

Our study aimed to compare the impact of water technology on the viability of bacteria released from biofilm in both water and aerosols. Our results revealed that the physiological status of bacteria did not significantly differ between the samples generated with the two showerhead systems. These findings suggest that the survival of bacteria in water and aerosols, especially *Legionella*, is not more affected by the water flow and pressure of the water atomization showerhead than by those of the continuous flow showerhead. This conclusion is consistent with a previous study (Niculita-Hirzel et al., 2022).

While our study has provided valuable insights, it is important to acknowledge its limitations. One such limitation is that we used only one showerhead model for each water technology, which may not fully represent the range of showerhead designs available. Therefore, further research using multiple showerhead models is needed to confirm our findings and determine the generalizability of our results. Despite these limitations, our study emphasizes the potential health risks associated with showering with energy-saving showerhead systems and highlights the importance of further research to better understand the health risks linked with exposure to shower aerosols and bioaerosols. We would like to emphasize that while the differences in droplet size distribution between showerhead systems based on different technologies may have limited implications for human exposure to infectious *Legionella*, they could significantly impact human exposure to airborne particles during showering. This is because most of the inhalable droplets generated during showering are in the nano scale and can reach the alveolar part of

the lung. Therefore, it is important to further investigate the potential health risks associated with exposure to aerosols during showering with water-saving showerheads and to develop strategies to reduce exposure to these aerosols. These findings suggest that different shower systems may have different impacts on human exposure to bioaerosols, and that further studies are needed to fully understand these impacts.

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CRediT authorship contribution statement

Hélène Niculita-Hirzel: Conceptualization, Formal analysis, Resources, Writing – original draft, Writing – review & editing, Supervision, Funding acquisition. **Marian Morales:** Conceptualization, Formal analysis, Investigation. **Priyanka Parmar:** Investigation.

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.

Data availability

Data will be made available on request.

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Supplementary materials

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