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RIGA TECHNICAL
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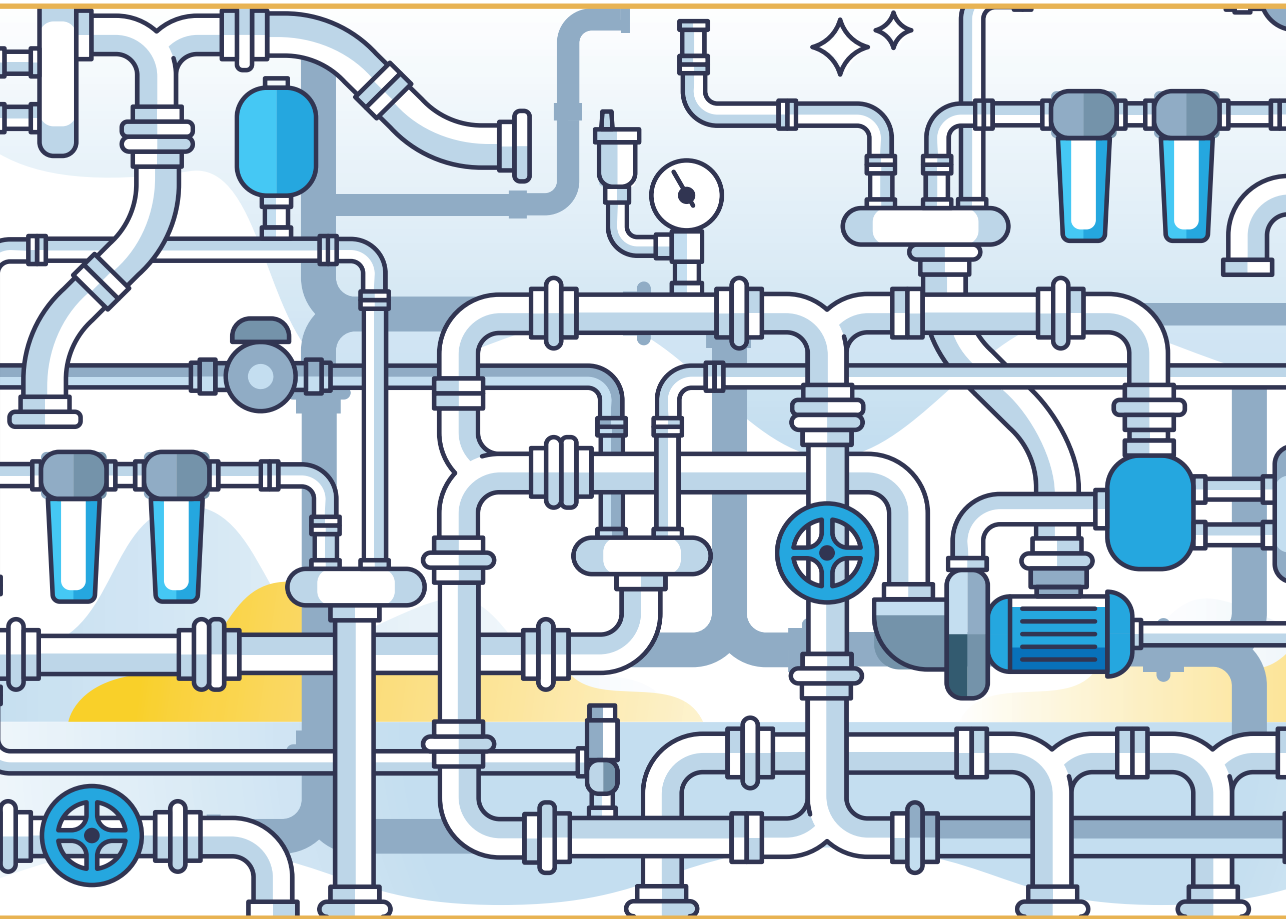
Marta Zemīte

EFFECT OF MICROBIALLY AVAILABLE PHOSPHOROUS LIMITATION ON MICROBIOLOGICAL WATER QUALITY IN INTERNAL DRINKING WATER SUPPLY

Summary of the Doctoral Thesis



RTU Press
Riga 2024

RIGA TECHNICAL UNIVERSITY
Faculty of Natural Sciences and Technology
Water Systems and Biotechnology Institute

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Scientific supervisor
Professor Dr. sc. ing.
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DOCTORAL THESIS PROPOSED TO RIGA TECHNICAL UNIVERSITY FOR PROMOTION TO THE SCIENTIFIC DEGREE OF DOCTOR OF SCIENCE

To be granted the scientific degree of Doctor of Science (Ph. D.), the present Doctoral Thesis has been submitted for defence at the open meeting of the RTU Promotion Council on 20 December 2024, at 13.00, at the Faculty of Natural Sciences and Technology of Riga Technical University, 6A Ķīpsalas Street, Room 143.

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DECLARATION OF ACADEMIC INTEGRITY

I hereby declare that the Doctoral Thesis submitted for review to Riga Technical University for promotion to the scientific degree of Doctor of Science (Ph. D.) is my own. I confirm that this Doctoral Thesis has not been submitted to any other university for promotion to a scientific degree.

Marta Zemīte ……………………………. (signature)
Date: ………………………

The Doctoral Thesis has been written in English. It consists of an Introduction, 7 chapters, Conclusions, 34 figures, and 21 tables; the total number of pages is 112. The Bibliography contains 130 titles.

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GENERAL OVERVIEW OF THE THESIS

Relevance of the study

Ensuring the provision of safe drinking water from source to tap is a fundamental public health objective. One widely accepted approach to achieving this is through the concept of biologically stable water (van der Wielen *et al.*, 2023). This approach focuses on controlling key nutrients, such as carbon – typically in the form of assimilable organic carbon (AOC) – and phosphorus, in the form of microbially available phosphorus (MAP), to maintain microbial stability within water distribution systems.

Phosphorus, being one of the major macrobiogenic nutrients essential for bacterial growth, plays a critical role in managing microbial proliferation. Its reduction is considered an effective measure to control microbial growth and subsequently reduce biofilm formation (Vrouwenvelder *et al.*, 2010), even in environments where carbon is plentiful. However, limited research has been conducted on how MAP limitation specifically influences microbial water quality at the consumer level. This is particularly relevant in internal drinking water supply systems, where a range of factors – including elevated temperatures, overnight water stagnation, dead-end pipes, high surface-to-volume ratios, and inadequate maintenance – can exacerbate water quality problems (Prest *et al.*, 2016b).

In Latvia, 65 % of residential buildings were constructed between 1961 and 2000, with 40 % built before 1980 (Centrālā statistikas pārvalde, 2021). While building managers may have replaced water mains over time, typically upgrading from iron pipes to plastic, internal apartment plumbing is the responsibility of individual owners. In practice, these internal systems are rarely maintained, which can increase the risk of opportunistic premise plumbing pathogen (OPPP) proliferation. Notably, higher levels of *Legionella* – one of the most common OPPPs (LeChevallier, Prosser and Stevens, 2024) – have been detected in residential apartment buildings compared to public buildings in Latvia (Valciņa *et al.*, 2019).

The central question of this study is whether reducing MAP levels in incoming water can improve the safety of water distribution in ageing residential infrastructure. Given the growing concern over OPPPs, such as *Legionella*, which remains a preventable threat in domestic plumbing, controlling nutrient levels within internal plumbing systems could be crucial for ensuring safe drinking water.

Objective and tasks

The **overall objective** of the Doctoral Thesis was to evaluate the impact of MAP limitation on enhancing water safety at a consumer level within the internal drinking water supply system.

The main **tasks** included:

1. **Adjustment of MAP quantification protocols** to suit local conditions.
2. **Determination of MAP reduction potential** of easy-to-implement treatment methods.
3. **Adjustment of the selected method** to ensure safe use within the drinking water supply system.

4. **Analyses of water usage patterns and incoming water quality characteristics** in residential buildings.
5. **Evaluation of MAP reduction potential of point-of-use filtration device** within the internal drinking water supply of a multi-storey residential building.
6. **Analyses of water quality changes** in both cold and hot internal drinking water supply systems.
7. **Assessment of centralised chemical flushing and disinfection** on *Legionella* prevalence.
8. **Evaluation of the impact of MAP reduction** on microbial water quality in internal drinking water distribution, using culturable *Legionella* bacteria counts and characteristics as an indicator of changes in the water safety level.

Scientific novelty and practical application

The **scientific novelty** of the Thesis lies in the implementation of an on-site MAP removal technology in a dynamic, real-world environment and the assessment of its effect on cultivable *Legionella* spp. Here, the author challenges the current concept of biostability, which has traditionally been narrowly defined from a nutrient perspective without considering microbial competition and the carrying capacity of the environment. To the author's knowledge, no previous study has addressed this interaction in a full-scale setting.

The hypothesis of this study is that limiting MAP, a nutrient essential for bacterial growth, will enhance the biostability of the internal hot drinking water supply and inhibit the growth of potentially pathogenic bacteria, specifically *Legionella* spp.

If the hypothesis is confirmed, **the practical implication** is the potential to adjust *Legionella* control measures – not only through temperature regulation but also by managing nutrient availability. This approach may allow for less energy-intensive strategies to prevent the regrowth of unwanted OPPPs.

Structure and scope

The Tesis consists of an Introduction, Background, Materials and Methods, five sections with Results and Discussion and the sections' Conclusions, addressing the main tasks, and general Conclusions. The scope of each section is as follows:

Section 1. The literature review, providing the **theoretical background**.

Section 2. **Materials and methods** used throughout the study.

Section 3. Adaptation and **adjustment of the MAP quantification protocol** to quantify the fraction of phosphate that is easily accessible to microorganisms.

This section primarily reflects the content of the paper "Approbation of microbially available phosphorus (MAP) determination method by flow cytometry" (Frolova *et al.*, 2017b) and includes unpublished calibration data with natural inoculum, supplemented with statistical analyses.

Section 4. Study on groundwater **MAP reduction potential** using electrocoagulation, biofiltration, and iron oxide sorption. This section evaluates the suitability of relatively simple methods for use in the pilot study and the **adjustment of the selected method** to maximize system robustness and material safety within an existing drinking water supply system.

This section primarily reflects the paper "Evaluation of pre-treatment technologies for phosphorous removal from drinking water to mitigate membrane biofouling" (Frolova *et al.*, 2017a) and, to a small extent, the content of the paper "Affordable pretreatment strategy for mitigation of biofouling in drinking-water systems" (Zemite *et al.*, 2022), with additional unpublished data.

Section 5. Assessment of **water usage patterns** and **incoming water quality characteristics**.

This section partially reflects the paper "Effect of microbially available phosphorous removal on Legionella spp. in multi-storey residential dwellings in Latvia" (Zemīte *et al.*, 2023) and includes unpublished data on water consumption and fluctuations in the electrical conductivity of inlet water, determined by an in-line probe.

Section 6. Evaluation of **MAP removal filter efficiency** and **water quality changes within the internal drinking water supply**.

This section partially reflects the paper "Effect of microbially available phosphorous removal on *Legionella* spp. in multi-storey residential dwellings in Latvia" (Zemīte *et al.*, 2023), with additional unpublished data on water quality differences between inlet water and outlet sampling points.

Section 7. Analyses on the **effectiveness of *Legionella* control measures, which included centralised chemical flushing and disinfection and MAP concentration reduction from the inflow water**.

This section primarily reflects the paper "Effect of microbially available phosphorous removal on *Legionella* spp. in multi-storey residential dwellings in Latvia" (Zemīte *et al.*, 2023).

1. BACKGROUND

Health-based targets ensure the provision of water that is safe for use without posing any health risks, such that is free from contaminants and pathogens (WHO, 2022). Although drinking water leaving water treatment facilities is typically of high quality, it is often subjected to secondary contamination during distribution within both external and internal water supply (Lehtola *et al.*, 2004, 2007; Zimoch and Paciej, 2020; Zimoch, Parafiński and Filipek, 2023). Household water quality is influenced by similar factors to those in external distribution systems, but internal water supplies often face more extreme conditions, including lower disinfectant residuals, higher temperatures, longer residence times, a wider variety of pipe materials, and significantly smaller pipe diameters compared to external networks (Prest *et al.*, 2016b), in such a way providing high surface-to-volume ratio and more favourable conditions for biofilms to develop and facilitate water quality deterioration.

To enhance drinking water safety, several water utilities in countries like the Netherlands, Switzerland, Denmark, Germany, and Belgium are focusing on the provision of biologically stable water (van der Wielen *et al.*, 2023). Biostable water is characterised by its inability to promote the growth of microorganisms during distribution, thereby protecting consumer safety, maintaining aesthetic water quality, and preventing technical failures at any stage of distribution (Rittmann and Snoeyink, 1984; Lautenschlager *et al.*, 2013; Prest *et al.*, 2016b).

The applied techniques to facilitate biostable water supply include integrated measures, e.g., end-point chlorination to inhibit bacterial growth, and nutrient limitation – particularly the limitation of organic carbon and phosphorus (Prest *et al.*, 2016a), which are essential for microbial growth. Nutrient control becomes especially critical during seasonal temperature changes, which can disturb biostability and increase bacterial growth in distribution systems (Nescerecka, Juhna and Hammes, 2018). The optimal stoichiometric molar ratio for microbial growth is approximately 100 C : 10 N : 1 P (LeChevallier, Schulz and Lee, 1991). Even though phosphorus constitutes only around 3 % of a bacterial cell`s dry mass (Kushkevych, 2022), it is vital for essential biological functions such as energy metabolism, membrane integrity, and genetic material inheritance (Santos-Beneit, 2015).

Though nutrient limitation for the decrease of bacterial growth in water supply has been extensively studied, phosphorus-related research remains limited. Partly, it is attributed to the fact that growth-promoting nutrients are place-specific and can vary even within one distribution network (Sathasivan and Ohgaki, 1999; Nescerecka, Juhna and Hammes, 2018). The fraction of phosphorus that can be easily utilised by microorganisms is called microbially available phosphorus (MAP), and it is quantified using bacterial bioassay (Lehtola *et al.*, 1999). MAP availability in drinking water network is dependent on several aspects, including the drinking water source, applied treatment train, and even seasonality, which can influence MAP reduction during water treatment (Lehtola *et al.*, 2002; Jiang, Chen and Ni, 2012; Nescerecka, Juhna and Hammes, 2018). Hydraulic retention time has been shown to reduce MAP concentrations during distribution (Jiang, Chen and Ni, 2011), while materials like new cross-linked polyethylene (PEX) pipes can leach phosphorus into the water (Inkinen *et al.*, 2014).

Moreover, MAP can enter systems through phosphate-based corrosion inhibitors and antiscalants, promoting biofilm formation (Vrouwenvelder *et al.*, 2010).

Various water treatment processes can either increase or decrease MAP concentrations. For instance, ozonation, disinfection, and liming can raise MAP levels, while chemical coagulation, granular activated carbon filtration, and soil filtration (or artificial re-charge) can reduce them (Lehtola *et al.*, 2001, 2002; Wen *et al.*, 2014). MAP consists of both soluble and particulate phosphorus, with treatment methods affecting the ratio of MAP to total phosphorus (Jiang, Chen and Ni, 2012; Wen *et al.*, 2014).

In internal water distribution systems, opportunistic premise plumbing pathogens (OPPPs) are a concern, particularly for individuals with compromised immune systems. OPPPs are both pathogens and normal inhabitants of drinking water that are adapted to growth and persistence in potable water plumbing (Falkinham, 2015).

Legionella pneumophila is one of the most widely known OPPPs. Legionnaires` disease remains a preventable health threat in Europe. It can be acquired through the inhalation of water aerosols containing *Legionella* bacteria. This can lead to severe pneumonia or a milder flu-like illness known as Pontiac fever (Fields, Benson and Besser, 2002). In 2021, the EU/EEA reported a total of 19 outbreaks, with 137 confirmed cases. Males over 65 years old were most affected, with a rate of 8.9 cases per 100 000 population (European Center for Disease Prevention and Control, 2023). Moreover, some models predict that only around 10 % of Legionnaires` diseases are being diagnosed (Cassell *et al.*, 2019).

Latvia had one of the highest rates (≥ 3.00 per 100 000) of Legionnaires` disease in Europe in 2021 (European Center for Disease Prevention and Control, 2023). The most common species found here are attributed to *Legionella pneumophila* (Valciņa *et al.*, 2019), which in general are responsible for around 90 % of clinical infections (Chauhan and Shames, 2021). Moreover, in Latvia, higher levels of *Legionella* have been observed in apartment buildings if compared to public buildings (Valciņa *et al.*, 2019). A further study reported the presence in 112 out of 200 samples from apartment buildings (56 %), and analyses of 58 isolates revealed 420 virulence genes, indicating high prevalence, extensive genetic diversity, and a wide range of virulence of *Legionella* in residential buildings (Valciņa *et al.*, 2023).

Reducing phosphorus, particularly MAP fraction, in water systems could help limit biofilm formation and control microbial growth, including that of *Legionella*. By limiting nutrient availability, phosphorus reduction has the potential to curb the proliferation of both planktonic microorganisms and biofilms, which could, in turn, contribute to more effective *Legionella* control within water distribution networks.

2. MATERIALS AND METHODS

2.1. Quantification of microbially available phosphorus

Microbially available phosphorus (MAP) was quantified by an adjusted bioassay method (Lehtola *et al.*, 1999; Wen *et al.*, 2016). The samples were subjected to filtration (0.2 μm), pasteurised for 1 h at 60 °C, and amendment with acetate-carbon and salts stocks. Further, samples were inoculated with 10^3 cells ml^{-1} and incubated at 30 °C with continuous shaking at 150 RPM for 5 days and then enumerated. All samples were prepared in triplicates. Disodium hydrogen phosphate (Na_2HPO_4) stock solution was used for calibration standards preparation.

For inoculum, either a single culture, specifically *Pseudomonas brenneri* P17 (ATCC 49642), or a mixed bacterial culture from a freshly opened bottle of natural mineral water (Evian, Danone, France) was used. For enumeration, either the heterotrophic plate count method or flow cytometry was used.

2.2. MAP reduction potential

The set of experiments aimed to evaluate the effectiveness of several cost-effective and easy-to-implement methods for removing MAP from drinking water. These methods included sorption, biofiltration, and electrocoagulation, and the study was conducted in three phases.

In the first phase, polyvinyl chloride (PVC) reactors with metal fittings (diameter: 7.5 cm, height: 50 cm) were set up at an artificially recharged groundwater station in Baltezers, Riga (Fig. 2.1). The **sorption reactor** contained plastic biomass carriers (Bioflow 9, RVT Process Equipment, Germany) naturally coated with ferric oxides from groundwater iron removal. The **biofiltration reactor** used similar biomass carriers covered with biofilm, cultivated for a month using a 1/3 river water (Daugava River) and 2/3 tap water mix. The blend was placed in sterilized glass bottles, covered with aluminium foil to prevent algal growth, and shaken weekly. The **electrocoagulation reactor** had four aluminium electrodes (60/60 grade), spaced 10 cm apart, with a combined surface area of 622 cm^2, powered by a laboratory power supply attaining a current density of 4 mA cm^{-2}.

Further, the effect of temperature and additional substrate on biofilter performance was evaluated (Fig. 2.2). As groundwater temperature is suboptimal for bacterial growth, increased temperature conditions were tested alongside the addition of acetate carbon to the biofilter to assess potential improvements in MAP removal efficiency.

Finally, the selected MAP removal method was tested in a scaled-up laboratory setup (Fig. 2.3), using commercially available materials certified for use in drinking water treatment processes.

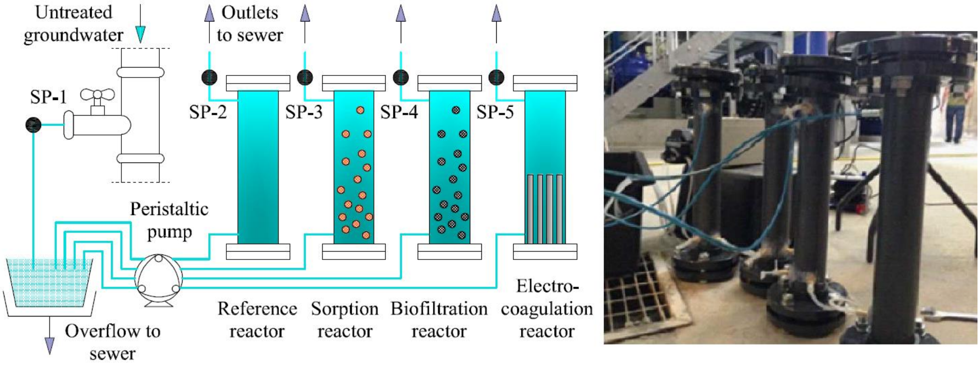

Fig. 2.1. Plug-flow configuration setup at an artificially recharged groundwater station in Baltezers, Riga, with marked sampling places (SP).

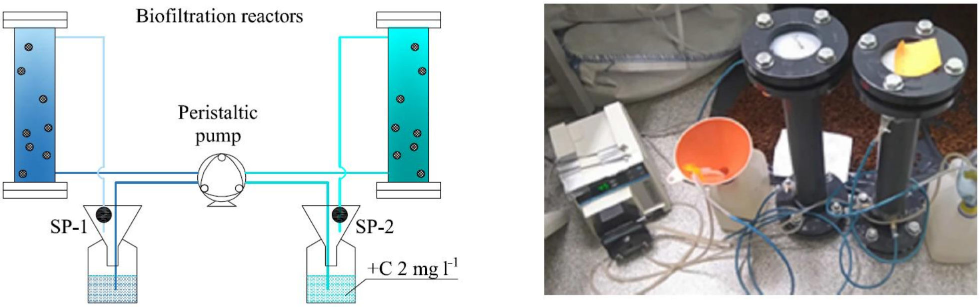

Fig. 2.2. Recirculation configuration setup for Biofiltration reactors with marked sampling places (SP).

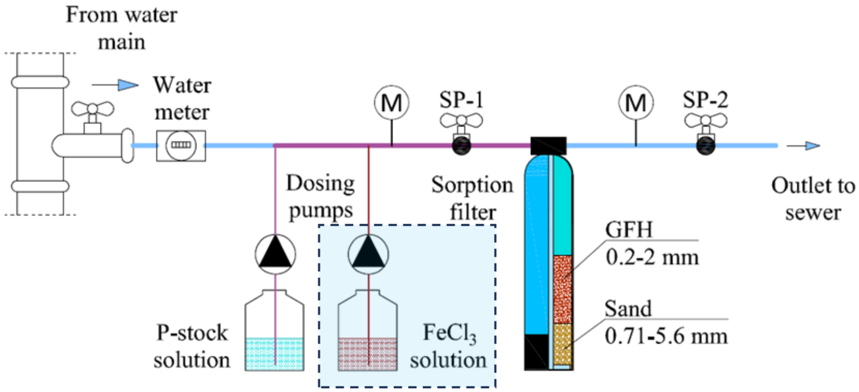

Fig. 2.3. Laboratory-scale filtration setup with granular ferric hydroxide (GFH) sorption filter with phosphate dosing and optional $FeCl_3$ coagulant dosing system for phosphorus (P) removal with marked sampling places (SP).

2.3. Case study

A pilot site consisted of two five-storey residential buildings within a proximity of around 100 meters. The internal water supply consisted of around 22-year-old polypropylene pipes conducting groundwater from the municipal water supply. To facilitate a similar starting point, both buildings were subjected to centralised chemical flushing and disinfection of internal water pipes. Initially, to remove the deposits, the domestic hot water (DHW) network was purified by an acidic phosphate-free reagent, with formic acid as an active component (ALBILEX®-KALK-EX, Germany). Subsequently, a disinfectant (ALBILEX®-SUPER-des, Germany) consisting of hydrogen peroxide and silver ions was introduced into the incoming cold water in such a way ensuring disinfection of both cold and hot drinking water systems.

Hot water was prepared by plate heat exchangers in individual heating substations located in the basement of each building. During the weeks 0–14, the heat exchanger was set to 57 °C. Then, along with the start of a heating season in mid-October 2022, the temperature setpoint was changed to a dynamic as an energy-saving measure during the energy crisis. This regime consisted of three interchanging temperature settings: 48 °C Monday through Friday at night-time (23:00–7:00), following 52 °C during the remaining hours and 57 °C during the weekends.

One of the buildings was equipped with a point-of-use (POU) filtration device for the reduction of MAP at the water inlet (Fig. 2.4) and denoted as “POU-device building”. The other one was used for comparison and denoted as “Reference building”.

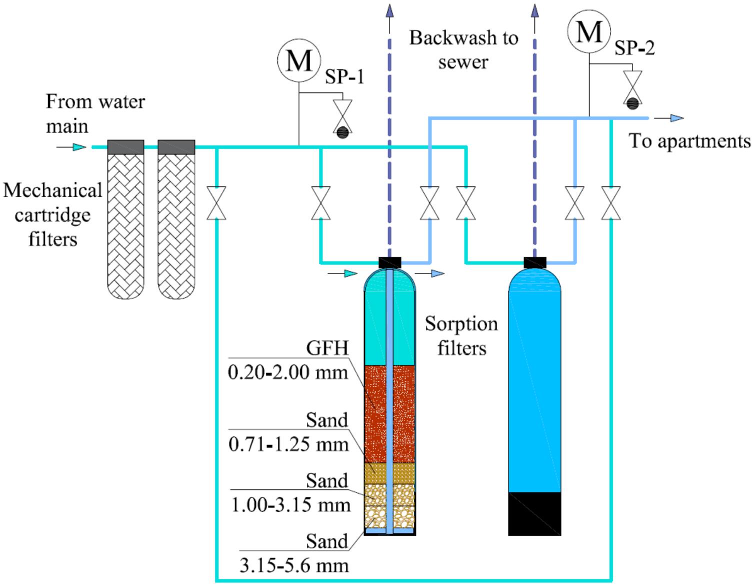

Fig. 2.4. The schematic of POU granular ferric hydroxide (GFH) sorption filters connected after existing cartridge filters with sampling places (SP) shown as black dots and mechanical manometers to monitor filter clogging (M). Figure adapted from (Zemīte *et al.*, 2023).

Within each building, there were four distinct sampling locations, such as kitchen taps as representative of the domestic cold water (DCW) system, showerheads and circulation return water before re-entry into the heat exchanger as domestic hot water (DHW) samples and the water inlet after the entry into the building. Additionally, in POU-building, the samples, after passing through the sorption filter, were collected. The first-draw samples from apartment sampling locations were collected by residents, following sampling protocol. Overall, all samples were mainly collected on the same day, with only rare exceptions due to the availability of inhabitants. The POU-device building had five apartments participating in sample collection: three on the 5th floor, one on the 2nd floor, and one on the 1st floor. In the Reference building, samples were collected from three participating apartments: two on the 5th floor and one on the 1st floor.

2.4. Analytical methods

Temperature was measured on the pilot site directly after sample collection. Such parameters as electrical conductivity, pH, total organic carbon (TOC), bacterial cell concentrations, and MAP concentration were determined in the laboratory of Water Systems and Biotechnology Institute by the author. During the pilot study, the concentration of Ca, Mg, Mn, Cu, Zn, Fe, and Pb, as well as cultivable colony counts and characteristics of *Legionella* spp. were determined by the Institute for Food Safety, Animal Health and Environment “BIOR” (Riga, Latvia).

2.5. Statistical analyses and data presentation

Statistical analyses were performed using IBM SPSS Statistics (v. 23), with a 2-tailed significance level of $\alpha = 0.05$. Independent sample t-tests were used for normally distributed data, while the Mann-Whitney U test was applied for non-normally distributed data. Dependent sample analyses in the sorption optimisation study employed paired t-tests for normal data, or Wilcoxon Signed Ranks tests for non-parametric data. Standard curve assessments were performed in MS Excel, while parametric correlations using Pearson's coefficient were also performed in SPSS.

Data were presented following SAMPL guidelines (Lang and Altman, 2015), showing mean value with standard deviation (SD) for normal distributions and medians (min-max) for non-normally distributed data. Graphical visualisation was done in MS Excel, Origin Pro 2019, and SPSS.

3. RESULTS AND DISCUSSION

3.1. MAP quantification using bioassay

Heterotrophic plate culture (HPC) and flow cytometry (FCM) enumeration methods, alongside the use of pure cultures and natural mixed bacterial inoculum, were evaluated for the quantification of microbially available phosphorus (MAP) under local conditions.

Comparison of cell-growth enumeration methods. HPC and FCM cell enumeration methods showed a statistically significant correlation with $R^2 = 0.996$. FCM was more robust due to its ability to quantify total cell concentration, enabling sample analysis at any point during the inoculum's stationary growth phase. In contrast, HPC required specific sampling times, limiting its flexibility, as well as it was significantly more time-consuming.

Impact of inoculum. FCM enumeration allowed for the use of natural inoculums, which encompass a diverse microbial community capable of utilising MAP to a greater extent. Under local conditions, the obtained yield factor using natural mineral water was 3.0×10^9, more than ten times higher than that obtained with pure cultures (Table 3.1), thereby significantly enhancing the sensitivity of the method. However, bioassays using natural consortia exhibited reduced consistency over time and across sample replications, necessitating the implementation of replicate data selection rules to enhance precision. To minimise the spread of measured total cell count (TCC) values and ensure more reliable conversion to MAP concentrations, it was chosen to be excluded from the calculation of the sample mean such sub-samples, where total cell count varied by more than 30 % logarithmic difference from the remaining two sub-samples of the triplicate assays.

Table 3.1

Microbial Yields of Pure and Mixed Culture Inoculums for MAP Assays

Inoculum	Yield factor	Enumeration	Reference
Ps. brenneri P17	1.3×10^8	HPC	This study
Ps. brenneri P17	3.2×10^8	HPC	(Polanska, Huysman and Van Keer, 2005)
Ps. brenneri P17	3.7×10^8	HPC	(Lehtola *et al.*, 1999)
Ps. brenneri P17	1.1×10^9	HPC	(Jiang, Chen and Ni, 2011)
Ps. brenneri P17	1.6×10^8	FCM	This study
Ps. brenneri P17	1.8×10^8	FCM	(Wen *et al.*, 2016)
Evian	9.4×10^8	FCM	(Wen *et al.*, 2016)
Evian	3.0×10^9	FCM	This study

3.2. MAP reduction methods

Three methods – electrocoagulation, sorption on iron-based media, and biofiltration – were tested for the removal of microbially available fraction of phosphorus (MAP). Further, the selected method was adjusted to ensure safe use within the drinking water supply of a pilot study building.

Efficiency of MAP removal in column reactors. Electrocoagulation achieved near-complete MAP removal (Table 3.2) but had several technical limitations, including significant sludge generation and the need for a backup power system to facilitate continuous operation in case of power shortages.

Biofiltration performed poorly under natural groundwater temperatures. However, at elevated water temperature, it was able to remove, on average, around 60 % of MAP, without further significant improvement after the addition of carbon (Mann-Whitney U test, $p = 0.686$).

Sorption, using iron-coated plastic carriers (a waste by-product from groundwater iron removal), achieved over 70 % MAP removal. As a relatively effective and easy-to-implement and maintain method, it was further adjusted to ensure safe use within a pilot setting.

Table 3.2

MAP Elimination Potential of Selected Treatment Methods

Method	Duration, days	No. of samples	Operation mode	Water temp., °C	Min / average / max MAP elimination, %
Electro-coagulation	17	7	PFM	8.1 ± 0.1	76 / 96 / 100
Sorption	17	9	PFM	8.1 ± 0.1	32 / 71 / 87
Biofiltration	17	9	PFM	8.1 ± 0.1	7 / 29 / 72
Biofiltration	1.9	6	RM	19.2 ± 0.3	0 / 61 / 92
Biofiltration	1.9	6	RM, + C	19.5 ± 0.5	11 / 68 / 88

PFM – plug-flow mode, RM – recirculation mode, +C – amended with acetate carbon (initial concentration 2 mg l^{-1}).

Sorption optimisation for use within the drinking water supply. Firstly, a certified iron-based sorption material was selected from commercially available solutions. Then it was assessed within a semi-pilot installation in a laboratory setting. Lastly, to evaluate a potential improvement of P removal efficiency, as well as provide a second barrier, an addition of $FeCl_3$ coagulant was monitored. For enhanced test speed and the possibility of rapid detection, phosphate was dosed into the system and analysed as orthophosphate P.

GFH sorption filter removed on average 94 % of dosed PO_4-P (Fig. 3.1), ensuring reduction from 0.55 mg l^{-1} to 0.01 mg l^{-1}. No additional improvement in PO_4-P removal was observed after the application of in-line chemical coagulation, which also increased system complexity and reduced robustness that was needed for nearly maintenance-free operation.

Consequently, a granular ferric hydroxide sorption filter was selected as an easy-to-implement, low-maintenance, point-of-use MAP removal unit for use in a pilot-scale setting.

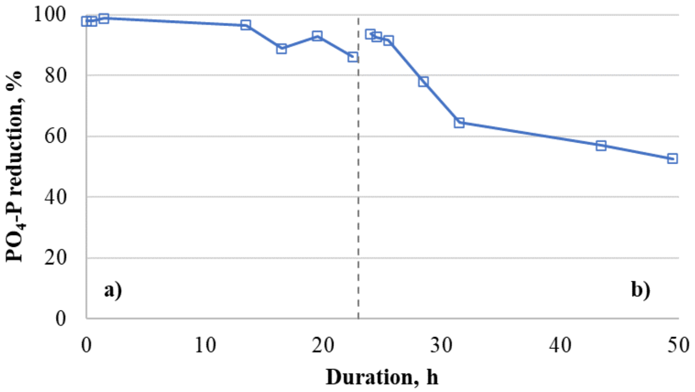

Fig. 3.1. PO$_4$-P removal efficiency of granular ferric hydroxide sorption a) without and b) with FeCl$_3$ coagulant addition.

3.3. Characteristics of inlet water

To assess whether both pilot buildings consumed water similarly, water usage patterns and volumes were analysed using the data from the inlet water meter, recorded by impulse reader (Zenner, Germany) on a portal with a timestep of 20 min by Metbox GSM/GPRS Remote Telemetry Unit (Teliko, Latvia). Also, to assess if both buildings received water with similar characteristics, the electrical conductivity (EC) probes (Comeco Control & Measurement, Bulgaria) were mounted in the water inlet main, and the readings were recorded every 10 min by GSM IoT Wireless Datalogger (COMET System, Czech Republic), as well as inlet water grab-samples were analysed for a set of water quality parameters.

Unevenness of water consumption. Both five-storey residential buildings exhibited typical similarly scattered variations in daily water consumption, as expected for internal water supply systems. However, the POU-device building consumed more water during the summer months (Fig. 3.2), resulting in a total of 250.6 m^3 greater water consumption at the end of the testing period compared to the Reference building.

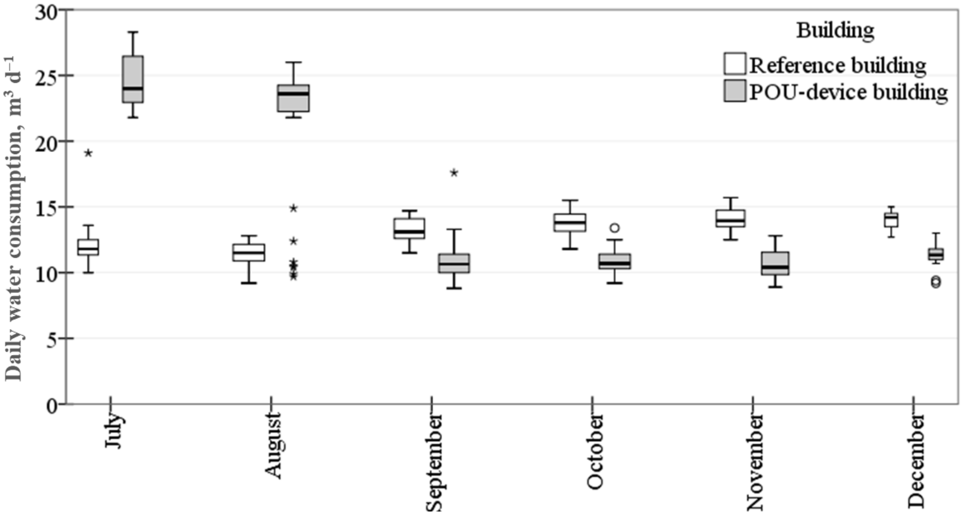

Fig. 3.2. Variations in daily water consumption for Reference and POU-device buildings. Dots represent outliers, and stars – extreme outliers. The width of the boxes is scaled to the number of cases (n = 13–31).

Mixed source water supply. The EC of incoming water fluctuated significantly both throughout the day (Fig. 3.3) and over the entire testing period, indicating a mixed water source supply. According to hydraulic modelling performed by the water provider, the water sources corresponded to artificially recharged groundwater plant “Baltezers”, which utilises biological iron and manganese removal with post-chlorination, and groundwater pumping stations “Zaķumuiža” and “Remberģi” that provide only chlorine disinfection without a necessity of any other treatment steps.

The higher EC values at the POU-device building’s inlet suggest potential differences in water origin between the buildings (Mann-Whitney U test, $p < 0.05$), although the EC parameter alone could not definitively determine the water source.

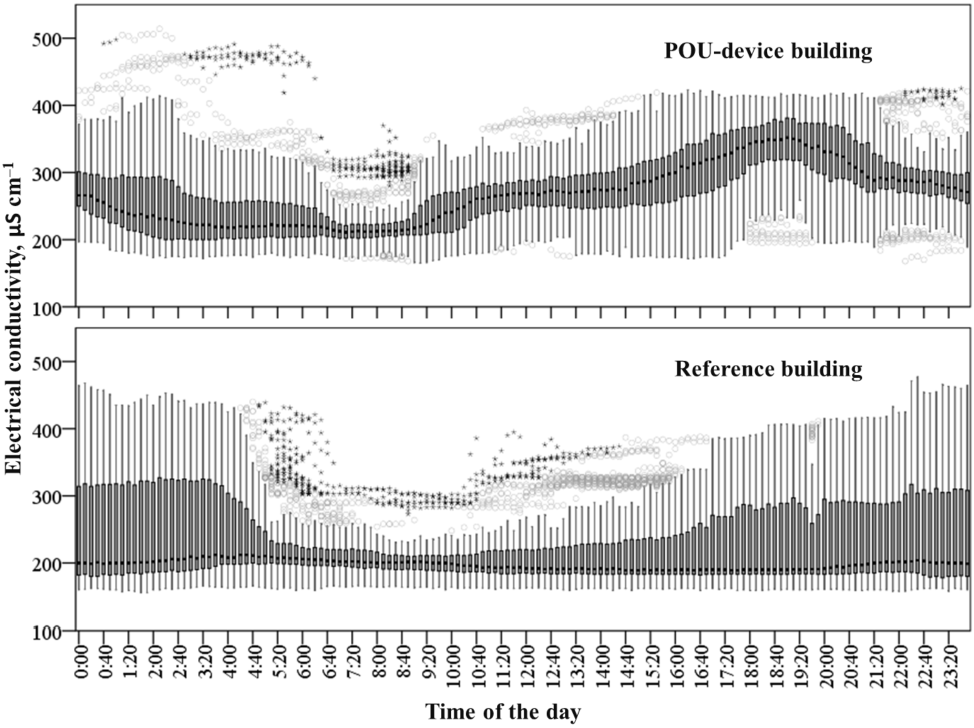

Fig. 3.3. Hourly variations in electrical conductivity values for the duration of 22 weeks in POU-device (top) and Reference (bottom) buildings. Dots represent outliers, and stars – extreme outliers.

Influent water composition. The analysis of grab samples, although most likely linked to a unique mixture of water from several sources that was present in the network at the time of sampling events, revealed seasonal variations in temperature, EC, and, to a small extent, also in pH. However, concentrations of Ca, Mg, Mn, Cu, Zn, Fe and Pb remained relatively stable. The POU-device building received slightly higher concentrations of calcium (by 3.23 mg l^{-1}) and magnesium (by 1.95 mg l^{-1}) but had lower copper levels (by 4.15 µg l^{-1}) compared to the Reference building.

Total organic carbon levels spiked in July but remained relatively constant thereafter, with the POU-device building showing a median TOC value 0.93 mg l^{-1} higher than the Reference building. MAP concentrations fluctuated throughout the sampling period, potentially indicating variability in MAP content from different water sources, with levels ranging from 3.3 µg l^{-1} to 16.3 µg l^{-1}, and being similar across both buildings.

Bacterial analysis showed a relatively stable ratio of LNA and HNA content cells over time, but an increasing proportion of damaged cells was observed, particularly in the POU-device building. *Legionella* was detected infrequently, with a maximum concentration of 400 CFU l^{-1}. Despite infrequent detection, the presence of *Legionella*, combined with potential underestimation from grab sampling, indicates periodic opportunistic premise plumbing pathogen inputs from municipal cold water mains. **In summary**, while both buildings exhibited similarly scattered water consumption patterns, the analysis revealed variability in overall water usage volume and incoming water quality characteristics, suggesting potential differences in inlet water sources between the buildings. This variability adds complexity to the study, introducing an additional unknown for pilot-scale analyses and complicating further evaluations. Such mixed water supplies could potentially serve as a nutrient exchange pool, influencing water quality within the internal supply systems, which may differ between the two studied buildings. Future studies should address the additional impact of such dynamic conditions on the overall safety of the water supply.

3.4. Water quality in internal drinking water supply

This section addressed the efficiency of POU sorption device performance and overall changes in inlet water composition compared to the samples collected at the outlets.

MAP reduction from inflow by ferric hydroxide sorption filter. A POU granular ferric hydroxide sorption filter was installed on the water main of a five-storey residential building to remove microbially available phosphorus (MAP) from the municipal water supply. The filter consistently removed MAP by an average of 70 % (Fig. 3.4), resulting in a mean concentration of 3.56 µg MAP l^{-1} (SD 1.5 µg l^{-1}) entering the internal distribution networks. It did not significantly affect other water quality parameters, except for iron, which was primarily reduced. However, most likely due to hydraulic fluctuations, occasional re-release of iron from the filter outlet into the water supply was observed.

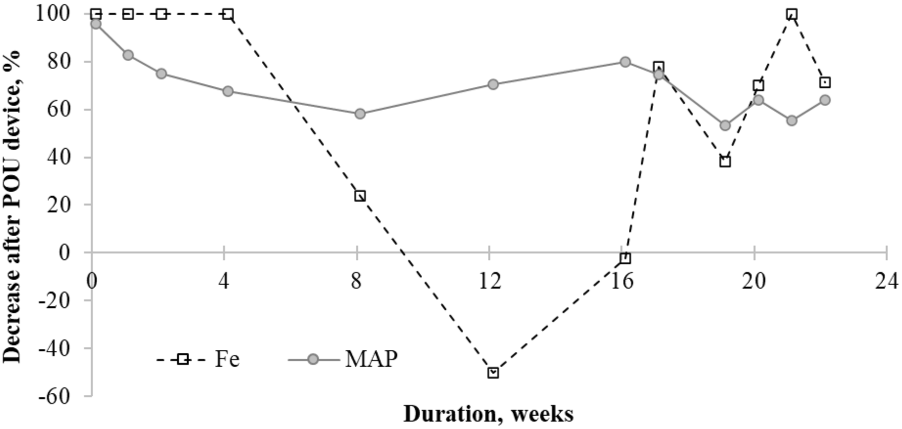

Fig. 3.4. Microbially available phosphorus (MAP) and iron reduction from inlet water by Point-of-use (POU) sorption filter. Points represent the average values of sample triplicates. Figure adapted from (Zemīte *et al.*, 2023).

Water quality changes within internal networks. The internal drinking water supply consists of two distinct systems: domestic cold water (DCW) and domestic hot water (DHW). The DHW system often includes a circulation line to reduce the time it takes for hot water to replace cooled water at the point of use. Drinking water entering internal networks was subjected to a change in several parameters (Table 3.3).

Table 3.3

Change in Inlet Water Quality Parameters ($p < 0.05$) During Internal DHW Supply (grab samples)

Parameter	POU-device building, showerhead	POU-device building, circulation	Reference building, showerhead	Reference building, circulation
Physical and chemical parameters				
Temperature	↑	↑	↑	↑
pH	→	↑	→	↑
Electrical conductivity	→	→	→	→
Ca	→	→	→	→
Mg	→	→	→	→
Mn	→	→	(↓)	→
Cu	↑	↑	↑	↑
Zn	↑	↑	↑	↑
Fe	(→)	↑	↓	↓
Pb	↑	(→)	↑	↑
Main microbial nutrients				
TOC	→	→	→	→
MAP	↓	↓	↓	↓
Microbiological parameters				
Total Cell Count	↑	→	↑	→
Damaged Cell Count	↑	→	↑	→
Intact Cell Count	↑	→	↑	→
Low Nucleic Acid content cells	↓	↓	↓	↓
High Nucleic Acid content cells	↑	↑	↑	↑
L. pneumophila	↑	↑	↑	↑

Brackets show potential attribution to outliers, as a relatively large difference in maximal value in comparison to the median was noted. No outliers were excluded due to the limited dataset size.

In the **DCW system**, water temperature increased significantly ($p < 0.001$) overnight due to thermal equalisation with ambient temperatures, which can negatively impact water quality by promoting microbial growth. While pH slightly decreased during distribution, electrical conductivity remained unchanged. However, intact cell counts increased by 35 % in the POU-device building, with a shift towards slower-growing LNA bacterial populations, indicating the proliferation of oligotrophic bacteria during overnight stagnation.

In the **DHW system**, water heating and distribution led to increased concentrations of copper (by 93–232 $\mu g\ l^{-1}$) and zinc (by 30–80 $\mu g\ l^{-1}$), with fluctuations in manganese, iron, and lead concentrations depending on the sampling location. The microbial composition shifted towards the HNA fraction, indicating the presence of more metabolically active bacterial populations.

Overnight stagnation in showerheads resulted in lower water temperatures and increased microbial growth, with intact cell counts rising by 38–48 %. However, *Legionella* counts showed no significant difference between showerheads and circulation return samples.

Distinct domestic hot water supply temperature regimes. During the duration of static hot water setpoint of 57 °C (samples from weeks 0–12), microbial concentration in both the DHW from showerheads and circulation was similar in both buildings. However, the shift to the dynamic temperature regime (samples from weeks 14–22) revealed notable differences. In the POU-device building, showerhead outflows exhibited larger concentrations of damaged cells and, consequently, higher total cell counts than in the Reference building. DHW circulation return samples reflected a similar trend, additionally with greater intact cell counts and a greater proportion of HNA intact cells compared to the Reference building.

MAP reduction within internal drinking water networks. Surprisingly, MAP concentrations decreased significantly in both cold and hot water systems in both buildings, regardless of the presence of the filter (Fig. 3.5). This suggests that hydraulic retention time, adsorption onto plumbing materials, and bacterial consumption may have played a role in reducing MAP levels.

Correlation analysis suggested that MAP could be a limiting nutrient for microbial growth, with microbial activity influenced by nutrient availability and temperature – particularly in the DHW system of the POU-device building. In contrast, certain sampling locations in the Reference building indicated potential carbon limitation, as expected.

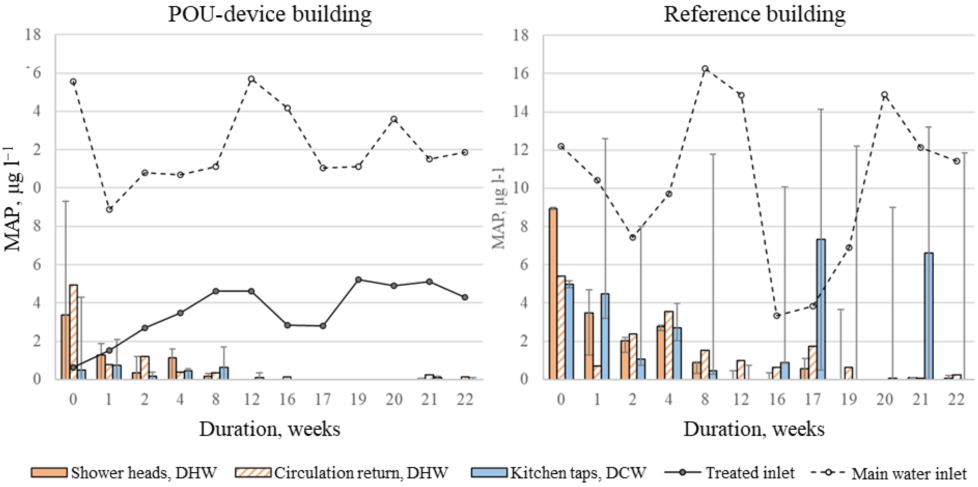

Fig. 3.5. Microbially available phosphorus concentration dynamics. For the apartment samples, data represent median values with bars corresponding to the range. POU – point-of-use; DHW – domestic hot water; DCW – domestic cold water. Figure adapted from (Zemīte *et al.*, 2023).

The fate of MAP in internal drinking water supply systems remains unclear, and further research is needed to understand the factors driving nutrient reduction within both hot and cold water piping networks. Future studies should investigate the relative contributions of biological and chemical processes to this phenomenon. However, the surface-to-water ratio plays a crucial role not only by promoting biofilm accumulation, which influences bacterial growth dynamics, but also in determining which nutrients become limiting.

3.5. *Legionella* spp. control in multi-storey residential buildings

This section focuses on evaluating the effectiveness of *Legionella* control measures, including centralised chemical flushing and disinfection, with a particular emphasis on the performance of the POU sorption filter. Additionally, the overall compliance of analysed water samples was assessed against guideline values.

Effect of centralised chemical flushing and disinfection. The centralised chemical flushing of the domestic hot water system with formic acid, followed by disinfection using hydrogen peroxide and silver ions, was effective in temporarily eliminating cultivable *Legionella* bacteria. However, the regrowth occurred already within a week (Fig. 3.6), with 36 % of samples showing contamination. In the Reference building, *Legionella* levels exceeded the EU Directive limit after two months, while the POU-device building experienced lower initial regrowth. These findings suggest that while centralised disinfection is effective in the short term, it is inadequate for sustained control of *Legionella*.

Effect of MAP removal unit and water heater setpoint. The POU sorption filter did not significantly reduce *Legionella* concentrations under normal temperature conditions but contributed to a nearly an order-of-magnitude increase during dynamic temperature settings (Fig. 3.6). This was likely due to factors such as:

1) nutrient-richer influent water (higher in total organic carbon, magnesium, and intact cells, compared to the Reference building);
2) shifts in microbial competition, induced by scarcer nutrient availability;
3) and fluctuations in DHW temperature, which promoted a mesophilic environment favouring *Legionella* growth.

Correlation analyses indicated weak to moderate relationships between *Legionella* concentrations and water quality parameters such as TOC, MAP, and magnesium. The study also found a positive correlation between *Legionella* and iron levels in the Reference building, suggesting the influence of trace elements on microbial dynamics.

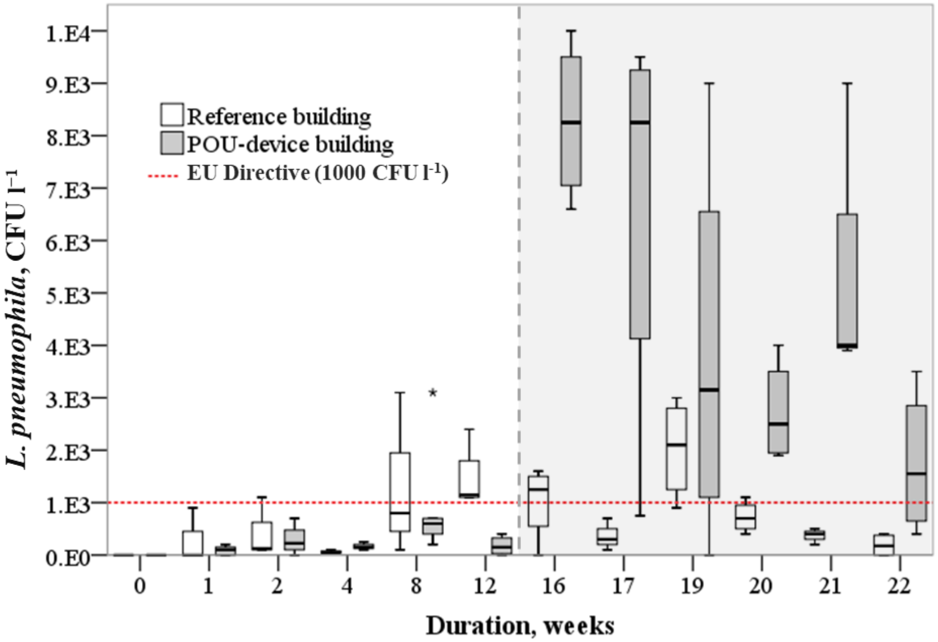

Fig. 3.6. Statistical data for *Legionella* spp. counts in DHW showerhead and circulation return samples during static (weeks 0–14) and dynamic (weeks 16–22) temperature setpoints. Star represents far-outlier, which in this case corresponds to the circulation return sample. Figure adapted from (Zemīte *et al.*, 2023).

Lowering the DHW temperature below the thermophilic threshold favoured *Legionella* growth by creating a more conducive mesophilic environment, while the periodic heat treatments may have facilitated nutrient release, further boosting *Legionella* proliferation. Despite regular heat treatments and dynamic temperature setpoints, nutrient dynamics and disruptions in microbial balance allowed *Legionella* to thrive in the POU-device building, underscoring the complexity of controlling this pathogen in fluctuating environmental conditions.

Characteristics of *Legionella* species. The most prevalent serogroup detected in both buildings was *L. pneumonia* SG 2, which was found in 51 % of samples taken from DHW sampling points in the POU-device building and 40 % of DHW samples in the Reference building (Fig. 3.7). The introduction of the POU sorption filter may have caused a shift in *Legionella pneumophila* species, favouring non-SG1 strains, as it was detected more often in the Reference building. This potential shift suggests that while the filter may reduce certain *Legionella* strains responsible for most clinical cases, the resulting nutrient limitation and its impact on the occurrence of potentially pathogenic strains need further investigation.

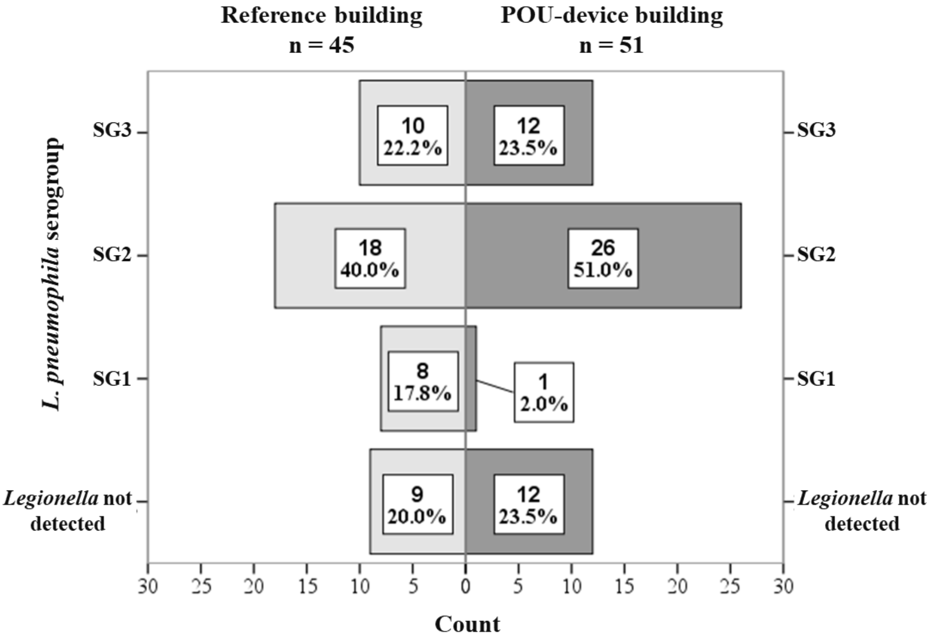

Fig. 3.7. The summary frequency of detected *Legionella pneumophila* serogroups in samples collected from both DHW sub-systems.

Legislative compliance. Ensuring access to clean drinking water is crucial for public health. Although municipal treatment usually meets safety standards, water quality can deteriorate in internal plumbing before consumption.

This case study revealed concerning levels of *Legionella pneumophila* exceeding the guideline of 1000 CFU l^{-1} in both the Reference building and POU-device building. Additional exceedances were noted for manganese, copper, iron, and lead, but those were mainly linked to pipeline flushing after chemical cleaning procedures and, in such a way, did not pose a longterm health risk. However, alarmingly, elevated levels were also found in the municipal water inflow.

To enhance water safety, mandatory monitoring of drinking water quality at the building level is needed to proactively identify and address potential health risks, ensuring that the final stages of water distribution maintain safety standards.

CONCLUSIONS AND FUTURE STUDIES

Addressing the general objective

The widely used concept of biostable water provision by reducing the availability of growth-promoting nutrients, among which is MAP, would prevent water quality from deterioration. As it states that no bacterial communities should change in time, the same principle can be addressed to the growth of co-occurring OPPP bacteria, including *Legionella* spp. However, such a principle might be valid only if other system conditions are stable.

In this study, dynamic water heater setpoints led to a significant increase in cultivable *Legionella* in a building with additional MAP removal, with concentrations exceeding those in control systems by over an order of magnitude. Consequently, the hypothesis that MAP limitation alone would inhibit *Legionella* growth was rejected.

Addressing the general objective, while MAP limitation may show potential under stable conditions where non-pathogenic bacteria dominate, maintaining these conditions in practice is challenging due to factors such as seasonal variations, maintenance works, and operational changes. Therefore, the focus should shift from simplistic nutrient limitation approaches to engineering selective environments that promote the growth of non-pathogenic organisms.

Main conclusions

1. 70 % MAP reduction to 3.56 µg MAP l^{-1} (SD 1.5 µg l^{-1}) by GFH sorption filter alone was not able to reduce *Legionella pneumophila* occurrence when the system was subjected to dynamic temperature (48 °C, 52 °C, and 57 °C), but it might have influenced *Legionella* species shift to non-SG1.
2. Buildings received different inflow water from various sources that varied in electrical conductivity, Ca, Mg, Cu, TOC, and DCC.
3. MAP decreased to low levels within DWS. Temperature, pH, Cu, Zn, Fe, Pb, and microbial parameters also showed changes during distribution.
4. Centralised chemical cleaning was effective for 2 months, after which average values for *Legionella* exceeded 1000 CFU l^{-1}, while it was detected in samples already after a week and sporadically reached the Directive limit after 2 weeks in the Reference building.
5. *Legionella* counts increased more than tenfold in the MAP-limited building but did not change much in the building without additional MAP removal. It potentially might be explained by r/K theory, when a temperature decrease provided optimal conditions, allowing *Legionella pneumophila* to outcompete nutrient-starved microorganisms. This condition was amplified by periodic heat disinfection at 57 °C, potentially inducing P-cycling in the P-starved system, further promoting rapid *Legionella* growth.

Study limitations

1. The small number of sampling sites, due to the limited willingness of residents to participate, hindered the ability to gather a statistically robust dataset and reduced the potential for identifying clear trends.
2. The limited number of buildings included in the study restricted the ability to compare results across different settings. A larger sample size would have provided better insights into the effect of location, water usage patterns, and incoming water quality on the outcomes.
3. Variability in the temporal composition of inflow water, stemming from different water sources, added complexity to the study's dynamic, real-world testing environment.
4. Insufficient sampling frequency due to limited sample handling and analysis capacity affected the ability to capture temporal dynamics in *Legionella* counts.
5. The limited scope of microbiological analyses – the inclusion of metagenomic techniques and q-PCR alongside plate culture for *Legionella* would have provided a deeper understanding of microbial species dynamics.
6. The limited range of chemical analyses; adding measurements such as AOC would have offered additional insights into nutrient dynamics within the system.

Broader implications and future studies

The pilot-scale nature of the study revealed complex interactions within internal water distribution systems, and the results challenge the assumption of direct interactions between MAP and other bacteria. The findings suggest that a more nuanced understanding of microbial competition and niche differentiation is required for predicting bacterial regrowth in water systems.

Future studies should focus on improving our understanding of microbial competition for growth-promoting nutrients within drinking water supply, particularly in the context of the r/K selection theory, originating from macroecology. The focus should be stirred towards determining beneficial conditions that favour "K-strategists" (harmless microorganisms) over "r-strategists" (opportunistic pathogens), thereby enhancing drinking water safety.

Some of specific questions that require further attention include:

- Exploring the impact of previously studied growth-promoting nutrients, such as carbon and phosphorus, on microbial competition, especially in dynamic environments characterized by water temperature fluctuations. This can be pursued through a combination of mathematical modelling and laboratory studies.
- Conducting habituation studies on oligotrophic drinking water bacteria to support future controlled experiments.
- Expanding the range of analyses to include techniques that describe both intracellular processes and overall microbial community dynamics.

APPROBATION

List of publications

1. **Frolova, M.**, Zemītis, J., Tihomirova, K., Mežule, L., Rubulis, J., Gruškeviča, K. and Juhna, T. (2017) 'Approbation of microbially available phosphorus (MAP) determination method by flow cytometry', in *Environment. Technology. Resources. Proceedings of the International Scientific and Practical Conference*. Rezekne, Latvia, pp. 89–92. Available at: https://doi.org/10.17770/etr2017vol1.2533.
2. **Frolova, M.**, Tihomirova, K., Mežule, L., Rubulis, J., Gruškeviča, K. and Juhna, T. (2017) 'Evaluation of pre-treatment technologies for phosphorous removal from drinking water to mitigate membrane biofouling', *IOP Conference Series: Materials Science and Engineering*, 251. Available at: https://doi.org/10.1088/1757-899X/251/1/012127.
3. **Zemite, M.**, Mezule, L., Gruskevica, K., Kokina, K., Rubulis, J., Juhna, T., Gottschalk, N., Dömer, F., Jagau, R., Röwe, K., Augustin, W., Scholl, S., Pereira, A., Barros, A. C., Machado, I., and Melo, L. F. (2022) 'Affordable Pretreatment Strategy for Mitigation of Biofouling in Drinking-Water Systems', *Journal of Environmental Engineering*, 148(2). Available at: https://doi.org/10.1061/(asce)ee.1943-7870.0001968.
4. **Zemīte, M.**, Pūle, D., Kiriļina-Gūtmane, O., Ķimse, L., Strods, M., Zemītis, J. J., Mežule, L., Valciņa, O. and Juhna, T. (2023) 'Effect of microbially available phosphorous removal on Legionella spp. in multi-storey residential dwellings in Latvia', *Environmental Science: Water Research and Technology*, 10(1), pp. 193–204. Available at: https://doi.org/10.1039/d3ew00588g.

List of conferences

- RTU 57th International Scientific Conference, Section 'Smart biotechnologies'; Presentation: **The elimination of membrane fouling by the use of biotechnologies**. 17.10.2016, Riga, Latvia.
- RAT 11th International Scientific-Practical Conference 'Environment. Technology. Resources', Presentation: **Approbation of Microbially Available Phosphorus (MAP) Determination Method by Flow Cytometry**. 15–17.06.2017, Rezekne, Latvia.
- 3rd International Conference 'Innovative Materials, Structures and Technologies', Poster: **Affordable technologies for mitigation of membrane (bio)fouling through optimization of pre-treatment methods**. 27–29.09.2017, Riga, Latvia
- RTU 58th International Scientific Conference, section 'Heat, gas, and water technologies', Presentation: **Phosphorus recirculation in pressure-driven membrane filtration systems**. 12.10.2017, Riga, Latvia.
- Seminar 'Smart biotechnologies', Presentation: **Bioreactors – pre-treatment method for membrane technologies**. 18.04.2018, Riga, Latvia.

- RTU 59th International Scientific Conference, section ‘Heat, gas, and water technologies’, Presentation: **Polyphosphate accumulation in drinking water bacteria**. 19.10.2018, Riga, Latvia.
- RTU 61st International Scientific Conference, section ‘Building science’, Presentation: **Increasing the biological stability of drinking water in internal networks**. 22.10.2020, online.
- Seminar ‘Bioenergy Technologies and Biotechnologies’, Presentation: **Drinking water biological stability**. 21.10.2021, online.
- RTU 63rd International Scientific Conference, section ‘Building science’, Presentation: **A pilot study of *Legionella* growth inhibition efficiency by iron hydroxide pre-treatment**. 10.11.2022, Riga, Latvia.

DECLARATION OF GENERATIVE AI

During the preparation of this work the OpenAI “ChatGPT” was used in order to improve readability and language. After using this tool, the content was reviewed and edited as needed.

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