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The Accumulation of Heavy Metals in Shower System Biofilms: Implications for Emissions and Indoor Human Exposure

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Abstract: Biofilms play a crucial role in absorbing various metals from water, including copper, lead, iron, chromium, nickel, zinc, cadmium, and barium. While their presence was revealed in shower system biofilms, the factors affecting metal accumulation in shower system biofilms were poorly explored. This study aimed to investigate the capacity of shower hose biofilms to accumulate heavy metals, in particular in biofilms growing in energy-efficient showerhead systems, and evaluate the potential emission in water and aerosols of metal pollutants during showering. The adsorption efficiency of various metals in biofilms was assessed by ICP/MS and revealed that biofilms accumulate metals as they age and as biofilm biomass increases, indicating a potential influence of heavy metals on biofilm ecology. Furthermore, the study examined the emission of heavy metals during showering and found that it was sporadic and limited primarily to copper and zinc. These findings raise concerns regarding the role of biofilms in both retaining and releasing metal contaminants in water distribution systems, as well as the associated risk of inhalation during showering. By shedding light on the accumulation dynamics of heavy metals in shower hose biofilms and their potential emission patterns, this research highlights the need for further investigation into the impact of biofilms on water quality and human exposure to metal pollutants. The findings underscore the importance of considering biofilm-related processes when addressing the overall management of heavy metal contamination in shower systems and its potential implications for public health.

Keywords: heavy metal adsorption; shower hose biofilms; inhalation risk; energy efficient showerheads

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1. Introduction

Biofilms, which are complex microbial communities adhering to surfaces, possess a highly adsorptive surface to metals due to the presence of extracellular polymeric substances (EPS) [1,2]. Numerous studies have demonstrated the capacity of diverse microbial biofilms to accumulate both soft and heavy metals. River biofilms have been shown to accumulate chromium (Cr^{3+}), nickel (Ni^{2+}), and copper (Cu^{2+}) [3], while lake biofilms have exhibited absorption of zinc (Zn^{2+}), cadmium (Cd^{2+}), lead (Pb^{2+}), and barium (Ba^{2+}), with an adsorption efficiency ranking of $Cu^{2+} \gg Pb^{2+} > Zn^{2+} > Cd^{2+} > Ba^{2+}$ [4]. Even freshwater biofilms have been shown to rapidly absorb Zn^{2+}, Cu^{2+}, and Pb^{2+} from water, with the highest absorption efficiency observed for Pb^{2+}, followed by Zn^{2+} and Cu^{2+} [5].

The biofilms also form in shower hoses and have been found to accumulate copper (Cu^{2+}), lead (Pb^{2+}), and iron (Fe^{2+}) [6]. The main issue with plastic pipes and shower hoses is their tendency to release substantial amounts of organic carbon into the water, a phenomenon known as "organic carbon migration" or leaching from the material. This organic carbon can be assimilated by bacteria—including *Legionella pneumophila*, the infectious bacterium responsible for the legionellosis disease—promoting their growth within the plumbing system [7,8]. Flexible hose materials exhibit varying degrees of carbon leaching,

and the level of carbon leaching has been observed to correlate with microbial growth and diversity in most of the studied pipes [9]. However, shower hoses, often made from polyvinyl chloride (PVC) with additional plasticizers, are particularly prone to carbon leaching due to the presence of low-molecular-weight compounds that easily leach into the water [8,9]. Consequently, biofilm growth and diversity are favored in these widely used hoses.

Biofilms that form on PVC pipes have been observed to accumulate aluminum (Al^{3+}) at a concentration of 1.5 ± 0.5 mg·m^{-1} and calcium (Ca^{2+}) at a concentration of 225 ± 36 mg·m^{-1} [10]. It has been reported that Ca^{2+} exhibits preferential binding to the extracellular matrix of *Bacillus subtilis* biofilms compared to other metal ions, such as Zn^{2+}, Mn^{2+}, and Fe^{2+}, and is uniformly distributed throughout the biofilms, while Zn^{2+}, Mn^{2+}, and Fe^{2+} specifically accumulate in biofilm "wrinkles" [11]. The presence of Ca^{2+} has been found to stabilize the biofilm structure, prevent bacterial dispersal, promote bacterial aggregation, and enhance biofilm formation [12,13]. Additionally, Zn^{2+}, Mn^{2+}, and Fe^{2+} ions are known to play pivotal roles in bacterial metabolism and sporulation [11].

In experimental studies, the presence of trace metals, including Fe^{2+}, Zn^{2+}, K^{+}, Mg^{2+}, Mn^{2+}, Ca^{2+}, and Cu^{2+}, has been shown to impact the growth of *Legionella pneumophila*. For instance, concentrations of Fe^{2+} or Zn^{2+} ranging from 0.5 to 1 mg·L^{-1}, as well as K^{+} concentrations at 1, 10, and 100 mg·L^{-1}, have been found to promote the proliferation of *L. pneumophila*. Conversely, concentrations of Al^{3+}, Cd^{2+}, Cu^{2+}, Fe^{2+}, Pb^{2+}, Mn^{2+}, and Zn^{2+} at 10 and 100 mg·L^{-1} significantly suppress the proliferation of *L. pneumophila* [14].

Regarding the dynamics of metal absorption and release from biofilms, they have been observed to vary over time. The absorption of metals from water enriched with these metals is most pronounced at the beginning of the exposure period, gradually decreasing until reaching equilibrium after 7–14 days, regardless of the specific metal [5]. When the metals are depleted from the water, the biofilms tend to release the accumulated metals, but at a slower release rate compared to their initial accumulation. However, even after 14 days, the biofilms still retain 10–16% of the accumulated Zn^{2+} and Cu^{2+} and 35% of Pb^{2+}, indicating a delayed release of Pb^{2+} [5]. These observations raise questions about the potential role of biofilms in the retention and release of metal contaminants within drinking water distribution systems (DWDs), highlighting the importance of considering biofilms as a potential source of metal exposure, particularly heavy metals, in indoor environments.

The research objective of this study was to determine the capacity of shower hose biofilms to accumulate both soft and heavy metals and thereby release these metals in water and aerosols during showering.

2. Materials and Methods

2.1. Shower System Used and Conditions of Biofilm Growth

The experimental setup for the study involved running showers in a shower cab with specific measurements (1.5 m × 1.5 m × 2.5 m). The shower cab was equipped with a mechanical extraction ventilation system and connected to both a drinking water supply system and the water-heating system of the Unisanté laboratory in Lausanne [15]. The shower system consisted of a plasticized polyvinyl chloride (PVC-P) flexible shower hose, measuring 180 cm in length and 9 mm in diameter, along with a water-atomization showerhead. The showerhead had a spray angle of 36° and six nozzles, each with a diameter of 1.1 mm, and allowed a water flow rate of 5.2 L·min^{-1}.

In the controlled experiments, the shower hoses were vertically hung in the experimental shower cab, allowing for replication by running nine independent shower systems simultaneously. Over the span of the experiment, a shower event was run for three minutes during working days (five days a week) with a water temperature of 38 °C ± 1 °C to allow biofilm growth in shower systems. The system does not allow for the draining of water in the shower system. Samples were collected at various biofilm ages, 5, 9, and 36 weeks, with each collection occurring 24 h after the last showering event.

Additionally, the same shower system (hose + showerhead) was distributed to three households located in different areas of the canton of Bern in Switzerland. Two households received the shower system in August 2020, and one household received it in August 2021. The shower hoses had been in daily use with a water temperature of 37 °C ± 2 °C. The three systems were collected back at the beginning of September 2022. Volunteers were instructed to detach the shower hoses from all fittings. After removal from the domestic showers, the hoses were filled with water to preserve moisture on the internal surfaces, sealed with screw caps, and transported to the laboratory within 24 h. Once in the laboratory, the hoses were installed one after another in the experimental shower cab to determine the quantity of metals and the number of bacteria released in water and aerosols, as well as the physiological status of the last ones during showering.

Three types of samples were collected from each shower system: the first liter of water released through the showerhead, the aerosols emitted during a 10 min shower event, and the hose biofilm.

2.2. Collection of Aerosols and Water Samples

Aerosols were collected during 10 min shower events using a Coriolis-μ air sampler (Bertin Instruments, Montigny-le-Bretonneux, France) at a flow rate of 300 min·L^{-1}. The collecting tube of the sampler was filled with 15 mL of sterile phosphate-buffered saline (PBS) and positioned approximately 45 cm from the showerhead on a sampling stand. Water samples corresponded to the first liter of water sprayed by the showerheads. To determine background levels of bacteria and metals, one liter of water was directly collected from the shower outlet, and an aerosol sample was obtained in the shower cab before initiating the showers. ICP-MS was conducted to quantify metals in samples. Flow cytometry (FC) analysis was conducted to determine the total number of bacterial cells and the proportion of live and dead cells. The number of metabolically active bacteria was estimated based on the Adenosine tri-phosphate (ATP) assay. Additionally, the number of cultivable bacteria was determined by plating the samples on R2A agar plates and the presence of Legionella screened on Glycine Vancomycin Polymyxin Cycloheximide (GVPC) agar.

2.3. Biofilm Extraction from Hoses

The hoses were divided into two sections and subjected to sonication. To prepare for sonication, the hose sections were filled with sterile glass beads (3 mm diameter) and 0.2 μm filtered bottled water (Evian, France). The ends of the sections were then sealed with sterile stoppers, and the sections were inverted ten times before being sonicated in a Branson ultrasonic water bath (model 5210, Branson, MO, USA) for 5 min. After the sonication, the biofilm suspension from each hose section was transferred into 50 mL Falcon tubes, and the sections were replenished with fresh filtered mineral water. This process of sonication and replacement was repeated five times for each hose to ensure complete dislodgement of the biofilm. Following the five rounds of sonication, the total biofilm suspension collected from one hose (with a volume ranging between 90 and 110 mL) was centrifuged at 4000× *g* for 10 min and then resuspended in 10 mL of 0.2 μm filtered Evian bottled water. The biofilm samples were submitted to the same analysis as the water and aerosol samples.

2.4. Elementary Analysis of Biofilms Suspensions

The samples were screened for the presence of metals, and those with significant concentration (^{44}Ca, ^{107}Ag, ^{57}Fe, ^{63}Cu, ^{66}Zn, and ^{208}Pb) were quantified using inductively coupled plasma mass spectrometry (ICP-MS) analyses. For this analysis, 2.5 mL aliquots of each kind of sample—biofilm suspensions, water, and aerosol—were subjected to acid digestion in 65% HNO_3 at a temperature of 95 °C for 25 min. After the digestion process, 25 mL of high-purity water MilliQ was added to each sample. The elemental concentrations were quantified using an ICP-MS (Thermo iCAP TQ). Standards were provided by SCP Science (Courtaboeuf, France) and Fluka (Merck KGaA, Darmstadt, Allemagne). The limits of detection and limits of quantification are indicated in Table 1. The ICP-MS analysis was

conducted by the Forensic Toxicology and Chemistry Unit at Vaud University Hospital (CHUV).

Table 1. Limit of detection (LOD) and limit of quantification (LOQ) of metals detected in samples.

	^{44}Ca [1]	^{57}Fe [1]	^{63}Cu [1]	^{66}Zn [1]	^{107}Ag [1]	^{208}Pb [1]
LOD	33.3	6.7	6.7	6.7	6.7	6.7
LOQ	100.0	20.0	20.0	20.0	20.0	20.0

[1] Expressed in $\mu g \cdot L^{-1}$.

2.5. Flow Cytometry Analysis

Flow cytometry analysis (FCA) was employed to assess the total cell count in the samples and determine the proportion of live/intact and dead cells. Gating for counting intact and damaged cells in biofilm, water, and aerosol samples was performed as described in [16], where the method was originally detailed. In brief, the samples were either used directly or diluted with 0.2 µm filtered bottled water prior to FCA to not exceed 10^5 cells$\cdot$ml^{-1}. Subsequently, 200 µL of each sample was stained with 2 µL of SYBR^{TM} Green I nucleic acid gel stain (SYBR) (Invitrogen S7563, Life Technologies Europe BV, Zug, Switzerland) diluted 100× and with 1 µL of propidium iodide (PI) at 1 mg$\cdot$mL^{-1} (Invitrogen P3566, Life Technologies Europe BV, Zug, Switzerland). PI is a fluorescent dye that can only penetrate damaged cell membranes and thus bind the DNA of bacteria that have a damaged cell membrane. SYBR green is a fluorescent dye that can penetrate all cells, whether they are damaged or intact, and bind to DNA. The FCA was performed for each sample at a flow rate of 30 µL$\cdot$min^{-1} with a CYTOFLEX S2 flow cytometer (Beckman Coulter, Inc., Brea, CA, USA). 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). To prevent cross-contamination, a 2 min bleach treatment followed by a 2 min wash with PBS was performed between samples. The obtained results were exported as FCS files and analyzed using FlowJo software (version 10.7, Ashland, OH, USA). Manually set gates were applied for each sample based on SYBR/PI fluorescence to establish the viability proportion.

2.6. Adenosine Tri-Phosphate (ATP) Quantification

ATP quantification assays were conducted to measure the metabolic activity of the microbial biomass in the samples. The measurement of ATP was performed using a luminometer (Tecan Infinite® M200, Tecan Trading AG, Switzerland) and the Intracellular ATP Kit HS (BioTherma, Handen, Stockholm, Sweden), following the supplier's instructions for microplate usage. The measurements were performed in triplicate to ensure accuracy and reproducibility.

2.7. Determination of Cultivable Biofilm Bacteria and Legionella Identification

One hundred microliters from each sample were serially diluted (1:10 to 1:100,000) with 2 µm filtered bottled water before being plated on R2A agar plate (Oxoid PO5149A, Thermo Fisher Diagnostics AG, Pratteln, Switzerland) and incubated at 36.5 ± 1 °C. Additionally, 500 µL of each sample were directly plated on GVPC (Oxoid, Thermo Fisher, Germany) for *Legionella* spp. detection. Confirmation of suspected *Legionella* spp. colonies was carried out by subculturing on Buffered Charcoal Yeast Extract (BCYE) agar with and without L-cysteine and by rRNA 16S sequencing of the *Legionella*-compatible phenotypes. GCPV and BCYE agar plates were incubated at 36.5 ± 1 °C in a humid atmosphere with 2.5% CO_2 for 7 days.

2.8. Statistics

The values obtained in $\mu g \cdot L^{-1}$ from biofilm samples (Table S1) were converted per unit surface ($ng \cdot cm^{-2}$) following Equation (1)

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

where Cb is the concentration of metals per L, Vb is the volume of biofilm solution recovered after the different steps of sonication, r is the rayon of the hose, and h is the length of hose section.

The values obtained in $\mu g \cdot L^{-1}$ from aerosol samples (Table S1) were converted per air volume ($\mu g \cdot m^{-3}$) following Equation (2):

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

where Ca is the concentration of metals in the aerosol sample, Va is the volume of solution recovered after the air sampling, De is the Coriolis air sampling debit, and t is the duration of the shower. Descriptive data were presented as mean ± standard deviation (SD). For further statistical analysis, data were log-transformed. Pearson's correlations were conducted to determine whether there was a high or moderate correlation between metal accumulation in biofilms and different biofilm determinants (biofilm age, total cell count, proportion of dead cells, and concentration of gDNA and ATP). One-way analysis of variance (ANOVA) and Bonferroni post hoc test were used to identify significant differences in soft and heavy metal emission in water or in aerosols during showering. The significance level in hypothesis testing was predetermined at p-value of <0.05. All analyses and graphs were carried out using STATA 14 software (StataCorp LLC., College Station, TX, USA).

3. Results

3.1. Metal Accumulation in Biofilms

The metals detected in biofilm samples included Ag, Fe, Cu, Zn, Pb, and Ca, with varying concentrations. Notably, Fe exhibited the highest levels, reaching concentrations as high as 799 $ng \cdot cm^{-2}$, followed by Cu at 348 $ng \cdot cm^{-2}$, Zn at 198 $ng \cdot cm^{-2}$, Ag at 4.9 $ng \cdot cm^{-2}$, and Pb at 41 $ng \cdot cm^{-2}$. Detailed metal concentrations in the shower hose biofilm samples are provided in Table 2.

Table 2. Mean concentration of metals in shower hose biofilms of different ages and from different geographic origins.

Metals Identified	Lausanne 5 Weeks	Lausanne 9 Weeks	Lausanne 36 Weeks	Nods 52 Weeks	Prêles 104 Weeks	Nidau 104 Weeks
Ca	2500 (2300–2700) [1]	3200 (2700–3700)	4300 (3900–4700)	3900 (3700–4100)	3600 (3000–4300)	4250 (4200–4300)
Fe	2.6 (1.9–3.1)	1.1 (0.3–2.4)	30.8 (9.5–53.3)	799.1 (754.7–843.5)	472.7 (303.3–645.6)	484.1 (479.6–488.6)
Cu	5.0 (4.2–5.8)	2.8 (0.3–7.7)	75.5 (20.2–133.9)	324.5 (306.4–342.5)	188.6 (47.6–332.7)	348.1 (344.9–351.3)
Zn	9.2 (7.5–10.7)	0.7 (0.1–1.5)	20.5 (8.9–32.3)	198.4 (187.4–209.4)	97.2 (47.0–148.4)	67.8 (67.2–68.4)
Ag	0.1 (0.0–0.1)	0.03 (0.02–0.06)	2.5 (0.7–4.3)	1.9 (1.8–2.0)	4.8 (1.0–8.8)	1.5 (1.5–1.5)
Pb	0.1 (0.1–0.1)	0.04 (0.01–0.10)	1.3 (0.4–2.3)	41.6 (39.3–43.9)	28.1 (22.3–34.0)	13.0 (12.0–13.1)

[1] Data are presented as mean (Min-Max) in $ng \cdot cm^{-2}$.

To further investigate the factors influencing metal accumulation, regression analyses were performed, considering several biofilm determinants such as biofilm age, total cell

count, proportion of dead cells, concentration of genomic DNA (gDNA), and ATP concentration. The results revealed high positive correlations between the concentration of each elemental metal and both biofilm age and gDNA concentration (Table 3). Similarly, Fe, Cu, Zn, Ag, and Pb concentrations showed a high positive correlation with the total cell count in the biofilms. These findings indicate that as biofilms mature and biomass increases, the accumulation of metals also increases.

Furthermore, high or moderate positive correlations were observed between the concentrations of Fe, Cu, Zn, Pb, and Ag, and ATP concentration or proportion of dead cells, suggesting that these metals favor the metabolic activity of the biofilm. A summary of the results from the linear regression analyses can be found in Table 3.

3.2. Release of Metals from Biofilms

To evaluate the release of metals from the biofilms, samples were collected from the first liter of water and aerosols emitted during showering. Metal concentrations were compared between samples collected with and without each shower system. Interestingly, the concentrations of Ag and Pb were found to be below the limit of detection (LOD) in all samples, and Fe concentrations were below the limit of quantification (LOQ), even in shower systems where biofilms contained these metals. The concentration of Cu presented a high positive correlation with that of Zn (r = 0.995). Although our results suggest a tendency for Ca, Cu, and Zn to be released from biofilms into the water and Ca and Zn into the air (Figure 1), the observed differences were not statistically significant. However, the Cu and Zn concentrations showed a negative positive correlation with the total cell count in water samples (r = −0.926 and r = −0.897, respectively).

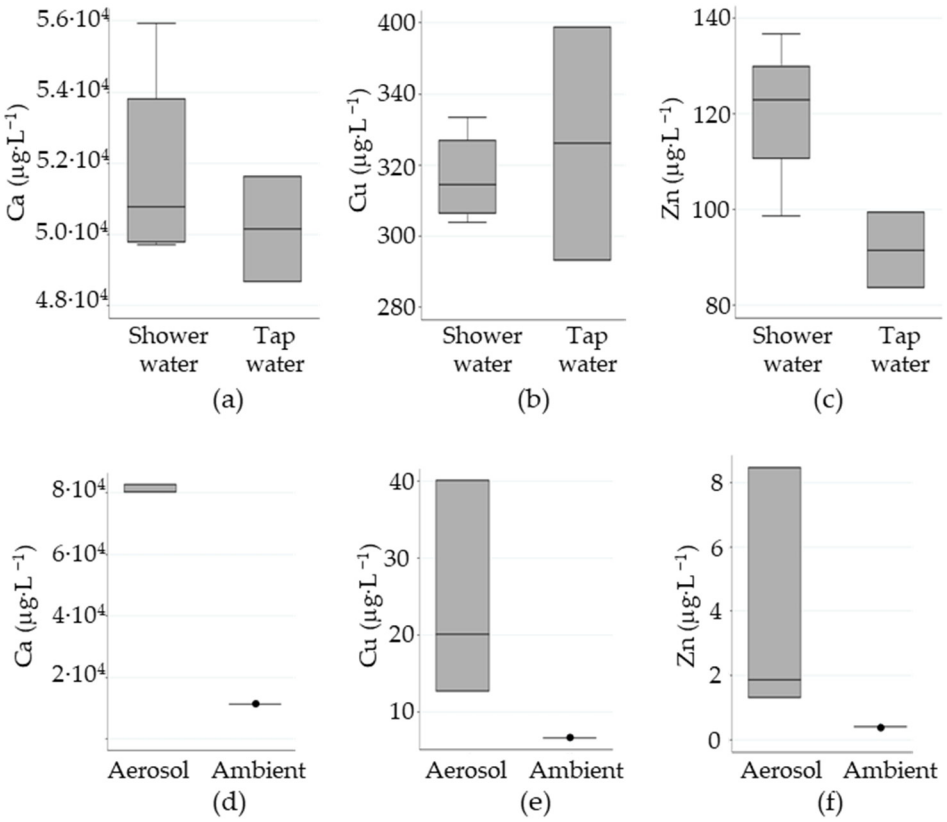

Figure 1. Variation in metals concentration in water (**a**–**c**) and aerosols samples (**d**–**f**). Metals concentration quantified in shower water and in the tap water for (**a**) calcium (Ca), (**b**) copper (Cu), and (**c**) zinc (Zn). Metals concentration quantified in the aerosols emitted during showering and in the ambient air for (**d**) calcium (Ca), (**e**) copper (Cu), and (**f**) zinc (Zn).

Table 3. Linear correlation coefficient *r* between metal concentration in samples and different biofilm determinants—age, total cell count, proportion of dead cells, and gDNA and ATP concentrations.

Metals Identified	Biofilm Age	Total Cell Counts	gDNA Concentration	ATP Concentration	Proportion of Dead Cells
Ca	0.737	0.435	0.604	0.081	0.159
Fe	0.601	0.869	0.945	0.471	0.653
Cu	0.976	0.856	0.949	0.567	0.505
Zn	0.974	0.812	0.908	0.728	0.563
Ag	0.642	0.840	0.911	0.386	0.575
Pb	0.950	0.866	0.950	0.459	0.691

4. Discussion

The findings of this study shed light on the significance of the observed metal concentrations in shower hose biofilms and their potential release into water and aerosols, which may have implications for the health of the users. Various metals were found to accumulate in mature biofilms of shower systems. However, only a few of them were released into the water and at limited concentrations, suggesting a low risk for users.

The potential for PVC to absorb metals has been demonstrated in various water systems. For example, studies have shown that PVC and PP particles in seawater exhibited higher absorbance of trace metals Pb, Cu, and Cd compared to other microplastics like polyamide (PA), PE, and polyformaldehyde [17]. Moreover, the adsorption of trace metals on microplastics can have significant effects on the structure and function of microbial communities within microplastic biofilms. This interplay between trace metals, microplastic surfaces, and biofilm-associated microorganisms can influence the further process of trace metal adsorption and accumulation [18].

The metabolic activity of microorganisms within the biofilm can also impact the fate of trace metals on microplastics. Microorganisms can produce extracellular polymeric substances (EPS) that act as binding sites for trace metals, enhancing metal sorption and retention on the microplastic surface. The differential accumulation rate of calcium compared to other metals, such as Fe, Cu, Zn, and Pb, suggests its preferential binding within biofilms during the early stages of biofilm formation. This aligns with previous studies that have reported calcium's affinity for binding in the extracellular matrix of biofilms [11]. Calcium plays a multifaceted role in biofilm dynamics and structure. Its ability to prevent biofilm dispersion or the detachment of cells from the biofilm matrix, has been well-documented [12]. The presence of calcium ions in the extracellular matrix strengthens the biofilm structure and acts as a binding agent, enhancing the cohesion and stability of the biofilm community [13]. This function of calcium is particularly crucial in the initial stages of biofilm development in shower hoses, where the establishment and aggregation of microbial cells are essential for biofilm formation and persistence. Moreover, calcium's ability to enhance biofilm aggregation and stability may contribute to the resilience of shower hose biofilms, making them more resistant to removal and disinfection. Therefore, further investigations into the interactions between calcium and other metals within biofilms will provide valuable insights into the ecological and functional implications of metal accumulation in shower hose biofilms, aiding in the development of targeted approaches for biofilm control and maintenance.

While the presence of calcium in drinking water is typically linked to water hardness, the occurrence of metals such as iron, copper, zinc, and lead in shower hose biofilms is commonly associated with the corrosion of plumbing systems [19]. Our study revealed significant regional variations in metal concentrations within shower hose biofilms, with notably high iron concentrations observed in biofilms from Nods (BE) compared to those from Lausanne. This regional disparity in metal availability can have a substantial impact on biofilm growth and composition, potentially influencing the prevalence of metal-accumulating bacteria in different locations and even the proliferation of pathogenic bacteria. The higher concentrations of metal, such as iron, in biofilms may promote the growth of metal-

accumulating bacteria, leading to distinct biofilm community structures and potentially influencing biofilm-related issues such as biofilm stability and resistance to disinfection treatments. For example, research has shown that increased iron concentrations can favor the growth of metal-accumulating bacteria, including members of the Acidobacteria phylum [11]. Additionally, zinc, manganese, and iron ions are known to play essential roles in bacterial metabolism and sporulation processes [11]. Moreover, iron and zinc concentrations have been identified to be essential in pneumococcal aggregation and early biofilm formation [20,21]. Thus, metal concentrations can alter the microbial dynamics and overall biofilm structure, which may have implications for water quality and increase the risk of exposure to bioaerosols, including potentially infectious organisms. Moreover, it is worth noting that low concentrations of certain metals, such as iron, zinc, or potassium, may also have implications for biofilm-related issues. Studies have demonstrated that low iron concentrations, for instance, can favor the proliferation of the pathogenic bacterium *Legionella pneumophila* in biofilms [14]. Therefore, regional variations in metal concentrations can impact biofilm development and composition in shower hoses, which, in turn, can affect water quality and increase the risk of exposure to bioaerosols, particularly those containing infectious microorganisms. In contrast to some previous findings [6], our study revealed significant correlations between metal accumulation and total cell count (TCC) in the biofilms. Specifically, concentrations of Fe, Cu, Zn, and Pb showed positive correlations with TCC, indicating that the presence of higher cell densities in the biofilms may contribute to the enhanced accumulation of these metals. This observation aligns with the notion that metal-accumulating bacteria, which are associated with higher cell counts, may play a key role in the uptake and retention of metals within the biofilm matrix.

Additionally, the release of metals from shower hose biofilms into the water during usage can have implications for human exposure and health, as it contributes to the overall metal content in water and aerosols generated during showering. The characterization of exposure to metals released from biofilms in shower systems is of significant importance as it provides insights into the potential risks associated with chronic metal exposure for the general population. Although our study demonstrated relatively low metal concentrations in the released water and aerosol samples, it is crucial to consider that biofilms have the capacity to accumulate much higher levels of metals than those described in our present study [6]. Moreover, previous research has indicated that metals accumulated within biofilms can be continuously released over time [5], potentially leading to chronic exposure to metals. Metal accumulation can occur at levels several orders of magnitude higher than the surrounding water, with subsequent release into the water flow [22]. This emphasizes the need for further investigation and comprehensive studies to better understand the potential risks associated with metal release from biofilms during showering.

To address the limitations of our study and provide a more robust assessment of exposure risk, future investigations should incorporate several key aspects. First, a more frequent and regular sampling approach for water and aerosols would enable capturing the dynamic nature of metal released from shower hose biofilms and its potential variations over time. This would provide a clearer understanding of the temporal patterns of metal accumulation and release in the shower system. Additionally, future studies should include a larger sample size encompassing diverse shower systems with a wider range of metals in water. By doing so, we can ensure a more comprehensive evaluation of the potential health risks associated with metal exposure during showering. Furthermore, expanding our knowledge in this area will inform strategies to mitigate potential health risks associated with metal exposure during showering, promoting safer practices for the general population.

The statement regarding the development of biofilm-resistant materials for shower hoses and the implementation of effective cleaning and maintenance protocols to reduce metal accumulation and subsequent release into the water flow is based on the general understanding of biofilm formation and management. While there is scientific evidence supporting the efficacy of biofilm-resistant materials and cleaning protocols in reducing

biofilm formation and its associated issues, specific studies on the reduction of metal accumulation and release in shower systems are limited. The development of biofilm-resistant materials aims to inhibit the initial attachment and growth of microorganisms, including bacteria that can contribute to metal accumulation. These materials often incorporate surface modifications or coatings that prevent biofilm formation or make the surface easier to clean. While studies have shown the effectiveness of biofilm-resistant materials in reducing overall biofilm formation and microbial colonization, their impact on metal accumulation, specifically in shower hoses, has not been extensively explored. Similarly, implementing effective cleaning and maintenance protocols can help minimize biofilm formation and remove accumulated materials, including metals. Regular cleaning practices, such as mechanical scrubbing or the use of disinfectants, can disrupt biofilm structures and reduce metal concentrations. However, the direct influence of cleaning protocols on metal accumulation and subsequent release in shower hoses has not been extensively studied. To support the affirmation that these approaches help reduce metal accumulation and release, further research specifically focused on shower systems and metal content is needed. Such studies would involve comparing the efficacy of biofilm-resistant materials and cleaning protocols in reducing metal accumulation in shower hoses, as well as assessing the impact on subsequent metal release into the water flow. These investigations would provide more robust scientific evidence to evaluate the effectiveness of these interventions in mitigating metal-related risks in shower systems.

Overall, while the concept of using biofilm-resistant materials and effective cleaning protocols to reduce metal accumulation in shower hoses is logical, further scientific studies are needed to validate their effectiveness, specifically in the context of metal release from shower hose biofilms. For instance, the development of biofilm-resistant materials for shower hoses and the implementation of effective cleaning and maintenance protocols have to be tested to see if they help to reduce metal accumulation and subsequent release into the water flow.

5. Conclusions

Overall, this study provides valuable insights into the composition and accumulation of metals in shower system biofilms. The significant correlations identified between metal concentrations and biofilm age, gDNA concentration, ATP concentration, and total cell count emphasize the complex interplay between biofilm dynamics and metal accumulation. Nevertheless, while our study revealed relatively low concentrations of metals in the released water and aerosol samples, it is important to acknowledge that biofilms have been reported to accumulate significantly higher levels of metals. By addressing the limitations of our study and conducting more comprehensive research, we can better inform strategies to mitigate potential health risks and ensure the safety of individuals exposed to metals during showering.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/pollutants3030027/s1. Table S1. Raw data generated in the study.

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