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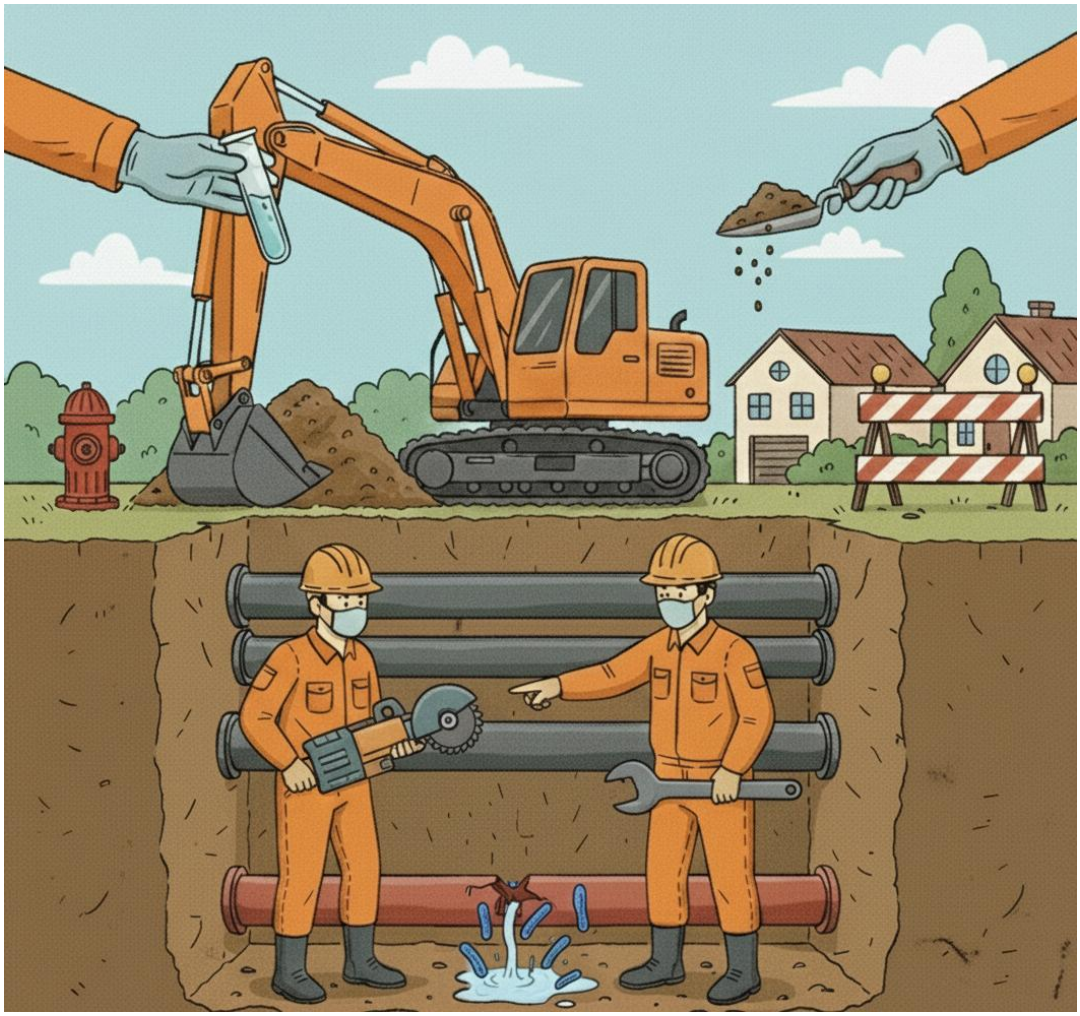

# Investigating The Role of Upstream Distribution Network Disruptions in Legionella Colonization of Downstream Water System

Master's Thesis in Infrastructure and Environmental Engineering

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CHALMERS UNIVERSITY OF TECHNOLOGY
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MASTER'S THESIS ACEX30

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Cover:
Illustration of excavation and maintenance work in a residential area, highlighting underground pipeline leakage maintenance and supporting infrastructure. The figure was generated using Gemini AI with the keywords: maintenance work, legionella bacteria, excavation, leakage, fire hydrant, water and soil samples.

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## ABSTRACT

This study investigates the relationship between upstream water distribution network disruptions, such as leakage and repair work, and the subsequent colonization of *Legionella pneumophila (L. Pneumophila)* in downstream water systems. Field samples were collected at various maintenance sites, including upstream, downstream, and pit locations, as well as from random high-risk environments such as public laundry rooms and gyms. The presence of *L. pneumophila* was assessed using the Legiolert test, alongside analyses of physicochemical water quality parameters including temperature, pH, conductivity, turbidity, Total Organic Carbon (TOC), and free chlorine. Soil samples from leakage areas were examined to assess the potential entry of bacteria into the system through surrounding soil. The study compares soil and water sample results to evaluate possible contaminant pathways and examines environmental conditions that support the survival and proliferation of *L. pneumophila*. Based on these findings, recommendations are proposed for targeted risk assessment, regular maintenance, and effective disinfection strategies to mitigate potential risks in drinking water distribution systems.

**Keywords:** *Legionella pneumophila, upstream network disruption, Legiolert test, water quality parameters, leakage and repair, disinfection methods, risk management, temperature control*

# Contents

# Preface

I would like to express my sincere gratitude to all those who have supported me in successfully completing this thesis work.

I am deeply thankful to my supervisor, **Jesper Knutsson**, for his continuous guidance, constructive feedback, and invaluable insights throughout the research process. He not only provided me with the fundamental and in-depth knowledge related to my thesis topic but also introduced me to various methods for defining the presence of bacteria, significantly enriching the scientific rigor of my work. His support was instrumental in structuring my research and maintaining its focus.

I am sincerely grateful to **Kretslopp och Vatten (KoV)** for providing the necessary information, resources, and support for sampling. My heartfelt thanks go to **Magnus Andersson** for granting me access to various sampling locations and connecting me with supervisors responsible for each repair project. I would like to extend special thanks to **Birthe Riisnes** for her profound knowledge, guidance, and assistance with industrial information and procedures at KoV, as well as for helping me refine the scope of my thesis. I am also thankful to **Amir Saeid Mohammadi** for his guidance in laboratory work and for ensuring safe and efficient use of laboratory facilities.

I am greatly appreciative of the **Department of Infrastructure and Environmental Engineering** for providing the laboratory facilities and essential resources that made this study possible.

Finally, I would like to thank my fellow students and my family for their unwavering support, encouragement, and insightful discussions, which helped me approach challenges with fresh perspectives. Their love, patience, and constant motivation gave me the strength to overcome obstacles—particularly during the sampling process—and made this research both rewarding and memorable

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POOJA RAGAVI SURESH

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# Abbreviations

AIDS - Acquired Immunodeficiency Syndrome

BCYE - Buffered Charcoal Yeast Extract

CDC - Centers for Disease Control and Prevention

CFU - Colony-Forming Units

DFA - Direct Fluorescent Antibody

DNA - Deoxyribonucleic Acid

EPS - Extracellular Polymeric Substances

FLA - Free-Living Amoeba

FSS - Filtered Soil Samples

GVPC - Glycine–Vancomycin–Polymyxin B–Cycloheximide

HIV - Human Immunodeficiency Virus

KoV - Kretslopp och Vatten (Gothenburg Municipality)

LERD - Light Emitting Radiation Disinfection

MPN - Most Probable Number

$Na_2S_2O_3$ - Sodium Thiosulfate

NIH - National Institutes of Health

PAS - Page's Amoebal Saline

PCR - Polymerase Chain Reaction

PEX - Cross linked Polyethylene

PMA - Propidium Monoazide

POU - Point-of-use

PTSD - Post-Traumatic Stress Disorder

PVC - Polyvinyl Chloride

PVC-C - Chlorinated Polyvinyl Chloride

PVC-U - Unplasticized Polyvinyl Chloride

RNA - Ribonucleic Acid

SGU - Geological Survey of Sweden (Sveriges Geologiska Undersökning)

SS - Unfiltered Soil Samples

TOC - Total Organic Carbon

UAT - Urinary Antigen Test

UV - Ultraviolet

VBNC - Viable But Non-Culturable

WET lab - Water Environment Technology lab (implied in Appendix A)

WHO - World Health Organization

# 1 Introduction

## 1.1 Background

Water-borne diseases are one of the major public health concerns around the world. The problem arises from contact with pathogenic microorganisms such as bacteria, viruses, parasites, and protozoa in water through direct consumption, bathing, or inhalation. These microorganisms cause symptoms such as gastrointestinal illness, acute respiratory illness, diarrhea, pneumonia, lung and urinary tract infections, and so on. The illness varies depending on the pathogens involved. Factors such as inadequate sanitation and hygiene, lack of clean drinking water, and climatic factors like changes in temperature are the major reasons that increase the vulnerability to water-borne diseases (Flor Yazmín Ramírez-Castillo, 2015).

*Legionella* species are rod-shaped gram-negative bacteria naturally found in freshwater ecosystems such as rivers, lakes, and groundwater, as well as in man-made water systems, causing serious threats to public health (Pascale, 2022). More than 60 *Legionella* species have been identified, including *L. pneumophila*, *L. longbeachae*, *L. feeleii*, *L. anisa*, *L. bozemanii*, *L. dumoffii*, etc (control)., with 15 different serogroups. Among these, serogroups 1, 4, and 6 are considered to be more harmful to human health. *Legionella pneumophila)* is regarded as the most clinically significant species, responsible for Legionnaires' disease and Pontiac fever, with over 80% of the reported serogroup 1 cases caused by *L. pneumophila* (Mahgoub, 2024). Their persistence and growth are favored by warm temperatures between 20 and 45 °C. Biofilm formation and water stagnation create the most favorable conditions (National Academies of Sciences, 2020).

In the United States, 24 outbreaks related to *Legionella* species were reported between 2001 and 2006, including 126 drinking water-related cases and 12 mortalities (Gunther F. Craun, 2010).

*L. pneumophila* is responsible for the cause of Legionnaires' disease. Affected individuals may experience mild to severe symptoms, in some cases, it leads to death (Monistero V, 2024). It was first identified in 1976, in Philadelphia, during an outbreak associated with American Legion Conference (National Academies of Sciences, 2020).

These bacteria are naturally found in freshwater ecosystems. They reside and multiply within free-living protozoa (Li Xu, 2013). *L. pneumophila* has the ability to survive both with and without host cells (National Academies of Sciences, 2020).

*Legionella* can survive in drinking water systems and the plumbing systems of buildings (Gary A. Burlingame, 2024). However, the concentration of the bacteria in drinking water systems is typically low, due to regular monitoring and control measures implemented on time to prevent outbreaks (LeChevallier, 2019). The most common exposure pathways identified are through man-made systems such as hot tubs, showerheads, cooling towers, air conditioning systems, and other building water

distribution systems, where contaminated aerosols are generated (National Academies of Sciences, 2020). These pathways are considered a major health concern.

Human beings are affected by inhaling these contaminated aerosols, which can subsequently lead to outbreaks. The infection does not spread from one person to another according to the studies (Li Xu, 2013). The bacterium responsible for the outbreak was named *Legionella pneumophila* after the American Legion conference, where the first recognized outbreak occurred. Infected individuals experienced complications primarily affecting the respiratory system and were diagnosed with a type of pneumonia, later termed Legionnaires' disease, named after the affected group (National Academies of Sciences, 2020).

## 1.2 Aim

The primary aim of this thesis is to investigate whether pipe leakages or repair work in the upstream distribution network facilitate the possible entry of *L. pneumophila* bacteria into the drinking water distribution system, and to examine how water chemistry is related to their growth under favorable conditions.

## 1.3 Research Question

The aim of this thesis can be addressed by focusing on the following research questions:

1. To investigate the prevalence of *L. pneumophila* in soil and water samples associated with disruptions in the water distribution system.
2. To analyze the impact of water flow disruptions and temperature variations caused by network issues on the proliferation of *Legionella*.
3. To examine the role of biofilms and changes in water chemistry in promoting *Legionella* growth following network disruptions.
4. To develop recommendations for water system management practices that can mitigate the risk of *Legionella* colonization associated with distribution network disruptions.

# 2 Literature Review

## 2.1 History and Discovery of Legionella

In 1976, almost 2,000 members of the American Legion attended a conference at the hotel named Bellevue-Stratford, located in Philadelphia, Pennsylvania, USA. Following this event, an outbreak of pneumonia-like fever was reported, caused by an unknown pathogen. From the group of attendees, 182 were diagnosed with the illness, and 29 fatalities were recorded as a result of the outbreak. This unknown disease raised national concern, prompting an urgent investigation to identify the pathogen responsible (National Academies of Sciences, 2020).

During the investigation, the transmission pathway was hypothesized to be airborne, as the rate of infection was correlated with the time attendees spent in the hotel lobby. It was also observed that hotel employees had been affected by this bacterium over an extended period of time, suggesting its presence in the hotel's environment (National Academies of Sciences, 2020).

Following this incident, scientists discovered new diagnostic methods, such as the urinary antigen test, to detect diseases in affected individuals. Epidemiological studies and laboratory experiments on animals were conducted to identify effective antibiotics for Legionellosis, leading to the use of macrolides and fluoroquinolones as treatment options (National Academies of Sciences, 2020).

These epidemiological studies also revealed that *Legionella* is a waterborne pathogen rather than an airborne one. This finding clarified that the outbreak of Legionnaires' disease in Pennsylvania was caused by water droplets contaminated with *L. pneumophila*, which were released from the hotel's cooling tower. These droplets then dispersed through the air, leading to the spread of the disease. It was later recognized that many *Legionella*-related outbreaks were linked to water exposure rather than direct airborne transmission (National Academies of Sciences, 2020).

The bacterium responsible for the outbreak was named *Legionella pneumophila* after the American Legion conference, where the first recognized outbreak occurred. Although this disease was formally identified following the 1976 incident, similar symptoms had been observed in earlier cases but had not been correctly diagnosed or documented at the time (National Academies of Sciences, 2020).

In 1957, an outbreak of respiratory disease occurred in Minnesota, suggesting the presence of *Legionella* in the United States long before its official identification in 1978. Several patients at the Pontiac County Health Department facility experienced a mild illness characterized by a slight fever, headache, and myalgia (muscle pain). This illness was later named Pontiac fever, after the location of the outbreak (National Academies of Sciences, 2020).

As a result of further studies, *Legionella* species have been found to evolve within microbial communities, giving rise to various species. According to the National Institutes of Health (NIH), more than 62 *Legionella* species had been identified by

2021. As per the ”*European Legionnaires' Disease Surveillance Network Annual Meeting 2019, unpublished data”*, in the EU/EEA, over 94% of the culture-confirmed hospitalized Legionnaires' disease cases were caused by *L. Pneumophila* in the year 2018 (Walker JT, 2021). *Legionella* bacteria has been divided into 16 serogroups (Folkhälsomyndigheten, 2015). Among the various *Legionella* groups, Serogroup 1 is considered the most virulent. The majority of reported *L. pneumophila* cases belong to this serogroup. Other *Legionella* species are also responsible but only in comparatively fewer cases; however, prevalence may vary depending on geographic location. Elderly people, especially men, smokers, and patients with underlying health conditions such as immunosuppression, are considered the most vulnerable group, and untreated cases might lead to death (National Academies of Sciences, 2020). This is further explained under the section ”Vulnerable Populations and Risk Factors”

## 2.2 Legionnaires' Disease and Pontiac Fever

Legionnaires' disease is caused by aerobic, Gram-negative rods of the *Legionella* species. Humans typically become infected through the inhalation of *Legionella*, allowing the bacteria to enter the respiratory system, where they replicate within pulmonary macrophages and monocytes. The incubation period varies depending on the patient's immune system but generally ranges between 2 to 12 days, or in some cases, even longer. Legionellosis is the collective term used to describe infections caused by *Legionella* bacteria (National Academies of Sciences, 2020).

Legionnaires' disease and Pontiac fever share similarities in symptoms with other respiratory infections. Patients with Legionnaires' disease commonly experience cough, fever, shortness of breath, and myalgia. However, in addition to these symptoms, they may also suffer from gastrointestinal issues, neurological abnormalities, and mental disturbances (National Academies of Sciences, 2020).

Similarly, Pontiac fever presents mild symptoms such as low-grade fever, headache, chills, and myalgia. However, unlike Legionnaires' disease, Pontiac fever does not cause pneumonia and is typically resolved on its own without requiring medical treatment (National Academies of Sciences, 2020).

The diagnosis of *Legionella* infections can be challenging and time-consuming, increasing the risk of missed or delayed detection. This, in turn, can lead to severe complications, particularly for patients suffering from *Legionella* pneumonia (National Academies of Sciences, 2020).

*Legionella* infections are most common among elderly individuals, those with weakened immune systems, and men rather than women. Additionally, people who smoke heavily are at higher risk of contracting the disease. *Legionella* species are found worldwide, but epidemiological data mainly focuses on major metropolitan cities in developed regions due to better data availability (National Academies of Sciences, 2020).

Legionnaires' disease is primarily associated with exposure to man-made water systems. However, most reported cases within a community are linked to travel or hospital exposure. Despite these known risk factors, the primary source of exposure often remains a mystery (National Academies of Sciences, 2020).

In most cases, when a person develops pneumonia, doctors initially prescribe empirical treatment based on their clinical experience without identifying the exact cause of the illness. As a result, many cases of Legionnaires' disease go undiagnosed. If an individual infected with *L. pneumophila* Serogroup 1 undergoes a urinary antigen test, results can be obtained within a day. However, a culture test can take up to a week or longer. This delay makes it difficult for doctors to diagnose the infection promptly and start appropriate treatment, increasing the risk of complications (National Academies of Sciences, 2020).

### 2.2.1 Legionellosis Symptoms

The severity of the disease ranges from mild to life-threatening conditions. The incubation period is approximately between 2 and 14 days. Early symptoms include headache, muscle aches, fatigue, and loss of appetite. Core symptoms include fever, usually greater than 38 °C. Cough may cause chest pain as part of respiratory complications, along with chills and shortness of breath (National Academies of Sciences, 2020). Gastrointestinal symptoms such as diarrhea, nausea, and abdominal pain may also occur, along with neurological issues like seizures. Recovery is often slow, and patients may experience lingering symptoms. Some may even suffer from post-traumatic stress disorder (PTSD) (Burke A Cunha, 2016). Most of the research biased and are primarily focused on l.pneumophila.

Similar symptoms can be found in Pontiac fever, which is a self-limiting, flu-like illness that does not involve pneumonia. The symptoms of Pontiac fever overlap with Legionnaires' disease, making diagnosis difficult. However, Legionellosis requires antibiotics due to respiratory complications, whereas Pontiac fever is mild and typically does not require antibiotic treatment (National Academies of Sciences, 2020).

### 2.2.2 Diagnosis of Legionellosis

Legionellosis can be diagnosed using several methods. Among these, urinary antigen tests (UAT) and culture tests are the most commonly used to detect the presence of *Legionella* in patients (National Academies of Sciences, 2020).

1. **Clinical Prediction**:

Clinically, the infection can be predicted using certain tools like the "*Winthrop-University Hospital Criteria*, *Japan Respiratory Society Score*, a six-parameter clinical score developed by Fiumefreddo and colleagues, and the *Community-Based Pneumonia Incidence Study Group Score"*. However, the sensitivity of these prediction tools is very low (National Academies of Sciences, 2020).

2. **Radiologic Imaging**:

Chest X-rays and CT scans can show pneumonia-related signs such as focal infiltrates, ground-glass opacities, and other patterns. However, these findings are not specific and cannot identify *Legionella* infections in particular (National Academies of Sciences, 2020).

3. **Culture Test**:

*Legionella* culture tests are typically conducted on Buffered Charcoal Yeast Extract (BCYE) agar. Although highly accurate, culture testing is slow, requiring between 3 to 14 days for results. Prior antibiotic treatment may suppress bacterial growth, making diagnosis more challenging (National Academies of Sciences, 2020).

4. **Urinary Antigen Test (UAT)**:

This is the most frequently used test. UAT is more sensitive to *L. pneumophila* serogroup 1 and is rapid, allowing it to be performed on-site at hospital laboratories. However, it fails to detect infections caused by non–serogroup 1 strains or by other *Legionella* species. In early stages of infection, the test may yield negative results, although antigens may remain detectable for months after infection (National Academies of Sciences, 2020).

5. **Polymerase Chain Reaction (PCR)**:

PCR is highly sensitive for detecting *Legionella* DNA in respiratory tract specimens. It is more sensitive than both culture and UAT. Currently, most PCR tests focus on serogroup 1, but newer methods have been developed that can identify non-*pneumophila* species. However, not every method is approved for clinical use, and it is difficult to distinguish between live and dead bacteria, as this method has the ability to amplify DNA from both (National Academies of Sciences, 2020). However, in advanced techniques, PCR tests can be combined with DNA-intercalating dyes such as propidium monoazide (PMA) to detect live cells among the combined dead and live bacteria (Yanzhe Zhu a, 2018).

6. **Serology**:

This method detects the presence of antibodies in a patient's blood. Its accuracy is relatively low. In the early stages of infection, detection is poor; results may improve during the later phase. However, it is difficult to distinguish between past and present infections in cases of reinfection due to lingering antibodies. Serology is mostly used in outbreak investigations rather than in acute diagnostics (National Academies of Sciences, 2020).

7. **Direct Fluorescent Antibody (DFA)**:

DFA is rarely used due to its very low sensitivity. It only detects *L. pneumophila* and fails to identify other species (National Academies of Sciences, 2020).

## 2.3 Ideal Built Environment Conditions

*Legionella* bacteria primarily exist in freshwater environments such as lakes, streams, and soil, including sediments. While several genera of these bacteria can be found in natural environments and have the potential to cause disease, they are generally not considered a significant threat under natural conditions (National Academies of Sciences, 2020).

*Legionella* bacteria naturally thrive in water sources like lakes, rivers, and groundwater, typically at concentrations less than 1,000 Colony forming units per liter (CFU/L), which comprises approximately only 1% of the total bacterial community. They can be detected either by the DFA method or by animal infection models. *L. pneumophila* is rare and not common below 25 °C. However, the concentration of *L. pneumophila* is high in hot springs and thermal vents where the temperature is greater than 27 °C, with concentrations up to 1,000,000 CFU/L, especially in summer. Thermal springs in spas are considered to be potential sources of Legionnaires' disease (National Academies of Sciences, 2020).

Twelve different types of *Legionella* species have been discovered from soil samples. *L.longbeachae* is common in potting and garden soils, which can also be found in water. It helps to degrade plant materials due to the presence of some specific enzymes (National Academies of Sciences, 2020). *L. pneumophila* detected in different soils is sometimes directly linked to human infections. Sometimes it even leads to Legionnaires' disease outbreaks (Lara Wallis, 2005). Protozoan hosts are common in soil, so *Legionella* can replicate even in soil; however, there is no direct proof, and more research is required to fully understand the ecology of the bacteria in soils (National Academies of Sciences, 2020).

However, the risk increases significantly in man-made water systems, such as cooling towers and drinking water distribution networks, where bacterial growth can become uncontrolled. These systems often provide favorable conditions for *Legionella* proliferation, particularly stagnant water and optimal temperature ranges maintained over extended periods (National Academies of Sciences, 2020).

Importantly, *Legionella* does not infect humans through drinking contaminated water. Instead, infection occurs via inhalation of aerosolized water droplets containing the bacteria. Additionally, person-to-person transmission has not been observed (National Academies of Sciences, 2020).

*L. Pneumophila* does not grow effectively as a free-floating (planktonic) bacterium. Rather, it thrives within amoebae and protozoa, which serve as host cells. The bacteria require external nutrients such as amino acids and minerals to replicate. Since they depend on host cells for survival, *Legionella* has adapted to a parasitic lifestyle (Fields, 2002)

For optimal growth, *Legionella* requires low oxygen conditions, ideally below 1 mg/L, and thrives in temperatures between 25°C and 43°C. These environmental parameters

are often found in artificial water systems such as plumbing networks, cooling towers, and water storage tanks (National Academies of Sciences, 2020)

Throughout its life cycle, *Legionella* alternates between three physiological states (Ashbolt N. J., 2015):

1. **Replicative form** – active multiplication occurs within the host cell.
2. **Infectious form** – the bacteria are released and become capable of infecting humans.
3. **Dormant form** – the bacteria enter a survival mode, allowing them to persist in harsh environments.

Although *Legionella* can survive without a host, replication is significantly reduced or halted in the absence of one. Inside amoebae, however, *Legionella* can multiply extensively, increasing the likelihood of human exposure when contaminated water becomes aerosolized, such as through showers, cooling towers, or humidifiers. This airborne route of transmission presents serious public health concern, particularly due to the potential for Legionnaires' disease (Ashbolt N. J., 2015).

Common disinfection practices, such as the use of chlorine, can kill the host cells (amoebae or protozoa), but often fail to eliminate *Legionella* hiding within them. Once the host cell is lysed or dies, the bacteria are released into the water system, where they remain viable. This makes complete eradication challenging and poses a continuous risk, especially when contaminated water is aerosolized and inhaled (Sílvia Cervero-Aragó, 2015).

*L. pneumophila* attaches to protozoa that reside within biofilms. These biofilms are slimy, structured communities formed on moist surfaces in the presence of water, including sediments, damp soil, and interior surfaces of water distribution systems. Biofilms primarily consist of a mixture of microorganisms such as bacteria, fungi, amoebae, nematodes, ciliates, and larvae (National Academies of Sciences, 2020).

In general, moist environments are highly favorable for *Legionella* survival and replication. Biofilms provide ideal conditions by offering a protective microhabitat where the bacteria can live within protozoa, shielded from environmental stresses and disinfectants. When water contains sufficient nutrients and remains within the optimal temperature range, biofilm formation is promoted, ultimately enhancing the ability of *Legionella* to multiply and persist in the system (National Academies of Sciences, 2020).

## 2.4 Exposure Pathways

Human exposure to *Legionella* primarily occurs through the inhalation of contaminated airborne droplets. Aerosols are tiny droplets, typically less than 100 µm in size, that remain suspended in the air. These droplets are generated when a fluid is disturbed, such as during sneezing or, in this case, the spraying of water, a process known as aerosolization (National Academies of Sciences, 2020).

Aerosolization differs from volatilization, as volatilization refers to the transformation of a substance from a solid or liquid state into a pure gaseous form, without forming droplets that contain bacteria or other particles (National Academies of Sciences, 2020).

However, not all aerosol particles can reach the human lungs. Only particles smaller than 10 μm can penetrate deeply into the lung tissue. These tiny droplets can contain microorganisms or other suspended particles from water or liquids. Due to their small size, these substances have a relatively large surface area, allowing them to capture specific materials, particularly greasy or hydrophobic (water-repellent) substances. The risk of Legionnaires' disease is highest in environments where both biofilm formation and aerosolization occur (National Academies of Sciences, 2020).

Investigations into Legionnaires' disease were typically conducted only when two or more people were affected simultaneously by the same source. However, only 4% (National Academies of Sciences, 2020) of outbreaks were thoroughly investigated, as those were the only sources that had been identified. Meanwhile, many unreported cases remain due to unidentified sources that were never properly investigated. This lack of thorough investigation makes it more difficult to trace the infection to specific sections of the water system (National Academies of Sciences, 2020).

Legionnaires' disease can be diagnosed using urinary antigen and sputum tests. However, these are not routinely included in standard diagnostic testing for pneumonia patients in hospitals. Additionally, the urinary antigen test detects only one of all *Legionella* serogroups, making it incomplete (National Academies of Sciences, 2020). This limitation leads to many undiagnosed cases and an underestimation of the disease's severity.

In water systems, traditional culture-based methods are commonly used to detect the presence of *Legionella* bacteria. However, this method is typically slow and may be biased, favoring the growth of other bacteria over *Legionella*. PCR techniques offer a faster approach, but they struggle to distinguish between live and dead bacteria, which raises concerns about result accuracy (National Academies of Sciences, 2020).

More reliable and precise methods are needed for effective monitoring of water systems. While a high concentration of *Legionella* in water systems requires corrective action, there is currently no known safe lower limit for the presence of the bacteria, as their potential to pose a health risk remains uncertain. That makes monitoring and risk assessments tricky in the water systems (National Academies of Sciences, 2020).

## 2.5 Sources of Legionella Contamination

### 2.5.1 Cooling Towers

The wet cooling towers (open and closed cooling towers) are a major cause of most *Legionella* outbreaks (National Academies of Sciences, 2020). The heat generated in circulating water by various heat-generating equipment, such as chillers, heat exchangers, heat pumps, compressors, and other devices, is removed in cooling towers

through evaporation. The evaporated droplets are released into the atmosphere as aerosols (CDC, Controlling Legionella in Cooling Towers, 2025).

If cooling towers are not cleaned or maintained properly, the warm temperature they maintain can promote biofilm formation in the pipes, which, in turn, fosters the growth of *Legionella* bacteria in both industrial and individual buildings (National Academies of Sciences, 2020). These aerosols can drift away from the cooling tower and be inhaled by workers and people nearby, leading to potential exposure to *Legionella*. Indoor exposure is also possible if contaminated air from cooling towers is drawn into buildings through ventilation systems or structural leaks (National Academies of Sciences, 2020).

Closed cooling towers pose a lower risk of *Legionella* exposure compared to open cooling towers, as the warm water circulates within a closed loop, preventing direct contact between evaporated aerosols and the air (CDC, Controlling Legionella in Cooling Towers, 2025).

### 2.5.2 Drinking Water Distribution Systems

Numerous species of *Legionella* have been found in drinking water systems. They can enter the system through various possible pathways (National Academies of Sciences, 2020). For example, *Legionella* may infiltrate the drinking water distribution system via microbial organisms associated with sand filters used during the backwash process in drinking water treatment facilities (Ting Xie, 2024).

Drinking water can also become contaminated with *Legionella* through the distribution network or building plumbing systems. Many aging water infrastructures—operating beyond their intended lifespan—can develop leaks due to pipe breaks, corrosion, and biofilm formation. End points where aerosols are produced, such as showerheads, also contribute to increased risk of *Legionella* colonization (María Concepción Almonacid Garrido, 2024).

*L. pneumophila* can live in biofilms even when no host cell like amoeba or protozoa is present. However, the presence of a host is mandatory for replication (Ricardo Murga, 2001). Free-living amoeba (FLA) plays a vital role among microbial society, especially in piped water environments, and can support the intracellular growth and help in the multiplication of *L. pneumophila* (Ashbolt M. S., 2019).

Protozoa containing *Legionella* can enter through premise plumbing and enable bacterial replication in pipe dead ends and stagnant zones, potentially leading to outbreaks of Legionnaires' disease (National Academies of Sciences, 2020). In certain locations, the temperature falls within the optimal range that promotes the growth of *Legionella*, which includes tap aerators, showerheads, and hot/cold water mixing valves (María Concepción Almonacid Garrido, 2024).

Furthermore, increased population density and the widespread use of modern amenities such as cooling towers, hot tubs, humidifiers, and similar systems raise the risk of daily

exposure to *Legionella*, providing favorable conditions for its growth (National Academies of Sciences, 2020).

Stagnant water in plumbing systems promotes the growth of *Legionella* due to several factors (Fischer-Hoch, 1982), including lowered concentrations of disinfectants and dissolved oxygen, reduced water temperature compared to other parts of the system, higher organic and biomass concentrations, and the presence of protozoa that support bacterial growth (National Academies of Sciences, 2020).

### 2.5.3 Recreational Water Features

There are several built environments that support the growth of *Legionella*. One of them is recreational water features, including hot tubs and spas, swimming pools, water parks, fountains, and splash pads, both indoors and outdoors (National Academies of Sciences, 2020). Biofilms can develop on surfaces in cases of poor maintenance, potentially leading to the release of contaminated aerosols. Several outbreaks of Legionnaires' disease have been linked to recreational water sources (National Academies of Sciences, 2020).

Some environments provide favorable conditions for the growth of *Legionella* and their hosts, such as protozoa within biofilms. As a result, the bacteria colonize these environments. Interior water features include waterfalls and green walls with plants, which are often used as aesthetic elements in interior design. Exterior features include green walls, green roofs, irrigated surfaces, and decorative fountains (National Academies of Sciences, 2020).

In the case of decorative fountains, water stagnation can occur if the system is turned off during winter or due to pump failure. Stagnant water poses a higher risk for the growth of *Legionella* compared to moving water. The use of stagnant water for lawn sprinkling and spray washing can increase the risk of Legionnaires' disease, as aerosols containing the bacteria can be released into the air, especially in windy conditions (Legionella Control).

In some countries, Legionnaires' disease has also been linked to wastewater treatment plants, particularly in facilities that collect industrial wastewater with temperatures above 25°C, which can create a favorable environment for *Legionella* growth (National Academies of Sciences, 2020).

## 2.6 Pipe Material Influence

Pipe materials play a significant role in the growth of *L. pneumophila*. The growth of *L. pneumophila* can be increased due to biofilm formation in the pipes, as biofilms support the protozoan hosts needed for the bacteria. Certain types of pipe materials promote the growth of biofilms, such as rubber (both synthetic and natural) and plastic pipes made of soft PVC, polypropylene, polyethylene, and polybutylene (Dick van der Kooij G. L., 2017). However, some types of pipe materials inhibit the growth of the

bacteria. These include stainless steel and certain types of PVCs, such as PVC-C and PVC-U (National Academies of Sciences, 2020).

Copper pipes have mixed effects on *L. pneumophila*. Copper kills the bacteria due to the release of copper ions, specifically in new pipes. However, over time, corrosion starts to occur, which reduces the availability of copper ions in the pipe and gradually decreases the inhibition rate (Dick van der Kooij H. R., 2005). The biofilm growth on copper pipes is comparatively less than on other plastic pipe materials (Dick van der Kooij G. L., 2017). However, the bacteria can grow on biofilms that contain the protozoan host that supports the lifecycle of the bacteria (Dick van der Kooij H. R., 2005).

Copper can also induce the bacteria to enter the VBNC (Viable But Non-Culturable) state, which means the bacteria can remain alive but cannot be detected using standard culture methods. However, they can still be detected by DNA-based methods (National Academies of Sciences, 2020). The pipe material has some effect on *L. pneumophila* growth, but at the same time, the biofilm growth rate indirectly influences it.

## 2.7 Biofilm and Its Role in Legionella Growth

Biofilm is a combination of microorganisms that are permanently attached and covered by a matrix of extracellular polymeric substances (EPS), which are produced by the microorganisms themselves. Biofilms can develop on different surfaces that are particularly in contact with non-sterile water (Wingender, 2011). It can be thin or patchy in structure or developed into multilayered surfaces and serves as a home for the microorganisms that require hygiene (Flemming, 2002). This allows the pathogens to attach and multiply, where the microorganisms get attached to and survive for a longer period of time. This condition makes the biofilm a main source of contamination and causes health-related risks to the consumers (Wingender, 2011).

This external protection makes the microorganisms like *L. pneumophila* stay safe and alive within the host (Ricardo Murga, 2001). They are usually seen on the sediments, suspended particles, and on the walls of the pipelines and containers of the drinking water distribution systems (Flemming, 2002). These biofilms can exist in both natural and man-made water environments (Priscilla Declerck, 2007).

It has been observed that biofilms are formed on various surfaces over different time intervals. For example, polyethylene takes 7 days for biofilm formation, while glass materials take around 14 days (Sibylle Kalmbach, 1997). Biodegradable substances act as the nutrient for the growth of biofilms, which is most abundant in non-sterile water compared to sterile water (Flemming, 2002).

Biofilm provides a protective environment for some microorganisms like *L. pneumophila*. However, these bacteria cannot replicate on their own without a host like amoeba and protozoa (Priscilla Declerck, 2007) and (Ricardo Murga, 2001). Retention time of water can be a major factor that influences the growth of bacteria. In stagnant water and in the dead ends of the pipes, higher amounts of biofilm are present, which

significantly increases the growth of *Legionella* in that region (Sibylle Kalmbach, 1997).

Sometimes, the bacteria enter into the VBNC state when residing on the biofilm, which is challenging during the analysis of water samples (Wingender, 2011). Ultraviolet (UV) treatment and conventional chlorination methods can be used as primary methods for disinfection (Långmark, Storey, Ashbolt, & Stenström, 2005).

## 2.8 Challenges in Legionella Detection

The global incidence of *Legionella* infection has increased in recent years. However, detection rates have also improved due to the availability of better diagnostic tools, such as urinary antigen tests, and greater awareness brought about by broader pneumonia treatment guidelines. Empirical antibiotic therapies, including macrolides and fluoroquinolones, commonly used for pneumonia, are also effective against *Legionella* species and *Mycoplasma pneumoniae*. Macrolides and fluoroquinolones recommended as first line therapy according to U.S and European guidelines (National Academies of Sciences, 2020).

Despite these improvements, many mild cases of Legionnaires' disease remain undiagnosed. Diagnostic testing is often reserved for patients with severe symptoms who are admitted to intensive care units, while individuals with milder symptoms may go untested. This selective testing contributes to significant underreporting of the disease (National Academies of Sciences, 2020).

Legionellosis can be diagnosed using several methods, such as clinical prediction, radiological imaging, culture test, UAT, PCR, serology, and DFA, which are detailed in the Appendix. (National Academies of Sciences, 2020).

The urinary antigen test detects *Legionella pneumophila* that belongs to serogroup 1. This means the urinary antigen test misses many positive cases caused either by other serogroups or by other *Legionella* species (National Academies of Sciences, 2020). This limitation can contribute to increased mortality due to delayed detection and diagnosis. Additionally, it results in lower testing sensitivity for identifying all possible infections. These limitations highlight the need for more accurate diagnostic tools to manage the spread of the disease. Culture and molecular tests can be performed to identify the presence of *Legionella* species. Among these two, molecular tests are generally more sensitive for detection (CDC, 2025).

## 2.9 Vulnerable Populations and Risk Factors

### 2.9.1 Understanding Host Vulnerability

Understanding the health effects caused by microbial exposure is essential, particularly in relation to three key factors: the level of microbial exposure, the pathogen's ability to cause harm, and the immune competence of the host. These factors collectively

determine the likelihood and severity of an infection (National Academies of Sciences, 2020).

The process by which *Legionella* and other pathogenic microbes cause infection can generally be divided into four stages. First, the microbe must come into contact with a susceptible individual—typically someone with a weakened immune system. In the case of *Legionella*, exposure occurs primarily through the inhalation of aerosols generated from contaminated water or soil (National Academies of Sciences, 2020).

Second, the pathogen must successfully enter the host's body. For *Legionella*, this involves deep penetration into the alveoli of the lungs following inhalation (National Academies of Sciences, 2020).

The third stage involves the pathogen overcoming the host's natural immune defenses. Damaged respiratory tracts—often due to underlying health conditions or environmental factors—can facilitate bacterial survival and proliferation (National Academies of Sciences, 2020).

Finally, the pathogen induces disease in the host. The illness may result from direct bacterial activity or from the immune system's response to the bacterial presence. Both bacterial toxins and the body's defense mechanisms can contribute to tissue damage within the lungs (National Academies of Sciences, 2020).

### 2.9.2 Immunocompromised and High-Risk Groups

Patients affected by Legionnaires' disease often require prolonged hospitalization, and in many cases, intensive care treatment is necessary. This not only increases the burden on healthcare systems but also leads to significant financial challenges for the patients and their families. Reported case fatality rates for Legionnaires' disease vary widely, ranging from approximately 2.9% to 33%. This variation is influenced by several factors, including the patient's overall health status—particularly immune function—the timeliness of diagnosis and initiation of antibiotic treatment, and the quality of medical care provided in hospitals. (National Academies of Sciences, 2020).

Changes in population growth and advancements in the medical system partly contribute to the increased risk of *Legionella* infections. Usually, aged people have a weakened immune system, which makes them more vulnerable to *Legionella* bacteria. People with serious health conditions like cancer, heart and lung problems, and organ transplants live longer due to advanced medical treatments. But at the same time, these people have weaker immune systems, which increases the risk of getting infected (National Academies of Sciences, 2020).

Cancer patients and organ transplant recipients undergo immune-suppressive therapies to avoid rejection—of bone marrow in cancer patients, and to keep the newly implanted organ healthy in the case of organ transplant recipients (Alex Gutierrez-Dalmau, 2007). People under these conditions are more prone to legionellosis. Patients receiving immunosuppressive therapies are also expected to increase in the future, which may

further raise the number of vulnerable individuals (National Academies of Sciences, 2020).

### 2.9.3 Other Risk Factors

Several risk factors, ranging from host characteristics to exposure pathways, are associated with an elevated risk of legionellosis. Adult males are affected more often than females. In the U.S., the proportion of affected men is over 50% higher compared to women (WHO, Legionellosis, 2022). This difference may be attributed to the higher smoking rates among men. Individuals who smoke more than 20 cigarettes a day for a period of over 20 years are at more than 25 times greater risk of contracting legionellosis compared to a person who do not smoke (National Academies of Sciences, 2020).

Individuals aged between 40 and 50 years have higher risk of infection compared to younger adults (National Academies of Sciences, 2020). Children are less commonly affected by legionellosis, and most pediatric cases occur in children with pre-existing health conditions. Many of these infections are hospital-acquired, particularly in neonates, whose immune systems are weak either due to premature birth or underlying complications (I Levy, 1998).

People affected by chronic illnesses such as cancer, Human Immunodeficiency Virus (HIV) or Acquired Immunodeficiency Syndrome (AIDS), or those who have received organ transplants are especially vulnerable, as they typically undergo immunosuppressive therapies that compromise their immune defenses (National Academies of Sciences, 2020).

Certain individuals, including the elderly, neonates, and those with neurological conditions, may be at risk of micro aspiration, a process through which *Legionella* bacteria can be inhaled directly into the lungs (National Academies of Sciences, 2020). However, the exposure pathway through aspiration is difficult to trace. Not everyone exposed to *Legionella* becomes ill—it is the combination of exposure intensity and individual susceptibility that ultimately determines disease outcome (National Academies of Sciences, 2020).

## 2.10 Climate Influence and Global Trends

Climatic changes, such as rising temperatures and increased rainfall, contribute to the rise in Legionnaires' disease (National Academies of Sciences, 2020). Studies show that seasonal trends, particularly during warm and humid periods, are associated with a higher number of *Legionella* cases, as these conditions favor the growth and spread of the bacteria (National Academies of Sciences, 2020).

Environmental aerosolization and fluctuating water system temperatures play a vital role in promoting the disease under various climatic conditions (Walker, 2018). Even a

small increase in rainfall has been linked to a heightened risk of the disease approximately two weeks later (Kelsie Cassell, 2018).

## 2.11 Case Studies and Outbreak Examples

In August 2004, a community outbreak of Legionnaires' disease was reported in Lidköping, Sweden, where 32 cases were recorded in the local hospital, and most of them were middle-aged men. It was found that the cooling tower was the significant source of *Legionella* exposure in that region. This incident created awareness among Swedish industries regarding the maintenance and control strategies for cooling towers (Ulleryd, 2012).

A national outbreak investigation was conducted between April and August in the year 2018. A total of 41 cases were reported, of which around 30 cases were related to *L.longbeachae*, linked to gardening activities and commercial bagged soils. This study indicated that the infection in Sweden can be sourced from non-pneumophila *Legionella* species as well (Löf, 2021).

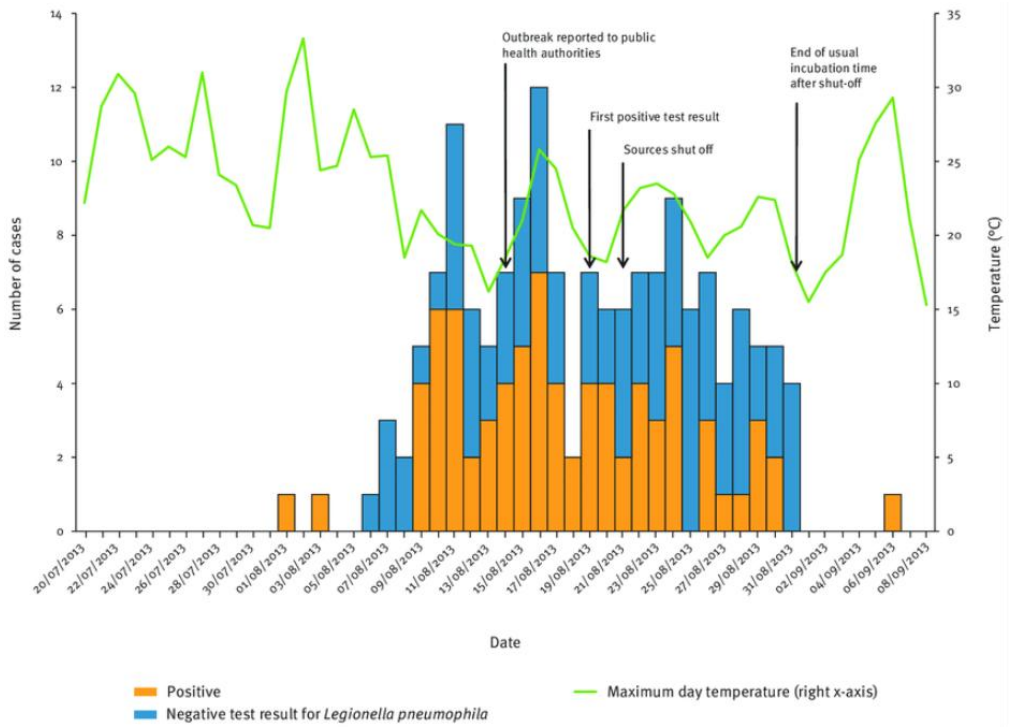


*Figure 1: Illustrates the ¨Epidemiological curve of the Legionnaires' disease outbreak in Warstein, Germany, August 2013¨*

A study conducted in Germany found that a Legionnaires' disease outbreak in 2013 was linked to a cooling tower. The study revealed that, in the 12 days leading up to the outbreak, temperatures remained warm, consistently exceeding 25 °C. This temperature range (25–30 °C) created optimal conditions for *Legionella* growth, contributing to the outbreak. The Figure 1 shows the trend of the outbreak in Warstein (Anna Maisa, 2015).

## 2.12 Risk Management

Mitigating the risk of *Legionella pneumophila* colonization due to disturbing factors in the drinking water distribution system requires a comprehensive and multi-layered approach. Water management plans are essential for implementing control strategies in building premises, which involve risk assessment, water quality monitoring, the implementation of control methods, and the evaluation and adjustment of those methods as needed (National Academies of Sciences, 2020). Several water management practices are recommended as follows.

### 2.12.1 Temperature Control

*Legionella* grows within a temperature range of 25–43 °C. Transmission is highest between 25–30 °C; however, the possibility of transmission is reduced at or above 37 °C. Studies have revealed that growth inside protozoa varies depending on the species' heat tolerance (National Academies of Sciences, 2020).

Heat shock treatments have been tested at different temperatures—50, 60, 70, and 80 °C—based on the decimal reduction time (i.e., time required to kill 90% of the bacteria):

- At 50 °C, it takes 100 to 1000 minutes to kill 90% of the bacteria, making it not very effective.
- At 60 °C, 2 to 5 minutes are sufficient to achieve 90% reduction, and it is considered more effective.
- At 70 °C, it only takes 1 to 1.6 minutes, making it the most effective among these temperatures.
- However, if the bacteria reside inside host cells like protozoa, they may survive even at 80 °C for 10 minutes, protected by the host cell environment.

To effectively reduce *Legionella* contamination, higher temperatures and consistent disinfection methods are required. Without such measures, *Legionella* may evolve and develop resistance to elevated temperatures (National Academies of Sciences, 2020).

It is recommended to maintain the temperature of water heaters above 60 °C and above 55 °C at distal points. Cold water should remain below 25 °C. In warmer climates, water temperature tends to rise, which becomes favorable for the growth of *Legionella*. Heat shock treatment around 70 °C can be used to reduce the bacterial count. However, bacteria residing inside protozoa require further disinfection practices (National Academies of Sciences, 2020).

### 2.12.2 Disinfection Methods

**a. Chlorine:**

Chlorine is effective against *Legionella* species in potable water systems, especially planktonic bacteria. A concentration of 2 mg/L of free chlorine can destroy the bacteria

within 3 minutes (M Miyamoto, 2000). Hyperchlorination is used to disinfect systems; however, its effectiveness is typically short-lived (National Academies of Sciences, 2020). For disinfection purposes, chlorine is usually added in the form of chlorine gas or sodium hypochlorite, as these produce hypochlorous acid, which then kills microorganisms effectively (Collivignarelli, 2018). When *Legionella* resides inside biofilms or protozoan hosts, the efficiency of chlorine disinfection decreases, requiring more aggressive methods of disinfection than chlorine alone. Higher organic content in the water also requires increased chlorination or longer contact time to improve the efficiency of *Legionella* disinfection (Rebekah L Martin, 2020).

**b. Monochloramine:**

Monochloramine is more effective than residual free chlorine in building water systems. It helps prevent the reproduction of *Legionella* within host cells by inducing encystment in amoebae, which makes the host less susceptible (National Academies of Sciences, 2020). Compared to free chlorine, monochloramine is less reactive with organic matter, which allows it to remain longer in the distribution system and thereby increases its effectiveness. This method is particularly useful in preventing *Legionella* outbreaks, especially in hospitals. However, its effectiveness depends greatly on the water chemistry, the amount of biofilm formation, and the condition of the water distribution system (Darren A Lytle, 2021).

**c. Copper-Silver Ionization:**

Cu-Ag ionization is a widely used method due to its relatively low cost and maintenance. Copper damages bacterial cell walls, allowing silver to enter and disrupt key functions such as RNA (Ribonucleic Acid), DNA (Deoxyribonucleic Acid), respiratory enzymes, and cellular proteins (National Academies of Sciences, 2020). While effective in many situations, its success depends on water chemistry, may be slow, and is often less effective when *Legionella* resides within biofilms or protozoa (Faucher, 2025).

**d. UV-C LERD (Light Emitting Radiation Disinfection):**

This method is effective in controlling different strains of *Legionella*, particularly at wavelengths between 255 and 280 nm. Its effectiveness varies depending on wavelength, water quality, and the *Legionella* strain present (Buse, 2022). It has shown promising results in laboratory settings and is commercially implemented in industrial operations. UV-C LERD does not leave residual disinfectants if used properly (National Academies of Sciences, 2020).

**e. Ozone:**

Ozone is a powerful oxidizing agent used in water treatment due to its disinfectant properties. However, its effectiveness is reduced when *Legionella* resides within biofilms or protozoa, requiring extended contact time or combination with other methods (Nadine Kotlarz, 2018). This method may produce harmful byproducts, such as bromate, if water contains high bromide levels. Ozone also does not provide residual

protection, requiring continuous monitoring. Its application is limited because of human toxicity and operational (National Academies of Sciences, 2020).

**f. Chlorine Dioxide:**

Chlorine dioxide is among the most effective chemical disinfectants. It penetrates biofilms and disrupts microbial cell membranes, effectively limiting the presence of *Legionella* in hot water systems. Its long-term efficiency makes it particularly suitable for hospital buildings, which pose higher risks due to patient vulnerability and operational complexity (Laurenti, 2019). Continuous residual monitoring is required, and its effectiveness may decline during prolonged stagnation or in cases of extreme water chemistry (EPA, September 2016).

### 2.12.3 Hydraulic Management

Hydraulic management usually focuses on regulating the temperature, flow, and stagnation of water in the distribution system to reduce bacterial growth and biofilm formation. Water stagnation should be avoided, particularly in dead legs of plumbing systems. Periodic flushing is recommended to reduce cell counts, remove biofilms, and lower *Legionella* concentrations, especially after long periods of disuse (National Academies of Sciences, 2020). Maintaining sufficient water flow velocity helps prevent sediment and biofilm formation, thereby limiting bacterial proliferation. Constant pressure should also be maintained within the system, as pressure drops allow contaminants to enter and promote microbial growth (Emilie Bédard, 2016)

Reducing water age decreases opportunities for bacterial growth in both the distribution system and premise plumbing. The implementation of recirculation lines ensures hot water reaches all parts of a building; these lines must be well-insulated and properly maintained to retain temperature, as otherwise they may support *Legionella* growth (National Academies of Sciences, 2020). Higher organic content in water requires more disinfectants, which in turn promotes biofilm development and increases the rate of *Legionella* occurrence in the distribution system. The occurrence of *Legionella* cannot be prevented by hydraulic management alone; however, when combined with temperature control, chemical disinfection, and scheduled maintenance, it can be highly effective.

### 2.12.4 Limiting Nutrients

Nutrient limitation plays a vital role in controlling the growth of *L. pneumophila* in drinking water distribution systems. Organic carbon is naturally present in organic matter, biofilms, and sediments of the water system. The availability of nutrients can be reduced by minimizing corrosion, organic content, and sediment and biofilm formation, which helps to reduce the proliferation of *Legionella* in the distribution system. Even small traces of some inorganic nutrients, such as potassium, iron, and zinc, can elevate the growth rate. However, *Legionella* can survive in a VBNC state

within biofilms and protozoan hosts even without nutrient supply (Emanuele Luigi Sciuto, 2021).

Protozoan hosts require nutrients for their survival. By reducing biofilm formation and limiting the availability of nutrient sources for hosts such as free-living protozoa and other supporting microorganisms, *Legionella* growth can be restricted. Lowering organic content in water can help suppress conditions favorable to *Legionella* colonization (National Academies of Sciences, 2020). Limiting nutrients alone, however, is not effective; it also requires water quality monitoring and proper maintenance of the distribution system, in combination with other methods, for greater effectiveness.

### 2.12.5 Pipe Materials

Plumbing material plays a crucial role in the growth of *Legionella*. Studies have shown that copper, stainless steel, and polybutylene pipe materials limit biofilm formation, thereby restricting the growth of *Legionella*. In contrast, materials such as rubber; certain types of polyvinyl chloride (PVC) such as soft PVCs, chlorinated polyvinyl chloride (PVC-C), and unplasticized polyvinyl chloride (PVC-U); as well as glass, iron (National Academies of Sciences, 2020), steel and polyethylene (Emanuele Luigi Sciuto, 2021), concrete pipes (Daniela Simina Stefan, 2023), and cross-linked polyethylene (PEX) promote biofilm formation, which supports *Legionella* growth in pipe systems (Sallamaari Siponen, 2025).

Copper pipes are considered the most effective in limiting biofilm formation, as copper releases $Cu^{2+}$ ions with antimicrobial properties. In comparison, plastic pipes do not release metal ions, which promotes biofilm growth and requires proper maintenance. Copper pipes initially reduce *Legionella* colonization; however, their effectiveness diminishes over time due to corrosion when reacting with disinfectants. This leads to increased organic content, supporting further biofilm formation (Sallamaari Siponen, 2025). Iron and steel pipes also undergo corrosion and are at risk of scale formation if not properly maintained. This can be mitigated through proper water quality management and the use of corrosion inhibitors such as poly- and orthophosphates (Emanuele Luigi Sciuto, 2021).

Pipe corrosion and the roughness of the surface of pipe materials contribute to nutrient and organic matter accumulation, which promotes biofilm development and supports *Legionella* growth. Metal pipes corrode and release metal ions, with scale formation depending on surface roughness, which can harbor biofilms. Other plastic and concrete pipe materials release fewer ions compared to metal pipes; however, biofilm growth is often higher due to their surface properties (Daniela Simina Stefan, 2023).

### 2.12.6 Aerosol Control

Aeration control technique focuses on minimizing the generation and inhalation of aerosols containing contaminants. Inhalation of aerosols is the primary transmission route for *Legionella* bacteria to cause infection to humans. Low-flow fixtures such as faucets and showers, although water is conserved, can increase water age and reduce disinfectant levels, thereby promoting *Legionella* (National Academies of Sciences, 2020). Faucets with laminar flow with minimal spray are highly recommended with regular cleaning to reduce scaling and biofilm formation. Aerosol prevention, by minimizing the generation and spread of contaminated droplets, directly reduces the risk of *Legionella* exposure (Aaron J Prussin II, 2017).

Point-of-use filters (POU) can be installed on the aerosol-generating fixtures like taps and showerheads to block *Legionella* from releasing as aerosols. That reduces the risk of exposure. POU is a membrane-based filter which filters the pathogens and microorganisms from water. Studies say that membrane-based POU are more efficient in filtering the *Legionella* especially in hospitals. These filters should be replaced once in 4 to 8 weeks, if not then the bacteria accumulate on the filter and get colonized (Kelsie M Carlson, 2020). High-quality 0.22 μm POU filters have been used in the outlets of taps and showerheads of the French hospital during an outbreak, POU filter usage prevented the transmission to patients in the hospital. POU can be used as a temporary solution during the outbreak events which immediately prevents the exposure and minimizes the risk (Philippe, 2018).

The deposition of organic content increases biofilm formation, which enhances *Legionella* persistence (National Academies of Sciences, 2020). The effectiveness of aeration control techniques can be improved by minimizing organic content and biofilm formation through proper water treatment methods in the distribution system, combined with the use of POU filters in high-risk areas.

# 3 Methodology

## 3.1 Field Sampling Strategies and Protocols

Field sampling was carried out at selected water pipeline maintenance sites where leakage events had occurred. However, sometimes the samples were collected from unused pipes that had not been in use for a prolonged period, where these unused pipes were connected to the currently used pipes. Technically, those samples were from the pipe maintenance site but not based on leakage.

Some random water samples were also collected from nearby publicly used gyms, laundry rooms, and from taps that had not been used for a prolonged period, as these locations were commonly used by many people and the risk of exposure is high. For example, saunas at gyms produce aerosols, and the temperatures maintained are suitable for the growth of *Legionella.* In some laundry rooms, several taps are available, especially in the folding rooms where taps are not frequently used, so there might be a possibility of scaling and stagnation of water within the pipe extensions. This situation possibly promotes the growth of *Legionella.*

In total, 46 water samples were collected at 16 different locations. Among them, 9 samples from 5 different locations were collected as random samples. The remaining 37 water samples were collected from 11 different locations related to repair or maintenance work. 11 soil samples were collected from 8 different locations, and all the soil sampling locations were related to repair or maintenance work (during the initial days of sampling, soil samples were not collected). The samples were collected over a period of 3 months' timeframe, between 19 February 2025 and 20 May 2025.

One soil sample and two or more water samples were collected from the leakage sites based on the availability as some repair work takes days and weeks for completion. In some locations, only soil samples with one or no water samples were collected, as those locations were based on extending pipe connections. This is explained in detail in the comments section of the Appendix A: Water Sample Data.

In the case of maintenance work, the water samples were systematically planned to include the upstream, downstream, leakage point on the pipe, and occasionally from the pit area beneath the leakage. Sampling at these locations helps to assess whether *L. pneumophila* potentially enters the system through leakage or through other parts of the drinking water system.

The selection of sampling sites was based on the municipality's scheduled maintenance notifications from the Gothenburg Stad's website "Vattenavstängningar" and direct communication with municipality personnel. Prior to each sampling, a contact person from the municipality was informed about the planned sampling for the upcoming day, then coordinated with the site supervisor of that particular maintenance work to ensure access, proper timing, and safety precautions if necessary for collecting samples on the day of maintenance work.

## 3.2 Maintenance Process Description

Water shutdowns during maintenance work typically last for 2 to 4 hours. The shutdown process involves the complete closure of the upstream valve to reduce water pressure on the pipe, followed by the closure of the downstream valve immediately after the leakage point. This “double shutdown” ensures that the maintenance work is not affected by upstream pressure or by backflow from the downstream side.

Once the valves are closed, the leaking pipe section is cut, and work begins on the section. Before replacing the pipe, workers use sandpaper to smooth the cut edges, including approximately 2 to 3 inches inside the pipe. Based on observations, this process has the potential to introduce particles into the system.

A new pipe section is then installed and securely tightened. The upstream valve is reopened, the pipe is refilled, and air, along with any contaminants or particles trapped within the repaired section, is removed. Flushing is performed through downstream openings or fire hydrants. During this stage, the replaced section is checked for leakage before the downstream valve is finally opened for public usage. Samples for this study were collected during this time frame.

After the shutdown, a pressure difference may occur in the pipe system. This can happen if there is leakage at the shutdown valves or if consumers—unaware of the repair work—draw water from the system. Such actions can create a pressure drop downstream of the repair site, potentially forming a void. Backflow of water into the downstream section could occur, but according to municipal guidelines, every customer’s water meter should be equipped with a check valve to prevent backflow between properties.

Because pressure is generally maintained at a constant level in the distribution system, shorter shutdowns carry a lower risk of *Legionella* spreading back into the pipeline. The stagnation time during these shutdowns is also insufficient for significant bacterial proliferation. Flushing in the repaired section, between the closed upstream and downstream valves, serves to remove trapped air along with water containing any contaminants or soil particles that may have entered during the repair process.

## 3.3 Water Sample Collection

Water and soil samples were collected from the selected leakage sites. For collecting water samples, sterile containers were used. The samples were collected directly from the upstream and downstream fire hydrants and the leaking point.

To neutralize residual chlorine in the sampled water (up to >0.5 mg/L), 300 µL of 0.05 M $Na_2S_2O_3$ (sodium thiosulfate) was added to 1000 mL of the collected water sample. Sodium thiosulfate should be added immediately after collection to be effective. However, since transportation time varied between sample locations, and to ensure more accurate results and avoid interpretation errors, $Na_2S_2O_3$ was pre-added to the sterile sample bottles before sample collection.

Water samples were then transported to the laboratory for analysis. Each sample bottle was labeled with the sampling site, sampling point, and collection date to avoid confusion during analysis.

### 3.3.1 Water Sample Analysis

In the laboratory, water samples were analyzed for the presence of *Legionella pneumophila* using the Legiolert test, according to the standard procedure, which requires an incubation period of one week. The test was selected specifically to identify the presence of *L. pneumophila* and cannot detect other bacterial species, including other species of *Legionella*.

In addition to the Legiolert test, the water samples were also analyzed for other basic physicochemical parameters such as pH, turbidity, conductivity, temperature, hardness, and the presence of free chlorine. These parameters were measured to characterize the water quality of the collected samples and assess whether any of these parameters influence bacterial growth or survival.

Apart from these tests, the samples were frozen and later analyzed for Total Organic Carbon (TOC). The organic content in the sample could potentially support the growth of microbes. All analyses were conducted using standard laboratory equipment and procedures.

#### 3.3.1.1 Legiolert Test Procedure

A total of 100 mL of water sample was transferred to a sterile container. The samples were measured for hardness to add the appropriate volume of potable Legiolert supplement to the water sample. As the water samples were taken from the drinking water distribution system, the hardness of most samples was between 0.3–0.7, and 0.33 g of Legiolert supplement was added.

Then, one pack of Legiolert blister powder was added to the 100 mL water sample. The bottle was closed and shaken until the blister powder was fully dissolved. However, the samples might still appear cloudy. The sample was then poured into the Quanti-Tray, which contains 90 small wells and 6 large wells. Trapped air bubbles were removed from the small wells and sealed with the Quanti-Tray Sealer PLUS. The trays were incubated for 7 days at 39 °C. The incubator must be provided with adequate humidity to reduce water loss in the sample tray.

After 7 days, the results were analyzed. Brown or turbid wells were considered positive for *L. Pneumophila.* The number of large and small positive wells was counted and quantified using the MPN (Most Probable Number) table.

## 3.4 Soil Sampling

Soil samples were collected randomly from the pit, primarily focusing on areas around the leakage. In cases of connection extension works, soil samples were also randomly collected to investigate whether the soil naturally contains *L. pneumophila*. The sampling was done manually and directly into sterile containers without using instruments. The depth of soil sample varies according to the location of drinking water pipe under the ground. The soil samples were then transported to the laboratory for analysis.

In most cases, the soil samples were collected before cutting the pipe section at the leakage site, and therefore, the majority of the samples were dry. However, in cases of severe leakage, the collected soil samples were wet.

### 3.4.1 Soil Sample Analysis

According to (van Heijnsbergen E, 2016) soil samples were analyzed using the amoebal co-culture method. In this method, 5 g of soil sample was suspended in 5 mL of distilled water, vortexed for around 10 seconds, and incubated for 1 hour at room temperature. *Acanthamoeba Castellani* amoebae were cultured in a 75 cm² culture flask, washed twice, and seeded into microplates with 12 wells. Each well received 1 mL of amoebae in Page's amoebal saline PAS and was injected with 100 µL of suspended soil, each in triplicate.

Then, they were incubated at 32 °C for 3 days, followed by subculturing to fresh amoebae for another 3 days at the same temperature. Samples were then serially diluted and plated on BCYE and GVPC (glycine–vancomycin–polymyxin B–cycloheximide) media. At 37 °C, after 4 and 7 days, the plates were examined for *Legionella*-like colonies (van Heijnsbergen E, 2016). However, several different *Legionella* species may be observed along with *L. pneumophila*, which must then be subjected to further identification and typing processes.

#### 3.4.1.1 Alternative Method Used

Due to limitations in laboratory facilities, the amoebal co-culture method—considered the standard method for detecting *Legionella* in soil matrices—was not used. Instead, a simplified method was used to detect the presence of *L. pneumophila* in soil samples.

Approximately 5 g of soil sample was mixed with 50 mL of distilled water in a conical flask. The mixture was centrifuged manually by hand for about 30 to 60 seconds and then left undisturbed at room temperature for about 1 hour to allow bacteria (if any) to suspend in the water. Then, 10 mL of the soil–water mixture was mixed with 90 mL of distilled water. The Figure 2 shows the collected soil sample along with the soil-water mixture in a conical flask.

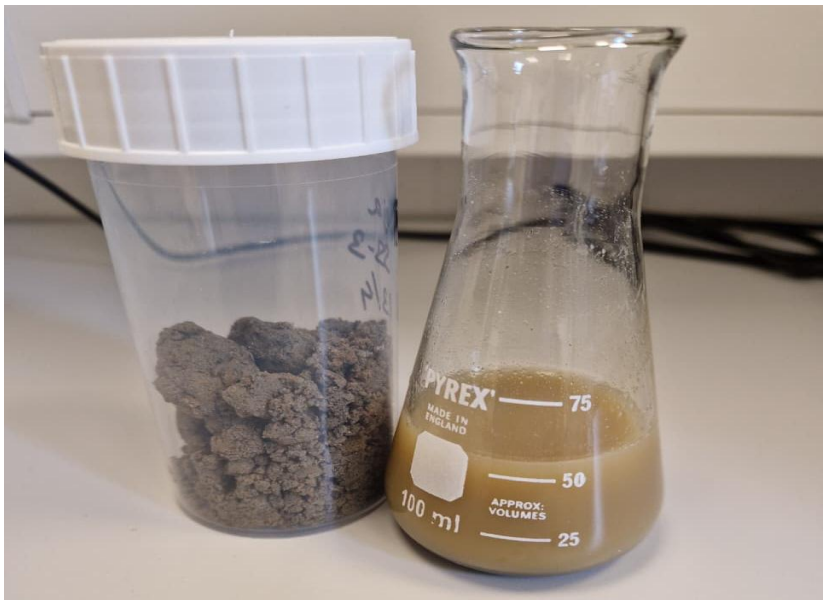


*Figure 2: Collected soil sample and dissolved soil sample in distilled water*

The diluted solution was subjected to the Legiolert test protocol (as described for water analysis). The 10 mL of sample mixed with 90 mL of distilled water was tested in two ways:

1. **Filtered sample** – passed through a 0.45 µm syringe filter before dilution.
2. **Unfiltered sample** – vortexed before dilution and tested without filtration.

This two-way analysis was performed to check whether filtration had any significant impact on the detection of *L. pneumophila* in soil samples. This method of soil sample analysis is *not* a standard or validated method for detecting *L. pneumophila* in soil. It was performed solely as a preliminary check to determine whether *L. pneumophila* was present in the soil samples. Additionally, in the unfiltered samples, it was harder to visually detect positive wells in the Legiolert test tray after 7 days of incubation at 39 °C.

## 3.5 KoV Data Collection and Use

### 3.5.1 Sampling Location

The sampling locations are categorized into codes A–F, L, and S. The purpose, location, and frequency of sampling for each category are summarized in Table 1. These samples are collected to analyze the physicochemical parameters of the water in the drinking water distribution system, which does not include testing for *Legionella* as part of the analysis.

*Table 1: Sampling Location Codes and Responsibilities*

| Code | Location Description | Purpose | Frequency |
|---|---|---|---|
| A | Pumping station (without reservoir) + 1–2 downstream homes | Monitor zone water quality | Every sampling round |
| B | Fixed points (long-term monitoring) | Detect long-term changes | Regular intervals |
| C | Remote/outer area pumping stations + 1–2 downstream homes | Check water quality in distant zones | As needed |
| D | Pumping station with reservoir + 1–3 downstream homes | Assess reservoir and nearby supply quality | 4–12 times per year |
| E | Homes in low-pressure city zones | Ensure all zones are included | 108 samples per year |
| F | User locations with full parameter testing (Group B) | Detailed trend monitoring | Regularly at fixed locations |
| L | Post-pipe repair sites | Assess water quality after repairs | ~40 times per year |
| S | Island homes with long sea pipelines | Monitor special risk areas | 10 times per year |

### 3.5.2 Sampling Procedure and Assessment Criteria

According to Kretslopp och Vatten (KoV) documents, water samples are analyzed for temperature, odor, and taste based on initial sensory impressions during the general routine of water sampling. These sensory parameters (odor and taste) were categorized as none, weak, clear, or strong based on initial sensory impressions. Following

collection, samples were stored in a cooling bag or a refrigerator to preserve their integrity and prevent biological changes. All samples were either analyzed immediately upon arrival at the laboratory or within a maximum of four hours, in accordance with standard protocols. Figure 3 shows the procedure of the sample collection to ensure consistency and to prevent contamination during sampling according to the municipality.

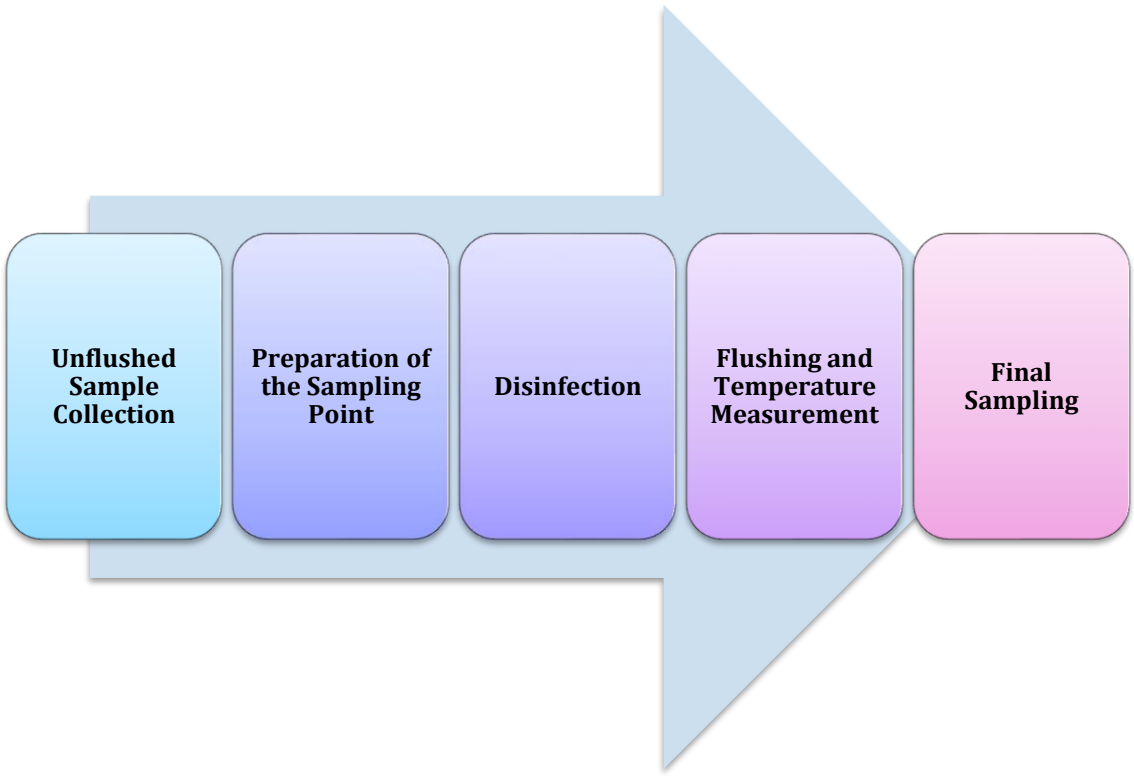


*Figure 3: Sample collection procedure followed by KoV*

All collected samples are categorized based on compliance with regulatory health standards:

- **Passed:** All measured parameters are within the acceptable limit values.
- **Failed:** One or more indicator or parameter values exceed the specified limits.
- **Unfit:** The water poses an acute health risk and must not be consumed without immediate remedial action.

To test for the presence of *Legionella*, water samples are collected from the reservoirs at 12 different locations around Gothenburg. The number of sampling times varied based on the risk at each location, with 4, 6, or 12 times per year. From 2021 until present, six *Legionella*-positive samples have been reported in the reservoirs by KoV, with values between 1 and 2 CFU/100 mL. In case a sample tested positive for the presence of *Legionella*, samples were retaken, and if they still showed positive results, reservoir cleaning was taken into consideration, and follow-up samplings were conducted until the results were negative for *Legionella*. According to KoV, the samples taken for the second time after a positive sample were always negative, and

none of them tested positive. In case of repeated positive samples, Miljöförvaltningen (the Environmental Administration) is notified.

### 3.5.3 Municipality (KoV) Data Insights

The Municipality of Gothenburg provided data on the bacterial growth in relation to temperature variance. Figure 4 below illustrates the general regrowth pattern of different kinds of bacteria in a reservoir.

The data in the graph has been smoothened using 11 point moving average to eliminate the random dips and spikes that may not reflect the true underlying trend. This approach is particularly useful for identifying seasonal patterns. The X-axis represents the sampling dates, ranging from January 2020 to May 2025.

The graph includes three Y-axes, each representing a different parameter. The black line shows the ambient temperature on the day of sampling. The blue line represents the logarithmic scale (base 10) of the number of slow-growing bacteria, measured in colony-forming units per milliliter (CFU/mL) after a 7-day incubation period. In this log scale, a value of 2 corresponds to 100 CFU/mL, a value of 3 corresponds to 1,000 CFU/mL, and so on. Although the blue line exhibits irregular fluctuations, the overall trend indicates higher bacterial counts during warmer periods.

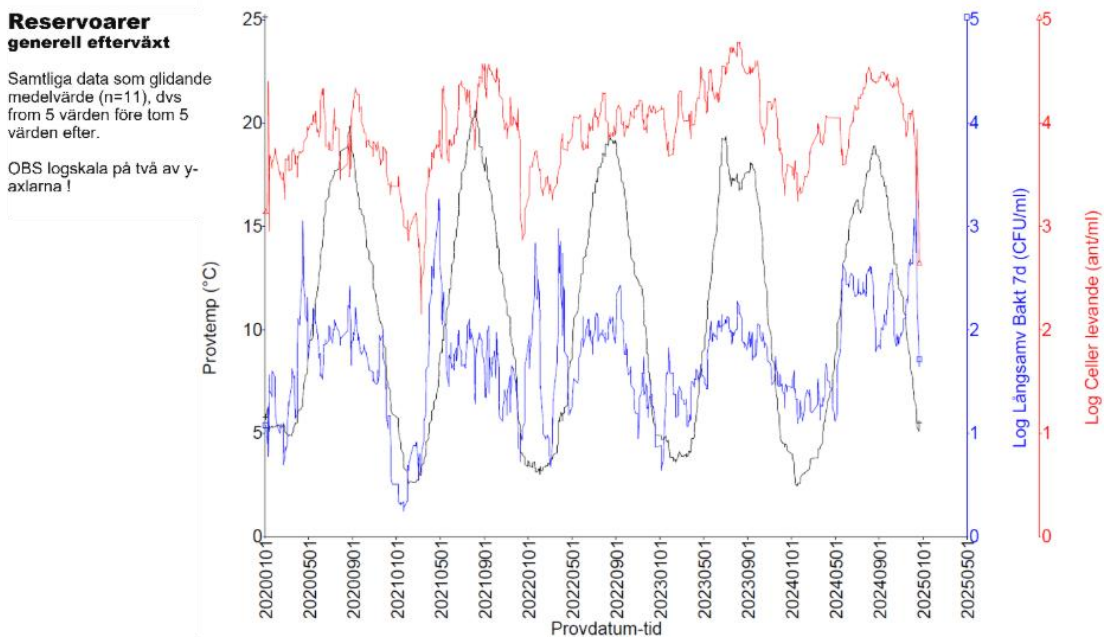


*Figure 4: Reservoir general regrowth data obtained from the municipality. The black, blue and red lines representsrepresent temperature fluctuations, number of slow growing bacteria after 7 days of incubation and number of living cells per millimeter respectivel*

The red line represents the logarithmic scale of living cells per milliliter. This includes bacteria, protozoa, algae, and other microorganisms. The red line closely follows the temperature curve, indicating that the total number of living cells increases during warmer periods and decreases during colder periods. This trend highlights the elevated biological activity typically observed in warmer conditions. It is important to note that this line does not account for dead microorganisms.

It is also observed that the temperature during the warmer periods is above 20 °C. Temperatures above 25 °C are considered to be the most favorable for the growth of *Legionella*; however, *Legionella* can proliferate at temperatures above 20 °C. In reality, during the warmer periods of summer, people migrate to summer houses or go on vacations. This situation increases the water age and stagnant zones, along with elevated temperatures in the reservoir due to the warm climate. These conditions create a favorable environment for the proliferation of microorganisms, including *Legionella*. This trend has also been observed in the graph. It is recommended to take a higher number of samples in the reservoir during the warmer periods than in the colder periods.

# 4 Result

## 4.1 Water sample Result

None of the water samples collected during maintenance work tested positive for *Legionella.* These negative results include samples taken from upstream, downstream, and the leakage point. To investigate positive cases, a set of random water samples was collected separately. Out of the nine random water samples, three tested positive. the below Figure 5 shows the *Legiolert* water sample test results after the incubation period of 7 days at 39 °C. Darker wells indicate the presence of *L. pneumophila*, while lighter wells indicate absence. The color change in the water is due to the addition of blister powder.

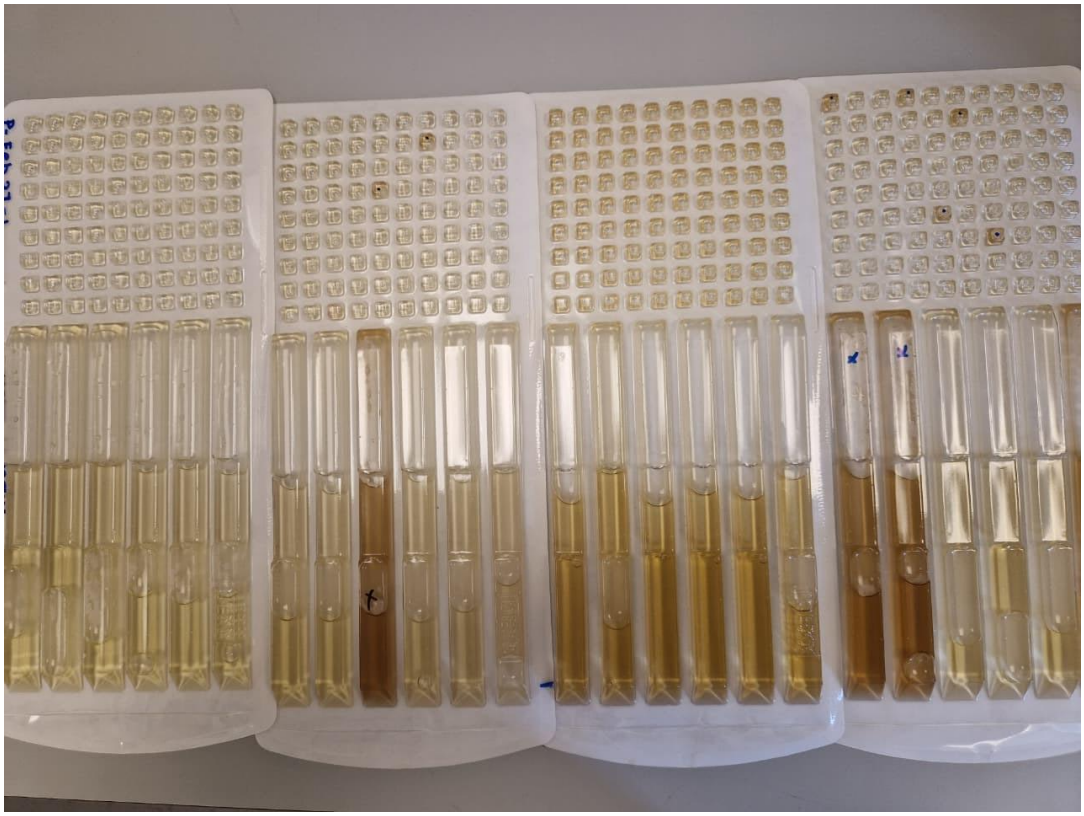

*Figure 5: Legiolert test result of water samples after 7 days of incubation*

The graph below Figure 6Figure 6 presents the Legiolert test results for these random water samples, collected from locations such as laundry rooms, gyms, and nearby areas. The positive samples indicate the presence of *L. pneumophila* bacteria in the collected water, with MPN values representing the probable concentration. The X-axis represents the sample number. Sample numbers are not sequential, as they were assigned according to the date of collection. The Y-axis shows the MPN values of the *Legionella* bacteria. Sample 9 and 11 displayed relatively low MPN values, whereas Sample 46 showed a significantly higher MPN value, indicating a notable presence of *L. pneumophila*. Locations with positive samples require immediate remediation to prevent bacterial spread and potential outbreaks. The presence of positive samples also

suggests that the water temperature and availability of protozoan hosts in these locations were favorable for bacterial growth and survival. Detailed information on the water samples is provided in the Appendix A: Water Sample Data and Appendix B: Legiolert Test Results.

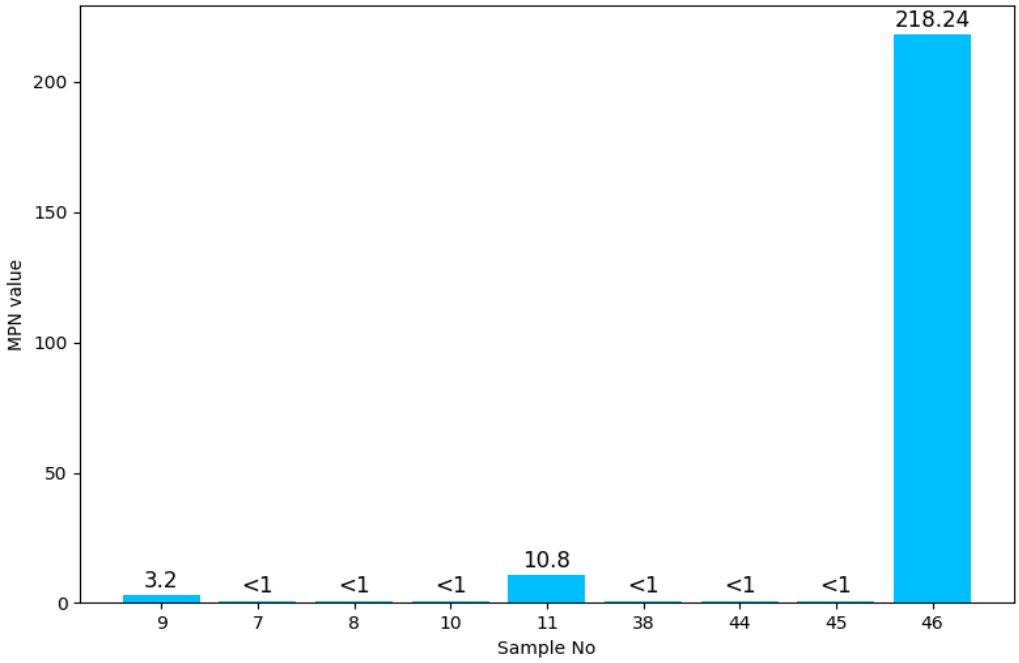


*Figure 6 : Legiolert test result of the random water samples*

## 4.2 Soil sample Result

Figure 7 shows a basic overview of the soil type at one of the sampling locations, obtained from the SGU (Geological Survey of Sweden) online map service. This information was used to understand the general properties of the soil in each area. The soil type classification is based on both field observations and SGU data. Detailed information on the collected soil samples is provided in the Appendix C: Soil sample datasheet.

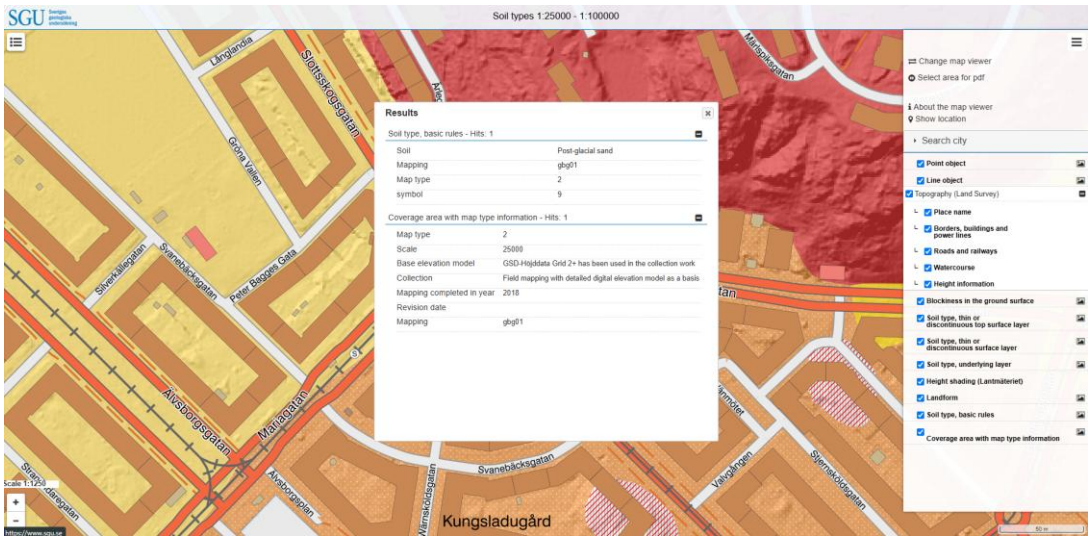


*Figure 7: Overview of soil types in the Kungsladugård area from the SGU online map*

Figure 8 shows the result of the Legiolert test method for different soil samples collected from various types of maintenance work, presented along the X-axis. These works include leakage repairs and pipe connections, with the Most Probable Number (MPN) values of *L. pneumophila* displayed on the Y-axis. SS represents unfiltered soil samples, while FSS represents filtered soil samples.

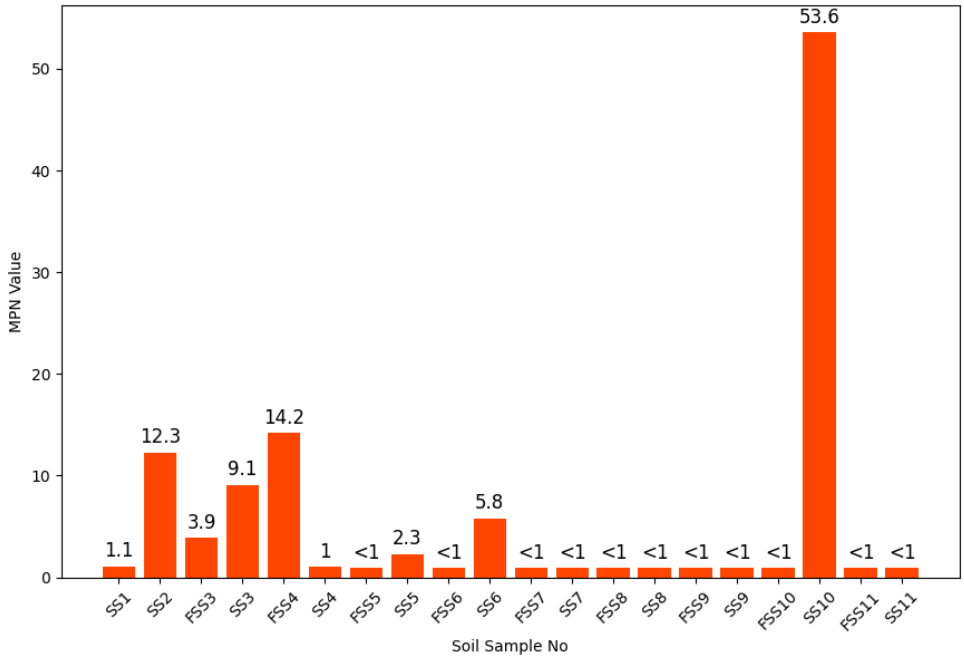


*Figure 8: Legiolert test results for different filtered (FSS) and unfiltered (SS) soil samples collected from various maintenance works*

Most soil samples showed MPN values below 15. Among these, sample SS10 exhibited a significantly higher concentration of *L. pneumophila*. In contrast, the corresponding filtered sample from the same site did not indicate the presence of *Legionella*. This suggests that the environmental conditions at that specific site—such as high moisture,

favorable temperature, availability of host cells, and organic content—were more conducive to *Legionella* growth. Figure 9 below illustrates the difference between the filtered and unfiltered soil sample test results obtained using the Legiolert method. The tray with darker wells represents the positive result for *L. pneumophila* (unfiltered sample), while the lighter wells represent the negative result for the filtered soil sample.

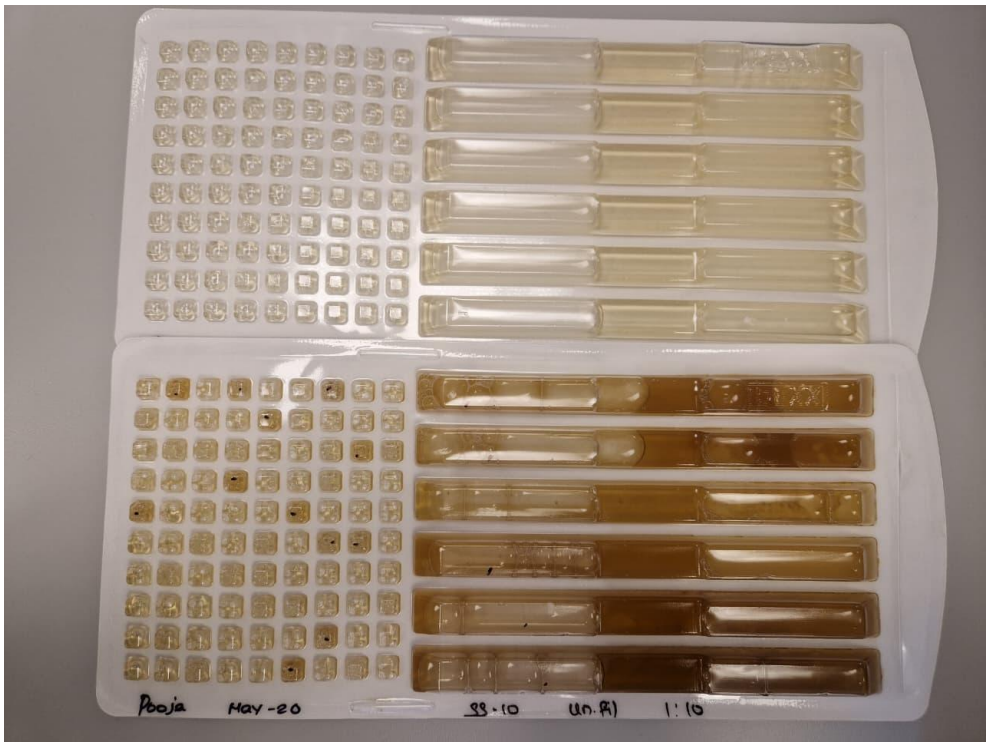


*Figure 9: Soil sample tested for Legiolert test. Above and below trays represent negative filtered sample and positive unfiltered samples.*

Both filtered and unfiltered soil samples were analyzed to assess the accessibility of bacterial load in the presence and absence of particulate matter. Only 2 of the 9 filtered samples tested positive for *Legionella*, whereas the remaining positive results were from unfiltered soil samples. This indicates that *Legionella* bacteria may be attached to particulate matter in the soil.

## 4.3 Water Quality Parameters

The physicochemical water quality parameters of the water samples were tested, and the results of the positive samples are presented in the following Table 2.

On comparing the key water quality parameters of the collected water samples, turbidity was significantly lower in the positive samples compared to the negative samples in the dataset. Lower turbidity is expected in potable water and indicates that cloudiness was not a factor contributing to the presence of *Legionella* in this study.

*Table 2: Water quality parameter result of positive samples*

| ***Sample number*** | **Turbidity** | **Temperature** | **Conductivity** | **pH** | **TOC** | **Free Chlorine** |
|---|---|---|---|---|---|---|
| *9* | Clear water, no visible solids | Within survival range, slightly below optimal growth range | Mid-range compared to dataset | Optimal for potable water | Low–moderate organic content, like negative samples | Not detected |
| *11* | Very low turbidity, clear water | Within survival range at lower rate | Low–mid range compared to dataset | Optimal for potable water | Low–moderate organic content, like negative samples | Not detected |
| *46* | Clear water, no visible solids | Within survival range at lower rate | Significantly higher than other samples | Optimal for potable water, higher pH may reduce chlorine effectiveness | Significantly higher | Minimal disinfectant residual |

The temperature of the water samples was within the optimal range for bacterial survival. All three positive samples were collected as hot water; however, they were analyzed after a time interval of approximately 10–12 hours. Samples 9 and 11 were collected in the evening of the previous day and analyzed the following morning. Sample 46 was collected in Stockholm and transported to Gothenburg for analysis. Despite the delay, the temperature remained within the survival range during the analysis period.

Higher conductivity indicates a higher ionic content in water, suggesting a greater presence of dissolved salts or minerals. Among all samples in the dataset, sample 46 had significantly higher conductivity, except for sample 8, where prolonged stagnation of water in the tap may have led to salt/mineral deposition.

The pH levels of the positive samples were within the optimal range, though sample 46 exhibited a notably higher pH. According to World Health Organization (WHO) recommendations, the pH of drinking water should range between 6.5 and 8.5 (WHO,

2011); therefore, pH alone does not have a direct correlation with *Legionella* presence. However, studies show that a rise in pH can reduce the effectiveness of chlorine disinfection in potable water systems (Yongji Zhang, 2021). Higher pH levels require an increased chlorine dosage or extended contact time to maintain disinfection efficiency.

Total organic carbon (TOC) levels in samples 9 and 11 were within the low-to-moderate range compared to other collected samples. TOC is naturally present in potable water but is controlled and monitored according to safety standards. Sample 46 recorded the highest TOC value, indicating a higher availability of nutrients to support bacterial growth.

Free chlorine was not measured for samples 9 and 11. In contrast, sample 46 showed minimal disinfectant residual. Based on these observations, it can be concluded that the combination of higher conductivity, elevated organic content, minimal chlorine residual, and optimal temperature in sample 46 created an environment favorable for the growth and survival of *L. pneumophila* compared to the other positive samples.

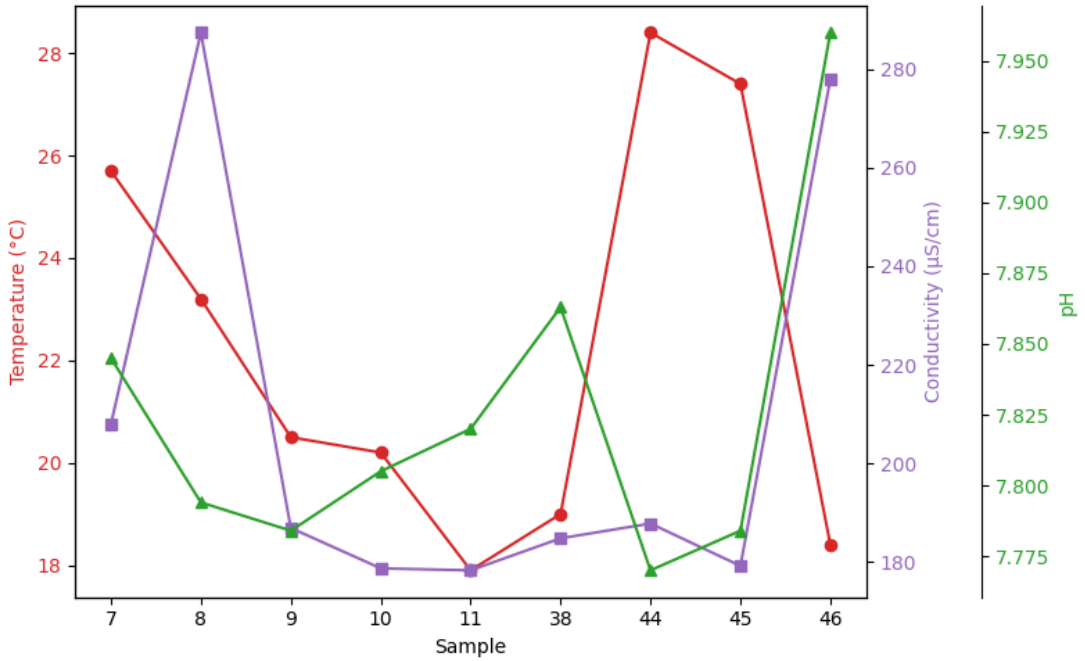


*Figure 10: Temperature conductivity and pH combined graph for random water samples*

Figure 10 shows a combined graph of temperature, conductivity, and pH for the random water samples, highlighting that sample 46 had significantly higher pH and conductivity values. Figure 11 shows a combined graph of turbidity, TOC, and free chlorine for random samples, indicating that turbidity had no notable effect, whereas TOC was high in sample 46. However, based on both graphs, the samples with minimal MPN values (samples 9 and 11) cannot be clearly distinguished from the negative

samples using these water quality parameters. Detailed comparative graphs of the three positive samples are attached in the Appendix D: Water Quality Parameters.

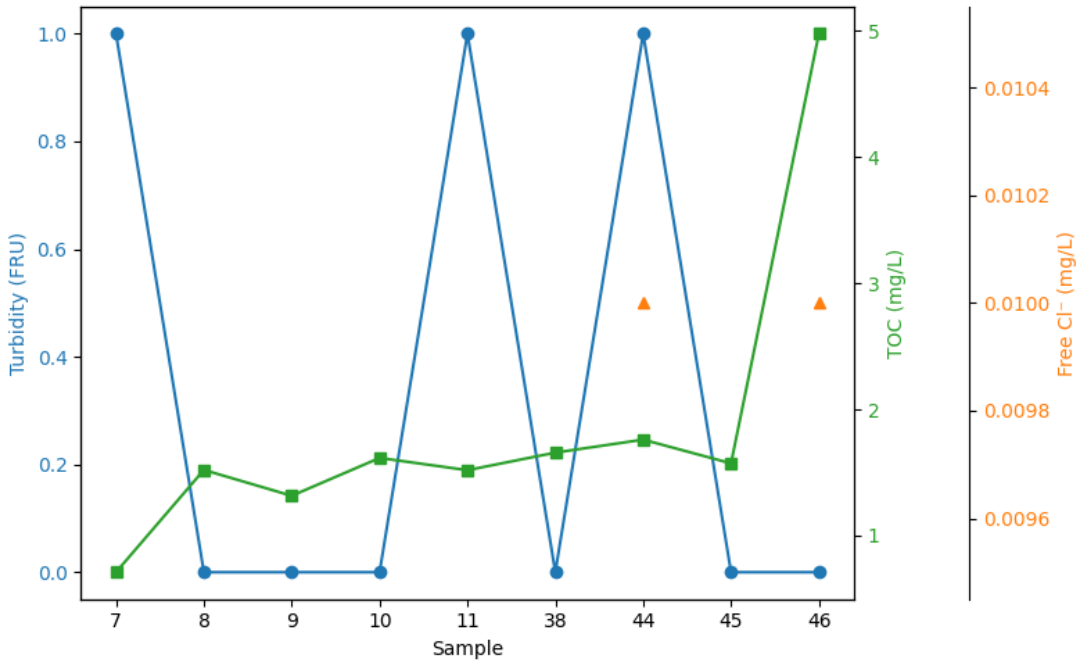


*Figure 11: Turbidity, TOC and Free Chlorine combined graph for random water samples*

# 5 Discussion

## 5.1 Interpretation of results

The primary goal of this study was to investigate the potential entry of *Legionella pneumophila* into the downstream water supply through leakage or repair work. Along with the Legiolert test, various physicochemical parameters were measured to understand the environmental conditions that might support bacterial growth. These parameters included pH, turbidity, conductivity, temperature, free chlorine, and TOC.

As the water samples related to the maintenance work tested negative for *L. pneumophila*, the direct correlation between these parameters, bacterial presence, and repair work remains unclear. However, the measured parameters were still useful in understanding the water quality before, during, and after repair activities at the upstream, downstream, and leakage pit locations.

9 random samples were also collected to explore possible relationships between *L. pneumophila* and physicochemical parameters. In summary, a combination of higher organic content, higher conductivity, minimal residual chlorine, and higher pH with optimal growth temperature appears to provide the most favorable environmental conditions for the survival and proliferation of *L. pneumophila*.

One possible reason for negative results in water samples could be the absence or delayed addition of sodium thiosulfate ($Na_2S_2O_3$) to the samples. In some cases, the solution was not added at all, and in others, it was added after a transport delay of approximately 45–60 minutes. For samples collected after sample number 30, the sodium thiosulfate solution was pre-added to the bottles before going to the site, ensuring immediate mixing with the collected water.

Turbidity levels showed little fluctuation between downstream and upstream samples. However, samples taken directly from the leakage pit often exhibited higher turbidity. Occasionally, turbidity spiked immediately after repair work in downstream samples but gradually decreased over time. TOC levels were generally lower in upstream samples, higher in leakage pit samples, and showed a localized spike in downstream samples following repair work. Within approximately a week, TOC values in downstream samples typically returned to levels similar to the upstream samples.

Residual free chlorine levels were observed to decrease in downstream samples after repair work compared to pre-repair levels. This reduction might be due to an increased chlorine demand caused by disturbances in the upstream flow during maintenance activities.

Interestingly, water samples collected from leakage pits or downstream sections were negative for *L. pneumophila*, whereas soil samples from the same pits were frequently positive. This indicates that *L. pneumophila* present in the soil did not enter the water system through leakage or maintenance work. Therefore, the source of *L. pneumophila* in the tested areas is unlikely to be soil from the leakage sites.

The positive soil sample might be explained by the fact that the soil environment can be more favorable for *Legionella*, with adequate nutrient availability and suitable temperature. Heat produced from wastewater pipes can provide conducive surroundings for *Legionella* growth, as the areas around such pipes usually have elevated temperatures. The presence of moisture and organic content further creates a suitable environment for their survival. It is also possible that the soil samples were taken from such locations—though not noted during the study, which may have had a higher likelihood of containing *Legionella.*

There is also a possibility of false-negative results in some of the water samples. Since biofilm formation was observed on the cut section of the pipe, there is a strong likelihood of *Legionella* presence; however, due to disinfection used in the water system, the protozoan host may not have been available, and the bacteria could have been in a VBNC state within the biofilm, which requires further study. Therefore, even if *L. pneumophila* was not detected immediately before or after repair work in the water samples collected from leakage or repair sites, there remains a potential risk of soil particles entering the system unless it is properly cleaned and measures are taken prior to reconnecting it for public use downstream. The risk of *L. pneumophila* entry into the system should therefore be considered greater than zero, as complete exclusion is never possible.

It has been observed that soil particles entered the distribution system during the repair work, and through soil sample analysis, it can be understood that the unfiltered soil sample number 10 indicated the presence of *Legionella*, whereas the filtered soil sample number 10 did not indicate the presence of *L. pneumophila*. This suggests that the bacteria are attached to the soil particles and do not dissolve completely into the water within a short period of time. The soil particles that entered during the repair work can attach to the biofilm, as the surface of the observed biofilm was not smooth. Even though flushing was carried out after the repair work, some soil particles with attached bacteria can still adhere to the biofilm, and when favorable temperature and growth conditions are met, the bacteria start to proliferate. Since the surface of the biofilm is not smooth, water flow can be disturbed and cause turbulence within the system, which in turn disturbs the biofilm, resulting in the release of bacteria into the system.

## 5.2 Research Question Answers

### 5.2.1 *L. pneumophila* and Distribution Disruptions

Previous studies and literature indicate that upstream distribution issues—such as pipe leakages and repair works—can create entry points for bacteria into drinking water systems. These disturbances can promote biofilm formation and facilitate bacterial colonization within host cells such as free-living protozoa and amoebae. Once bacteria travel downstream and reach favorable conditions—such as stagnant zones or warm areas within hot water systems—they may proliferate under optimal temperature conditions.

In this study, water samples were collected from upstream, downstream, and leakage points associated with repair works to assess the potential entry of *L. pneumophila*. The analysis revealed that *L. pneumophila* was not detected in the water samples, suggesting that the bacteria did not enter the distribution system through leakage events or repair work. However, soil samples collected from the leakage site did test positive for *L. pneumophila*, indicating the environmental presence of the bacteria near the distribution infrastructure.

Although there was no immediate evidence of bacterial intrusion through the leakage, it is possible that the bacteria may require time to proliferate and establish within the system. Therefore, further studies are recommended, including follow-up sampling at intervals—such as one week and one month after the initial repair—to monitor potential bacterial growth over time.

### 5.2.2 Flow Disruptions and Temperature Fluctuations

Disruption of water flow and temperature fluctuations due to network issues promote the growth of *Legionella* by creating stagnant zones, including dead ends and areas with reduced circulation. Low disinfectant levels, temperatures within the optimal range for bacterial growth (28 °C to 45 °C), high organic and microbial content, and increased biofilm formation create ideal conditions for *Legionella* proliferation. These conditions support the survival and multiplication of *L. pneumophila* within host cells such as free-living protozoa. However, it is important to note that the bacteria cannot replicate in the absence of a host cell.

### 5.2.3 Biofilms, Water Chemistry, and Legionella Growth

Biofilms act as a protective environment for *Legionella*, especially when the bacteria reside within protozoa. Due to network disruptions, such as leakages or maintenance work, the flow of water becomes disturbed, which in turn disrupts the biofilm. The observed surface of the biofilm was not smooth, and this can also induce slight turbulence in the system. Such disturbances can result in the release of *Legionella* into the water system.

However, the factors that determine *Legionella* colonization remain unclear, as all of the water samples from the repair work were collected either from the hydrant or from the pit. As shown in Figure 12 biofilm formation was observed on the inner surface of the cut section of the pipe, providing a safer environment for *Legionella* to survive and colonize. However, these biofilms were not analyzed further. The colonization of *Legionella* in the drinking water system therefore remains unclear, as the water samples tested negative. This makes it difficult to conclude whether *L. pneumophila* was truly absent or simply missed in the analyzed samples.

*Figure 12: Biofilm buildup in the cut section of the pipe during repair work*

Network disruptions also alter water chemistry by reducing disinfectant levels, increasing turbidity, and introducing organic matter into the water distribution network. These changes create favorable conditions for the growth of *Legionella*. Such disruptions create vulnerable situations particularly in stagnant zones, dead legs, and areas with low temperature and oxygen levels.

However, *Legionella* bacteria can replicate only inside protozoan host cells; replication does not occur in the biofilm alone without the host cell. Additionally, the bacteria can enter a VBNC state under these conditions, which makes it difficult to detect their presence without host cells. This situation might lead to false-negative results in the samples.

### 5.2.4 Mitigation Strategies for Legionella

Some of the mitigation strategies must be carried out by the municipality for the entire drinking water distribution system, while at the same time, some strategies must be implemented by building owners to protect the plumbing water systems within buildings. Apart from these, certain strategies can be carried out by both municipalities and building owners. These are explained further.

#### 5.2.4.1 In Drinking Water Distribution System

**a. Disinfection Methods:**

Use disinfectants such as chlorine, monochloramine, chlorine dioxide, copper-silver ionization, UV-C LERD, and ozone. Combining methods (e.g., UV-C LERD with ozone) can improve disinfection effectiveness.

**b. Hydraulic Management:**

Avoid water stagnation by implementing flushing in the reservoir, especially after long periods of disuse or limited use, as water age increases in the system, which results in proliferation of microorganisms.

**c. Pipe Materials:**

Materials such as stainless steel and copper pipes are used by the municipality for drinking water distribution. However, the use of copper pipes has become limited in practice, as they are not cost-efficient.

**d. Nutrient Limitation:**

Reduce organic matter to restrict the growth of biofilms and protozoa that support *Legionella* proliferation in the drinking water distribution system.

#### 5.2.4.2 In Building plumbing system

**a. Temperature Control:**

Maintain hot water temperatures above 60°C and cold water below 25°C. Apply heat shock treatment at 70°C following network disruptions within the building plumbing system.

**b. Hydraulic Management:**

Avoid water stagnation by implementing periodic flushing of pipes, especially after long periods of disuse, particularly in summer, as the water temperature rises and provides a favorable environment for bacterial proliferation. Use recirculation lines to ensure consistent water flow within the building.

**c. Pipe Materials:**

Prefer materials like stainless steel and certain types of PVC. Avoid glass, rubber, soft PVC, and iron, which promote *Legionella* growth.

**d. Nutrient Limitation:**

Reduce organic matter in the pipes, tanks, and circulation system within the building plumbing system through proper cleaning and maintenance. Poor maintenance leads to the growth of biofilms and protozoa, which support *Legionella* proliferation.

**e. Aerosol Control:**

Minimize aerosol generation in high-risk areas, such as cooling towers, to reduce exposure, and reduce the use of low-flow showers in buildings, as they generate aerosols.

#### 5.2.4.3 Suggestions from the study

Most of the strategies listed above focus on distribution system water management or on the plumbing system within buildings, managed by the municipality and building

owners respectively. For localized mitigation, attention should be given to the water inside the pipe on the downstream side and to preventing soil from entering the system during leakage and repair works.

Water samples can be tested with molecular methods like PCR to detect the presence of *Legionella* in a very short duration of time. It is preferred to collect water samples both prior to work and after completion of the work to indicate if there is a presence of *L. pneumophila*. If it indicates presence, then the water downstream should be treated, which prevents further outbreaks.

Before performing large-scale repairs, soil samples can be analyzed for the presence of *Legionella* in that particular region. In case of positive results, the work can be carried out with precautions, such as the application of disinfectants around the excavated area before cutting the pipe section, and preventive measures should be taken to avoid dust inhalation, like continuous wetting of soil to suppress dust generation. Workers are also advised to wear proper masks to avoid inhalation of contaminants during repair work and soil excavation. However, disinfectant effectiveness may vary depending on the soil type and the availability of organic content in the soil.

In case of significant soil entry into the system during repair work, a higher dosage of disinfectant can be applied for a short duration of time in the repaired section, and samples can be tested after a certain interval.

## 5.3 Limitations of the Study

- **Sampling timing restrictions:** Samples were collected effectively during maintenance work; however, follow-up sampling to monitor changes in water chemistry over time in relation to *Legionella* growth was limited due to delayed or denied building access.
- **Study boundary:** The geographical location of the sampling sites was limited to the Gothenburg region and did not include other regions, restricting the generalizability of the findings.
- **Difficulty contacting building owners:** Particularly in the case of individual housing units, privacy concerns made it challenging to reach owners, hindering the assessment of *Legionella* growth risk rates, especially during follow-ups.
- **Short notice for site access:** Access to maintenance sites depended on municipality notification times, which were sometimes given only a day before or immediately prior to the work, reducing preparation time.
- **Limited detail in notifications:** The notification website often lacked sufficient information, resulting in sampling during maintenance activities that were not always related to leakage events.

- **Incomplete network coverage:** Water samples were collected from upstream, downstream, and leakage points; however, critical network locations such as dead ends and reservoirs were not included in the sampling process.
- **Testing limitations:** The Legiolert test detected only *L. pneumophila* and did not identify other *Legionella* species, potentially underestimating overall *Legionella* presence.
- **Soil sample constraints:** Soil samples were analyzed using the same procedure as water samples due to limited laboratory facilities, which may not have been optimal for detecting *Legionella* in soil environments.
- **Unclear soil survival patterns:** Although soil samples tested positive for *L. pneumophila*, the survival and growth behavior of the bacteria around leakage areas remain poorly understood.

# 6 Conclusion

This study investigates whether upstream pipe disruptions, such as leakages and repair work, lead to the potential entry of *Legionella pneumophila* into the drinking water distribution system. The findings reveal that these disruption events do not allow the *Legionella* bacteria to enter the system. The disruption is not directly linked to *Legionella* intrusion before or immediately after the repair work. However, it may promote the possibility of entry for other contaminants and microorganisms, which were not the focus of this study. There is a possibility of false-negative results for *L. pneumophila*, as the presence of biofilm in the cut sections was not investigated further. The bacteria might survive in the biofilm under the VBNC state, which makes it difficult to identify their presence through culture-based tests like Legiolert.

The soil samples collected from the same pit of the leakage areas frequently tested positive for *L. pneumophila*, which indicates that the soil is the potential environmental reservoir of the bacteria at the repair site. However, the bacteria did not enter the system, possibly due to water pressure acting as a barrier to intrusion. There is a higher possibility of bacterial entry into the repair section during the repair work, but such entry can be prevented through proper flushing before reconnecting the system for public use.

Additionally, random samples were collected from common laundry and gyms, and some of them tested positive. This provided a better understanding of the environmental conditions and physicochemical parameters favorable for *L. pneumophila* to thrive and replicate.

Overall, the study concludes that there is no direct link between upstream disturbance and the entry of *Legionella* into the system. However, the risk of *L. pneumophila* entry into the system cannot be considered as zero, and it is always greater than 0. If *L. pneumophila* already exists within the system, it can thrive under favorable conditions like higher organic content, conductivity, minimal residual chlorine, elevated pH, and optimal growth temperature, leading to increased growth. This highlights the need for careful maintenance and effective disinfection to prevent outbreaks.

**Future Work**:

- Follow-up sampling should be conducted to improve accuracy and to understand water chemistry changes over time related to the growth of *Legionella* in the system.
- Soil samples should be analyzed using proper laboratory procedures to determine how *Legionella* growth is associated with leakage environments.
- Additional testing methods should be applied to detect the presence of other *Legionella* species in the water distribution system.
- Further research should focus on the possibility of entry through downstream sections.
- Studies should be conducted on different soil layers and surrounding environmental conditions.

- Future studies should investigate the survival of *Legionella* in biofilms to address uncertainties related to false-negative results.
- A greater number of water samples must be tested during shutdowns and in reservoirs during the summer period, as temperatures increase above 20 °C.

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# Appendix A: Water Sample Data

*Table 3: Sampling locations and physical observation*

| Sample No | Date | Repair Location | Sampling time (repair work) | Sampling Location | Condition | Warm/ cold | Comments |
|---|---|---|---|---|---|---|---|
| 1 | 19-Feb | Lille Danska Vagen | During | upstream | clear | cold | |
| 2 | 19-Feb | Lille Danska Vagen | During | pit | dirty | cold | mixed with mud |
| 3 | 26-Feb | Annaedal | Before | Sharon Kyrkan-WC | clear | warm | 1st floor near prayer hall |
| 4 | 26-Feb | Annaedal | Before | Sharon Kyrkan-Kok | clear | warm | ground floor kitchen |
| 5 | 26-Feb | Annaedal | Before | SATS, Landala | clear | warm | WC |
| 6 | 26-Feb | Annaedal | Before | BVC, Landala | clear | cold | WC |
| 7 | 27-Feb | Random sampling | - | Meteorgatan | clear | warm | laundry room |
| 8 | 27-Feb | Random sampling | - | Meteorgatan, Bergsjon | clear | warm | shower from my home |
| 9 | 26-Feb | Random sampling | - | Antons Gym, Kortidala | clear | warm | shower |
| 10 | 26-Feb | Random sampling | - | Antons Gym, Kortidala | clear | warm | shower near sauna |
| 11 | 26-Feb | Random sampling | - | Antons Gym, Kortidala | clear | warm | WC |
| 12 | 28-Feb | Bö | Before | örgryte Kykan-underground | clear | warm | kind of ventilation room |
| 13 | 28-Feb | Bö | Before | örgryte Kykan-underground | clear | warm | ground floor kitchen |
| 14 | 28-Feb | Bö | During | maintenance work pit | dirty | cold | mixed with some particles (kind of rust of around 1mm ) |
| 15 | 28-Feb | Bö | During | upstream | | cold | mixed with some particles (kind of rust of around 1mm ) |
| 16 | 28-Feb | Bö | After | örgryte Kykan-underground | | warm | ground floor kitchen |
| 17 | 28-Feb | Annaedal | After | Sharon Kyrkan-WC | clear | warm | 1st floor near prayer hall |
| 18 | 28-Feb | Annaedal | After | Sharon Kyrkan-Kok | clear | warm | ground floor kitchen |
| 19 | 13-Mar | Kalandersvagen, kortidala | Before | office, common Kok | clear | warm | All the samples were taken from the |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 20 | 13-Mar | Kalandersvagen, kortidala | Before | Office women changing room | clear | warm | familja bostader office at kortidala and the upstream from the building 42 quite far away from the office building. The water from the kitchen and women's changing room was clear before the repair work but after the maintenance work the watercolor changed to slightly yellowish color. |
| 21 | 13-Mar | Kalandersvagen, kortidala | Before | Office workshop | clear | cold | |
| 22 | 13-Mar | Kalandersvagen, kortidala | During | Pit (inside pipe) | little dirty | cold | |
| 23 | 13-Mar | Kalandersvagen, kortidala | During | upstream | clear | cold | |
| 24 | 13-Mar | Kalandersvagen, kortidala | After | office, common Kok | slightly yellow | warm | |
| 25 | 13-Mar | Kalandersvagen, kortidala | After | Office women changing room | slightly yellow | warm | |
| 26 | 13-Mar | Kalandersvagen, kortidala | After | Office workshop | clear | cold | |
| 27 | 21-Mar | Kalandersvagen, kortidala | 1 week later | common kök | clear | warm | at the same time the building was constructed in 1960s, and the radiators are connected to the hot water system |
| 28 | 21-Mar | Kalandersvagen, kortidala | 1 week later | women's changing room | clear | warm | |
| 29 | 21-Mar | Kalandersvagen, kortidala | 1 week later | workshop | clear | cold | |
| 30 | 11-Apr | Marklandsgatan | Before | downstream | clear | cold | |
| 31 | 11-Apr | Marklandsgatan | During | upstream | clear | cold | |
| 32 | 11-Apr | Marklandsgatan | During | downstream | clear | cold | After repair work-test flush before opening the valve to public usage |
| 33 | 17-Apr | Gränsvägen | During | upstream | clear | cold | |
| 34 | 17-Apr | Gränsvägen | Before | downstream | slightly yellow | cold | |
| 35 | 17-Apr | Gränsvägen | During | Pit (pipe) | slight muddy | cold | |
| 36 | 17-Apr | Gränsvägen | after | downstream | muddy | cold | After repair work-test flush before opening the valve to public usage |
| 37 | 23-Apr | Påskbergsgatan | Before | upstream | clear | cold | Not leakage, the section of the pipe is closed for usage to add extra valve in the existing pipe section |
| 38 | 8-May | Random sampling | - | Tamburingatan, Laundry room | clear | warm | unused for long time and water collected from the initial flow. |
| 39 | 13-May | Älvsborg | Before | downstream | clear | cold | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 40 | 13-May | Älvsborg | During | pit | muddy | cold | |
| 41 | 14-May | lunden | During | pit | blackish grey | cold | no upstream fire hydrant as the area was too wide, entire section was closed for usage. And the repair work happened in the middle of the road |
| 42 | 20-May | Klareborgsgatan, majorna | During | downstream | yellow | cold | unused for a long time |
| 43 | 20-May | Klareborgsgatan, majorna | During | pit | yellowish mixed with sand | cold | |
| 44 | 4-Apr | Random sampling | - | Chalmers WET lab | clear | warm | with Na2S2O3 |
| 45 | 4-Apr | Random sampling | - | Chalmers WET lab | clear | warm | without Na2S2O3 |
| 46 | 6-May | Random sampling | - | Stockholm | clear | cold (maybe hot) | samples were sent from Stockholm, which is definitely positive. Mixed all together and analyzed in 5 different trays result based on mean of all samples togather. |

# Appendix B: Legiolert Test Results

*Table 4: Legiolert test result of water sample*

| Sample No | Date of incubation | Date of analysis | Small wells positive | Large wells positive | MPN value | Dilution |
|---|---|---|---|---|---|---|
| 1 | 2/19/2025 | 2/26/2025 | 0 | 0 | <1 | - |
| 2 | 2/19/2025 | 2/26/2025 | 0 | 0 | <1 | 1:10 |
| 3 | 2/26/2025 | 3/5/2025 | 0 | 0 | <1 | - |
| 4 | 2/26/2025 | 3/5/2025 | 0 | 0 | <1 | - |
| 5 | 2/26/2025 | 3/5/2025 | 0 | 0 | <1 | - |
| 6 | 2/26/2025 | 3/5/2025 | 0 | 0 | <1 | - |
| 7 | 2/27/2025 | 3/6/2025 | 0 | 0 | <1 | - |
| 8 | 2/27/2025 | 3/6/2025 | 0 | 0 | <1 | - |
| 9 | 2/26/2025 | 3/6/2025 | 2 | 1 | 3.2 | - |
| 10 | 2/26/2025 | 3/6/2025 | 0 | 0 | <1 | - |
| 11 | 2/26/2025 | 3/6/2025 | 5 | 3 | 10.8 | - |
| 12 | 2/28/2025 | 3/7/2025 | 0 | 0 | <1 | - |
| 13 | 2/28/2025 | 3/7/2025 | 0 | 0 | <1 | - |
| 14 | 2/28/2025 | 3/7/2025 | 0 | 0 | <1 | - |
| 15 | 2/28/2025 | 3/7/2025 | 0 | 0 | <1 | - |
| 16 | 2/28/2025 | 3/7/2025 | 0 | 0 | <1 | - |
| 17 | 2/28/2025 | 3/7/2025 | 0 | 0 | <1 | - |
| 18 | 2/28/2025 | 3/7/2025 | 0 | 0 | <1 | - |
| 19 | 3/13/2025 | 3/20/2025 | 0 | 0 | <1 | - |
| 20 | 3/13/2025 | 3/20/2025 | 0 | 0 | <1 | - |
| 21 | 3/13/2025 | 3/20/2025 | 0 | 0 | <1 | - |
| 22 | 3/13/2025 | 3/20/2025 | 0 | 0 | <1 | - |
| 23 | 3/13/2025 | 3/20/2025 | 0 | 0 | <1 | - |
| 24 | 3/13/2025 | 3/20/2025 | 0 | 0 | <1 | - |
| 25 | 3/13/2025 | 3/20/2025 | 0 | 0 | <1 | - |
| 26 | 3/13/2025 | 3/20/2025 | 0 | 0 | <1 | - |
| 27 | 3/21/2025 | 3/28/2025 | 0 | 0 | <1 | - |
| 28 | 3/21/2025 | 3/28/2025 | 0 | 0 | <1 | - |
| 29 | 3/21/2025 | 3/28/2025 | 0 | 0 | <1 | - |
| 30 | 4/11/2025 | 4/18/2025 | 0 | 0 | <1 | - |
| 31 | 4/11/2025 | 4/18/2025 | 0 | 0 | <1 | - |
| 32 | 4/11/2025 | 4/18/2025 | 0 | 0 | <1 | - |
| 33 | 4/17/2025 | 4/24/2025 | 0 | 0 | <1 | - |
| 34 | 4/17/2025 | 4/24/2025 | 0 | 0 | <1 | - |
| 35 | 4/17/2025 | 4/24/2025 | 0 | 0 | <1 | - |
| 36 | 4/17/2025 | 4/24/2025 | 0 | 0 | <1 | 1:10 |
| 37 | 4/23/2025 | 4/30/2025 | 0 | 0 | <1 | - |
| 38 | 5/8/2025 | 5/15/2025 | 0 | 0 | <1 | - |
| 39 | 5/13/2025 | 5/20/2025 | 0 | 0 | <1 | - |
| 40 | 5/13/2025 | 5/20/2025 | 0 | 0 | <1 | 1:10 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 41 | 5/14/2025 | 5/21/2025 | 0 | 0 | <1 | 1:10 |
| 42 | 5/20/2025 | 5/27/2025 | 0 | 0 | <1 | - |
| 43 | 5/20/2025 | 5/27/2025 | 0 | 0 | <1 | - |
| 44 | 4/4/2025 | 4/11/2025 | 0 | 0 | <1 | - |
| 45 | 4/4/2025 | 4/11/2025 | 0 | 0 | <1 | - |
| 46 | 5/6/2025 | 5/13/2025 | 6 | 30 | 218.24 | - |

**Hardness**: 0.3–0.7 for all water samples, as the samples are potable and taken from the drinking water distribution system.

**Supplement for Legionella**: 0.33 g, calculated based on the water hardness. **Test volume**: 100 mL of sample water was used for the Legiolert test procedure. **Table notation**: Samples highlighted in green indicate random samples; positive samples are highlighted in red cells.

Sample number 46 was collected from Stockholm. Four bottles of water samples were taken from the same location at different time intervals. All samples were combined and analyzed using five Quanti-Trays. The mean MPN value obtained from these analyses was used as the final result presented in the above table.

*Table 5: Mean calculation of sample 46*

| Tray no. | Positive large well | Positive small well | MNP value |
|---|---|---|---|
| 1 | 6 | 30 | 204.8 |
| 2 | 6 | 7 | 41.6 |
| 3 | 6 | 50 | 409.6 |
| 4 | 6 | 31 | 213.3 |
| 5 | 6 | 32 | 221.9 |
| **Mean:** | **6** | **30** | **218.24** |

# Appendix C: Soil sample datasheet

*Table 6: Soil sample datasheet with Legiolert test result*

| Soil sample number | Sample Location | Soil type SGU | Soil type | wet/dry | Positive large wells | Positive small wells | MPN table |
|---|---|---|---|---|---|---|---|
| SS1 | Marklandsgatan | postglacial clay | sandy gravel | wet | 1 | 0 | 1.1 |
| SS2 | Gränsvägen | postglacial clay | clay | dry | 3 | 6 | 12.3 |
| FSS3 | Påskbergsgatan | bedrock (Urberg) | silty sand | dry | 3 | 0 | 3.9 |
| SS3 | Påskbergsgatan | bedrock (Urberg) | silty sand | dry | 0 | 9 | 9.1 |
| FSS4 | Kungsladugård | postglacial sand | sandy gravel | dry | 0 | 14 | 14.2 |
| SS4 | Kungsladugård | postglacial sand | sandy gravel | dry | 0 | 1 | 1 |
| FSS5 | Kungsladugård | postglacial sand | sandy silt | dry | 0 | 0 | <1 |
| SS5 | Kungsladugård | postglacial sand | sandy silt | dry | 2 | 0 | 2.3 |
| FSS6 | Älvsborg | postglacial sand | sand | dry | 0 | 0 | <1 |
| SS6 | Älvsborg | postglacial sand | sand | dry | 4 | 0 | 5.8 |
| FSS7 | Älvsborg | postglacial sand | gravel | dry | 0 | 0 | <1 |
| SS7 | Älvsborg | postglacial sand | gravel | dry | 0 | 0 | <1 |
| FSS8 | Lunden | glacial clay | silt | dry | 0 | 0 | <1 |
| SS8 | Lunden | glacial clay | silt | dry | 0 | 0 | <1 |
| FSS9 | Ullevi Norra | postglacial clay | sandy gravel | dry | 0 | 0 | <1 |
| SS9 | Ullevi Norra | postglacial clay | sandy gravel | dry | 0 | 0 | <1 |
| FSS10 | Majorna | glacial clay | sandy clay | wet | 0 | 0 | <1 |
| SS10 | Majorna | glacial clay | sandy clay | wet | 5 | 16 | 53.6 |
| FSS11 | Majorna | glacial clay | sand | dry | 0 | 0 | <1 |
| SS11 | Majorna | glacial clay | sand | dry | 0 | 0 | <1 |

# Appendix D: Water Quality Parameters

*Table 7: Physiochemical water quality parameters of all collected samples*

| | | | | | | with $Na_2S_2O_3$ | without $Na_2S_2O_3$ |
|---|---|---|---|---|---|---|---|
| **Sample No** | **Turbidity (FRU)** | **Temperature 0C** | **Conductivity (µS/cm)** | **pH** | **TOC (mg/L)** | **Free cl- (mg/L Cl2-)** | |
| 1 | 0 | 17.9 | 201 | 7.567 | 2.063 | - | - |
| 2 | 504 | 17.9 | 154.7 | 7.593 | 2.704 | - | - |
| 3 | 1 | 15.7 | 191.2 | 7.778 | 2.05 | - | - |
| 4 | 1 | 15.7 | 183.3 | 7.827 | 1.595 | - | - |
| 5 | 1 | 16.5 | 179.2 | 7.826 | 1.553 | - | - |
| 6 | 1 | 16.7 | 180.7 | 7.845 | 1.502 | - | - |
| 7 | 1 | 25.7 | 208 | 7.845 | 0.7098 | - | - |
| 8 | 0 | 23.2 | 287.4 | 7.794 | 1.52 | - | - |
| 9 | 0 | 20.5 | 186.9 | 7.784 | 1.315 | - | - |
| 10 | 0 | 20.2 | 178.7 | 7.805 | 1.615 | - | - |
| 11 | 1 | 17.9 | 178.3 | 7.82 | 1.518 | - | - |
| 12 | 1 | 20.5 | 182.4 | 7.656 | 1.375 | - | - |
| 13 | 0 | 20.4 | 183.4 | 7.752 | 1.595 | - | - |
| 14 | 7 | 19.8 | 182.9 | 7.483 | 2.124 | - | - |
| 15 | 2 | 20 | 183.4 | 7.715 | 2.68 | - | - |
| 16 | 0 | 20.6 | 183.7 | 7.672 | 1.266 | - | - |
| 17 | 1 | 27.6 | 187.9 | 7.764 | 1.488 | - | - |
| 18 | 1 | 24.3 | 184.6 | 7.927 | 1.33 | - | - |
| 19 | 0 | 20.5 | 194.1 | 7.762 | 1.372 | - | - |
| 20 | 1 | 20.3 | 178 | 7.732 | 1.405 | - | - |
| 21 | 1 | 19.9 | 177.1 | 7.671 | 1.4 | - | - |
| 22 | 28 | 20 | 177.7 | 7.813 | 1.8 | - | - |
| 23 | 0 | 11.4 | 186.5 | 7.446 | 1.429 | - | - |
| 24 | 11 | 11.4 | 177.3 | 7.507 | 1.642 | - | - |
| 25 | 8 | 11.8 | 177.5 | 7.488 | 1.536 | - | - |
| 26 | 11 | 12.1 | 177 | 7.545 | 1.333 | - | - |
| 27 | 0 | 22.9 | 180.7 | 7.801 | 1.738 | - | - |
| 28 | 1 | 24.8 | 176.5 | 7.728 | 1.603 | - | - |
| 29 | 1 | 22.5 | 177 | 7.642 | 1.604 | - | - |
| 30 | 3 | 12.6 | 184.7 | 7.647 | 1.863 | 0.03 | 0.01 |
| 31 | 0 | 14.5 | 179.2 | 7.818 | 1.954 | 0.04 | 0.01 |
| 32 | 5 | 15.1 | 179.4 | 7.818 | 2.015 | 0.01 | 0.02 |
| 33 | 0 | 17.4 | 178.8 | 7.92 | 2.045 | 0.03 | - |
| 34 | 6 | 17.9 | 179.5 | 7.89 | 1.913 | 0.06 | - |
| 35 | 5 | 18.5 | 178.3 | 7.949 | 2.48 | 0.07 | - |
| 36 | 99 | 18.1 | 181.3 | 7.989 | 2.246 | 0.04 | - |
| 37 | 2 | 15 | 183.7 | 7.78 | 1.858 | 0.05 | - |
| 38 | 0 | 19 | 184.8 | 7.863 | 1.657 | - | - |
| 39 | 0 | 18.8 | 215 | 7.972 | 2.654 | - | - |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 40 | 376 | 18.8 | 188.8 | 8.01 | 2.307 | - | - |
| 41 | 436 | 17.8 | 250 | 7.772 | 3.291 | - | - |
| 42 | 183 | 19.7 | 158.7 | 7.767 | 2.796 | - | - |
| 43 | 81 | 19.5 | 164.6 | 7.748 | 1.921 | - | - |
| 44 | 1 | 28.4 | 187.8 | 7.77 | 1.76 | 0.01 | - |
| 45 | 0 | 27.4 | 179.2 | 7.784 | 1.572 | - | 0.01 |
| 46 | 0 | 18.4 | 278 | 7.96 | 4.976 | 0.01 | - |

*Table 8: Water quality parameters of random samples*

| Sample No | Turbidity (FRU) | Temperature C | Conductivity (µS/cm) | pH | TOC (mg/L) | Free cl- (mg/L Cl2-) |
|---|---|---|---|---|---|---|
| 7 | 1 | 25.7 | 208 | 7.845 | 0.7098 | - |
| 8 | 0 | 23.2 | 287.4 | 7.794 | 1.52 | - |
| 9 | 0 | 20.5 | 186.9 | 7.784 | 1.315 | - |
| 10 | 0 | 20.2 | 178.7 | 7.805 | 1.615 | - |
| 11 | 1 | 17.9 | 178.3 | 7.82 | 1.518 | - |
| 38 | 0 | 19 | 184.8 | 7.863 | 1.657 | - |
| 44 | 1 | 28.4 | 187.8 | 7.77 | 1.76 | 0.01 |
| 45 | 0 | 27.4 | 179.2 | 7.784 | 1.572 | - |
| 46 | 0 | 18.4 | 278 | 7.96 | 4.976 | 0.01 |

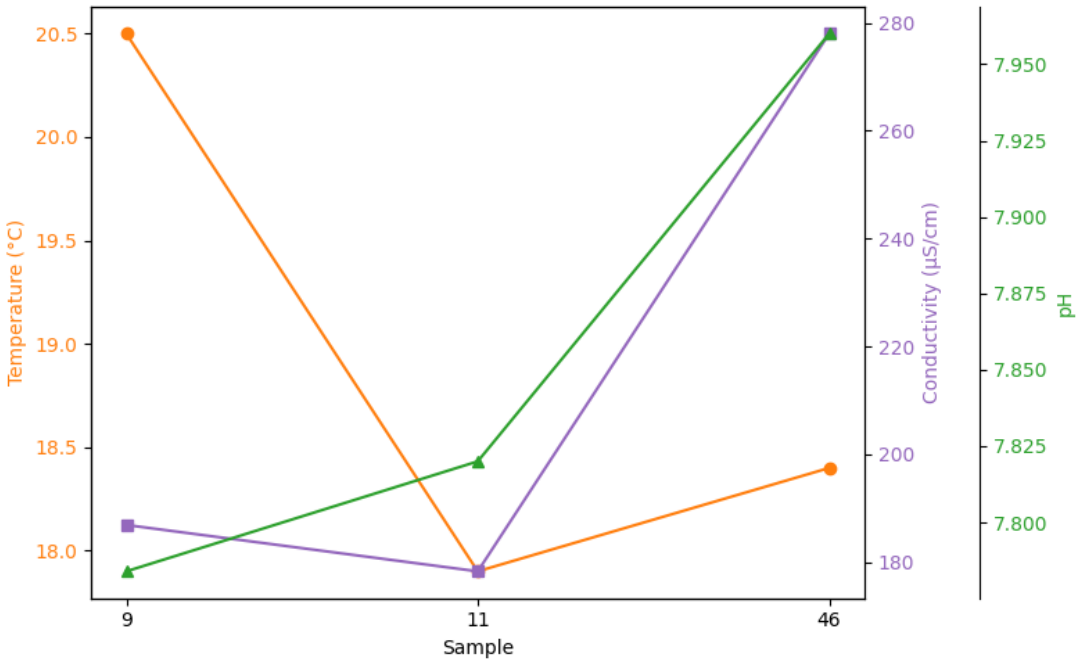


*Figure 13: Combined graph of temperature, conductivity and pH of the positive random samples*

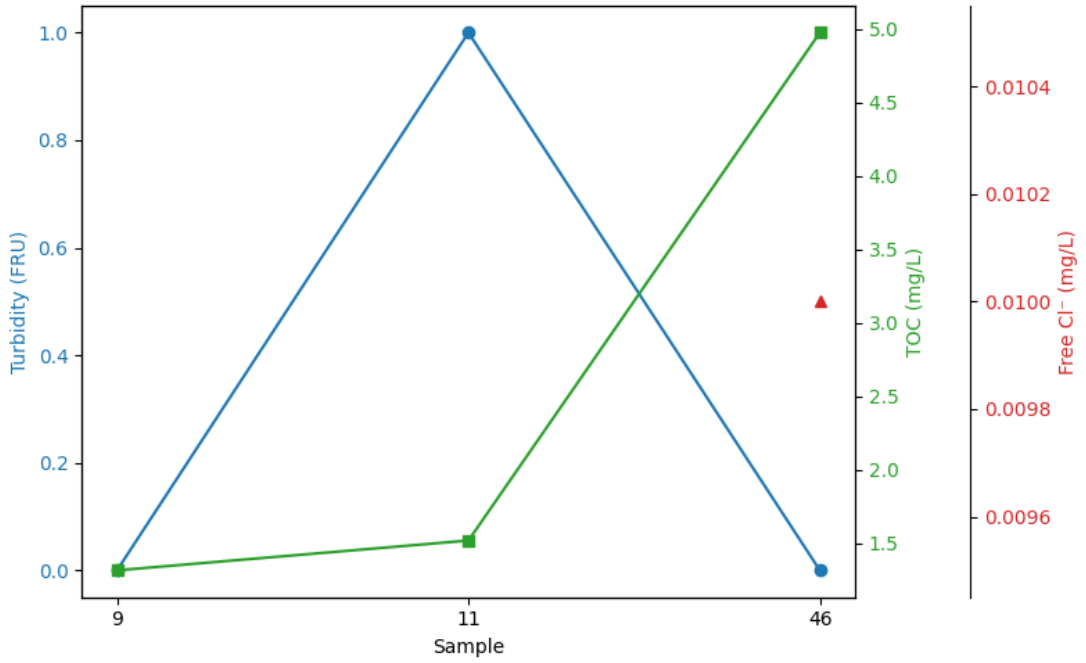


*Figure 14: Combined graph of turbidity, TOC and free chlorine of the random positive samples*

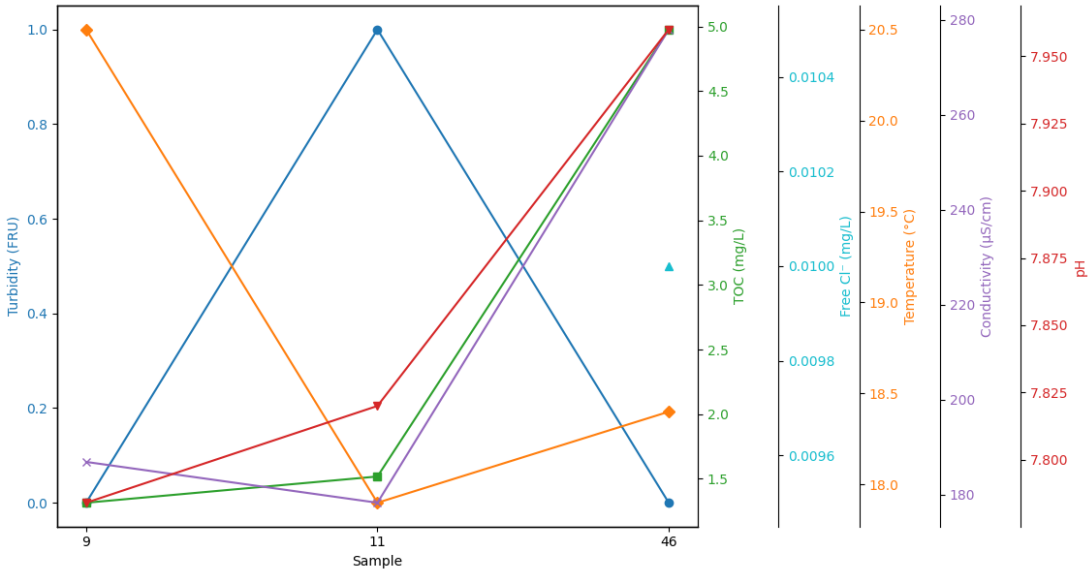


*Figure 15: Combined graph of water quality parameters (turbidity, TOC, free chlorine, temperature, conductivity and pH) of random positive samples*

# Appendix E: Field Work Images

*Figure 16: Flushing of excess water from the pit during the maintenance work*

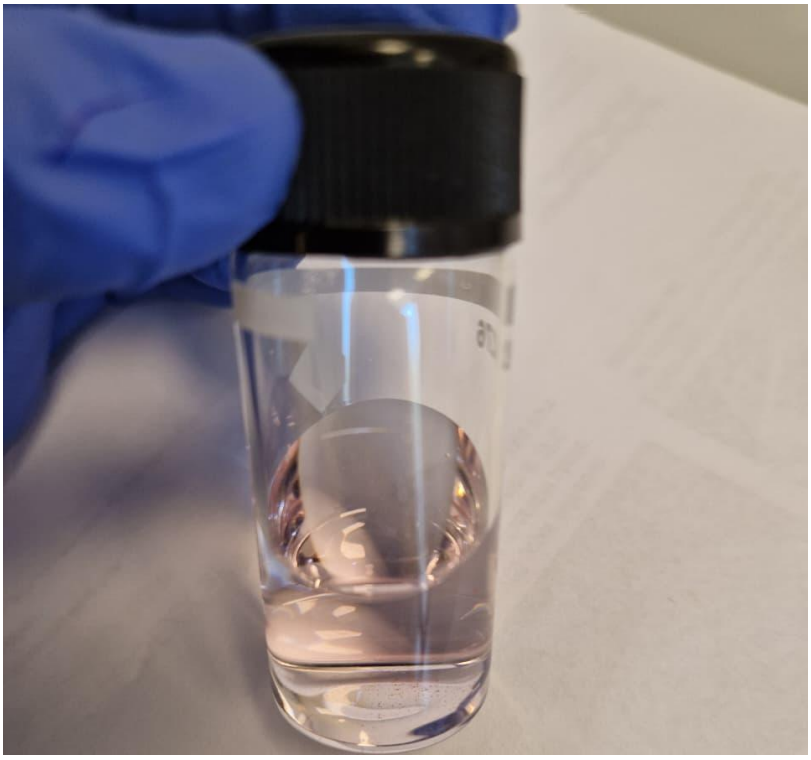

*Figure 17: Color changed water indicating the presence of free chlorine*

*Figure 18: Leakage section after repair work*

*Figure 19: Connecting currently in usage pipe section with already existing unused pipe section*


*Figure 20: Defrost frozen water samples for TOC analysis. samples were marked with name, sample number and date of collection.*


*Figure 21: Preparing samples for TOC analysis 20ml distilled water with 20ml water sample*

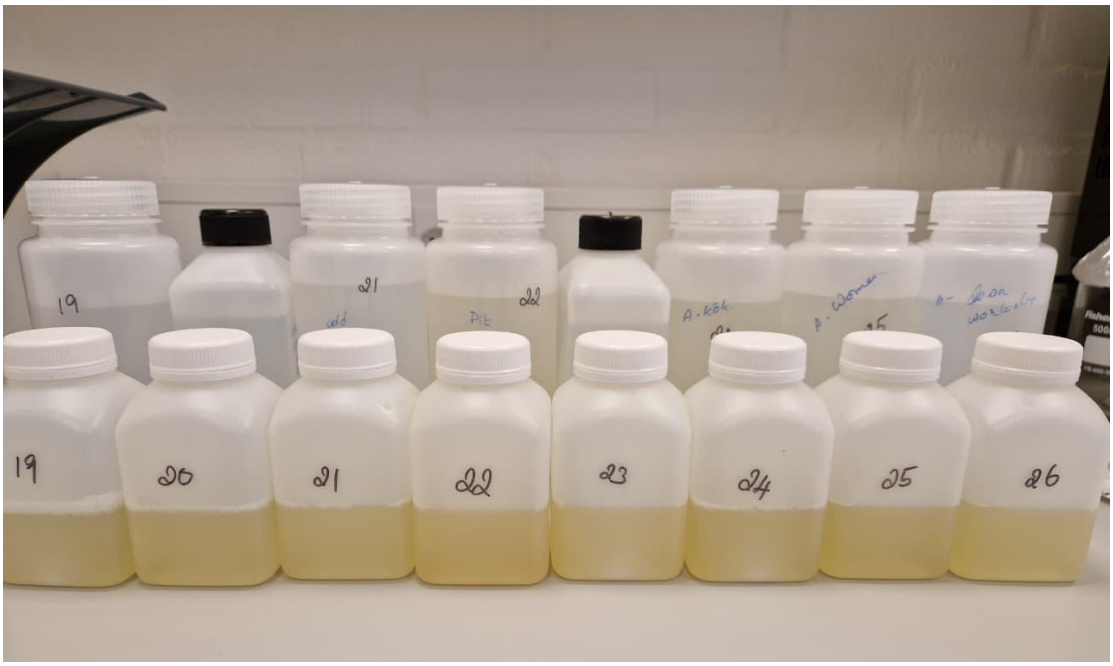


*Figure 22: Prepared samples for Legiolert test procedure*

*Figure 23: Upstream and Downstream fire hydrants where the water samples were collected.*