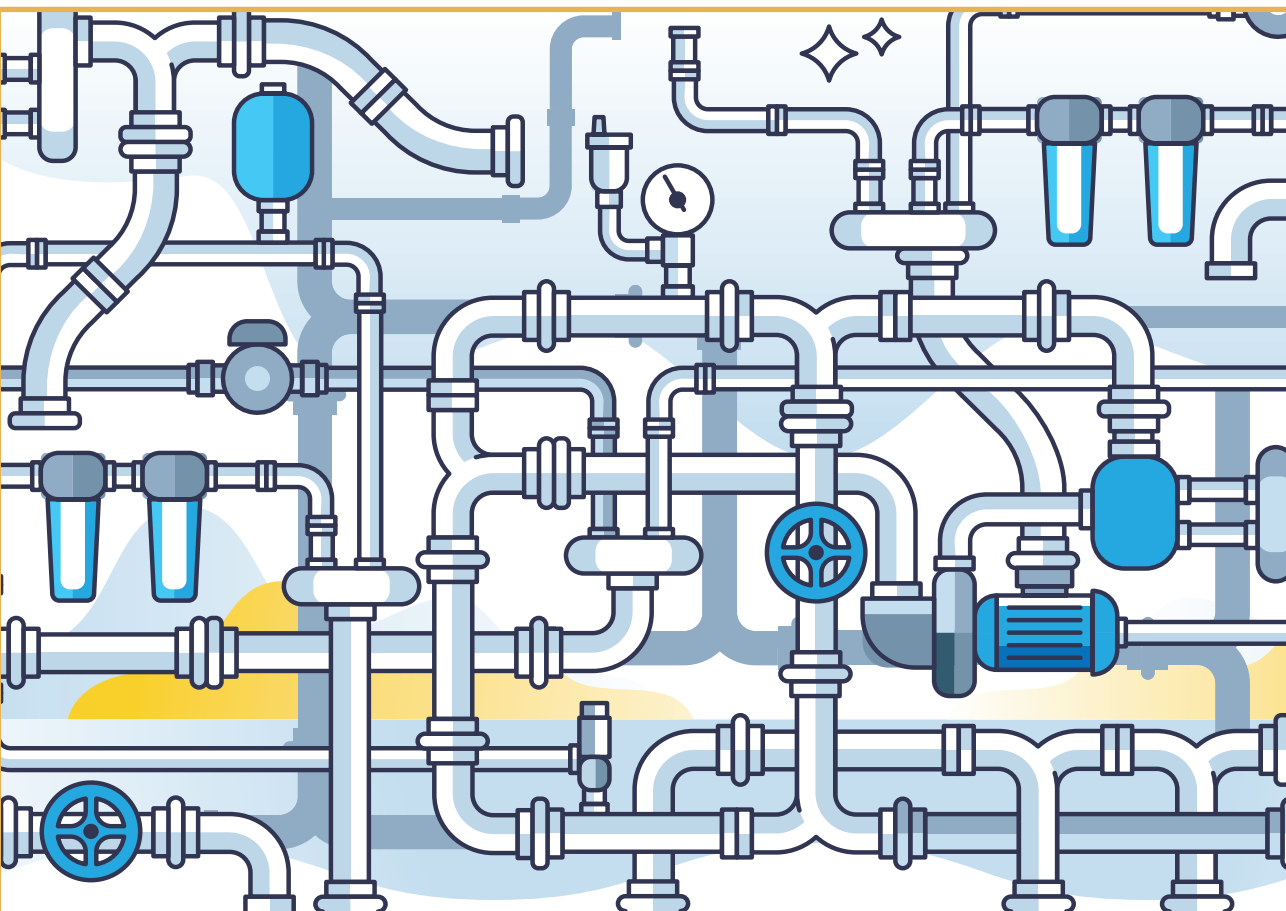RIGA TECHNICAL
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Marta Zemīte

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

Doctoral Thesis

RIGA TECHNICAL UNIVERSITY

Faculty of Natural Sciences and Technology
Water Systems and Biotechnology Institute

Marta Zemīte

Doctoral Student of the Study Programme "Heat, Gas and Water Technology"

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Scientific supervisor
Professor *Dr. sc. ing.*
TĀLIS JUHNA

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ABSTRACT

Microbially available phosphorus (MAP) contributes to the concept of biologically stable water. Phosphate is a vital element for bacterial cell proliferation, it is a component in the cell wall and membrane, links DNA and RNA sections, and is the main component involved in energy transport. When available, it is stored within cells in granules and inclusion, from where it is utilised when the conditions are less favourable. In organic carbon-rich waters, a limitation of MAP slows biofilm formation and restricts bacterial growth in the planktonic phase. Phosphorus limitation reduces bacterial growth both in bulk phase and in biofilms, and in such a way it can be beneficial also for potentially pathogenic microorganisms` growth control.

However, there is limited information on the implications of MAP reduction within internal drinking water supply, where the main problems with water quality deterioration often occur due to elevated temperatures, high surface-to-volume ratios, dead-end pipes, and poor pipeline maintenance activities. Therefore, this work focuses on the implication of MAP limitation within the internal drinking water supply and assesses its impact on such opportunistic premise plumbing pathogens as *Legionella* bacteria.

Initially, the MAP measurement method was tested and adjusted for local conditions. Subsequently, methods such as electrocoagulation, sorption, and biofiltration were evaluated for their efficiency in removing MAP from groundwater, which is the main source of water supply in Latvia. Further, the selected method was adjusted to ensure safe use within the drinking water supply system. Finally, this method was tested in the internal drinking water system of a multi-storey residential building, and its impact on microbiological water quality, particularly in the hot water supply, was assessed.

The results indicated that the granular ferric hydroxide point-of-use sorption filter reduced MAP to $3.6 \ \mu g \ l^{-1}$ (SD $1.5 \ \mu g \ l^{-1}$), achieving an average reduction of 70 % in a pilot-scale setting. It may have influenced the identified *Legionella* bacteria species within the hot drinking water system, leading to a decreased frequency of detected *Legionella pneumophila* serogroup 1. However, it did not significantly affect *Legionella* counts at the regular temperature setpoint of 57 °C. When the setpoint was changed, the temperature was dynamically varied between 48, 52, and 57 °C, *Legionella* counts increased more than tenfold. The lowered temperature setpoint fostered an environment conducive to *Legionella* proliferation, enabling it to outcompete other nutrient-starved biota.

This study is the first to demonstrate, in a full-scale domestic hot water system, that bacterial regrowth, including *Legionella*, is influenced by both nutrient concentrations and interactions between competing species based on niche differentiation. Disruptions to the system's existing balance can lead to undesirable outcomes and compromise water safety. Therefore, the theory of biostability should be revised to incorporate a competition component. However, creating a selective environment that eliminates the proliferation of opportunistic premise plumbing pathogens remains a challenge.

The doctoral thesis is written in English and structured as a dissertation, it comprises 112 pages, containing 34 figures, and 21 tables. The bibliography includes 130 references.

ANOTĀCIJA

Mikrobiāli pieejamam fosforam (MAP) ir būtiska loma bioloģiski stabila ūdens koncepcijā. Fosfors ir būtisks elements baktēriju šūnās, tas ir šūnu sieniņu un membrānu sastāvdaļa, saista DNS un RNS sekcijas, un ir galvenā sastāvdaļa, kas iesaistīta enerģijas transportā. Kad fosfors ir pieejams, tas var tikt uzkrāts šūnās granulu veidā, no kurām tas tiek izmantots, kad apstākļi kļūst mazāk labvēlīgi. Organiskā oglekļa bagātos ūdeņos MAP ierobežošana palēnina bioplēves veidošanos un ierobežo baktēriju augšanu planktoniskā veidā. Tā kā fosfora ierobežošana samazina baktēriju augšanu gan šķidrā fāzē, gan bioplēvē, tādā veidā tā var būt labvēlīga arī potenciāli patogēnu mikroorganismu augšanas kontrolei.

Tomēr ir ierobežota informācija par MAP samazināšanas sekām iekšējās dzeramā ūdens apgādes sistēmās, kur galvenās ūdens kvalitātes pasliktināšanās problēmas bieži rodas paaugstinātas temperatūras, augstas virsmas-tilpuma attiecības, izzaru līniju un nepietiekamas cauruļvadu uzturēšanas darbību dēļ. Tādēļ šis darbs koncentrējas uz MAP koncentrācijas samazināšanas ietekmi iekšējās dzeramā ūdens apgādes sistēmās un izvērtē tās ietekmi uz tādiem oportūnistiskiem patogēniem kā *Legionella* baktērijas.

Sākotnēji tika pārbaudīta un pielāgota vietējiem apstākļiem analītiskā MAP koncentrācijas noteikšanas metode. Pēc tam tika izvērtētas tādas metodes kā elektrokoagulācija, sorbcija un biofiltrācija, lai novērtētu to efektivitāti MAP daudzuma samazināšanā no pazemes ūdens, kas ir galvenais ūdens apgādes avots Latvijā. Turpmāk izvēlētā metode tika pielāgota drošai lietošanai dzeramā ūdens apgādes sistēmā. Beidzot, šī metode tika pārbaudīta daudzstāvu dzīvojamās ēkas iekšējā dzeramā ūdens sistēmā, un tika novērtēta tās ietekme uz mikrobioloģisko ūdens kvalitāti, īpaši karstā ūdens apgādē.

Rezultāti parādīja, ka lokāli uzstādīts granulētais dzelzs hidroksīda sorbcijas filtrs samazināja MAP līdz 3.6 μg l^{-1} (SD 1.5 μg l^{-1}), pilotpētījuma ietvaros sasniedzot vidējo samazinājumu par 70 %. Tas, iespējams, ietekmēja karstā dzeramā ūdens sistēmā identificēto *Legionella* baktēriju konkrētu serogrupu izplatību, izraisot samazinātu *Legionella pneumophila* 1. serogrupas konstatēšanas biežumu. Tomēr tas būtiski neietekmēja *Legionella* baktēriju skaitu pie standarta siltummaiņa temperatūras iestatījuma 57 °C. Kad iestatījums tika mainīts, temperatūru dinamiski mainot starp 48, 52 un 57 °C, *Legionella* skaits pieauga vairāk nekā desmitkārtīgi. Pazeminātais temperatūras iestatījums veicināja vidi, kas bija labvēlīga *Legionella* vairošanās iespējām, ļaujot tai pārspēt citus barības vielu trūkuma nomocītos organismus.

Šis pētījums ir pirmais, kas pilna mēroga karstā ūdens sistēmā dzīvojamās ēkās parāda, ka baktēriju, tostarp *Legionella*, augšana ir atkarīga gan no barības vielu koncentrācijām, gan no sugu savstarpējās mijiedarbības, kas balstās uz nišu diferenciāciju. Sistēmas līdzsvara traucējumi var radīt nevēlamas sekas un apdraudēt ūdens drošību. Tāpēc biostabilitātes teorija būtu jāpārskata, iekļaujot konkurences komponentu. Tomēr paliek izaicinājums radīt selektīvu vidi, kas novērstu nosacīti patogēnu mikroorganismu augšanu ūdensapgādes sistēmās.

Doktora disertācija ir rakstīta angļu valodā un strukturēta kā monogrāfija. Tā sastāv no 112 lapām, ietver 34 attēlus un 21 tabulas. Bibliogrāfija ietver 130 avotus.

Atslēgvārdi:

Mikrobiāli pieejamais fosfors, iekšējā dzeramā ūdens apgāde, kultivējamā *Legionella pneumophila*, karstā ūdensapgāde, daudzstāvu dzīvojamās ēkas, granulētais dzelzs hidroksīds.

TABLE OF CONTENTS

NOMENCLATURE

ADP	adenosine diphosphate
AOC	assimilable organic carbon, $\mu g \, l^{-1}$
ATP	adenosine triphosphate, $\mu g \, l^{-1}$
BDOC	biodegradable dissolved organic carbon
C	carbon, $mg \, l^{-1}$
CFU	colony forming units
DCC	damaged cell count, cells ml^{-1}
DCW	domestic cold water
DHW	domestic hot water
DNA	deoxyribonucleic acid
EC	electrocoagulation
EPS	extracellular polymeric substances
FCM	flow cytometry
GAC	granular activated carbon
HNA-ICC	intact high nucleic acid content cells
HPC	heterotrophic plate count, CFU l^{-1}
ICC	intact cell count, cells ml^{-1}
LNA-ICC	intact low nucleic acid content cells
MAP	microbially available phosphorus, $\mu g \, l^{-1}$
P	phosphorus, $\mu g \, l^{-1}$
PE	polyethylene
PO_4	orthophosphate, $mg \, l^{-1}$
POU	point-of-use
TP	total phosphorus, $mg \, l^{-1}$
poly-P	polyphosphate
PVC-P	plasticized polyvinyl chloride
RNA	ribonucleic acid
RPM	rotations per minute
SD	standard deviation
SDGs	Sustainable Development Goals
TCC	total cell count, cells ml^{-1}
TOC	total organic carbon, $mg \, l^{-1}$
WHO	World Health Organization
WSPs	water safety plans

INTRODUCTION

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 concept focuses on controlling nutrients such as carbon, 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. Specifically, microbial communities should remain stable in terms of their diversity and cell concentration during distribution (Prest *et al.*, 2016b).

In the humic-rich natural waters of boreal regions, where carbon is abundant, phosphorus often acts as the limiting factor for microbial growth within the drinking water supply (Miettinen, Vartiainen and Martikainen, 1997). Similarly, in drinking water distribution systems, carbon can become readily available due to its release from materials in contact with drinking water, such as soft plastic connections, which are known to leach carbon (Proctor *et al.*, 2016). However, the element limiting microbial growth may vary within the same distribution system. Several factors determine nutrient availability during water supply, particularly in systems where water is sourced from multiple sources and subjected to different treatment technologies (Nescerecka, Juhna and Hammes, 2018). For example, in Riga, extensively treated surface water is phosphorus-limited, while groundwater, subjected mainly to chlorination, is carbon-limited (Nescerecka, Juhna and Hammes, 2018).

Phosphorus, being one of the major macrobiogenic nutrients required for bacterial growth, plays a critical role in controlling microbial proliferation. Reducing phosphorus levels is often associated with limiting bacterial growth, even in environments with abundant carbon. For example, reducing MAP concentrations to approximately 0.3 µg l^{-1} has been shown to restrict biofilm formation on pressure-driven membrane spacers (Vrouwenvelder *et al.*, 2010). However, even at low phosphorus concentrations (below the detection limit of 1 µg MAP l^{-1}), biofilm formation can still occur within drinking water distribution system simulators (Rubulis and Juhna, 2007).

Although phosphorus reduction is an effective measure for controlling microbial growth and subsequently reducing biofilm formation (Vrouwenvelder *et al.*, 2010), limited information is available regarding the influence of MAP limitation on microbial quality at the consumer level. This is especially relevant in internal drinking water supply systems, where various factors such as elevated temperatures, overnight water stagnation, dead-end pipes, high surface-to-volume ratios, and inadequate maintenance can exacerbate water quality issues (Prest *et al.*, 2016b).

In Latvia, the majority (65 %) of residential buildings were constructed between 1961 and 2000, with 40 % built before 1980 (Centrālā statistikas pārvalde, 2021). While water mains in these buildings may have been periodically replaced by building managers – most often switching from old iron pipes to plastic ones – the internal apartment connections remain the responsibility of individual owners. In practice, these internal plumbing systems are rarely

maintained, potentially increasing 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 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 on a full-scale.

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.

The study contributes to the United Nations Sustainable Development Goals (SDGs), particularly Goal 6, "Clean Water and Sanitation," and Goal 11, "Sustainable Cities and Communities," by focusing on improving drinking water quality within the distribution network at the consumer level. It also aligns with Goal 3, "Good Health and Well-being," and Goal 10, "Reduced Inequalities," by addressing the impact of pilot-scale MAP limitation on *Legionella* spp., which poses significant health risks, especially to immunocompromised individuals.

Structure and scope

The Thesis 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 (Fig. 1):

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 pre-treatment 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).

Chapter 1
Theoretical Background

Chapter 2
Materials and methods

Lab-scale tests

Chapter 3
Microbially available phosphorus measurement protocol adjustments

Chapter 4
Selection of treatment method to use within the Pilot study

Pilot-scale studies

Chapter 5
Characteristics of water received from municipal distribution network

Chapter 6
Microbially available phosphorus reduction efficiency and water quality changes within internal drinking water supply

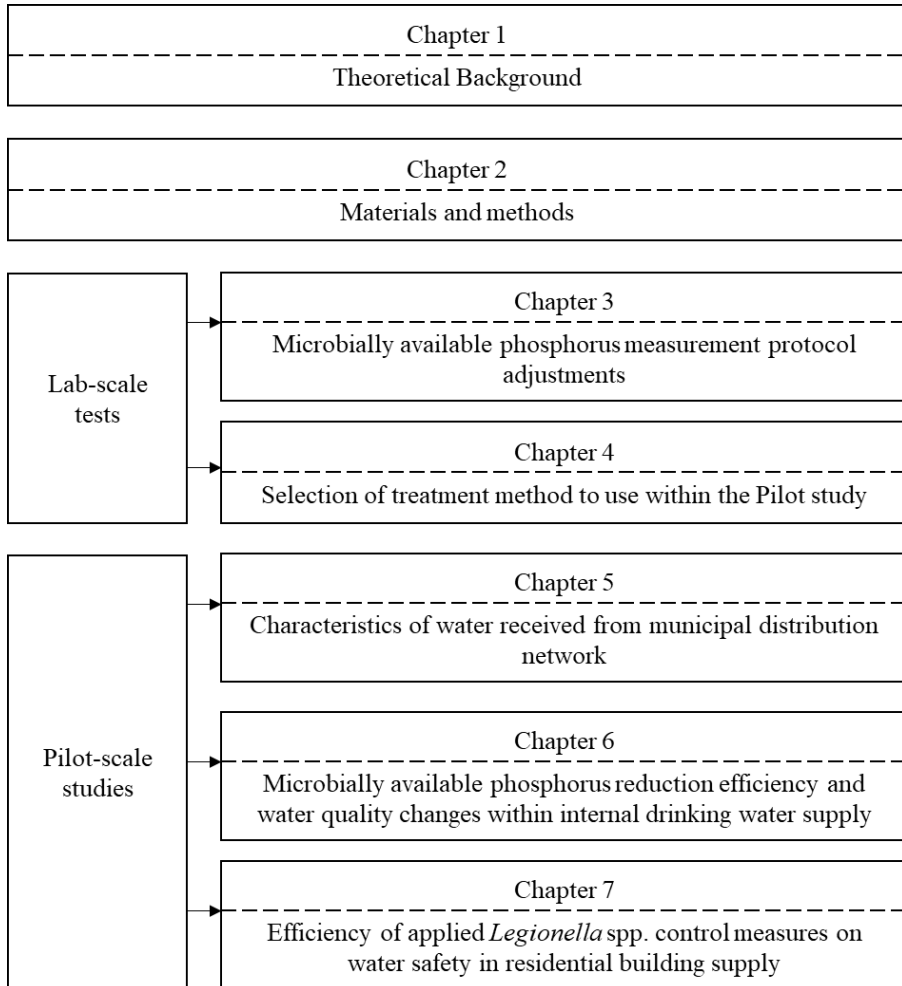| Chapter 7 |
| --- |
| Efficiency of applied *Legionella* spp. control measures on water safety in residential building supply |

Fig. 1. Outline of the thesis.

1. BACKGROUND

1.1. Provision of safe drinking water

1.1.1. Regulatory framework

The fundamental and crucial requirements to ensure the safety of drinking water include a comprehensive framework, which consists of health-based targets set by a competent health authority, adequately maintained systems (including sufficient infrastructure, proper monitoring, and effective planning and management), and a system of independent surveillance (WHO, 2022).

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. In Europe, along with the World Health Organization guidelines, the quality of water intended for human consumption is regulated by the European Union Directive 2020/2184 (The European Parliament and the Council, 2020). It sets minimum requirements for the chemical and microbiological composition of drinking water to be safe for human health. In Latvia, accordingly, the Directive requirements are adapted in the Cabinet of Ministers Regulatory Act No. 547 "Mandatory safety and quality requirements for drinking water, monitoring and control procedures" (Ministru Kabinets, 2023). The regulations apply to both raw or treated surface and groundwater and provide for a maximal concentration of various polluting substances for potable water, as well as ensure a regulatory basis for monitoring frequency for proper water quality control both after the treatment and during distribution.

Adequate maintenance of water supply systems is ensured through the implementation of Water Safety Plans (WSPs). Developed by water suppliers, these plans serve as the foundation for the protection and control of the water supply system, from source to tap. WSPs ensure that pathogen levels and chemical concentrations pose negligible risks to public health and that the water remains acceptable and safe for consumers (WHO, 2022). Monitoring water quality is a critical component of WSPs, but challenges exist. Legally, municipal or external drinking water networks end at the entry point of a building. Beyond this, the internal water network becomes the responsibility of building owners, residents, or designated managers. According to legislation, water quality within internal networks should be tested in accordance with risk management plans, which must be reviewed at least once every six years. These plans require monitoring for *Legionella* spp., which must be maintained below 1000 CFU l^{-1}, and for lead, which must not exceed a temporary limit of 10 µg l^{-1} (The European Parliament and the Council, 2020). Unfortunately, under the Regulatory Act, such testing is mandatory only for priority locations, such as hospitals and social care facilities providing accommodation services (Ministru Kabinets, 2023). Other types of buildings are encouraged to monitor water quality within internal networks on a voluntary basis, which, in practice, often means only reacting post-factum or after problems arise.

The final step involves independent surveillance of the drinking water supply, which should be in-place to assure proper functioning of previously described measures. This includes public

health oversight, investigations, reporting, and compiling information on waterborne disease outbreaks. In Latvia, these activities are conducted by the Health Inspectorate, which operates under the Ministry of Health. In a 2021 surveillance study (Veselības inspekcija, 2022), 90 % of surveyed inhabitants received water that met regulatory standards, while 10 % were supplied with water that exceeded acceptable limits for certain parameters. Among non-compliant samples, 12.8 % were attributed to chemical parameters and 2.3 % to microbiological issues.

1.1.2. Water quality deterioration

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 supplies (Lehtola, Miettinen, *et al.*, 2004; Lehtola *et al.*, 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). Also, the internal network consists of two distinct systems – domestic cold water supply (DCW) and domestic hot water supply (DHW), where DHW often also contains a recirculation loop to lower the time hot water reaches water outlets.

The smaller diameters of the internal water supply result in a high surface-to-volume ratio, which is one of the main factors facilitating a rapid increase in microbial cells even after just one hour of stagnation (Bédard *et al.*, 2018). Additionally, the mixture of different pipe materials that are present in the internal water supply, as well as spatio-temporal water temperature changes, can create various habitats for bacterial growth. E.g., copper pipes can suppress *L. pneumophila* growths at temperatures below 41 °C but have no effect at higher temperatures, while cross-linked polyethylene (PEX) can leak organic carbon and sustain better growth at lower temperatures (Proctor *et al.*, 2017). By elevating the temperature by 2.5–4 °C every 5–7 weeks, they also found that the temperature induced shifts in hot water microbial structure. Another study (Ji *et al.*, 2017) found that the environment in distal taps is distinct from that of the recirculating DHW line due to stagnation and cooling and that a stagnation period as short as 8 hours was enough to cause a shift away from the core microbes that defined the bulk water or biofilm composition in the recirculating line.

Drinking water typically contains complex microbiomes, comprising up to a diversity of 48 phyla, with concentrations ranging from 10^3 to 10^5 cells ml^{-1} of bacteria in a planktonic phase and 10^4 to 10^7 cells cm^{-2} growing in pipe-associated biofilms (Proctor and Hammes, 2015). The formed biofilms can lead to water quality deterioration, e.g., by the release of odour-potent volatile organic compounds (Skjevrak *et al.*, 2004), in such a way negatively affecting organoleptic characteristics of supplied water.

The bacterial community profile is affected by the used treatment strategy and can be stable over time (El-Chakhtoura *et al.*, 2015), however, it becomes more abundant during distribution (El-Chakhtoura *et al.*, 2015), and contains different core bacterial communities in bulk phase

and biofilm, with biofilm communities exhibiting higher relative abundances of single phylotypes and lower overall richness compared to bulk water (Henne *et al.*, 2012).

It takes several years for the biofilm to mature and house a high-diversity community structure, including both heterotrophic and autotrophic organisms (Martiny *et al.*, 2003), among which also potential pathogens can be found (Parsek and Singh, 2003). Special attention must be addressed towards such places where human interaction with pathogens in biofilms occur, i.e., shower hoses in internal drinking water supply, especially, as they can contain more than 100-fold greater sequences compared to content in the background of potentially pathogenic microorganisms, such as *Mycobacterium avium*, as well as host other known pathogens, such as *Legionella* species (Feazel *et al.*, 2009).

1.1.3. Biological stability in water distribution

To enhance drinking water safety during distribution, several water companies, e.g., in the Netherlands, Switzerland, Denmark, Germany, and parts of Belgium, have been aiming for the provision of biologically stable water (van der Wielen *et al.*, 2023). The essence of drinking water biostability is that it does not promote the growth of microorganisms (Rittmann and Snoeyink, 1984), and a change in the microbial community during water distribution (Lautenschlager *et al.*, 2013), "at least not to a degree that could negatively affect either consumers` safety and aesthetic perception or cause any technical failure at any point during water distribution, neither on spatial nor on temporal scales, at any drinking water distribution location including the point of use and consumption" (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 organic carbon, as well as an inorganic nutrient limitation (Prest *et al.*, 2016a). Alongside reduced chlorine residual and excess nutrients, also seasonal changes, affect water temperature, and cause disturbances in biostable water, supporting an increase in bacterial numbers during distribution (Nescerecka, Juhna and Hammes, 2018). Although disinfection is effective and widely used and needed to control bacterial growth, it should be used with caution, as it can be the cause of water quality deterioration. Chlorine-based disinfection methods produce harmful disinfection by-products, like trihalomethanes, haloacetic acids, chlorites, chlorates etc. (Kim *et al.*, 2002). Additionally, nutrients can be released into the drinking water distribution system from chlorine-damaged bacteria, e.g., in the form of extracellular adenosine triphosphate (ATP), and in such a way be further utilised as a phosphorus source during bacteria proliferation (Nescerecka, Juhna and Hammes, 2016).

Regarding organic carbon, the fraction that can be utilised by microorganisms, is called easily assimilable organic carbon (AOC), which ranges from 0.03 % to 27 % of dissolved organic carbon (van der Kooij, Visser and Hijnen, 1982). Also, biodegradable dissolved organic carbon (BDOC) can be used as an indicator of bacterial regrowth potential (Escobar and Randall, 2001), determining a different fraction of carbon. However, in drinking water systems biodegradable carbon can be released from pipes (e.g. PE, PVC-P), especially from soft plastic connections of the shower heads (Proctor *et al.*, 2016), making it abundant.

Another bacterial regrowth control option is connected to the removal of inorganic nutrients, such as phosphorus, which is more suitable in carbon-rich environments. Additionally, phosphorus is the limiting element for microbial growth in Boreal regions, e.g., Finland, and other countries where water is naturally humic-rich (Miettinen, Vartiainen and Martikainen, 1997; Lehtola, Juhna, *et al.*, 2004; Polanska, Huysman and Van Keer, 2005). Also, a study from Japan stated inorganic nutrients to be limiting tap water bacteria growth (Sathasivan *et al.*, 1997). Therefore, the removal of phosphorus is a focus-point in waters with high organic concentrations. 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).

In general, the nutrient that limits bacterial growth can be site-specific. An example from Tokyo shows that drinking water was limited by phosphorus in more than half of the analysed samples, while biologically available organic carbon was limited in the remaining samples (Sathasivan and Ohgaki, 1999). Similarly, in Riga, a part of the distribution network, supplied by surface water that passes extensive chemical and biological treatment, after passing the chlorination step, was phosphorus-limited, while chlorinated groundwater was carbon-limited (Nescerecka, Juhna and Hammes, 2018). Additionally, while nutrients might be limited in the treatment plant outflow, the ratio of elements can change during distribution, providing suitable conditions for microbial growth (Nescerecka, Juhna and Hammes, 2018). In 2014, a city-wide sampling showed bacterial re-growth in 50 % of samples from a maximum of 2.1×10^4 cells ml^{-1} leaving water treatment plants to over 1.06×10^4 cells ml^{-1} at sampling points, in such a way highlighting biologically instable water supply (Nescerecka *et al.*, 2014).

The measures to assess the biological stability of water, in general, contain a variety of predictive and direct indicators, such as evaluation of growth-promoting properties (control of nutrient limitation), assessment of growth-promoting properties of materials in contact with water, bacterial abundance, viability and activity, community composition (Prest *et al.*, 2016b). Moreover, a recent study introduced a wide set of parameters with guidance values, determining both the growth and biofouling potential to describe the biological stability of water (van der Wielen *et al.*, 2023). For unchlorinated drinking water distribution that has a low regrowth potential, the authors suggested the following parameters:

- If a source is surface water:
  - maximum biomass growth during the first seven days of incubation in the biomass production potential for water test (MBG$_7$) < 4.5 ng ATP l^{-1},
  - particulate and/or high molecular organic carbon (PHMOC) < 47 µg C l^{-1},
  - iron accumulation rate determined with the continuous biofilm monitor (FeAR) < 0.34 mg Fe m^{-2} day^{-1}.
- If a source is groundwater:
  - total organic carbon (TOC) < 4.1 mg C l^{-1},
  - maximal biomass concentration during the first seven days of incubation in the biomass production potential for water test (MBC$_7$) < 8.6 ng ATP l^{-1},
  - cumulative biomass production during 14 days of incubation in the biomass production potential for water test (CBP$_{14}$) < 110 d ng ATP l^{-1}.

1.2. Phosphorus in natural processes

1.2.1. Role of phosphorus in bacterial cell

Although phosphorus (P) is needed in relatively small amounts, accounting for approximately 3% of bacterial cell`s dry mass (Kushkevych, 2022), it is a crucial element in microbial cell functioning. It is vital in various biological processes, including the inheritance of genetic material, energy metabolism, maintaining membrane integrity, and intracellular signalling (Santos-Beneit, 2015). It is constitutive of nucleic acids, phospholipids, teichoic acids, membranes, highly phosphorylated nucleotides, and phosphorylated proteins, as well as it participates in the respiratory chain and signalling cascades (Martín and Liras, 2021).

Bacterial membrane. P is an essential component of the cell membrane, where it is present in the form of phospholipids that enclose the bacterial cell. In gram-negative bacteria, it forms a phospholipid bilayer of an inner membrane, encompassing the cytoplasm, and inner leaflet of the outer membrane (Silhavy, Kahne and Walker, 2010).

Gram-positive bacteria, however, have no outer membrane. Their cellular membrane is comprised of a similar phospholipid bilayer, encompassing cytoplasm. The outer part, however, is covered by a thick peptidoglycan layer, threaded by long anionic polymers, called teichoic acids, which are composed mostly of glycerol phosphate, glucosyl phosphate, or ribitol phosphate repeats (Silhavy, Kahne and Walker, 2010). In P-limited conditions, some bacteria can replace teichoic acids with Pi-free polymers – teichuronic acids (Santos-Beneit, 2015).

DNA and RNA. P is a part of the structural framework of nucleic acids, such as DNA and RNA. Phosphodiester bonds link deoxyribose (a pentose sugar) molecules (Kushkevych, 2022), forming a "backbone" of the nucleic acid composition, and linking nucleotides to form DNA and RNA sections (Butusov and Jernelöv, 2013).

Some **organic phosphorus compounds**, such as adenosine triphosphate (ATP), phosphoenolpyruvate, acyl phosphate, and others can accumulate energy through macroenergetic bonds to provide energy for biosynthesis processes (Kushkevych, 2022).

Adenosine triphosphate. P is involved in the energy system of a cell, as a part of ATP, which is a coenzyme that carries out most of the intracellular energy transport. When energy is required, these compounds undergo oxidation, transferring energy from the storage molecules to adenosine phosphate. In the most common reaction, this energy is captured when adenosine diphosphate (ADP) gains an additional phosphate group, forming ATP. Further, the energy is used to transport substances across cell membranes (Butusov and Jernelöv, 2013).

ATP is often used to assess cell viability, by detection of external ATP (eATP) as evidence of cell destruction. However, ATP leakage can also occur during the bacterial exponential growth phase (Ihssen *et al.*, 2021).

Phosphoenolpyruvate : sugar phosphotransferase system (PTS). PTS uses phosphoenolpyruvate as an energy source and phosphoryl donor (Deutscher, Francke and Postma, 2006), and is further involved in a variety of cell processes. The primary PTS functions

involve catalysis of sugar transport, phosphorylation and chemoreception, while some secondary regulatory functions include control of the activities of catabolic enzymes, transcription factors and other activities, as well as control of such physiological processes as ionic homeostasis (potassium transport), glycogen accumulation, polyhydroxybutyrate storage, nitrogen utilization, phosphorous metabolism, biofilm formation, virulence and transposon-mediated directed mutation (Saier, 2015).

1.2.2. Microbial response to P-limited conditions

Both carbon and phosphorus, used in nutrient limitation to enhance the biostability of drinking water, are among the main macrobiogenic elements sustaining microbial growth. The optimal stoichiometric molar ratio for microbial growth is approximately 100 C : 10 N : 1 P (LeChevallier, Schulz and Lee, 1991), resulting in a concentration ratio of $1 \text{ mg C l}^{-1} : 0.117 \text{ mg N l}^{-1} : 0.026 \text{ mg P l}^{-1}$ (Selbes *et al.*, 2016).

The availability of macrobiogenic elements may induce various processes in microorganisms. E. g., Danhorn *et al.*, 2004 reported that P-limiting conditions trigger the PhoR-PhoB regulatory system in plant pathogen A*grobacterium tumefaciens*, significantly enhancing its adherence. Huang, Voutchkov and Jiang, 2019 reported an accelerated formation of seawater reverse osmosis bacterial biofilm during conditions when the amount of C was increased, while N and P were low.

Microorganisms, living in diverse environments, have evolved adaptive mechanisms to cope with phosphate scarcity or abundance, utilizing intracellular phosphorus transport systems that vary in affinity and mechanism of action (Kulakovskaya, 2014). Bacteria transport inorganic phosphate either by the low-affinity PitH transporters or by the high-affinity phosphate transport system PstSCAB (Martín and Liras, 2021). When P is abundant, low-affinity inorganic phosphate transport (Pit) is in action, which requires less energy for phosphate transport (Martín and Liras, 2021). When subjected to P-limiting conditions or P starvation, a high-affinity phosphate transport system takes place, and it requires more energy for phosphate transport as it is energized by ATP hydrolysis (Martín and Liras, 2021). The availability of phosphate controls the expression of hundreds of genes, which constitute Pho regulon (Martín and Liras, 2021).

The Phosphate (Pho) regulon is a global regulatory mechanism, which includes extracellular enzymes capable of obtaining Pi from organic phosphates, Pi-specific transporters, and enzymes involved in the storage and saving of P (Santos-Beneit, 2015).

To sustain cell functions during P-limited conditions, bacteria can store Pi within their cells in the form of polyphosphate granules. Polyphosphate is a stress response molecule, which is synthesized in a Pho-dependent manner – most bacteria can induce the expression of polyphosphate kinase (PpK), which accumulates polyphosphate (poly-P) as a Pi reservoir that can be reused when needed (Santos-Beneit, 2015). The formation of poly-P granules can be influenced by oxygen availability. Saia *et al.*, 2017 found a significantly higher percentage of

cells with intracellular poly-P granules during aerobic than anaerobic conditions, when testing P release under alternating aerobic/anaerobic water column.

In natural water, periphetic biofilms form the buffers for P release and precipitation, entrapping P from water, attenuating its release and storing P as a sink (Lu *et al.*, 2016). It can utilise a variety of P species (TP, TDP, DIP, PP, DOP), and, by increasing water pH, favour co-precipitation of P and metal salts, in such a way reducing the release of some sedimentary P like Fe/Al–P and Ca–P to overlying water (Lu *et al.*, 2016). Freshwater biofilms accumulate P when it`s scarce, restricting algal growth, biomass production, and potentially also community structure, but it does not always affect bacterial biofilm community structure (Li *et al.*, 2016).

1.3.   MAP limitation to control bacterial growth

1.3.1.   MAP in engineered systems

Nutrient limitation has been extensively studied since the end of last century, however, the major part is related to organic carbon investigations. Although MAP is another crucial parameter, the number of studies addressing it is rather 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).

Jiang, Chen and Ni, 2011 reported MAP values from 0.69–8.01 µg l^{-1} in the city water distribution network in China, and that MAP concentration can slightly decrease with an increase in hydraulic retention time. It can also leach from new PEX pipes (Inkinen *et al.*, 2014).

Moreover, 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). Additionally, a change in oxygen availability can potentially facilitate P release from existing drinking water biofilms. Saia *et al.*, 2017 reported that alternating aerobic/ anaerobic conditions produce patterns of dissolved orthophosphate uptake and release in stream biofilm, inducing P-cycling. Such a process was found to be similar to the one employed in wastewater treatment, however, the authors did not find any known phosphate-accumulating organisms.

While it is generally expected that the addition of P would support bacterial growth in P-limited systems, sometimes it does not provide the expected results. Rahman *et al.*, 2016 tried to improve natural organic matter removal by biofilters to reduce membrane fouling. But such an amendment only initially slightly lowered dissolved organic carbon and humic substances, subsequently diminishing over time, and not influencing membrane fouling. Similarly, a study in a local drinking water treatment plant in Riga did not improve biofilter performance after amendment with P, but it potentially increased the detachment of bacterial cells, causing larger numbers in the outflow (Rubulis and Juhna, 2005). Such observation might be similar to the one reported by Selbes *et al.*, 2016 that "phosphate amendment enhanced the microbial activity during cold-temperature months, which resulted in higher particle counts compared with

conventional BAF effluent". Moreover, the addition of P at certain levels greatly increases the metabolic potential of cells in biofilm and promotes cell growth, while it also induces thicker and less homogeneous biofilms with around 80 % lower extracellular polymeric substances (EPS) production (Fang, Hu and Ong, 2009).

MAP reduction to ultra-low levels can reduce biofilm formation. Vrouwenvelder *et al.*, 2010 showed that by reducing MAP concentration to ~0.3 µg l^{-1}, it was possible to restrict the pressure drop increase and biomass accumulation in membrane fouling simulator (a tool used for spiral wound reverse osmosis membrane antifouling studies), even at high organic carbon concentrations. However, other studies show that such removal to ultra-low levels (below the detection limit of 1 µg MAP l^{-1}) does not significantly reduce biofilm formation in drinking water distribution system simulators (Rubulis and Juhna, 2007).

Javier *et al.*, 2021 reported that a balanced P amount was beneficial in membrane filtration – P in low doses (3 µg P l^{-1}) balanced the ratio of EPS production per cell (in the study it corresponded to 60 % cells and 40 % EPS), making the biofilm removal easier during cleaning procedure, while lower concentrations stimulated greater EPS production (for 0 µg l^{-1} the ratio was 5 % cells and 95 % EPS), and higher P doses (6 µg P l^{-1}) worsened biofilm removal, although the ratio was 80 % cells and 20 % EPS, explaining it by biofilm stratification and denser biofilms at the membrane surface, when more P was present, and more uniform structural properties and easier to clean biofilm structure at lower P concentration.

Some drinking water providers use technical chemicals, such as phosphate-based corrosion inhibitors to protect water distribution pipes, but it can serve as nutritional input into the distribution system. The membrane fouling studies showed that dosing phosphonate-based antiscalants induced biofouling but no biofouling was observed when acids or phosphonate-free antiscalants were used (Vrouwenvelder *et al.*, 2010). Therefore, additional attention needs to be addressed to the choice of appropriate supplementary substances, which include disinfectants, corrosion inhibitors, antiscalants, descaling and cleaning agents, as their chemical composition should not provide additional nutrient inputs. Additionally, trace P inputs (up to 0.6 mg l^{-1}) can bind with corrosion scales, shifting zeta potential towards more negative values, in such a way increasing electrostatic repulsion among bacteria and corrosion scales and contribution to microbial release from scales to surface biofilms due to such change in physicochemical properties of corrosion products (Xing *et al.*, 2021).

In the study of MAP limitation effect on anaerobic groundwater treatment by nitrifying trickling filter, De Vet, van Loosdrecht and Rietveld, 2012, showed that the nitrification and maximum growth rate of ammonia-oxidizing bacteria (AOB) reduced at MAP concentrations under 100 µg l^{-1}, and was close to zero when MAP was lower than 10 µg l^{-1}, explaining it with AOBs relatively low affinity for phosphate, resulting in high sensitivity to the growth of competing microorganisms with higher P-affinity.

1.3.2. Microbially available phosphorus reduction

During drinking water preparation, there is a variety of technologies that are implemented to achieve a water of desirable quality that is safe for further consumption. The effect of the

most common water treatment technologies on specifically MAP concentration has been studied mostly by Markuu Lehtola *et al.*, and to a smaller extent by very few other groups.

MAP, as well as AOC concentrations, increase after ozonation, providing MAP increase by 0.08–0.73 mg P l^{-1} (Table 1.1), which, consequently resulted in an increase of 80 000–730 000 CFU ml^{-1} in water samples (Lehtola *et al.*, 2001). Further, an increase in MAP is provided also by disinfection, coupled with liming, as well as liming alone. However, such technologies as chemical coagulation, granular activated carbon filtration, and artificial re-charge (or soil filtration) facilitate MAP reduction. Wen *et al.*, 2014 reported P limitation and consequent reduction of microbial regrowth potential by coagulation, with the efficiency dependent on coagulant dose – the lowest dose (1 mg l^{-1}) was needed for aluminium sulphate, a higher dose of 3 mg l^{-1} was sufficient for poly-aluminium chloride, and at a dose of 5 mg l^{-1} also ferric chloride was effective; however, poly-aluminium chloride still provided a better reduction of MAP fraction.

Table 1.1. Microbially available phosphorus reduction by conventional treatment.

Process	MAP levels	Comment	Reference
Ozonation	↑	Increase by 13–200 %, on average 94 % from initial av. 0.24 μg l^{-1} (< 0.08–0.39 μg l^{-1}) to av. 0.40 μg l^{-1} (< 0.08–0.73 μg l^{-1}), TP did not change.	(Lehtola *et al.*, 2001, 2002)
Coagulation[*]	↓	Reduced from initial av. 7.09 μg l^{-1} (< 0.08–20.8 μg l^{-1}) to av. 0.20 μg l^{-1}(< 0.08–0.39 μg l^{-1}).	(Lehtola *et al.*, 2002)
Coagulation	↓	Reduced from initial 6 μg l^{-1} to 0.19 μg l^{-1}	(Wen *et al.*, 2014)
Coagulation and sedimentation	↓	27.7 % reduction in July (from 16.67 μg l^{-1} to 12.03 μg l^{-1}), 76.1 % reduction in August (from 12.03 μg l^{-1} to 6.92 μg l^{-1}).	(Jiang, Chen and Ni, 2012)
Filtration	↓	8.6 % reduction in July (from 12.03 μg l^{-1} to 11.00 μg l^{-1}), 31.8 % reduction in August (from 6.92 μg l^{-1} to 4.72 μg l^{-1}), 50.7 % reduction in September (from 7.59 μg l^{-1} to 3.74 μg l^{-1}).	(Jiang, Chen and Ni, 2012)

Process	MAP levels	Comment	Reference
GAC	↓	Decrease by 50–79% (to 0.27 µg l^{-1}).	(Polanska, Huysman and Van Keer, 2005)
GAC		Decreased by 47 %, from initial av. 0.43 µg l^{-1} (0.14–0.79 µg l^{-1}) to av. 0.23 µg l^{-1} (< 0.08–0.70 µg l^{-1}), TP below detection limit of 2 µg l^{-1}.	(Lehtola *et al.*, 2002)
Disinfection (+ liming)	↑	Increase from initial av. 0.23 µg l^{-1} (0.08–0.58 µg l^{-1}) to av. 0.60 µg l^{-1} (0.2–1.15 µg l^{-1}).	(Lehtola *et al.*, 2002)
Infiltration on soil	↓	Reduced by 67 % from initial av. 1.48 µg l^{-1} (0.99–1.92 µg l^{-1}) to av. 0.49 µg l^{-1} (0.35–0.68 µg l^{-1}), TP below detection limit of 2 µg l^{-1}.	(Lehtola *et al.*, 2002)
pH-adjustment with lime	↑	Increase from initial av. 0.50 µg l^{-1} (0.18–1.02 µg l^{-1}) to av. 1.05 µg l^{-1} (0.34–1.79 µg l^{-1}).	(Lehtola *et al.*, 2002)

[*] Poly-aluminium chloride, Fe and Al salts

MAP – microbially available phosphorus, TP – total phosphorus

Jiang, Chen and Ni, 2012 reported that MAP fraction was greater than that of total soluble phosphorus, in such a way attributing some microbial availability also to particulate phosphorus. The proportion of MAP to total phosphorus varied from around 9 to 76 %, mainly depending on the amount of total soluble phosphorus. Similarly, Wen *et al.*, 2014 reported changes in MAP to TP ratio during the water treatment process – the highest observed was in raw water (12.8 %), and it decreased to 1.3 % after coagulation. Therefore, as MAP is a part of total P, also other methods targeting P removal, especially targeting dissolved fraction, might be considered effective in removing its microbially available fraction. As such, a promising technique is adsorption. It can remove P under 10 µg l^{-1} (Kumar *et al.*, 2019), therefore it is expected that MAP fraction removal would be achieved at relatively low levels.

In general, conventional treatment methods are capable of decreasing the microbially available fraction of phosphorus. It also results in the fact that extensively treated surface waters, and artificially recharged groundwaters contain lower MAP concentrations compared to natural groundwater supply (Lehtola *et al.*, 2002; Nescerecka, Juhna and Hammes, 2018), in such a way posing a potentially smaller risk of bacterial re-growth.

1.3.3. Analytical methods

The quantification of growth-promoting nutrients is in general performed using a bioassay, inoculated either by pure culture or by mixed natural bacterial consortium. Initially, the research was focused on the studies of assimilable organic carbon. The protocol for determining the amount of AOC was developed by van der Kooij, Visser and Hijnen, 1982. The essence of the method was the maximum growth of *Pseudomonas fluorescens* P17 and *Spirillum sp.* strain NOX in the water sample, which was further adopted with the addition of inorganic salts to differentiate among AOC_{native} and $AOC_{potential}$ specifically in humus-rich waters (Miettinen, Vartiainen and Martikainen, 1999). Further, the bacterial regrowth potential method was developed at the University of Tokyo (Sathasivan and Ohgaki, 1999), to identify the limiting nutrient for the specific water within five days using indigenous inoculum.

Similarly to analyses of AOC, also MAP determination is based on the use of bioassay. Initially, *Pseudomonas fluorescens* P17 was used due to its phosphatase activity, while the samples were amended with nutrients and carbon in excess (Lehtola *et al.*, 1999). Bioassay was incubated at 15 °C and spread-plated after 4, 5, 6, 7, and 8 days to determine heterotrophic cell count. In such bioassays, $1\,\mu g\,PO_4\text{-}P\,l^{-1}$ corresponded to $3.20–3.73\times10^8\,CFU$ of *Ps. fluorescens*, while $1\,\mu g\,AOC\,l^{-1}$ resulted in $2.04–4.10\times10^6\,CFU$ of *Ps. fluorescens* (Miettinen, Vartiainen and Martikainen, 1999; Polanska, Huysman and Van Keer, 2005).

In 2005, along with flow cytometry protocol adjustment for drinking water analyses, a group of scientists proposed improvements to the AOC determination method (Hammes and Egli, 2005). They suggested the use of natural microbial consortium instead of pure cultures, and its quantification by flow cytometry instead of culture method.

De Vet, van Loosdrecht and Rietveld, 2012, adjusted the MAP protocol for the application on nitrifying bacteria – they used natural inoculum, collected from filtrate water of a nitrifying trickling filter, and ATP coupled with qPCR for general biomass measurements and ammonia-oxidizing bacteria quantification. By comparing different inoculums from cultures originating from a wastewater plant and several drinking water plants, they found that initial cell numbers and their preloading with phosphate were more important factors than the origin of the inoculum (De Vet, van Loosdrecht and Rietveld, 2012).

Wen *et al.*, 2016 proposed improvements to MAP quantification protocol, suggesting the use of flow cytometry for cell enumeration, natural bacterium consortium from Evian mineral water for inoculum, increased incubation temperature to 30 °C to speed bacterial growth, and variation in supplement concentration to extend the measurement range.

1.4. *Legionella* in internal water supply

1.4.1. Opportunistic premise plumbing pathogens

Some microbial residents of drinking water distribution systems within the buildings, or so-called premise plumbing, can cause various diseases in people with weakened immune systems. Such organisms are called opportunistic premise plumbing pathogens (OPPPs), and they are both pathogens and normal inhabitants of drinking water that are adapted to growth and

persistence in potable water plumbing (Falkinham, 2015). The most common OPPPs are *Legionella pneumophila*, *Mycobacterium avium*, and *Pseudomonas aeruginosa*, while less frequently found opportunistic pathogens include *Aeromonas hydrophila*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Burkholderia pseudomallei*, *Acinetobacter baumannii*, *Stenotrophomonas maltophilia*, *Arcobacter butzleri*, and several free-living amoebae including *Naegleria fowleri* and species of *Acanthamoeba* (LeChevallier, Prosser and Stevens, 2024).

Legionella pneumophila is one of the most widely known OPPPs. *Legionella* bacteria are commonly found in freshwater aquatic habitats and natural hot-springs, at temperatures below 63 °C, including their presence in isolates obtained from frozen rivers (Fliermans, 1983). A recent review showed the presence of different concentrations of *Legionella* in a wide variety of both natural and man-made environments (Schwake, Alum and Abbaszadegan, 2021), which include both pristine and anthropogenically polluted surface freshwaters, rainwater collection tanks, and even rain puddles, especially on asphalt roads (Sakamoto *et al.*, 2009), ground freshwater, saltwater of both marine and inland sources, and drinking water, with detection not only in distribution system but also in drinking water treatment plants. In addition to domestic plumbing, it can also be inhaled through aerosols generated by other artificial water systems, such as hot water tanks, hot tubs or spas, cooling towers, and decorative pools or fountains.

In laboratory culturing, it is very fastidious, requiring specific supplements added to the growth medium. However, *L. pneumophila* can grow without amendment with supplements if grown with several other heterotrophic bacteria, by forming satellite colonies around them (Abu Khweek and Amer, 2018).

Legionellae are known to be intracellular pathogens of freshwater protozoa (Fields, Benson and Besser, 2002), and *L. pneumophila* is known to be able to exhibit necrotrophic growth (Temmerman *et al.*, 2006). In plumbing systems, *Legionella pneumophila* can exist in planktonic form, but it mainly persists in biofilms mostly formed by specific microorganisms (Mampel *et al.*, 2006). The persistence of *L. pneumophila* in a biofilm can be promoted by the presence of such bacterial species as *Klebsiella pneumoniae*, *Flavobacterium sp.*, *Empedobacter breve*, *Pseudomonas putida*, and *Pseudomonas fluorescens*, while such species as *Pseudomonas aeruginosa*, *Aeromonas hydrophila*, *Burkholderia cepacia*, *Acidovorax sp.*, and *Sphingomonas sp.* have an inhibitory effect (Abu Khweek and Amer, 2018).

1.4.2. *Legionella* bacteria prevalence in Europe

Legionnaires` disease remains a preventable health threat in Europe. It can be acquired by individuals with weakened immune systems 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 (Fig. 1.1). 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, Pūle, Ķibilds, Labecka, *et al.*, 2023). Apart from the apartment buildings, a study conducted between 2016 and 2022 found that 55 % of 47 surveyed hotels across various municipalities, had at least one *Legionella*-positive sample exceeding the EU-stated threshold value for *Legionella*-related risks control of 1000 CFU l^{-1} (Valciņa, Pūle, Ķibilds, Lazdāne, *et al.*, 2023).
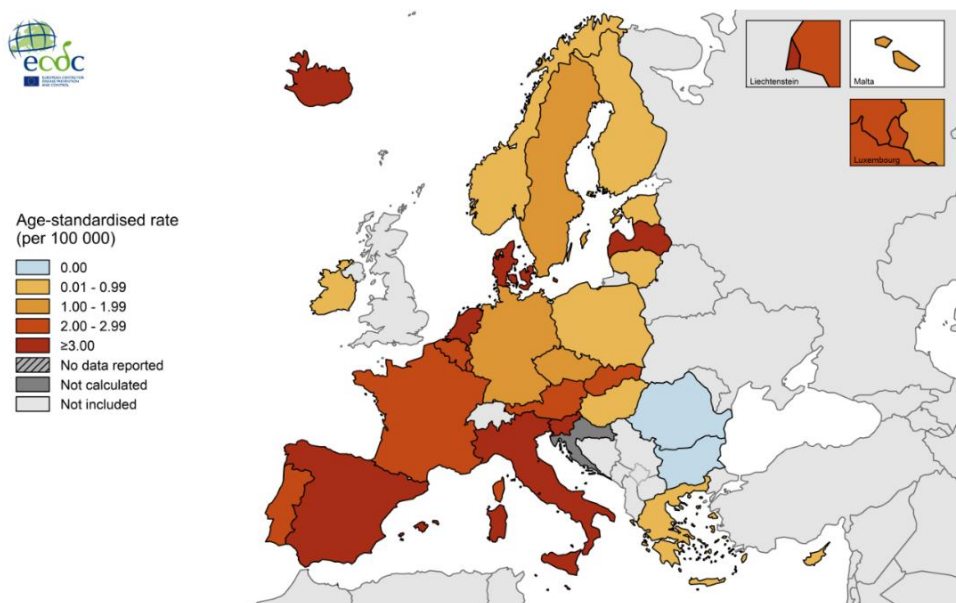

Fig. 1.1. Distribution of cases of Legionnaires' disease per 100 000 population by country, EU/EEA, 2021. Figure from (European Center for Disease Prevention and Control, 2023).

1.4.3. Potential control measures

As stated before, the EU Directive on the quality of water intended for human consumption (The European Parliament and the Council, 2020) has established a *Legionella* control value of 1000 CFU l^{-1} for assessing the risk in domestic water distribution systems. However, there is no common practice to monitor it regularly, especially in residential dwellings.

Water temperature in general has a high impact on the microbiome of drinking water systems. E. g., *Legionellaceae* and *Mycobacteriaceae* are preferentially prevalent in warmer waters, while *Pseudomonadaceae* prevails in colder waters (e.g., 22 °C) (Aloraini, Alum and Abbaszadegan, 2023). Proctor *et al.*, 2017 reported that a temperature of 53 °C uniformly reduced *L. pneumophila* and its key host *V. vermiformis* to below detection limits, regardless of the amount of AOC or pipe material (cupper or PEX). Currently, the hot water temperature remains the main control strategy to limit unwanted growth in the internal drinking water supply. Some of the suggested values include sustaining 55 °C at hot water outlets (Ministru kabinets, 2015), or water heater setpoints of above 60 °C along with recirculation loop water temperatures above 50 °C (Singh *et al.*, 2022). However, in practice, these temperatures often fall significantly lower. A study from the USA reports that both setpoint and circulation loop temperatures are on a median of 6 °C lower than the recommended values (Singh *et al.*, 2022), while the average hot water temperature in Latvian hotels was measured to be 49.8 °C (Valciņa, Pūle, Ķibilds, Lazdāne, *et al.*, 2023).

Other existing control methods are related to periodic high-temperature flushing at 70 °C (Ministru kabinets, 2015), regular disinfection of pipes using chlorine, chlorine dioxide, chloramines, ozonation, UV irradiation or other disinfectants, biocidal treatment, disinfection or ultrafiltration at the building entry (World Health Organization, 2007). However, they are not sufficiently effective over the long-term, as they are unable to affect bacteria in biofilms (Kim *et al.*, 2002), but if colonisation is in place, such measures might be beneficial as *Legionella* control measures not only to reduce the severity of unwanted growth but also to induce shifts in the species. E. g., the hydrogen peroxide treatment can induce a prevalence shift from *L. pneumophila* serogroup 1 to less virulent or non-pathogenic non-*pneumophila* species in hospital water networks (Marchesi *et al.*, 2016).

Another potential measure to control OPPPs prevalence might be a growth-promoting macrobiogenic nutrient limitation. Like the removal of organic carbon to ultra-low levels is stated to decrease *Legionella* risks (Escobar, Randall and Taylor, 2001; Rożej *et al.*, 2015; Learbuch *et al.*, 2019), also removal of MAP can be suggested. A similar strategy applies to the choice of technical agents, e.g., water softeners used to prevent scale formation in drinking water distribution, as phosphate-based chemicals can stimulate *L. pneumophila* growth (Jereb *et al.*, 2022). In general, the prevention of biofilm formation would be a favourable measure, as biofilm protects *L. pneumophila* from the environment and provides nutrients to other biofilm inhabitants (Abu Khweek and Amer, 2018).

As P reduction can reduce biofilm formation, as well as the growth of planktonic microbial cells, it also has the potential to growth *Legionella* control within the distribution system not only by direct nutrient availability but also by potentially reducing the extent of biofilm formation in drinking water distribution system in general.

2. MATERIALS AND METHODS

2.1. Quantification of microbially available phosphorus

2.1.1. Glassware preparation

To prevent traces of phosphate from influencing MAP analysis results, firstly all necessary glassware underwent extensive cleaning procedures. At first, all glassware and plastic caps, used for stock solution and sample preparation, were thoroughly washed to ensure the removal of all traces of phosphate. Firstly, it was soaked in 2 % HCl solution for at least 2 hours. Then it was washed in the dishwasher with phosphate-free detergents, following additional rinsing several times with deionized water and air-drying. Further, the glassware was sealed with aluminium folium and muffled in the oven for three hours at 500 °C. The plastic caps were sealed into the aluminium foil and autoclaved at 121 °C for 20 min.

2.1.2. Sample preparation

Microbially available phosphorus (MAP) was quantified by an adjusted bioassay method (Lehtola *et al.*, 1999; Wen *et al.*, 2016). Initially, the sample was either filtered through a 0.2 μm syringe filter to remove the bacterial background or directly proceeded to the next step, where it was pasteurized in a water bath at 60 °C for at least 50 minutes. Once cooled to room temperature, the samples were amended with either 2 or 4 mg C l^{-1} from a sodium acetate (CH_3COONa) stock solution. Further, to ensure that only phosphorus limits the bacterial growth within the samples, 250 μg N l^{-1}, 10 μg Mg l^{-1}, 27 μg Ca l^{-1}, 53 μg K l^{-1} and 40 μg Na l^{-1} was added from salts stock solution, which contained 42.7 g l^{-1} NH_4NO_3, 6.1 g l^{-1} $MgSO_4$ x $7H_2O$, 5.9 g l^{-1} $CaCl_2$ x $2H_2O$, 6.1 g l^{-1} KCl and 6.1 g l^{-1} NaCl in demineralised water. Then samples were inoculated with 10^3 cells per millilitre of sample and incubated at 30 °C with continuous shaking at 150 RPM for 5 days and then enumerated by flow cytometry (Chapter 2.4.2). All samples were prepared in triplicates.

2.1.3. Inoculum

Single culture. *Pseudomonas brenneri* P17 (ATCC 49642) were used for MAP quantification as a single-culture inoculum due to its phosphatase activity (Lehtola *et al.*, 1999). To ensure the cell freshness, one eyelet of the microbial culture, stored on an R2A agar plate at +4 °C, was dispersed into a liquid R2A medium and incubated for 24 hours at +30 °C, using an orbital shaker at 150 RPM. Then, to remove the traces of the cultivation medium, the cells were washed several times with 0.1 μm filtrated mineral water, using a micro-spin centrifuge for the cell separation at the setting of 6000 RPM for 2 min. Further, to utilize the remaining traces of nutrients, cells were resuspended in mineral water, amended with sodium acetate (CH_3COONa) as a carbon source (1 mg C l^{-1}), and incubated at +30 °C at 150 RPM for 24 hours.

Mixed bacterial culture. A freshly opened bottle of natural mineral water (Evian, Danone, France) was used as a natural bacterial consortium without additional preparation.

2.1.4. Calibration standards and controls

To convert bioassay cell concentration to the amount of MAP, a set of calibration standards was prepared in various phosphorus concentrations, using disodium hydrogen phosphate (Na_2HPO_4) stock solution in demineralised water.

In general, the procedure was the same as in the case of sample preparation, only, as demineralised water was used, the concentrations of salts were 60x larger in the case when added acetate carbon concentration was 2 mg C l^{-1} and 90x times larger when added acetate carbon concentration was 4 mg C l^{-1}.

Alongside the sample preparation, a set of triplicate standards in several concentrations, including without the addition of phosphate, was used as a quality control measure. The standard without added phosphate was used as a control for background MAP concentration when mixed bacterial inoculum was used. An average "0-MAP" control cell concentration was subtracted from all samples before converting to MAP concentration.

When pure culture was used as inoculum, the sample was processed without previous filtration, therefore initial bacterial enumeration followed directly after inoculation to control for sample background. The gained value was subtracted from the final cell concentration after the incubation period.

2.2. MAP reduction potential

2.2.1. Experimental outline

A set of experiments was conducted to determine the potential for MAP removal of several easy-to-implement and affordable methods. The study was performed in three phases. Firstly, it was conducted at an artificially recharged groundwater station in Baltezers, Riga, to compare the selected methods for their ability to eliminate MAP in groundwater. Such methods as sorption, biofiltration and coagulation were chosen due to their potential to remove phosphate to low concentrations in water treatment plants. However, mostly they are used for surface water treatment, which is usually at higher temperatures, and their impact on microbially available fraction removal from colder temperature groundwater, which is used as a drinking water source in almost all Latvian cities, except for a part of a capital, was unknown.

Secondly, the effect of temperature and additional substrate on biofilter performance was evaluated. As the groundwater temperature is not optimal for bacterial growth, increased temperature conditions were evaluated alongside the biofilter amendment with acetate carbon to determine potential improvements in MAP removal potential.

Finally, a chosen MAP removal method was tested in an enlarged laboratory set-up, using commercially available materials certified suitable for drinking water treatment processes.

2.2.2. Column reactors and selected methods

To test the three chosen methods, custom-made column reactors were used. They consisted of polyvinyl chloride (PVC) pipes with a diameter of 7.5 cm cut to a length of 50 cm and

enclosed by metal fittings. The water volume within each reactor was from 1.3 to 1.8 litres, depending on the filling.

The sorption reactor contained plastic biomass carriers (Bioflow 9, RVT Process Equipment, Germany), which were a by-product of iron removal processes from groundwater, where they were naturally covered with ferric oxides.

The biofiltration reactor was filled with similar commercial biomass carriers, which were covered with biomass. The biofilm was cultivated for a duration of one month using a mixture of river and drinking water (Tihomirova, 2011). Specifically, a combination of 1/3 river water sourced from the Daugava River, filtered through a 1.2 µm pore filter (Millipore, Germany), was blended with 2/3 tap water obtained from Riga's city water supply. This water blend was filled into heat-sterilized borosilicate glass bottles, and biomass carriers were inserted, their tops covered with aluminium foil to prevent algae growth. The bottles were positioned on an orbital shaker, with the water being renewed every week.

The electrocoagulation reactor was equipped with four cylindrical-shaped 60/60 grade aluminium electrodes, placed within 10 cm of each other. They were 2 cm in diameter, with a combined electrode surface area of 622 cm^2. A laboratory power supply unit (EA-PS 2084-10B, Elektro-Automatik, Germany) ensured a direct current of 2.5 A that was applied in a monopolar-parallel connection, attaining a current density of 4 mA cm^{-2}.

2.2.3. Experimental layout and test water

Methods comparison test. An experimental set-up to compare the MAP removal potential of the selected methods at the artificially recharged groundwater pumping and treatment station contained four column reactors (Fig. 2.1). There was one reactor dedicated for each water treatment method and one remained without any inserts and was utilised as a control.

A flexible plastic pipeline transferred the raw groundwater (main parameters summarised in Table 2.1) from the distribution pipeline into a plastic vessel that was located on the sewer floor drain grid, allowing for operation with continuous overflowing. Further, the water was pumped with a peristaltic pump (MasterFlex 77202-50, Cole-Parmer Instrument Co., USA) at a flow rate of 65 ml min^{-1}. The pump was equipped with four cartridges, ensuring individual water supply to each reactor. The inlet water was distributed using silicone tubing, and at reactors` outflows, flexible plastic tubing was used. The contact time in each reactor was around 20 minutes.

The test was carried out in a plug-flow configuration for over two weeks at an ambient temperature of 10.9 °C (SD 0.8 °C, n = 9), with a sampling frequency three times per week. The samples were taken from the common inlet and at each outflow.
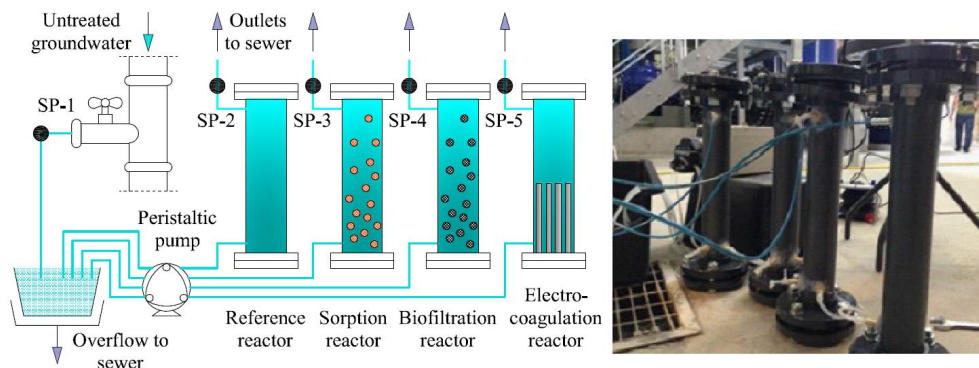
Fig. 2.1. Plug-flow configuration setup at an artificially recharged groundwater station in Baltezers, Riga with marked sampling places (SP).

Temperature and carbon limitation test. The performance of the biofilm reactor was monitored under elevated temperature conditions. Moreover, an addition of substrate was tested for the ability to better utilise MAP. For this purpose, the study was performed in a controlled environment within a climatic chamber at an ambient temperature of +21 °C (SD 0 °C, n = 12).

Biomass carriers from previous tests at natural groundwater temperature were equally divided among two column reactors (Fig. 2.2), in such a way as to ensure the use of the same biota for performance evaluation. The flow rate was adjusted to 60 ml min^{-1}, providing a contact time of around 25 min.

The groundwater from the same artificially recharged groundwater station Baltezers, Riga, was used for the test. Sodium acetate was used as an additional substrate for one reactor at the initial amendment of 2 mg C l^{-1} (main parameters summarised in Table 2.1).

The test was carried out in a recirculation mode configuration for two days (45 hours), and MAP concentration was monitored at the outflow of reactors before re-entry into the feed water vessel.
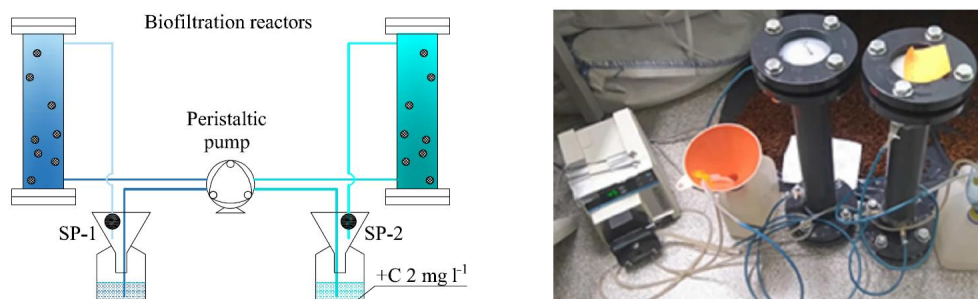

Fig. 2.2. Recirculation configuration setup for Biofiltration reactors with marked sampling places (SP).

Table 2.1. Inlet water parameters.

Parameter, unit	Plug-flow configuration			Recirculation configuration		
	Number of samples	Mean value	SD	Number of samples	Mean value	SD
Temperature, °C	5	8.1	0.10	12	19.32	0.41
Turbidity, NTU	5	0.82	0.25	-	-	-
pH	5	8.04	0.32	12	8.11	0.31
Oxidation-reduction potential, mV	5	41.4	50.30	12	68.50	51.59
Electrical conductivity, $\mu S\ cm^{-1}$	5	910	11.60	12	967.58	11.26
Fe (total), mg l^{-1}	2	0.29	0.04	-	-	-
Fe^{+2}, mg l^{-1}	1	0.07	0.07	-	-	-
O_2, mg l^{-1}	-	-	-	12	9.14	0.22

2.2.4. Ferric hydroxide sorption filter

Granular ferric hydroxide (GFH) was chosen as a commercial alternative (GEH 102, GEH Wasserchemie, Germany) to iron oxide-coated sands to ensure the use of safe materials in further pilot-scale studies. GFH is mostly used for arsenic removal from drinking water, and similar materials are utilised in wastewater treatment to mitigate phosphate levels. GFH consists mainly of ß-FeOOH and $Fe(OH)_3$, containing 58% (± 10%) dry solids with an iron content of 600 g kg^{-1} (± 10%). The particle size ranges from 0.2–2.0 mm, with a specific surface area of around 300 $m^2\ g^{-1}$. The backwashed bulk density is 1150 kg m^{-3} (± 10%).

A laboratory-scale system (Fig. 2.3) consisted of one 25-litre filter casing filled with 9.3 litres of GFH sorbent and 6.2 litres of supporting sand layer of various grain sizes, obtained from iron removal filter maintenance. The water flow was adjusted to 0.55 $m^3\ h^{-1}$ and disodium phosphate (Na_2HPO_4) solution was injected into the system with a dosing pump Etatron 5-5 (Etatron D.S., Italy) to enlarge orthophosphate concentration to rapidly detectable values.

Alternatively, to evaluate possible improvements in filter P-removal efficiency, iron-based coagulant was dosed into the system.
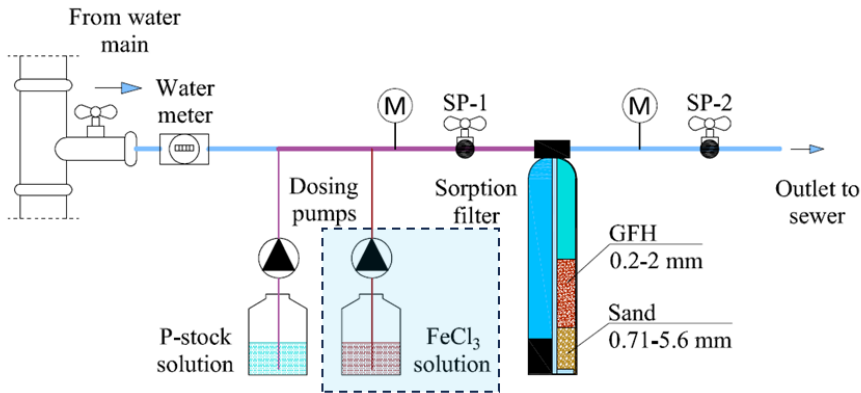
Fig. 2.3. Laboratory-scale filtration setup with granular ferric hydroxide (GFH) sorption filter with phosphate dosing and optional FeCl$_3$ coagulant dosing system for phosphorus (P) removal with marked sampling places (SP).

2.3. Case study

2.3.1. Pilot site description

A pilot site consisted of two five-storey residential buildings within a proximity of around 100 meters (Fig. 2.4). Both buildings were commissioned in 1976. Within the framework of building maintenance works, the communal internal water pipelines were replaced with polypropylene pipes in the year 2000. Thus, the age of the internal water supply pipelines was 22 years. Both buildings were supplied with municipal drinking water of groundwater origin. Hot water was prepared by plate heat exchangers in individual heating substations, located in the basement of each building.

During the study time, the domestic hot water supply (DHW) encounter two distinct temperature regimes. Firstly, the water temperature at the exit of the plate 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 and denoted as "POU-device building". The other one was used for comparison and denoted as "Reference building".
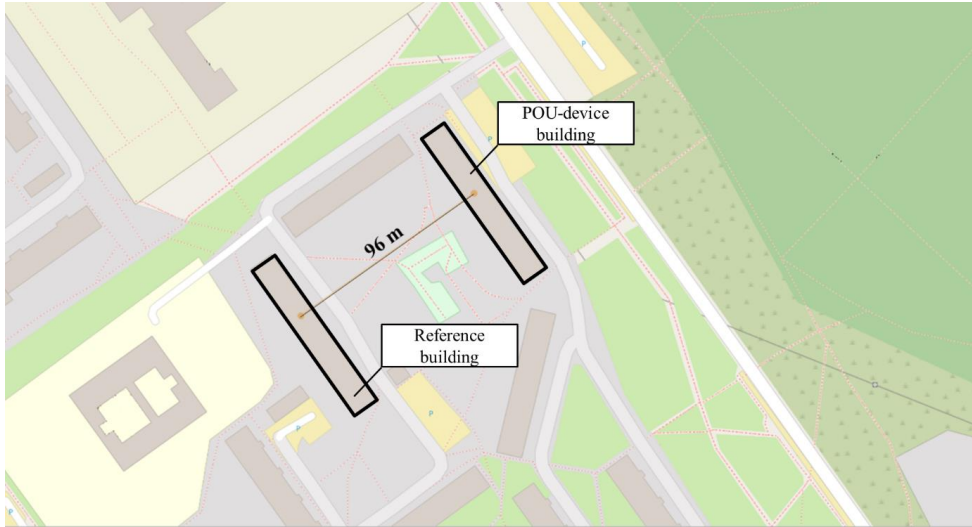
Fig. 2.4. Map layout of the pilot site with distance between pilot buildings.

2.3.2. Point-of-use treatment unit

The POU filtration device was located inside the basement of one of the buildings, close to the location of the water mains inlet of the building.

The unit incorporated two 145-litre filter housings (Fig. 2.5). Each of them was filled with 60 kg of granular ferric hydroxide (GFH) sorption media (GEH Wasserchemie, Germany) supported by three 17-litre layers of AQUAGRAN quartz sand in sizes of 0.71–1.25 mm, 1.00–3.15 mm, and 3.15–5.6 mm (Euroquarz, Germany).

The sampling taps were located before and after the filtration set-up. During the sampling events, a change in pressure was monitored to assess the occurrence of filter clogging. However, during the five-month study period, there was no significant pressure drop observed and therefore there was no need for filter backwashing to clean or maintain its performance.
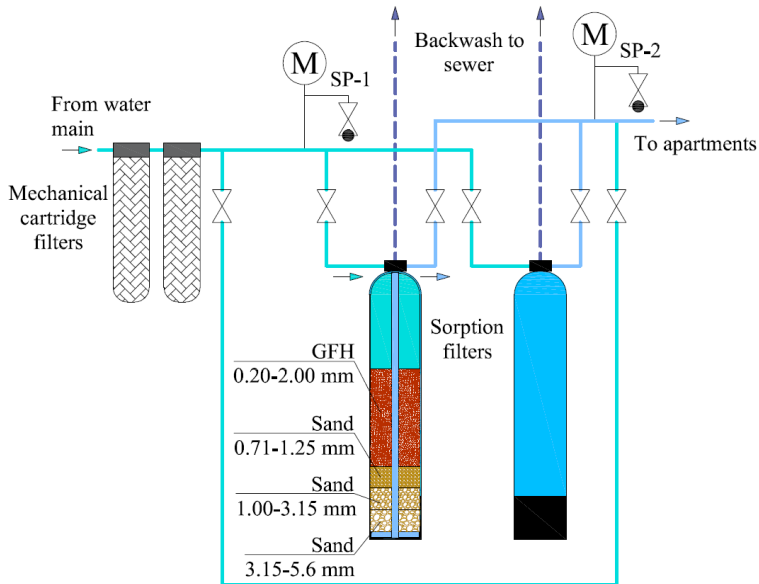
Fig. 2.5. The schematic of POU granular ferric hydroxide (GFH) sorption filters connected after existing cartridge filters with sampling places (SP) shown as black dots and mechanical manometers to monitor filter clogging (M). Figure adapted from (Zemīte *et al.*, 2023).

2.3.3. Internal network chemical flushing and disinfection

Before the testing period, both buildings underwent centralised chemical flushing and disinfection of the internal water supply to remove existing deposits and provide pipeline disinfection, ensuring a uniform starting point for both the POU-device and Reference buildings. This process was carried out by a specialist company, priory informing the residents about forthcoming procedures and necessary actions.

Initially, to initiate the removal of the deposits from hot water pipelines, the 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.

To encourage thorough disinfection of sanitary devices, and ensure the required reagent concentrations in the outflows, the specialists surveyed all apartments. However, not all residents were available for such a procedure, therefore a substantial number of apartments did not perform this step. Generally, controlled disinfectant monitoring was feasible in the sanitary devices` outflows of 51 % of POU-device building apartments and 68 % of Reference building apartments (Fig. 2.6). The remaining apartments faced unsuccessful specialist visits due to absent or unwilling occupants. Due to the ageing network, there was one pipeline leakage caused by scale removal from the acid treatment step in the Reference building. This event

caused subsequent downstairs apartment flooding and water pipe replacement by the building manager in the affected area).
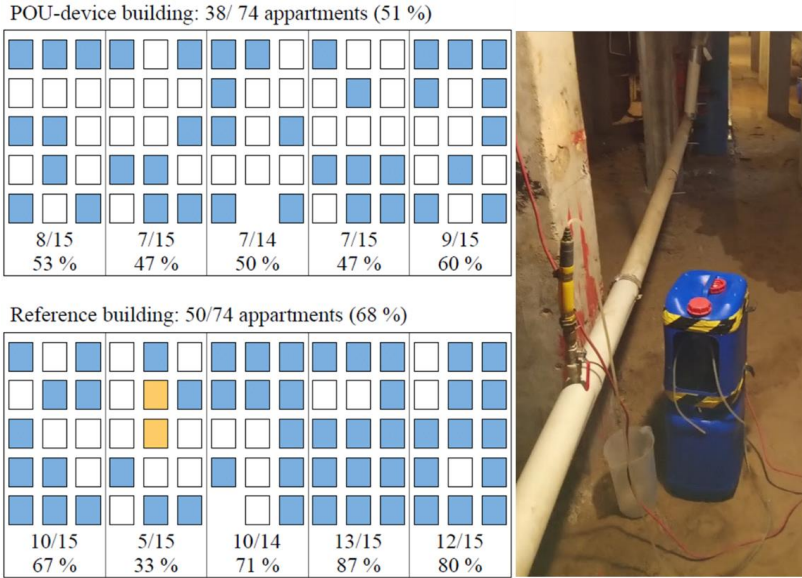

Fig. 2.6. The distribution (blue rectangles) and number of apartments in each stairwell that responded to specialist visits while performing centralised building chemical flushing of domestic hot water supply and disinfection of both hot and cold-water supply networks. Orange rectangles depict apartments with emergency leakages.

2.3.4. Apartment selection

The building's DHW supply system is designed such that heated water from the heating substation in the basement is distributed through hot water mains on the basement level. The piping then enters the shafts with riser pipes, which are connected to individual apartment water distribution pipelines. Additionally, the DHW plate heat exchanger has an additional connection for circulating hot water to reduce the travel time once the apartment`s water usage point is open. However, due to heat loss, the circulating hot water cools down as it moves upwards, leading to a higher risk of unwanted bacterial growth on higher floors. To gain a better overview of *Legionella* growth, apartment sampling was based on their location. Generally, only ground-floor and top-floor apartments were considered, but due to residents' willingness to participate, one apartment from the 2nd floor was also included.

All participants in the study were using hot water prepared inside the building's heating substation by a centralized DHW heat exchanger. However, the shower usage frequency within apartments varied from several times per day to once every few days, depending on the number of residents in each apartment and their habits.

Throughout the sampling period, the number of apartments included in the study varied due to the varying involvement of inhabitants. In total, the POU-device building had three apartments on the 5th floor, one on the 2nd floor, and one on the 1st floor. Meanwhile, the Reference building had two apartments on the 5th floor and one on the 1st floor. The spatial location of apartments relative to the water inlet and heating substation is marked in Fig. 2.7.
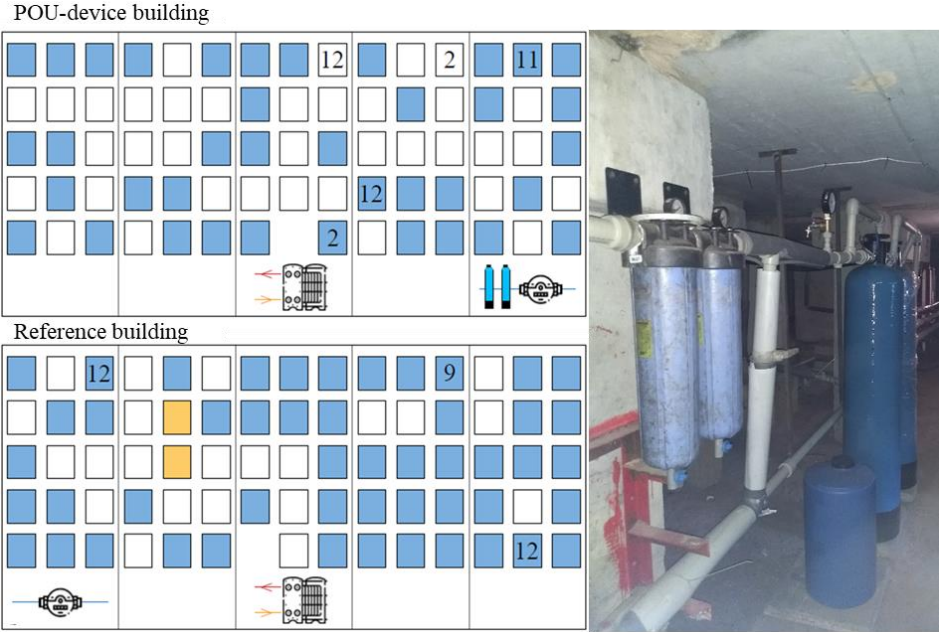

Fig. 2.7. The number of sampling times and spatial distribution of involved apartments in relation to the drinking water inlet meter, POU filtration device (on the right) and domestic hot water preparation unit, and successful disinfection survey (blue rectangles). Icons made by Freepik on Flaticon.com.

2.3.5. Sample collection

The sample collection was organised over a period of five months, starting from mid-July 2022 until mid-December. There were four distinct sampling locations (Fig. 2.8), 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. Overall, all samples were mainly collected on the same day, with only rare exceptions due to the availability of inhabitants.

For water collection, autoclavable polypropylene two-litre bottles were used. These bottles were machine-washed with phosphate-free detergent and heat-sterilized at 121 °C for 20 min. Then, they were distributed among involved inhabitants one day before the sampling event. The residents were instructed to collect the first-draw samples directly from the showerheads early in the morning, before using the shower, and further to collect kitchen tap water similarly. On the sampling day, the bottles were collected from the residents, as well as water was sampled from the remaining sampling locations. Further, the samples were transferred to the BIOR laboratory, where they were divided into separate borosilicate bottles to be spread among the laboratories.
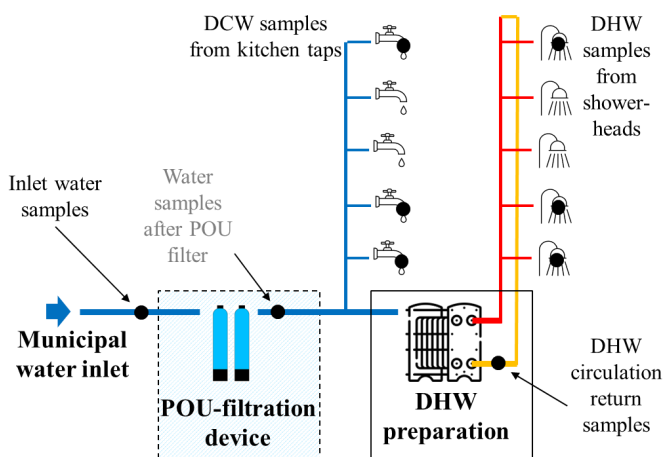

Fig. 2.8. Schematic of the sampling locations (marked with black dots) for both domestic cold water (DCW) and domestic hot water (DHW) systems. Figure adapted from (Zemīte *et al.*, 2023).

2.4.   Analytical methods

2.4.1. Physical and chemical analyses

Temperature was measured with digital thermometers (accuracy ± 1°C in the range of −30°C ~ +150°C). Before the POU filtration start-up, all participating residents were provided with identical thermometers and instructed to record the temperature of the first-draw showerhead and tap samples by pouring the liquid from the sampling bottle into the provided 12-ml vial immediately after sample collection. Further, a one-minute flushed temperature was also recorded. The temperature of inlet water and circulation return samples was measured directly in a sample bottle, first rinsing the thermometer with the flowing sample water from sampling taps.

After the sample transition to a laboratory, **electrical conductivity** and **pH** were measured with HQ40D Portable Multi Meter (HACH, ASV) with corresponding sensors attached.

Online electrical conductivity measurements were done by in-line mounted Conductivity Probes with a measuring range of 0.02–2000 µS cm^{-1} (Comeco Control & Measurement, Bulgaria). The logging was done every 10 minutes by GSM IoT Wireless Datalogger (COMET System, Czech Republic).

Water consumption was read from an existing inlet water meter amended with an impulse reader (Zenner, Germany) and recorded on a portal with a timestep of 20 minutes by Metbox GSM/ GPRS Remote Telemetry Unit (Teliko, Latvia).

Total organic carbon (TOC) was quantified by high-temperature catalytic combustion using Formacs^{HT} TOC Analyzer with LAS-160 Autosampler module (Skalar Analytical B.V., The Netherlands). The calibration curves were adjusted to correspond to the values of control samples of various concentrations and analysed with each set of samples.

Orthophosphates (PO$_4$-P) concentration was determined by DR/890 Portable Colorimeter (HACH, USA), using PhosVer 3$^®$ Ascorbic acid method reagent kit.

Ca, Mg, Mn, Cu, Zn, Fe and Pb were quantified by ICP-MS by the Institute for Food Safety, Animal Health and Environment "BIOR" (Riga, Latvia).

2.4.2. Cell enumeration

Initially, for the MAP protocol testing, a comparison of enumeration methods was performed. The inoculated MAP standards with known P concentrations were subjected to both – classical cultivation, and cell enumeration by flow cytometry. For the Heterotrophic Plate Count method, an inoculated sample was spread-plated on R2A agar medium (Reasoner and Geldreich, 1985) plates in several dilutions and incubated for 2 days at 30 °C before enumeration.

In general, flow cytometry was used for bacterial cell enumeration throughout the study. Initially, PartecCyFlow® SL (Partec, Germany) was used. Further, during the time of the case study, it was changed to CyFlow® Cube 6 (Sysmex, Germany). Both cytometers were equipped with a blue (488 nm) solid-state laser for excitation with a power of 25 mW for the first one and 50 mW for the latter.

The two-channel density plots (FL1 vs. FL3) were used for data acquisition, where FL1 represented green emission (520 nm ± 10 nm or 536 nm ± 20 nm) and FL3 represented red emission (630 nm long pass). The trigger was set on the FL1 channel with a threshold of 10.

CyFlow® Cube 6 had the voltage set at 325 for the FL1 channel, and for the FL3 channel, 500 for total cell count (TCC) or 375 for intact/damaged cell count (ICC/DCC). True Volumetric Absolute Counting (TVAC) was employed for measurements, and a flow rate of 5.0 µl s^{-1} was utilised.

Bacterial cell count was determined as **intact cell count (ICC)** and **damaged cell count (DCC)**. The **ICC** was divided into two categories: **high nucleic acid content (HNA) cells** and **low nucleic acid (LNA) content cells** according to the positioning on acquired density plots (Fig. 2.9, left). The same gating of cell regions on density plots was used throughout the study

with some variation among original and diluted samples due to changes in cell positioning on the plot. For cell enumeration, **total cell count (TCC)** was determined by summing up ICC and DCC numbers, however, in the case of MAP measurements, it was measured as a single parameter (Fig. 2.9, right).

For sample staining, a working fluorophore stock of SYBR Green I nucleic acid stain in DMSO (Sigma-Aldrich, USA) was diluted to 100x concentration with 10 mM TRIS buffer (pH 8). For TCC, an additional 100x dilution of the working fluorophore stock was used for staining within the sample. For ICC/DCC sample staining, a working stock was prepared with an additional 0.6 mM Propidium iodide. The staining process was carried out in a dark environment using a thermo-block at 35 °C for 10 min (TCC) or 15 min (ICC/DCC) (Nescerecka, Hammes and Juhna, 2016).
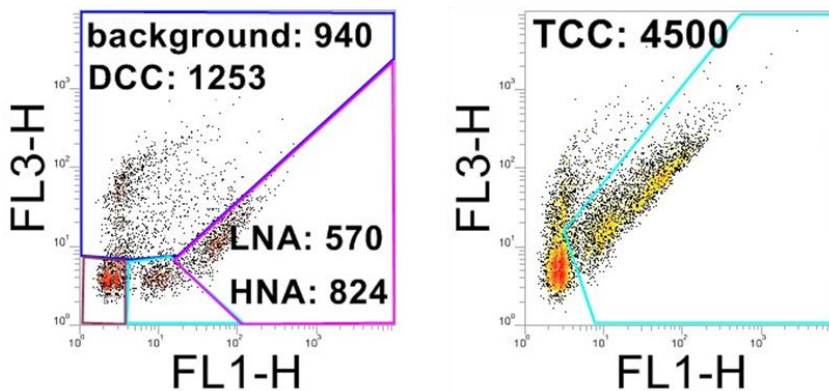

Fig. 2.9. Flow cytometry density plots with microbial cell counts per 200 µl of True Volumetric Absolute Counting (TVAC) for SYBR Green I + Propidium iodide (left) and solely SYBR Green I (right) staining.

2.4.3. Legionella analysis

Legionella spp. counts and serotyping were performed by the "BIOR". The isolation and identification of *Legionella* spp. was performed according to the ISO 11731 standard (European Committee for Standardization, 2017). Further, *L. pneumophila* was confirmed by an agglutination test (Thermo Fisher Scientific, Bred, Netherlands). Then, the detection of serogroup was performed with individual latex reagents (Pro-Lab Diagnostics, Richmond Hill, Canada). In summary, colonies from all plates were counted and confirmed, and the estimated number of *Legionella* was expressed as CFU l^{-1} of *Legionella* species and serogroup.

2.5.   Statistical analyses and data presentation

Statistical analyses were performed using IBM SPSS Statistics software (version 23). A 2-tailed significance with $\alpha = 0.05$ was used throughout the study.

Assumptions of normal distribution were evaluated using Shapiro-Wilk's test of normality in most cases, while the Kolmogorov-Smirnov test of normality was employed for online data related to electrical conductivity. The homogeneity of variances was assessed using Levene's test. No outliers were excluded from the analysis.

For establishing statistical significance, an independent sample t-test was employed for data with normal distribution. Alternatively, the non-parametric Mann-Whitney U test was applied for data not meeting necessary assumptions, where a comparison of mean ranks between groups was executed.

The dependent sample analyses were used only in the sorption optimisation study. A paired samples t-test was used for normally distributed data, or otherwise Wilcoxon Signed Ranks Test was used for a non-parametric rank test data comparison.

Assessment of the standard curve and corresponding coefficients was performed in MS Excel, utilizing a scatter plot with a linear trendline. Furthermore, for parametric correlation analysis, bivariate correlation involving the Pearson coefficient was carried out using IBM SPSS software.

For data presentation, the suggestions from SAMPL guidelines were used (Lang and Altman, 2015). The descriptive statistics in tables were summarised in two ways – either as a mean value with standard deviation (SD) or a median value with minimum and maximum. The mean value was used for normally distributed data and the median was used for non-normal distribution, indicating both the characteristics of the data set and the choice of statistical tests for data normality assumption.

The graphical data visualisation was performed in MS Excel, Origin Pro 2019, and IBM SPSS Statistics (version 23) software.

3. MAP QUANTIFICATION USING BIOASSAY

3.1. Comparison of cell-growth enumeration methods

To determine the interchangeability of cell enumeration techniques, the comparison between the conventional heterotrophic plate count (HPC) method and more rapid flow cytometry (FCM) measurement technique was performed with 4 different microbially available phosphorus (MAP) assays, containing P concentrations of 0, 1, 5 and 10 $\mu g\, l^{-1}$. To ensure bacterial growth during plate culture, only the single-strain bacterial cells (*Pseudomonas brenneri* P17) were used for the bioassays. The inoculated assays were subjected to daily sampling and further bacterial enumeration by both techniques.

The results (Fig. 3.1) show similar trends for both quantification methods. Various P concentrations samples taken shortly after inoculation reached an average of 2.1×10^3 cells ml^{-1} (SD 360 cells ml^{-1}) when analysed by FCM and 6.5×10^2 cells ml^{-1} (SD 95 cells ml^{-1}) when analysed by HPC. After 24 hours the cell concentration showed a 0.07–0.38 $\log_{10}$ increase when quantified by FCM or a 0.42–0.76 $\log_{10}$ increase when quantified by HPC.

Days 1–3 display exponential cell growth with the most rapid increase detected after 48 hours. The $\log_{10}$ increase for this sampling time reached 0.91–1.53 cells ml^{-1} for FCM and 1.10–1.92 cells ml^{-1} for HPC and the corresponding calculated maximal exponential growth rate μ_{max} was 0.09–0.15 h^{-1} and 0.11–0.18 h^{-1}, respectively. However, it should be noted that samples were taken only once per day and the actual μ_{max} can differ.

Both cell enumeration methods performed similarly with no statistically significant differences for all different MAP assays (Mann–Whitney U test p values 0.59–0.82). Additionally, they showed with very high correlation (Fig. 3.2) with $R^2 = 0.996$.

Although the pure culture inoculum ensures methods interchangeability, it shows a week correlation when natural water biota needs to be quantified. Other authors (Siebel *et al.*, 2008; Nescerecka *et al.*, 2014; Wen *et al.*, 2016) reported R^2 values of 0.18–0.31 when mixed bacterial consortium was used. As culturable cells represent less than 1% of the total cell count value (Amann, Ludwig and Schleifer, 1995), it underestimates the quantities of bacterial counts.

Additionally, FCM quantification is more robust in terms of timing, as it requires sample acquisition during the microbial stationary phase while HPC needs precise determination of the day of maximum growth. Also, FCM requires minutes for analyses compared to days in the case of HPC.

Therefore, due to the method's rapidness, timing robustness and versatility of variable inoculum enumeration, FCM is the preferred cell quantification technique for MAP analyses and thus is further used throughout the study.
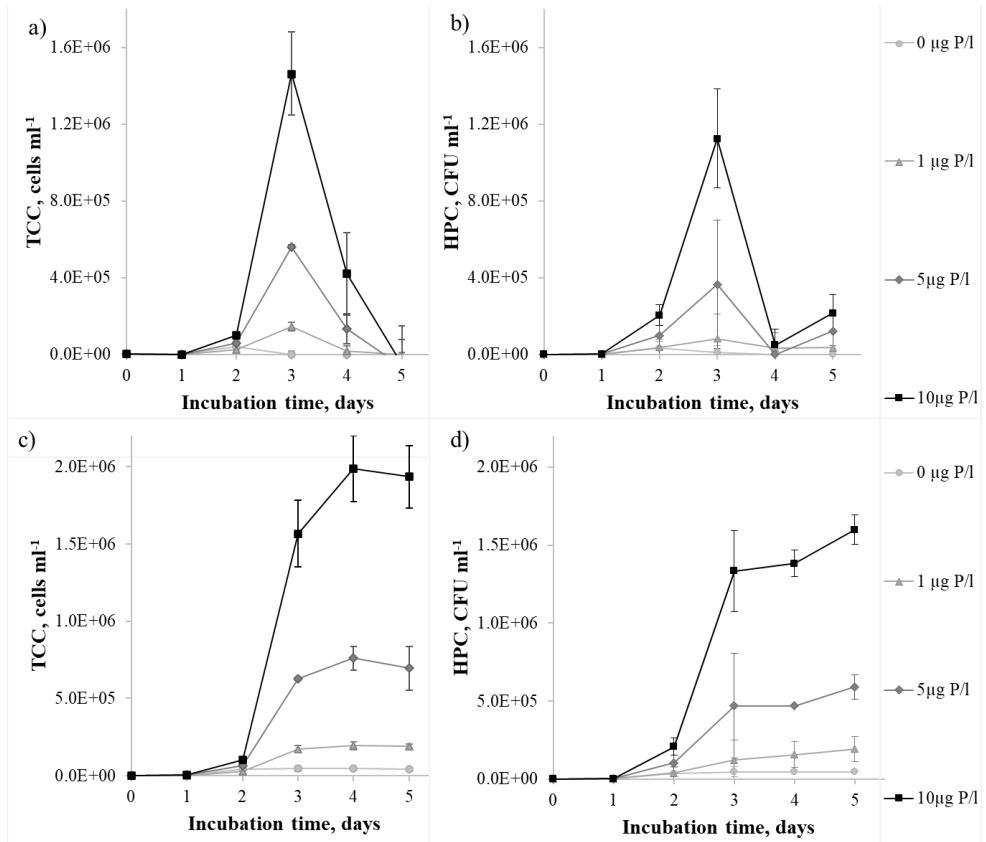
Fig. 3.1. Daily (a, b) and cumulative (c, d) quantity of *Ps. brenneri* in MAP bioassays enumerated by FCM (a, c) and HPC (b, d) with bars for stand. dev. of triplicate samples.
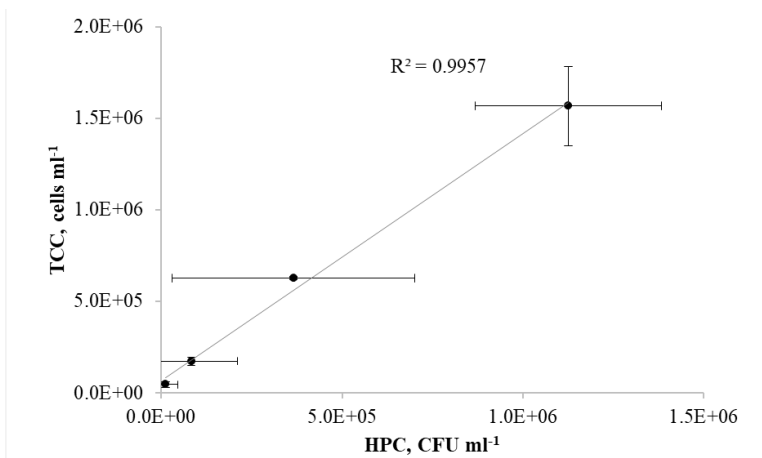

Fig. 3.2. Relationship between FCM total cell count and HPC colony forming units of *Ps. brenneri* from various MAP bioassays with bars for stand. dev. of triplicate samples.

3.2. Impact of inoculum

To determine the impact of inoculum on MAP quantification, a natural bacterial consortium from mineral water (Evian, Danone, France) was compared to a single-organism cell culture (*Pseudomonas brenneri* P17). Flow cytometry was used for cell enumeration, and MAP assays for each inoculum were prepared independently.

The results (Fig. 3.3) indicate high linear correlation ($R^2 > 0.95$) for both types of inoculums with corresponding yield factors of 1.6×10^8 cells of *Ps. brenneri* per μg of MAP with Pearson correlation of 0.978 ($p < 0.001$, n = 8) (Fig. 3.3 a) and 3.0×10^9 cells of natural mineral water consortium per μg of MAP with Pearson correlation of 0.973 ($p < 0.001$, n = 59) (Fig. 3.3 b).
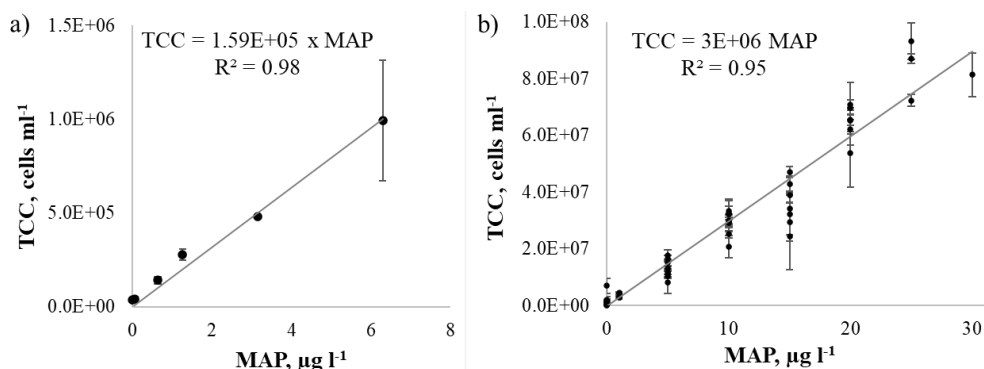

Fig. 3.3. Correlation between the growth of a) *Ps. brenneri* and b) natural bacterium consortium cells in MAP assays, enumerated by FCM, with bars for stand. dev. of triplicate samples. The data points are replicates in time.

In general, yield factors range between 1.3×10^8 to 1.1×10^9 cells per μg of P for single-strain culture inoculum and 9.4×10^8 to 3.0×10^9 cells per μg of P for mixed bacterial consortium inoculum (Table 3.1).

The use of mixed bacterial consortium in our case resulted in over an-order-of-magnitude higher cell yield for the same amount of phosphate if compared to single strain inoculum. That corresponds to the findings by Wen *et al.*, 2016, who reported 0.7 log cells per μg of P higher yield achieved by mixed microbial inoculum. Such a higher yield is more favourable as it increases the method`s sensitivity.

Additionally, an increase in measurement range can be achieved when increasing spiked acetate carbon concentration (Wen *et al.*, 2016), though it might require also enlarged salts solution addition for MAP calibration standards preparation to ensure sufficient nitrogen and other elements content when demineralised water is used as MAP assay medium (data not shown).

There were some limitations noted when mixed bacterial consortium was used as inoculum for MAP bioassays:

1) Although a freshly opened inoculum bottle was used at every inoculation, calibration revealed variability in correlation in PO$_4$-P consumption (Fig. 3.3 b), which is especially visible at a P concentration of 15 mg l^{-1};

2) There were noted greater inconsistencies among the sample triplicates. To minimise the spread of measured 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

3.3.   Section conclusions

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.

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. Furthermore, 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 was 3.0×10^9, more than ten times higher than that obtained with pure cultures, 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.

4. MAP REDUCTION METHODS

4.1. Efficiency of MAP removal in column reactors

Selection of test methods. The column reactors were used to monitor electrocoagulation, sorption onto ferric oxides and biofiltration efficiency of MAP removal from natural groundwater. The selected methods were known to be effective in overall phosphorus removal and were chosen to assess their impact on the elimination of microbially available fractions of P. Moreover, they had to be potentially easy to implement as point-of-use water treatment devices.

Electrocoagulation (EC) was used instead of conventional coagulation as it generates less sludge, minimizes the direct handling of corrosive chemicals, preserves the water's natural buffering capacity (alkalinity), and can be easily implemented in portable water purification units (Mollah *et al.*, 2004; Holt, Barton and Mitchell, 2005; Gamage and Chellam, 2011). Sorption, although mainly addresses a nutrient limitation in wastewater studies, showed a potential 70–90 % reduction in total dissolved P also from stormwater runoff (O'Reilly *et al.*, 2012), while biofilters, in general, utilise nutrients present in the inflow water to support their activity.

Initially, the study was conducted at an artificially recharged groundwater plant, operating in a plug-flow mode (Fig. 2.1), but afterwards biofiltration reactor was moved to a climatic chamber and operated in recirculation mode to test for potential temperature and carbon limitation (Fig. 2.2).

Plug-flow configuration setup. When treating groundwater at about +8 °C temperature, the outflow MAP concentrations for the electrocoagulation reactor reached 0–5.05 µg l^{-1} (Fig. 4.1). It showed 76 % MAP elimination directly after the start-up, while further it should be noted that there was no EC reactor outflow sampled on days 2 and 3 due to interruptions in power supply. However, later on, the EC reactor ensured nearly complete MAP removal.

Sorption provided MAP reduction from 18.92 µg l^{-1} (SD 2.15 µg l^{-1}) to 2.35–13.22 µg l^{-1} with a median value of 4.19 µg l^{-1}, ensuring 41 % MAP removal directly after start-up and further providing > 72 % elimination, except for day 10, when 32 % elimination was shown. A decrease in removal might be attributed to partial MAP desorption (Neely and Nairn, 2011), especially, if the pH of inflow water would slightly decrease.

The biofiltration ensured reduction only to an average of 13.25 µg l^{-1} (SD 3.85 µg l^{-1}), showing the lowest MAP reduction if compared to other technologies. Such performance was most likely attributed to the temperature, however, the availability of carbon source was also not excluded, therefore additional tests at elevated temperatures were done.

In addition to column reactors dedicated to specific water treatment methods, an empty reactor was used as a control. Initially, it showed MAP reduction from 20.65 µg l^{-1} to 4.45 µg l^{-1}, or around 80 %, directly after the start-up. It could have been attributed to sorption onto reactor materials. On the third day, however, an empty column outflow contained higher

MAP concentration than inflow into the reactors, which might have indicated potential accumulated MAP de-sorption. Statistically, throughout the test time the control reactor provided MAP concentrations similar to the water inflow (Wilcoxon Signed Ranks Test, $p = 0.26$), so there was no impact on MAP concentration from materials used in column reactor fabrication.
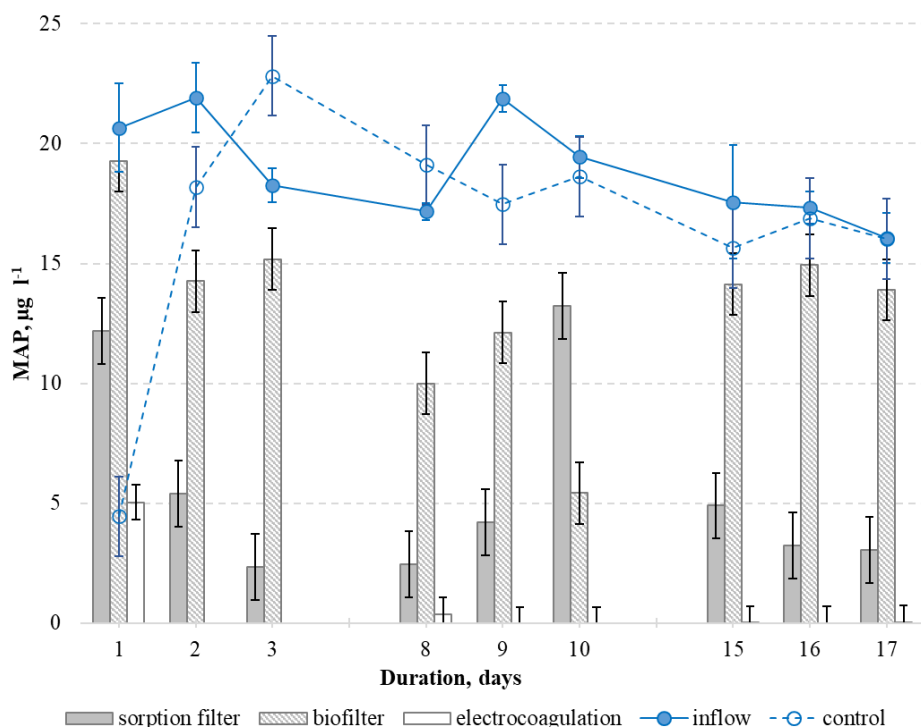

Fig. 4.1. MAP concentrations in inflow and outflow of column reactors with bars representing stand. dev. of triplicate samples. Detection limit 6.27×10^{-3} µg P l^{-1}.

Recirculation configuration setup. When operating at elevated water temperatures of about +19 °C, the biofiltration reactor showed reduced MAP concentration in samples after 21 hours of operation. In general, it ensured MAP elimination from 24.3 µg l^{-1} up to 1.6 µg l^{-1} and from 23.5 µg l^{-1} up to 2.7 µg l^{-1} when initially amended with carbon source (Fig. 4.2). The analysis showed that only the temperature was limiting biofilter performance at the groundwater treatment plant (Mann-Whitney U test, $p = 0.04$) and the addition of carbon did not additionally improve biofilter performance (Mann-Whitney U test, $p = 0.686$).
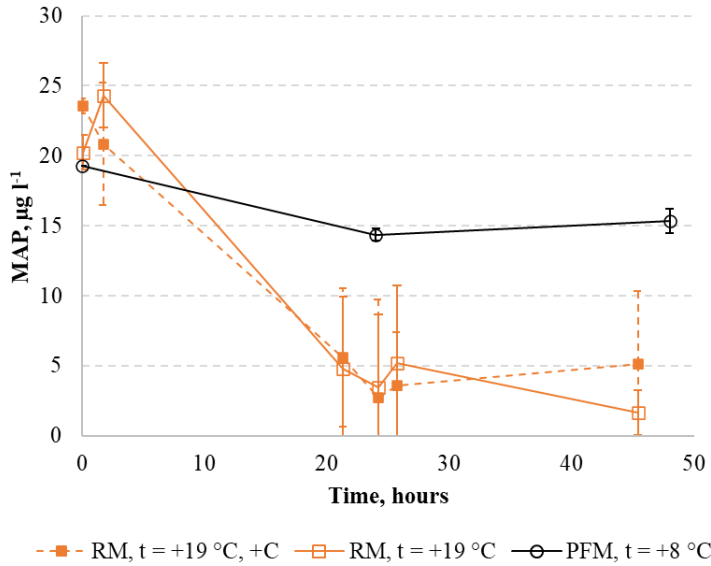
Fig. 4.2. Biofilter reactor performance during operation in plug-flow mode (PFM) and recirculation mode (RM) at different water temperatures without and with (+C) amendment with acetate carbon. Bars represent stand. dev. of triplicate samples. Detection limit 6.27×10^{-3} µg P l^{-1}.

Overall comparison. All treatment methods significantly decreased MAP concentration (Wilcoxon Signed Ranks Test, $p = 0.008$–0.018). Overall, the electrocoagulation reactor provided 76–100 % MAP removal from groundwater (Table 4.1), while 32–87 % removal was achieved by sorption onto ferric oxides-coated plastic media and 7–72 % MAP elimination was provided by biofilm-covered plastic biocarriers. Once the water temperature was elevated, the biofilter had an improved performance, reaching MAP reduction up to 88–92 %.

Table 4.1. 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}).

The pervious tests (results published in (Zemite *et al.*, 2022)) with several sorption materials, addressing surface water quality improval, determined that phosphorus sorption was more effective on iron-covered sand collected from iron removal filters if compared to commercial reagents, providing a 90 % reduction within 1 min, as determined in kinetic studies. The sorption capacity performed in a plug-flow column reactor resulted in an average of 11.4 mg g^{-1} of P sorbed from 100 litres of groundwater.

Fe and Al-based drinking water treatment residues have been studied also in other P-binding applications, e. g., in soil (Makris *et al.*, 2004), with sorption capacity over 9.1 mg g^{-1} of P. Metal hydroxides typically exhibit initial rapid sorption phase, which is followed by slow phase, providing stable P immobilization over long periods, once entrapped into sorbents micro-pores (Makris *et al.*, 2004).

As expected, among the tested methods, electrocoagulation achieved the best results for MAP removal. Lehtola *et al.*, 2002 reported that chemical coagulation was able to reduce MAP below the detection limit. In this study the electrochemical coagulation provided similar results, most of the time ensuring non-detectable MAP levels. However, the method produced a substantial amount of sludge, which might have additionally impacted MAP content, affecting it during sample transportation, as no specific sludge removal was performed at the sample collection site. Overall, the method would require an extra filter for sludge removal, as well as finances for additional sludge handling expenses, an electrical power backup system to ensure smooth operation and periodic replacement of electrodes. Moreover, electrocoagulation could potentially induce the formation of some toxic chlorinated organic compounds in the presence of chlorides (Mollah *et al.*, 2004) Therefore, it was established to be unsuitable for further use within a case-study setting.

Biofiltration was only effective at elevated water temperatures and therefore was unfeasible for use at groundwater supply temperature. Additionally, apart from low water temperature, biofilters can be negatively affected by chlorine (Liu, Huck and Slawson, 2001), and in Riga, preventive disinfection or chlorination of water supply networks happens periodically.

Although sorption did not ensure complete MAP removal, it was relatively stable in operation, robust, and did not require extensive maintenance activities.

Ferric oxide is a promising material to use in MAP removal in sorption filters. However, for scale-up tests in DWDS, it was important to ensure material safety, therefore a commercial material, certified for use within drinking water systems, with similar characteristics was chosen for further studies.

4.2. Sorption optimisation for use within the drinking water supply

To further optimise the selected method and ensure its suitability for the use within 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 (Fig. 2.3) in a laboratory setting. Lastly, to evaluate a potential improvement of P removal efficiency, an

addition of $FeCl_3$ coagulant was monitored. For enhanced test speed and the possibility of 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. 4.3), ensuring reduction from 0.55 mg l^{-1} to 0.01 mg l^{-1} (Table 4.2). However, when further the process was enhanced by the addition of in-line coagulation with diluted $FeCl_3$ coagulant at a mean concentration of 0.62 mg l^{-1} (SD 0.14 mg l^{-1}), the P reduction decreased to an average of 76 % removal, with mean inflow of 0.62 mg P l^{-1} and outflow of 0.14 mg P l^{-1}. The dynamics of PO_4-P removal (Fig. 4.4) showed a slight decrease in performance after 16.5 hours of operation in P-rich conditions when only sorption was present. However, when in-line coagulation was activated, a 16 % decrease was registered after 4.5 hours of operation and after 25.5 hours a decrease reached a value of 41 %.

In addition to phosphate removal, granular ferric hydroxide filtration influenced such parameters as dissolved oxygen, increasing it on average by 3 mg l^{-1} ($p < 0.001$) and pH, decreasing it by around 0.09 units ($p = 0.046$). Once the coagulant was added, iron, turbidity and electrical conductivity reduction could have been observed with values decreased by 0.48 mg Fe l^{-1}, 1 NTU and 9.6 µS cm^{-1}.
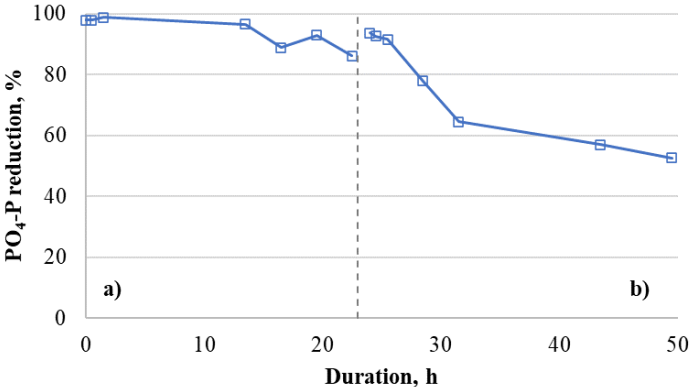

Fig. 4.3. PO_4-P removal efficiency of granular ferric hydroxide sorption a) without and b) with $FeCl_3$ coagulant addition.

Table 4.2. Effect of granular ferric hydroxide sorption on water quality without and with FeCl₃ in-line coagulation.

Parameter	GFH in	GFH out	GFH + FeCl$_3$ in	GFH + FeCl$_3$ out
PO$_4$-P, mg l^{-1}	0.55 (SD 0.16)	0.01 (0.01–0.1)	0.62 (SD 0.15)	0.14 (SD 0.10)
	$p = 0.018$		**$p < 0.001$**	
Fe, mg l^{-1}	0.05 (SD 0.03)	0.02 (0–0.15)	0.62 (SD 0.14)	0.13 (SD 0.03)
	$p = 0.344$		**$p < 0.001$**	
Turbidity, NTU	1.71 (SD 0.95)	0.86 (SD 0.90)	1.00 (0–3.00)	0 (0–2.00)
	$p = 0.111$		**$p = 0.038$**	
Dissolved oxygen, mg l^{-1}	15.9 (SD 1.1)	18.8 (SD 1.0)	10.6 (10.2–15.9)	11.1 (10.3–18.8)
	$p < 0.001$		$p = 0.063$	
pH	6.81 (6.66–6.84)	6.72 (SD 0.03)	6.92 (SD 0.15)	6.85 (SD 0.19)
	$p = 0.046$		$p = 0.134$	
Electrical conductivity, μS cm^{-1}	391 (SD 11)	383 (SD 3)	616 (SD 100)	606 (SD 94)
	$p = 0.128$		**$p = 0.007$**	

Mean (standard deviation, SD) values are presented for normally distributed data or otherwise, Median (min−max) is used. Number of samples = 7 for each test. Paired samples t-test was used for normally distributed data or otherwise Wilcoxon Signed Ranks Test was used.

4.3. Section conclusions

Three methods – electrocoagulation, sorption on iron-based media, and biofiltration – were tested for the removal of microbially available fraction of phosphorus (MAP).

Electrocoagulation achieved near-complete MAP removal but had several technical limitations, including significant sludge generation and the need for a backup power system. Biofiltration performed poorly under natural groundwater temperatures. Sorption, using both iron-coated plastic carriers (a waste by-product from groundwater iron removal) and commercial granular iron hydroxide sorbent, achieved over 70 % MAP removal. 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 for 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.
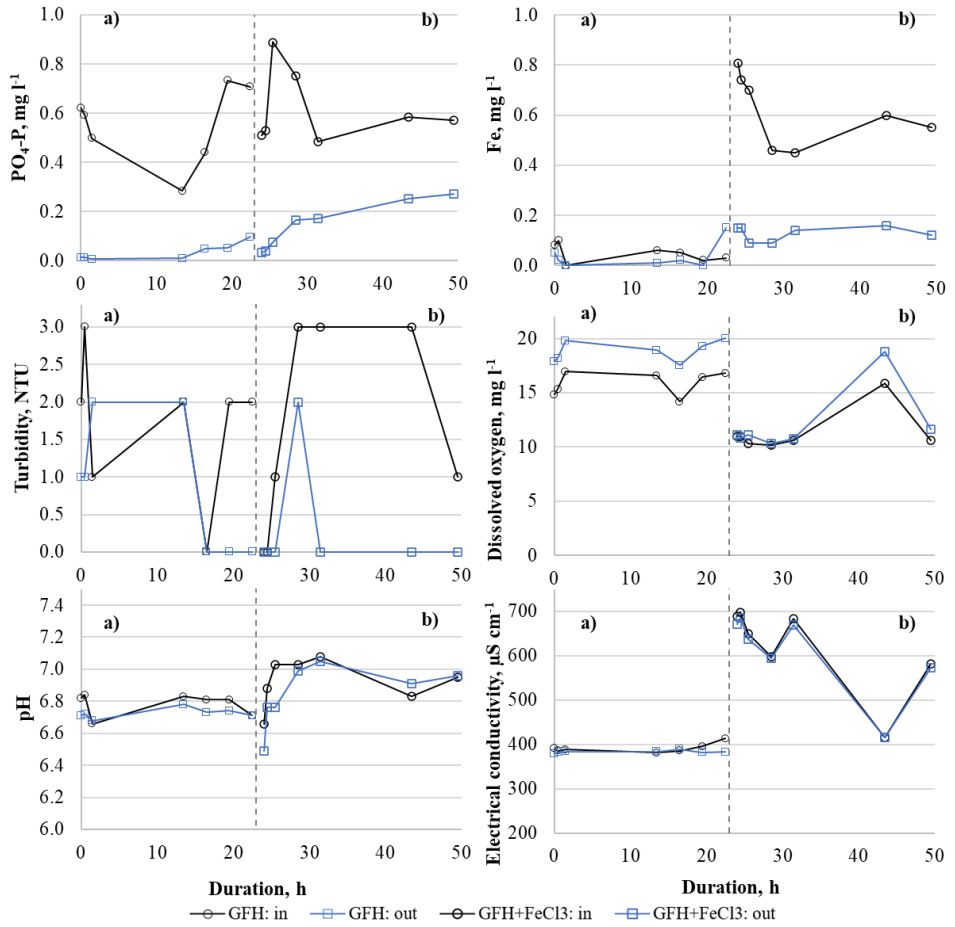
Fig. 4.4. Granular ferric hydroxide (GFH) sorption inflow and outflow values of various parameters a) without and b) with FeCl₃ in-line coagulation.

5. CHARACTERISTICS OF INLET WATER

5.1. Unevenness of water consumption

The pilot site, consisting of two five-storey residential buildings, was subjected to water flow monitoring throughout the study to assess the differences in overall water consumption and daily water usage patterns.

The water consumption varied from 9.2 to 19.1 m^3 d^{-1} with a median value of 13.1 m^3 d^{-1} for the Reference building and from 8.8 to 28.3 m^3 d^{-1} with a median value of 11.4 m^3 d^{-1} for the POU-device building. The POU-device building daily consumed more water in July and August (Fig. 5.1). However, starting from September, the consumption was more similar but still significantly different (for each month), with the Reference building daily consuming slightly more water. In general, there was a non-linear cumulative water consumption pattern for POU-device building, consisting of more rapid consumption during the first 6 weeks and less rapid water consumption during the remaining period, compared to the evenly linear pattern for Reference building (Fig. 5.2), with a total of 250.6 m^3 water consumption difference at the end of the sampling period.
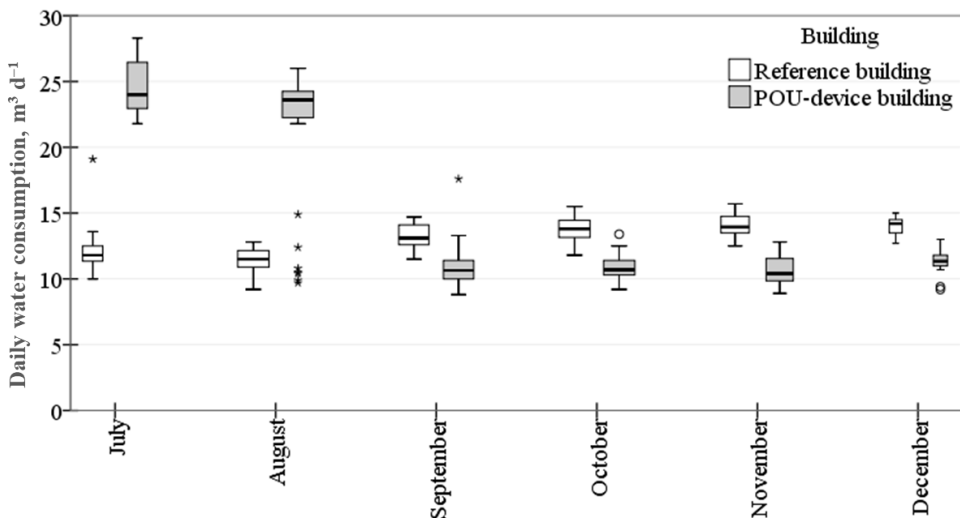

Fig. 5.1. Variations in daily water consumption for Reference and POU-device buildings. Dots represent outliers and stars – extreme outliers. The width of boxes is scaled to number of cases (n = 13-31).

The general pattern of daily water usage was similarly scattered for both buildings (Fig. 5.3). POU-device building showed flow rates from 0 to 2.3 m^3 h^{-1}, with median value of 0.6 m^3 h^{-1}, while Reference building reached maximal value of 4.8 m^3 h^{-1} with same median value of 0.6 m^3 h^{-1} when analysed for the whole sampling duration of 22 weeks.
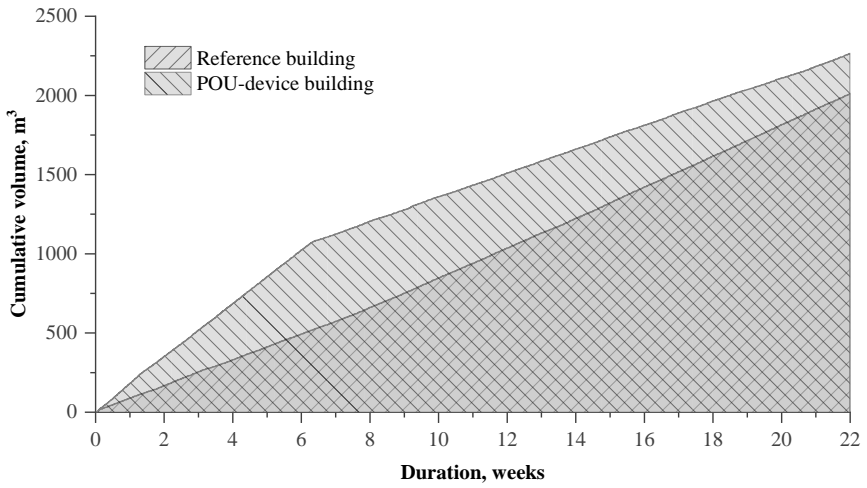
Fig. 5.2. Cumulative water consumption for the sampling period.
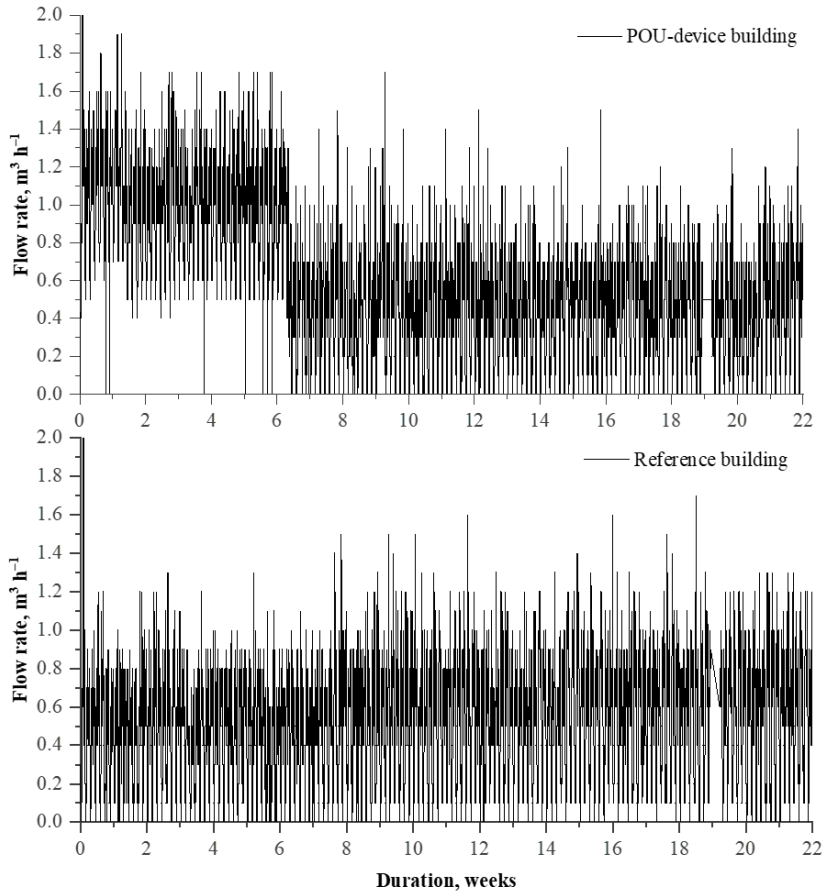

Fig. 5.3. Water consumption pattern.

Regular daily dynamics in water consumption patterns are normal in drinking water distribution, and unevenness of consumption increases with a decrease in the number of water consumers. If compared to an external drinking water distribution, such dynamic flow conditions are mostly responsible for particle deposition and subsequent resuspension, which result in a significant impact on drinking water turbidity, while the change in concentration of suspended microbial cells by such fluctuations in hydraulic conditions can stay below detection (Prest *et al.*, 2021). However, higher water velocities, present during daily peak consumption, can also increase the bacterial growth in biofilm (Torvinen *et al.*, 2007). (Prest *et al.*, 2021) found that bulk bacteria concentration was mostly influenced by water age and concentration of the disinfectant residual, and not by hydraulics, however, there was a weak correlation of flow velocity and ATP, indicating incidental resuspension of particle-bound bacteria either from biofilm detachment or from random release from sediment.

5.2. Mixed source water supply

In Riga drinking water is extracted from six locations, providing most of the left Daugava bank inhabitants with Daugava River water, which undergoes extensive chemical treatment with successive sedimentation, filtration and disinfection with ozone. The majority of the right bank inhabitants, in turn, mainly receive groundwater from five underground boreholes – "Baltezers", "Baltezers 1", "Baltezers 2", "Zaķumuiža" and "Remberģi". The lake Little Baltezers is used for groundwater recharge in the water intakes "Baltezers" and "Baltezers 2".

Both pilot five-storey residential buildings received water from municipal water supply networks. 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.

These water sources can be partially differentiated by their electrical conductivity (EC) values (Table 5.1). Plant "Baltezers" has an average value of 695 µS cm^{-1}, while the remaining water extraction sites have more similar average values of 337 µS cm^{-1} and 305 µS cm^{-1}.

Table 5.1. Electrical conductivity at 25 °C (µS cm^{-1}) for water pumping sources supplying the study site (data from January – May 2022, obtained from drinking water provider).

"Baltezers"			"Remberģi"			"Zaķumuiža"		
min	average	max	min	average	max	min	average	max
646	695	779	302	337	356	250	305	338

Both pilot buildings were equipped with an online electrical conductivity sensor on the water main to track EC changes in the water inflow. The EC values in both buildings varied greatly throughout the day and during the test time (Fig. 5.4). POU-device building received the water with a minimal EC value of 165 µS cm^{-1} and maximal value of 514 µS cm^{-1}, while

the median values for each week varied from 210 to 316 μS cm^{-1}. The Reference building, however, received in general water with smaller EC values, corresponding to a minimal EC value of 156 μS cm^{-1}, maximal value of 477 μS cm^{-1}, and median values for each weak of 163 to 326 μS cm^{-1}. Mann-Whitney U test showed significantly different EC values ($p < 0.05$) between the inflow water of both buildings for every test week, as well as for each separate day, except for the July 30 and 31, and August 2–5, 9, 21, and 22 (study days 18, 19, 21–24, 28, 40 and 41).
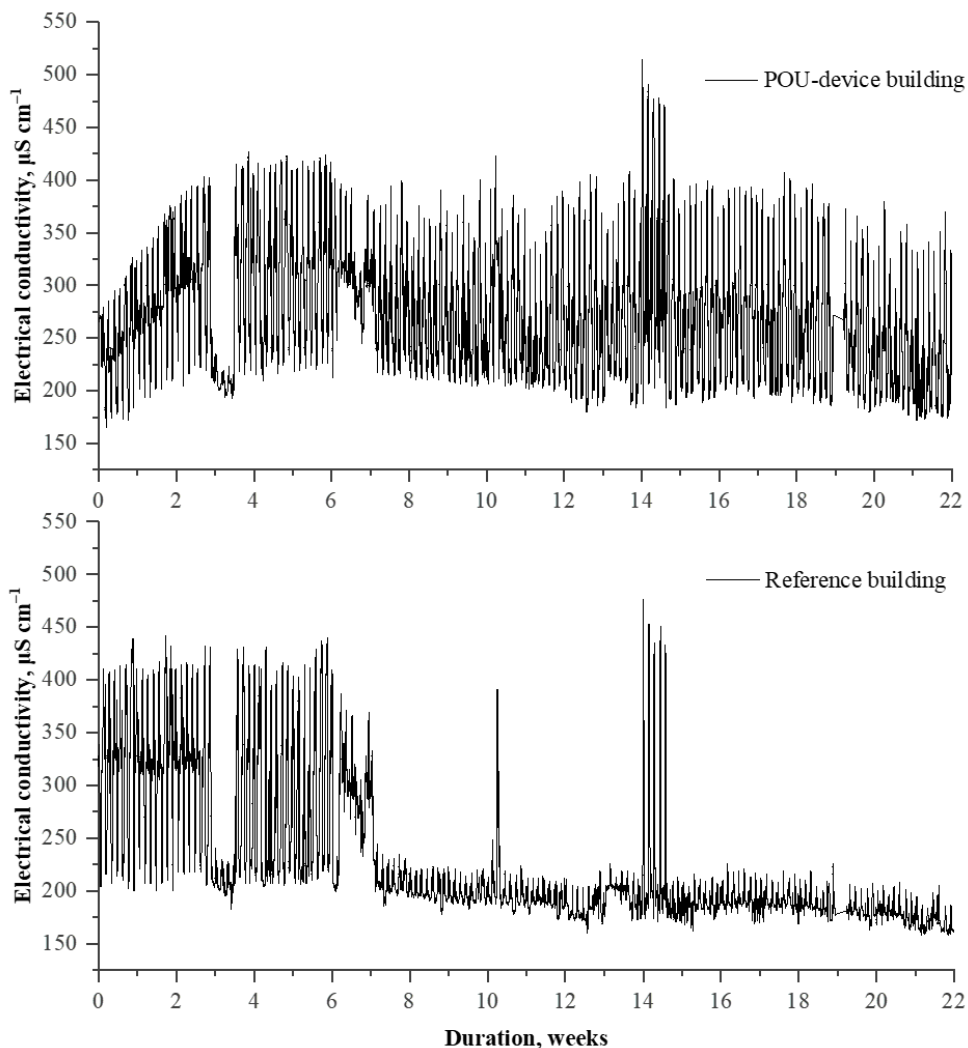

Fig. 5.4. Daily and weekly variations in electrical conductivity values of inflowing water of POU-device (top) and Reference (bottom) buildings.

Similarly, both buildings received water of different EC measurements throughout the day ($p < 0.05$) except from 3 am to 5 am, during which no significant difference in readings was shown. In general, both buildings received water with a smaller variance in EC values during the morning hours (Fig. 5.5). The median values of EC were somewhat stable for the Reference building during the day with greater variation at night and in the afternoon. POU-device building, however, received higher EC water in the afternoon with the peak values during around 5–8 pm.
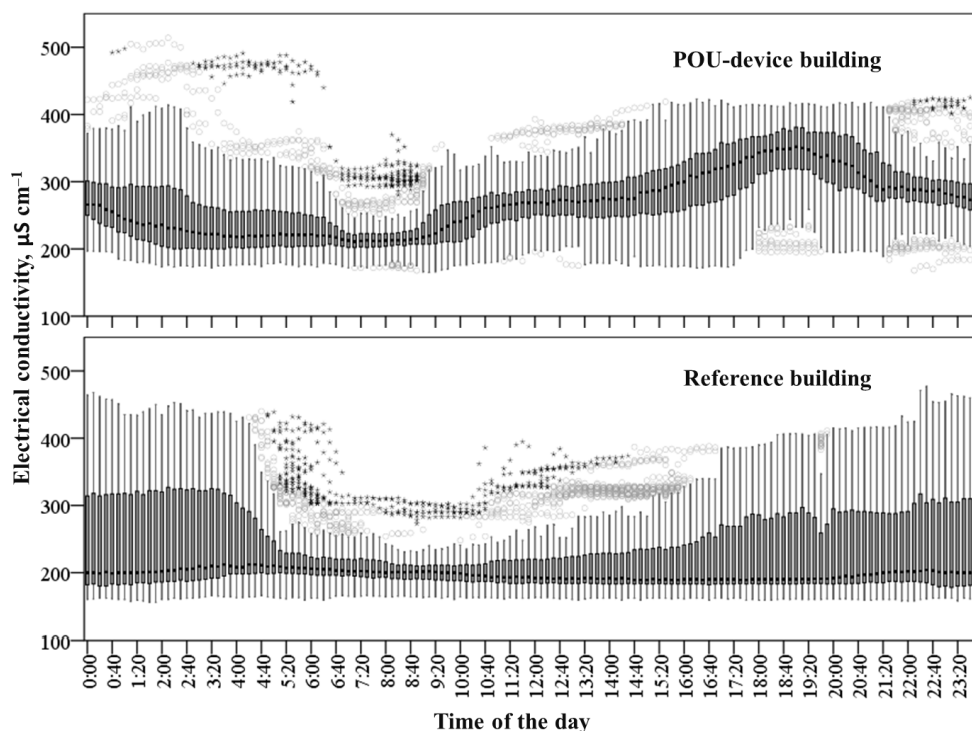

Fig. 5.5. Hourly variations in electrical conductivity values for the duration of 22 weeks in POU-device (top) and Reference (bottom) buildings. Dots represent outliers and stars – extreme outliers.

The correlation analyses reveal very weak but significant (at 0.01 level) relationships between water consumption and EC. When analysed for both buildings jointly, the Pearson correlation coefficient (R) is 0.086, but it is slightly higher for each building analysed separately, with values of R = −0.243 for the Reference building and R = −0.331 for the POU-device building. The peak EC values might be expected after several hours of increased consumption (Fig. 5.6). It might be around 4 to 8 hours of increased or decreased water flow, until a visually distinct change is noticeable in the incoming water EC measurements,

depending on the amount of water used in the timestep, with more rapid usage causing steeper EC change.

According to the EC values of potential groundwater sources, the pilot buildings received mixed water possibly from all of them. However, it is not possible to determine the volume of each source water received for the study time. 40 % of median measurements, when compared by the time of the day for the course of 22 weeks, in the POU-device water main show smaller EC values than the minimal stated water source "Zaķumuiža" value of 250 µS cm^{-1}, while for the Reference building same is true for 100 % of daytime median values. The data suggest that there might be water from another source reaching the pilot area. Also, the data shows EC values higher than the maximal value of 356 µS cm^{-1} for the source "Remberġi" but never reaches the minimal value of 649 µS cm^{-1} for the source "Baltezers". This also suggests a mixed water composition entering the water mains, which is impossible to differentiate solely by EC measurements.

A part of the distribution network, where the pilot site is located, according to (Nescerecka, Juhna and Hammes, 2018), is carbon-limited, while phosphorus-limited treated surface water, according to municipality hydraulic models, should not reach the site. However, such variability in inlet water from mixed sources, as revealed by EC measurements, might have an impact on the provision of safe drinking water. Water from different sources might potentially represent distinct chemical compositions, in such a way negatively affect drinking water quality due to the cross-provision of both – macrobiogenic elements and additional supplementary substances, such as metal elements etc., needed for bacterial growth.
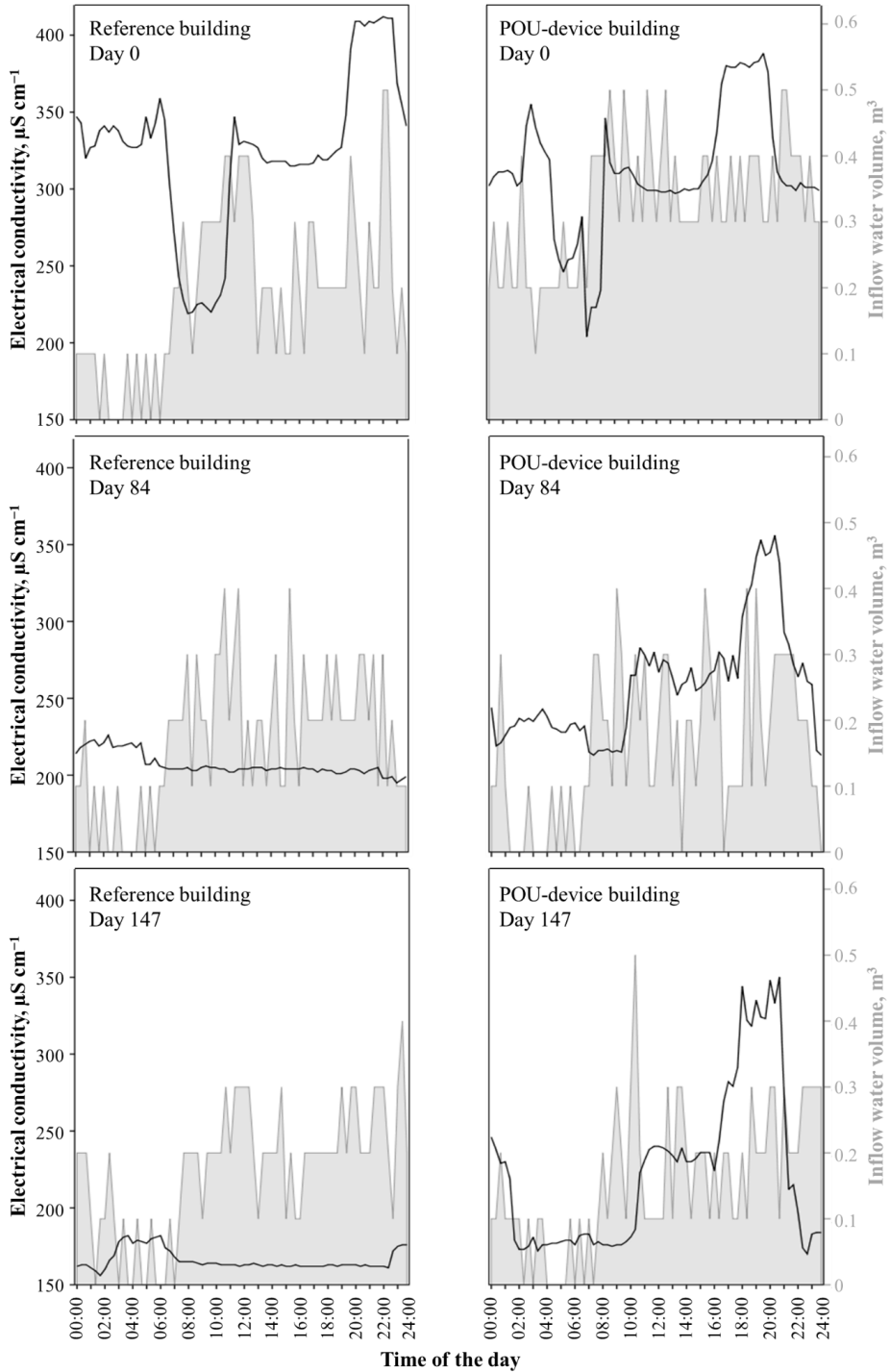
Fig. 5.6. Water consumption (grey area) and water source or electrical conductivity variation (black line) pattern for three Wednesdays.

5.3. Influent water composition

The inlet water was periodically sampled from cold water mains close to the entry point within the buildings. As the buildings received different source water mixtures throughout the daytime, it's important to note that these samples were taken as grab samples, thus not providing a comprehensive representation of the daily incoming water composition.

The inlet water characteristics and composition showed some changes throughout the sampling period. Although the supplied water source was groundwater, the temperature decreased with the change of seasons (Fig. 5.7), most likely during municipal water distribution, from 12.4–14.1 °C in July/August to 7.9–8.4 °C in December. Electrical conductivity also showed a gradually decreasing tendency during the sampling period duration, showing a maximal value of 521 µS cm^{-1} in the POU-device building and 356 µS cm^{-1} in the Reference building in July and minimum values of 344 µS cm^{-1} and 265 µS cm^{-1} in December in both buildings, respectively. That could be attributed either to seasonal variations or also to the shift in the main groundwater source. The pH values showed similar, however very slight, tendency, with maximal values of 8.12 and 8.25, and minimal values of 7.66 and 7.80 within the study duration in the POU-device building and Reference building, respectively.
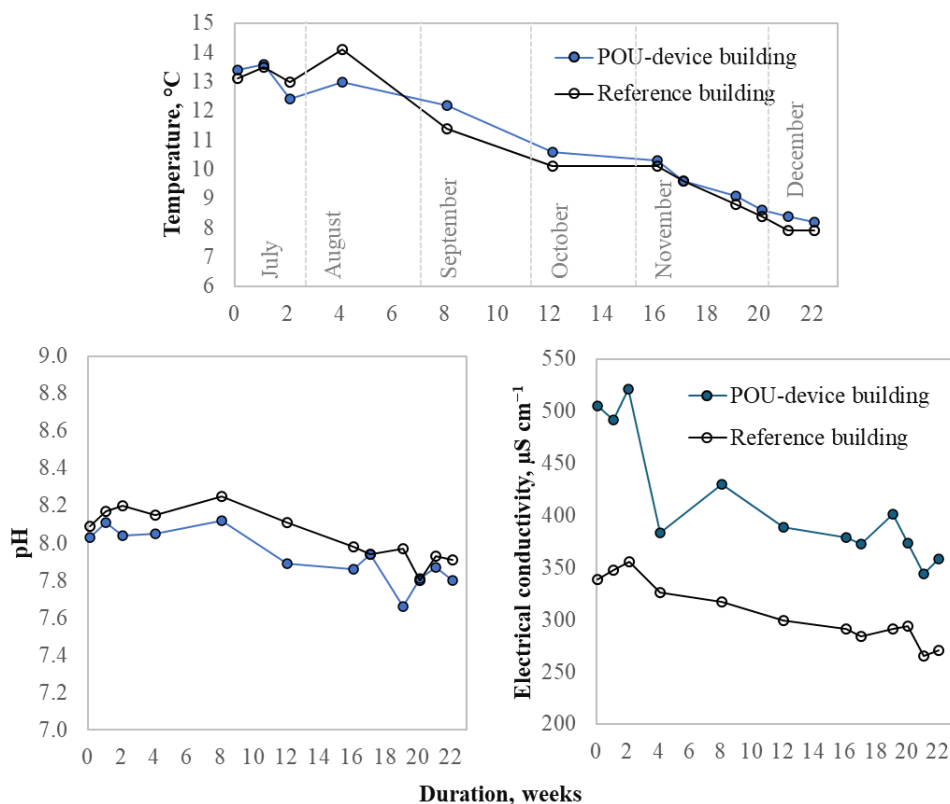

Fig. 5.7. Temperature, pH and electrical conductivity in inlet water grab-samples.

Elemental analyses (Fig. 5.8) showed peak values of manganese, copper, zinc, iron and lead in samples collected from the Reference building at the beginning of the sampling period. It might be attributed to the preceding hydrogen peroxide disinfection procedure inducing possible metals release from plumbing components or deposits. The fact that a similar relationship does not appear in the POU-device building is due to the location of a sampling tap. The POU-device building inlet water sampling tap was located on the filter unit line, which was bypassed during the disinfection process (Fig. 2.5).

Further elemental concentration was relatively stable except for week 21, where POU-device building`s inlet showed a peak copper concentration of 210 µg l^{-1}, and weeks 4 and 16 for peaks in iron concentration in Reference building (0.224 mg l^{-1} and 0.652 mg l^{-1}, respectively) and week 16 with high manganese values (0.102 mg l^{-1}), which might have been caused by hydraulic imbalances in the distribution network, causing resuspension of deposits with sorbed elements.

Total organic carbon (TOC) content in inflow was higher during the first two sampling weeks (Fig. 5.9) with a maximal concentration of 4.96 mg l^{-1} and 5.76 mg l^{-1} in POU-device and Reference building, respectively. Further this parameter was relatively stable with median values for the sampling duration of 2.24 mg l^{-1} and 1.31 mg l^{-1}, respectively.

Microbially available phosphorus (MAP), however, fluctuated throughout the study time from 8.9 µg l^{-1} to 15.7 µg l^{-1} and a median value of 11.3 µg l^{-1} in POU-device building and from 3.3 µg l^{-1} to 16.3 µg l^{-1} with a median value of 10.9 µg l^{-1} in Reference building.

These concentrations provided a TOC : MAP ratio with median values of 183:1 for POU-device building (min 116, max 560), and 167:1 for Reference building (min 67, max 472), with higher values at the beginning of the sampling period following higher TOC concentration in inflow water and an elevated value in Reference building during week 16 due to reduced MAP concentration.

The characteristics of cells contributing to total cell count were similar in the distribution of intact low nucleic acid and high nucleic acid content cells (Table 5.2) that was somewhat stable throughout sampling time (Fig. 5.10), while the share of damaged cells seemed to increase as a seasonal change towards winter. The total microbial cell count (TCC) in POU-device building`s inlet water was on median 2.45×10^5 cells ml^{-1} (min 1.5×10^5 cells ml^{-1}, max 3.5×10^5 cells ml^{-1}) with a median damaged cells concentration of 49 % (min 25 %, max 61 %), intact cells concentration with low nucleic acid content of 22 % (min 16 %, max 34 %) and intact cells concentration with high nucleic acid content of 28 % (min 21 %, max 41 %). For the Reference building the median total microbial cell concentration was 2.07×10^5 cells ml^{-1} (min 1.3×10^5 cells ml^{-1}, max 3.4×10^5 cells ml^{-1}), containing on median 40 % (20–53 %) damaged cells, 26 % (20–47 %) intact low nucleic acid content cells and 32 % (21–42 %) intact high nucleic acid content cells.

The inflow water samples mostly did not show any cultivable *Legionella spp.*, except for week 16, when 400 CFU l^{-1} of *Legionella pneumophila* serogroup (SG) 3 were found in POU-device building`s inflow, and weeks 20 and 21, when 200 CFU l$^{-1}$ of *L. pneumophila* SG 2 and 350 CFU l$^{-1}$ of *L. pneumophila* SG 3, respectively, were found in Reference building`s inflow cold water main samples.

Similarly to the continuous monitoring data, also grab-sample analyses revealed significant differences in EC values ($p < 0.001$), with higher measurements in POU-device building samples (Table 5.2). Additionally, POU-device inlet samples showed higher calcium ($p = 0.021$), magnesium ($p < 0.001$), total organic carbon (TOC) ($p = 0.007$) content, and concentrations of damaged cells ($p = 0.003$), while Reference building inlet contained higher copper ($p = 0.014$) concentration.
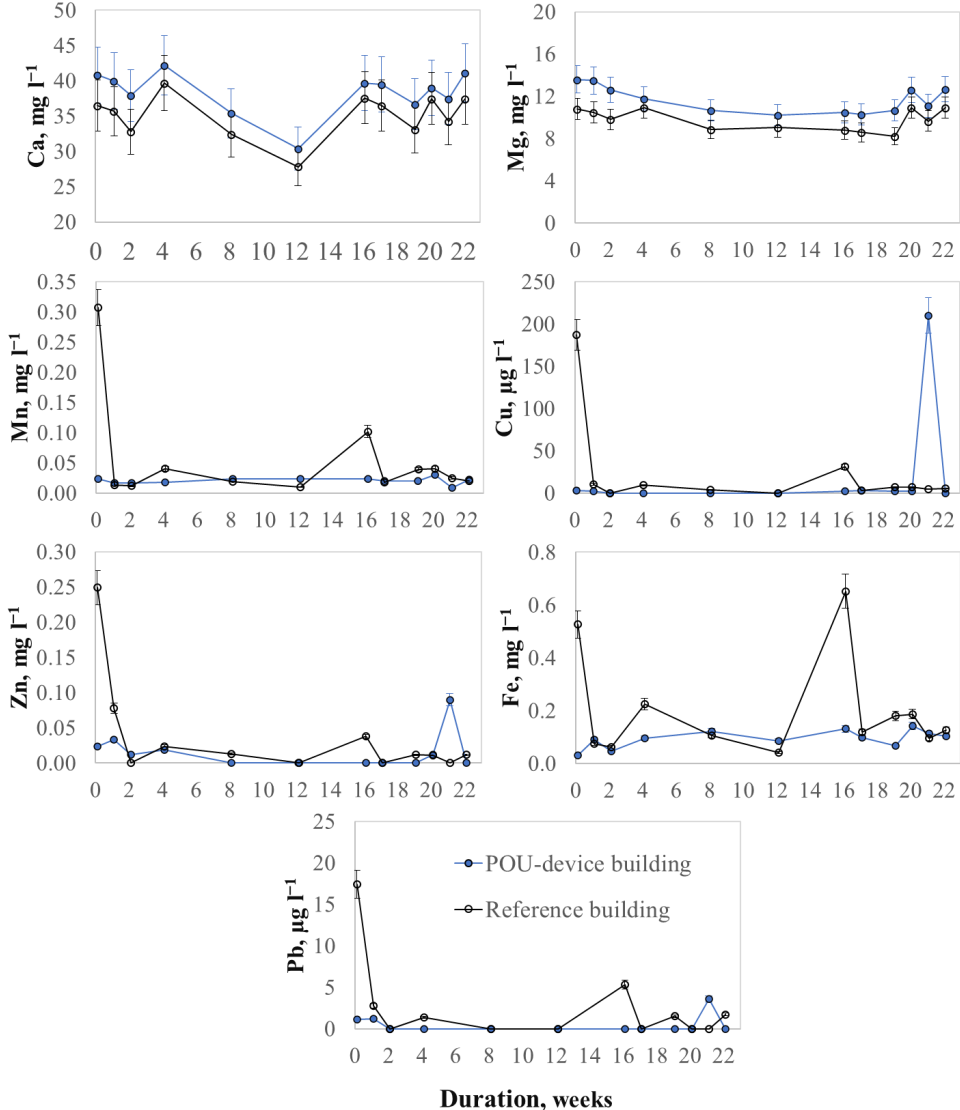
Fig. 5.8. Elemental composition of inlet water (grab-samples). The bars represent standard deviation of three replicates.
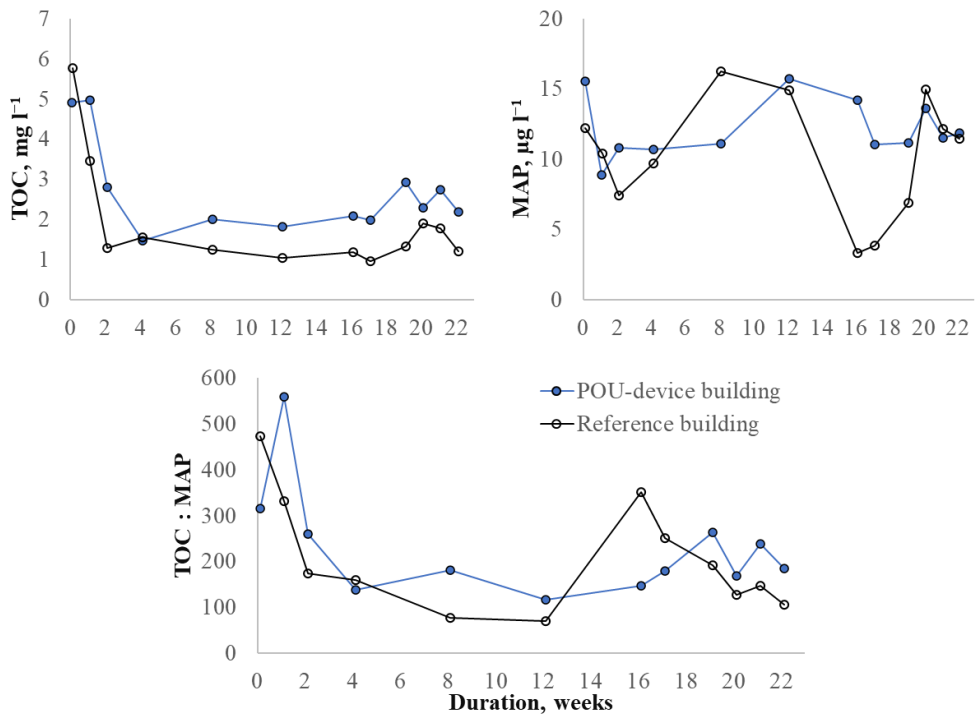
Fig. 5.9. Amount of total organic carbon (TOC) and microbially available phosphorus (MAP) in inflow water, and the respective TOC:MAP ratio.
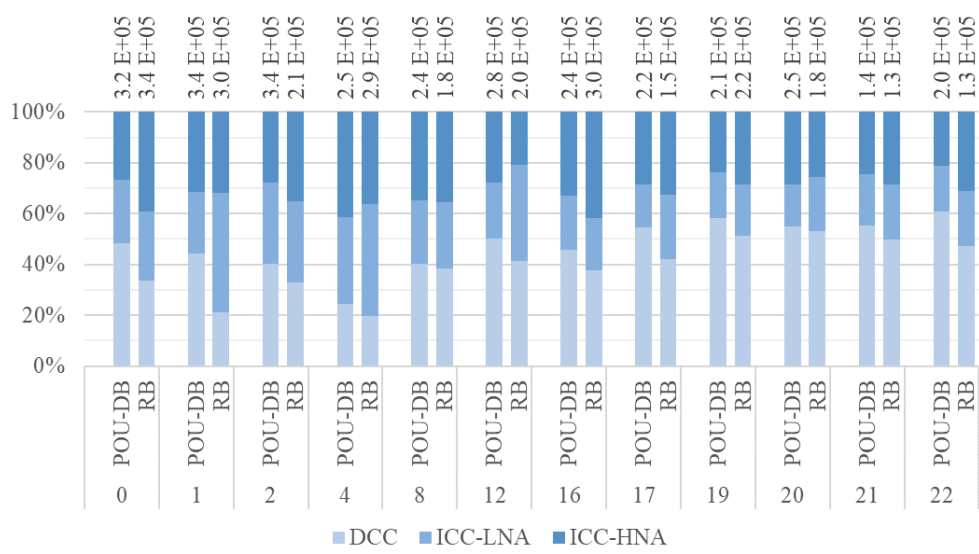

Fig. 5.10. Total cell count (top row, cells ml^{-1}) and the share of damaged cells (DCC), and intact cells (ICC) with high nucleic acid content (HNA) and low nucleic acid (LNA) content.

Table 5.2. Characteristics of inlet water (grab samples).

Parameter	POU-device building [*]	Reference building [*]	p-value [**]
Physical and chemical parameters			
Temperature, °C	10.78 (SD 2.04)	10.66 (SD 2.28)	0.889
pH	7.93 (SD 0.14)	8.04 (SD 0.14)	0.067
Electrical conductivity, µS cm^{-1}	386.0 (344–521)	306.75 (SD 30.00)	**< 0.001**
Ca, mg l^{-1}	38.35 (SD 3.17)	35.12 (SD 3.22)	**0.021**
Mg, mg l^{-1}	11.69 (SD 1.26)	9.74 (SD 1.03)	**< 0.001**
Mn, mg l^{-1}	0.021 (SD 0.005)	0.023 (0.010–0.307)	0.410
Cu, µg l^{-1}	2.05 (0–210)	6.2 (0–187)	**0.014**
Zn, mg l^{-1}	0.006 (0–0.090)	0.012 (0–0.249)	0.514
Fe, mg l^{-1}	0.094 (SD 0.033)	0.123 (0.042–0.652)	0.128
Pb, µg l^{-1}	0 (0–3.6)	0.685 (0–17.4)	0.198
Main microbial nutrients			
TOC, mg l^{-1}	2.24 (1.47–4.96)	1.31 (0.96–5.76)	**0.007**
MAP, µg l^{-1}	12.17 (SD 2.11)	10.29 (SD 4.23)	0.186
Microbiological parameters			
Total Cell Count, cells ml^{-1} [***]	2.53×10^5 (SD 5.77×10^4)	2.19×10^5 (SD 7.22×10^4)	0.225
Damaged Cell Count, cells ml^{-1}	1.19×10^5 (SD 2.83×10^4)	6.83×10^4 (5.77×10^4 – 1.14×10^5)	**0.003**
Intact Cell Count (ICC), cells ml^{-1}	1.33×10^5 (SD 4.54×10^4)	1.39×10^5 (SD 6.54×10^4)	0.810
Low Nucleic Acid content cells, % ICC	44 (SD 4.8)	47 (SD 8.9)	0.319
High Nucleic Acid content cells, % ICC	56 (SD 4.8)	53 (SD 8.9)	0.315
L. pneumophila, CFU ml^{-1}	0 (0-400)	0 (0-350)	0.799

[*] Mean (standard deviation, SD) values are presented for normally distributed data, or otherwise Median (min-max) is used. Number of samples = 12 for each building.

[**] Statistically significant p-values are highlighted in bold; 2-tailed significance used for normally distributed data or exact significance value for non-parametric independent samples t-test.

[***] Total Cell Count = Intact Cell Count + Damaged Cell Count.

5.4. Section conclusions

To assess whether both pilot buildings received similar water and consumed it similarly, water usage patterns and incoming water quality were analysed.

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, resulting in a total of 250.6 m^3 greater water consumption at the end of the testing period compared to the Reference building.

The electrical conductivity (EC) of incoming water fluctuated significantly both throughout the day and over the entire testing period, indicating a mixed water source supply. The higher EC values at the POU-device building's inlet suggest potential differences in water origin between the buildings, although the EC parameter alone could not definitively determine the water source.

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.

6. WATER QUALITY IN INTERNAL DRINKING WATER SUPPLY

6.1. MAP reduction from inflow by ferric hydroxide sorption filter

To provide MAP reduction in an existing internal drinking water network, a POU sorption filter, containing granular ferric hydroxide active layer and sand support layers, was installed on a water main of a five-storey residential building (Fig. 2.5), denoted "POU-device building".

The POU filtration unit did not affect any other analysed inflow water parameters than MAP ($p < 0.001$) and Fe ($p = 0.010$) concentrations (Table 6.1). On average, a 70 % MAP reduction was achieved, resulting in a mean concentration of 3.56 µg MAP l^{-1} (SD 1.5 µg l^{-1}) entering the internal water networks. Such reduction level corresponded to the one achieved in smaller-scale tests with filtration through ferric oxides-coated biomass carriers filled column (Section 4). Initially, the MAP reduction level decreased from 96 % to 83 % during the first week, and further, it was somewhat stable, fluctuating in a range from 53–80 % removal (Fig. 6.1).

The iron, however, was removed below the detection limit during the first month of filter operation. In a sample taken two months from start-up, it showed only a 24 % reduction, reaching a concentration of 0.092 mg l^{-1}. After another month, the outflow iron concentration showed a 50 % increase from 0.086 mg l^{-1} in the building`s inflow to 0.129 mg l^{-1} after the POU filter. Further, the iron decrease gradually enlarged. In general, the median iron concentration was 0.094 mg l^{-1} (SD 0.033 µg l^{-1}) for the whole sampling duration of 22 weeks, with a median reduction of 75 %.

Table 6.1. Characteristics of water before and after POU-filter unit (grab samples).

Parameter	before POU-device [*]	after POU-device [*]	p-value [**]
Physical and chemical parameters			
Temperature, °C	10.78 (SD 2.04)	11.04 (SD 1.83)	0.748
pH	7.93 (SD 0.14)	7.91 (SD 0.12)	0.723
Electrical conductivity, µS cm^{-1}	386.0 (344–521)	406.08 (SD 59.21)	0.671
Ca, mg l^{-1}	38.35 (SD 3.17)	38.37 (SD 2.94)	0.989
Mg, mg l^{-1}	11.69 (SD 1.26)	11.55 (SD 1.25)	0.785
Mn, mg l^{-1}	0.021 (SD 0.005)	0.014 (SD 0.013)	0.139
Cu, µg l^{-1}	2.05 (0–210)	0.0 (0–6.1)	0.755
Zn, mg l^{-1}	0.006 (0–0.090)	0.017 (0–0.064)	0.378
Fe, mg l^{-1}	0.094 (SD 0.033)	0.026 (0–0.134)	**0.010**
Pb, µg l^{-1}	0 (0–3.6)	1.255 (0–32.9)	0.101

Parameter	before POU-device [*]	after POU-device [*]	p-value [**]
Main microbial nutrients			
TOC, mg l^{-1}	2.24 (1.47–4.96)	2.06 (1.38–7.62)	0.291
MAP, µg l^{-1}	12.17 (SD 2.11)	3.56 (SD 1.49)	**< 0.001**
Microbiological parameters			
Total Cell Count, cells ml^{-1} [***]	2.53×10^5 (SD 5.77×10^4)	2.55×10^5 (SD 6.23×10^4)	0.912
Damaged Cell Count, cells ml^{-1}	1.19×10^5 (SD 2.83×10^4)	9.71×10^4 (SD 3.11×10^4)	0.080
Intact Cell Count (ICC), cells ml^{-1}	1.33×10^5 (SD 4.54×10^4)	1.58×10^5 (SD 5.05×10^4)	0.214
Low Nucleic Acid content cells, % ICC	44 (SD 4.8)	40 (SD 10.7)	0.365
High Nucleic Acid content cells, % ICC	56 (SD 4.8)	60 (SD 10.7)	0.373
L. pneumophila, CFU ml^{-1}	0 (0-400)	0 (0-150)	0.977

[*] Mean (standard deviation, SD) values are presented for normally distributed data, or otherwise Median (min-max) is used. Number of samples = 12 for each building.

[**] Statistically significant p-values are highlighted in bold; 2-tailed significance used for normally distributed data or exact significance value for non-parametric independent samples t-test.

[***] Total Cell Count = Intact Cell Count + Damaged Cell Count.
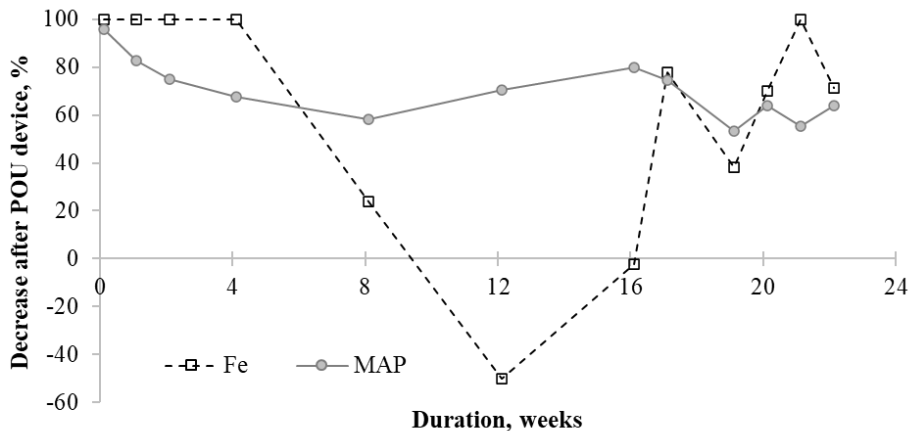

Fig. 6.1. Microbially available phosphorus (MAP) and iron reduction from inlet water by Point-of-use (POU) sorption filter. Points represent the average values of sample triplicates. Figure adapted from (Zemīte *et al.*, 2023).

One possible reason for iron release could be changes in the hydraulic conditions, potentially linked to variations in water consumption patterns. Another factor contributing to

desorption might be fluctuations in the pH of the incoming water. Initially, the pH averaged around 8.1 during the first eight weeks, but it slightly decreased to an average of 7.8 between weeks 12 and 22.

As the pH was greater than 7.5 throughout the sampling period, most likely Fe(II) was sorbed onto the ferric oxo-hydroxide with consequent surface oxidation, resulting in a hydrolysed Fe(III)(OH)2 complex with an electron transfer to the solid, instead of an adsorbed Fe2+ ion form that could have been more likely at lower pH levels (Hiemstra and van Riemsdijk, 2007), if assuming similarities in sorption mechanisms between different Fe hydroxide materials.

6.2.   Water quality changes within internal networks

6.2.1.   Domestic cold water supply

In most buildings, the drinking water from the inlet water main is divided into two systems: domestic cold water (DCW) and domestic hot water (DHW). Generally, DCW is supplied directly from the water main without additional treatment. However, in the POU-device building, a filter unit is installed on the cold water main, which processes the incoming water before it is divided into cold and hot water supplies. Consequently, the outlet from the filter unit was used for subsequent analyses in this building.

Cold water samples were collected from kitchen taps in the morning, before any water usage, and further subjected to analyses of a limited number of parameters. Such water was on average around two times warmer than water in the water mains ($p < 0.001$) both in the POU-device (Table 6.2) and Reference buildings (Table 6.3). This temperature increase can be attributed to overnight thermal equalisation with ambient temperatures. Additionally, after a one-minute flush, the temperature showed an increase of around 40 % compared to the inlet, which can largely be explained by the individual flushing procedures of apartment residents.

While overnight temperature increases in water pipes are unavoidable, they are undesirable as they may negatively impact water quality. A study by (Torvinen *et al.*, 2007) showed that a temperature increase from 7 °C to 20 °C facilitates an increase in the number of heterotrophic bacterial counts and supports the survival of potential pathogens, such as *Mycobacterium avium*, in biofilm.

The pH of DCW decreased slightly during distribution. In the Reference building, the pH decreased by 0.16 units ($p < 0.001$), while in the POU-device building, it dropped by 0.11 units ($p = 0.013$) compared to the inlet water. It is attributed to an elevated formation of H^+ ions caused by an increase in temperature during DCW supply.

Further, no significant changes in electrical conductivity were observed. Regarding microbial nutrients, there was no change in TOC, but surprisingly MAP levels decreased in both buildings, regardless of passage through the filter.

Concerning microbial cell counts, no significant changes were noted in total microbial concentration or the number of damaged cells during distribution. However, the intact cell count

increased by 35 % in the POU-device building, with no similar change observed in the Reference building. Interestingly, the distribution of cell types shifted during the distribution process. In both buildings, the proportion of LNA content intact cells rose slightly – by 23 % in the POU-device building and by 17 % in the Reference building. This increase in LNA cells was accompanied by a decrease in the proportion of HNA content intact cells, by 16 % in the POU-device building and 15 % in the Reference building. This shift towards a greater proportion of LNA cells suggests the dominance of slower-growing, less metabolically active, oligotrophic bacterial populations (Hu *et al.*, 2022) within the DCW supply, with the increase attributed to overnight stagnation (Lautenschlager *et al.*, 2010).

Table 6.2. Comparison of water quality parameters of water inlet and kitchen taps outlet points in POU-device building (grab samples).

Parameter	Inlet, after POU-device (n = 12) [*]	DCW taps (n = 39) [*]	*p*-value [**]
Physical and chemical parameters			
Temperature (direct), °C	11.04 (SD 1.83)	22.42 (SD 3.24)	**< 0.001**
Temperature (1 min flushed), °C	11.04 (SD 1.83) [****]	16.00 (11.30–26.80)	**< 0.001**
pH	7.91 (SD 0.12)	7.80 (4.68–8.16)	**0.013**
Electrical conductivity, μS cm^{-1}	406.08 (SD 59.21)	393.74 (SD 68.48)	0.577
Main microbial nutrients			
TOC, mg l^{-1}	2.06 (1.38–7.62)	2.16 (1.06–6.78)	0.929
MAP, μg l^{-1}	3.56 (SD 1.49)	0 (0–4.28)	**< 0.001**
Microbiological parameters			
Total Cell Count, cells ml^{-1} [***]	2.55×10^5 (SD 6.23×10^4)	3.04×10^5 (SD 8.58×10^4)	0.077
Damaged Cell Count, cells ml^{-1}	9.71×10^4 (SD 3.11×10^4)	8.38×10^4 (3.41×10^4 – 2.23×10^5)	0.281
Intact Cell Count (ICC), cells ml^{-1}	1.58×10^5 (SD 5.05×10^4)	2.14×10^5 (SD 6.42×10^4)	**0.009**
Low Nucleic Acid content cells, % ICC	40 (SD 10.7)	50 (SD 7.9)	**0.002**
High Nucleic Acid content cells, % ICC	60 (SD 10.7)	50 (SD 7.9)	**0.002**

[*] Mean (standard deviation, SD) values are presented for normally distributed data, or otherwise Median (min-max) is used.

[**] Statistically significant *p*-values are highlighted in bold; 2-tailed significance used for normally distributed data or exact significance value for non-parametric independent samples t-test.

[***] Total Cell Count = Intact Cell Count + Damaged Cell Count.

[****] Due to large diameter, assumed same as directly measured.

Table 6.3. Comparison of water quality parameters of water inlet and kitchen taps outlet points in Reference building (grab samples).

Parameter	Inlet (n = 12) [*]	DCW taps (n = 33) [*]	p-value [**]
Physical and chemical parameters			
Temperature (direct), °C	10.66 (SD 2.28)	21.10 (18.20–25.70)	**< 0.001**
Temperature (1 min flushed), °C	10.66 (SD 2.28) [****]	15.04 (SD 323)	**< 0.001**
pH	8.04 (SD 0.14)	7.88 (7.15–7.98)	**< 0.001**
Electrical conductivity, $\mu S\ cm^{-1}$	306.75 (SD 30.00)	317.00 (270–607)	0.156
Main microbial nutrients			
TOC, mg l^{-1}	1.31 (0.96–5.76)	1.71 (0.74–7.68)	0.135
MAP, $\mu g\ l^{-1}$	10.29 (SD 4.23)	1.06 (0–14.14)	**0.001**
Microbiological parameters			
Total Cell Count, cells ml^{-1} [***]	2.19×10^5 (SD 7.22×10^4)	2.55×10^5 ($1.28 \times 10^5 - 1.37 \times 10^6$)	0.551
Damaged Cell Count, cells ml^{-1}	6.83×10^4 ($5.77 \times 10^4 - 1.14 \times 10^5$)	7.24×10^4 ($4.12 \times 10^4 - 9.60 \times 10^5$)	0.950
Intact Cell Count (ICC), cells ml^{-1}	1.39×10^5 (SD 6.54×10^4)	1.53×10^5 ($7.15 \times 10^4 - 4.05 \times 10^5$)	0.381
Low Nucleic Acid content cells, % ICC	47 (SD 8.9)	54 (SD 10.5)	**0.025**
High Nucleic Acid content cells, % ICC	53 (SD 8.9)	46 (10.5)	**0.025**

[*] Mean (standard deviation, SD) values are presented for normally distributed data, or otherwise Median (min-max) is used. Number of samples = 12 for each building.

[**] Statistically significant p-values are highlighted in bold; 2-tailed significance used for normally distributed data or exact significance value for non-parametric independent samples t-test.

[***] Total Cell Count = Intact Cell Count + Damaged Cell Count.

[****] Due to large diameter, assumed same as directly measured.

6.2.2. Domestic hot water supply

In the buildings studied, a portion of the inlet water is directed to heating substations located in the basements. Here, it is heated in heat exchangers and then distributed to apartments via the DHW system. To minimize the heat loss effect and expedite the replacement of cold water with hot water, a circulation loop is often implemented, ensuring continuous flow between the riser pipes and the heat exchanger.

DHW samples were collected from two locations: showerheads and the circulation return pipeline just before re-entry into the heat exchanger.

Changes after heating and distribution. Water, undergoing thermal heating, is subjected to a change in its characteristics (Table 6.4). The most obvious change is the increase in temperature, which depends on the water heater setpoint. A slight increase in pH (by 0.18 units) was observed in samples from the circulation loop, but no significant pH change was noted in samples collected from showerheads.

In terms of elemental composition, there was a notable increase in copper, on average by 93–232 µg l^{-1}, and zinc, by 30–80 µg l^{-1}, depending on the building and DHW system type. Manganese, iron and lead showed different characteristics based on the sampling place. Mn in general did not reveal significantly different amounts in all DHW sampling places except for showerheads in the Reference building, where its value slightly decreased on average by 10 µg l^{-1}, compared to water inlet. Iron decreased on average by 60 µg l^{-1} and 90 µg l^{-1}, in circulation and showerhead samples of the Reference building, respectively, while it increased on average by 130 µg l^{-1} in POU-device building circulation loop samples and did not change significantly in showerhead samples. Lead, however, showed a significant increase on average by 1.52 µg l^{-1}, in the showerheads of both buildings, but there was no significant change determined in the circulation return of the POU-device building ($p = 0.089$).

Regarding microbial nutrients, similarly to the DCW supply, there was no change in TOC, but, also in the DHW system MAP levels decreased in both buildings. Microbial cell counts were generally higher in showerhead samples, including both intact and damaged cells, as well as *Legionella* numbers. Bacterial communities in the DHW system shifted towards the HNA fraction, indicating more active bacterial populations (Lebaron *et al.*, 2001).

Differences between showerheads and circulation return. Although samples were taken from the same DHW system, water quality parameters differed between apartment showerheads and circulation water (Table 6.5 and Table 6.6). Prolonged stagnation in showerheads resulted in significantly lower water temperatures (< 0.001) and slightly lower pH values than in the circulation loop samples.

Elemental analysis showed divergent patterns across buildings. In the POU-device building, showerhead samples contained 7 % more calcium and 19 % more zinc than circulation samples, while iron levels were below detection limits in the showerheads but had a median concentration of 0.155 mg l^{-1} in the circulation samples. In the Reference building, copper concentrations were around 30 % higher in showerhead samples compared to circulation water.

Microbial counts in the showerhead samples were 38–48 % higher ($p = 0.001$) for intact cells and 24–32 % higher for total microbial cells, likely due to overnight microbial growth during stagnation. This effect was more pronounced in the Reference building, where a shift towards more HNA-intact cells was observed, indicating active microbial growth (Lebaron *et al.*, 2001). The POU-device building did not show a similar shift in cell proportions.

Table 6.4. 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.

Potential impact of stagnation on microbial communities. Prolonged stagnation at the tap level, especially overnight, can promote microbial growth. Studies by (Lautenschlager *et al.*, 2010) found that water stagnation at the tap level (in their case for 12 hours) can lead to a cell increase at the rate of $0.22\ \text{h}^{-1}$, supported by available nutrients. Similarly, (Ji *et al.*, 2017) noted that even 8 hours of stagnation caused significant shifts in microbial communities. This highlights the variability of microbial ecosystems within the same water system.

Microbial communities are not uniform within a building's water network. Variations in microbial composition can be attributed to differences in water temperature and chemistry at different points in the system. For instance, stagnation results in cooler water temperatures at showerheads compared to circulation return pipes. These temperature differences, typically at least 4°C, create distinct ecological niches, supporting diverse microbial communities (Proctor *et al.*, 2017; Meyer *et al.*, 2023).

Interestingly, despite the cooler water in apartment plumbing after overnight stagnation ($p < 0.001$ for both buildings), no significant differences in *Legionella* counts were found between circulation return and showerhead DHW samples ($p = 0.409$ and $p = 304$). However, these conditions favoured the proliferation of other microorganisms, resulting in a 38-48 % increase in intact cell counts over the total sampling period ($p = 0.001$ for both buildings).

The potential impact of metal accumulation on microbial growth. Metal accumulation in biofilms and plumbing materials can influence microbial growth. Studies of biofilms in shower hoses have detected significant metal build-up from upstream pipes, including iron (up to 5 µg Fe cm^{-2}), lead (75 ng Pb cm^{-2}), and copper (460 ng Cu cm^{-2}) (Proctor *et al.*, 2018). Metal leaching from plumbing materials, such as iron, zinc, and potassium, is known to enhance the growth of *Legionella pneumophila* (States *et al.*, 1985).

The inconsistencies observed with manganese, iron, and lead concentrations (Table 6.4) could likely be attributed to outliers, as a relatively large difference in maximal value in comparison to the median either in inlet water or DHW water sample was noted. No outliers were excluded due to the limited dataset size.

Table 6.5. Comparison of water quality parameters in DHW samples taken from showerheads and circulation return in POU-device building (grab samples).

Parameter	DHW, showerhead (n=39) [*]	DHW, circulation return (n=12) [*]	*p*-value [**]
Physical and chemical parameters			
Temperature (direct), °C	30.40 (15.9–43.9)	45.85 (SD 2.06)	**< 0.001**
Temperature (1 min flushed), °C	43.48 (SD 0.15)	45.85 (SD 2.06) [****]	**0.035**
pH	7.98 (SD 0.15)	8.09 (SD 0.11)	**0.025**
Electrical conductivity, µS cm^{-1}	384 (293–524)	373.5 (SD 51.3)	0.222
Ca, mg l^{-1}	38.80 (31.4–42.3)	37.47 (SD 3.03)	0.339
Mg, mg l^{-1}	11.75 (SD 1.12)	10.98 (SD 0.97)	**0.036**
Mn, mg l^{-1}	0.008 (0–0.397)	0.11 (0.005–0.099)	0.131
Cu, µg l^{-1}	232 (38.8–2421)	157.8 (SD 103.5)	0.079
Zn, mg l^{-1}	0.097 (0.046–0.627)	0.078 (SD 0.042)	**0.012**
Fe, mg l^{-1}	0 (0–1240)	0.155 (0.041–0.553)	**< 0.001**
Pb, µg l^{-1}	2.77 (0–133)	4.29 (SD 2.64)	0.689
Main microbial nutrients			
TOC, mg l^{-1}	2.21 (1.46–8.57)	1.97 (1.37–4.29)	0.152
MAP, µg l^{-1}	0 (0–9.31)	0.2 (0–4.95)	0.307

Parameter	DHW, showerhead (n=39) [*]	DHW, circulation return (n=12) [*]	p-value [**]
Microbiological parameters			
Total Cell Count, cells ml^{-1} [***]	4.12×10^5 (1.28×10^5 – 9.57×10^5)	3.12×10^5 (SD 7.39×10^4)	**0.008**
Damaged Cell Count, cells ml^{-1}	1.13×10^5 (7.50×10^4 – 5.09×10^5)	1.17×10^5 (SD 3.25×10^4)	0.318
Intact Cell Count (ICC), cells ml^{-1}	3.13×10^5 (SD 1.67×10^5)	1.95×10^5 (SD 6.65×10^4)	**0.001**
Low Nucleic Acid content cells, % ICC	25.29 (12.78–74.03)	24.47 (18.11–58.15)	0.756
High Nucleic Acid content cells, % ICC	74.64 (26.15–87.28)	75.41 (42.15–81.53)	0.657
L. pneumophila, CFU ml^{-1}	400 (0–9500)	1925 (0–10 000)	0.304

[*] Mean (standard deviation, SD) values are presented for normally distributed data, or otherwise Median (min-max) is used.

[**] Statistically significant p-values are highlighted in bold; 2-tailed significance used for normally distributed data or exact significance value for non-parametric independent samples t-test.

[***] Total Cell Count = Intact Cell Count + Damaged Cell Count.

[****] Due to large diameter, assumed same as directly measured.

Table 6.6. Comparison of water quality parameters in DHW samples taken from showerheads and circulation return in Reference building (grab samples).

Parameter	DHW, showerhead (n=33) [*]	DHW, circulation return (n=12) [*]	p-value [**]
Physical and chemical parameters			
Temperature (direct), °C	21.94 (SD 2.29)	44.35 (SD 2.62)	**< 0.001**
Temperature (1 min flushed), °C	40.60 (35.3–49.9)	44.35 (SD 2.62) [****]	0.128
pH	8.00 (SD 0.12)	8.23 (SD 0.11)	**< 0.001**
Electrical conductivity, µS cm^{-1}	303 (270–602)	315.42 (SD 40.12)	0.409
Ca, mg l^{-1}	36.93 (SD 4.27)	35.47 (SD 3.04)	0.282
Mg, mg l^{-1}	10.8 (8.5–16.3)	10.0 (8.5–11.3)	0.424
Mn, mg l^{-1}	0.014 (0.008–0.178)	0.014 (0.011–0.081)	1.000
Cu, µg l^{-1}	142 (73–289)	99 (30)	**0.002**
Zn, mg l^{-1}	0.052 (0.027–0.142)	0.042 (0.033–0.076)	0.052
Fe, mg l^{-1}	0.032 (0–0.122)	0.061 (SD 0.047)	0.115
Pb, µg l^{-1}	2.20 (1.45–9.87)	2.84 (1.70–6.36)	0.109

Parameter	DHW, showerhead (n=33) [*]	DHW, circulation return (n=12) [*]	p-value [**]
Main microbial nutrients			
TOC, mg l^{-1}	1.57 (1.04–8.14)	1.42 (1.03–4.18)	0.409
MAP, μg l^{-1}	0.33 (0–9.01)	1.45 (SD 1.64)	0.367
Microbiological parameters			
Total Cell Count, cells ml^{-1} [***]	3.31×10^5 (1.11×10^5 – 8.71×10^5)	2.25×10^5 (SD 9.47×10^4)	**0.007**
Damaged Cell Count, cells ml^{-1}	9.61×10^4 (6.06×10^4 – 3.31×10^5)	8.43×10^4 (SD 2.36×10^4)	0.142
Intact Cell Count (ICC), cells ml^{-1}	2.73×10^5 (SD 1.59×10^5)	1.41×10^5 (SD 7.97×10^4)	**0.001**
Low Nucleic Acid content cells, % ICC	24.65 (6.32–68.13)	35.76 (SD 10.99)	**0.009**
High Nucleic Acid content cells, % ICC	75.35 (31.87–93.81)	64.32 (SD 11.01)	**0.009**
L. pneumophila, CFU ml^{-1}	400 (0–3100)	100 (0–2600)	0.409

[*] Mean (standard deviation, SD) values are presented for normally distributed data, or otherwise Median (min-max) is used.

[**] Statistically significant p-values are highlighted in bold; 2-tailed significance used for normally distributed data or exact significance value for non-parametric independent samples t-test.

[***] Total Cell Count = Intact Cell Count + Damaged Cell Count.

[****] Due to large diameter, assumed same as directly measured.

6.3. Distinct domestic hot water supply temperature regimes

Due to energy-saving measures, the study site operated with two distinct DHW temperature regimes (Section 2.3.1). Initially, for the first 14 weeks, the system maintained a static setpoint of 57 °C. Following this period, a dynamic temperature regime was introduced, with varying setpoints of 48 °C, 52 °C, and 57 °C. Consequently, analyses were conducted either for the entire study duration (weeks 0–22) or specific heating regimes (samples from weeks 0–12 and 16–22).

During the static temperature regime, no significant changes in microbial composition were observed in either the DHW from showerhead outflows (Table 6.7) or in the DHW circulation (Table 6.8). This finding applied to both the Reference building and the POU-device building, regardless of additional nutrient removal processes.

However, when the system transitioned to the dynamic temperature regime in the later weeks, notable differences emerged. In the POU-device building, the showerhead outflows exhibited higher concentrations of damaged cells, which also led to an overall increase in total cell concentrations. The DHW circulation return samples from the POU-device building

showed a similar trend, with additional higher intact cell counts and a greater fraction of HNA intact cells compared to the Reference building.

Furthermore, both DHW systems displayed higher *Legionella* concentrations during the dynamic temperature regime, with the POU-device building showing higher values, compared to the Reference building.

Table 6.7. Microbiological composition of DHW showerhead first-draw samples.

Parameter	POU-device building [*]	Reference building return [*]	*p*-value [**]
Regular temperature setting (samples from weeks 0-12), n=17 (RB) and 22 (POU)			
Total Cell Count, cells ml^{-1} [***]	5.09×10^5* (2.56×10^5)	4.75×10^5* (2.36×10^5)	0.675
Damaged Cell Count, cells ml^{-1}	1.11×10^5 (7.50×10^4 – 5.09×10^5)	1.03×10^5 (6.06×10^4 – 3.31×10^5)	0.347
Intact Cell Count (ICC), cells ml^{-1}	3.50×10^5* (1.99×10^5)	3.38×10^5* (1.73×10^5)	0.837
Low Nucleic Acid content cells, % ICC	25.28 (12.78–74.03)	21.99 (6.32–68.13)	0.210
High Nucleic Acid content cells, % ICC	74.48 (26.15–87.28)	77.67 (31.87–93.81)	0.232
Changed temperature setting (samples from weeks 16-22), n=16 (RB) and 17 (POU)			
Total Cell Count, cells ml^{-1} [***]	4.02×10^5* (1.34×10^5)	2.51×10^5 (1.62×10^5 – 6.53×10^5)	**0.028**
Damaged Cell Count, cells ml^{-1}	1.18×10^5 (7.61×10^4 – 2.53×10^5)	8.47×10^4 (6.09×10^4 – 2.21×10^5)	**0.025**
Intact Cell Count (ICC), cells ml^{-1}	2.64×10^5* (9.93×10^4)	1.68×10^5 (9.06×10^4 – 4.51×10^5)	0.081
Low Nucleic Acid content cells, % ICC	26.12* (2.25)	27.63* (10.32)	0.605
High Nucleic Acid content cells, % ICC	73.92* (5.25)	72.41* (10.30)	0.606
L. pneumophila, CFU ml^{-1}	4335* (3202)	550 (0–3000)	**< 0.001**

[*] Mean (standard deviation, SD) values are presented for normally distributed data, or otherwise Median (min-max) is used.

[**] Statistically significant *p*-values are highlighted in bold; 2-tailed significance used for normally distributed data or exact significance value for non-parametric independent samples t-test.

[***] Total Cell Count = Intact Cell Count + Damaged Cell Count.

Table 6.8. Microbiological composition of DHW circulation return samples.

Parameter	POU-device building [*]	Reference building return [*]	p-value [**]
Regular temperature setting (samples from weeks 0-12), n=6			
Total Cell Count, cells ml^{-1} [***]	2.94×10^5* (8.48×10^4)	2.82×10^5* (1.03×10^5)	0.825
Damaged Cell Count, cells ml^{-1}	1.03×10^5* (2.77×10^4)	9.44×10^4* (2.35×10^4)	0.555
Intact Cell Count (ICC), cells ml^{-1}	1.90×10^5* (8.28×10^4)	1.87×10^5* (8.95×10^4)	0.948
Low Nucleic Acid content cells, % ICC	30.89* (14.29)	36.02* (15.41)	0.564
High Nucleic Acid content cells, % ICC	69.05* (14.08)	64.04* (15.41)	0.569
Changed temperature setting (samples from weeks 16-22), n=6			
Total Cell Count, cells ml^{-1} [***]	3.31×10^5* (6.34×10^4)	1.69×10^5* (4.05×10^4)	**< 0.001**
Damaged Cell Count, cells ml^{-1}	1.31×10^5* (3.30×10^4)	7.43×10^4* (2.06×10^4)	**0.006**
Intact Cell Count (ICC), cells ml^{-1}	2.00×10^5* (5.32×10^4)	9.51×10^4* (2.96×10^4)	**0.002**
Low Nucleic Acid content cells, % ICC	23.68 (21.87–33.38)	35.50* (5.31)	0.065
High Nucleic Acid content cells, % ICC	76.27 (66.86-78.26)	64.59* (5.41)	**0.026**
L. pneumophila, CFU ml^{-1}	6042* (3790)	900* (1008)	**0.020**

[*] Mean (standard deviation, SD) values are presented for normally distributed data, or otherwise Median (min-max) is used.

[**] Statistically significant *p*-values are highlighted in bold; 2-tailed significance used for normally distributed data or exact significance value for non-parametric independent samples t-test.

[***] Total Cell Count = Intact Cell Count + Damaged Cell Count.

6.4. MAP reduction within internal drinking water networks

The water supplied to the internal drinking water networks had an average of 3.56 µg MAP l^{-1} (SD 1.49 µg l^{-1}) after the inlet filter in the POU-device building and on average 10.29 µg MAP l^{-1} (SD 4.23 µg l^{-1}) in Reference building inlet. Initially, MAP values higher than inflow were observed in some sampling locations in the POU-device building (Fig. 6.2), reaching up to 4.28 µg l^{-1} for DCW and up to 9.31 µg l^{-1} for DHW samples, potentially indicating system flushing. In the following weeks, the measured concentrations did not exceed 2.1 µg l^{-1} for DCW and 1.87 µg l^{-1} for DHW samples.

In the Reference building, the initial MAP concentrations reached up to $5.15\ \mu g\ l^{-1}$ for DCW and up to $9.01\ \mu g\ l^{-1}$ for DHW samples. Over the subsequent weeks, the maximal concentrations for the cold-water samples varied from 0.75 to $14.14\ \mu g\ l^{-1}$, while DHW samples had a maximal concentration of $4.67\ \mu g\ l^{-1}$ throughout the entire period.
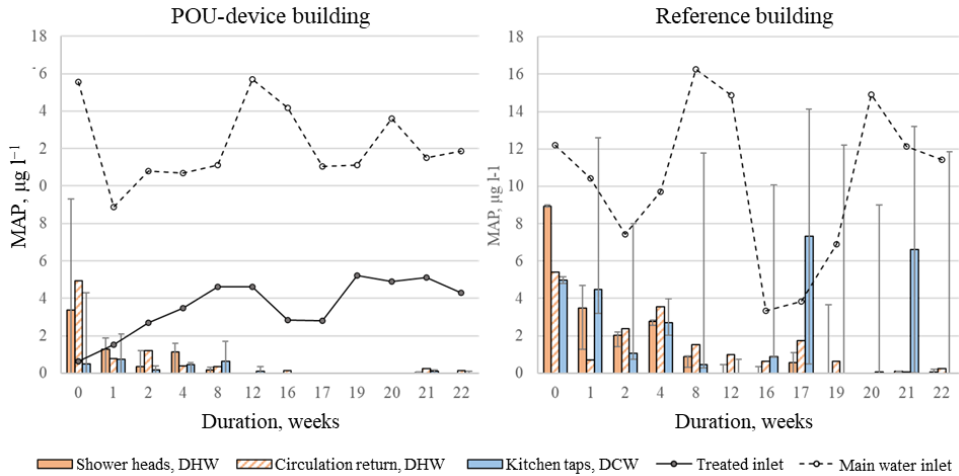
Fig. 6.2. Microbially available phosphorus concentration dynamics. For the apartment samples, data represent median values with bars corresponding to the range. POU – point-of-use, DHW – domestic hot water, DCW – domestic cold water. Figure adapted from (Zemīte *et al.*, 2023).

As expected, Reference building in almost all sampling locations showed higher MAP concentrations ($p < 0.05$) (Table 6.9), as no specific removal was employed. An exception was circulation return during regular temperature setpoint ($p = 0.132$), as well as if analysed for the whole duration ($p = 0.060$). However, the values in both buildings were very small.

Surprisingly, MAP levels decreased significantly in both buildings, regardless of whether the inlet water passed through the MAP removal filter unit. In DCW kitchen tap samples from weeks 0–12, the POU-device building showed an average MAP decrease of 86 %, while the Reference building exhibited a 77 % decrease, compared to the inlet. During the sampling period from weeks 16–22, the reduction increased to 100 % and 98 % in the POU-device and Reference buildings, respectively.

A similar trend was observed across all sampling locations, though with a slightly smaller average decrease during weeks 0–12. For example, the POU-device building showed an 88 % MAP reduction in DHW showerheads and an 81 % reduction in DHW circulation return, while the Reference building showed reductions of 83 % and 80 % in these respective locations. During the dynamic hot water heating setpoint phase (weeks 16–22), the MAP decrease was even more pronounced, resulting in non-detectable average levels in all POU-device outlets and DHW showerhead samples in the Reference building. DHW circulation return samples from the Reference building showed a 94 % reduction during this period.

Table 6.9. Concentration of microbially available phosphorus (MAP), µg l^{-1} (grab samples).

Sampling time	POU-device building [*]	Reference building [*]	p-value [**]
Water inlet (from water main (Ref. building) or after sorption filter (POU-dev. building)			
weeks 0–22, n=12 for each building	3.56 (SD 1.49)	10.29 (SD 4.23)	**<0.001**
weeks 0–12, n=6 for each building	2.93 (SD 1.63)	11.81 (SD 3.32)	**<0.001**
weeks 16–22, n=6 for each building	4.19 (SD 1.12)	8.76 (SD 4.77)	0.066
DCW, kitchen taps			
weeks 0–22, n=33 (RB) and 39 (POU)	0 (0–4.28)	1.06 (0–14.14)	**<0.001**
weeks 0–12, n=17 (RB) and 22 (POU)	0.41 (0–4.28)	2.69 (0–12.60)	**0.001**
weeks 16–22, n=16 (RB) and 17 (POU)	0 (0–0.18)	0.67 (0–14.14)	**0.001**
DHW, showerheads			
weeks 0–22, n=33 (RB) and 39 (POU)	0 (0–9.31)	0.33 (0–9.01)	**0.012**
weeks 0–12, n=17 (RB) and 22 (POU)	0.36 (0–9.31)	2.01 (0–9.01)	**0.018**
weeks 16–22, n=16 (RB) and 17 (POU)	0 (0–0.08)	0 (0–3.66)	**0.014**
DHW, circulation return			
weeks 0–22, n=12 for each building	0.20 (0–4.95)	1.45 (SD 1.64)	0.060
weeks 0–12, n=6 for each building	0.57 (0–4.95)	2.41 (SD 1.79)	0.132
weeks 16–22, n=6 for each building	0 (0–0.26)	0.49 (SD 0.72)	**0.001**

[*] Mean (standard deviation, SD) values are presented for normally distributed data, or otherwise Median (min-max) is used.

[**] Statistically significant p-values are highlighted in bold; 2-tailed significance used for normally distributed data or exact significance value for non-parametric independent samples t-test.

[***] Total Cell Count = Intact Cell Count + Damaged Cell Count.

[****] Due to large diameter, assumed same as directly measured.

One possible explanation for the observed decrease in MAP concentrations within the distribution network could be related to hydraulic retention time, as many samples were collected after overnight stagnation. (Jiang, Chen and Ni, 2011) demonstrated a reduction in total phosphorus with increasing hydraulic retention time, attributing this to potential

consumption by both bulk water and biofilm-associated bacteria, as well as adsorption onto particles and biofilms.

Correlation analyses (Table 6.10 and Table 6.11) revealed moderate to very strong positive correlations between MAP and TOC, along with their correlations with other elements at DHW system sampling points. This suggests that both nutrients, along with other elements, are simultaneously required to support microbial growth. Additionally, the negative correlations between MAP and both LNA and HNA content microbial cells at certain DHW system sampling points imply that MAP may have been consumed for microbial cell production, indicating that MAP could have been a limiting nutrient in those cases within the POU-device building.

In contrast, in the DCW system of the Reference building, only TOC showed a positive correlation with LNA, HNA content intact cell concentrations, and damaged cells. This suggests that bacterial growth in the Reference building was likely driven primarily by organic carbon. A limitation of this study is the lack of data on the AOC fraction of TOC and bacterial regrowth potential. Therefore, it remains unclear which nutrient primarily contributed to bacterial regrowth. However, it was expected that the Reference building would be carbon-limited, consistent with previous findings that the groundwater supplied in Riga is carbon-limited (Nescerecka, Juhna and Hammes, 2018).

The positive correlation between MAP and temperature (in direct, unflushed samples) could indicate either reduced MAP consumption at higher temperatures or increased MAP release, leading to elevated concentrations. This may have influenced the nutrient cycling necessary for *Legionella* growth, as the *Legionella* pattern did not align with total microbial cell counts but instead negatively correlated with both carbon and phosphorus in the Reference building's showerheads.

Additionally, MAP had a strong correlation with metals such as manganese, which typically originate from the water source rather than the pipes, and accumulate in biofilms. This suggests that MAP is adsorbed onto metal oxides during transport along the pipes.

In the POU-device building, MAP levels gradually diminished to undetectable levels after three months. This suggests that the internal piping, with its high surface-to-water ratio, serves as a reservoir for bacterial nutrients, promoting biofilm formation and creating "hot spots" for opportunistic bacterial growth. Furthermore, some MAP may have been removed through adsorption onto deposits formed during water heating. Although MAP levels in the DCW system of the Reference building showed greater variability, no significant differences were observed between MAP concentrations in the DCW and DHW systems (combining showerhead and circulation return samples) throughout the entire sampling period ($p = 0.057$). The smaller pipes, with their larger surface-to-water ratio, appear to be more selective for MAP rather than AOC, shifting the limiting factor from larger service pipes to smaller distribution lines.

Table 6.10. Significant Pearson correlations (R) with MAP content.

	DHW, showerheads		DHW, circulation return		DCW, kitchen taps	
	RB (n=33)	POUB (n=39)	RB (n=12)	POUB (n=12)	RB (n=33)	POUB (n=39)
TOC	0.887** very strong	0.487** moderate	0.685* strong	0.652* strong	–	–
T, °C	0.626** strong	–	0.783** strong	0.621* strong	–	–
El. cond.	0.637** strong	–	0.627* strong	–	–	–
LNA cells	–	–	–	–0.619* strong	–	–
HNA cells	–	–0.395* weak	–	–0.642* strong	–	–
Ca	0.463**	–	–	–	n.a.	n.a.
Mg	0.588**	–	–	–	n.a.	n.a.
Mn	0.837** very strong	0.392* weak	0.655* strong	0.931** very strong	n.a.	n.a.
Cu	–	–0.328* weak	–	–	n.a.	n.a.
Zn	–	–	–	0.720** strong	n.a.	n.a.
Fe	–	–	–	0.702* strong	n.a.	n.a.
Pb	–	–	–	0.837** very strong	n.a.	n.a.
Legionella	–0.378* weak	–	–	–	n.a.	n.a.

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

DHW – domestic hot water, DCW – domestic cold water, POUB – POU-device building, RB – Reference Building, n. a. – not analysed.

Table 6.11. Significant Pearson correlations (R) with TOC content.

	DHW, showerheads		DHW, circulation return		DCW, kitchen taps	
	RB (n=33)	POUB (n=39)	RB (n=12)	POUB (n=12)	RB (n=33)	POUB (n=39)
MAP	0.887** very strong	0.487** moderate	0.685* strong	0.652* strong	–	–
T, °C	0.596** moderate	–	0.681* strong	–	0.478** moderate	0.334* weak
El. cond.	0.578** moderate	0.413** moderate	–	–	0.453** moderate	–
LNA cells	–	–	–	–	0.420* moderate	–
HNA cells	–	–	–	–	0.455** moderate	–
DCC cells	–	0.333* weak	–	–	0.812** very strong	–
Ca	0.466** moderate	0.318* weak	–	–	n.a.	n.a.
Mg	0.553** moderate	0.464** moderate	0.584* moderate	–	n.a.	n.a.
Mn	0.768** strong	0.790** strong	0.674* strong	0.596* moderate	n.a.	n.a.
Cu	–	0.356*	–	–0.578* moderate	n.a.	n.a.
Zn	–	0.649** strong	–	–	n.a.	n.a.
Fe	–0.361* weak	0.587** moderate	–	–	n.a.	n.a.
Pb	–	0.652** strong	–	0.685* strong	n.a.	n.a.
Legionella	–0.406* moderate	–	–	–	n.a.	n.a.

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

DHW – domestic hot water, DCW – domestic cold water, POUB – POU-device building, RB – Reference Building, n. a. – not analysed.

6.5. Section conclusions

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

MAP reduction potential of the point-of-use filtration device

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 %, 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.

Water quality changes during distribution

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.

In the **DCW system**, water temperature increased significantly 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 µg l^{-1}) and zinc (by 30–80 µg 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.

Interestingly, MAP concentrations decreased significantly in both cold and hot water systems in both buildings, regardless of the presence of the filter. 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.

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.

7. *LEGIONELLA* SPP. CONTROL IN MULTI-STOREY RESIDENTIAL BUILDINGS

7.1. Effect of centralised chemical flushing and disinfection

A centralised chemical flushing of the DHW system with formic acid-containing reagent (Section 2.3.3) was performed before the start-up of the testing period to ensure the removal of scale deposits in the networks, and followed by disinfection with reagent containing hydrogen peroxide and silver ions.

Initially, such chemical network cleaning resulted in no cultivable *Legionella* spp. in the DHW samples taken the next day after the procedure (Fig. 7.1), but it did not prevent *Legionella* bacteria regrowth. Already 36 % of samples taken one week later revealed the presence of culturable *Legionella*, which resulted on average in 2.3×10^2 CFU l^{-1} (max 9.0×10^2 CFU l^{-1}) in the DHW samples of the Reference building, and on average 90 CFU l^{-1} (max 2.0×10^2 CFU l^{-1}) in the POU-device building.

Furthermore, just another week later, some DHW sampling locations in the Reference building exceeded the EU Directive guideline value of 1000 CFU l^{-1}, while an average of all DHW samples exceeded the guideline value after around two months in the Reference building, which aligned with first exceedance of EU guideline value in circulation return sample of POU-device building.

During these centralised cleaning procedures, the most challenging aspect is ensuring that all water outlets receive the correct dosage of disinfectant. To maintain control, the specialists conducting the procedure visit apartments and measure the disinfectant concentration at each outlet to verify the effectiveness of the process. However, it is generally impossible to ensure full compliance across all apartments due to residents' availability or willingness to cooperate. In the POU-device building, the apartment survey success rate was 51 %, which included 3 out of 5 apartments that participated in the sampling. The Reference building achieved a higher success rate of 68 %, including all 3 sampled apartments (Fig. 2.6 and Fig. 2.7).

Overall, the number of apartments involved in the study was too limited to make meaningful comparisons about the effectiveness of disinfectant dosage control at the outlets on the presence of *Legionella* in water samples. The collected dataset did not reveal any statistically significant effect of proper disinfection on *Legionella* counts or serogroups nor on the characteristics of total microbial cells (LNA, HNA, and DCC).

Another study reported that regular dosing of hydrogen peroxide at 25 mg l^{-1} has been shown to promote a shift in *Legionella* species from *L. pneumophila* serogroups 2–15 to non-*pneumophila* species, as well as *L. pneumophila* serogroup 1. The prevalence of *Legionella* was, however, eliminated with the addition of food-grade polyphosphates (Casini *et al.*, 2017).

In this pilot study, the purpose of hydrogen peroxide disinfection was to establish a fresh baseline in both buildings, which was successfully achieved. While centralised chemical flushing and disinfection of a building's internal water network is a useful strategy to address existing *Legionella* colonisation, it proved ineffective for long-term *Legionella* control.

7.2. Effect of MAP removal unit and water heater setpoint

Legionella count in the internal plumbing system was determined by plate counting for samples taken during both static and dynamic DHW heat exchanger setpoints. The change of temperature setpoint aligned with the start of a heating season (week 14), shifting from a 57 °C heating setpoint for the samples taken during weeks 0–12, to a dynamic setting of varying temperature setpoints at 48 °C, 52 °C and 57 °C for the samples taken during weeks 16–22.

During weeks 0–12, when operating at a regular static temperature setting, the DHW samples from both buildings contained similar concentrations of cultivable *Legionella* ($p = 0.124$ for showerhead samples and $p = 0.394$ for circulation return samples). The DHW showerhead samples contained on median 1.5×10^2 (0–7.0×10^2) CFU l^{-1} in the POU-device building and 1.5×10^2 (0–3.0×10^3) CFU l^{-1} in the Reference building. Similarly, the circulation return loop samples contained 1.3×10^2 (0–3.1×10^3) CFU l^{-1} and 75 (0-1.2×10^3) CFU l^{-1} in POU-device and Reference buildings, respectively.

Further, as the heating setpoint was changed to the dynamic setting, there was more cultivable Legionella detected in the POU-device building ($p < 0.001$ for showerhead samples and $p = 0.020$ for circulation return samples) compared to the Reference building. Surprisingly, there was nearly an order-of-magnitude increase of *Legionella* bacteria concentration in the samples taken from the building, where MAP removal unit was installed at water inlet, resulting in average values of 4.3×10^3 (SD 3.2×10^3) CFU l^{-1} in the showerhead and 6.0×10^3 (SD 3.8×10^3) CFU l^{-1} in circulation return samples, while the Reference building had on average 5.5×10^2 (0-3.0×10^3) CFU l^{-1} in the showerhead and 9.0×10^2 (SD 1.0×10^3) CFU l^{-1} in circulation return samples.

Overall, the concentration of cultivable *Legionella* bacteria in DHW samples was relatively stable in Reference building throughout the whole sampling duration (Fig. 7.1), while for POU-device building it was relatively stable during the first temperature setting period and highly scattered during the dynamic DHW temperature setting. It showed weak to moderate correlations with electrical conductivity and magnesium content, as well as TOC and MAP concentrations, while Reference building also showed a positive correlation to iron levels and negative – to calcium (Table 7.1).

Influent water composition provided growth-promoting substances. Apart from temperature adjustments, other factors likely contributed to the rapid *Legionella* regrowth. Various parameters, such as total chlorine concentration, pH, phosphorus content, sulphate (SO_4^{2-}), and magnesium can influence the variations in the bulk water microbiome (Ji *et al.*, 2015). During the study, the POU-device building received water with 55 % (0.76 mg l^{-1}) higher TOC content ($p = 0.003$), 9 % (3.2 mg l^{-1}) more calcium ($p = 0.009$), and 17 % (1.58 mg l^{-1}) more magnesium ($p = 0.040$) compared to the Reference building. Additionally, the POU-device building had a 90 % (7.54×10^4 cells ml^{-1}) greater inflow of intact cells ($p = 0.041$), with around 10 % higher shift towards HNA bacterial cells ($p = 0.004$). In contrast, it received 55 % (0.02 mg l^{-1}) smaller manganese concentration ($p = 0.026$), 100 %

(6.20 µg l^{-1}) less copper ($p = 0.015$), and 70 % (0.11 mg l^{-1}) less iron ($p = 0.015$) in the inflow water.

The observed correlations between *Legionella* concentrations and certain water parameters, such as electrical conductivity, magnesium, total organic carbon, and microbially available phosphorus, can be potentially explained by nutrient dynamics. The negative correlation with phosphorus and carbon likely reflected nutrient depletion, as these elements were consumed by *Legionella* bacteria, which in turn stimulated its growth. Similarly, such an assumption can be addressed also to magnesium concentration, as well as to iron and calcium utilisation in Reference building. Increased levels of calcium and magnesium are known to enhance the risk of *Legionella* colonisation (Rakić *et al.*, 2022), while iron is an essential nutrient for *Legionella* growth (Portier *et al.*, 2016). The positive correlation between *Legionella* and iron in the Reference building supports this.

The weak to moderate correlation with electrical conductivity values most likely was attributed to the specific water source, as this parameter was linked to variation in inlet waters entering from the municipal system (Section 5).
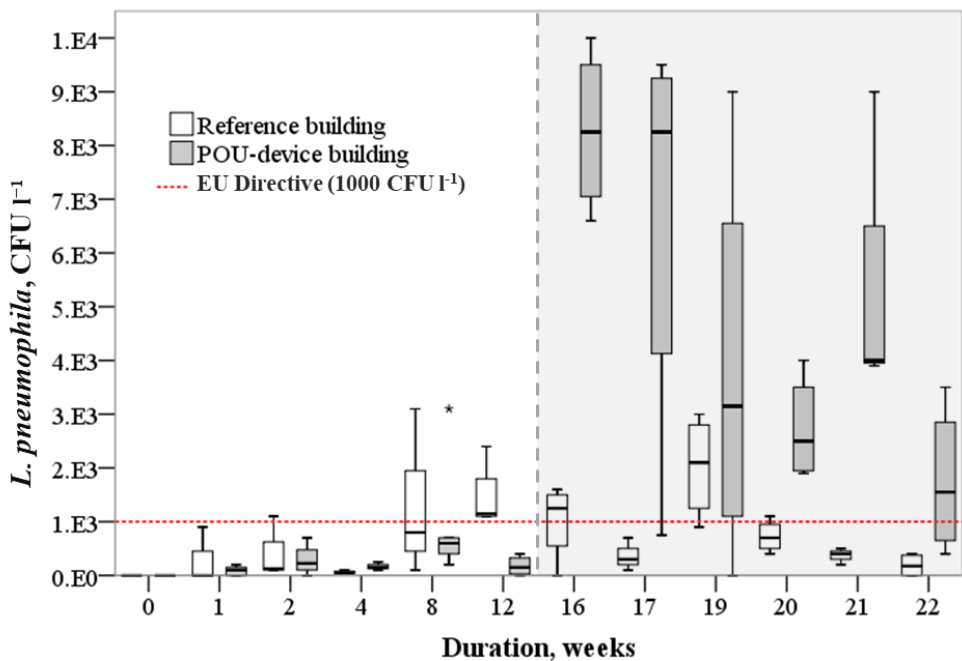
Fig. 7.1. Statistical data for *Legionella* spp. counts in DHW showerhead and circulation return samples during static (weeks 0–14) and dynamic (weeks 16–22) temperature setpoints. Star represents far-outlier, which in this case corresponds to the circulation return sample. Figure adapted from (Zemīte *et al.*, 2023).

Table 7.1. Significant Pearson correlations (R) with *Legionella* bacteria concentration in DHW samples.

	POU-device building (n=51)		Reference building (n=45)	
	R	*p*-value	R	*p*-value
Electrical conductivity	**–0.553** **moderate**	**<0.001**	–0.313 weak	**0.037**
TOC	–0.289 weak	**0.040**	–0.387 weak	**0.009**
MAP	–0.309 weak	**0.027**	–0.373 weak	**0.012**
Ca	–	–	**–0.522** **moderate**	**<0.001**
Mg	**–0.535** **moderate**	**<0.001**	**–0.503** **moderate**	**<0.001**
Fe	–	–	0.369 weak	**0.013**

The internal microbial competition. Another explanation for the higher concentrations of *Legionella* in buildings with point-of-use (POU) filters may be attributed to competition between different bacterial species, with or without evolutionary changes. If bacterial evolution is involved, the r/K selection theory could serve as an explanation. This concept, originating from macroecology, suggests that a dynamic equilibrium should be considered, where nutrient availability is evaluated relative to the abundance and composition of the native bacterial community. (Favere *et al.*, 2021).

Drinking water distribution systems are highly complex environments, populated by a wide range of bacteria both in planktonic form and within pipe-associated biofilms (Proctor and Hammes, 2015). They form balanced ecosystems, with different microorganisms occupying specific ecological niches. However, disturbances can disrupt this balance, enabling the proliferation of undesirable bacteria. The r/K-strategy concept classifies microorganisms as either r-strategists, which thrive in high-nutrient environments with rapid growth rates, or K-strategists, which dominate in low-nutrient environments by having a high affinity for limited resources. When nutrients, such as phosphorus, levels are low and conditions become favourable for opportunistic bacteria (e.g., optimal temperature (Sharaby *et al.*, 2017)), r-strategists can become dominant and outcompete the more beneficial K-strategists.

Phosphorus availability is a key factor in microbial competition within biofilms. Torvinen *et al.*, 2007 demonstrated that a 10 µg l^{-1} increase in phosphorus could boost the number of heterotrophic bacteria in biofilms, while simultaneously reducing the colonisation potential of pathogens like *Mycobacterium avium*. This was attributed to enhanced bacterial competition, as phosphorus availability itself was not critical for *M. avium* survival.

In this study, the additional phosphorus removal most likely reduced the competitiveness of beneficial bacteria, providing a niche for *Legionella* to thrive.

Lowered temperature induced a shift from a thermophilic to a mesophilic environment. Initially, the temperature at the entry of the DHW heat exchanger averaged 46.3 °C in the Reference building and 47.6 °C in the POU-device building, while the heat exchanger setpoint was 57 °C. After lowering the setpoint, the temperatures decreased to an average of 42.1 °C and 44.1 °C, respectively, with the lowest heating setting at 48 °C. Ji *et al.*, 2017 observed a significant shift in the bulk microbiota at a temperature threshold of around 51 °C, indicating a transition from a mesophilic to a thermophilic environment. By reducing the DHW temperature, conditions became more favourable for *Legionella*, allowing it to outcompete beneficial bacteria, as demonstrated in other water pathogen studies performed under laboratory conditions (Vital *et al.*, 2010). This shift resulted in elevated *Legionella* levels in shower hose outflows. Although the temperature of DHW circulation return grab samples was on average 2 °C higher in the POU-device building, it became more susceptible to favouring unwanted bacterial growth once the previous microbial balance was disturbed.

Periodic heat treatment facilitated nutrient release. Periodic heat treatments applied to the DHW system, such as night-time increases from 48 °C to 52 °C and elevated setpoint during weekends (57 °C setpoint), likely contributed to *Legionella* proliferation. *Legionella pneumophila* is known to exhibit necrotrophic growth by consuming heat-sterilised bacteria (Temmerman *et al.*, 2006). Under P-limited conditions, irregular heat treatments may have released nutrients from the biota, inducing phosphorus cycling.

Despite no initial differences in bacterial counts between the two buildings during constant temperature setpoint conditions (Table 6.7 and Table 6.8), the P-limited building had 10^5 (110 %) more intact cells ($p = 0.002$) in DHW circulation return loop samples, with a 12 % higher shift towards HNA-ICC microbial cells ($p = 0.026$), indicating active bacterial growth. However, no significant differences were observed in showerhead samples, even after the change in temperature regime ($p = 0.081$). Both systems showed a notable increase in damaged cells concentration during the altered temperature regime ($p = 0.025$ for showerheads and $p = 0.006$ for circulation return samples), with an average increase of 39 % (3.3×10^4 cells ml^{-1}) for showerheads and 77 % (5.7×10^4 cells ml^{-1}) for circulation return samples.

7.3.   Characteristics of *Legionella* species

The samples containing *Legionella* bacteria were subjected to further characterisation. The only detected species during the testing time were attributed to *Legionella pneumophila*, with serogroups (SG) 1, 2 and 3.

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. 7.2). It was followed by *L. pneumophila* SG 3, with a detection frequency of 24 % and 22 % of samples in POU-device

and Reference buildings, respectively. *L. pneumophila* SG 1 was detected in 2 % of DHW samples in the POU-device building and 18 % of Reference building DHW samples.

Percentagewise, there was a similar distribution of SG 2 detection frequency (around 50 % of samples) in both showerhead and circulation return samples of POU-device building (Fig. 7.3), while circulation return showed a greater frequency of SG 3, compared to showerheads. Among the showerhead samples, however, was the only *L. pneumophila* SG 1 sample. The Reference building, however, showed a very similar distribution of detected serogroups in both showerhead and circulation return samples (Fig. 7.4).

Temporally, the *L. pneumophila* SG 2 occurred in the POU-device building next week after centralised chemical disinfection (Table 7.2) and was detected every further sampling week, resulting in the presence in overall 66 % of *Legionella*-positive samples, while SG 3 was detected after almost a month, prevailing in 31 % of positive samples. There was only one occurrence of SG 1 presence in the POU-device building DHW sample during week 12 (3 months after the filtration start-up).

Meanwhile, the prevalence of specific serogroups in the Reference building was more scattered. SG 3 was detected next week after testing start-up, while SG 2 was detected one more week later. SG 1 was detected after 1 month, and it was in total found in samples collected at four different sampling times, accounting for 22 % of *Legionella*-positive samples in the Reference building.
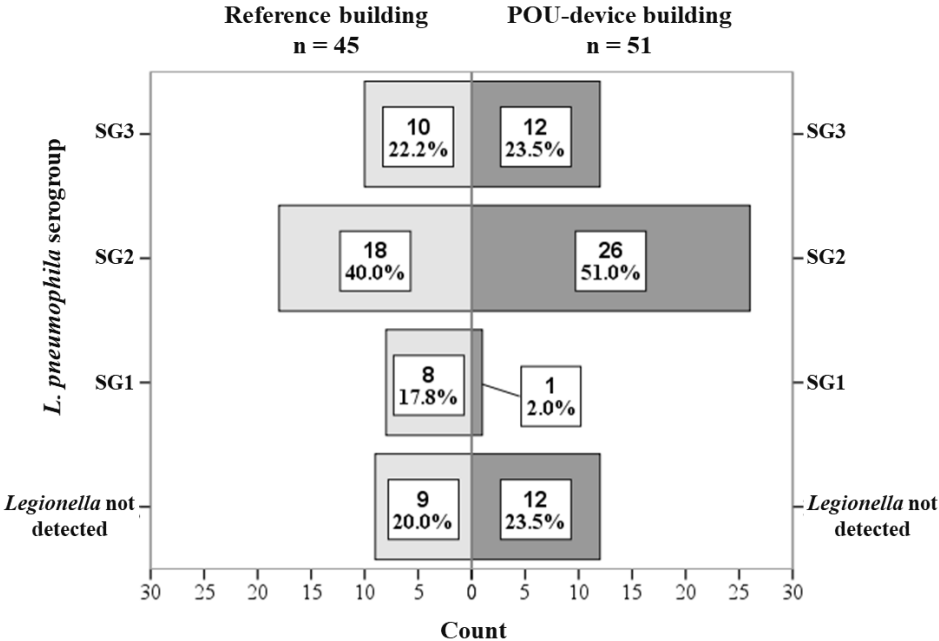
Fig. 7.2. The summary frequency of detected *Legionella pneumophila* serogroups in samples collected from both DHW sub-systems.
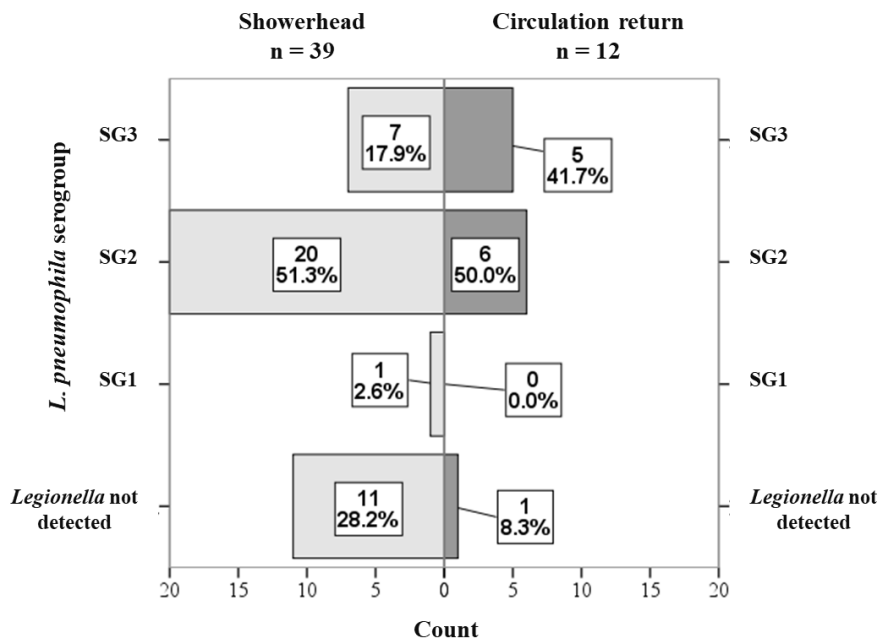
Fig. 7.3. The frequency of detected *Legionella pneumophila* serogroups in samples collected from the DHW system of POU-device building.
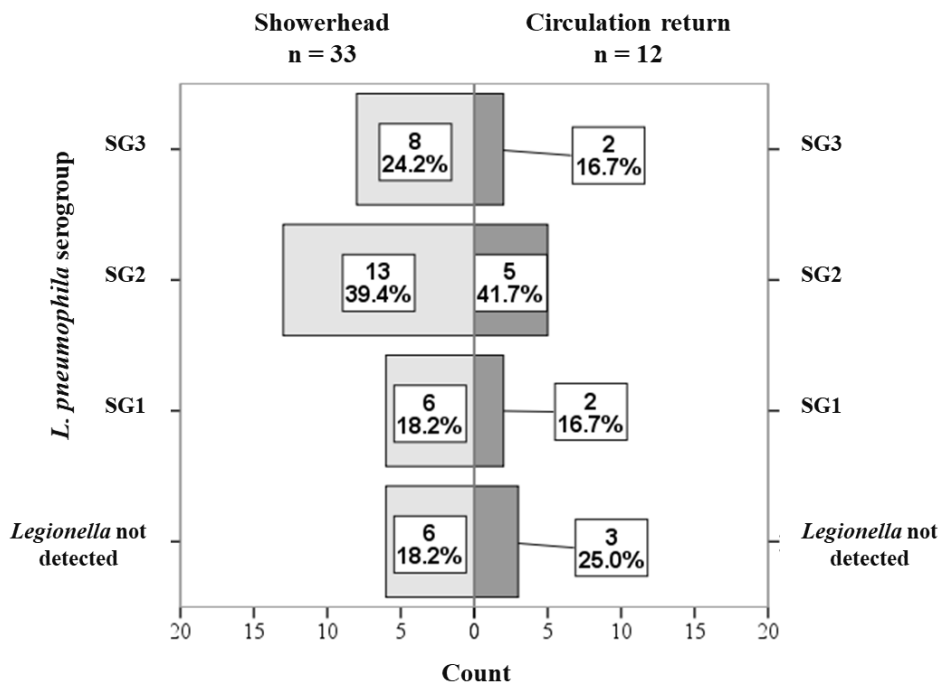
Fig. 7.4. The frequency of detected *Legionella pneumophila* serogroups in samples collected from the DHW system of Reference building.

Table 7.2. The temporal variation of detected *L. pneumophila* serogroups in DHW samples.

Duration, weeks	POU-device building				Reference building			
	Total/ *Legionella*-positive	Serogroup			Total/ *Legionella*-positive	Serogroup		
		SG 1	SG 2	SG 3		SG 1	SG 2	SG 3
0	6/0	-	-	-	3/0	-	-	-
1	5/3	-	3	-	4/1	-	-	1
2	4/2	-	2	-	4/4	-	3	1
4	4/4	-	3	1	4/4	2	-	2
8	5/5	-	3	2	4/4	2	-	2
12	4/3	1	1	1	4/4	-	4	-
16	4/4	-	4	-	4/3	-	-	3
17	4/4	-	3	1	3/3	1	2	-
19	4/3	-	1	2	4/4	-	3	1
20	4/4	-	1	3	4/4	3	1	-
21	3/3	-	2	1	3/3	-	3	-
22	4/4	-	3	1	4/2	-	2	-
Total	51/ 39	1 (3 %)	26 (66 %)	12 (31 %)	45/ 36	8 (22 %)	18 (50 %)	10 (28 %)

Note: Percentages are attributed as values from positively tested samples. Table adapted from (Zemīte *et al.*, 2023).

Mainly, the only noticeable difference between both buildings was the prevalence of *L. pneumophila* serogroup 1, which was more often detected in the Reference building, where no MAP removal was specifically employed. Even during weeks 16–22, when total *Legionella* counts increased greatly in the POU-device building, there was only non-SG 1 *L. pneumophila* detected during this time. In contrast to the overall 3 % of total *Legionella* positive samples attributed to SG 1 in the MAP-limited building, nearly one-fifth (22 %) of total positive samples were attributed to SG 1 in the Reference building.

L. pneumophila SG 1 is frequently associated with health threats – this serogroup is linked to more than 90% of community-acquired Legionnaires' disease cases (Beauté *et al.*, 2020). Therefore, further studies of growth-promoting nutrient limitation for specific sub-species control are necessary.

7.4.   Legislative compliance

Clean and safe drinking water is a priority in the drinking water supply. Water should be safe for use without posing any health risks – such that is free from contaminants and pathogens.

Although treated accordingly and monitored during municipal distribution, it can worsen in quality within its last steps before consumption, i. e., within internal plumbing.

During the case study, the pilot site buildings encountered *L. pneumophila* numbers larger than the guideline value of 1000 CFU l^{-1} (Fig. 7.1) in a total of 10 samples collected from Reference building (RB) DHW showerheads (SH), 14 samples from POU-device building (POUB) SH, 3 samples from RB DHW circulation return (CR), and 6 samples from POUB DHW CR.

Other parameters with exceeding values included Mn, Cu, Fe, and Pb (Table 7.3), one lower value of pH, and some seasonal change in TOC content. However, the majority of cases were attributed to system flushing after the centralised chemical cleaning procedure (samples from week 0) with some occasional other cases. Surprisingly, also inflow from municipal networks showed such exceedances upon entry into the building.

The results show that more extensive monitoring of the drinking water system is needed, especially at the building level, preferably on a regular and mandatory, not only voluntary, basis.

7.5.   Section conclusions

This section focused 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.

Effectiveness 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* spp. However, the regrowth occurred already within a week, 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*.

Impact of POU sorption filter

The POU sorption filter did not significantly reduce *Legionella* concentrations under normal temperature conditions but contributed to a substantial increase during dynamic temperature settings. This was likely due to factors such as nutrient-richer influent water (higher in total organic carbon, magnesium, and intact cells), shifts in microbial competition, induced by nutrient availability, 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.

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.

However, the introduction of the POU sorption filter may have caused a shift in *Legionella pneumophila* species, favouring non-SG1 strains. This potential shift suggests that while the filter may reduce certain *Legionella* strains responsible for the majority of clinical cases, the resulting nutrient limitation and its impact on the occurrence of potentially pathogenic strains need further investigation.

Overall water quality 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 long-term 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.

Table 7.3. Regulatory compliance violation cases during sampling time.

Param.	Limit value [*]	Exceedance
pH	6.9–9.5 pH units	4.68 (POUB–DCW tap, floor 5, disinfected, week 2)
TOC	Without significant change	higher in summer (around weeks 0-4)
Mn	0.05 mg l^{-1}	0.081 (RB–DHW CR, week 0)
		0.099 (POUB–DHW CR, week 0)
		0.115 (POUB–DHW SH, floor 1, disinfected, week 0)
		0.127 (RB–DHW SH, floor 5, disinfected, week 0)
		0.154 (POUB–DHW SH, floor 2, disinfected, week 0)
		0.178 (POUB–DCW tap, floor 5, **not** disinfected, week 0)
		0.178 (RB–DHW SH, floor 1, disinfected, week 0)
		0.180 (POUB–DHW SH, floor 5, **not** disinfected, week 0)
		0.249 (POUB–DHW SH, floor 5, **not** disinfected, week 0)
		0.307 (RB-**inlet, untreated**, week 0)
		0.397 (POUB–DHW SH, floor 5, disinfected, week 0)
		0.102 (RB-**inlet, untreated**, week 16)
Cu	2000 µg l^{-1}	2421 (POUB–DHW SH, floor 5, disinfected, week 0)
Fe	0.2 mg l^{-1}	0.250 (POUB–DHW SH, floor 5, **not** disinfected, week 0)
		0.473 (POUB–DHW SH, floor 5, **not** disinfected, week 0)
		0.526 (RB-**inlet, untreated**, week 0)
		0.553 (POUB–DHW CR, week 0)
		1240 (POUB–DHW SH, floor 5, disinfected, week 0)
		0.202 (POUB–DHW CR, week 22)
		0.224 (RB-**inlet, untreated**, week 4)
		0.236 (POUB–DHW CR, week 20)
		0.372 (POUB–DHW CR, week 16)
		0.652 (RB-**inlet, untreated**, week 16)
Pb	10 µg l^{-1}	10.8 (POUB–DHW CR, week 0)
		17.4 (RB-**inlet, untreated**, week 0)
		23.8 (POUB–DHW SH, floor 5, **not** disinfected, week 0)
		133.0 (POUB–DHW SH, floor 5, disinfected, week 0)
		11.0 (POUB–DHW SH, floor 5, disinfected, week 8)
		11.3 (POUB–DHW SH, floor 5, disinfected, week 1)
		11.3 (POUB–DHW SH, floor 5, disinfected, week 2)
		14.2 (POUB-**inlet, after filter**, week 12)
		32.9 (POUB-**inlet, after filter**, week 16)

[*] (Ministru Kabinets, 2023)

POUB – POU-device building, RB – Reference building, DCW – domestic cold water, DHW – domestic hot water, SH – showerheads, CR – circulation return, disinfected or not disinfected – attributed to the success of specialist`s visit during centralised chemical flushing and disinfection, ensuring there was a proper disinfection of specific water outlet point.

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 temperature decreases 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.

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LIST OF FIGURES

LIST OF TABLES

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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Marta Zemīte (née Frolova) was born in 1990 in Rēzekne. She received a Bachelor's degree and an engineer's qualification (2013) and a Master's degree (2014) in Heat, Gas and Water Engineering Systems from Riga Technical University. Since 2013, she has worked at MEP Solutions engineering systems design company. Since 2016, she has been a research assistant and, since 2019, a researcher at Riga Technical University. Her research interests include the biological stability of drinking water, technologies to reduce membrane fouling in water treatment, and water quality of urban runoff.