MANAGING THE RISK OF LEGIONELLA IN DRINKING WATER AERATION PROCESSES

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MANAGING THE RISK OF *LEGIONELLA* IN DRINKING WATER AERATION PROCESSES

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ABSTRACT

Experimental and probabilistic methods were used to assess the risk of exposure to *Legionella sp* from aerators used in groundwater treatment plants. Factors considered include an assessment of conditions conducive to *Legionella* growth, detachment and inhalation by operators; the use of coupon studies to understand temporal changes and biofilm formation; and modelling the risk of *Legionella* using iterative Bayesian networks (BNs).

A survey of 13 groundwater treatment plants (GWTPs) aerators, including tray, open and semi-enclosed systems were identified to feature design and operational risk factors favouring elevated levels of nutrients, water stagnation, challenging water quality, aerosolisation, and inconsistent operation and maintenance. Based on these observations, design considerations for the next generation of safer aerators that can overcome identified *Legionella* risks factors were outlined.

Analysis of 300 sampling events from the aerators over five years indicated an average of 7% increase in colony counts between the inlet and outlet, indicating growth of *Legionella* within the aerators. In total, 28% of all samples collected from aerator surfaces tested positive for *Legionella*. However, there was no correlation between the type of aerator and *Legionella* positivity.

Coupons were placed in aerators to assess temporal changes in fouling developed after 6 weeks of operation. The biological activity per unit area (ATP/cm^2) was higher for samples collected on the sprayed (vertically placed) coupons (277 ng ATP/cm^2) compared with the submerged (horizontally laid) (73 ng ATP/cm^2) coupons. Concentrations of dissolved organic carbon (DOC) in the biofilm formed on the coupons were statistically similar for the two tested conditions. Comparing fouling characteristics from the lab and full-scale coupons confirmed the impact of surface orientation and influent characteristics on biofilm formation. In terms of cleaning of the fouled surface, NaOCl at (concentration $\geq$ 6%) was found to achieve 99.9% efficiency in biofilm inactivation. Oxalic acid (concentration >1%) significantly removed inorganic materials like iron and manganese. Combining biocides and antiscalants was therefore recommended to efficiently address fouling challenges in aerators.

A BN which considered risk of exposure due to growth and transmission was developed using a fishbone diagram and bowtie analysis. The initial iterative output BN model was elicited deterministically through expert weighted scoring process and discretisation approach and defined relative contributions of risk variables. The BN model also efficiently categorised and differentiated *Legionella* risk thresholds. A revised BN model conceptually mapped and estimated the causes and consequences of *Legionella* aerosolisation separately. The *Legionella* growth sub-model showed weak prediction accuracy with a negative kappa coefficient, signifying inconsistency in predicted and observed *Legionella* occurrence.

The effect of water quality was further explored with a data-driven learning approach using diverse historical water quality records. The optimised BN model utilised the greedy thick thinning approach, complemented with domain knowledge, and achieved superior performance accuracy exceeding 90%. The results indicated that water temperature, free chlorine, season, and heterotrophic plate count can be utilised to track *Legionella* occurrence in water systems.

LIST OF PUBLICATIONS

Danladi Yunana, Stuart Maclaine, Keng Han Tng, Luke Zappia, Ian Bradley, David Roser, Greg Leslie, C Raina MacIntyre, Pierre Le-Clech (2021). Developing Bayesian networks in managing the risk of *Legionella* colonisation of groundwater aeration systems. Water Research, 193, DOI: 10.1016/j.watres.2021.116854

Danladi Yunana, A Branch, K H Tng, I Bradley, L Zappia and P Le-Clech (2019). Legionella Exposure Risk in Groundwater Treatment Plants Using Bayesian models to assess exposure risks for aeration systems. Australian Water e-Journal Vol 4 No 4 2019. https://doi.org/10.21139/wej.2019.024

Danladi Yunana, Pierre Le-Clech (2022). Student research summary -Managing the Risk of Legionella in groundwater aeration systems Australian Water e-journal

Conference presentations:

Application of Bayesian belief networks to better characterise the risk of *Legionella* colonisation of groundwater aeration unit," presented at Tenth Annual Conference of the Australasian Bayesian Network. Modelling Society (ABNMS), December 4 – 5, 2018 Adelaide South Australia

Bayesian approach to managing and predicting *Legionella* colonisation of aeration systems in groundwater treatment", presented at National Science Foundation (NSF) Legionella Conference, September 11 – 13, Los Angeles, USA.

Characterisation of deposits in groundwater aerators: implications for colonisation by Legionella," presented at the International Water Association – Young water Professional (IWA -YWP) Conference, June 23 – 27, Toronto, Canada.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS AND NOMEMCLATURE

ANOVA	Analysis of variance
AOC	Assimilable Organic Carbon
AS/NZS	Australian/New Zealand Standards
ATP	Adenosine Triphosphate
AUC	Area Under Curve
BB	Building Blocks
BP	Biopolymers
BNs	Bayesian Networks
BYCE	Buffered charcoal yeast extract
CA	Citric acid
CFU	Colony Forming Units
CPTs	Conditional Probability Tables
DOC	Dissolved organic carbon
DNA	Deoxyribonucleic Acid
DOM	Dissolved Organic Matter
EDTA	Ethylenediaminetetraacetic Acid
EMA	Ethidium Monoazide
EPS	Extracellular polymeric substances
FEEM	Fluorescence Excitation Emission Matrix
FD	Fishbone diagram
GTT	Greedy Thick Thinning
GU	Genomic Units
GWTPs	groundwater treatment plants
HCl	Hydrochloric Acid
HIDRA	Hazard Identification and Risk Assessment
HPC	Heterotrophic Plate Counts
HVAC	Heating Ventilation, and Air Conditioning
HS	Humic-like Substances
IDEA	Investigate Discuss Estimate Aggregate
ISO	International Standard Organisation
ISO/TS	International Standard Organisation Technical Specifications
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry

K	Cohen's Kappa
LHLRA	*Legionella* High Level Risk Assessment
LC-OCD	Liquid Chromatography – Organic Carbon Detection
LMWA	Low Molecular Weight Acids
LMWN	Low Molecular Weight Neutrals
LRV	Log removal value
MC	Moisture Content
NaOH	Sodium Hydroxide
HNO_3	Nitric acid
NOM	Natural Organic Matter
PA	Prediction accuracy
PMA	Propidium Monoazide
PVC	Polyvinyl Chloride
PVDF	Polyvinylidene fluoride or polyvinylidene difluoride
PBS	Phosphate Buffer Solution
PARAFAC	PARAllel FACtor analysis
RLU	Relative Light Units
ROC	Receiver Operator Characteristic
TS	Total solids
QCM	Quartz Crystal Microbalance
QPCR	Quantitative Polymerase Chain Reaction
VR	Variance Reduction
WCWA	Water Corporation of Western Australia
XRD	X-ray powder diffraction

<u>CHAPTER 1</u>

THESIS INTRODUCTION

1 INTRODUCTION

1.1 Background

Legionella bacteria in natural and engineered water environments are considered one of the leading causes of waterborne disease outbreaks (van Heijnsbergen *et al*., 2015). In natural settings including groundwater, the bacterium is considered benign posing little or no threat to public health. However, *Legionella* bacteria tend to proliferate to high densities in engineered systems that operate in warm conditions, and also support periodic stagnation, biofilm formation as well as elevated nutrients (Buse *et al*., 2012a; Völker *et al*., 2016a). Inhalation or aspiration of contaminated aerosols from these systems can lead to Legionnaires' disease. Efforts to manage the frequency of Legionnaires' disease outbreaks have been, so far, focused on air and water handling systems in buildings, overlooking drinking water treatment assets (Bentham, 2000; Cline *et al*., 2020).

Typically, groundwater is characterised by lower levels of microbial pathogens, thus, groundwater treatment is less advanced compared to surface water (Costa *et al*., 2005; Gydesen and Tkker, 2013). Despite the generally low levels of harmful microbes, *Legionella* has been shown to inhabit groundwater sources (De Giglio *et al.,* 2019). The occurrence of *Legionella* in groundwater can be linked to geothermal activity that can create optimal temperature for pathogen growth. Although *Legionella* occurrences are lower in groundwater and often lack the path for direct human contact, they can potentially increase within the drinking water treatment processes where favourable habitat is provided (Wullings *et al.,* 2011a).

A drinking water treatment plant supplied by groundwater demonstrated drastic increases in *Legionella* contamination during treatment (Wullings *et al*., 2011a). While *Legionella* was not detected in the feed water, several treatment processes, including rapid sand filter, pellet softener, and treated water samples, were contaminated with the pathogen (Wullings *et al*., 2011a). The increased detection of *Legionella* within the treatment plant suggests the critical need to assess aeration systems. From a health risk perspective, aeration systems are important in treatment processes because of their ability to create aerosol, thus establishing the potential for direct human exposure to *Legionella*.

Aeration units commonly used as a pre-treatment process in groundwater treatment plants (GWTPs) are considered one of such overlooked systems at risk of *Legionella* colonisation. Aeration systems are used as the first step for treatment of groundwater intended for drinking water applications. Aerators are designed to facilitate the contact of groundwater and air to remove undesirable water components and gas (for example, CO_2), correct pH, and oxidise dissolved components (such as ammonia, sulphide, or metal ions) to more desirable (or treatable) species. Aeration systems also help to lower water temperature (Duranceau and Smith, 2016; Siabi, 2008; Yoakum and Duranceau, 2018).

This relatively simple and efficient process has been widely implemented in many drinking water treatment plants, but with little (or no) consideration for monitoring, maintenance, and cleaning. The generally poorly defined strategy for designing, operating, and maintaining drinking water aeration systems has resulted in fouling, biofilm and microbial growth, including *Burkholderia pseudomallei* in Northern Australia (Inglis *et al*, 2000). The presence of biofilms have been strongly correlated to increased water contaminations further promoting the viability of opportunistic pathogens (Bédard *et al*., 2018). The efficient contact between air and water in aeration systems is an additional factor that can accelerate the rate at which opportunistic microbial pathogens may grow within these systems and may result in the generation of aerosols contaminated with *Legionella.* Presently, there is limited knowledge regarding the presence and fate of *Legionella* in GWTPs aerators.

Specifically, in Western Australia, operational aerator assets, including open spray and tray aerators employed as pre-treatment processes in GWTPs, has been deemed to pose an unacceptable *Legionella* risk. However, these assets have been overlooked as sources of *Legionella* exposure, a situation that potentially presents a health risk to operators of treatment plant and residents of local communities (Zappia, 2015). This potential risk is expected to be significantly under-recognised across drinking water treatment assets in regions of hot and temperate climates worldwide. The limited understanding and absence of a practical standard for *Legionella* monitoring and management in drinking water systems underscore the low consideration of the health risk of this pathogen by water utilities (LeChevallier, 2019a).

Literature and standards exist regarding the occurrence and control of *Legionella* in a variety of engineered air-water handling systems (AS/NZS 3666), but little information is available for groundwater aeration technologies specifically. Due to low concentrations and sporadic occurrence, the relevance of *Legionella* contamination within drinking water treatment processes are overshadowed by that in buildings and cooling towers (Bentham, 2000; Cline *et al*., 2020). While total elimination of *Legionella* in drinking water treatment including aerator may not be feasible, better understanding is needed to develop appropriate strategies and monitoring guidelines within these assets.

As a result, this thesis systematically investigates and provides an increased understanding of water quality parameters and environmental factors that promote and contribute to *Legionella* growth and transmissions in these assets through a range of sampling and advanced analysis. Additionally, characterisation of biofilms considered to be strongholds for *Legionella,* will be conducted within aeration assets. This research project will use advanced risk characterisation activities to close an important industry gap by creating a reliable risk assessment that specifically account for the potential presence and transmission of *Legionella* from within the aeration systems.

1.2 Thesis aim and objectives

This research project aims to provide a better understanding of mechanisms and factors influencing the occurrence of *Legionella* pathogens in aerators used in GWTPs and to identify measures to mitigate associated health risk. The research addresses the potential for *Legionella* growth, assess presence of pathway to human exposure, explore risk assessment for better management of current assets and provides new information for improved and safer designs.

In order to achieve the established aims, the following research objectives were defined:

1. Establish current knowledge on *Legionella* risk in the aeration systems used in drinking water treatment (Chapter 2).
2. Investigate risk factors associated with *Legionella* growth, persistence, and transmission in operating GWTPs aerators (Chapter 3).
3. Monitor and characterise temporal changes in aerator fouling deposition and biofilm formation and to optimise cleaning and control measures for existing aerators (Chapter 4).
4. Develop risk assessment tools for monitoring *Legionella* in aerator systems
    a. Develop a Bayesian network (BN) model for assessing *Legionella* risk (Chapter 5).
    b. Develop variables and parameters from water quality that can be continuously monitored to predict *Legionella* occurrences (Chapter 6).
5. Synthesise the main findings of this research and propose opportunities for future work and significance in the water industry (Chapter 7).

1.3 Chapter descriptions

This thesis is subdivided into nine chapters covering the areas of study completed to address the risk of *Legionella* in GWTP aeration systems. The introduction of this thesis (Chapter 1) describes the problem of *Legionella* bacteria in groundwater aerators. This chapter also outlines the research objectives and summarises the thesis structural outline.

Chapter 2 reviews existing literature on managing *Legionella* pathogen colonisation of aeration systems used in GWTPs. The chapter explores different aeration design configurations, functions, operational characteristics, and limitations considered favourable risk factors for *Legionella* growth and transmissions. The implications of *Legionella* pathogen colonisation of aerators for public health, the absence of management guidelines and control measures needed to minimise risk are identified.

Chapter 3 involves a preliminary assessment of the risk of *Legionella* in groundwater treatment aerators with the aim to assess the presence of risk factors associated with *Legionella* growth and transmission. Existing aerator assets were surveyed to identify design and operational constraints that can be considered favourable to the risk of *Legionella*

The analysis of historical influent water quality parameters and grab samples of effluents and fouling characteristics confirms the source and fate of nutrients for *Legionella* growth in GWTPs.

Chapter 4 covers the monitoring and characterisation of fouling from the laboratory and full-scale aerators with the aim of better understanding *Legionella* ecology in aerators and to control the risk of growth. Temporal changes in fouling deposition and biofilm formation patterns are established through long term coupon monitoring, and chemical cleaning cleaners optimised as *Legionella* mitigation strategies.

Chapter 5 develops BNs to better manage the risk of *Legionella* colonisation of groundwater aeration systems. Fishbone diagram and bowtie analysis are used to implement conceptual frameworks and to split the risk factors into growth and transmission categories that are subsequently modelled in iterative BNs. The models are evaluated through sensitivity analysis and scenario testing.

Chapter 6 describes the integration of data-driven learning and expert knowledge to improve the accuracy and stability of BN modelling of *Legionella* occurrence. Data-driven learning using diverse historical water quality records is explored to strengthen the predictive power of *Legionella* occurrence model. The optimised BN model utilises the Greedy Thick Thinning approach, complemented with domain knowledge to achieve superior performance accuracy.

Chapter 7 summarises the major outcomes of this thesis and provides recommendations for future research activities and practical implications for the water industry.

Chapter 8 presents the list of references used throughout this thesis.

Chapter 9 details the appendices covering additional relevant information and data for:

1. Chapter 3 (Appendix 1)

2. Chapter 4 (Appendix 2)

3. Chapter 5 (Appendix 3)

4. Chapter 6 (Appendix 4)

5. Chapter 7 (Appendix 5)

CHAPTER 2

LITERATURE REVIEW

2 MANAGING THE RISK OF *LEGIONELLA* IN DRINKING WATER AERATION PROCESSES

2.1 Introduction

Legionellosis is the collective term for diseases caused by human exposure to *Legionella*-contaminated water droplets or aerosols from engineered water systems (Prussin *et al.,* 2017). In recent years, the frequency of sporadic and clustered Legionellosis outbreaks in air and water handling systems (particularly in the cooling towers) has been on the rise across the world, including in the US and Australia (MacIntyre *et al*., 2018). This increase is likely due to improvements in surveillance methods, expansion of ageing populations, and rise in global temperature (Beauté, 2017; Paranjape *et al.,* 2020).

Over 60 *Legionella* species have been identified. *Legionella* pneumophila is considered the leading cause of Legionellosis of all the different species (Brooks *et al.*, 2004; Yu *et al.,* 2002). Recently, new pathogenic *Legionella* species, including some previously considered benign, are being implicated in Legionellosis outbreaks (De Giglio *et al.*, 2019).

Assessing *Legionella* risk levels can be variable depending on the asset environment and the vulnerability of population at risk of exposure. While the monitoring of *Legionella spp* in some existing standards (European regulations) have been shown to trigger unnecessary health alerts, such measurement will be deemed appropriate for vulnerable clientele such as occupants of long-term care and hospitals.

The assessment and considerations made in this study is to support the design of risk management measures that address the broad and general *Legionella* genera in drinking water systems. As such, this study views the entire bacteria genera to be opportunistic pathogens and considers the positive occurrence of *Legionella* as an enumeration of any species (De Giglio *et al.*, 2019; Zhang *et al.,* 2021).

Numerous engineered systems have been identified to support conditions favourable for the growth and transmission of *Legionella*. These systems included plumbing sources, respiratory devices (e.g., humidifiers, vaporizers, and nebulizers), swimming pools, cooling towers, and a myriad of devices that operate with warm water, including hot tubs and fountains (van Heijnsbergen *et al.*, 2015). As a result, efforts to manage the frequency of Legionnaires' disease outbreaks have been focused on these systems (mostly air and water handling) in buildings, overlooking other "at-risk" settings such as drinking water treatment assets (Cline *et al.*, 2020).

Aeration units in groundwater treatment plants (GWTPs) are one such potentially overlooked system (Zappia, 2016). Aerators are designed to facilitate contact between air and groundwater and, in doing so, remove undesirable water quality components and gases (e.g., CO2), correct pH, or oxidise components (such as ammonia, sulphide, or metal ions) to more desirable (or treatable) species, and facilitate water temperature reduction (Ghernaout, 2019; Siabi, 2008). This relatively simple and efficient process has been widely implemented in many drinking water treatment plants, but with little (or no) specific consideration for monitoring, maintenance, and cleaning optimisation for the risk of *Legionella*.

An assessment of the occurrence, persistence, growth, and transmission of *Legionella* within groundwater aeration systems will allow for better management of the associated health risk. This chapter provides an overview of *Legionella* pathogen colonisation of aeration systems used in GWTPs and its implications for public health. The review assesses (i) groundwater treatment for potable uses, (ii) aeration technologies' design, configurations, and operational limitations, (iii) *Legionella* occurrence in source and groundwater treatment plants' systems, and (iv) *Legionella* risk factors and successful management measures in various engineered water systems. The synthesis and development of this knowledge is expected to form the basis for improved risk assessment and to guide the development of appropriate modelling tools and maintenance protocols.

2.1.1 Groundwater treatment for drinking purpose

Groundwater accounts for about half of the global potable water supply (Smith, 2016). In Australia, the use of groundwater for drinking purposes is widely distributed across the states. While the national average stands at 13.5%, the consumption of groundwater in Western Australia is significantly higher, measured at 70% (Harrington and Cook, 2014). In Western Australia, groundwater is preferred over surface water for a number of reasons, including its proximity to the point of use and its relatively pristine physio-chemical and microbial characteristics, requiring little or no treatment to meet drinking water standards (Aksever *et al*., 2015; Giordano, 2010).

Global dependence on groundwater for drinking purposes is projected to rise and possibly become the dominant source of potable water worldwide by 2050 (Piesse, 2020). Increasing water demand and the growing contamination of surface water due to pollution, urbanisation, and economic growth underscore the attraction of groundwater as a reliable source of water supply. This situation is further complicated by climate change pressures (Boretti and Rosa, 2019).

2.1.2 Physio-chemical and microbial hazard associated with groundwater

Groundwater has intrinsically favourable physio-chemical and microbial characteristics for drinking applications. The natural systems of the subsoil profile actively protect and filter impurities and contaminants to create an improved source with acceptable constituents for drinking purposes (Aksever *et al.*, 2015; Giordano, 2010). Despite the intrinsic protection from the subsoil profile, groundwater quality is under extrinsic contamination threat from increasing anthropogenic effects. These threats result from rapid development in agricultural activities and industrial expansion that has increased the chances of infiltration of groundwater with organic, inorganic, and microbial contaminants (Giordano, 2010).

Groundwater contamination has resulted in numerous reports of elevated concentrations of dissolved metals, including iron and manganese, dissolved gas, and volatile organic substances (VOCs). Elevated concentrations of dissolved metals deteriorate water quality, impact on taste, create bad odours and raise aesthetic concerns (Aksever *et al*., 2015; Subramani *et al*., 2005). Additionally, the presence of iron and manganese in groundwater has been linked to the stimulation of microbial and biofilm growth (Bargellini *et al.*, 2011a; Ginige *et al.*, 2011a). Hence, national drinking water regulation stipulates that the concentrations of iron and manganese in potable water be kept under 0.3 and 0.1 mg/L, respectively (NHMRC-ADWG, 2011).

A variety of microbial pathogens, including *Legionella,* Norovirus, Campylobacter, shigella, hepatitis A, salmonella, Cryptosporidium, and Giardia, have been traced to groundwater sources (Macler and Merkle, 2000; Murphy *et al.*, 2017). The presence of these pathogenic microbes in groundwater contributes to quality deterioration and can pose a health risk to the public.

Legionella occurrence in groundwater is often sporadic and generally low in concentration (Costa *et al.*, 2005; Riffard *et al.*, 2001). The aetiology of *Legionella* includes low concentrations of the bacteria in the natural environment and increased growth to critical densities in engineered water systems that create optimal growth conditions including high nutrients, warm temperatures, and stagnation (Prussin *et al.*, 2017). The increased use of aeration technologies for a groundwater treatment suggests the need for better understanding of conditions that can support *Legionella* occurrence in this type of environment.

2.2 Aeration technologies for drinking water treatment

Specific treatment processes are usually introduced to ensure the compliance of groundwater with drinking water standards (NHMRC-ADWG, 2011). Conventionally, groundwater treatment involves aeration as the first line of treatment followed by rapid sand filtration, softening process, and activated carbon filtration (*Figure 2.1*). Disinfection is usually the last step applied before the treated water is supplied to reservoir or directly into the distribution system (Crittenden *et. al*., 2012).

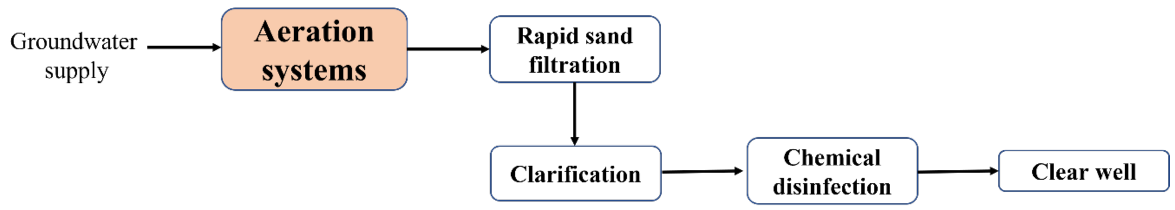

Figure 2.1: Typical groundwater treatment flow process

Aeration treatment processes have a long history of application in water treatment. First usage of the process can be traced to the early eighteenth century, when aeration was introduced to improve oxygen levels in rainwater on a household scale (Scott *et al*., 1955). Aeration has since become a big part of the traditional municipal water treatment process. The principle and functions of aeration are still about the same.

The process of aeration facilitates the contact of air and water for the purpose of diffusing unwanted pollutants that include oxidation of iron and manganese, removal of colour, turbidity, odour and taste (Siabi, 2008). Aeration can also displace dissolved gases, including hydrogen sulphide (H_2S) and carbon dioxide (CO_2), from water, a process referred to as air stripping. Air stripping has also been successfully used in water treatment to reduce the concentration of VOCs that cause taste and odour (Duranceau and Smith, 2016; Yoakum and Duranceau, 2018).

The following section reviews different aeration designs and operational limitations that can support the occurrence of opportunistic pathogens such as *Legionella.* Aerator technologies can be grouped into two types: water-in-air and air-to water configurations.

2.2.1 Water-in-air designs

The water-in-air aerators are designed and operated so that water is introduced into the air to achieve the desired treatment. Examples of this type of design include spray, tray, cascade, and packed/column aerators.

2.2.1.1 Spray Aerators

A spray aerator consists of one or more spray nozzles attached to a piping manifold arranged in a fountain and is contained in tanks, large basins, or reservoirs (Crittenden *et. al*., 2012). Water is fed into the pipe under pressure and forced out of the nozzles in a fine spray. Spray aeration nozzles point water upward, vertically, or at an inclined angle. The water is broken into small drops during flight (Ghernaout, 2019). Specific design dimensions of spray aerator nozzles include diameters of between 2.5 and 3.8 cm, discharge ratings of 17-34 m^3/h, and nozzles spaced at 60-370 cm apart.

In industrial applications, spray aerators come in two design variations: open-air and semi-enclosed spray types *(Figure 2.2).* In the open-air spray design, the nozzles are installed on a pipe grid in tanks or reservoirs and are open to the atmosphere. The semi-enclosed system, on the contrary, has the nozzles enclosed. The enclosures are usually designed to allow adequate air flow and minimise aerosol dispersion.

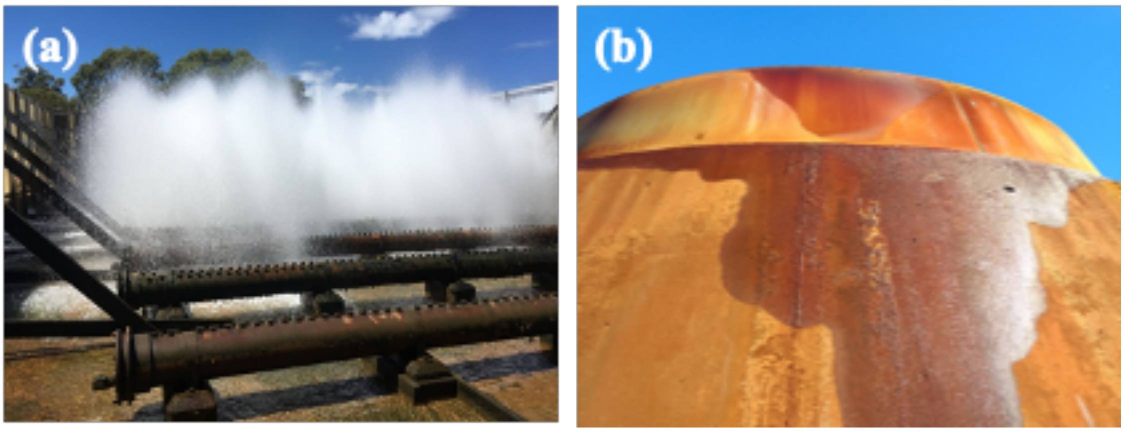

Figure 2.2: Open-air (a) and semi-enclosed (b) spray aerator types in groundwater treatment plants.

The performance efficiency of spray aerators is dependent on nozzle characteristics (i.e., size, orientation, spray velocity) and pressure in the line, driving water droplet sizes. Externally, the atmospheric conditions, including airflow impact the rise and fall of water droplets, contributing to the aerator efficiency (Ghernaout, 2019).

The benefits of open spray include higher performance efficiency, no packing material costs, and lower maintenance requirements (EPA, 1999). The disadvantages of this type of aerator involve the necessity for a large functioning area, which translates into increased construction costs, high-pressure requirements, the susceptibility of spray nozzles to clogging following prolonged operations, and reduced performance under cold weather conditions (EPA, 1999).

2.2.1.2 Tray Aerators

Tray aerators are a low profile (relatively smaller types of aerators) aeration class. They are designed to operate in a passive manner. Water is lifted to an elevated point above the distribution tray using the available head in the influent, from which it then flows under gravity over a series of horizontal perforated plates (*Figure 2.3).* Tray aerators may be filled with course media to increase the contact area between the water and air (Baruth, 2005).

Figure 2.3: Tray aerator operated in groundwater treatment plant

A tray aerator can have four to ten perforated trays arranged vertically underneath each other with regular spacing between trays. When water falls over a tray, a thin film layer of water forms over the tray before exiting from the several holes. The performance efficiency of tray aerators is determined by the contact time between the air, feedwater flow rate, and the asset geometry (i.e., the number and size of the trays).

The advantages of tray aerators include the passive nature of the operation, which requires less energy than spray types. Compared to spray aerators, tray aerators have smaller footprints than spray aerators.

These marked features make tray aerators the preferred option for modular designs, particularly in smaller treatment plants (Ghernaout, 2019). However, tray aerators can be susceptible to precipitation and biological fouling. Additionally, access for cleaning and maintenance can be very difficult (Krupińska, 2016). A more detailed discussion on fouling in aerators is provided in section 2.2.4.

2.2.1.3 Cascade Aerators

The cascade aerator consists of a series of sequential weirs that allow water to fall as a thin stream from one level to another, with aeration accomplished during the fall and due to mixing and entrainment in the splash zones (Razif *et al*., 2020). The mechanisms behind the cascade aerator system are based on the strong water turbulence occurring after a series of steps from near the crest to the toe, large residence time, and air bubble entrainment. The turbulent variations cause the entrainment of air bubbles when the kinetic energy exceeds both the forces of gravity and the surface tension.

Cascade aerators are passive (no energy requirement), low cost, simple to design and operate with little maintenance requirements (Toombes and Chanson, 2020). The downside to the cascade design is related to the drop in performance efficiency when used for large scale water treatment applications (Yoakum and Duranceau, 2018).

2.2.1.4 Packed Bed/Column Aerators

The principles of packed bed aerator are based on flow under gravity. In packed bed aerators, water is introduced at the top of the column. From the column top water then flow over bed of randomly packed media. Key design criteria for packed bed aerators properties of the feedwater and operating conditions of the system. Properties of feedwater that shape the performance of this type of aerator are air and water temperatures, and physiochemical and contaminants load. Similarly, relevant operating conditions include height of packing in the column, surface area for mass transfer, nature of the packing media, air to water ratio as well as the water loading rate (Baruth, 2005).

Packed bed aerators have been reported to be more economical for larger flows, typically exhibiting better removal efficiency and having a greater turn-down and operability range when compared to tray aerators (EPA, 1999). The common downside is susceptibility to fouling in packed beds due to their high mass transfer efficiency, which can accelerate scaling and attachment of foulants.

2.2.2 Air-in-water designs

The air-in-water aerators are designed and operated in a way that introduces air into the water to achieve the desired treatment. Examples of this type of design include diffused air and jet aerators.

2.2.2.1 Diffused air aerators

The diffused air aeration system involves using diffusers to introduce pressurised air water. They are mostly applied in smaller capacity groundwater treatment schemes (reported under 63 L/sand) are selected where removal of CO_2, VOCs, gasoline components, H_2S, methane, and radon is desired (Baruth, 2005).

Bubble size, air flowrate, diffuser position, and air/water mixing are the primary factors that influence mass transfer in diffused-air aerators. Diffused-air aeration systems are usually characterised based on the bubble size as either coarse or fine bubble diffusers and based on water column depth, as either low profile, having a water depth of 46 – 92 cm or conventional, with typically over 3 m water depth.

The diffused aerators are compact, have a small footprint, and require no packing bed. However, diffused aerators have limitations such as energy requirements and the potential for requiring a higher air-water ratio to ensure efficient mass transfer (Baruth, 2005).

2.2.2.2 Jet Aerators

Jet aerators inject air into the throat of a venturi in order to create fine bubbles. Jet aerators consist of air suction pipes that protrude above the water surface and a venturi-like nozzle and diffuser pipe serving as the ejector. At water depths of between 3 and 7 m, jet aerators are suitable for aeration at small to medium-sized treatment plants and are more often found in industrial applications than in municipalities (Erbisti, 2014).

Jet aerators are reliable, low-cost, and have improved performance efficiency. The downsides to this aerator design can include noise pollution and high technical expertise required for operation and maintenance.

2.2.3 Operational performance of aerators

The different configurations of the aeration system have implications for the performance efficiency in the treatment of groundwater. To better contrast the various designs, details about different aerator applications are discussed below. Aeration treatment processes can remove dissolved gases and adjust the pH, VOCs, oxidising and precipitating metals, which can then be removed by subsequent filtration units.

2.2.3.1 Dissolved gases and pH adjustment

Aerators were studied for the purpose of stripping i.e., the removal of unwanted gases from groundwater. The concentration of CO_2 in groundwater varies significantly. Deep well waters typically have less than 50 mg/L, whereas shallow wells can have levels ranging from 50 to 300 mg/L. Levels of CO_2 (i.e., above 5–15 mg/L) can cause several operational issues in water treatment facilities. These include increasing the acidity of the water, making it more corrosive (La Motta, 1995). Aerators through oxidation can effectively reduce CO_2 to low levels, (as low as 4.5 mg/L) and, as a result, increase pH levels (Crittenden, *et al*., 2012).

Hydrogen sulphide (H_2S) is another gas that is effectively removed through the aeration process. H_2S gas is common in groundwater. Aesthetic and odour issues have been associated with elevated concentrations of H_2S. Additionally, H_2S can contribute to operational issues such as reduced disinfection efficiency due to its chlorine demand and accelerated corrosion (Daud *et al*., 2013). Drinking water guidelines state that the concentration of H_2S should be kept below 0.05 mg/L (NHMRC-ADWG, 2011).

2.2.3.2 Precipitation of metals

The aerator treatment process has been effective in precipitating elemental concentrations of iron, manganese, arsenic, and radon (Ghernaout, 2019; La Motta, 1995). The need for elemental reduction from the groundwater is based on aesthetic, taste, and health considerations (NHMRC-ADWG, 2011).

Iron can occur at elevated concentrations in groundwater. Iron is an important mineral for human health. However, high iron concentration in groundwater can be problematic. High load of iron can create aesthetic concern because of discoloration effect, increase odour, turbidity, and metallic taste. Additionally, iron staining of hydraulic and plumbing fixtures have detrimental effect on engineered water assets (Munter *et al*., 2005). For example, iron concentrations exceeding 0.3 mg/L have health and aesthetic implications (NHMRC-ADWG, 2011).

High concentrations of manganese in water can have both aesthetic and adverse health effects. Manganese concentration greater than 0.2 mg/L has been suggested to create unpleasant tastes and stimulate microbial growth in water systems (Bruins *et al*., 2014). When the concentration of manganese exceeds 0.5 mg/L, it is considered to pose some health risk. To limit the aesthetic and health effect, the allowable concentration of manganese should not be greater 0.1 mg/L (NHMRC-ADWG, 2011).

2.2.3.3 Volatile Organic Compounds

Air stripping using aeration processes has been effective in removing VOCs from groundwater (Toombes and Chanson, 2020). In practice, air stripping process involve aerating water with high concentration of VOCs thereby increasing the surface area. The increase surface area as a result of the contact of water with air can lead to stripping of the high organic compounds contained in the water. The stripping occurs through diffusion process and will continue until equilibrium is achieved (Brooke and Collins, 2011; Smith, 2015).

Trihalomethanes is one of the VOCs that the aeration treatment process is designed to remove. Local safe water guidelines recommend permissible trihalomethanes concentration at below 0.25 mg/L for health reasons (NHMRC-ADWG, 2011). Aeration has been shown to be an effective method of removing dissolved trihalomethanes (Brooke and Collins, 2011).

2.2.4 Design and operational limitations in aeration systems

Aerator systems, by their design and operation, can feature conditions that promote the occurrence and transmission of *Legionella*. First, this section contextualises the common limitations associated with the increased *Legionella* risk from air and water handling systems to aerators. Then, fouling and biofilm accumulation, which have been reported as the dominant challenges in aerators, are discussed in detail, including strategies for their control.

2.2.4.1 *Factors promoting the growth and transmission of Legionella risk in engineered water systems*

The commonly available management guidelines grouped the many risk factors considered favourable for *Legionella* growth and transmission into five areas. These five areas include water quality, water stagnation, nutrient availability, system deficiencies and location and access (AS/NZS 3666:2011; AS/NZS 5059: 2006). The characteristics of the different risk areas and their impact on *Legionella* are discussed in the context of aerators. Broadly, stagnation, system deficiencies, location and access, water quality, and nutrient availability are the areas considered to drive the growth and transmission of *Legionella* in engineered systems.

2.2.4.1.1 *Water stagnation*

Stagnation of water is a main risk area for *Legionella* growth. Stagnation in engineered systems facilitates the loss of disinfectant concentrations, increases the deterioration of water quality and allows bacteria, including *Legionella,* to grow through sedimentation that fosters adherence and biofilm evolution (Nisar *et al.*, 2020).

Existing regulations recommend removal of any structural factor within engineered water systems that can breed water stagnation as a control measure for *Legionella* proliferation (Wang *et al.,* 2012). Current design of aerators can facilitate stagnation particularly when operated intermittently. The stagnation conditions which can become favourable growth environments for *Legionella*.

2.2.4.1.2 *System deficiencies*

System deficiencies are considered inappropriate design and configuration that support the formation and dispersion of aerosols. Operational aerators usually create and disperse water droplets and aerosols. While water droplets usually fall off within a short distance from the source, aerosols can spread further beyond the water treatment plant. Aerosols have been reported to travel and cause infection at long distances downstream of emission source. A case of infection at 7 km away from source of aerosols have been reported (Nhu Nguyen *et al.,* 2005).

Aerators generally lack barriers that limit the ingress of foreign debris and the egress of aerosols. This makes aeration susceptible to cross-contamination of foreign debris, including from surrounding soil, which could be a favourable habitat for *Legionella* (Schalk *et al.*, 2014). Similarly, aerosols are expected from operational aeration systems in the absence of a limiting barrier (

Figure 2.4*)*. The aerosols from the aerators establish a pathway for the *Legionella* pathogen that could lead to exposure of plant operators and nearby communities.

Figure 2.4: Aerosol formation and dispersion from an operational open spray aerator.

Bacteria contaminated aerosols have been reported to survive in the air for an extended time (hours). This suggest *Legionella* in aerosols can survive for an extended time period posing a risk of human exposure (Benoit *et al.*, 2021). Appropriate assessment of the aerosols produced by the aerator, their travel distance, and mechanism will determine the level of potential risk (Principe *et al*., 2017).

2.2.4.1.3 Location and access

Location and access risk factors must also be studied as important parameters impacting the potential of contaminated aerosols to reach human beings (AS/NZS 3666:2011; AS/NZS 5059:2006). Aerators in most treatment plants come in varying elevations. In particular, the tray aerator placed at an elevated height can increase the risk of aerosol dispersal downwind.

Two potential human exposure scenarios are likely in aerating systems. The plant operators access the systems for cleaning and maintenance. The other scenario will involve residents that come into proximity to the treatment plants. Understanding the duration and how often these assets are accessed for maintenance and cleaning operations will allow for estimation of exposure risk to plant operators (Bradley, 2015). In addition, determining the distance between aerator and nearby residences will support modelling of the risk of exposure to *Legionella.*

2.2.4.1.4 Poor water quality

Growth of *Legionella* has been strongly linked to nutrient availability in different engineered water environments. *Legionella* has been readily recovered in nutrient rich wastewater environment (Caicedo et al., 2019). Contrary, free cells of *Legionella* was reported not to be able to compete successfully with oligotrophic bacteria in poor nutrient environments such as drinking water (van Der Kooij, 2014). To survive in poor nutrient environment, *Legionella* enter into protozoan hosts that that will provide the required nutrient for survival and growth.

Organic matter measured as assimilable organic carbon (AOC) was observed to correlate to biofilm and *Legionella* in a drinking water system (Learbuch et al., 2019; van der Kooij et al., 2017). These studies established that *Legionella* numbers in drinking water are indirectly correlated to the AOC concentration measured in the water. A biofilm concentration of 50 µg ATP/cm^2 and a threshold AOC concentration of 1 µg C/L are critical to initiate growth of *Legionella* in water systems. An AOC concentration of >1 µg C/L is likely in both groundwater sources and water sampled after the treatment processes. This increases the potential for *Legionella* growth in engineered water systems (Volk et al., 2000). Other additional water quality parameters and environmental factors have been shown to also contribute to *Legionella* growth in water system.

Numerous water parameters have been identified as favourable for and promoters of *Legionella* growth (Buse *et al.*, 2012a). Elevated water temperature (20–37^{0}C), pH, free chlorine, hardness, heterotrophic plate counts (HPC), and certain trace elements such as iron and manganese are all linked to *Legionella* contamination (Bargellini *et al*., 2011b; Pierre *et al*., 2019).

Manganese, zinc, iron, and HPC bacteria were all found to be positively correlated with *Legionella* in a study of hot water systems (35 – 53°C). Manganese concentrations less than 6 mg/L and HPC concentrations below 150 CFU/mL (incubated at 37°C) have been suggested to be good predictors of *Legionella* absence (Bargellini *et al.*, 2011b). Regrettably, it is likely that manganese and iron concentrations in groundwater will be high enough to support *Legionella* growth. pH values greater than 7.8 were correlated with positivity of *Legionella* serogroups 2–14 in water systems, although the exact pH range observed was not specified (Goutziana *et al*., 2008).

The characteristics of groundwater were consistent with critical thresholds for water parameters associated with *Legionella* growth (Bargellini *et al.*, 2011b). Thus, understanding the characteristics of the groundwater that enters the aerator will provide insight into the resulting effects on *Legionella* growth.

2.2.4.1.5 Nutrient availability

Legionella can survive in a low nutrient environment. Certain specific nutrients, including trace elements such as iron and manganese, have been considered suitable for their proliferation to high densities (Cianciotto, 2007; Portier *et al*., 2016). Corrosion products and fouling materials within the water systems are a source of these trace elements and nutrients that stimulate *Legionella* growth.

An abundance of iron from corrosion products increased *Legionella* growth in drinking water systems in Flint, Michigan (Zahran *et al*., 2018). The increased availability of trace metals in corrosion products, such as iron, can create a favourable environment for *Legionella* growth, increase virulence, and stimulate general biofilm growth (Buse *et al.*, 2012a; Wang *et al.*, 2017). The susceptibility of surfaces of aerators to corrosion due to the interaction of air and water on metallic components suggests the need to understand the abundance of critical nutrients for *Legionella* growth (Bradley, 2017; Brazeau and Edwards, 2013).

2.2.5 Fouling challenges in aerators

Aerators have an enormous propensity for fouling, given their typical operation of precipitating dissolved metals from the influents on their surfaces. Fouling is the umbrella term for the materials deposited and accumulated in the aerators following prolonged operations. This includes scale, corrosion products, precipitates (dissolved metals, suspended solids, general dirt, and debris), organic and biological matters (

Figure 2.5).

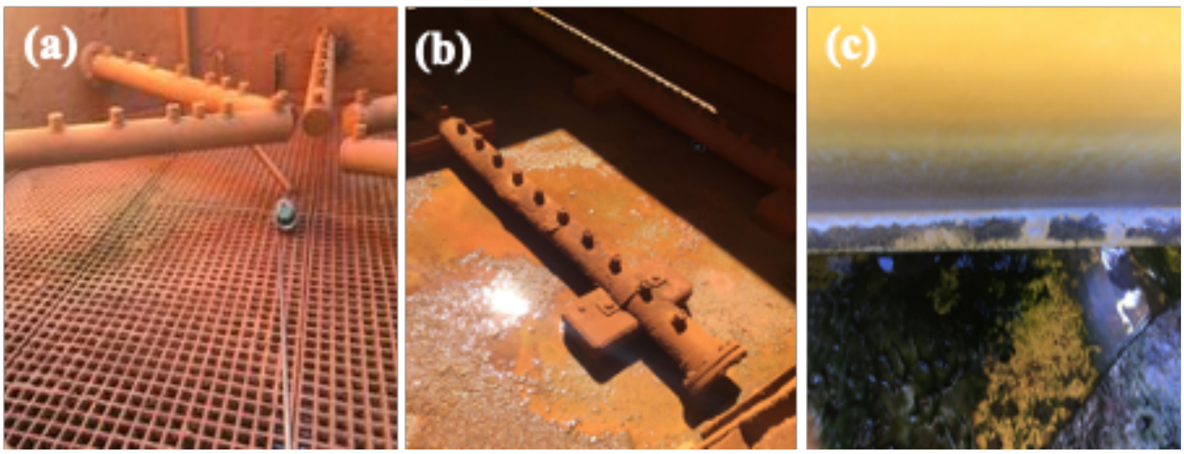

Figure 2.5: Semi-enclosed, open spray aerator and tray type aerators showing visible fouling build-up

Fouling in the aerator results from a combination of inorganic and organic debris, scaling and biofilm development (Diaz-Bejarano *et al.,* 2017; Müller-Steinhagen, 1999).The fouling collected from the aerators in GWTPs is a complex mixture in different states, including slurries and solid, which can be viewed broadly under chemical and biological components in terms of the process of their formation.

Chemical fouling comprises corrosion and scaling products. Scaling occurs when hardness salts (calcium and magnesium) in the groundwater are deposited onto the aerator surface as the solubility of the salts reduces with changes in temperature as a result of aeration process. Corrosion fouling occurs when a layer of corrosion products builds up on the surfaces of the aerator components made with metals, forming an extra layer of materials.

2.2.5.1 Corrosion

Corrosion is a common design and operational challenge in aerators as a result of the contact of air and water on metallic surfaces (Bradley, 2017; Volk *et al*., 2000). Aerator processes introduce oxygen into the water, which is expected to accelerate the rate of corrosion. On the other hand, aeration also decreases CO_2, increases the pH and the corrosiveness of the water, which are additional catalysts for corrosion (Brazeau and Edwards, 2013; Rolston, 2004).

Along with water characteristics, the type of construction materials used plays a significant role in corrosion. Aerators' metallic components, such as nozzles, diffusers, and grid pipes, make them corrosive. It is strongly recommended that corrosion-resistant materials such as stainless steel, aluminium, or plastic (including fibreglass reinforced polyester) be used in aerators (Baruth, 2005). Certain corrosion-resistant materials, such as those used in aerators, may promote biofilm formation and subsequently harbour *Legionella* and other opportunistic pathogens (Bartram *et. al*., 2007).

2.2.5.2 Scaling

Scaling is the process by which inorganic substances precipitate onto a surface. When a compound's saturation limit in water is exceeded, scale crystals form on the surface (Baruth, 2005). Aeration process alters pH conditions and CO_2 concentrations of the feed water, which can facilitate an increase in scale formation.

The common hardness constituents in groundwater (calcite and aragonite) can cause scale build-up on surfaces. Scale can alter typical surface characteristics such as roughness and provide sufficient mineral deposits to promote the rapid development of biofilms, thereby improving *Legionella* survival and multiplication (Bartram *et. al*., 2007).

2.2.5.3 Biological fouling

Biological fouling is caused by organisms such as algae and biofilm slimes growing in the fluid and depositing on the aerators' surfaces. Biofilms are a significant issue for any potable water system because they can shield pathogenic species from commonly used disinfectants and, in the case of *Legionella,* promote growth and pathogenicity (Declerck *et al.*, 2007). A biofilm is a cluster of structured microbial cells contained in extracellular polymers. Biofilms provide a community for microorganisms, increasing their access to nutrients and making them more resilient to environmental stress (Flemming *et al*., 2011).

The following section discusses the process of biofilm formation, its implications for *Legionella* colonisation, and practises for limiting their growth.

2.2.5.4 Biofilm formation and growth

Biofilms can form on aerators' various surfaces, including the sprinkler pipe, the walls, and the bottom floor. Biofilms have reported to live and grow in loose and crystal fouling in engineered water systems environment (Lehtola *et al*., 2004; Xia *et al*., 2004).

The formation of biofilms on surfaces and within fouling entailed a series of developmental stages. Typically, the growth of biofilms begins with a stationary phase characterised by a transient and weak attachment. The exponential phase follows, during which cells proliferate and form multilayered cells. Additionally, the stable phases associated with biofilm maturation. Unfavourable environmental conditions can cause cells to detach and disperse from biofilms (Kierek-Pearson and Karatan, 2005).

Multiple environmental factors, including the water characteristics and surface conditions of the systems, can influence the pattern and extent of biofilm growth (Liu *et al.*, 2016). Water characteristics such as nutrient availability, pH, and temperature all have an effect on biofilm development (Boe-Hansen *et al*., 2002; Villanueva *et al*., 2011).

Additionally, surface characteristics can have a substantial effect on microbial adhesion. These properties include the composition of the material, the wettability of the material, and the orientation of the material's surface. Bacterial biofilms adhere well to a variety of plastics commonly used in plumbing, but copper inhibits their adhesion (van der Kooij *et al*., 1995a). Similarly, increased biofilm growth and resistance to detachment were observed when comparing vertically positioned, fully immersed surfaces to horizontal, partially immersed surfaces (Jha *et al*., 2021; Waines *et al*., 2011). Increased biofilm formation on the horizontally positioned surface was most likely caused by bacteria accumulating on horizontal surfaces via sedimentation. Due to the dynamic nature of the aerator systems, it is possible for different surface positions and wettability to exist, which may facilitate variable biofilm growth.

As biofilms are thought to be a stronghold for *Legionella* colonisations, their growth on aerator surfaces can increase the risk of opportunistic pathogens. Biofilms have been shown to provide the ideal ecological niche for *Legionella* growth, increase virulence, and act as a protective barrier against disinfectants (Abdel-Nour *et al*., 2013).

2.2.5.5 Strategies for the control of biofilm.

Two main strategies are predominantly considered for the control of biofilms. The first strategy is focused on preventing initial microbial attachment to surfaces, thereby reducing potential for biofilm development. The second strategy targets the removal of matured biofilms from surfaces.

2.2.5.5.1 Prevention of initial cell attachment

Prevention of initial attachment to surface is considered a proactive practice in limiting biofilm development. The initial cell attachment to surfaces first process in the biofilm development. Initial attachments usually occur within the first two days into the process (Flemming *et al*., 2011). The prevention of initial attachment is usually achieved by targeting the factors considered to mediate cell attachment and biofilm evolution. For example, rough and more hydrophobic surfaces have usually shown an increase biofilm formation (Lorite *et al*., 2011). Thus, altering the chemical or physical properties of surfaces of materials within the aerators can prevent the initial attachment of cells.

2.2.5.5.2 Removal of biofilm

When microbial cells attach, grow and mature into biofilms, they become firm and resistant to removal in some instance even under cleaning actions or disinfectant effects (Di Pippo *et al*., 2018). Increased tolerance of biofilms to removal has been attributed to the altered growth rate, emergence of resistant subpopulations, as well as surface properties and position of the surface within the biofilms (Jha *et al*., 2022; Subhadra *et al*., 2018). Given that the biofilm within aerators is embedded with fouling, detailed cleaning and removal methods and their application within the drinking water treatment are further discussed in section 2.3.5.

Ultimately, a better understanding of the effects of surface and water characteristics on biofilm formation within aerators will contribute to developing effective strategies for their control to limit *Legionella* colonisation.

2.2.5.6 Fouling and biofilm cleaning practice

WCWA currently employs a hybrid cleaning technique to dislodge and remove foulants from aerator assets. They include the use of a power washer to remove scale and biofilm from all surfaces, ensuring that they are clean and devoid of solid build-up. When hydraulic cleaning is unable to resolve fouling issues, manual scrappers are introduced to remove the firmly attached build-up mechanically. Following cleaning, a 12 percent sodium hypochlorite solution is applied and allowed to stand for 30 minutes to disinfect completely (Bradley, 2015).

The chemical agent is deemed appropriate and effective because it is readily available, can inhibit pathogen within a short period and is safe on surfaces of aerators. While NaOCl is a frequently used disinfectant and cleaning agent for biofilm removal, the optimal dosage for effective *Legionella* inhibition has varied across sources (Fliermans, 1995; Scheikl *et al*., 2014).

While NaOCl is effective at destroying a biofilm that is predominantly organic, it is ineffective at removing inorganic scale. Acid cleaning solutions such as oxalic, nitric, and citric acid are typically used to remove inorganic scale. As a result, optimising the current physical and chemical methods for cleaning fouled deposits in aerators assets remains critical.

2.2.5.6.1 Optimisation of current aerator cleaning practice

Traditionally, mechanical cleaning and/or the use of chemical agents have been used to clean foulants in industrial systems. Scraping (brushes), abrasive, abrasive hydraulic, hydraulic, steam shocks, hydroblasts, and thermal cleaning are the broad categories of mechanical cleaning (Liang *et al*., 2008).

Commonly use chemical agents for fouling removal include citric acid (CA), sodium hydroxide (NaOH), hydrochloric acid (HCl), and ethylenediaminetetraacetic acid (EDTA). Sodium hypochlorite (NaOCl) is usually applied for disinfection (Liang *et al*., 2008). Any chemical agents to be considered in addressing fouling in aerator must first be approved as safe for application in drinking water systems. In addition, they must also be able to react with or dissolve solid mineral scales, be non-flammable, non-toxic, environmentally friendly, and economically viable (Chauhan *et al*., 2015).

The combination of various cleaning agents — acids, NaOH, and NaOCl — has been shown to be more effective at removing fouling than their individual applications (Liang *et al*., 2008). For example, inorganic fouling could be removed via chelation with citric acid; organic fouling could be cleaned using NaOH's hydrolysis and solubilization functions; and oxidants such as high concentrations of NaOCl could oxidise NOM, increase hydrophilicity, and inhibit and destroy the EPS gel layer (Liang *et al*., 2008; Metzger *et al*., 2007). When combined, NaOH and NaOCl have been shown to increase the solubility of solutes through hydrolysis and solubilisation, altering the configuration of the biofilm and allowing NaOCl to penetrate the gel and reach the inner layer of fouling materials more easily. This may address the conundrum of the *Legionella* pathogen being protected from disinfectant effects by a biofilm. The composition of fouling and biofilm in drinking water treatment assets can vary across sites, depending on the characteristics of the feedwater, operating conditions, and hydraulic materials used (Peng *et al*., 2010). As a result, monitoring and characterising aerator fouling and biofilm formation will aid in the selection of appropriate chemical cleaners.

2.3 *Legionella* occurrence in drinking water treatment systems

Significant research works have explored the occurrence of *Legionella* in natural and engineered water environment. Cooling tower and plumbing systems in building have received the greater emphasis with lesser focus given to drinking water treatment environment (Cline *et al*., 2020). This section discusses the various *Legionella* detection methods and the prevalence of *Legionella* in groundwater samples taken from source, treated, and distribution systems. The following sections compare and contrast three major *Legionella* enumeration techniques.

2.3.1 Measurement of *Legionella* in water samples

Currently different methods exist for the measurement of *Legionella* in water samples, and new ones are continuing to emerge. Despite this advancement, accurate enumeration of *Legionella* from water samples can still be a challenge due to low sensitivity of available techniques (Collins *et al.*, 2015). The two primary methods for measuring *Legionella* in samples are (i) culture methods and (ii) real-time polymerase chain reaction (qPCR). A critical review of these two techniques is presented in Table 2.1, along with their respective advantages and disadvantages, in order to provide guidance on the most appropriate technique for enumerating samples from groundwater treatment plants.

2.3.1.1.1 Culture method

Culture methods continue to be the gold standard method due to their ability to detect and count viable and culturable *Legionella* in environmental samples (Reischl *et al.*, 2002). ISO 11,731:2017 provides a detailed procedure for *Legionella* quantification using culture method for water sample, with results expressed in colony forming units (CFU).

Culture methods provide information about viable bacteria and generate results that can be compared to established guidelines and standards (AS/NZS 3666; AS/NZS 5059). However, the disadvantages of culture methods include a lengthy turnaround time (up to 14 days), the possibility of underestimating the concentration of bacteria in samples due to viable but nonculturable (VBNC) bacteria presence (and fast-growing microorganisms that over shadow *Legionella*) (Conza *et al*., 2013).

Despite these limitations, the culture method is widely used because there is no alternative that expresses *Legionella* measurement in a manner comparable to standard guidelines values. Additionally, there is scarce evidence that VBNC are effective in infecting cells. A recent comprehensive study, demonstrated that VBNC *L. pneumophila* starved for one year reduced the efficacy of human infection (as measured by lower fractions of infected cells) compared to bacteria obtained from broth cultures (Dietersdorfer et al., 2018). In general, after prolonged stress, stationary phase L. pneumophila lose culturability, yet are still able to cause infection (Dietersdorfer et al., 2018; Ducret et al., 2014; Kirschner, 2016). The environmental factors that trigger *L. pneumophila* differentiation into a VBNC-like cell type has been partially understood. Thus, more research is required to determine whether, or under what conditions, VBNC-like L. pneumophila can establish infections in humans.

To overcome the limitations of the culture method, agar formulations and pre-treatment processes involving heat and acids are frequently used to promote *Legionella* growth and to inhibit the growth of undesirable microorganisms (Bartie *et al.*, 2003). While the pre-treatment processes improved *Legionella* cultivations, they were deemed to be too harsh on the samples, excludes certain species, lacked reproducibility and consistency (Delgado-Viscogliosi *et al*., 2009; McCoy *et al.*, 2012).

Legiolert emerged as an improved alternative to culture method that was developed recently. Legiolert requires less expertise to perform compared to the conventional culture methods. The Legiolert method has been widely validated and standardised in the last few years. Although the method only measures

L. *pneumophila* (not *Legionella spp*) and provides results in 7 days, the superior accuracy of this method in industrial waters has been established and accepted (AFNOR, 2019).

Recently, numerous studies on *Legionella* monitoring in drinking water treatment indicate that Legiolert results are comparable at low *Legionella* concentrations and outperform traditional culture methods at high *Legionella* pneumophila concentrations (Barrette, 2019; LeChevallier, 2019a).

2.3.1.1.2 Molecular (qPCR) methods

A common molecular method for measuring *Legionella* is the quantitative polymerase chain reaction (qPCR). This method represents a quicker alternative identification and quantification of *Legionella*. The procedure for the (qPCR) method is detailed in ISO/TS 12869:2019. The qPCR method has been successfully used to quantify *Legionella* from samples collected from source, treated, and distribution systems supplied with groundwater (De Giglio *et al*., 2019; Valciņa *et al.*, 2019).

The qPCR principle of measurement involves amplifying and quantifying a target DNA. The result is presented in genomic units (GU). The qPCR method offers a rapid and sensitive analysis (within hours) compared to the culture technique. Its rapid response is its major advantage.

The downside to the qPCR includes the inhibition of its reaction by substances in the sample and potential for overestimation due to its the inability to differentiate dead and living bacteria cells (Mansi *et al.*, 2014).

Another challenge for the qPCR techniques is the interpretation of the risk associated with its results. The qPCR return result in genomic units (GU/L). But most standards are designed using the CFU/mL concentration reported using the culture method. The use of PCR methods is becoming more widespread for clinical surveillance and outbreak detection and, if applied appropriately, could also be used for routine monitoring of water systems (NASEM, 2019).

There has been significant growth in the use of molecular methods to detect and characterise *Legionella* in environmental samples. More recently, an extensive characterisation of *Legionella* in different building water samples (Donohue et al., 2022). Similarly, a study compared the molecular and culture methods to develop an algorithm for risk interpretations using *Legionella* results from the different techniques, (Lee *et al*., 2011). The study concluded that, the recommended action within guidelines and regulatory documents should be enforced when both culture and the qPCR returned positive *Legionella* results. Alternatively, further investigation should be pursued to uncover the true source of variability when the qPCR method returned a positive result and the culture method returned negative

Table 2.1: Advantages and disadvantages of primary methods used for Legionella enumeration from drinking water treatment samples

Techniques	Advantages	Disadvantages
Culture	• Standardised with inter-laboratory precision • Results can be compared with historical samples • Acceptable measure of viability • Isolate bacteria for epidemiologic investigations	• *Legionella* cultivability can be impacted by the pre-treatment processes. • Reliable for detecting *L. pneumophila* SG1, but can omit some *Legionella* spp. • Low consistency and reproducibility of results. • *Legionella* presence can be overshadowed by other fast-growing bacteria in the sample. • May underestimate VBNC, other serogroups and species
Molecular (qPCR)	• Rapid (compared to culture) • Higher sensitivity and specificity • Detects genetic materials from viable but non-culturable bacteria. • Shorter turnaround time (results can be available within hours)	• Subject to inhibition by constituents of the water sample (potential for assay to be invalidated). • Results are not readily interpretable • Potentially estimate count due inability to differentiate between living, and dead bacteria.
Alternative culture technique (Legiolert)	• Much simpler to conduct than other culture assays. • Better performance than other culture assays (e.g., plating on BYCE agar) for high concentration samples • Results are comparable to those from other culture methods and appropriate for comparison against standards. • Technique have been validated and standardised	• Relatively new and unfamiliar to the drinking water community. • Might be subject to not counting VBNC organisms (though some reports indicate Legiolert is less prone to undercounting • VBNC organisms compared with methods such as plating on BYCE agar.

2.3.2 *Legionella* occurrence

2.3.2.1 Groundwater

Groundwater is typically free of water-borne pathogens, necessitating less sophisticated treatment than surface water. However, the presence of *Legionella* in water and biofilm sampled from groundwater sources have been widely documented (De Giglio et al., 2019; Riffard *et al*., 2001). The concentration of *Legionella* measured in the water samples were relatively low compared to biofilm samples (Riffard *et al*., 2001).

Legionella contamination of groundwater has been linked to geothermal activity, which can create an optimal temperature for pathogen growth. *Table 2.2* summarises the incidences of *Legionella* occurrence at treatment plant outlets as reported in the literature. Numerous studies have established that *Legionella* can survive in varying depths of groundwater and at varying temperatures. *Legionella* has been isolated from both water and groundwater biofilms.

Numerous strains and species have been identified in groundwater, including *L. pneumophila.* 50 CFU/mL (culture method) and 2,870,000 GU/L were the highest concentrations observed (qPCR method) (De Giglio *et al.,* 2019). In this study, *Legionella* occurrence in groundwater was rare. Results from about 80 percent of the samples tested were below limit of detection.

Although *Legionella* occurrences are rare in groundwater and frequently lack a path for direct human contact, they may increase in drinking water treatment processes that create optimal growth conditions (Wullings *et al.,* 2011a). Increase *Legionella* colonisation in the engineered water environment was attributed in part to biofilms development that support pathogen growth (Wullings *et al.,* 2011a).

Table 2.2: Occurrence of Legionella in groundwater source

Sampling point	**T [^{0}C]**	**Samples**	**Depth** (m)	**Methods**	**Species**	***Legionella* concentration**	**Reference**
Groundwater	30-35	Water biofilm	-	Culture qPCR	*Legionella spp.* *L pneumophila* *L steigerwaltii* *L rubrilucens*	Water 10^2 – 8.4 x 10^4 CFU/L Biofilm 2 – 267 CFU/cm^2	Riffard *et al*. (2001)
Groundwater bores	15 - 25	Water Biofilms	-	Culture qPCR	*Legionella spp*	Water 10^2 - 10^5 CFU/L Biofilm 1.2 x 10^2 CFU/cm^2	Brooks *et al.* (2004)
Boreholes	35 - 36	Water	63. 5 307	qPCR	*L.pneumophila, L.oakridgensis, L. sainthelensi, L. londiniensis*	10^2 x 10^4 CFU/L	Costa *et al.* (2005)
Well water	-	Water	-	Culture	*L. longbeache L. bozemanii L. dumoffi i, L. gormanii, L.jordanis, L. micdadei L. anisa*	< 100 CFU/100 ml	Stojek and Dutkiewicz (2011)
Wells	-	Water	-	qPCR	*Legionella spp., L. pneumophila*,	8.4 1 to 1.8 × 10^4 GC/mL	El-liethy *et al.* (2016)
Wells	-	Water	-	Culture qPCR	*L. pneumophila* sg 2-15 *L.bozemanii; L. gormannii*	261 – 2870000 GU/L, 300 – 50,000 CFU/L	De Giglio *et al.* (2019)
Groundwater	2 – 24	Water	-	Legiolert	*Legionella spp. L. pneumophila*	114 MPN/100MPN/mL	LeChevallier, (2019)

Table 2.3: Occurrence of Legionella in groundwater treatment plant outlets and within the distribution systems

Sampling point	T [^{0}C]	Samples	Methods	Species	*Legionella* concentration	Reference
Groundwater in distributed systems		Water	Culture	*Legionella spp, L.penuemophila sg2-14*	25 - 10^4 CFU/L	Borella *et al.* (2004)
Treated groundwater	55	Water	Culture	*L. pneumophila sg 1 -15*	1.2-1000 CFU/ mL	Mathys *et al.* (2008)
Treated groundwater	10 – 18	Water	Culture qPCR	*L. bozemanii, L.worsleiensis, L. quateirensis, L. waltersii, L. pneumophila*	10^3 to 10^5 cells /L	Wullings *et al.* (2011)
Treated and untreated groundwater	-	Water	Culture	*Legionella spp. L. pneumophila*	**Untreated** 11 to 9.3x10^3 CFU/100mL **Treated n.d**	El-liethy *et al.* (2016)
Treated groundwater	30 - 35	Water	Culture	*L.pneumophila sg 2–14*	10 – 1000 CFU/L	Barna et al. (2016)
Treated groundwater	8 - 33	Water, Biofilm	Culture qPCR	*Legionella spp. L. pneumophila sg 1-15*	-	Richards *et al.* (2018)
Treated well drinking water	35 - 55	Water	Legiolert PCR	*Legionella spp. L. pneumophila*	1·1 - 4·74 MPN/ mL	Mapili *et al.* (2020)
Groundwater distribution systems	-	Water	PCR	*Legionella spp. L. pneumophila, L. anisa, L. micdadei, L. bozemanii, a L. longbeachae*	1.3-2.9log10 GC/100mL	Logan-jackson and Rose (2021)

2.3.2.2 Drinking water treatment plant and distributions

Table 2.3 summarises studies that characterised *Legionella* from samples collected from within drinking water treatment plants and distribution networks. While it is assumed that proper drinking water treatment is sufficient to reduce waterborne pathogen concentrations to a safe level, *Legionella* frequently defies this assumption. *Legionella* was detected in a variety of drinking water treatment processes and throughout the distribution system.

A groundwater-supplied drinking water treatment plant demonstrated dramatic increases in *Legionella* contamination during treatment (Wullings *et al*., 2011a). *Legionella* was not detected in raw groundwater, but was detected during several treatment processes, including rapid sand filtering, pellet softening, and in treated water (Wullings *et al*., 2011a). Similarly, the frequency of *Legionella* detection increased by a factor of two in the treated water sample compared to the raw groundwater sample (Richards *et al*., 2018). While water treatment plants are generally designed to address water-borne pathogens, little consideration have been given to understanding and addressing the risk of *Legionella,* which frequently precludes the use of conventional treatment processes.

In summary, these studies established that drinking water treatment processes can promote *Legionella* growth. The increased detection of *Legionella* within the treatment plant demonstrates the importance of characterising the potential for human exposure from aeration systems.

2.3.3 Managing *Legionella* risk in drinking water treatment

Standards and governing documents that provide guidance for safe management of *Legionella* growth and release of contaminated aerosol release exist for air-water handling systems (AS/NZS 3666:2011). Regrettably, there is no specific guidance available regarding drinking water treatment processes such as aerator technologies. This section summarises the risk management approach for *Legionella* that has been published in the literature for air water handling systems and may be applicable to aeration technologies. The application of surrogate indicators and mathematical models to the management of microbiological risk.

2.3.3.1 Inappropriateness of existing standard for managing Legionella in drinking aerator processes

In the absence of *Legionella* management guidelines for drinking water systems, control strategies from the air and water handling industries are considered. This standard has been successfully adapted for use with recirculating water in power systems and can provide a foundation for managing the risk of *Legionell*a in drinking water treatment systems (AS/NZS 5059).

Quarterly *Legionella* monitoring using the culture method is required by the standard for air and handling systems (ISO 11,731). *Legionella*, like HPC, was classified into three thresholds by the standard. Each threshold initiates a unique control action (*Table 2.4)*. *Legionella* and HPC concentrations of 10 and 100,000 CFU/mL, respectively, do not require action. When *Legionella* and HPC concentrations are equal to or greater than 10,000 and 100,000 CFU/mL, the standard requires determining the cause of the increase and confirming that corrective measures have been taken. The final threshold, which requires immediate system decontamination, is reached when *Legionella* exceeds 1000 CFU/ml and/or HPC exceeds 5,000,000 CFU/mL

Table 2.4: Strategies for controlling the presence of Legionella adapted from local standard for managing air and water handling systems (AS/NZS 3666)

***Legionella* (CFU/mL)**	**HPC (CFU/mL)**	**Required control strategy**
<10	< 100,000	1. Continue with established water treatment plan and cycle of *Legionella* monitoring.
≥ 10	≥ 100, 000	2. Investigate the source of *Legionella* • Evaluate the water treatment plan • Introduce corrective actions include disinfecting the system and extend to measure outlined in control strategy (3).
		3. Adjust the frequency of *Legionella* testing to 3 to 7 days: • Maintain the 3 to 7 days *Legionella* testing cycle until two consecutive negative results is obtained and then return to control strategy (1) • If *Legionella* and HPC is detected at (≥ 10 or ≥ 100, 000) CFU/mL repeat control strategy (2).
≥ 1000	≥ 5 000 000	4. Investigate the source of *Legionella* • Evaluate the water treatment plan • Introduce corrective actions including decontamination of the system as highlighted in this guide and undertake strategy (5)
		5. Adjust the frequency of *Legionella* testing to 3 to 7 days: • Maintain the adjusted 3 to 7 days *Legionella* monitoring cycle until two consecutive samples return negative results readings and then return to control strategy (1) • If *Legionella* and HPC detected at (<100 and < 5,000,000) CFU/mL return to control strategy (2) • If *Legionella* and HPC detected at (≥ 1000 and ≥ 5 000 000) CFU/mL investigate and adjust water treatment plan, decontaminate the system, and repeat control strategy (5).

Despite the widespread applicability of *Legionella* air and water handling standards, a number of challenges are considered to be impeding the monitoring protocol's effectiveness (Whiley, 2017). To begin, as described in Section 2.4.1.1, the culture method recommended for *Legionella* enumeration is time consuming and subject to limitations. Second, the thresholds for initiating control actions (for both HPC and *Legionella*) are determined solely by their concentration in the water sample, without regard for biofilm. This will almost certainly understate the true level of contamination. Thirdly, there is a dearth of standardised protocols specifying sampling frequency and site selection. The frequency of HPC and *Legionella* testing is impacted by different factors, including changes to water treatment program; recent high result of HPC and/or *Legionella*, necessitating an increased testing cycle to ensure that the water treatment system's subsequent changes are effective (AS/NZS 3666).

When microbial monitoring and management standards are unavailable or ineffective, the use of surrogate indicators has been deemed extremely beneficial in providing guidance for monitoring and intervention (Sadiq *et al.*, 2006). *E. coli, total coliform*, and HPC have been used as indicators for evaluating faecal contamination and microbial control effectiveness in water treatment samples (Mapili *et al.*, 2020).

2.3.3.2 Surrogate indicators for monitoring Legionella occurrence

Surrogate indicators are used to monitor difficult-to-measure microbial organisms. At the moment, various microorganisms are used as indicators to determine the biological contamination level of water samples. Microorganisms that are not pathogenic, such as total coliforms, faecal coliforms, enterococci, and E. coli, are the primary indicators of faecal and enteric pathogens contamination (Wen *et al.*, 2020).

Attempts to establish a link between *Legionella* and conventional indicators have been fruitless (Whiley *et al*., 2015). Given that faecal indicators can only detect enteric pathogens and/or faecal contaminants, they cannot be used to track the presence and concentrations of opportunistic pathogens found in water, such as *Legionella* (Whiley *et al*., 2015).

Legionella's lack of correlation with conventional indicators necessitated the investigation of more diverse microbial alternatives. HPC can be used as an alternative microbial indicator for *Legionella* growth, as HPC has a moderate association with *Legionella* presence (De Filippis *et al*., 2017; Donohue, 2021). While HPC testing is generally regarded as a reliable indicator of microbial control, its moderate association with *Legionella* was attributed to the pathogen's slow growth rate and proclivity for shielding itself by amoeba commonly found in water (Donohue, 2021).

Similarly, we looked for a correlation between adenosine triphosphate (ATP) levels and the presence or absence of *Legionella.* So far, no reasonable correlation have been established between ATP (a measure of broad microbial composition) and *Legionella* (Arroyo *et al.*, 2017; Duda *et al.*, 2015).

Apart from microbial indicators, research into water quality parameters as predictors of *Legionella* positivity is gaining momentum (Lasheras *et al.*, 2006a; Pierre *et al.,* 2019). Numerous studies have established significant correlations between water quality, operating and environmental parameters such as pH, and residual chlorine and *Legionella* (Pierre *et al*., 2019; Zhang *et al*., 2021). These studies established that interactions between *Legionella* and these parameters are not always linear or positive. Regardless of these established relationships, no attempt has been made to integrate the numerous parameters used to characterise *Legionella* outbreaks.

Interestingly, the successful application of empirical models to predict microbial pathogens based on routine water quality and operational parameters is increasing (Carvajal *et al.*, 2015a; Panidhapu *et al.*, 2020a).

2.3.3.3 Potential of empirical models as alternative to microbial indicators

Microbial interactions in environmental systems are complex and influenced by many variables (Bradshaw *et al*., 2016). Empirical models with capacity to accurately integrate and define qualitative and quantitative relationship between the many variables and microorganism can provide potential for alternative indicators. A wide variety of empirical models have been used to model microbial organisms in water systems. Example of such empirical models include Bayesian networks (BNs) and machine learning algorithm (data-driven) models (Panidhapu *et al., 2*020a).

2.3.3.3.1 Bayesian networks

BNs are a popular tool for modelling complex systems, across a range of fields, including environmental and water systems (Korb and Nicholson, 2011). BNs are probabilistic graphical models. They consist of conceptual maps that define the interactions and links (using arcs) between the different factors (nodes). BNs have been successful in modelling causal relationships between different variable in complex systems and facilitate inferential reasoning. Two related variables with connection in a BN model are considered as “parent” and “child” nodes (Korb and Nicholson, 2011).

The strength of the relationship between the nodes is specified using joint a probability distribution. A set of conditional probabilities are used to quantitatively define the relationship between a parent and child node. Each variable referred to as node in BN is divided into states. These states can be categorical or range of values. Each combination of the different states between the connecting variables is assign a probability value. These probabilities can be derived from diverse sources, including observed or experimental data, simulation models, and expert-elicited opinion. Nodes without parents are referred to as root nodes. Root nodes do not have probability but only require marginal probabilities (Uusitalo, 2007). Further description of the steps in BN modelling in provided in chapter 5 and 6 where the method in applied in *Legionella* risk characterisation.

BN has several unique and attractive features that can be explored to create sophisticated and reliable models. These unique features of BN models include the creation of a visual decision tool to improve risk-based decision making, the ability to account for all data in an unbiased fashion and the ease of continual refinement of BN through addition of newly collected data. BN are also capable of incorporating expert knowledge as well as learning relationship from data. BN have been successfully used in mine from databases both conceptual and conditional probabilities relationship (Chen and Pollino, 2012a; Uusitalo, 2007).

BNs have previously been used in modelling microbial interactions with water quality variables for different objectives (*Table 2.5).* In particular, Wijesiri *et al*., (2018) used BN modelling to develop surrogate health risk indicators from environmental and anthropogenic factors from poor water quality. The surrogate indicators developed enabled the identification of different risk levels (Wijesiri *et al.,* 2018). In a related fashion, water quality parameters were modelled using BN to provide rapid and easy alternative for *E. coli* and faecal coliform in monitoring microbial quality (Panidhapu *et al.*, 2020a).

Table 2.5: Summary of the Bayesian network application for microbial risk assessment

Target microbes	Objective	Environmental systems	References
Biofilm development	Model the interaction of water quality variable with biofilm development	Pilot drinking water system	(Bois *et al*. 1997)
Giardia lamblia* and *Cryptosporidium parvum	Represent relationship monitored feed characteristics and operating conditions with pathogen reduction	Biological treatment process	(Carvajal et al., 2015b)
Escherichia coli	Predict contamination of drinking water with *Escherichia coli* using demographic and management factors	Drinking water sourced from wells and rainfall.	(Hall and Le 2017)
	Predict microbial water quality using *Escherichia coli* monitoring and hydrometeorological data	Drinking water sourced from the river.	(Sokolova *et al*. 2022)
Cyanobacterial bloom	Integrate physio chemical water characteristics to determine cyanobacteria bloom	Lake water	(Shan *et al*. 2019)
Faecal coliform and *Escherichia coli*	Integrate water quality measures and historical weathers data to predict faecal coliforms and *Escherichia coli*	Lake and river	(Panidhapu *et al.* 2020).
Total Bacteria	Integrate weather and season variables to predict total bacteria	Drinking water distribution system	(Mohammed *et al*. 2021)
Health risk	Develop surrogate indicators for predicting health risk	Urban water systems	(Wijesiri et al., 2018)

Legionella risk has been modelled using BN modelling in air and water handling systems. Wilmot *et al*. 2000, using expert opinion and BNs, establish a relationship between *Legionella* and variables considered favourable for growth and transmission in cooling towers (Wilmot *et. al*., , 2000). Another attempt used BN to model and connect the relationships and dependencies between various water quality parameters to predict the risk of *Legionella* contamination of condensers (Armero *et al.*, 2011). Both studies relied on the qualitative aspect of BN to establish relationships between variables but were unable to establish a reliable quantitative definition and model validation.

2.3.3.3.2 Data driven modelling of microbial risk

Data-driven approaches to risk modelling have successfully characterised and estimated conditions that support microbial occurrence in different environments (Sokolova *et al*., 2022). The study of microbial interactions in water is complicated and highly dependent on a variety of variables and the precision of measurement techniques (Wijesiri *et al.*, 2018). As such, data-driven modelling approaches can contribute to the advancement of knowledge about the microbial interaction with water quality in systems.

Numerous attempts have been made to model microbial occurrence using a variety of machine learning algorithms from monitored water quality datasets. For instance, a model that forecast occurrence of *E.coli* in water have been developed using Artificial Neural Networks (ANN) (Avila *et al*., 2018). Other attempts were made to model microbial occurrences using different machine learning algorithms including adaptive neuro-fuzzy system, random forest and zero-inflated regression (Mohammed *et al*., 2018). In a more recent study Greedy Thick Thinning (GTT) machine learning algorithm was successfully utilised to develop BN models that predict occurrence of *faecal coliform* and *E. coli* in water systems (Panidhapu *et al*., 2020).

The studies cited above used either supervised or unsupervised methods to complete their data-driven machine learning algorithms. Supervised learning methods build on partial known relationship and target in the input data. Unsupervised methods are frequently used to uncover latent relationship that existing within a dataset (by extension the system) and can assist in identifying sample groupings without prior knowledge of how to categorise the data (Mckee, 2019).

The supervised learning approach, which combines machine learning with prior system knowledge and is a promising way to address significant data-driven method challenges, such as ensuring models adhere to known physical laws and have logical validity (Mckee, 2019).Thus, data-driven learning based on monitored water quality data can help improve the interdependence of variables and aid in the identification

2.3.4 Managing *Legionella* in GWTPs aeration: A case study of WCWA

WCWA operates multiple groundwater and wastewater treatment plants throughout the state's regional and metropolitan areas. WCWA deemed the potential for *Legionella* growth and persistence in the aerator treatment system environment, as well as the risk of human exposure, to be unacceptable (Zappia, 2015).

To address knowledge gaps created by the absence of a standardised framework for managing *Legionella* risk in drinking water treatment plant assets such as aerators, the WCWA developed two guiding documents. The *Legionella* High Level Risk Assessment (LHLRA), followed by a fouling characterization and the development of a cleaning protocol, addressed the challenge of fouling and biofilm formation in aerator environments. This section reviews these guiding documents and identifies opportunities for improvement as a starting point for developing a *Legionella* risk assessment.

2.3.4.1.1 Legionella High Level Risk Assessment

In bridging the gap for the absence of appropriate *Legionella* standards for aerators systems, WCWA develop an internal risk assessment of framework entitled the *Legionella* high level risk assessment (LHLRA). The LHLRA evaluated existing aerator assets on based on the five critical risk areas defined in air handling systems (AS/NZS 3666).

The five risk areas listed in the AS/NZS-3666 standard (nutrients availability, location and access to system, system design, hydraulic conditions, and water quality) were contextualised by listing all the factors (or indicators) capable of impacting on *Legionella* growth and transmission in aerators. Factors relevant to aerators were streamlined and grouped into their interdependence and connection justified by the detailed literature review and systematic assessment of industry and regulatory guidelines for water quality governance.

Shortlisted factors were defined by observable states or critical measurable levels. The impacts of the different factors were scored based on their relative expected influence on growth and transmission of *Legionella*. The output is a ranking tool that efficiently estimates and ranks *Legionella* risk across the aerators in a semi-quantitative manner. Periodically aerators are surveyed and observations for the risk factors are entered as scores into the outputs framework and the aerator assets are ranked. Based on the outputs score control measures for limiting *Legionella* risk are introduced.

2.4 Conclusions

The literature review demonstrates that GWTPs aerators are favourable for the growth of *Legionella*. Even though groundwater aeration assets could grow and spread *Legionella* bacteria because of their design and operational limitations, there are no current management guidelines for these assets. This suggests that these assets are an overlooked source of *Legionella* infections.

The following section presents identified knowledge gaps that should be tackled to provide a better understanding and develop measures to minimise the risk of *Legionella* in drinking water treatment aerators.

Knowledge gaps and need for research.

- Design and operational limitations in GWTPs aerators can create conditions that harbour, promote growth and facilitate the transmission of *Legionella.* Research is needed to assess operating and environmental conditions of aerators and a measure of the nutrient levels, water temperature, stagnation and aerosols emitted from these systems (a research plan to address this gap is presented in chapter 3).
- A quantitative assessment of *Legionella* from the aerator treatment process is needed. *Legionella* has been measured in groundwater and across different treatment processes within a drinking water plant, including filtration systems, but no specific measurement has been made in the aerator treatment units. In addition, the impact of aerators treatment process on water quality and implication on microbial growth is unknown (a research plan to address this gap is documented in chapter 4).
- Fouling deposition within the aerations systems are unavoidable but can be controlled. Characterisation of biofilm and impact of aerator operating conditions will allow for system optimisation to limit fouling and biofilm growth. There is the opportunity to determine the constituents of biofilm and fouling within aerators and optimise the most appropriate methods for fouling removal.
- LHLRA provides an initial step in assessing the risk of *Legionella* growth in aerators. There is an opportunity to improve this current risk framework, as well as to study available advanced risk assessment frameworks that can overcome current challenges. This can allow a transition from the current semi-qualitative outcome to a quantitative estimation of *Legionella* risk (research plan to address this gap is documented in chapter 5).

- Understanding the correlations of *Legionella* with water characteristics and operating conditions can provide insight into conditions under which the pathogens can grow, as well as determine better management practices to limit risk. There is an opportunity to explore the capability of using a hybrid of data-driven and expert knowledge to improve our understand of relationship between *Legionella* growth and water quality parameters to identify correlative indicators to track *Legionella* in aerators (a research plan attempting to address this gap is presented in chapter 6).

CHAPTER 3

PRELIMINARY ASSESSMENT OF THE RISK OF *LEGIONELLA* IN GROUNDWATER TREATMENT AERATORS

3 PRELIMINARY ASSESSMENT OF THE RISK OF *LEGIONELLA* IN GROUNDWATER TREATMENT AERATORS

3.1 Introduction

The presence of organic matters and dissolved metals have been confirmed to stimulate *Legionella* growth in groundwater treatment systems (Bargellini *et al.*, 2011). *Legionella* in the natural groundwater environment is considered benign, posing little or no public health risk (Costa *et al*., 2005). But engineered water treatment systems, such as aerators can provide optimal conditions for *Legionella.* In such optimal conditions, low *Legionella* numbers can grow into high density thresholds that can be considered to pose significant risk of inhalation to humans and could cause Legionnaire's disease (Ginige *et al*., 2011; Lasheras *et al.*, 2006).

In Chapter 2, critical risk factors considered to provide favourable environment for *Legionella* proliferation in engineered systems such as aerators have been documented. In aeration systems, the potential for elevated levels of available nutrients, periodic water stagnation, and water quality such as warm temperature present an ideal ecological niche habitat for biofilm formation that harbours *Legionella* growth. When combined with aerosol generation, and system deficiencies linked to poor aerator design and operation as well as the lack of consistent maintenance can exacerbate the growth and increase the risk of *Legionella* transmission.

During water treatment, aerators precipitate dissolved constituents in the influents creating residues and by-products that can accumulate to form fouling layers. Fouling accumulation combined with water stagnation in aerators can promote a rapid proliferation of *Legionella* population and aerosolisation of water droplets that can provide an exposure pathway. This scenario potentially put plant operators and nearby communities at increased risk of legionellosis transmission from aerators (Ginige *et al*., 2011a; Lasheras *et al.*, 2006b). To date, no study has assessed and measured the potential risk for *Legionella* growth and transmission in operational aerators utilised in GWTPs.

This chapter aims to assess the presence of risk factors associated with *Legionella* growth and transmission in groundwater treatment aerators. This was achieved through inspection of existing operational aerators in GWTPs with the following objectives:

i. Survey groundwater treatment aerators to assess the presence of the five identified *Legionella* risk factors,
ii. Establish the source and fate of nutrients in groundwater aerators by examining the characteristics of aerator influent, effluent, and accumulated fouling materials,
iii. Correlate incidence of *Legionella* occurrence with the presence of the risk factors in aerators,
iv. Develop initial considerations to mitigate *Legionella* risk in aerators.

3.2 Materials and Methods

3.2.1 Site inspection of aeration assets in GWTPs

Between July 2017 and May 2019, 13 GWTPs that spanned the mid-west and south-west Perth regions in Western Australia, were inspected. The aerators inspected include the tray, semi-enclosed and open-spray type as detailed in chapter 2 (Section 2.2.1). Inspection of the aerators identified design flaws and asset conditions that can possibly increase *Legionella* growth and transmission risk during and after operation. A *Legionella* risk register was developed to consolidate the observations from the site inspections and relevant information such as water safety plans, historical water quality data, site-specific limitations, and issues raised during interviews and discussions with relevant stakeholders. Characterisation of the influent was determined from the historical records collected from the GWTPs monitored water quality parameters. For confidentiality reasons, the detailed information and location of these plants were not provided.

3.2.2 Aerator effluents and fouling sampling

Aerator effluents and fouling materials were sampled and characterised to assess their resulting implications for *Legionella* growth. Aerator effluents were sampled into sterile polyethylene bottles for the analyses. Effluents samples for the spray type aerators (open and semi-enclosed) were collected from off the sprinklers while those for the trays were taken from sumps within the collection basins.

Fouling sampling was completed in four cycles — twice in the summer and winter seasons, respectively. Fouling depositions were sampled from multiple spatial locations within each aerator using a scrapper. Typically, samples for tray aerators were taken from the bottom sump and splash plate wall (*Figure 3.1*). For the open spray (*Figure 3.2*) and semi-enclosed spray (*Figure 3.3*) systems, samples collected ranged from wet slurries to dry solid residues depending on the aerator operational status.

In total, 56 samples were collected across the aerators inspected. 26 samples were collected from the open spray, 18 in the trays and 12 in the semi-enclosed aerator types. The samples were stored and transported in an Eski to the laboratory and analysed immediately.

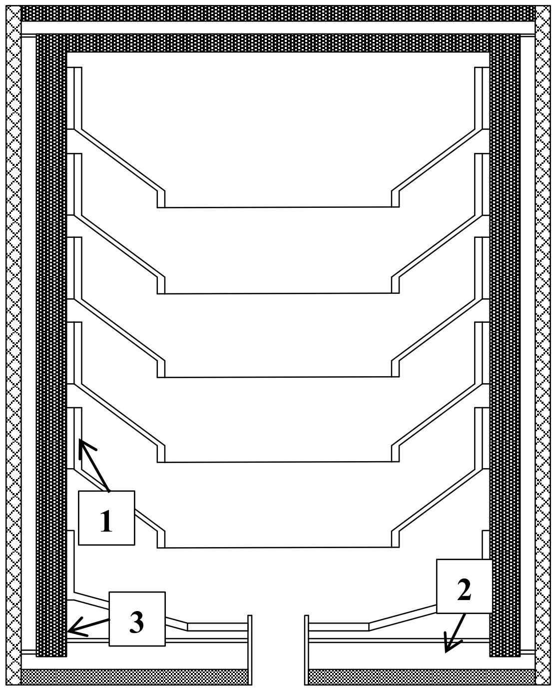

Figure 3.1 – Schematic of a tray aerator showing the typical sampling locations of the fouling materials including (1) Underside of aerator plate (2) Topside of last aerator plate and (3) Corner of bottom collection basin.

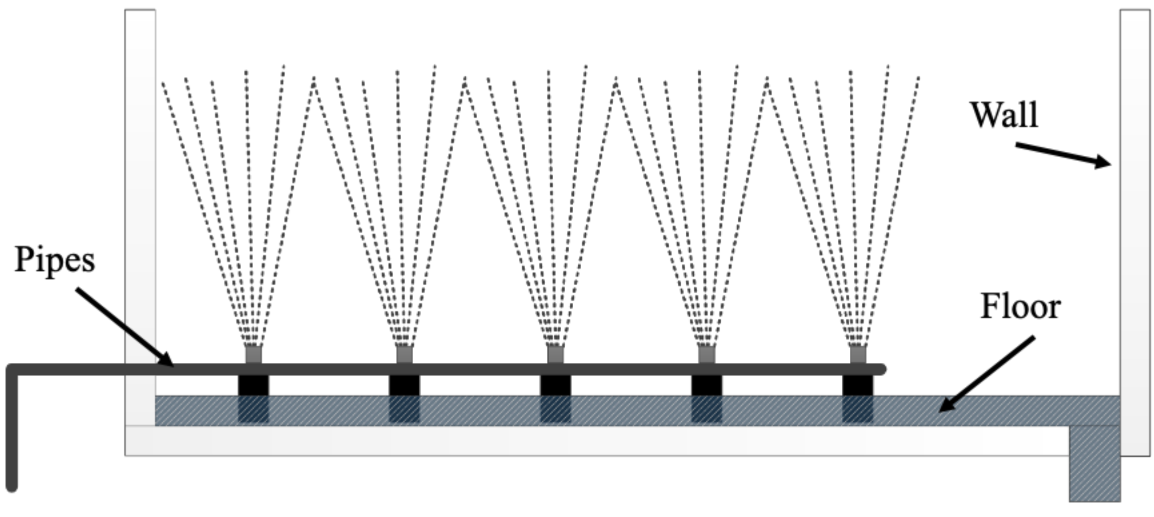

Figure 3.2 – Schematic of open tray aerator showing the typical sampling locations of the saturated sump solution as well as fouling materials on the aerator concrete base, steel, sprinkler pipes, Perspex wall and floor of spray aerator wall.

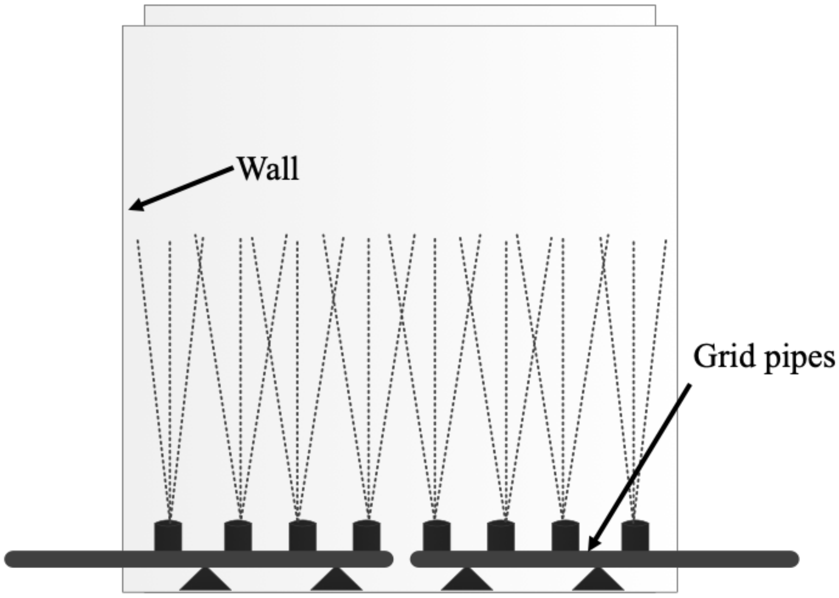

Figure 3.3 – Semi-enclosed spray aerator with typical sampling location of a mesh steel base and aluminium wall. The semi-enclosed configuration is similar to the open tray but with the spray (sprinkler) enclosed in a cylindrical tank.

3.2.3 Sample characterisation

Aerator effluent samples were prepared and characterised into inorganic and organic components. On the other hand, the fouling materials were first grouped into physical constituent types, and subsequently, organic, and inorganic distributions and element compositions were described using gravimetry and elemental analysis. The methods are described in full detail below.

3.2.3.1 Gravimetric analysis

The inorganic and organic contents in the fouling samples were determined using gravimetry analysis. First, the samples (slurry and solid) were weighed and dried in a laboratory oven (105^0C). Slurry samples were shaken vigorously to ensure homogeneity, and 30 mL of solution was placed in a pre-weighed crucible, and the new mass was measured and recorded as wet mass (M_{wet}). For solid samples, approximately 1 g each were transferred into the pre-weighed crucibles using a laboratory spatula, and the new mass was measured and recorded. The crucibles containing both slurry and solid samples were dried overnight at 105^0C. Subsequently, the mass of total dry solids was recorded (M_{dry}). The moisture content (MC) and total solids mass (TS) were then calculated with equations 1 and 2, respectively.

$$\text{MC}\ (\%) = \frac{M_{Wet} - M_{Dry}}{M_{Wet}}\ x\ 100\% \qquad (1)$$

$$\text{TS}\ (\%) = \frac{M_{Dry}\,(mg)}{M_{Wet}\,(g)}\ x\ 100\% \qquad (2)$$

The dried samples were then volatilised in a furnace set to 550°C for 30 min, and the residual ash contents weight was measured (M_{550}). Mass difference between the original dry sample and residual ash after volatilisation at 550°C represents the organic contents. The residual ash represents the inorganic contents in the samples. The proportion of inorganic and organic in the samples is expressed using equations 3 and 4.

$$Inorganic\ content\ (\%) = \frac{M_{550}}{M_{dry}}\ x\,100\% \qquad (3)$$

The organic percentage is equal to 100% - inorganic percentage and is typically representative of organic components of the sample.

$$Organic\ content\ (\%) = \frac{M_{dry} - M_{550}}{M_{dry}}\ x\ 100\% \qquad (4)$$

3.2.3.2 Inorganic analysis and quantification

Inductively Coupled Plasma - Optical Emission Spectrometry (ICP-OES), was used to identify and quantify inorganic elements present in the influents and fouling materials samples. A shortlist of 20 elements with high concentrations in typical groundwater sources and have implications for biofilm growth and development were targeted (Ginige *et al.*, 2011; Rutlidge *et al.,* 2015).

All the samples were acid digested using 5% nitric acid (HNO_3). The effluents samples were blended with an acid ratio that achieved the desired acid concentration. Known masses of the fixed weight of the foulants were dissolved in 15 mL of acid and sonicated for 1 hr to promote dissolution, decompose sample matrix, and solubilise the analytes of interest for nebulisation by ICP-OES (Phan-Thien *et al.*, 2012). The samples (effluents and fouling) were shaken at 100 rpm overnight at 40°C to ensure complete dissolution. The samples were sonicated again and passed through 0.45 µm filters (Whatman GD/X PVDF) pre-washed with milli-Q water. Assuming a pure chemical component, the mass concentration of the elements in the mixture are reported as density (mg/g). The same procedure for inorganic elemental analysis was applied throughout the whole research.

3.2.3.3 Organic analysis and quantification

The total dissolved organic carbon (DOC) for the sampled effluents were characterised using Liquid Chromatography - Organic Carbon Detector (LC-OCD). Before analysis, samples were filtered through 0.45 µm (Whatman GD/X PVDF). The filters were pre-washed with milli-Q water before applying to the samples.

First, organic compounds in the samples were separated into hydrophilic and hydrophobic compositions. Subsequently, the hydrophilic compositions were further fractionated into biopolymers (BP) humic substances (HS), building blocks (BB) low molecular weight acids (LMWA, and neutrals (LMWN). Detailed descriptions of the different fractions are summarised in the literature (Huber *et al.*, 2011). The fractionation process was completed using LC-OCD (Model 8DOC- LABOR, Germany) and the quantified using DOC-LABOR ChromCalc software. The DOC and LC-OCD analysis was applied for organic characterisation of samples through this research work.

3.2.4 Statistical analysis

The results of the influent, effluents and foulants characterisation were expressed in mean and standard deviations. Difference between grouped datasets was determined using test statistics. Two groups of datasets that are normally distributed were evaluated using student's t-test. For non-normally distributed sets, the nonparametric Wilcoxon signed-rank test was completed. For more than two groups of datasets, analysis of variance (ANOVA) was used for comparison.

The null hypothesis considers the means of the samples to be equal. The critical t-value was set at p-levels 0.05, corresponding to a 95% confidence interval (2-tailed). A statistically significant difference between two data sets is established when the calculated P-value is below 0.05. A P-value above 0.05 implies no difference between the mean of the samples. The statistical analyses were completed using GraphPad Prism Software. The statistical definitions established in this section apply throughout the whole of this thesis.

3.3 Results and Discussion

Surveyed aerator types operating in the GWTPs were classified based on their design configurations. Aerators from the 13 plants fell into three broad categories: open spray, semi-enclosed spray, and tray aerators. The tray-type was the most widely implemented, with nine operating assets, followed by open spray with three, and only one semi-enclosed spray aerator configuration. The photographic images of the internal and external views of the aerators are provided in the *Appendix 1, Figure A1.1*. The constituents of the water quality (influent and effluent), risk factors created by the design and operational limitation and characteristics of fouling with relation to *Legionella* from the three aerator types are discussed.

3.3.1 Aerator water quality

This section assesses the suitability of physical water quality parameters measured in the influent and effluent of the aerators with respect to *Legionella* colonisation. Concentrations of critical quality parameters in the influent and effluent to aerator were assessed and contrasted with threshold limits reported in the literature to either increase or decrease the likelihood of *Legionella* colonisation of water systems. Concentration threshold values in this context referred to the limit above which there is a significantly increased positive or negative impact of the quality parameter on *Legionella* colonisation of the water system defined through statistical regressions.

The historically monitored water quality records collected from the 13 GWTPs spanning 2011 and 2018 were summarised for the influent water characteristics. The effluents quality on the other hand were determined through inorganic and organic characterisation of samples taken during the survey of the aerators between 2017 and 2019.

3.3.1.1 Influents water characteristics

Mean and standard deviations of the selected critical parameters and trace elements extracted from historical water quality parameters monitored at the 13 GWTPs plants are presented in *Table 3.1*. Additionally, threshold values for the selected parameters indicated to have positive or negative impact on *Legionella* occurrence gathered from different studies are provided alongside.

Table 3.1: Descriptive statistics mean ± standard deviation (number of samples) of the influent streams quality parameters from the historical water quality records sourced from the GWTPs, alongside the reported concentration thresholds values above which increase or decrease impact on Legionella colonisation was observed.

	Open spray	**Semi-enclosed**	**Tray**		
	Aerator measured concentration (mg/L)			Reported concentration thresholds (mg/L)	References
Alkalinity	103.34 ± 59 (180)	82.2 ± 35 (9)	107.03 ± 50 (187)	100 (+ve)	States et al., 1987
Chloride	188.60 ± 75 (152)	148.75 ± 71 (4)	136.26 ± 136 (182)	100 (-ve)	Tulkki *et al*., 2003
Hardness	158.26 ± 56 (289)	82.44 ± 18 (9)	108.069 ± 32 (244)	300 (-ve)	Kyritsi *et al*., 2018.
Trace elements					
Calcium	29.69 ± 19 (45)	18.0 ± 7 (4)	12.15± 9 (190)	n. r	*Crabtree et al*., 1999
Copper	0.17 ± 0.4 28)	-	0.04 ± 0.1 (70)	0.06 (+ve)	-
Iron	2.36 ± 1.7 (267)	13.65 ±11.5 (55)	1.94 ± 3.1 (733)	0.103 (+ve)	Rakić *et al*., 2012
Magnesium	7.89 ± 4.5 (49)	10.5± 0.6 (4)	15.76 ± 8.5 (190)	n.r	-
Manganese	0.06 ± 0.7 (261)	0.17 ± 0.01 (8)	0.07 ± 0.07 (700)	0.006 (+ve)	Bargellini *et al*., 2011
Potassium	10.24 ± 3.6 (152)	18± 6.4 (4)	8.41 ± 6.1 (271)	n.r	Crabtree *et al.*, 1999
Sodium	69.07 ± 43.7 (152)	74.5 ± 3 (9)	98.12 ± 59.7 (271)	n.r	-
Zinc	0.04 ± 0.03 (5)	-	0.04 ± 0.02 (12)	0.375 (+ve)	Bargellini *et al*., 2011

(+ve) denoted threshold above which a positive relationship was observed and (-ve) denote value above which a negative impact relationship was observed with Legionella occurrence and n.r denote non-reported indicated parameters whose direct impact on Legionella have not been considered in the literature.

The mean concentration of alkalinity varied widely across the aerator types. The mean value observed in the semi-enclosed aerator influent was below the 100mg/L alkalinity concentration threshold correlated with *Legionella* positivity. Chloride concentrations were consistently greater than the critical threshold value (100mg/L) correlated to *Legionella* positivity in influents from all the aerator types.

Alkalinity and chloride concentrations above 100 mg/L have been correlated to reduce cases of *Legionella* positivity due to the toxicity of associated metals and compounds (States *et al.*, 1987; Tulkki *et al*., 2003). The distribution of alkalinity concentration that include values below the cut off represented risk to *Legionella* occurrence in the aerators.

The mean concentrations of hardness were below the value (300 mg/L) correlated with reduced positivity of *Legionella* (Kyritsi *et al*., 2018). Expectedly, elevated concentrations of hardness can be deemed to contribute to abundance of calcium and magnesium in the influents. While the concentrations of calcium and magnesium have not been directly correlated with *Legionella,* their abundance when precipitated can contribute to scaling, creating a favourable ecological niche for *Legionella* through fouling formation (Liao *et al.*, 2018; van der Kooij *et al*., 2015).

Concentrations of trace elements consistently represented thresholds positively correlated with increased *Legionella* occurrence. Evidence from pioneer studies postulated that low concentrations (0.005 - 0.5 mg/L) of iron, magnesium, copper, manganese, and zinc stimulate *Legionella* (Reeves *et al*., 1981), and higher concentrations (10 - 100mg/L) featured inhibitory characteristics (States *et al.,* 1985). Specifically, strong correlation of *Legionella* positivity was established with elemental concentration cut-off of 00.58 mg/L for zinc, 0.13 mg/L for iron and greater than 0.006 mg/L for manganese (Bargellini *et al.,* 2011a; Rakić *et al*., 2012; Rakić and Štambuk-Giljanović, 2016). The concentration of iron, zinc and manganese monitored in aerator influents reflected the values considered to be correlated with increased *Legionella* positivity in water samples (Bargellini *et al*., 2011; Rakić and Štambuk-Giljanović, 2016).

Influent water temperatures calculated from the monitored records and grouped into seasons gave average values for summer as $25 \pm 4\ ^0$C; autumn $24 \pm 4\ ^0$C; winter $22 \pm 4\ ^0$C and spring $23 \pm 3\ ^0$C. *Legionella* have been isolated from a wide range of water temperature in the environments. These temperatures ranged from 0^0C to 45^0C. But water temperatures between for 25^0C and 30 ^{0}C are considered as the optimal range for *Legionella* growth (Anand *et al*., 1983; Wullings and Van Der Kooij, 2006). The mean temperature reported in the influent and effluent can be considered to the lower limit for optimal growth. While maintaining water temperatures below 25^0C is a standard preventative measure in water systems, this measure is impractical in the influents and effluents to aerator and as such these systems are likely to continue to provide suitable *Legionella* growth temperature.

Reinforcing this optimal temperature, a two year long weekly sampling of cooling tower in Australia revealed water temperature as the dominant *Legionella* determinant variable, with measures above

20°C strongly correlated with frequent occurrence (Bentham, 1993). This suggested aerator influent water temperature present conditions favourable for *Legionella* growth all seasons.

Overall, the influent water characteristics can be deemed favourable for hosting and stimulating *Legionella.* The unexpectedly elevated concentration of the environmental parameters of trace elements, alkalinity, chloride, and hardness considered ideal for *Legionella* can be traced to the slightly brackish nature of the catchment groundwater in Western Australia. Analysis of the effluent streams will allow for the assessment of the impact of the aeration process in altering the water characteristics.

3.3.1.2 Effluent water characteristics

3.3.1.2.1 Inorganic characterisation

Summary of concentrations of inorganic elements measured in the effluents is presented in *Table 3.2*. The abundance of critical trace elements in the effluents in decreasing order were sodium, calcium, magnesium, potassium, iron, zinc, copper, and manganese. The mean concentrations of the elements in the effluents were variable across the different aerators. The highest concentrations of calcium, potassium, sodium, zinc, and magnesium was observed in the effluents of the tray aerators, while concentrations of iron, and manganese were higher in the semi-enclosed and open spray aerators. Magnesium concentration varied greatly, from below detection in the semi-enclosed to 5.22 mg/L and 679 mg/L in open spray and tray aerators. Similarly, the concentrations of sodium range from 40.7 to 588.5 mg/L.

Table 3.2: Characteristics of the inorganic elements of the effluents from the open, semi-enclosed spray and tray aerators.

	Mean concentrations +/- standard deviation (mg/L)		
Elements	**Open spray**	**Semi-enclosed spray**	**Tray**
Calcium	19.64 ± 22.26	134.44	317.48 ± 12.58
Copper	0.01	3.08	4.56 ± 1.2
Iron	**0.77** ± 0.79	**48.31**	**< 0.02**
Magnesium	5.22 ± 3.88	< 0.05	679.43 ± 2.56
Manganese	0.01 ± 0.02	4.42	< 0.02
Potassium	3.10 ± 2.33	195.35	519.79 ± 153.73
Sodium	40.66 ± 25.59	182.45	588.53 ± 663.04
Zinc	< 0.02	3.37	3.66 ± 3.0

Comparing the elemental concentrations of the influent and effluent show no clear trend. The elemental concentrations in the effluent either remained relatively consistent or slightly increased in the effluents with large variation between the different aerator configurations. A possible explanation could be that aerators are expected to oxidise metals from the influents with the separate occurring in the filtration treatment process further downstream. As such no clear differentiation could be observed between the influent and effluent streams elemental concentrations.

Another possible explanation particularly, for the large variations and inconsistent trends between the influent and effluent concentrations could be the difference in sampling period. The difference in sampling and characterisation periods for the influents (2010 – 2018) and effluent (2017 – 2019) can introduce variability that makes it difficult to assess the exact impact of aerator process. To better assess aerator impact, a pairwise sampling of the influent and effluent and a concurrent characterisation will be discussed in chapter 4.

3.3.1.2.2 Organic characterisation

Concentrations of organic constituents of the influents measured as DOC and LC-COD fractions for the open spray aerators are presented in *Table 3.3*. The organic matter was highly variable across the different open spray aerators (with up to 50% standard deviations).

Table 3.3: Characteristics of the organic contents of the influents to the aerators (three open spray aerators were sampled and analysed).

	Concentration (mg/L)
DOC including:	16.30 ± 9.25
Hydrophilic	13.03 ± 10.32
Hydrophobic	3.25 ± 2.73
LC- OCD (hydrophobic):	
BP	0.15 ± 0.10
HS	0.46 ± 0.61
BB	1.53 ± 1.06
LMWN	10.0 ± 8.10
LMWA	0.01 ± 0.01

The organic matter was primarily hydrophilic, representing 75% of the concentration. The hydrophilic/hydrophobic ratio is 4. A quarter of the LC-COD fraction was LMWN, followed by BB, HS, BP, and a negligible amount of LMWA.

The organic matter concentration and fraction distribution in the influents falls within the range reported in groundwater sources in Australia (Rutlidge *et al*., 2021). Groundwater treatability is significantly impacted by the ratios of hydrophilic/hydrophobic contents. The high hydrophilic in the influents will mean difficulty of organic matter removal due to higher concentrations of non-polar compounds (Ghernaout, 2014).

On the other hand, high organic matter loads in water sample has been implicated for creating optimal conditions for increased *Legionella* growth (van der Kooij *et al*., 2017). Earlier research demonstrated that higher concentrations of dissolved organic compounds can support microbial growth including amoeba which are considered to shield *Legionella* from disinfectant effect and increase their virulence (Proctor *et al*., 2017; van der Kooij *et al.,* 2017). Several Legionellosis incidences have been traced to water characteristics with a large organic load that make disinfectant levels challenging to maintain (LeChevallier, 2019).

Collectively, the combined effect of different water quality parameters of temperature, total hardness, alkalinity, organic matter and trace elements in the influents and effluents can significantly influence *Legionella* proliferation in aerators. Monitoring these water quality parameters in aerators can be integrated as a useful part of risk assessment programs in water safety plan to help track the possibility of *Legionella* contamination. This can provide insight into the mediating role of water quality parameters in stimulating ecological niche for *Legionella.*

3.3.2 Design and operational limitations in aerators

3.3.2.1 Open spray

Six limitations that have the potential to increase the risk of *Legionella* growth and transmission were observed in the open tray type aerators. The limitations are both design and operation related. The design related limitations include open exposure to direct sunlight, large flat floored configuration, and susceptibility of the component materials to high biofilm formation. Firstly, the open spray consisted of multiple spray nozzles on pipes that were contained within a rectangular bund. The assets were open to the atmosphere and exposed to direct sunlight, which provided favourable conditions for biological growth (Jørgensen *et al*., 1998). Secondly, its large space footprint and flat floor allowed for locations with dead – stagnant zones, particularly at points further away from the outlet. Thirdly, the construction materials in aerators including concrete, galvanised steel and wood have demonstrate increased susceptivity to corrosion, microbial biofilm formation that can harbour pathogen (Marques *et al*., 2002; Waines *et al.,* 2011).

The other three limitations were related to the operation of aerators. During operation, water droplets were created and dispersed widely within and beyond the aerator walls. Operation of the aerators was intermittent (8 to 18 hr daily runtime) resulting in water stagnation in different locations of the open spray aerators when offline. Following prolonged operations, fouling materials depositions were observed in the internal surfaces of aerators.

The distribution of fouling materials in the aerator surfaces were non-uniform (*Figure 3.4*). The greater surface area of open spray aerator increased their propensity for fouling. Different fouling patterns were observed: thin sludge film covering the floors and thick sludge cake layers coated the sprinkler pipes and walls. The fouling includes reddish corrosion and precipitated deposit layers and dense green algae coated formations.

Figure 3.4 – Fouling materials on all the aerator types: the open spray (a) floor and (b) sprinkler pipe (c) walls; semi-enclosed spray (d) lower section of the cylindrical wall (e) middle section (f) upper section and in the tray (g) plate underside (h) plate topside (i) bottom of the collection basin

Interview with stakeholders confirmed the observed lack of system isolation from downstream processes during cleaning or incidences of contamination as well as influent and influents sampling points for aeration performance monitoring.

3.3.2.2 Semi-enclosed spray

The semi-enclosed spray has similar design principles to the open tray aerator but with a slightly improved design. Unlike the open tray, the semi-enclosed aerator consists of a series of nozzles enclosed within a cylindrical tank offering a smaller footprint. The sprinkler pipes are rested on a grated base that drains off the treated water eliminating stagnation.

Compared to the open spray type, lesser aerosols were observed to be emitted from the top of the cylindrical wall. A significant proportion of the water droplets is contained within the enclosed cylindrical tank, limiting aerosolisation.

Like the open spray, visible deposition of fouling layers was observed on the different surfaces. Various thicknesses and colourations were observed on the wall sections, sprinkler pipes, and grated floor. Sampling and isolation points were noted to be lacking. The semi-enclosed aerators only had man-hole access making sampling and cleaning more difficult (see image in the *appendix 2, Figure A3.2*).

3.3.2.3 Tray aerator

The tray type of aerators presented several design flaws and operational constraints. The raised outlet flange and square-shaped trays created dead zones that slowed water drainage. This design limitation translated to incidences of water stagnation when the system was offline. In the current design, the exterior mesh does not limit aerosol egress during operation or prevent foreign debris from entering the aerator.

Similar to open and semi-enclosed spray aerators, layers of fouling depositions were observed in the trays. The tray aerators were constructed with metal, which becomes rusted under continuous contact with water during aeration treatment process. The inside of the tray showed thick slurry fouling in moist and solid crystal solid deposition in dried aerators conditions.

In addition to the identified *Legionella* risk factors, access for sampling and cleaning operations are challenging. Interviews with operators confirmed the observed lack of sampling points for influent, influents, and the improper allowance for downstream process isolation. Access for proper cleaning operations is difficult. An in-depth cleaning requires dismantling of the system and cutting out the grills of the aerator to restore the surfaces to their initial conditions using pressure jets and chemical cleaners (*Figure 3.5*).

Figure 3.5 – Tray aerator mesh decoupled and cleaned at floor level (photo provided by WCWA)

3.3.3 *Legionella* risk factors created by aerators assets limitations

The design flaws and operational constraints in current aerator assets facilitate five risk factors for *Legionella* growth and transmission. Four of the five factors directly impacting *Legionella* risk were periodic water stagnation, nutrient availability, biofilm and nutrient-rich fouling, and aerosols emission. The impact of inadequate maintenance was considered indirect. Based on these observations, recommendations on more appropriate design, operation and maintenance of aerator systems are provided.

3.3.3.1 Water stagnation

Water stagnation after operation occurred in both tray and open spray aerators. The improper outlet flange and square-shaped tray-type aerators created dead zones in corners of the systems. The small sloping floor of the open spray facilitated slow drainage, leading to water stagnation. The presence of water stagnation in aerators can support and harbour the growth of biofilm and *Legionella* (Bédard *et al.*, 2018; Paranjape *et al.*, 2020; Wang *et al.*, 2012). Water stagnation in aerators can increase the susceptibility to scale formations and settling of precipitated sediments leading to fouling (Völker *et al.*, 2016b). As such, optimal aeration asset design should consider angled sumps and floors to facilitate water drainage and minimise water stagnation.

3.3.3.2 Biofilm and nutrient-rich fouling

Biofilm and fouling deposition were common in all aerator types. The presence of biofilms can create an environment favourable for *Legionella* to persist and to thrive (Shen *et al.*, 2015). Therefore, biofilms in aeration systems could exacerbate pathogenic *Legionella* persistence and associated risks.

The fouling characteristics confirmed that biofilms were a nutrient-rich source for *Legionella* growth. The availability of essential elements (such as iron) and organic matter measured in the aerator fouling can be considered as critical factors for microbial growth and attachment to aerator surfaces. Similarly, the uneven layering of fouling deposition within aerators can exacerbate water stagnation (Walser *et al*., 2014). The colonisation of aerators by biofilm and the availability of nutrient-rich fouling increases the risk of *Legionella.*

3.3.3.3 Water droplet and aerosols emission

Water droplets were observed dispersing from the aerators during operation. Water droplets break into aerosols as they travel away from the source. These aerosols will represent a potential source of Legionellosis, in instances of contamination in the water coming from the aerators (Schoen and Ashbolt, 2011).

The distances water droplets can travel away from the aerator is dependent on the size distributions. While water droplets usually fall to the ground within short distance from the source, aerosols (<5µm) from operational aerators have the potential to spread further off beyond the water treatment plant (Blatny *et al*., 2011; Nhu Nguyen *et al.*, 2005). Open spray aerators have the highest propensity to generate aerosols.

The tray and open spray type aerators are linked with increased emission and dispersion of aerosols relative to the semi-closed where the cylindrical tank provides containment that minimises aerosolisation distance.

With the potential for the aerosols to travel further distances (up to 7km) downwind, plant operators, maintenance personnel and residents of nearby communities can be considered at risk of *Legionella* exposure (Blatny *et al*., 2011; Nhu Nguyen *et al.*, 2005). The semi-open and open spray aerators were placed further away from resident communities (>2km). Tray aerator, on the contrary, was placed less than 500 m to settlement potentially susceptible to delivering aerosols to individuals in proximity.

The water droplets (mix of mist and aerosols) from the aerators establish a pathway for *Legionella* pathogen that could lead to exposure of plant operators and nearby communities. With the potential for

Legionella growth in aerators, combined aerosols have made them a potential occupational setting for legionellosis (Principe *et al*., 2017).

3.3.3.4 Monitoring and cleaning constraints

The lack of easy access for cleaning and maintenance operations in the aerators can be considered an indirect risk factor for *Legionella* growth with legionellosis clusters and outbreaks being linked in part to improper water systems maintenance and irregular cleaning of cooling towers (Zahran *et al.*, 2018). Regular maintenance activities including flushing of fouling and periodic cleaning of the surfaces of engineered water systems recommended as a preventive measure for biofilm growth and exposure to *Legionella* (Leoni *et al*., 2018).

The current aerator designs for tray and semi-enclosed systems present constraints for access for cleaning and maintenance except for the open spray. Therefore, appropriate design considerations that enable easier access for effective cleaning of the different components can reduce fouling, eliminate biofilms, and contribute to mitigate risk of *Legionella* associated with the aerator mediated transmission from GWTPs.

Similarly, the aerators lack sampling points for influent and effluents monitoring. Therefore, measuring parameters that can be correlated to define the impact of aeration performance was impossible. The inability to monitor water quality, aerator performance parameters made it challenging to identify the exact source of *Legionella*, either external infiltration or direct from feedwater (Costa *et al.*, 2005; Schalk *et al*., 2014).

3.3.4 Composition of fouling materials

Aerator fouling was observed on different surfaces in all the three aerator types: from the floor, sprinkler pipes, and walls. Fouling in the aerator results from a combination of inorganic and organic debris, scaling and biofilms development (Diaz-Bejarano *et al.*, 2017; Müller-Steinhagen, 1999). Fouling collected from the aerators in GWTPs were a complex mixture in different states including slurries and solid (*Figure 3.6*). Slurry fouling were soft (loose) mostly in regions of stagnant water. Crystal fouling are mostly corrosion scale products and/or precipitation fouling that had dried up.

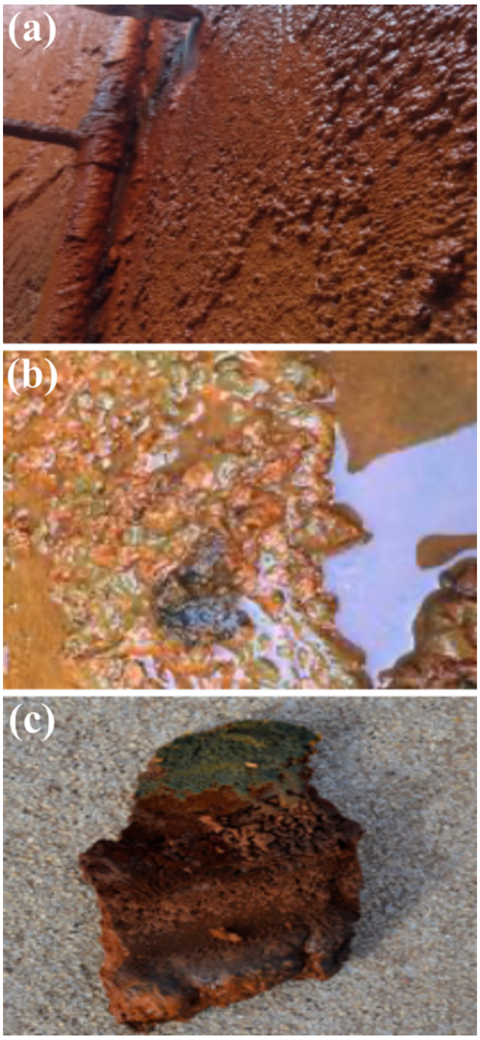

Figure 3.6 – Illustration of the fouling materials (a) slurry reddish sludge (b) green biofilm slimes (c) solid corrosion scales sampled from the open, semi-enclosed spray and tray type aerators.

The fouling collected from the aerators in GWTPs was composed of a mixture of organic and inorganic materials. The inorganic composition which can be traced to chemical fouling comprises corrosion and scales products. The precipitation components comprise reddish-brown aerator treatment residues, and debris materials conveyed into the aerators. The organic composition will result from the biological components comprising of deposition and growth of living matter: biofilm slime layers and algae films. Composition of the fouling samples were further characterised into inorganic, organic and element compositions.

3.3.4.1 Organic and inorganic content distribution

The gravimetrical analysis assessed the inorganic and organic contents distributions of the fouling materials. For all results, at least ten samples were reported ($n > 10$). Firstly, samples were grouped into their aerator types to assess the spatial variability of the inorganic and organic contents distributions of the fouling materials. Secondly, the samples were grouped into winter and summer under each aerator type to assess the impact of seasons on the fouling materials.

3.3.4.1.1 Spatial distribution of the organic and inorganic contents

Spatial distribution of the organic and inorganic contents in the different aerators fouling is presented in *Figure 3.7*. The greatest variability in the inorganic and organic fouling contents was observed in the semi-enclosed spray aerator sample. The variance in the semi-enclosed was about twice those in the open spray and more than triple those in the tray-type aerator fouling.

The fouling materials from the open spray show the most consistent and abundant organic contents. The median organic content was 83%, which is about two and ten folds higher than for semi-enclosed and tray aerators. On the other hand, tray aerator fouling materials contained the most inorganic content, with a median value of 91% which is about double and quadruple those for the semi-enclosed and open spray aerators.

The varying content distribution within and across the aerators was expected because of the distinct patterns visually observed, suggesting the prevalence of different types of fouling. The dominance of organic contents in semi-open spray aerators confirmed the visual observation of mostly precipitation and biological constituent materials. The prevalence of inorganic contents in the tray was an indication of the increased chemical constituents (scaling and corrosion products).

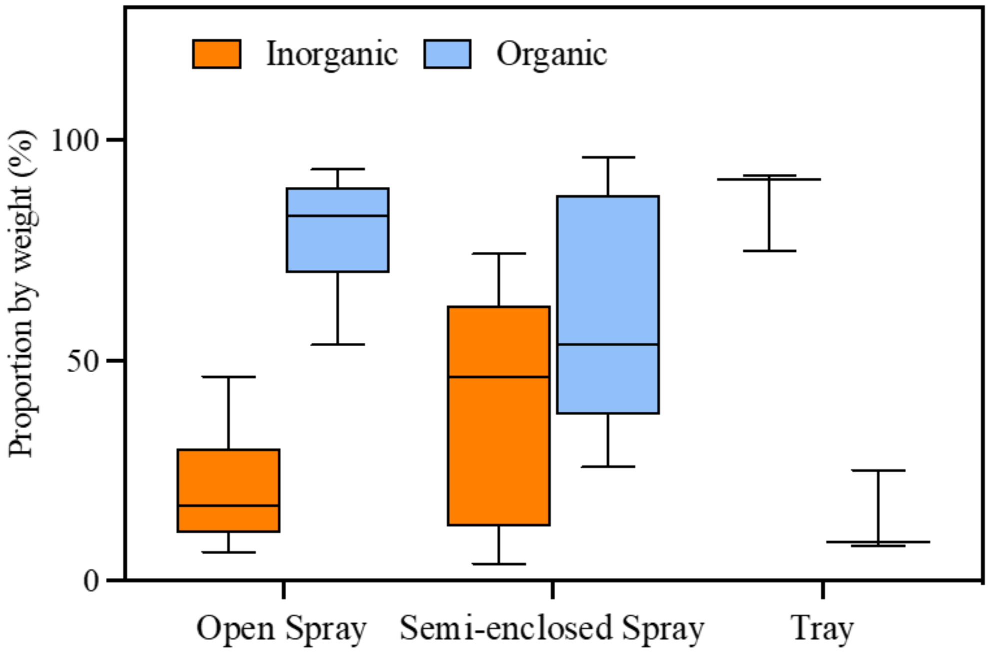

Figure 3.7 – Comparison of the distribution of inorganic and organic contents of the fouling materials sampled across different locations of the three different aerator types.

The inorganic and organic content distribution in the samples varied with aerator types. The organic content was significantly higher in samples for the open and semi-enclosed spray aerator. Tray samples were dominated by inorganic content. The higher organic contents in the open spray suggest dominance of biological fouling. In comparison, higher mean inorganic contents in tray aerators confirmed the dominance of chemical and precipitation (reddish-brown) constituents. Previous studies reported organic and inorganic contents distribution for groundwater treatment and distribution systems fouling materials at 5 and 24% and 65 – 80%, respectively (Echeverría *et al.*, 2009; Ociński *et al.*, 2016). Thus, organic contents obtained for trays were similar to other studies but considerably higher for the open and semi-enclosed spray aerators.

Contents of fouling materials in water systems depend on the feed characteristics and impact of the treatment process (Yang *et al.*, 2012). Inorganic contents in the fouling can be traced largely to dissolved metals and ions from influents and deposition of scales and corrosion (Peng *et al.*, 2010).

The differences in the distribution of the organic and inorganic contents in the aerators can be explained by the influence of constituents of the influent as well as design configurations and modes of operation of the aerators. Firstly, variability was observed in concentration of trace elements measured in the effluents of the different aerator types as highlighted in *section 3.3.1.1.* The effluents of the tray aerator contained higher concentration of at least four (calcium, magnesium, potassium, and sodium) of the eight critical trace elements characterised (*Table 3.3*). The significantly higher inorganic composition in the tray aerator compared to the semi-enclosed and open spray can be partly attributed to the elevated concentrations of certain key trace elements in the effluents which precipitated into the fouling (Yang *et al.*, 2012).

Secondly, the distinctive design configurations and operational patterns of the different aerators can possibly impact the organic and inorganic content distribution of fouling. The open spray by design was the most susceptible of the aerator types to allochthonous content. This is due to the open spray's relatively wider setting and larger footprint, making them more susceptible to cross-contamination with external constituents such as plant litter and soil, thereby increasing organic contents load. Similarly, the wide nature of the open spray allows for a higher amount of radiation hitting systems that can accelerate microbial activity. Increased microbial activity, as evident in the dominant biological fouling in the open spray can translate to higher organic matter through excreted metabolism (Villacorte *et al.*, 2015). The low inorganic content in the open spray compared to tray aerators can be attributed to the difference in corrosion-resistant indices and biofilm formation potential of the component materials. The tray built using metal components is expected to experience a higher corrosion rate than open spray with mixed steel, wood, and large concrete elements.

On the other hand, the difference in the modes of operation of the aerators can also contribute to drive variation in distribution of fouling contents. The varying operational pattern of the aerators created different surface conditions and subsequently fouling formation. For example, the operating condition of the open spray created distinct patterns of surface wettability within the aerator environment, including wetted and moist base and relatively drier panel walls. The wet conditions of sprinkler steel pipes made them highly susceptible to biological growth. For instance, algae flourishes under moist and illuminated conditions and their abundance on the sprinkler pipes are expected (Bott, 1995).

Similarly, the semi-enclosed spray created varying surface wetness. The positioning of nozzles at the bottom section of the cylindrical tank facilitated variable surface wettability. Variable surface wettability contributed to the formation of fouling of varying thickness across the walls. Subsequently, the variability observed in the organic and inorganic contents distribution in fouling sampled across cylindrical sections.

3.3.4.1.2 Impact of seasons on the fouling materials

The inorganic and organic content distribution in the samples varied with season across the aerator types (*Figure 3.8*). The mean organic content was significantly higher in summer for the open and semi-enclosed spray aerator compared to the winter seasons ($P = 0.003$). No reasonable seasonal difference was observed in tray aerators for mean organic contents measured in the summer and winter periods. The seasonal changes in the mean inorganic content in the samples of the aerators were not significantly different in all the aerator types ($P > 0.05$). But in general, the mean inorganic content for the summer season were higher for the open and semi-enclosed spray and lower for the tray aerators. The summer samples from the tray and open spray aerators showed the least inorganic and organic contents of 25% and 30% respectively.

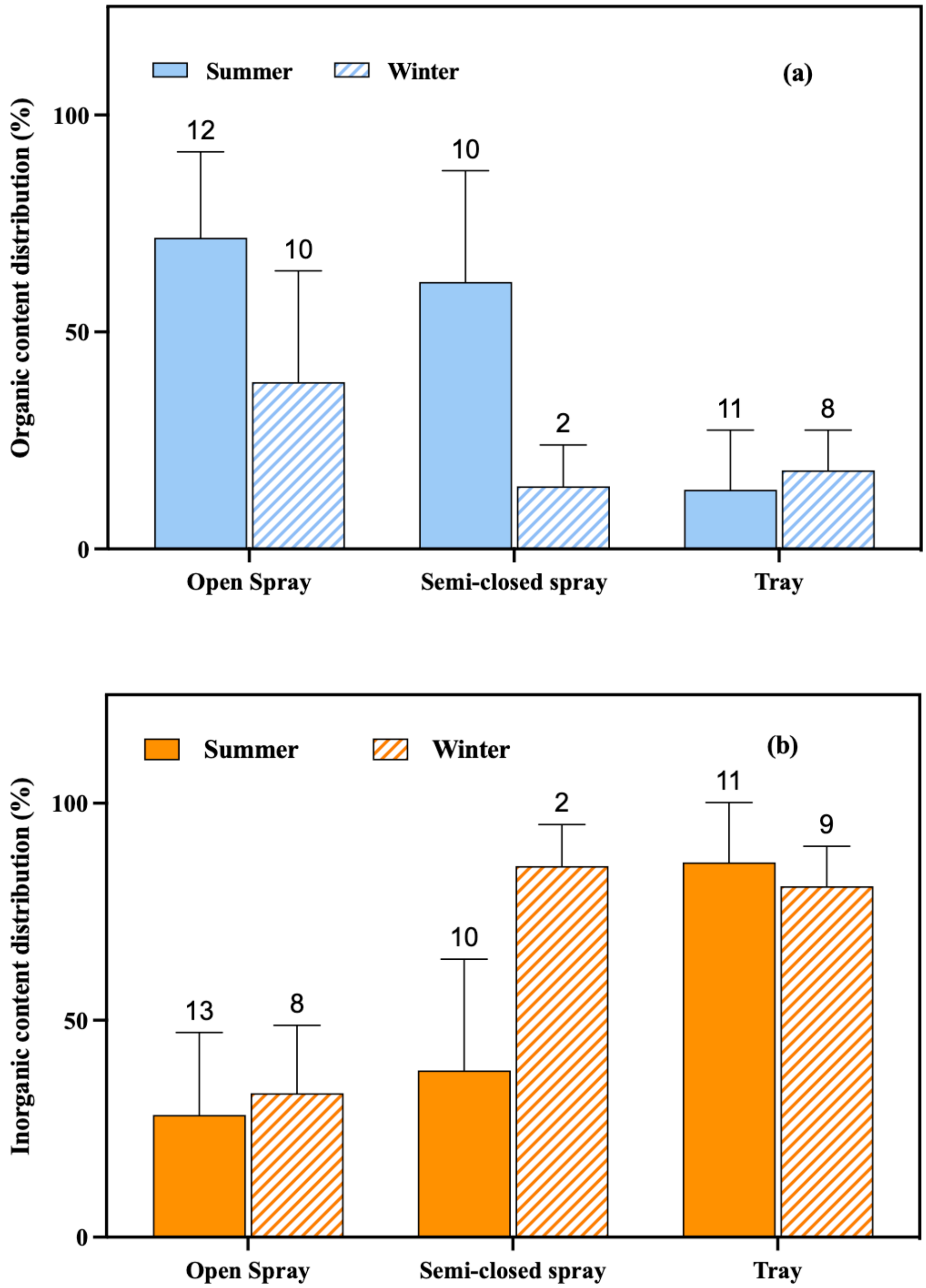

Figure 3.8 – Mean distribution of inorganic and organic proportion in the fouling materials across three different aerator types for summer and winter.

In addition to season fouling, the compositions were also impacted by the aerator types. In all samples, the highest mean organic content was 92% observed in summer in the open spray, and the highest inorganic contents of 88% in the tray in the winter. The mean organic contents of the samples across all seasons stand at 61 ± 23%, 53 ± 30 %, and 14.0 ± 9% for the open spray, semi-enclosed spray, and tray aerators, respectively. The mean inorganic content of the tray samples was about twice those for the open and semi-enclosed spray. The organic content was significantly higher in samples for the open spray aerator (P = 0.001) and the inorganic in the aerators (P = 0.002). The higher mean inorganic contents in the trays confirmed the dominance of chemical and precipitation (reddish-brown). In comparison, the higher organic contents in the open spray confirmed the dominant of biological fouling.

The distribution of organic contents in the fouling samples vary significantly with the seasons, but the inorganic contents do not. The mean organic contents in fouling are higher in summer season for all the aerator types. The high organic contents in the summer season were expected. Compared to other seasons, summer created more favourable conditions for increased biological activities. The elevated temperatures (as characterised by warmer influent from the historical water quality records) and radiation can enhance biological fouling, particularly in open spray aerators (Villanueva *et al.*, 2011).

3.3.4.2 Inorganic element analysis

The concentrations of the ten most abundant elements from the 20 analysed from the foulants are presented in *Table 3.4.* The critical trace elements accounted for between 95% and 99% of the total concentrations. Iron was the most prevalent of all the elements by a considerable margin (36 – 392 mg/g), followed in decreasing order by aluminium, calcium, phosphorus, magnesium, manganese, and potassium.

Table 3.4: The density of inorganic elements in aerator fouling materials (mg/g).

	Mean concentrations (mg/g) and standard deviations		
Elements	**Open spray**	**Semi-enclosed spray**	**Tray**
Calcium	77.79 ±101.74	11.58 ± 5.23	8.29 ± 15.29
Copper	12.38 ± 18.29	0.52 ± 0.01	537.30 ± 581
Iron	**134.42 ± 124.20**	**392.29 ± 181.69**	**36.61 ± 78.30**
Potassium	0.68 ± 0.51	0.54 ± 0.31	0.58 ± 0.43
Magnesium	1.64 ± 1.30	2.68 ± 0.99	0.73 ± 0.77
Manganese	0.56 ± 0.99	3.28 ± 2.03	0.59 ± 1.11
Sodium	6.10 ± 4.95	0.48 ± 0.17	0.34 ± 0.15
Phosphorus	3.35 ± 9.97	12.61 ± 5.30	2.61 ± 4.74
Zinc	78.36 ± 195.47	46.57 ± 20.29	18.21 ± 13.13

Iron, aluminium, and calcium mass concentrations were highly variable within the samples and across the different aerators. The highest mean concentration of iron was observed in the semi-enclosed spray, which is more than two and tenfold compared to those for the open spray and tray aerators. For aluminium, the highest mean concentration by a considerable margin was observed in the tray samples. However, potassium and magnesium concentration did not vary much across the aerators, with 0.54 to 0.68 mg/g and 0.73 to 2.68 mg/g observed, respectively.

The concentration of inorganic element compositions of fouling materials mirrors those of the influent constituents. The top 10 most abundant elements were the same for the influents and fouling materials. But the elements with the dominant concentration were different. While calcium was the most abundant in water, iron was most prevalent in the fouling materials. This suggests a transformation in the elements from the influents into the fouling materials.

The prevalence of iron in aerator fouling is unsurprising. Aerators are employed in groundwater treatment to oxidise and precipitate dissolved metals including iron. The prevalence of iron further explains reddish-brown stains observed on aerator surfaces. The mean concentrations of the iron in the foulants and influent show a positive correlation. The semi-enclosed aerators have the highest mean iron concentrations in foulants (392 mg/g) and the influent (48.31mg/L). Similarly, the tray aerator had the lowest iron concentration in the foulants and influents. This confirmed that characteristics of the feed water and impact of aeration process are key determinant of the concentration of the resultant elements in the fouling materials (Bajda, 2021; El-Zahaby and El-Gendy, 2016).

Although potassium and magnesium have a relatively higher concentration in the influents (*Table 1*), only a small measure was characterised in the foulants. This can be explained by the higher solubility index of salts (NaCl) with low tendencies to form insoluble crystal precipitates of the aeration treatment process that contribute to fouling material deposition (Al-Hadhrami and Quddus, 2010).

Unlike potassium and magnesium, the concentration of phosphorous show an increased abundance in the foulants against the extremely low values in the influent (below detected limit in two of the three aerator sources). This suggests a potential increase in microbial activity in the fouling materials against the influents as abundant phosphorus can be associated with biofilms uptake.

3.4 Implications of the risk factors on *Legionella* occurrence

3.4.1 Aerators systems as potential occupational assets for *Legionella* exposure

The potential influence of the recognised risk factors reported in AS/NZS 3666 on *Legionella* colonisation and transmission was examined in the context of the different aerator configurations. At least four of the five identified critical *Legionella* risk factors confirmed in other engineered systems were observed in the different aerator types (*Table 3.5*). The poor design and operational constraints in current aerators make them favourable for harbouring and supporting proliferation of the *Legionella* pathogen. The abundance of essential growth elements and organic matter in the aerator influent, effluent and fouling materials combine to create suitable niche habitats for *Legionella* growth. Aerosols and water droplets become a possible pathway for *Legionella* transmission during operation, implicating aerators as occupational exposure systems.

Table 3.5: Summary of the shortlisted Legionella risk factors observed in the aeration configurations surveyed.

Risk factors	Open tray	Semi-enclosed spray	Tray
Water quality	Optimal growth temperature (25 ^{0}C) Favourable concentrations of trace elements & organic matter		
Presence of nutrient sources	Yes	Yes	Yes
Presence of water stagnation	Yes	No	Yes
Aerosol dispersion	Excessive	Minimal	Moderate
Location and access	Isolated from communities	Elevated height and isolated from communities	Elevated height and in proximity to communities

Risk factors that can be considered to support either the growth or transmission of *Legionella* were observed in all the different aerator design configurations. These risk factors however take different forms across the different aerators. This makes the risk levels variable across the different type of aerators. The semi-enclosed spray will be considered to present the least risk factors due to the presence of mesh bottom eliminating periodic and the cylindrical tank helping to curtail aerosols. On the other hand, the open spray can be considered as the most at-risk aerator configuration for two reasons. First, the larger surface area in the open tray increases the tendency for stagnation and biofilm development that has been shown to complement *Legionella* growth with these systems (Bentham, 1993). Second, the forced pressure system created and dispersed relatively more massive aerosols and an increased risk of spreading contaminated particles. The presence of nutrients, abundant stagnation and excessive aerosols in the open spray will suggest these types of aerators as the most susceptible to risk factors considered favourable for *Legionella.*

3.4.2 Occurrence of *Legionella* in aerator systems

Historical records were assessed to determine the frequency and density of *Legionella* occurrence in the influent, effluent and fouling across the different aerator types. The distribution of *Legionella* occurrence in the influent, effluent and fouling were grouped based on the aerator types and season of sampling (*Figure 3.9*).

Legionella enumerated from over 300 samples collected in five years revealed the frequency of occurrence at 8% in the influents and 15% in the effluents with median density of 10 CFU/mL in both streams. The sporadic occurrence in the influent is similar to those reported for groundwater source (De Giglio *et al*., 2019). The frequency of *Legionella* occurrence in the effluent is two-fold more than those observed in the influent. The increased frequency in the effluent suggests *Legionella* is more likely to grow within the aerator than occurring within the feed groundwater. The influent and effluent water were not sampled in pair as such comparison for *Legionella* positivity was not evaluated from the records.

The swab of fouling resuspended in solution reported 28% *Legionella* occurrence frequency. The median density of *Legionella* in the swab was 100 CFU/mL. The likelihood of *Legionella* occurring in the fouling was four and six-fold compared to influent and the effluent respectively. The persistence and more frequent detection of *Legionella* in the fouling, compared to the water (influent and effluent) is expected. Fouling composition in aerator include biofilm which are considered favourable habitat for *Legionella* (Buse *et al*., 2012b; Tulkki *et al*., 2003). Recently, evidence from aerator faucet modelling show that fouling can drastically increase the quantity of contaminated aerosol particles released, increasing risk of human exposure (Benoit *et al*., 2021). Hence, the observed higher *Legionella* occurrence confirmed fouling within aerators as due to growth niche habitat.

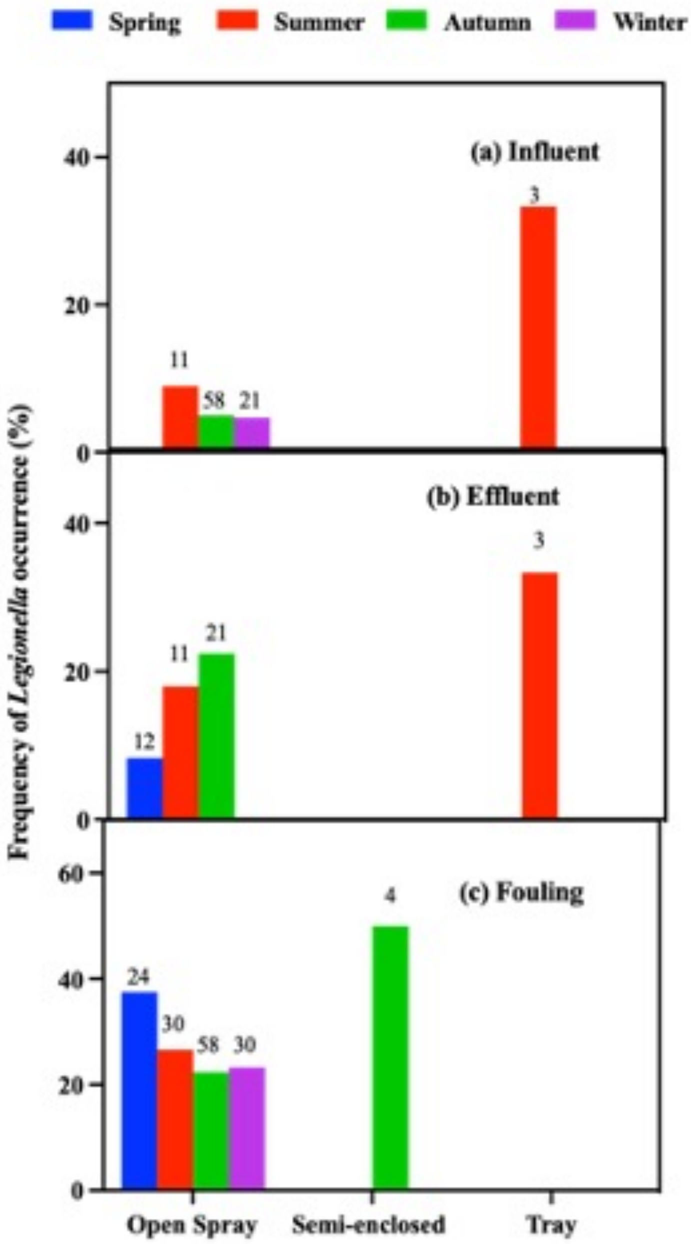

Figure 3.9: Frequency of Legionella positive detection in the (a) influent, (b) effluent and (c) fouling sampled from the three different aerator configurations spanning a five-year period grouped into the four seasons (The concentrations of Legionella in the aerators ranged from undetected to 100 CFU/ml in some biofilm sample. Number of samples analysed (n) shown above the bars).

The distribution of *Legionella* occurrence was variable across the aerator types. The occurrence of *Legionella* in the open spray was for the influent 5/89 (5.6%), effluent 6/45 (13.3%) and fouling 37/142 (26.1%). In the tray aerators as influent 8/99 (8%), effluent 9/58 (15.5%) and fouling 41/146 (28.1%). The semi-enclosed was sampled once. *Legionella* occurred only in the fouling and not detected in the influent and effluent. Although the distribution of sampling was skewed (with uneven sampling across the aerator types), the open spray tends to be more susceptible to *Legionella* persistence. The higher susceptibility of open spray can be linked to the larger surface area promoting greater biofilms formation and the more periodic water stagnation relative to the semi-enclosed and tray aerator configurations.

Seasonally, the frequency of *Legionella* detection shows no significant variability. Evidence on the prevalence of *Legionella* with season have been inconsistent. Both summer and winter seasons have been associated with increased *Legionella* positivity in Australia and internationally (Bentham and Broadbent, 1993; Zhang *et al.*, 2021). In Western Australia, the mean influent temperature was over 20^0C across all seasons representing hot conditions for optimal *Legionella* growth. In an earlier study by Bentham and Broadbent observed that water temperatures regardless of ambient temperature (seasonal temperature) is the major determinant in *Legionella* colonisation (Bentham and Broadbent, 1993). Presumably, the relatively warm influent water temperature regardless of the seasons in Western Australia explains the lack of significantly higher seasonal variability in *Legionella* occurrence.

The increased frequency of occurrence in the effluent and fouling confirmed aerator environment as favourable systems that can promote and harbour *Legionella.* Based on this, continuous monthly monitoring of *Legionella* alongside HPC influent, effluent and fouling as recommended in local standards would be prudent for better tracking of occurrence. In additional to monitoring, local standards should recommend performance based remedial actions for the detection of *Legionella* and HPC measurement as triggers which will be discussed in chapter 4.

3.4.3 Approaches to mitigate *Legionella* risk in aerator

This preliminary assessment has demonstrated that GWTPs aerators present risk factors and are frequently colonised by *Legionella* that can increase potential for release of contaminated aerosols. Two approaches to *Legionella* mitigation were considered. The first involved consideration for improved aerator design that address the identified limitations in current systems. The second explored improved approaches to operations, better monitoring, and appropriate maintenance strategies for current assets.

3.4.3.1 Consideration for improved aerator design to minimise Legionella risk

The identified deficiencies present the opportunity for an improved aerator design. Aerator technologies are useful in the groundwater treatment toolbox, with the main downside being the risk of harbouring and transmission of the *Legionella* pathogen. A next-generation design that overcomes current constraints should mitigate *Legionella* growth risk whilst still achieving its designed purpose. Considerations for the improved aerator design that overcome all the *Legionella* related limitations in the different aerator types are summarised in *Table 3.6*.

Table 3.6: Summary of design requirements, proposed features, and associated justifications to overcome limitations identified in three aerator types.

	Design Requirements	Aerator type	Proposed Design Features	Justification
1.	Addition of sampling points	All	Sampling points located on the inlet and outlet to the aerators.	To allow for monitoring of water quality and aerator performance parameters.
2.	Implement pre and post aerator isolation valves	All	Implementation of isolation and drainage by-pass lines on aeration assets.	To allow for isolation of aerator from downstream processes.
3.	Minimise water stagnation	▪ Open spray ▪ Tray	Installation of sloping aerator base for water drainage.	To allow direct flows to outlet points, minimise dead zones and water stagnation.
4.	Minimise fouling and scale formation	Tray	Introduce chamfered circular trays built out of fibreglass and lined with Polyvinylidene fluoride or polyvinylidene difluoride (PVDF) material	To provide a smooth surface using materials resistant to corrosion and biofilm/fouling adhesion and formation.
5.	Limit aerosol egress	All	Implementation of mist eliminators panels and/or octagonal housing to allow for complete enclosure of aerator	To reduce the risk of *Legionella* transmission through aerosols. Panels of octagonal housing can be sealed to quantify the minimum amount of passive airflow required
6.	Optimise design to improve access cleaning and maintenance	▪ Tray ▪ semi-enclosed	Design light-weighted trays (no longer than 120cm in diameter) for easy removal and manual handling for cleaning.	To improve access to aerator components and ease of maintenance

3.4.3.2 Further assessment and management of risk in current aerators assets

Employing appropriate cleaning strategies can effectively address the fouling accumulation within the surface of the aerators and reduce *Legionella* occurrence. However, a better understanding of temporal changes of fouling across different surface positions and wettability conditions within the aerator as well as the interaction of biofilms will provide insight for more effective cleaning operations (Madaeni *et al*., 2009; Obot *et al.*, 2019) as will be demonstrated in chapter 4.

Monitoring and integrating the recognised *Legionella* risk factors in current aerators can create a more robust risk assessment technique. Advanced risk management tools such as Bayesian networks have been proven to integrate different variables to prioritise risk levels in environmental uncertainty (Aguilera *et al*., 2011; Uusitalo, 2007). There is the opportunity to introduce Bayesian network to integrate the different risk factors of water quality, nutrient sources, stagnation, location and access to quantitatively establish the risk of *Legionella* in aerators as we will see later demonstrated in chapter 5 and 6.

3.5 Conclusions

- This preliminary assessment has demonstrated that GWTPs aerators present risk factors and are frequently colonised by *Legionella*. Therefore, aerators should be recognised as a possible source for an outbreak Legionnaires' disease.
- Improved aerator design and construction should consider appropriate materials that are less susceptible to fouling, configuration that eliminate water stagnation, minimise aerosol production and provide ease of maintenance to minimise *Legionella* risk in GWTPs.
- Monitoring water quality parameters such as iron, zinc manganese, temperature, hardness, and alkalinity with strong links to *Legionella* growth as part of the water safety plans can be considered a useful measure to track the possibility of proliferation within current aeration systems.
- Fouling materials occurring on all components within the internal aerator surfaces are a potential nutrients source for *Legionella* growth and can provide a niche habitat for harbouring microbial adhesion.
- Implementing adequate mechanical and/or chemical cleaning measures can mitigate prevalence of fouling and curb the opportunity for biofilm colonisation.
- So far, the compositions of the water and fouling were determined through assessment of historically monitored quality parameters and grab sampling. In the next chapter, a more precise and predictable characterisation of water and fouling was completed using a continuous monitoring of coupon study design to provide insights on the effects of surface orientation and location on the characteristics of temporal changes of biofilms formation and deposit accumulation in a controlled lab and full-scale aerators.

CHAPTER 4

FOULING MONITORING AND CHARACTERISATION TO DEVELOP INDICATOR PARAMETERS FOR BIOLOGICAL GROWTH ON SURFACES OF AERATOR

4 FOULING MONITORING AND CHARACTERISATION TO DEVELOP INDICATOR PARAMETERS FOR BIOLOGICAL GROWTH ON SURFACES OF AERATORS.

4.1 Introduction

The survey of GWTPs completed in Chapter 3 confirmed the varying degrees of biofilm/fouling layer formation on the aerators' surfaces. Aerator surfaces coated with biofilm and fouling included walls, sprinkler pipes, and floors. The variously fouled aerator surfaces occurred in a variety of orientations (e.g., vertical and horizontal) and were exposed to a variety of wettability conditions, including sprayed and fully submerged.

Different surface orientations in engineered water systems have significant effect on biofilm and fouling formation patterns, structure, composition, and resistance to cleaning operations (Faille *et al.*, 2018; Giaouris and Nychas, 2006). As a result, it is possible to gain a better understanding of the effect of temporal changes on fouling deposition and biofilm formation on various aerator surfaces. Understanding the effect of surface properties on fouling deposition, biofilm formation, and *Legionella* colonisation will guide in selecting the most effective mitigation approach.

Swabbing is the technique that has traditionally been recommended for sampling fouling and biofilms on surfaces. Despite its widespread use, the swabbing method is prone to sampling errors and inaccurate estimates of surface contamination. This is due to differences in the surface areas of the swabs, as well as the type and amount of biofilm effectively absorbed during sample collection (Faille *et al.*, 2020). Coupon sampling was proposed as a means of circumventing the limitations of swabs and providing a more representative measure of surface microbial contamination (Gagnon and Slawson, 1999).

In addition to the surface conditions, the compositions of the influent water are considered to contribute to the kinetic of fouling and microbial growth (Rakić *et al.*, 2012). The inorganic (e.g., iron, manganese, and phosphorous) and organic (i.e., dissolved organic moieties) properties of specific water constituents can impact on microbial growth, fouling, and biofilm development. Understanding the impact of the aeration process on specific influent constituents will provide further clarity on the role of the influent in fouling and biofilm development.

This chapter aims to increase the understanding of temporal changes in fouling and biofilms formation on sprayed and submerged wetted surfaces conditions positioned horizontally and vertically within spray aerators. This was accomplished by monitoring changes in feedwater characteristics over time and assessing the formation and composition of biofilms and fouling in both natural and controlled laboratory environments.

This was achieved through long-term monitoring of changes in feedwater characteristics alongside an assessment of the formation and composition of biofilms and fouling in a field and controlled laboratory environment.

The objectives of this study were as follows:

i. Assess the formation and composition of fouling in a controlled laboratory environment,
ii. Evaluate the effectiveness of chemical cleaning agents in the laboratory scale for fouling removal,
iii. Assess the formation and composition of fouling in a full-scale GWTP aerator,
iv. Determine the correlation between physical parameters and biological activity in feedwater and fouling materials and,
v. Develop initial *Legionella* risk management strategies.

4.2 Materials and Methods

Two studies were completed to monitor and understand temporal changes in fouling formation in the aerator systems. First, fouling formation was monitored in a controlled laboratory environment. A series of coupons were exposed to synthetic feedwater in a laboratory-scale aeration system at UNSW. The effectiveness of chemical agents on fouling cleaning was assessed on the aged coupons. Second, fouling was monitored in a full-scale, uncontrolled environment. A series of cement coupons were exposed to a full-scale open spray aerator in a GWTP in Western Australia.

4.2.1 Coupon preparation

Cement coupons were used in the study to mimic the material composition of the aerator environment. Coupons were made in the laboratory using a commercially prepared mix of blended sand, cement, and aggregate (Bastion general purpose cement, conforming to AS 3972 Australia). Water was added gradually into a measured concrete, mixed thoroughly to a workable consistency, and cast in silicone moulds. The concrete cast were cured for 14 days via immersion in water.

The total effective area of the cement coupons was 34 cm^2 (length: 85 mm, width: 40 mm and thickness: 10 mm). The coupons were used to monitor fouling formation in laboratory and full-scale aerators and to optimise the chemical cleaning agents' removal efficiencies.

4.2.2 Experimental aerator set-up for fouling monitoring

The laboratory-scale setup was designed to monitor fouling formation in a controlled and accelerated growth environment. Six-week-long monitoring and sampling of fouling deposition were conducted in a laboratory-scale spray aerator.

4.2.3 Configuration of the experimental set-up

A closed-loop laboratory-scale aerator was set-up to simulate a spray aeration system *(Figure 4.1).* The set-up included an automatic pump system that dispensed the feed solution for 6 hr a day (24 cycles of 15 min online and 30 min offline) to mimic the average operational period of the spray aeration system. A light source was controlled using an electric timer to provide 12 hr of artificial sunlight daily. Autoclaved cement coupons were secured to PVC rods and laid at the bottom of the container to represent the sprayed and submerged conditions, respectively. The temporal changes in the formation and composition of fouling materials were investigated under two different orientations and surface wettabilities – sprayed (vertically positioned) and submerged (horizontally positioned) coupon exposure conditions. The setup was operated in a warm room with high humidity and an average temperature of 30^0C.

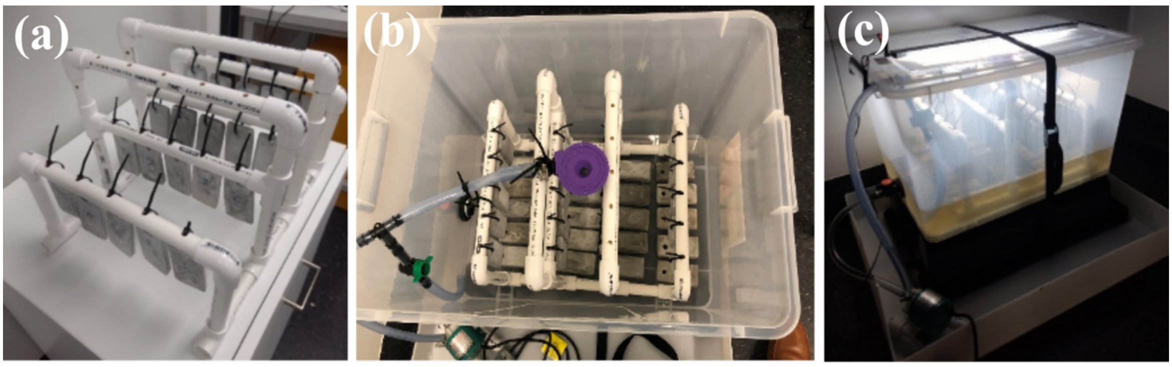

Figure 4.1: (a) Side view of coupons vertically positioned to represent the sprayed surface exposure conditions (b) Coupons horizontally placed to represent submerged exposure conditions (c) The operational Laboratory-scale experimental set-up with coupons in the sprayed and submerged exposure conditions.

4.2.4 Preparation of synthetic recirculation solution

To accelerate fouling formation, a laboratory-prepared enriched nutrient solution with increased microbial and organic concentrations was prepared. For the recirculation solution, synthetic water with similar characteristics to the influent stream of the aeration systems was considered. To begin, tap water from a UNSW laboratory was dechlorinated and analysed for its elemental composition and microbial load. The ICP-OES analysis revealed that key elements had very low inorganic concentrations. As a result, inorganic salts were added to the influent groundwater stream to maintain similar concentrations to the full-scale aerator.

An inoculant derived from biofilm recovered from the full-scale aerator surface was cultured in a growth medium for microbial activity. Biofilm development in the laboratory was accelerated by dosing a nutrient solution comprising of iron phosphate (0.1 g), glucose (1 g), yeast extract (1 g), and peptone (5 g) dissolved in a litre of Milli-Q water (Han *et al*., (2016)) before being spiked into the synthetic solution. The spiked synthetic solution was blended with 50% growth media and used as the recirculating solution to ensure sustained nutrients for accelerated microbiological growth. The initial microbial load in the solution at the start of the experiment was 2000 ATP/mL and was 10 times higher than the full-scale influent samples. The conditions applied in the lab (nutrient-rich, warm operating environment, increased microbial load) were designed to encourage accelerated fouling formation.

4.2.5 Samples collection

The synthetic solution was continuously circulated as described in *Section 4.2.3.* The coupons (under sprayed and submerged surface conditions) were sampled thrice during the first two weeks and once weekly after that (spanning from August to October 2020). The recirculation solution was also monitored throughout the experiment. The recirculation solution and the fouling layers developed on the coupons were collected and analysed during each sampling event.

4.2.5.1 Fouling sample processing

The fouling attached to the coupon surfaces was removed and placed into a laboratory-made phosphate buffer solution (PBS) with a concentration of 10mM. PBS constituents including potassium phosphate monobasic (KH_2PO_4), sodium chloride (NaCl), sodium phosphate dibasic (Na_2HPO_4), and sodiumazide (NaN_3) were sourced from Sigma-Aldrich.

In preparing the PBS, NaCl (8 g), Na_2HPO_4 (1.38 g), KH_2PO_4 (0.19g) and NaN_3 (0.2 g) were dissolved in a litre of distilled water. HCl and NaOH were used to adjust the pH of the prepared solution to 7.4.

The method considered to be efficient and consistent in optimal recovery and resuspension of fouling materials was adapted (Gagnon and Slawson, 1999). The proposed method was a hybrid of manual scrapping and stomaching, followed by sonication for optimal fouling recovery. The approach recovered optimal fouling materials but is considered to have some destructive impact on the structure of the biofilm. The coupon surface was first scrapped using a sterilised utility knife, detaching the visible fouling materials from the coupon surface into a zip-lock bag. Together with the coupon, additional PBS was introduced to fully submerge the coupon in the zip lock bag before it was gently scrubbed for 30 s to completely remove the remaining biofilm attachments into the zip-lock bag. The zip-lock bags were sonicated in water bath (Bransonic 220, USA) for 5 min at 40 kHz to dislodge attached fouling. Following this, the suspensions were then decanted into a new beaker. Aliquots of fouling layer suspensions and bulk water samples were each subjected to a comprehensive suite of analyses.

4.2.5.2 Chemical cleaning of fouled coupons

The cleaning experiment was carried out on coupons from the laboratory scale set-up. Coupons fouled from the laboratory aerator model were processed using the physical removal method (*sections 4.2.5.1.)* to assess the effectiveness of chemical cleaners. First, the baseline characteristics of the aged coupon was established using Milli-Q water.

NaClO and oxalic acid were selected as chemical cleaners based on the characteristics of foulants detailed in Chapter 3, (including XRD of the fouling materials provided in the appendix). The cleaning procedures include immersing the fouled coupon into the chemical solution for 30 min (

Figure 4.2*).* After 30 min, the coupons were removed from the solutions, rinsed in Milli-Q, and further characterised using the physical recovery methods described in *Section 4.2.5.1.*

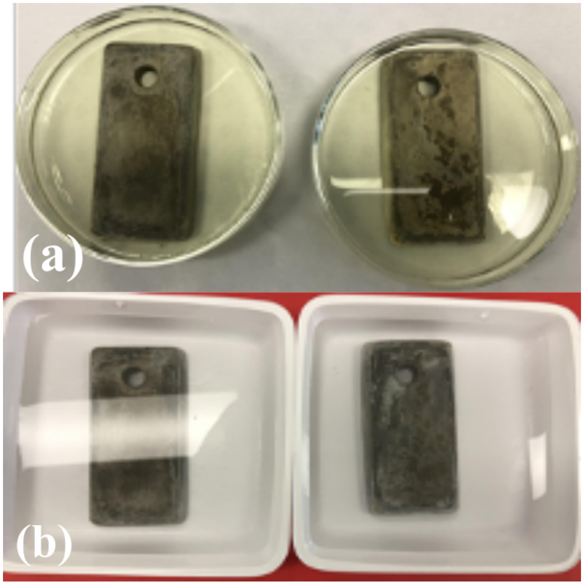

Figure 4.2: Aged coupons immersed in (a) NaOCl and (b) oxalic acid solutions.

The effect of different concentrations of NaOCl (1%, 3%, 6%, 12%) and oxalic acid (1%, 2%, 5%, 10%) on fouling removal was assessed. The characteristics of the recovered solution are compared to the baseline to ascertain the chemical agent's effect on overall fouling removal.

On sprayed and submerged coupons, the cleaning efficiency of NaOCl and oxalic was evaluated qualitatively (physical impact) and quantitatively (chemical and biological effect). Qualitative comparisons were made between the fouling observed on aged coupons after week 6, when the fouling is considered stable, and after application of the chemical agents.

4.2.6 Full-scale open spray aerator fouling monitoring

The full-scale experiment was designed to monitor fouling formation and investigate a more comprehensive range of factors affecting the aerators' mode of operations, quality of influent, environmental and seasonal conditions. A 9-month-long monitoring and sampling of fouling deposition was conducted in a full-scale open spray aerator.

4.2.6.1 Coupon installation

The coupons were installed in a Western Australian GWTP's open spray aerator. The open spray aerator was selected due to its higher susceptibility to fouling (including biological fouling), increased *Legionella* transmission risk and easier access for sampling. A series of 28 coupons were installed on the first header after a routine cleaning event with coupons collected at various time periods for characterisation (

Figure 4.3).

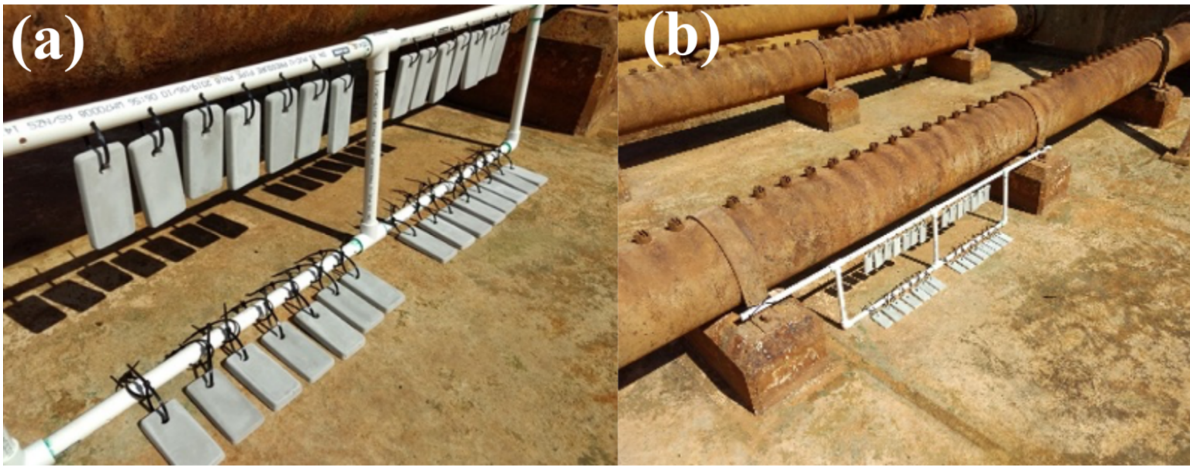

Figure 4.3: Two rows of newly installed coupons in the open spray aerator (a) Close view of the coupons two different surface wettabilities — vertically oriented for sprayed and horizontally placed submerged coupon exposure conditions (b) Distance view showing the coupon attachment to the sprinkler pipe.

The coupons were evenly divided between the top and bottom rows. The top row coupons were located vertically above the waterline (sprayed position), and the horizontally placed ones at the bottom floor were fully submerged underwater during aerator operation (submerged position). The position and orientation of the two-immersion scenarios were to account for fouling formation patterns and ease of removal under different wetted surface conditions.

4.2.6.2 Samples collection

Bulk water — influent and effluent streams to the aerator — and coupons (one sprayed and one submerged conditions) were sampled weekly during the first month, fortnightly during the second month and monthly after that (spanning from October 2019 to June 2020). This corresponds to the start of spring, through summer and autumn seasons in Australia. The coupons were collected during periodic sampling events and the fouling layer developed on them was analysed as described in *Section 4.2.5.1*.

The influent samples were collected at the upstream inlet point of the aeration process, and effluent downstream of the aerators. 500 mL of influent and outlet streams from the aerator were collected. Upon collection in the field, the samples are placed in a plastic container and chilled for transport to the laboratory for analysis.

4.2.7 Sample analysis

4.2.7.1 Biofilm activity (ATP)

The ATP analysis was performed on fouling and feed solutions using a Hygiena Aqua SnapTM (Total ATP probe and Hygiena Systems SURE luminometer) according to the manufacturer's procedures. After fouling extraction, 100µL was collected onto the tip of the ATP sampling probe and placed into the Hygiena luminometer for 10 s, with a value in relative light units (RLU) obtained. ATP analysis was carried out in triplicates. The limit of detection of the Ensure System Ultra Snap utilised for the analysis is one femtomoles ATP based on calibration. The concentration of 1 ng/L ATP was a reasonable estimate of the limit of quantification since it has been proven to be statically different from milli-Q water and 0.2 ng/L ATP (Ochromowicz & Hoekstra, 2005).

4.2.7.2 Characterisation of organic matter

The organic compounds in the resuspended solution were analysed by two techniques. The total organic carbon (TOC) assessed through the Shimadzu TOC-V analyser and the dissolved organic carbon and associated fractions using LC-OCD. Total dissolved organic carbon (DOC) measurements were carried out to quantify organic matter contained in the fouling and feedwater (influent, effluent, and recirculation solutions) samples. Following the fractionation process, each group of organic materials was quantified using DOC-LABOR ChromCalc software.

4.2.7.3 Fluorescence excitation-emission matrix (FEEM) analysis

Fluorescence Excitation – Emission Matrices (FEEMs) were used to characterise the present of natural organic matter in the fouling and water samples. The EEM spectra were analysed, and the "Peak-picking" technique was used to select individual excitation-emission peaks based on their peak intensities to characterise the yield as both qualitative and quantitative compositions of key functional groups. The samples were passed through a 0.45 µm filter (Whatman GD/X PVDF) before fluorescence analysis. The analysis was undertaken in an Aqualog spectrofluorometer (HORIBA Scientific., USA). The intensities of five commonly observed key fluorescence peaks in aquatic and fouling samples classified as B, T, A, M and C were tracked (Coble, 1996). Peaks B is Tyrosine-like and Peak T Tryptophan-like. Collectively Peaks B and T represent the protein like substances. Peaks A, M, and C are humic-like substances. Due to the limited fluorescence datasets, the conventional PARAllel FACtor analysis (PARAFAC) used in EEMs analysis was not utilised (Murphy *et al.*, 2013).

4.3 Results of laboratory coupon analysis

4.3.1 Characteristics of the synthetic feed solution

4.3.1.1 Microbial and dissolved organic carbon concentration

The changes in microbial and organic matter concentrations in the recirculation solutions show opposite trends at the start of the experiment (*Figure 4.4*). The microbial concentration showed an exponential increase followed by a decrease. On the contrary, the organic matter gradually decreased from the onset of the experiment.

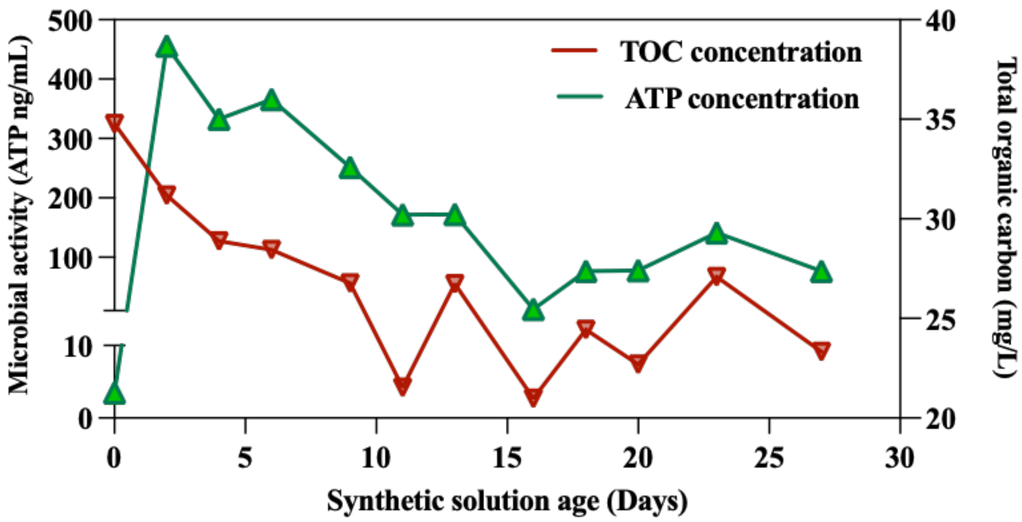

Figure 4.4: Temporal change in the microbial load and organic carbon in the recirculation solution.

Within 24 hr, the microbial concentration increased rapidly from 3.5 to 456 ng ATP/mL. A decline in the concentrations of ATP was observed over time, decreasing from 456 to 60 ng ATP /mL from Day 2 to 28. The starting level of organic matter (~ 35 mg/L) was intentionally increased to concentrations higher than expected in groundwater, so as to accelerate the formation and the growth of the biofilm on the coupons. TOC of the recirculation solution showed a gradual decrease with time to a relatively stable concentration midway into the monitoring period (measuring ~ 20mg/L).

After two weeks, the TOC and ATP measurements indicate that the systems have achieved operational pseudo-stability. Due to the absence of additional feed or nutrients during the sampling period, the decrease in TOC concentration was attributed to the utilisation of organic nutrients during biofilm metabolism and development (van der Kooij *et al.*, 1995b).

Analyses of microbial and organic trend data indicated that nutrients were not the sole determinant of biological growth. Due to the high concentration of nutrients (both organic and inorganic) in the synthetic solution, the growth of the microbial inoculant peaked after one day and then began a steady decline.

The fractional distributions of the DOC of the synthetic solution are shown in *Figure 4.5*. The measure of the fractional distributions remained reasonably stable across the 18 days. The components of fractional distribution for the different sampling cycles were summed together and expressed as a percentage and standard deviations.

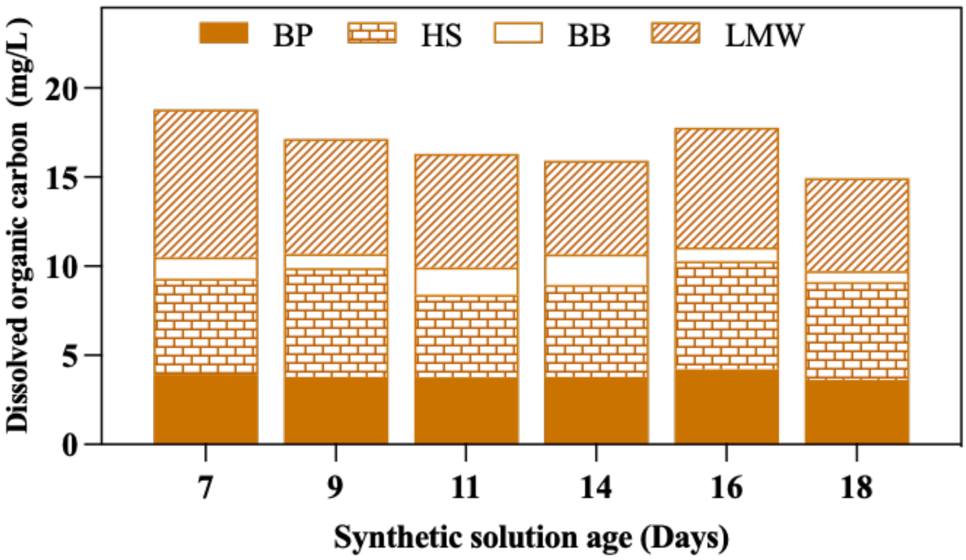

Figure 4.5: Organic fractions of the recirculation solution (BP -Biopolymer; HS - Humic substances; LMW-N -, Low molecular weights Neutrals and BB – Building blocks).

By distribution, the concentration of building blocks within the recirculation solution at 6.6 ± 2.8% accounts for the fraction with the least proportion, followed by biopolymer at 23.0 ± 1.3%, and humic substances at 32.7 ± 3.6%. LMW (neutral and acid) represents the predominant fraction at 37.8 ± 5.9%. The abundance of LMW compounds signified an increase in labile organics that contributed to microbial growth. Specifically, the easily biodegradable organic compounds in the feed water will be considered highly influential to biofouling development.

The changed concentrations of the individual fractions from the LC-OCD analysis remained relatively similar. While the TOC decreased, the relative abundance of the fractional compositions remained constant with slight fluctuations across the sampling cycles, indicating that no component was taken up preferentially during biological processes. While assimilable organic carbon (AOC) was not quantified in the recirculation solution, previous research has shown that AOC is the fraction of carbon that bacteria can be easily consume and accounts for a small fraction (0.1–8.5%) of DOC (Nescerecka *et al.*, 2014). Thus, the large variation in TOC and DOC concentrations in comparison to the relative stability observed on the LC OCD fractions.

4.3.1.2 Inorganic (elemental) composition

The elemental analysis of the recirculation solution's composition revealed that the concentrations of inorganic compounds remained relatively stable over the monitoring period (*Table 4.1*). Despite the wide variation in the concentrations of the various elements, their distribution remained relatively stable over the sampling period. Calcium, potassium, and sodium concentrations are expected to be high because these salts are the predominant constituents of groundwater, which has been mimicked in the recirculation solution.

Table 4.1: Inorganic (elemental) composition in the recirculation solution (n = 18)

Elements	Concentration (mg/L)
Calcium	27.4 ± 8.9
Iron	0.55 ± 0.01
Potassium	80.8 ± 31.7
Magnesium	9.1 ±1.2
Manganese	0.019 ± 0.005
Sodium	228.3 ± 57.2
Phosphorus	1.0 ± 0.4
Sulphur	22.2 ± 1.5

A high concentration of inorganics was chosen to accelerate the formation of biofouling. Indeed, elevated trace element concentrations in drinking water, including iron, phosphorus, and manganese, have been linked to a greater biofilm in fouling biomass (Ginige *et al., 2011; Prévost, et al., 2014).* Similarly, elevated concentrations of calcium and magnesium are necessary components of scale formation, contributing to an increase in fouling density (Gomes e*t al*., 2014).

4.3.2 Characteristics of fouling under sprayed and submerged conditions

First, visual examinations of the physical changes caused by fouling development on coupons were conducted. The fouling development profile was then comprehensively characterised using a variety of microbiological, organic, and inorganic analyses. Following that, the cleaning efficiency of chemical agents was evaluated on the fouled coupon.

4.3.2.1 Visual changes on the coupon surfaces

Visual observation of fouling formation patterns on the coupons includes initial, partial, and transient build-up of layers that gradually shift towards complete surface coverage with age (*Figure 4.6*). From a visual inspection, changes in fouling development patterns were different for the coupons under the sprayed and submerged exposure conditions. The submerged coupons surface was covered with reddish, hydrogel-like, loose materials indicating biofilm development (Kierek-Pearson and Karatan, 2005). In contrast, the surfaces of sprayed coupons were covered with whitish precipitate, an indicator of inorganic scale formation.

Cunault *et al*. observed denser and more fragile biofilm/fouling in a horizontal position or orientation surface exposure against the vertical sprayed coupons. This variance could be attributed to increased accumulation of suspended particulate in the solution through sedimentation on horizontal surfaces (Cunault *et al*., 2019a).

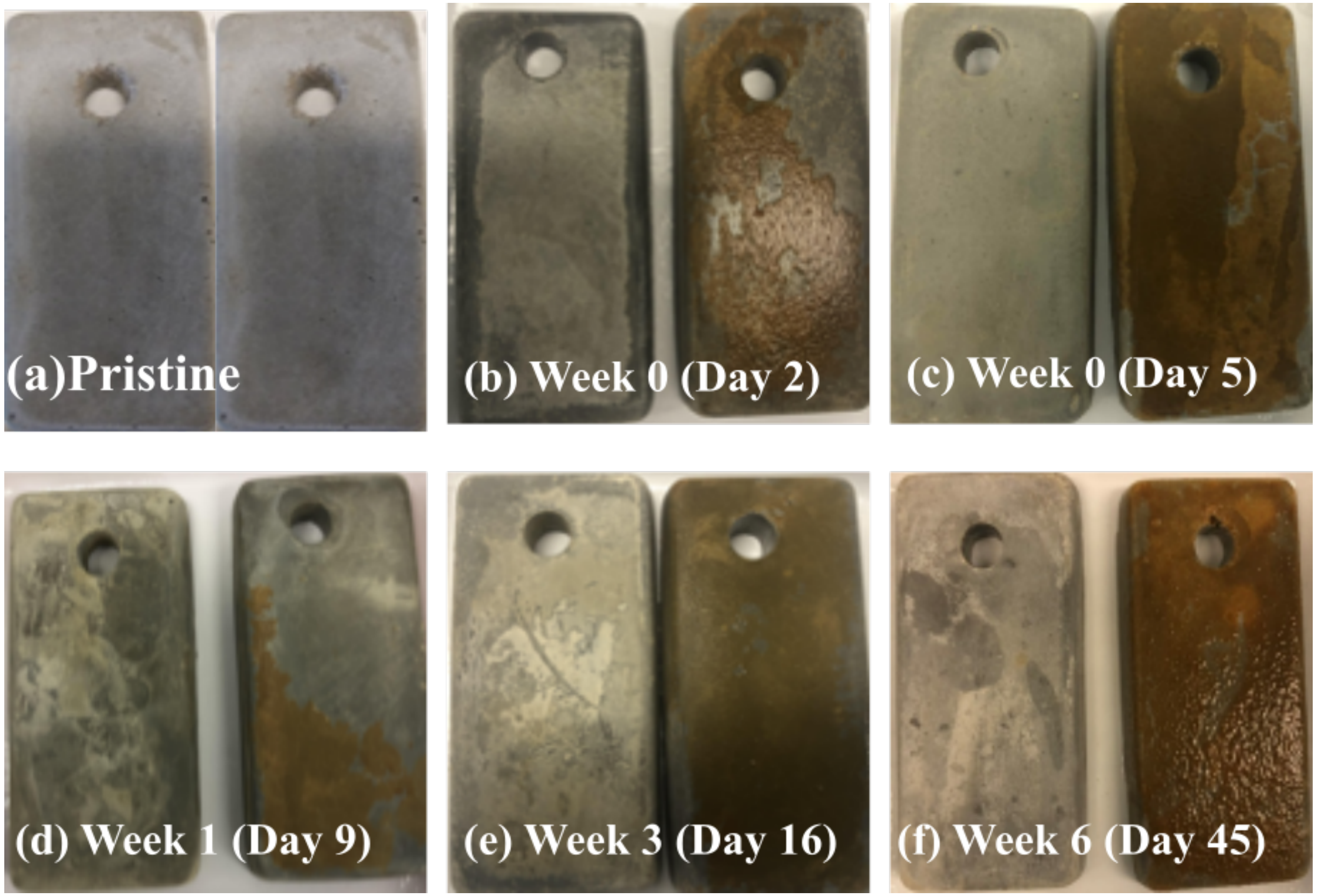

Figure 4.6: Images showing temporal changes on the coupon surfaces at different exposure times. From left to right, sprayed and submerged coupons exposure conditions.

4.3.2.2 Biofilm activity measured as ATP on the sprayed and submerged surfaces

The fouling on sprayed and submerged coupons was biologically active throughout the sampling period (*Figure 4.7*). Biological activity (measured as ATP) confirmed two growth phases of biofilm development under both coupon exposure conditions. There was an initial, sudden exponential growth phase (from day 0 to 7). Beyond day seven, a quasi-steady state was observed.

The observed biofilm formation pattern of sudden exponential increase and a transition to a quasi-steady-state phase is consistent with other studies in nutrient-rich environments (Camper *et al*., 1991; Liu *et al*., 2016). A high concentration of growth nutrients, and elevated temperature resulted in sudden exponential growth and rapid attainment of a pseudo-stability phase in less than seven days (Camper *et al*., 1991; Manuel *et al*., 2007; van der Kooij *et al*., 2017). The absence of the traditional pattern of initial stationary growth before sudden exponential growth observed in the lab coupons can be attributed to the nutrient-rich environment of the recirculation solution (DOC 17 mg/L).

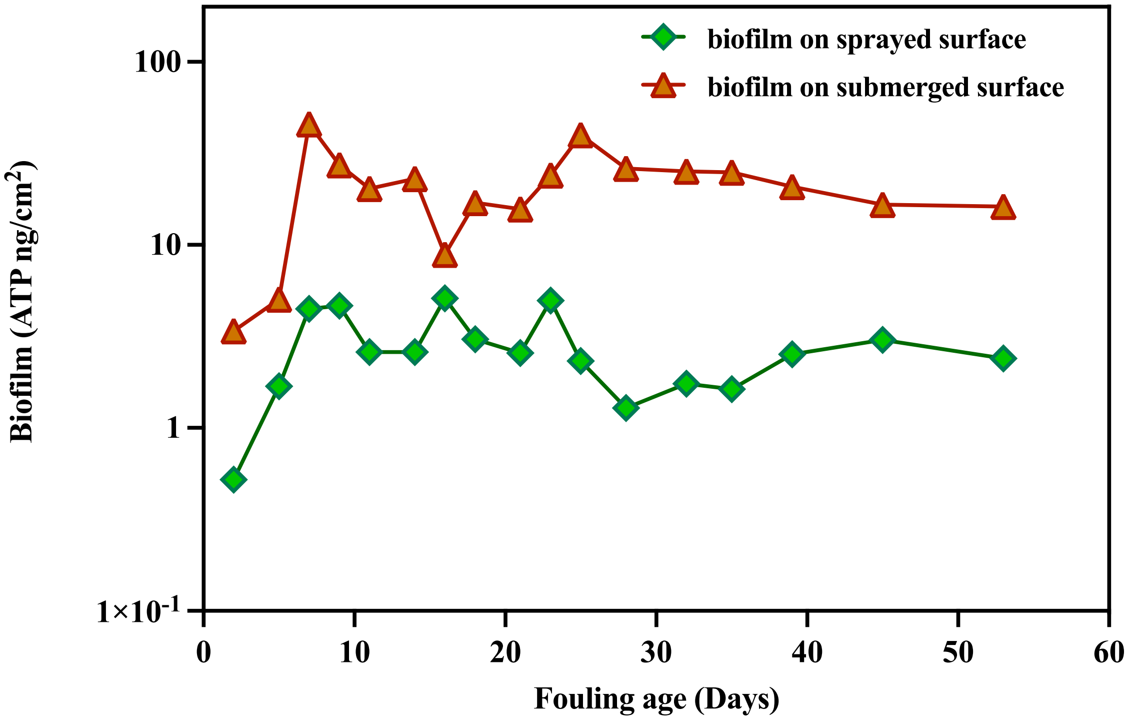

Figure 4.7: Comparison of the biofilm concentration on the sprayed and submerged coupons exposure conditions for full-scale samples.

In both growth phases, biological activity measured as ATP was significantly higher on submerged coupons compared to the sprayed ($p < 0.001$). The ATP measured on submerged coupons reached 45.7 ng/cm^2 compared to 4.5 ng/cm^2 of the sprayed coupons after the exponential growth phase. The mean measure of ATP for the sprayed and submerged coupons in the steady phase were summed together.

The concentration of ATP dropped in the pseudo-stability phase to reach a mean ATP of 21.9 ± 7.0 ng/cm^2 and 3.0 ± 1.2 ng/cm^2 for the submerged and sprayed coupons, respectively. The higher ATP concentration in the submerged exposure conditions confirms the observed aqueous hydrogel layer on the submerged coupons to be indicative of increased microbial activity. The presence of softer conditioning films such as the visible thin layer of hydrogel on the submerged coupons has been confirmed to facilitate better microbial cell adherence (Kierek-Pearson and Karatan, 2005; Rasmussen and Østgaard, 2003; Saha *et al.*, 2013). However, the overall trend of higher microbial activity in the submerged compared to sprayed conditions is a deviation from earlier studies on the effect of surface wettability (Kierek-Pearson and Karatan, 2005; Rasmussen and Østgaard, 2003; Saha *et al.*, 2013).

Partially immersed surfaces can increase biofilm formation and bacterial adhesion. Moist surfaces, similar to those sprayed in this study, may provide more favourable access to oxygen and nutrients for microbial growth (Liu *et al.*, 2016; Wijman *et al*., 2007). *Salmonella enteritidis* biofilms on partially immersed stainless-steel coupons were two to three logs larger than those on fully immersed coupons (Giaouris and Nychas, 2006). Similarly, for partially immersed surfaces, a significantly greater difference in *Pseudomonas fluorescens and P. grimontii* biofilm growth was observed (Cunault *et al*., 2019a; Faille *et al.*, 2018).

4.3.2.3 DOC and LC-OCD fractions

DOC and LC-OCD fractions were measured for 18 days of fouling to reflect the exponential and steady (stability phase) biofilm phases. Organic matter measured as DOC on the sprayed and submerged coupon surfaces was statistically similar at both the exponential and pseudo stability microbial growth stages (*Figure 4.8*). The initial phase of biofilm formation is usually characterised with accumulation of conditioning film under all exposure conditions. Conditioning films are primarily the accumulation of organic matter in the suspension and, hence, the similarities in the concentrations of DOC under both immersed and sprayed exposure conditions were expected (Lorite *et al*., 2011). As the biofilm transitioned from exponential to steady state, the DOC concentration was reduced by more than half.

The DOC measures were grouped into exponential and steady phases. The results for each of the phases were summed together. Average DOC concentration on the exponential phase (days 2 – 7) and steady phase (beyond day 7) of the sprayed decreased from 8.3 ± 3.6 to 3.3 ± 0.3 µg/cm^2 and, for the submerged coupons, from 9.0 ± 4.0 to 4.0 ± 1.7 µg/cm^2.

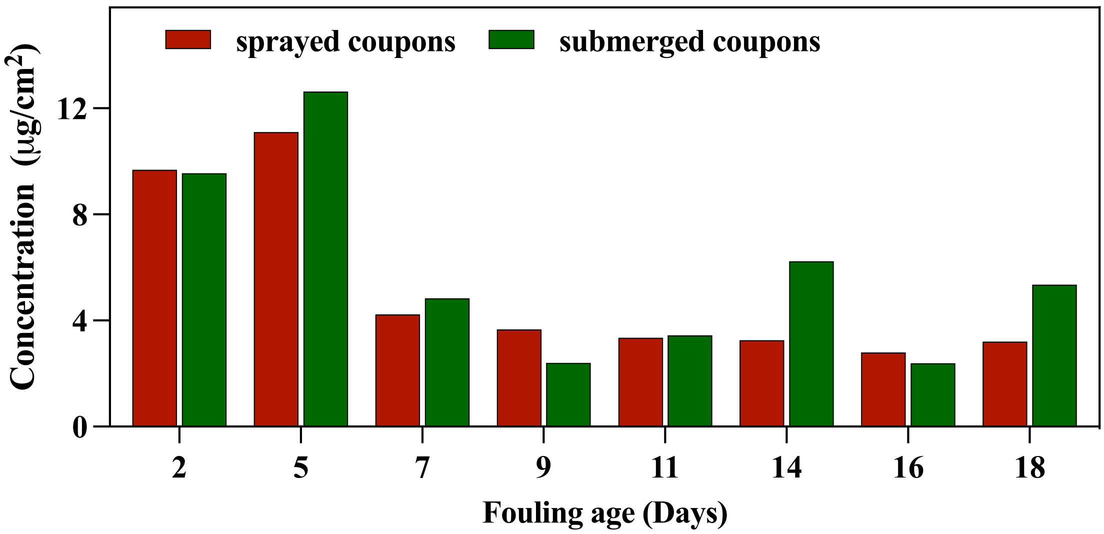

Figure 4.8: Concentration of temporal changes in DOC on the sprayed and submerged coupons.

Changes in the concentration of organic matter have been correlated with microbial growth phases. In this study, microbial growth tends to be higher in the initial days of the monitoring when organic compounds in the recirculation solution was high. The relatively high nutrient levels (DOC) in the early stages (days 0 – 7), are expected to encourage the increased growth. As the DOC declines in the recirculation solution, the microbes tend to get into the biofilms where there are potentially accumulated and elevated nutrients to support their metabolism. This could potentially explain the reduced concentrations of organic matter measured in the biofilms in the steady phase (Pick *et al*., 2021).

On the other hand, the characteristics of fouling organic fractions showed different trends from the overall DOC. Contrary to the DOC profile, changes in organic fractions were statistically similar during the exponential phase, but different in the pseudo-steady state (*Figure 4.9).* A significantly higher concentration of BP, BB and LMW (acids and neutrals) was observed for the submerged exposure condition in the pseudo-steady state. This suggests that different surface wettability can create different characteristics in the nature of the conditioning films (Loeb and Neihof, 1975).

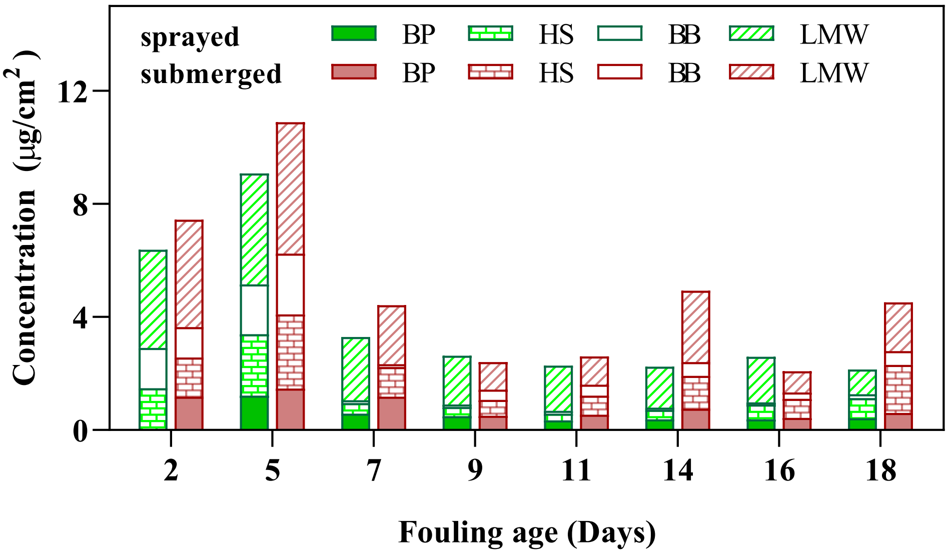

Figure 4.9: Distribution of the LC-OCD fractions measured in the fouling recovered from the sprayed and submerged coupons. Fouling aged 2 and 5 showed exponential growth, beyond which a relatively stable growth pattern was observed.

In this study, biopolymers and low molecular weight compounds appear to be the fractions that decreased more significantly between the exponential and steady growth states. Given that LC-OCD classified organic matter components according to their molecular weight, there was a high likelihood that higher molecular weight components such as biopolymers and humic substances would be broken down into smaller fractions as a result of the sonication process used to extract biofilm from coupons (Huber *et al.*, 2011; Stewart *et al.,* 2013). This additional sonication step was likely to affect the true concentration assessment of the various molecular sizes.

4.3.2.4 Inorganic elemental concentration

The inorganic elements were characterised in order to determine their correlation with and potential as biofilm growth indicators. Overall, no discernible trend in the concentrations of the various elements measured in the fouling over time was observed, as illustrated by iron and manganese (*Figure 4.10*). The elemental deposition concentrations on the sprayed and submerged coupons were comparable (full data provided in the *Appendix 2, Figure A2.2*).

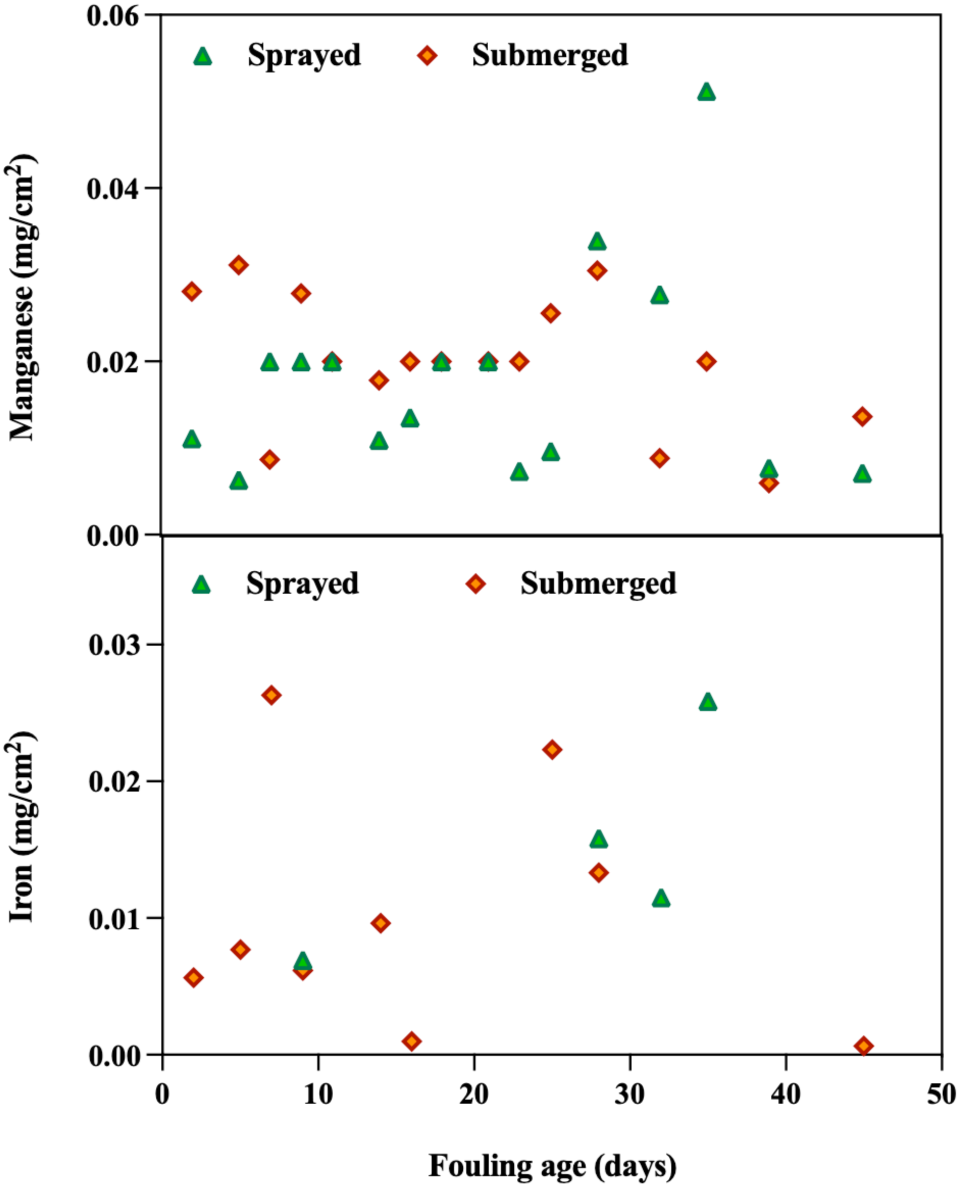

Figure 4.10: Temporal changes in inorganic deposits (manganese and iron) on the sprayed and submerged coupons with fouling age

Consistently, higher concentrations of calcium, phosphorus, and sodium were observed in the fouling materials compared to other elements measured. While calcium, phosphorus, and sodium are the most abundant elements detected, their origin may not be entirely due to fouling deposited on the coupons. Calcium concentrations are frequently elevated as a result of leaching of cement materials used in coupons into the extracted biofilm (Liang *et al*., 2013). Similarly, the increased sodium and phosphorus loads are due in part to the abundance of these elements in the PBS used to recover the fouling materials from the coupons. This made determining their precise concentrations from the temporal changes in the fouling materials difficult. As a result, calcium, sodium, and phosphorus concentrations were not considered in further analysis.

Two critical trace elements for biofilm development were examined in detail, namely iron and manganese (*Figure 4.10*). Manganese and iron concentrations tended to deposit on the coupons' surface over time, but with considerable variability and a lack of discernible trend. The general trend toward increased iron and manganese deposition suggests that micronutrients may accumulate within biofilm matrixes (Kurniawan and Yamamoto, 2019). In general, the lack of a trend between inorganic element concentrations and fouling age could be attributed to the constantly evolving biofilms and their frequent detachment and sloughing from coupon surfaces (Fang *et al.*, 2009; Ginige *et al.*, 2011b).

Given the similar variations observed in biofilms (as ATP), there is an opportunity to evaluate the suitability of manganese and iron concentrations as possible indicators of biological activity in aerator environment.

4.3.3 Cleaning efficiency of chemical agents

On sprayed and submerged coupons, the cleaning efficiency of NaOCl and oxalic was evaluated qualitatively (physical impact) and quantitatively (chemical and biological effect). Qualitative comparisons were made between the fouling observed on aged coupons after week 6, when the fouling is considered stable, and after application of the chemical agents.

The efficiencies of the chemical actions were quantified by comparing the amount of constituent compounds (microbial and inorganic) recovered from the aged coupon (after the sixth week) to the amount recovered following the chemical agent cleaning step (oxalic acid and NaOCl). The efficiency of the chemical action was determined by changes in physical, chemical, and biological properties.

4.3.3.1 Visual changes on coupons

Pronounced visual changes were observed between the fouled and cleaned coupons (*Figure 4.11*). Despite considerable fouling layer removal, coupons were not fully restored after the combined effects of chemical and mechanical cleaning actions. This confirmed that fouling on the coupons has reached an irreversible state. The synergistic use of chemical treatment (NaOCl and oxalic acid) and mechanical/physical action was observed to restore fouled laboratory fouled coupons to a nearly pristine condition.

(a) Fouled coupon (b) cleaned coupons

Figure 4.11: (a) Aged coupon indicating fouling layers before physical removal (b) cleaned coupon using combined action of chemical and mechanical effects.

4.3.3.2 Removal of microbial compounds

A significant decrease in ATP concentrations in the fouling layer was observed after NaOCl cleaning compared to a lower reduction following the use of oxalic acid on the fouled sprayed and submerged coupons (*Figure 4.12*). The reduction in microbial compounds was significantly higher with NaOCl cleaning compared to oxalic acid on the sprayed and submerged coupons (p = 0.005).

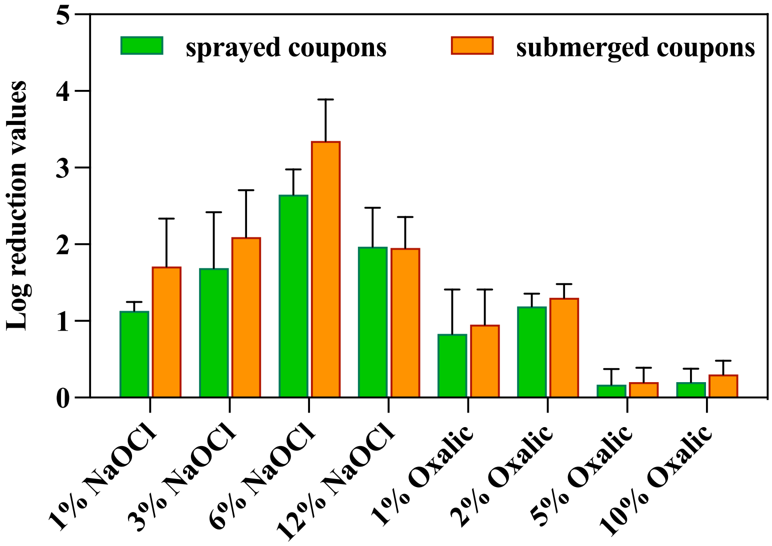

Figure 4.12: Reduction of microbial activity resulting from the effect of the chemical agents on the aged sprayed and submerged coupons (n = 3) for all the samples.

The four different NaOCl concentrations (1%, 3%, 6% and 12%) resulted in varying reductions of microbial compounds in the fouling. A statistically lower reduction threshold was observed at 1% NaOCl compared to 3%, 6% and 12%. A comparison of the levels of microbial compounds reduction for different concentrations of NaOCl (3%, 6% and 12%) does not demonstrate significant difference. For the different concentrations, ATP removal efficiency (regarded as a microbial inactivation efficiency) span between log removal value (LRV) of 1 (equivalent to 90% removal of pathogen), LRV of 2 (99% removal) and an LRV of 3 (99.9% removal). As expected, both the 6 and 12% NaOCl, at 30 min exposure time, achieved enough penetration to destroy the biofilm matrix, with an efficiency of up to 99% microbial removal (Piasecka *et al.,* 2015; Zand *et al.*, 2012).

Similarly, both NaOCl and oxalic acid showed a significantly higher reduction of microbial compounds in submerged exposure conditions than sprayed coupons ($p = 0.043$). This trend was consistent with earlier studies, indicating that biofilm formed on partially immersed surfaces (like the sprayed) exhibited firmer adherence and subsequently higher resistance to cleaning actions than fully immersed surfaces (Cunault *et al*., 2019b). Similarly, comparing different positions showed that biofilms formed on horizontal surfaces were denser and more resistant to detachment compared to those formed on vertical surfaces (Jha *et al*., 2022).

Considering all these observations, surface orientation and wettability properties can be considered to greatly impact on biofilm formation and resistant to detachment from cleaning actions. The increased resistance observed on biofilms formed on the vertically placed sprayed coupons can be used as a marker to the susceptibility of these regions within aerators to tougher microbial adherence. On a practical level, regions within operational aerators exposed to intermittent spray can be monitored more closely and use as to benchmark the maximum level of microbial adherence. This can also be extended to confirm effectiveness of routine cleaning operations in GWTPs.

The present study demonstrated that there was a reduction in microbial compounds upon NaOCl treatment. The efficiency of NaOCl in microbial inactivation is due to the synergism of the ability of ^{-}OH to dissolve organic materials and the oxidising power of ^{-}OCl to destroy microbial gel. On the contrary, oxalic acid was not expected to remove microbial compounds significantly, but the slight inhibition of the pathogen could be traced to the acidic effect in the solution.

While local standards (such as AS/NZS 3666) recommend 0.5 mg/L free chlorine to disinfect *Legionella* in water systems, no specific value exist for biofilms/fouling. Cooper and Hanlon, (2010) observed a 1.57 LRV when a two-month-old biofilms of *L. pneumophila,* was exposed to 200 mg/L of NaOCl solution for 1 hr (Cooper and Hanlon, 2010). This suggests that greater residual chlorine may be required for cleaning and inactivating *Legionella* inside the biofilm. In the present study, a NaOCl concentration of above 6% (equivalent of 7500 mg/L) was effective to achieve 3 LRV in steady biofilms and can be considered a good guide for industrial recommendation.

4.3.3.3 *Removal of inorganic compounds (measured as elemental composition)*

After oxalic acid cleaning, a significant decrease in elemental concentrations in the fouling layer was observed, compared to a weak reduction after NaOCl cleaning in fouled sprayed and submerged coupons (*Complete data is provided in Appendix 2, Figure A2.1*). Oxalic acid had a major impact on the reduction of critical metals such as iron and manganese on sprayed and submerged coupons when compared to NaOCl ($P = 0.007$). The reduction in elemental concentration was not significantly different for oxalic acid concentrations of 1%, 2%, 5%, and 10%. Similarly, no significant difference in inorganic compounds removal was observed between the sprayed and submerged conditions. The different concentrations of oxalic acid and sodium hydroxide have a variable effect on the fouling elements measured.

There was no discernible and consistent reduction observed with the various NaOCl concentrations. However, oxalic acid exhibits a significant reduction in a number of critical elements. As expected, the greatest reduction occurred at a 10% concentration, with up to 29% and 47% removal of iron and 43% and 60% removal of manganese in the sprayed and submerged coupons, respectively. Magnesium was reduced by 43 and 90 percent, aluminium by 26.8 and 45 percent, and zinc by 78 and 35 percent, respectively, in sprayed and submerged coupons.

Oxalic acid's relative superiority in terms of element reduction can be attributed to its reductive dissolution mechanism and chelating properties, which facilitate the removal of metallic oxides from fouling. This finding adds to previous evidence that oxalic acid is an effective cleaner for dissolving metallic oxides (Panias *et al*., 1996). On the other hand, NaOCl had a negligible to no effect on the elemental concentration. This was expected, as the anion and cation of NaOCl inhibit the growth of biofilms (OCl) and organic components (OH) but have little effect on the inorganic fractions present in the fouling layer.

To further understand mechanism and patterns of biofilm formation, the lab-scale study was supplemented by a full-scale monitoring of coupons under similar sprayed and submerged coupon conditions.

4.4 Coupon analysis of the full-scale aerator

4.4.1 Characteristics of influent and effluent

4.4.1.1 Microbial activity (measured as ATP)

The concentration of ATP in the influent was 180 ± 145 pg/mL (*Table 4.2*). The observed concentration was comparable to that observed for groundwater sources (van der Kooij *et al.*, 2017; Wullings *et al.,* 2011b). Variability in the influents can be considered indicative of fluctuation that is expected in the source bore water.

Table 4.2: Characteristics of the microbial, organic matter and inorganic matter for the influent and effluent

	Influent	Effluent	P - value
n value	13	12	
Microbial activity			
ATP (pg/mL)	180 ± 145	229 ± 185	0.31
Organic matter (mg/L)			
DOC	2.4 ± 0.4	2.5 ± 2.1	0.69
Hydrophilic	2.04 ± 0.4	1.72 ± 0.6	0.126
Hydrophobic	0.26 ± 0.1	0.26 ± 0.1	0.592
LC-OCD fractions (mg/L)			
Biopolymer	0.3 ± 0.06	0.2 ± 0.2	0.89
Humic substances	0.8 ± 0.1	0.7 ± 0.2	0.28
Building blocks	0.1 ± 0.04	0.1 ± 0.05	0.55
LMW (neutrals & acids)	0.9 ± 0.4	1.0 ± 1.9	0.72
Inorganic elements (mg/L)			
Iron	0.34 ± 0.22	0.28 ± 0.16	0.3203
Calcium	58.35 ± 3.5	57.75 ± 4.1	0.3917
Sodium	64.61 ± 3.2	64.9 ± 2.4	0.4628
Silicon	6.86 ± 2.2	9.70 ± 2.0	0.4022
Magnesium	9.26 ± 0.6	9.08 ± 0.8	0.4559
Potassium	4.60 ± 1.1	4.46 ± 1.1	0.3061

The mean concentration of ATP in the effluent was 229 ± 185 pg/mL. However, microbial concentrations in the effluent showed a slightly wider variability compared to the influent during the sampling period. This observation could be attributed to physical forces of disturbance and redistribution of sediments and sloughing of intact biofilms and other organic and inorganic material from within the aerator, leading to increased value in the outlet stream. The study by Bédard *et al.* attributed increased microbial load in effluent streams to biofilm detachment during the process of treatment (Bédard *et al.*, 2018).

Comparably, the values of microbial activity measured as ATP in the influent and effluent streams were statistically similar (P = 0.31). Due to the lack of statistical difference between influent and effluent, the aeration process was deemed to not have a measurable impact on the microbial constituents.

4.4.1.2 DOC and LC-OCD fractions

Concentration of DOC in the influent was 2.4 ± 0.4 mg/L and the effluent was 2.5 ± 2.1 mg/L. These values are relatively similar and hydrophilic in nature (82 – 88% of DOC concentrations) (*Table 4.2*). The LC-OCD fraction distribution of BB was 11%, building blocks 5 –7%, HS 33 – 41% and the LMW (neural and acids) 28 – 39% respectively. The organic matter distribution is consistent with the typical observations of an increased amount of hydrophilic NOM in Australian waters, suggesting a greater contribution by autochthonous sources, such as algae and bacteria (Leenheer, 2000).

The LMW (neutrals and acid) and humic substances represented fractions with dominant concentrations in both the influent and effluent samples. The dominant distribution of LMW compounds represented an abundance of labile organics that can contribute to microbial growth in the feed water as measured in the ATP (Wullings *et al.*, 2011b).

4.4.1.3 FEEM spectroscopy intensity

The fluorescent organic components in both influent and effluent were similar, with peak intensities of the five components remaining relatively consistent (

Figure 4.13*)*. Monitoring of influent and effluent over a six-month period concluded that there were no significant changes in peak intensities. The peak intensities of the five components of interest, in decreasing order, were A, B, M, C and T. The similarity observed in peak intensities of the influent and effluent streams further confirmed that aeration process has no measurable impact on the fluorescent components and the organic matter in the feedwater.

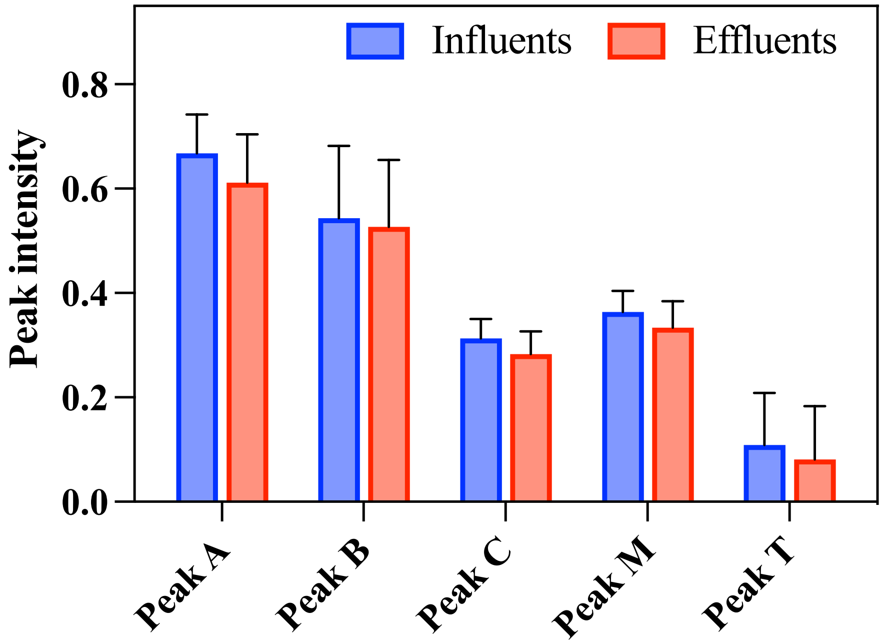

Figure 4.13: Peaks for the five identified components in the influent and effluent streams.

A correlation of organic fractions and EEM peaks shows some apparent associations. In the influent samples, biopolymer was moderately correlated with Peaks B and T (*Table 4.4*). This was expected as these peaks are known to be representative of protein-like components. The humic substances fraction identified by LC-OCD moderately correlated with peaks B and T ($r^2 = 0.5 – 0.6$) and had a poor relationship with A, C and M ($r^2 < 0.1$). This was unexpected given that the humic substance component should correlate to peaks A, C and M. As expected, a strong correlation was observed for building block with peaks T which is indicative of tryptophan and protein like components ($r^2 = 0.75$). LMW shows a poor correlation with all the peaks in the influents.

The measure of correlations of fluorescent components and organic fractions were calculated for influent and effluent (*Table 4.3*). In the effluents, a slightly different relationship was observed between the organic fractions and the fluorescent peak components. All the correlations ranged from poor to moderate, with no good indicators between the components.

Table 4.3: Correlations (r^2) between fluorescent peaks and *LC-OCD fraction concentrations of influent and effluent water samples.*

	Peak A	**Peak B**	**Peak C**	**Peak M**	**Peak T**
		Influent			
LC - OCD					
Biopolymer	- 0.007	0.599	0.053	0.037	0.528
Humic substances	0.019	0.614	0.082	0.062	0.519
Building blocks	0.344	0.417	0.383	0.383	0.753
LMW	- 0.526	- 0.205	- 0.576	- 0.511	0.053
		Effluent			
Biopolymer	0.309	-0.185	0.287	0.338	0.351
Humic substances	0.569	-0.200	0.589	0.552	0.267
Building blocks	0.117	0.538	0.110	0.103	-0.017
LMW	0.567	-0.191	0.518	0.568	0.304

A moderate correlation was observed between the humic substances and the humic-like peaks of A, C and M. As expected, the building block was moderately associated with Peak B. Overall, the correlation between fluorescence and organic matter observed in the influent and effluents was not significant. Generally, attempts to link changes in fluorescence in drinking water treatment to other bulk organic parameters have been achieved with mixed success (Peleato *et al*., 2016). This does, however, show some promise as a potential influent monitoring technique for aeration assets.

4.4.1.4 Inorganic elemental concentration

The proportional distribution of the elements was similar in both streams ($P > 0.05$) (*Table 4.2*). The five most abundant elements in the two streams were sodium (42%), calcium (38%), silicon (6%), magnesium (6%) and potassium (2%), and the remainder of the quantified elements accounted for 4% of the total composition. Surprisingly, iron was not amongst the most abundant elements, however a mean concentration of 0.34 and 0.28 mg/l was observed. The concentration threshold of the critical trace element in influents and effluents represents those correlated to the increased positivity of *Legionella* as discussed in *Section 3.3.1.*

The similarity in the elemental concentration of influent and effluent is expected because while aeration systems are designed primarily to oxidise metal concentration in the feedwater, the separation usually occurs in the subsequent filtration units within the treatment process.

4.4.1.5 HPC and Legionella characteristics in the influents and effluents

Performance based measures in guidelines use HPC and *Legionella* concentration as two criteria for triggering control strategies as highlighted in section 2.6, *Table 2.7.* Historically, monitored concentrations of HPC in aerator used for the coupons study ranged from (2 – 15) CFU/mL in the influent and (6 – 8500) CFU/mL in the effluent samples over the 5-year period. The influent and effluent HPC concentrations were considered below the critical values required to trigger remedial action *(100, 000 CFU/mL-* AS/NZS 3666).

In the case of *Legionella* measurements, the frequency of occurrence was 16% and 29% in the influent and effluent, with median concentrations of 10 and 15 CFU/mL, respectively. With guidelines (like AS/NZS 3666) requiring review of treatment process when *Legionella* is detected (usually with a level of detection above 10 CFU/mL), a moderately high occurrence frequency of 29% indicates the need for disinfection and review of the aerator's process performance.

Similarly, detection of *Legionella* in influent and effluent, despite the low levels of HPC, further suggested the lack of established microbial indicators that can be correlated with *Legionella* (Critchley and Bentham, 2009). As such, there is an opportunity to explore ATP and other physical parameters that can be used as surrogates for *Legionella*.

4.4.2 Correlation of physical parameters and microbial activity in the bulk water

Given the inability of microbial indicators to be established as surrogate for *Legionella,* there is the opportunity to explore other possible parameters. One approach is to explore other physical parameters measured in water. In this initial attempt, we explore ATP as measure of broad microbes to established potential physical parameters. Once established for the bulk microbial, these parameters can be further explored for the presence of *Legionella.*

ATP concentration can be considered a good indicator for measuring activity and changes in microbial compounds. Hence, the potential for its correlation to other physical parameters was explored to identify additional, and easier to measure, microbial indicators. Microbial activity was not well correlated with any inorganic elements in the influent and effluent streams ($r^2 \leq 0.3$) (*Table 4.4*). The correlations of iron and phosphorus with microbial density were inconsistent. Evidence from earlier studies showed a correlation between increased phosphate concentrations in water and a higher biofilm in fouling (Critchley *et al*., 2001; Lehtola *et al*., 2002). In contrast, Prévost *et al*. reported an association between higher phosphorus concentration with weaker biofilm (Prévost, *et al.*, 2014).

Table 4.4: Correlation coefficient (r^2) between microbial activity (measured as ATP) and measured parameters on the influent and effluent.

	Influent	**Effluent**
Inorganic including:		
Iron	0.008	0.04
Manganese	0.006	0.04
Phosphorus	0.3	0.2
Organic including:		
DOC	-0.194	0.62
Biopolymer	0.049	0.61
Building blocks	0.410	-0.27
Humic substances	0.054	-0.43
LMW	-0.072	0.55

The correlation between DOC and biological activity (through ATP) was poor in the influents but showed increasing strength in the effluent stream. The higher correlation in the effluent can be explained as carryover effect of biofilm detachment into the water and measured in the effluent. The biopolymers and LMW showed stronger positive correlations (but still weak) with concentration of microbial activity. The increased correlation of biopolymers and LMW with microbial ($r^2 > 0.55$) reflected their stable characteristics and potential to be explored as microbial activity indicators.

4.4.3 Characteristics of fouling on sprayed and submerged coupons

4.4.3.1 Visual changes of fouling on the coupon surfaces

Temporal changes from thin towards thick and more compact fouling layers were observed on the sprayed and submerged coupon surfaces (*Figure 4.14*). The visual appearance of the fouling layers differs between the two coupon surface conditions. The sprayed conditions show a reddish-brown submerged coupon with darker greenish colouration. Dark greenish biofilm forming on coupons has been associated with the presence of algae, and grazing organisms, including ciliates and amebae, rotifers, and nematodes (Taylor *et al*., 2013).

Clear and varied changes can be observed between the new coupon and its state at weeks 8, 12 and 33. Visually, the fouling layer varied significantly but in a non-uniform pattern, particularly under the sprayed exposure conditions. The non-uniformity in the sprayed coupon conditions could be explained by the more complex environment experienced by the coupons placed just underneath the distribution pipe, which was susceptible to biofilm interference from algae growing on the header pipes (*Images of algae trickling down on sprayed vertically placed coupons, Appendix 2, Figure A2.3).*

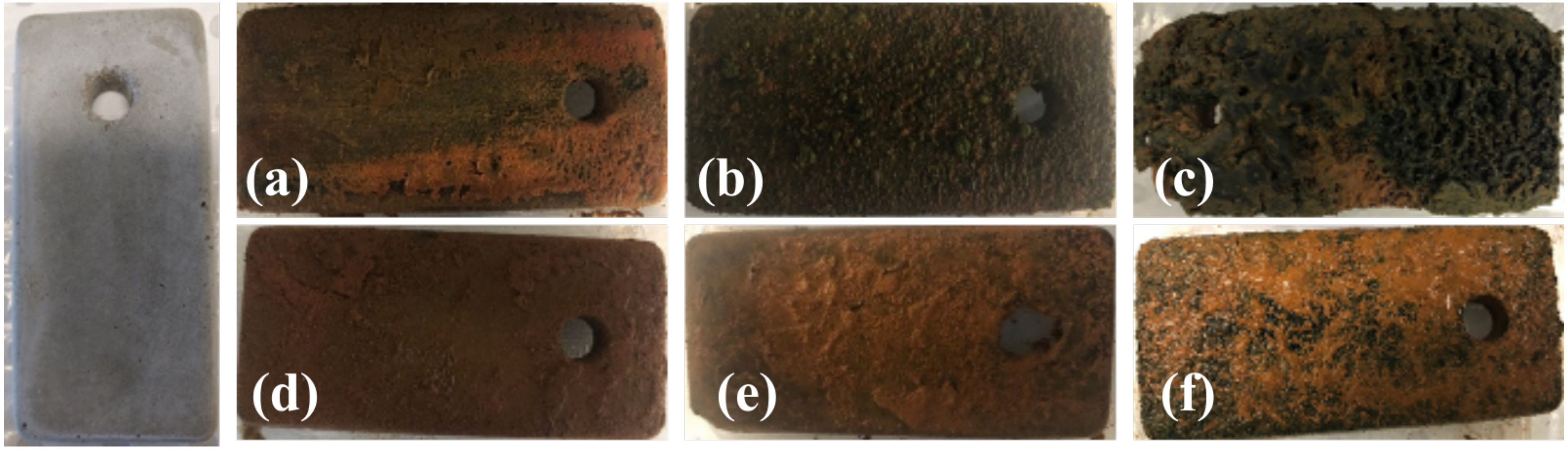

Figure 4.14: Digital images of pristine and aged coupons taken at weeks 8, 12 and 33 with (a – c) for the sprayed and (d-f) submerged surface exposure conditions.

4.4.3.2 Biofilm activity (measured as ATP)

The coupon surfaces (sprayed and submerged) were observed to be biologically active throughout the 33 weeks of monitoring (*Figure 4.15).* The concentration of biofilm was significantly higher on sprayed coupon surface (P = 0.04). The median density concentrations on the sprayed and submerged coupons under the exposure conditions were 277 and 73 ng ATP/cm^2 respectively. The median concentration of biofilm density observed on the coupons indicates a substantially higher profile than the value in other studies (van der Kooij *et al.*, 1995b).

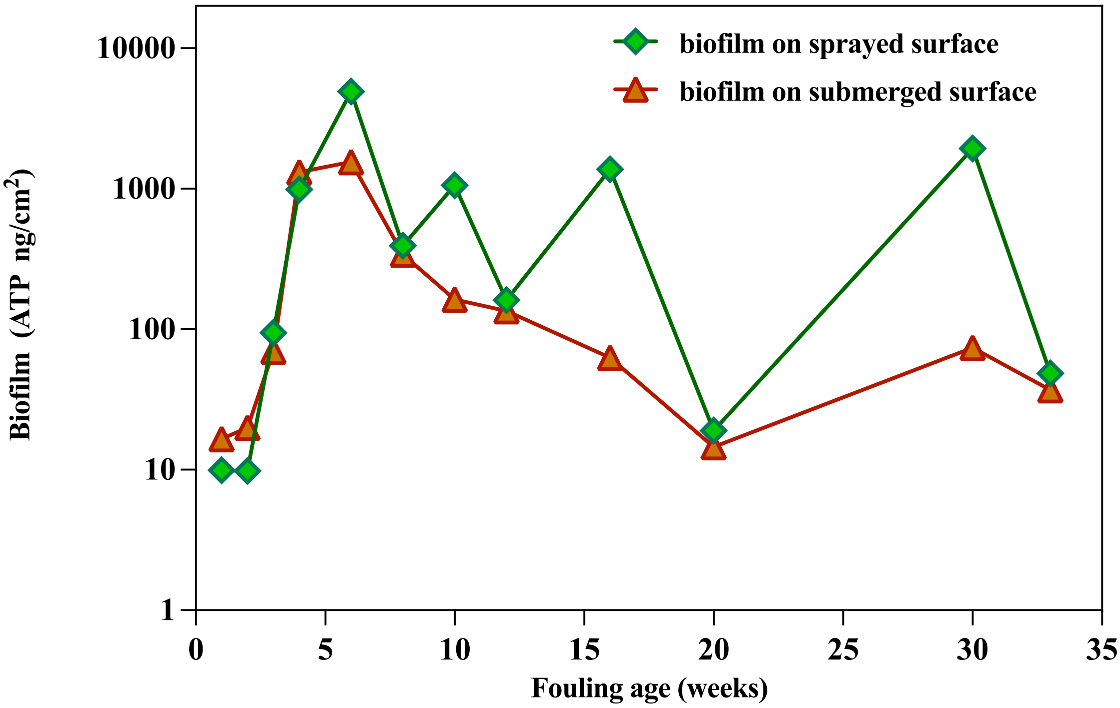

Figure 4.15: Biofilm concentration on the sprayed and submerged coupons conditions in full-scale plant.

Biofilm formation on the coupons followed the traditional patterns of initial stationery, exponential and steady phase for sprayed and submerged surface conditions. Under both conditions, biofilm accumulation is initially lagged (up to 2 weeks), increases exponentially before peaking and maintains a relatively steady growth pattern. The first two weeks (mostly initial attachments) indicated higher growth in the submerged coupon, after which a sustained higher growth occurred on the sprayed surface. The exponential rise indicates most growth occurs within the first four weeks. Beyond the exponential stage is the transition to the pseudo-steady-state or plateau phase (at the sixth week), which can be described by a fluctuation of the biofilm thickness (Chan *et al.*, 2019). From week 6, a pseudo-steady state could be considered, although this period still featured a large (apparently randomly distributed) variation in ATP values. Since biofilms constantly evolve through detachment, achieving a steady state, particularly in non-chlorinated systems can be a challenge.

4.4.3.3 DOC and LC-OCD Fractions

A significantly higher measure of DOC concentration was observed on the submerged coupon ($P = 0.01$) compared to the sprayed conditions. In the sprayed coupons, the concentrations of DOC (6 – 17 $\mu g/cm^2$) in the biofilm stationary growth phase nearly quadrupled (17 – 81 $\mu g/cm^2$) as the growth transitioned into the steady phase. The submerged, on the other hand, witnessed an increase from 4 – 24 $\mu g/cm^2$ at the initial growth phase to 24 and 39 $\mu g/cm^2$ at the steady phase, excluding the outlier of 169 $\mu g/cm^2$ on the submerged surface. The temporal changes in the DOC loading stabilised earlier in the sprayed condition in the sixth week compared to the eighth week for the submerged surface.

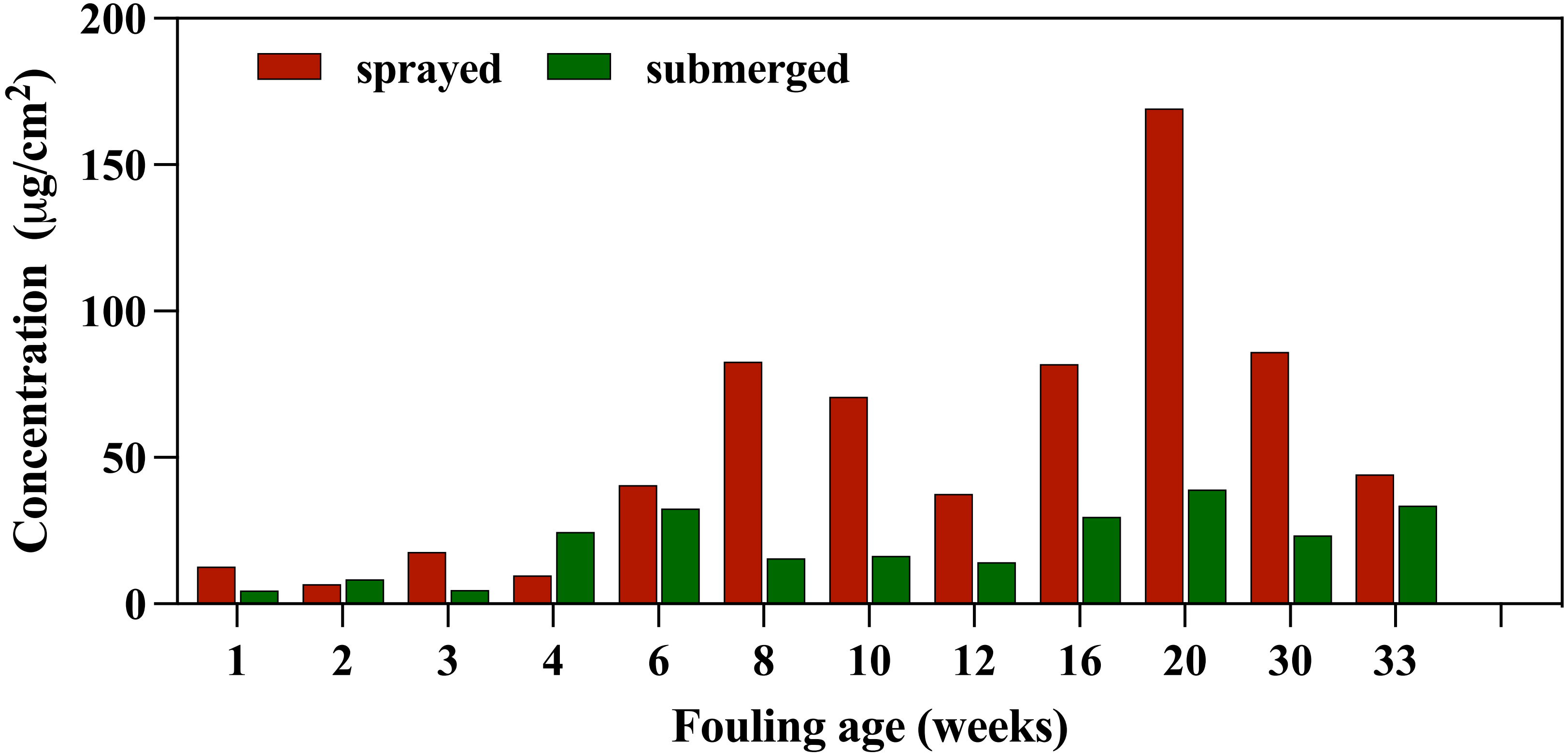

Figure 4.16: Concentration of temporal changes in DOC in fouling sampled from the sprayed and submerged coupons.

The DOC concentrations were dominated by hydrophilic fractions. The sprayed and submerged exposure conditions comprised of hydrophilic (93 ± 7 %) and hydrophobic (7 ± 7 %) and (88 ± 8%) and (12 ± 8 %) respectively. Contrary to the DOC concentrations, the distribution of the different fractions was relatively similar for the sprayed and submerged coupon exposure conditions. The distribution of fractional components was dominated by LMW, with building blocks having the smallest fraction (*Figure 4.17)*.

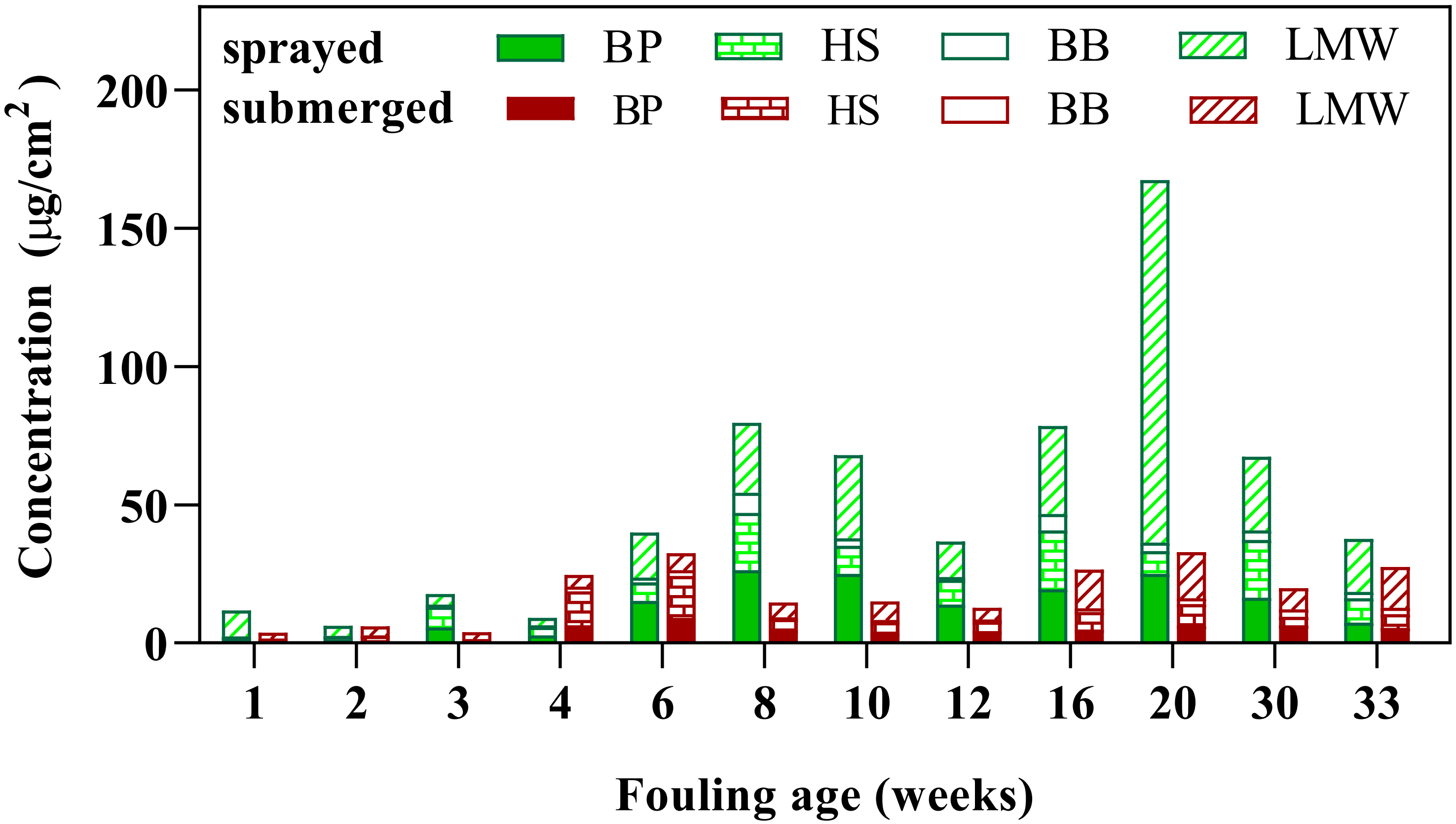

Figure 4.17: Concentration of temporal changes in LC-OCD fractions on the sprayed and submerged coupon.

Humic substances and LMW neutral were the dominant components (over 40% total organic compounds). The biopolymer concentration increased with time and was significantly higher on the sprayed coupon surface (P = 0.04) (*Appendix 2, Figure A2.2*). The high biopolymer concentration in the submerged coupon was not surprising given its higher biofilm growth pattern, since biopolymers have been reported as substrates can support biofilm growth in the context of drinking water (Sack *et al*., 2014; van der Kooij *et al.*, 2015).

With time, the humic substances were altered to follow a similar trend as the DOC but showed no significant difference across the coupons (P = 0.06). This was not surprising given that humic substances were the major component (approximately 30%) in the DOC. The concentration of building blocks increased slightly during the lag phase and then suddenly increased to a level comparable to the biofilm formation under both exposure conditions (P = 0.11). LMW neutrals seem to follow a gradual and steadily increasing trend with age, closely mirroring the DOC loading. This suggests the biofilm does not adsorb the component accumulating in this fraction.

4.4.3.4 FEEM spectroscopy intensity

Similar peak patterns were featured on fouling materials recovered from the coupons exposed to the two conditions. The mean strengths of the intensities of the peaks in decreasing orders are T, B, A, M and C respectively (*Figure 4.18*). Peak T and B show stronger intensity with fouling age than A and M. The peak shows similar trends to biofilm formation patterns in the fouling. The peak intensities like the biofilm featured a similar initial lagging phase in the first four weeks and a sudden increase was observed in the subsequent week with higher intensity in the sprayed coupons.

Peak intensities of all five components increased as the biofilm transitioned from the stationery through a steady phase similar to trend observed in the DOC measurements. The significantly increased intensities can be attributed to the higher concentrations of humic and protein-like substances arising from the more substantial accumulation of biofilm and organic matter on the coupons after the 4-week biofilm lag growth phase (Ahimou *et al.*, 2007).

Comparatively, significant increases in intensity in protein-like peaks B and T were observed in sprayed fouling coupons. The higher peak intensities featured on the sprayed coupons could be linked to the greater amount of biofilm and organic matter deposition measured as ATP and DOC respectively

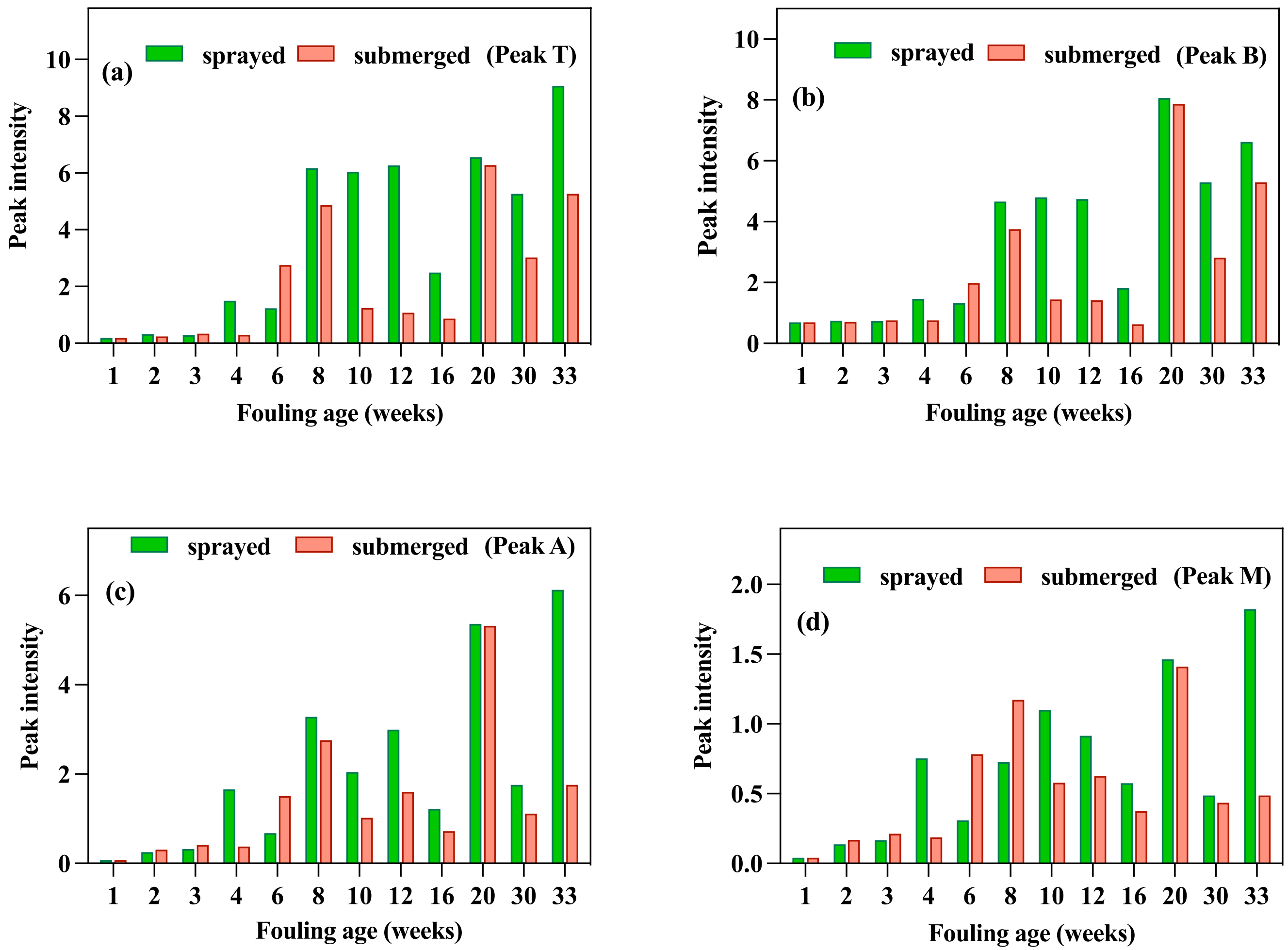

Figure 4.18:Main peak intensity in fouling materials recovered from sprayed and submerged coupons

An assessment of the correlations of fluorescent components and organic fractions for the samples from the sprayed and submerged coupon is presented in *Table 4.5.* Biopolymers showed a poor relationship with all five fluorescent peaks under both coupon conditions. A lack of relationship was observed between biopolymer and Peaks M and T. This was contrary to expectations as both are considered to indicate protein like materials as such should exhibit some correlation.

Table 4.5: Correlations (r^2) between fluorescent peaks and LC-OCD fraction concentrations of sprayed coupons.

	Peak A	Peak B	Peak C	Peak M	Peak T
			Sprayed coupons		
LC - COD					
Biopolymer	0.134	0.026	0.114	0.209	- 0.005
Humic substances	0.367	0.365	0.356	0.374	0.333
Building blocks	0.520	0.540	0.472	0.524	0.471
LMW	0.737	0.693	0.705	0.742	0.608
			Submerged coupons		
Biopolymer	0.252	0.173	0.228	0.334	0.235
Humic substances	0.424	0.412	0.433	0.502	0.518
Building blocks	0.389	0.547	0.414	0.319	0.576
LMW	0.657	0.749	0.661	0.522	0.703

Similarly, the association of the five fluorescent peaks components with humic substances and building blocks was moderate for both sprayed and submerged coupon conditions ($r^2 = 0.3 - 0.50$). A reasonably strong correlations were observed among the three components fluorescent Peak A, B, C and T and LMW ($r^2 = 0.66 – 0.75$).

This could be attributed to the sonication process breaking down the large components into smaller components, once altering the size distribution the fraction and hence the higher correlation observed for the smaller components of LMW ($r^2 > 0.6$).

Overall, the trend observed in the study revealed a weak correlation between the apparently larger fractions of biopolymers (considered as a combination of protein and polysaccharide constituents) and the fluorescent peaks against the relative stronger relationship indicated in the smaller fractions of LMW. This could be attributed to the sonication process breaking down the large components into smaller pieces once the size distribution is altered, hence the higher correlation observed in the smaller components of LMW ($r^2 > 0.6$).

4.4.3.5 Inorganic elemental concentration

The rate of accumulation of these elements was initially low. The accumulation gradually increased with time, showing slight variability (*Figure 4.19)*. The most abundant elements in the analysed samples were calcium and magnesium, however, iron and manganese were also shortlisted as elements of interest given their potential to be correlated in the growth of biofilm (Ginige *et al.*, 2011b). Complete distribution for the temporal changes in all the elements measured is provided *appendix 2, Figure A2.3.*

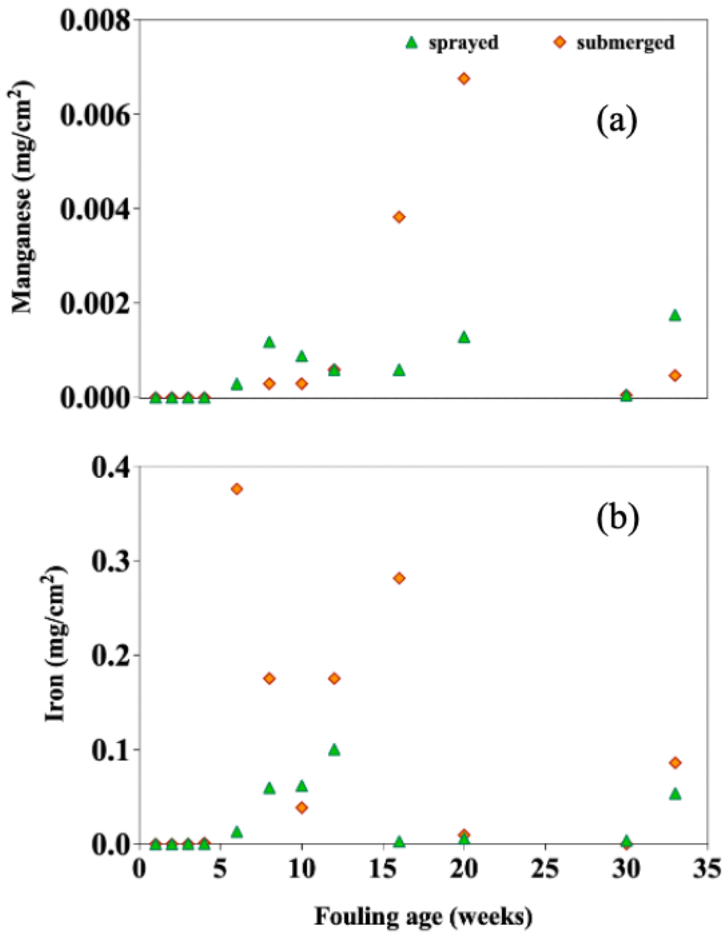

Figure 4.19: Temporal changes in inorganic deposits on the sprayed and submerged coupons (a) Manganese and (b) Iron

The trend observed for the accumulation of iron and manganese closely mirrors the biofilm formation pattern on the coupon. Both patterns for the biofilm and elemental accumulation involved an initial low concentration, followed by a sudden increase and subsequent attainment of a quasi-steady state with wide variability. The higher iron and manganese concentrations on fouling suggest potential micronutrient accumulation within the biofilm matrixes and subsequent deposition on the coupon (Kurniawan & Yamamoto, 2019).

While the iron concentration levels in the feed water were negligible, iron oxides appeared to precipitate and accumulate onto the fouling on the coupon surfaces, resulting in the reddish-brown fouling layer observed. The increased iron and manganese concentration in the fouling was expected due to the aeration process' precipitation of metals from the groundwater and their subsequent deposit on the coupon surfaces (Sharma *et al*., 2005).

4.4.3.6 HPC and Legionella in fouling

Similar to the influent and effluent, the control trigger criteria for HPC and *Legionella* was assessed for the biofilm measurements. The concentrations of HPC and *Legionella* measured in the resuspended fouling for the open spray aerator used for coupon monitoring were analysed over the 5-year period. The mean HPC in the fouling ranged from 2200 to 390,000 (with a median of 35,000) CFU /cm^2. The resuspended biofilm exceeded the critical threshold (100,000 CFU/mL) by 27% in the samples.

This density was considered below critical threshold values (10^5 CFU) in most standards and guidelines designed for the control of *Legionella (*AS/NZS 3666). In the case of *Legionella* measurement, the frequency of *Legionella* occurrence was 35% in the fouling with a median concentration density of 100 CFU/ mL. The higher frequency of *Legionella* occurrence in fouling (35%) compared to the effluent (29%) suggested that the pathogen was more persistent in the biofilms. A microbial model linked extracellular growth and multiplication of *Legionella* within biofilms to adherence and interactions with heterotrophic bacteria in the presence of increased sources of nutrients (Schoen and Ashbolt, 2011; Taylor *et al.*, 2009)

The current Australian standard recommends corrective measures for positive detection (≥10 CFU/mL). The guidelines recommend a review of the treatment process (particularly disinfection dose) to identify the cause of the increased occurrence. Given that aerator systems are pre-treatment processes with design impractical for disinfection. The alternative measure to the aerator will be to proactively control biofilm formation and reduce nutrient levels.

4.4.4 Correlation of biofilm activity and physical characteristics

Given the persistence of *Legionella* and HPC in the fouling, a correlational assessment of the overall microbial activity (measured as ATP) and physical parameters (organic and inorganic composition) was determined (*Table 4.6*).

The inorganic elemental measure did not correlate strongly with microbial activity in the fouling samples. The inorganic elements of iron and manganese displayed low correlational strength with microbial activity in the fouling ($r^2 \leq 0.4$). The correlation for phosphorus was not considered due to the potential interference from the PBS used in the fouling removal and resuspension. The low correlation can be partly traced to the wide variability observed in the monitoring of the inorganic compounds both in the controlled laboratory and in the full-scale coupon study.

The correlation between DOC and biological activity was weak but showed increasing strength in the fractional components. The biopolymer showed the strongest positive correlation with the concentration of microbial activity (*Table 4.6*).

Table 4.6: Correlation coefficient (r^2) between microbial activity (measured as ATP) and measured parameters on the sprayed and submerged coupon.

	Sprayed	**Submerged**
Inorganic including:		
Iron	-0.17	0.43
Manganese	-0.23	- 0.23
	-	-
Organic including:		
DOC	0.04	0.30
Biopolymer	0.203	0.85
Building blocks	0.092	0.31
Humic substances	0.164	0.65
LMW	-0.115	-0.16

The submerged coupon fouling showed stronger correlations compared to that of the sprayed coupons for the DOC and the fractional components. The relatively weaker correlations in the sprayed coupon conditions can be linked to wide variability observed in the biofilms and DOC concentration, which indicates the high susceptivity of the sprayed conditions to increased oxidation potential.

The LC-OCD fractions featured poor correlation in the spray, but moderate to high correlations were observed with humic and biopolymer components for the submerged coupon conditions. Humic substances showed a moderate correlation ($r^2 = 0.65$) and biopolymers a strong association ($r^2 = 0.85$). The biopolymers featured significant correlations, indicating their potential to be explored as microbial activity indicators.

In comparison, organic components of biopolymers, building blocks, and humic substances demonstrated a stronger correlational potential with observed microbial activity in water (influent and effluent) and fouling (biofilm). This suggests that general measures show potential for consideration as a better predictor of microbial activity than inorganic elements. However, further studies are required to strengthen the significance of this association.

4.5 Biofilm control for managing microbial pathogenicity in aerators

4.5.1 Contrasting biofilm growth under different conditions and environments

Fouling observed in the full-scale aerator is composed of relatively denser and thicker layers. While the accelerated laboratory feed solution contained a high substrate, the full scale with low substrate operated for about six times longer. Biofilms grown under low substrate concentration have demonstrated higher surface adherence, leading to denser layers compared to those grown under high nutrient conditions (Chun *et al.*, 2015).

The characteristics of recovered fouling materials assessed through biofilms (measured as ATP), organic matter quantified in terms of DOC, and the sub-fractions evaluated using LC-OCD were higher in the full-scale coupons compared to the laboratory model. Excessive nutrient conditions are usually associated with unconventional biofilm growth patterns (Manuel et al., 2007). The excessive nutrient and controlled laboratory environment resulted in a small initial stationary growth phase, swift transition to exponential and subsequent attainment of steady state in about a week. On the contrary, biofilm growth in the low nutrient (and uncontrolled) full-scale environment followed the traditional pattern (stationary-exponential-steady state formation phases), with a significant fluctuation in concentration at the end of the experiment in week 33. The relative higher variability in the concentration of biofilm at full scale, particularly at the pseudo-steady state, can be related to the complex interaction of processes within the aerator that includes observed algae growing on the header of sprinkler pipe and trickling down on the coupon, contributing to increased microbial density (*Appendix 2, Figure A2.2*).

Table 4.7: Comparison of the full and laboratory monitoring of fouling formation in aerator environment

	Full scale	**Laboratory-scale**
Feed solutions	• Nutrient limited feed water with low organic matter (2.5 mg/L) and microbial concentration (0.2 pg/mL). • Operated for a longer time (33 weeks)	• Nutrient excess synthetic feed water with organic matter (20 mg/L and microbial load (4.5 – 600 ng/mL). • Operated for a short period (a fifth of the time of the full scale).
Biofilm formation	• Followed the traditional pattern of stationary, exponential, and steady phase. • Slow biofilm growth peaked in six and eight weeks for the sprayed and submerged.	• Traditional growth pattern (stationary, exponential, and steady phases) was not detected. • Fast biofilm growth conditions, with a peak concentration achieved in 1 week.
Biofilm concentration	• The concentrations of biofilms were significantly higher in the sprayed (1,238 ± 1,534 ng/cm^2) compared to submerged coupons (298 ± 484 ng/cm^2). • The growth in an uncontrolled environment includes interference from algae detachment from the sprinkler header.	• The concentrations of biofilms are two logs less compared to the full scale. • Significantly higher in submerged (22 ± 7 ng/cm^2) compared to sprayed (3 ± 1.2 ng/cm^2) conditions.
Organic matter	• The organic matter and fractions were higher in sprayed coupons, similar to biofilm concentration. • The organic matter followed a similar of trend of gradual increase in the stationary phase and relative stability in the biofilm growth steady phase.	• The organic matter and fractions were lower than the full scale and higher in submerged coupons similar to biofilm concentration. • The organic matter followed a similar trend observed for biofilm formation and concentration.

In the case of fouling formation under the different exposure conditions, higher biofilm growth was observed in the submerged coupon in the laboratory model and on the sprayed surfaces on the full scale. The complex and variable nature of biofilm formation on the different coupon conditions makes it difficult to assess the exact reason for the discrepancies.

4.5.2 Biofilm removal to limit *Legionella* risk

The potential of biofilm to harbour *Legionella* in aerators presents an increased risk and an important health challenge. Like other industrial/municipal water systems, preventing the formation of biofilm within aerators is difficult. A realistic approach will be a focus on control measures targeted at different stages of their development (Critchley and Bentham, 2009; Subhadra *et al.*, 2018). Currently, the control strategy is the disruption of matured biofilm during cleaning action when aerators become too dirty with fouling.

As discussed in this Chapter, aerator fouling is a complicated matrix that requires careful consideration when selecting the most effective biofilm control strategy. In *Figure 4.20,* several of the major actors and mechanisms involved in fouling formation and removal are depicted, including the organic matter in the solution that forms the conditioning film, the inorganic composition that results in precipitation and scaling, and the microbial compounds that induce biofilm formation on the surface of aerators.

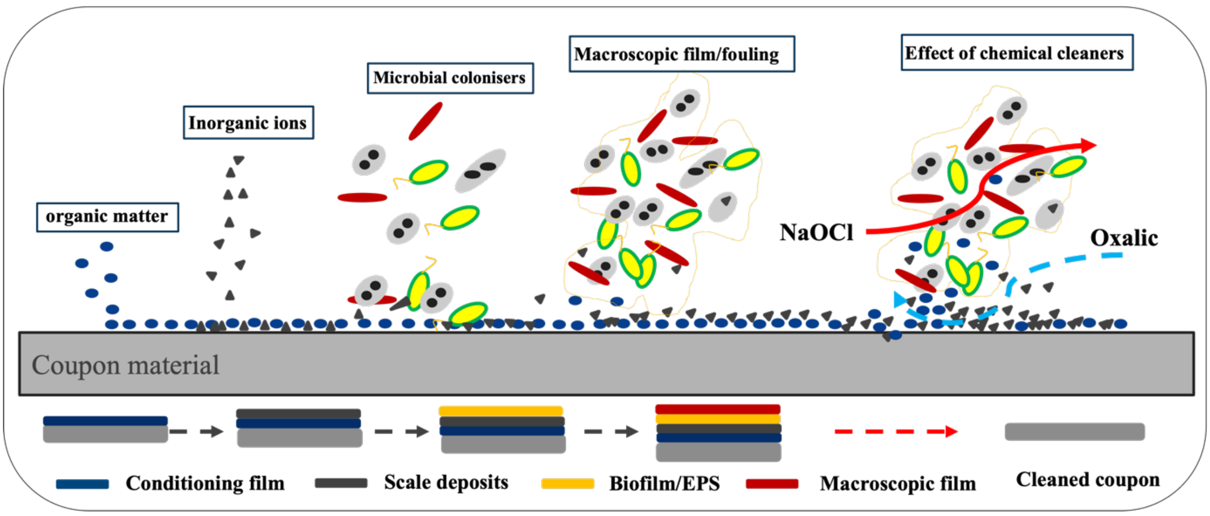

Figure 4.20: Schematic representation of the biofouling process and impact of chemical cleaning actions

Effective cleaning of fouling in aerators requires mechanical action (through high pressure water jets) and the combined effect of chemical agents. With the chelating effect of the oxalic acid, the inorganic scaling is removed, and the biocides inactivate the biological compounds (*Figure 4.20).*

4.6 Conclusions

- Fouling in aerators generally consists of organic, inorganic and microbial compounds formed into a matrix that can be considered a niche habitat for *Legionella* persistence.
- Continuous monitoring and characterisation of influent, effluent and fouling provides more reliable insight than the grab sampling approach.
- Organic compounds like biopolymers, building blocks and humic substances show initial promise of being explored as additional indicators for microbial activity in water and fouling.
- Typical laboratory-scale fouling experiments are not a good representative of full-scale conditions that include biofilm and algae interaction.
- Tracking the development of biofilms and the efficacy of removal methods can be considered as a measure for mitigating *Legionella* in aerator systems.
- ATP offers a quick and reliable technique for general indicator of microbial population.
- The laboratory experiment demonstrated that hypochlorite and oxalic acid synergistic effects are a good strategy for recovering aerator fouling with the ATP measure utilised to indicate inhibition efficiency. However, further long-term optimisation experimenting with the realistic feed water in a full-scale aerator is recommended to validate the cleaning protocol to improve current industrial practice.
- Beyond developing alternative microbial indicators, integration of multiple environmental parameters instead of a single variable has proven to improve the reliability of predicted *Legionella* risk outcomes (Bédard *et al.*, 2015; Lasheras *et al.*, 2006b). Advanced risk management tools such as Bayesian networks have been proven to integrate different variables to prioritise risk levels in systems with high uncertainty (Aguilera *et al.*, 2011; Uusitalo *et al.*, 2015). As such, there is the opportunity to explore the capabilities of BN models to integrate the various risk factors of water quality, nutrient sources, stagnation, location and access to quantitative establish the risk of *Legionella* in aerators as will be demonstrated in Chapter 5.

CHAPTER 5

DEVELOPING BAYESIAN NETWORKS IN MANAGING THE RISK OF *LEGIONELLA* COLONISATION OF GROUNDWATER AERATION SYSTEMS

This chapter is based on the following published article and presentations given at local and international conferences.

Publications:

Danladi Yunana, Stuart Maclaine, Keng Han Tng, Luke Zappia, Ian Bradley, David Roser, Greg Leslie, C Raina MacIntyre, Pierre Le-Clech (2021). Developing Bayesian networks in managing the risk of *Legionella* colonisation of groundwater aeration systems. Water Research, 193, DOI: 10.1016/j.watres.2021.116854

Conference presentations:

1. Application of Bayesian belief networks to better characterise the risk of *Legionella* colonisation of groundwater aeration unit," presented at Tenth Annual Conference of the Australasian Bayesian Network. Modelling Society (ABNMS), December 4 – 5, 2018 Adelaide South Australia

2. Bayesian approach to managing and predicting *Legionella* colonisation of aeration systems in groundwater treatment", presented at National Science Foundation (NSF) *Legionella* Conference, September 11 – 13, Los Angeles, USA.

5 DEVELOPING BAYESIAN NETWORKS IN MANAGING THE RISK OF *LEGIONELLA* COLONISATION OF GROUNDWATER AERATION SYSTEMS

5.1 Introduction

In Chapter 4, it was concluded that the occurrence of *Legionella* in aerator systems is complex and dependant on multiple variables including water characteristics, outcomes of structural deficiencies like stagnation and fouling, which are sources of nutrients for growth. Due to this complexity, conventional microbial indicators are unable to predict *Legionella* occurrence. To bridge these gaps, Bayesian networks modelling was introduced to develop an assessment tool that integrate the different variables contributing to growth and exposure of *Legionella* in aerator system.

The literature review (Chapter 2) has confirmed the absence of standards for managing health risks associated with the occurrence of *Legionella* pathogens in drinking water treatment processes. In a major step towards a risk-based approach, WCWA employed the traditional Hazard Identification and Risk Assessment (HIDRA) to assess the risk of *Legionella*. The outcome is a preliminary risk management register referred to as *Legionella* High Level Risk Assessment that defines the five critical risk areas, including hydraulic conditions, nutrients availability and growth, water quality, system design (and maintenance), and location and access using empirical observation and expert knowledge (Bradley, 2017). The classifications of the states of the shortlisted risk variables were based on industry (process control points) and regulatory criteria commonly applied to water quality governance (i.e. aesthetic, pathogen especially *Legionella,* HPC [22^{0}C and 37^{0}C] and total coliforms) as well as assets conditions (AS/NZS 4276.3.1:2007; AS/NZS 3666, 2011; DoC CoP WA, 2010; NHMRC, 2011).

The shortlisted dependent and interacting variables were assigned weighted scores and the resultant risk levels in each of the areas were determined through ordinary linear combination (MacKenzie, 2014). The LHLRA tool requires the user to select from qualitative statements and/or to provide quantitative data concerning the relevant factors. The assessment converts the qualitative/quantitative inputs into numerical values and percentages using simple calculations (Linkov *et al.*, 2009). So far, the results have been successfully used to build a knowledge base and to make informed decisions about the conditions of the assets, prioritise minor and major capital work plan and determine appropriate mitigative and remedial actions.

5.1.1 Limits of LHLRA under high uncertainty

The current practice using LHLRA has systematically assembled, weighted, and assessed the multiple factors believed to promote the risk of *Legionella* in support of improved decision making. However, this approach to risk assessment has been historically criticised to be subjective, lacking in transparency and reproducibility resulting in risk estimates without quantified uncertainties (Linkov *et al.*, 2009). As such methods that are likely to be more successful at assessing the complex interactions between variables and improve the understanding of *Legionella* risk are being explored (Cox *et al.*, 2005; Linkov *et al*., 2009).

The introduction of international standard ISO 31000 (2018) has expanded the risk management framework, provided more robust tools, and broadened its applicability across all industries (IEC/ISO, 2018). Recently, more comprehensive approaches have been employed in the water industry including bowtie diagram, fault tree, event tree analysis (Lindhe *et al.*, 2012), and BNs (Trinh *et al*., 2017). Of the available techniques, stochastic approaches to risk modelling are preferred because of their probabilistic nature that accounts for the elements of uncertainties (Ross and Sumner, 2002). Particularly, BNs have become increasingly popular for modelling conditions with a high degree of uncertainty and complexity such as in ecological systems and water management (Bertone *et al*., 2016; Moe *et al.,* 2020; Wijesiri *et al*., 2018). The advanced features of BNs have been explored to successfully improve microbial risk modelling of *Giardia lamblia* and *Cryptosporidium parvum* (Carvajal *et al.*, 2015), as well as faecal coliforms and *Escherichia coli* (Panidhapu *et al.,* 2020b). Although the mode transmission of these agents like *Legionella* is via ingestion, there is the opportunity to use same principle of BNs to document and quantify belief system surrounding the mechanistic relation of risk factors that are considered to contribute to the potential exposure to *Legionella* in groundwater aeration units.

The concept of BNs offer an alternative approach for relating the risk of *Legionella* exposure with the critical variables and incorporate parameters of uncertainty and variability. Besides, BNs offer a convenient means for performing scenario exploration and inference, as well as the prediction of *Legionella* exposure under diverse conditions. A BN structure is defined by graphical nodes with the relationship between variables specified using arcs. The variables are presented as "child" nodes, which have input connections from other nodes known as "parents" nodes. The strength of relationship between a child and its parent node (s) is quantified through conditional probability distributions, with one probability for each combination of possible states of the parents. These probabilities are defined in Conditional Probability Table (CPT) for each child node. The CPT of each node captures the associated uncertainty, i.e., the higher the uncertainty, the wider the probability distribution. Consequently, Bayesian modelling was explored as tool to integrate the risk variables and quantity the risk of *Legionella* growth and transmission in groundwater treatment aerators.

In this chapter, we assessed the capabilities of BNs as a quantitative tool to better capture the implications of reasoning along the current management paradigm and to move beyond qualitative risk assessment. First, we extend and directly map the basic mechanistic understanding of the system regarding causality into a BN. The strength and causal relationships between variables were determined through scenario exploration and sensitivity analysis. Subsequently, we invest in improving the existing causal relationship of *Legionella* proliferation and human exposure through a conceptual map using bowtie principles (Khakzad *et al*., 2013; Li *et al.*, 2016). Next, the revised model developed the predictors of *Legionella* growth and the risk of human exposure through the interaction of operational, water quality monitoring and asset conditions and the predictive ability was explicitly tested.

5.2 Development of the BN models

The stepwise approach for developing the BNs are summarised in *Figure 5.1,* detailing the sources of data and techniques employed in the conceptual modelling as well as the subsequent translation into BNs.

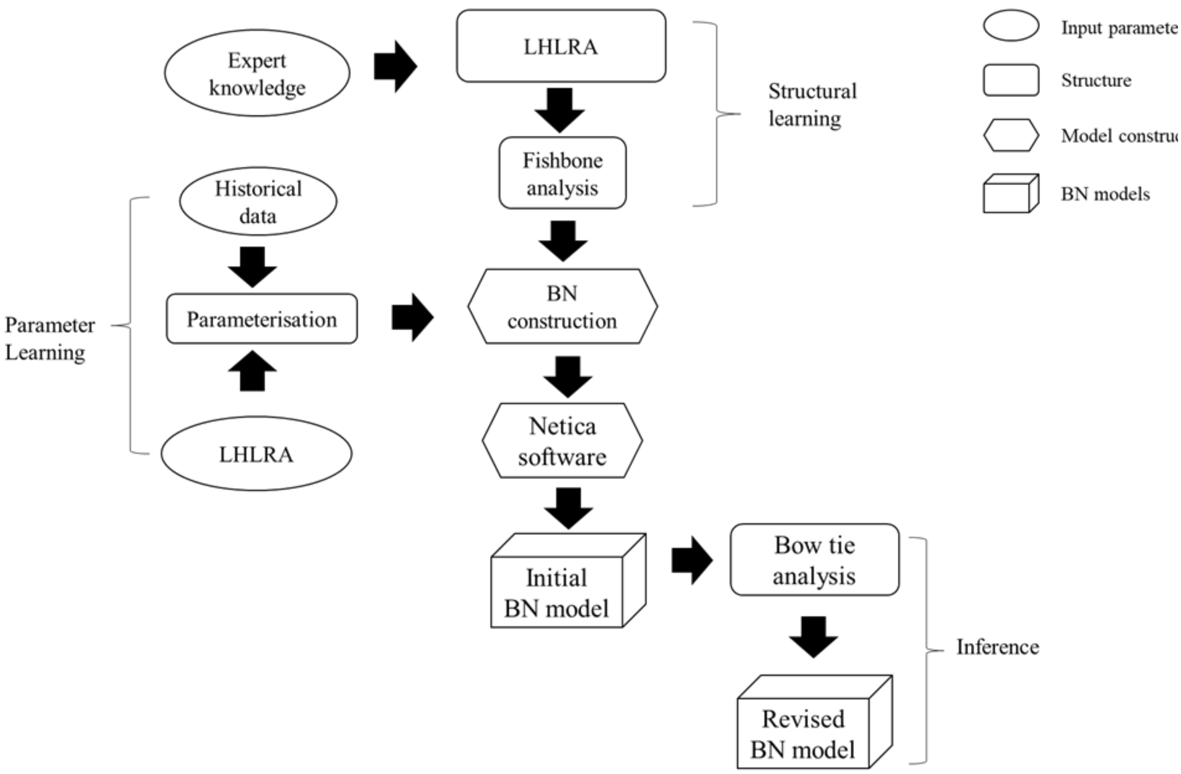

Figure 5.1: Flow chart describing the BN model development and evaluation adapted from (Chai et al., 2020; Trinh et al., 2017).

The existing basic mechanistic understanding of the system regarding causality defined in LHLRA was considered in the model. The relations and interconnections between the variables were primarily set based on expert knowledge. *Table 5.1* summarises common terminology, acronyms, and abbreviations.

Table 5.1: Table Key Bayesian belief network abbreviations and terminology relevant to model validation

Abbreviations /Acronyms	**Meaning**	**Explanation/use/comments**	**Reference**
LHLRA	*Legionella* High Level Risk Assessment	An expert elicited scoring system that correlated the risk factors contributing to *Legionella* in ground water aeration units	
FD	Fish bone diagram	Causal diagrams that show the potential causes of a specific event.	(Luo *et al.*, 2018)
BNs	Bayesian networks	Probabilistic graphical models formed by nodes (variables) and arcs (connections) in a directed acyclic graph.	
PA	Prediction accuracy	Quantifies the number of correctly predicted values divided by the total number of cases.	(Marcot *et al.*, 2006).
Netica	-	Bayesian network modelling software.	(Norsys, 2015)
BowtieXP		Bowtie software for analysis and risk assessment	(CGE Risk Management Solutions, 2020)
CPTs	Conditional probabilities tables	A table that has one probability for every possible combination of parent and child states.	
Deterministic CPTs	-	Tables that the child node has exactly only one possible value for each possible configuration of parent states. This means that in the child's CPT, in each row, one column will have a value of 1.0, and all the other columns will have a value of 0.0.	(Baldi and Rosen-Zvi, 2005; Barber, 2003)
Probabilistic CPTs	-	Tables that provide the probability distribution for each state of the child for each possible configuration of parent values.	(Marcot, 2017)
Nodes	-	Variables formatted in Bayesian belief net format.	
Latent variables	-	Unobserved variables derived from the interrelations of immediate parameters.	(Marcot, 2017)

5.2.1 Project scoping and conceptual modelling

A tier wise approach to BN modelling was adopted (Marcot *et al.*, 2006). This approach includes (1) creating conceptual diagrams of key factors affecting the outcome of interest, (2) mapping the conceptual diagram into the initial, alpha-level BN model, (3) revising the model after expert review; testing and calibrating to create the beta-level model, and (4) updating the structure and conditional probabilities with new validation data, to create the final-application gamma-level model (Marcot *et al.*, 2006). In a first step, the processes and dependencies of the variables in the five critically defined risk areas were mapped into a conceptual diagram using a fishbone analysis, as outlined in Luo *et al.* (2018). In an attempt to convert the LHLRA risk assessment into BNs, that allow for direct comparison of inputs and outputs, all relationship between the variables were initially considered as potentially causal.

An instantiated *Legionella* exposure variable that combines the scores accrued from the five different areas was created. The output conceptual map with the basic mechanistic relationship was directly mapped into the first (alpha level) BN, representing input and output variables. Subsequently, the structure of the initial BNs was revised through an expert workshop, comprising academics, microbiologist, and water treatment plant operators. Using bowtie principle, the expert review improves the causality pathway defined in basic mechanistic understanding. Bowtie analyses have been successful as conceptual maps for BNs (Khakzad *et al.*, 2013; Li *et al.*, 2016) In its traditional representation, the left-hand side includes a list of potential hazards leading, through different pathways, to a specific top (critical) event (*Legionell*a colonisation), whilst the right-hand side includes the different consequences of such event. The improved relationship was based on bowtie principles and conceptually mapped using bowtie analysis software (BowtieXP, CGE Risk Management Solutions, 2020). The revised (beta-level) BNs model was then built based on the modified causality relationship defined by the new conceptual map.

5.2.2 Model parameters, discretisation, and learning

The summative scores in each of the five critically defined areas and the *Legionella* exposure variable was categorised through a uniform distribution into three ranking schemes intervals to represent low, medium, and high-risk levels. The low range indicates the contributing factors are present in states that result in a minimal risk. The medium level represents the threshold of potential concern and the high range indicates the variables are present in a state that pose an unacceptable risk level (Brien *et al.*, 2017). From operational standpoint, the minimum risk (required no action from the plant operator), medium threshold (require the review and close monitoring of operational parameters) and the high state (represent the point for intermediate management intervention).

For the *Legionella* exposure variable, a low state requires no mitigative action; the medium state prompts the review of operational parameters, and the high state calls for immediate decontamination (AS/NZS 3666). The description of discretisation style for all the risk factors that translated as input variables for the initial and revised models based on the weighted scores are provided in the supplementary data (*Appendix 3).*

The prior probability distributions for the root nodes in the water quality and nutrient availability and growth variables were defined using historical data sourced from the GWTPs, while hydraulic conditions, system design, and location and access were captured from LHLRA records and statistical considerations utilised for variables with missing records (Beaudequin *et al*., 2016). Several practical ways in which CPT values can be established in the initial, alpha-level BNs have been outlined (Marcot *et al.*, 2007). The expert elicited scores were set to deterministic value and filled as CPTs for the BNs. A deterministic BN has conditional probability distributions as Kronecker or Dirac delta functions (Baldi and Rosen-Zvi, 2005; Barber, 2003). This implies that only one occurrence of a possible combination is represented when the relationships between parents and child variables are arranged in a tabular format (in one row per dataset). The complete deterministic CPTs used in the models are available on request. The BN models were developed with the software Netica 5.18 (Norsys, 2015).

5.2.3 Model evaluation and validation

The BN models were evaluated through sensitivity, scenario analyses and validated through different performance metric including prediction accuracy and Cohen Kappa coefficient. Sensitivity analyses were performed on the initial and revised BNs to identify the variables that are most influential on the risk of *Legionella*. Scenario testing was performed on the initial model to understand the triggers of the different risk levels and model behaviour. On the revised model, the validation option "testing the net using cases", was employed to grade the BNs predictions against the actual outcome (Norsys, 2015). A recent independent dataset from the LHLRA assessment of 23 plants was compiled, and the observed states of the input variables were tested as net cases. In order to evaluate the agreement between predicted and observed outcome, performance accuracy as well as error rates were calculated for false positives (Type I error, rejecting a true hypothesis i.e., failing to correctly identify positive cases) and false negatives (Type II error, failing to reject a false hypothesis i.e falsely identifying negative cases as positive) (Othman and Al-Hamadi, 2018). In addition, performance metrics like Cohen's Kappa, prevalence index and bias effect were computed to assess the symmetry of the validation datasets.

5.3 Results and Discussions

5.3.1 Conceptual model and Input variables

The shortlisted variables and the relative contribution of the five different critical areas to the risk of *Legionella* exposure is summarised in *Table 5.2.* The summative scores from the different risk areas differ significantly. While the hydraulic conditions factor had the lowest score, accounting for 11% of the total contribution to risk, both nutrients availability and growth, and location and access also featured small contributions. On the other end of the spectrum, water quality was identified as a major contributor to risk (35%). The fishbone diagram (FD), illustrating the interactions of input variables and the integrated risk of *Legionella* in the system, is presented in *Figure 5.2*.

Table 5.2: Summary of the five risk areas and the corresponding input variables translated into the LHLRA

Risk area	**Description**	**Risk factors**	**Numeric scores & proportion (%)**
Nutrients availability and growth	Treatment process contamination and assets deterioration can provide nutrients favourable for algae and protozoa in the presence of sunlight. These microbes could conceal and protect *Legionella* from biocides.	Sources of nutrient (sludge, biofilm, rust, sludge, organic matter), Radiation, Temperature	25 (14%)
Hydraulic conditions	Lack of circulation allows the settling of more solids as sludge creating ecological niche for bacteria growth and *Legionella* proliferation.	Time in line, Dead leg*	20 (11%)
Water quality	Feedwater is likely to contain high presence of nutrients supporting microbial and biofilm development as well as the likelihood of *Legionella* multiplication.	Water sampling, Process system deficiencies, Total coliform, HPC, *Legionella* count	65 (35%)
System design	System design and configuration, particularly the absence of effective drift eliminator, present risk of contaminated aerosols leaving the aeration unit.	Aerosol generations, Systems height, Materials susceptivity to corrosion, Cross-contamination, Windblown contamination, Aerosol generation.	45 (24%)
Location and access to system	Aerators in most treatment plants are located at high elevations increasing the risk of aerosol dispersal. Equally, the frequency of access to the aerator for cleaning and maintenance impacts on the risk of exposure.	Frequency of access, *Legionella* occurrence in aerosol, Proximity of more susceptible people	30 16%)

*Dead legs are sections of the aerator that do not allow the circulation and/or flow of water.

The systematic integration of different risk areas established a clear structural endpoint variable for an easy BNs modelling of uncertainties in the system (Luo *et al.,* 2018; Pollino and Henderson, 2010). Subsequently, the illustrated potential causal connections establishing functional relationship from the FD were retained as a conceptual map and developed into the initial BNs model.

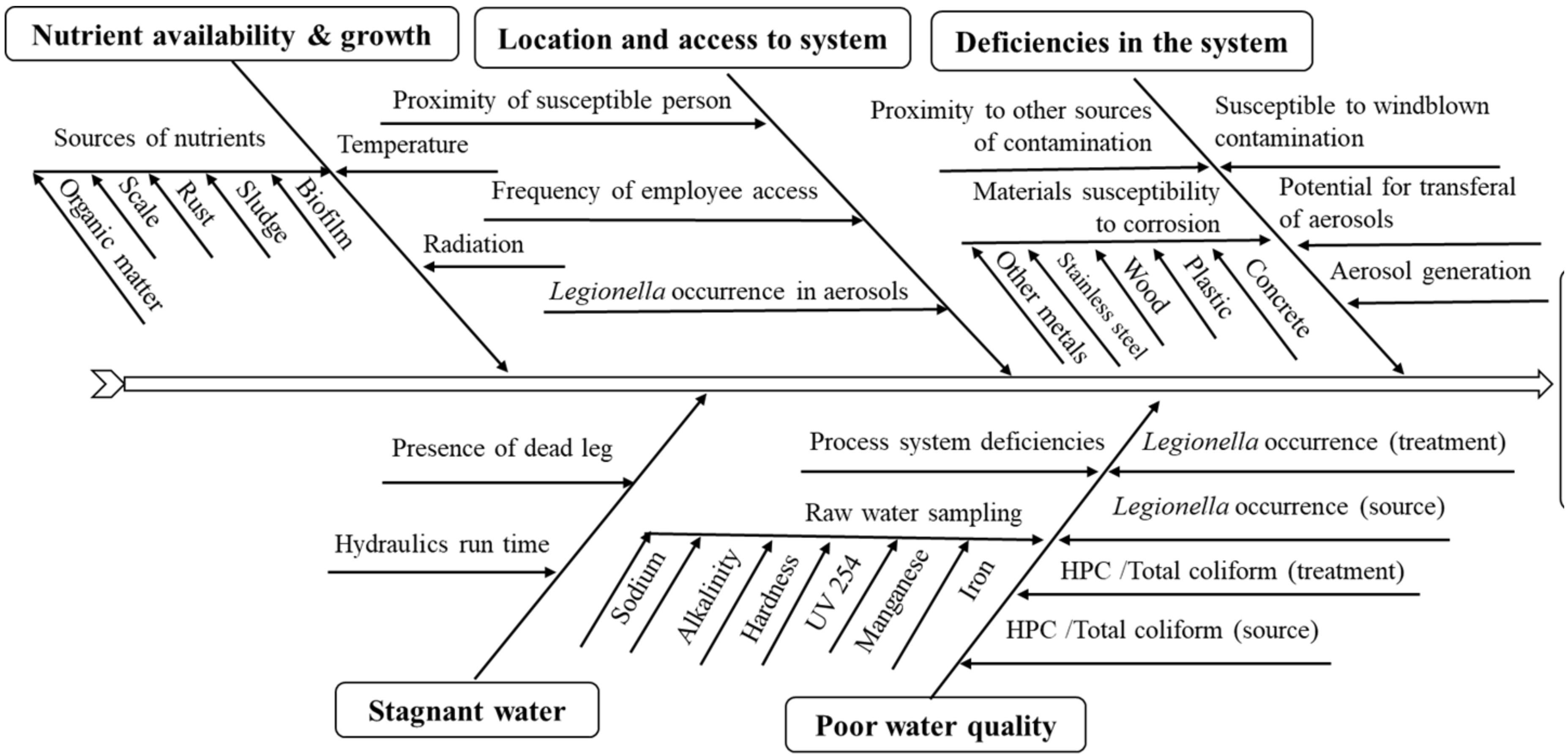

Figure 5.2: FD representing causal connections among the five different areas, and establishing the functional variables in each category as follows: Nutrients availability and growth (8), Location and access to system (3), System design (10), Hydraulic conditions (2) and Water Quality (12).

Table 5.3 summarises water quality data sourced from 25 GWTPs utilised for deriving prior probabilities for the nutrients availability and growth and the water quality variables. The average temperature was 24 ^{0}C (ranging from 14 to 34 ^{0}C) and representative of hot dry climates. *Legionella* counts were a qualitative indication of the presence and absence of the pathogenic bacteria with limits of (>10 CFU/mL) and (<10 CFU/mL) respectively. Subsequently, the graphical representation of causal *connections among variables in the conceptual FD and discretised variables were converted to a BNs.*

Table 5.3: Descriptive statistics of data computed as prior probabilities for the BN models.

Variables	**Total counts**	**Mean**	**Standard deviation**	**Minimum**	**Maximum**
Temperature (^{0}C)	1711	23.5	3.4	13.7	34
Manganese (mg/L)	1060	0.05	0.07	< 0.002	0.4
Alkalinity(mg/L)	377	79.8	62.8	2.1	291
Na(mg/L)	685	97.7	57.4	23	355
Hardness(mg/L)	536	137.6	62.7	15	270
HPC (CFU/mL)	29	37.7	73.4	<10	220
Legionella (CFU/mL)	43	-	-	< 10	>10

*HPC: Heterotrophic plate counts at 37^{0}C

5.3.2 Model development and analysis

5.3.2.1 Initial model output

The initial output BN model is presented in *Figure 5.3*, in a labelled bar style with colour coding created to categorise variables into distinct groups in line with good BNs practice (Marcot, 2017). The five risk areas (orange), primary and immediate risk factors (tan) and endpoint point variable represented as the

Legionella exposure (cyan). The model consisted of 42 nodes, 43 links, and 9461 conditional probabilities. Of the 42 nodes, 33 were inputs variables (*Supplementary Materials A, Figure S1*). The prior distributions of parent nodes, captured through the BN, provided a posterior probability estimate of the risk parameters. The BN model, in its simplest form (without inputs of observed evidence), depicts the marginal probability distributions for each state in all the nodes as summarised in *Table 5.4.* This display is a visual representation of potential causality between the nodes leading to the risk of *Legionella* exposure. While the relationships between variables in BN are not necessarily, by definition, causal, the graphical portion has been useful for representing presumably causal understanding (Carriger and Parker, 2021).

Table 5.4:Summary of the probability distributions of the BN model predictions for five risk areas

Variables	**Model states**		
	Low	**Medium**	**High**
Nutrients availability and growth	42.3	56.4	1.3
Stagnant water	13	64	22
Water quality	7	61	32
Location and access	23.6	56.1	20.3
System design	6.9	63.6	29.5
Legionella risk	32	61	7.03

The distribution probabilities across the states tends to exhibit a normality pattern. The conditional probabilities tend towards the medium states in the five risk areas, as seen with the high probability values. The distribution for the end variable (*Legionella* exposure) tends to be skewed to medium and high-risk (accounting for over 99%).

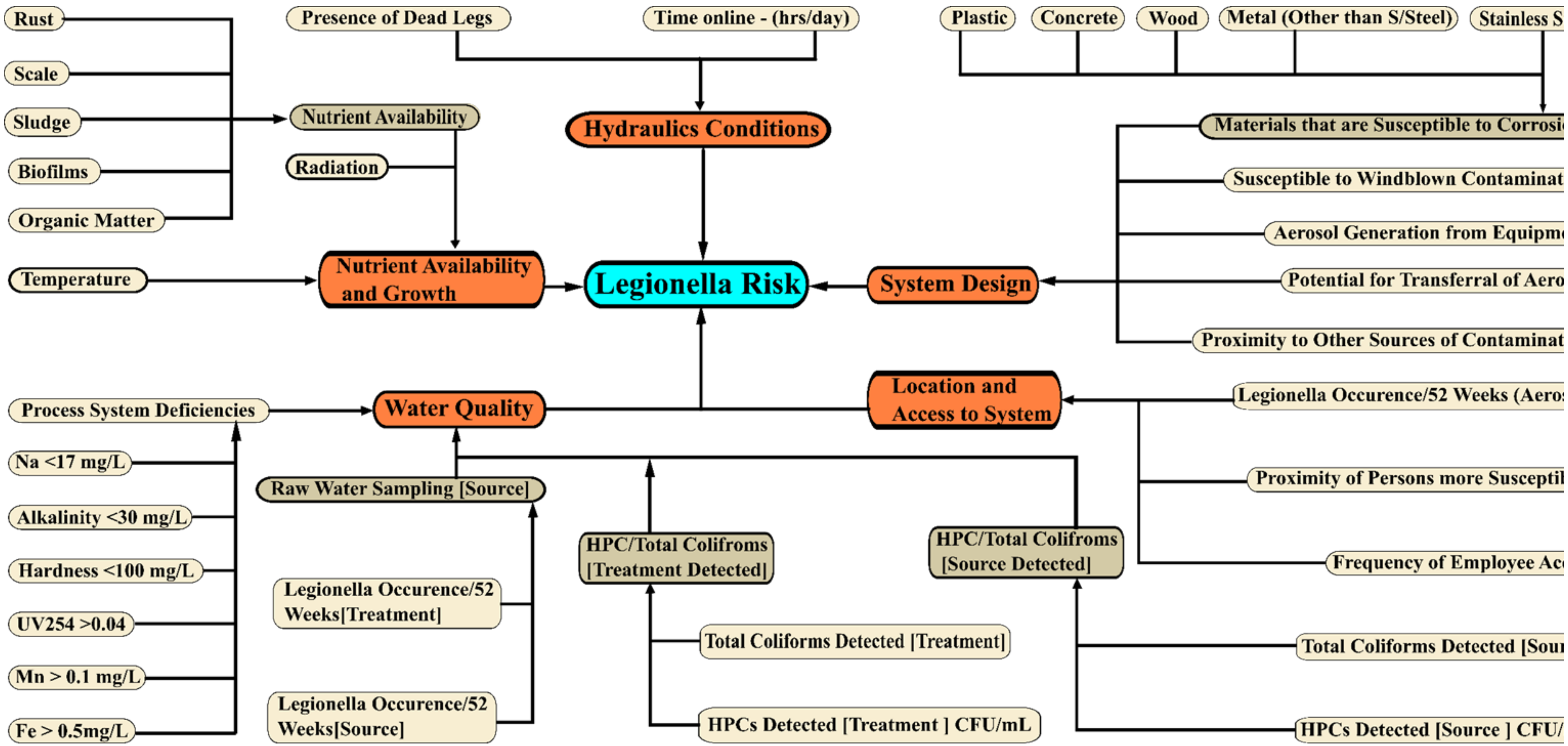

Figure 5.3: Initial BN model with the proposed illustration of potential causal relationship directly adapted from the LHLRA

The explicit visualisation of the interactions between variables enabled the exploration of model behaviour and interrogation of the reasoning behind the model output, thus providing a more transparent approach as compared to the LHLRA. This is not the case with most traditional forecasting methods like the LHLRA. Going a step forward, by inputting findings into the nodes (i.e. setting evidence), the BNs can update the prior probabilities in the model to propagate new posterior probabilities based on the structural relationships and the conditional probabilities. With this feature, we can analyse the effects of different scenarios on the model variables (Pollino *et al.*, 2007).

5.3.2.2 Scenario assessment

A scenario assessment is a BNs feature that helps to clearly understand the characteristics of each variable and its relationship with others in the model. One of the advantages of BNs is the ability to perform predictive (forward) and diagnostic (backward) inferences. Two diagnostic scenarios were developed to illustrate the capability of the model to examine the probabilities of factors that would result in a high and low risk of *Legionella* exposure. By applying a backward (diagnostic) inference all the way to the top parents, the original triggers for risk scenario can be assessed. In these scenarios, a high state was selected (set as hard evidence) in the *Legionella* exposure node (setting the probability to 100%) with the same procedure repeated for the low and the probabilities for all the nodes were examined. *Figure 5.4* summarises the estimated occurrence probabilities of the worst states of the variables across all the nodes. By worst, we refer to the probability of delivering the state of a given variable with the most impact on the risk of *Legionella* exposure. The five risk areas are all likely to be in the worst state (high) under the high scenarios. It is also obvious that the chance of worst states occurring is greater in the high-risk scenario for all the variables, except for the hydraulic runtime node. For high risk of *Legionella* exposure, the five risk areas appeared with dominant probabilities in the worst states: nutrient availability and growth (0.72), hydraulic conditions (0.70), water quality (0.68), location and access (0.60), and system design (0.57), while in the low-risk scenario, only the proximity of other sources of cross-contamination has up to 50% chance of being in the worst-case.

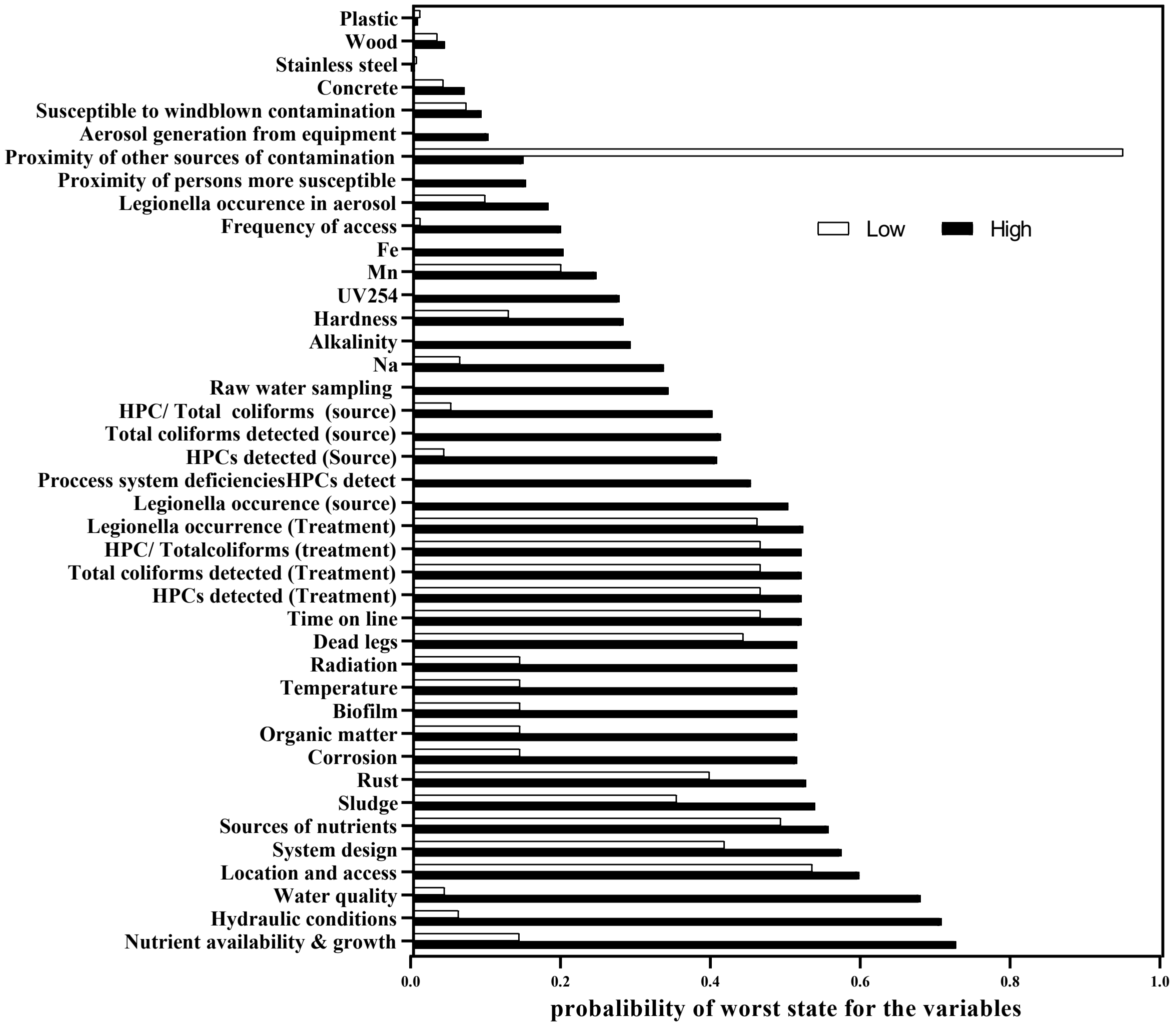

Figure 5.4: Probability of prediction of the worst state in each of the variables contained in the model for the high and low risk of Legionella scenarios (a low state requires no mitigative action; and the high state calls for immediate decontamination).

These predictions are expected because high risk of *Legionella* exposure is generally the product of worst conditions present in the different individual variables. This implies that monitoring nutrient availability, hydraulic conditions, and water quality will be a reasonable surveillance approach to detect elevated risk in the system (*Figure 5.4*). This agrees with both LHLRA and standards for *Legionella* managing in other systems (AS/NZS 3666, 4276). These dynamic responses of BNs through the demonstration of the interaction of the different variables with observed evidence set them aside from other models. While scenario assessment provided system interaction in a very transparent manner, the approach is not able to rank the contribution of each of the variables to the overall risk in this modelling software. But through sensitivity analysis, we can begin to identify which variables in the model have the greatest impact on the model endpoints, as well as the order of importance, strength, and relevance of the inputs in determining the variation in the output.

5.3.2.3 Sensitivity analysis

An analysis of sensitivity to findings was performed on the endpoint variable (*Legionella* exposure) in the BN to identify the most important variables in the system. The sensitivity analysis ordered quantitatively the degree of variance reduction (VR) or uncertainty (entropy) associated with input variables in a specified outcome (Chen and Pollino, 2012b; Pollino and Henderson, 2010). As shown in *Figure 5.5* the system design (the potential of the aeration system to release contaminated aerosols) is the variable with the most influence (0.225) , followed by the location and access (the height and duration of exposure as a result access to the aerator environment) (0.166), *Legionella* occurrence in the aerosols (0.097) and susceptibility of the systems to windblown contamination (0.073). Unsurprisingly, the nodes with the greatest impacts are the risk areas. This is expected as the risk areas are intermediate (i.e., neither 'root' nor 'leaf') nodes that represent multiple risk factors. Of the primary ('root') nodes, *Legionella* occurrence in aerosol has the most significant impact. This could be attributed to the expert weighted score influenced by risk aversion. This behaviour in the model demonstrates a reasonable representation of the interactions between input and output variables that aligns with experts understanding of the risk of *Legionella* exposure. The complete summary of the sensitivity analysis data is provided in the (*Appendix 3, Table A3.2*).

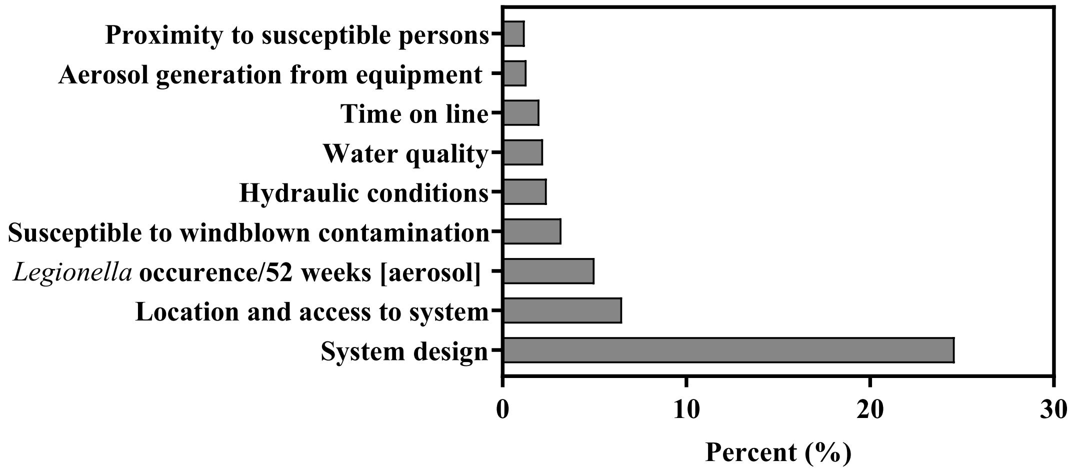

Figure 5.5: Sensitivity analysis profile for initial model represented as percentage variance reduction of Legionella concentration as the target variable (only variables with VR greater than 1% shown)

Although temperature is expected to have a significant influence on *Legionella* exposure , the current analysis showed a minimal impact (0.01) (Rakić and Štambuk-Giljanović, 2016). This suggests possible deficiencies in the relationship between variables adopted as causality from the LHLRA to be mostly correlational and resulted in too many CPTs (9461). Where CPTs are higher than the number of samples available (particularly with respect to endpoints like *Legionella* exposure) for model evaluation, the accuracy of how well the model represents the system can be poorly assessed (Pollino and Henderson, 2010).

In the current adaptation, the model has a deep structure — with many intermediate (10) and primary (19) nodes — which can facilitate more uncertain propagation of uncertainties from input to output nodes. Equally the asymmetric structure of the model can contribute to making distant input nodes with many intervening nodes less sensitive (Marcot *et al.*, 2006).

Good practice recommends the depth of model — the number of layers of nodes — be kept relatively shallow (four or fewer, if possible) to create minimal associated CPTs that are tractable and understandable when specified by the experts (Marcot *et al.*, 2006). Collectively, these findings suggest the need for a relatively shallow depth model in the revised (beta) iteration. Breaking up multifaceted systems into two or more model networks is recommended as a good approach to increase accuracy and assemble diverse variables in a coherent and systematic environment with moderate depth (Marcot *et al.*, 2006).

5.3.3 Revised BN models

5.3.3.1 Modifying the model causal structure

The experts' driven workshop facilitated a conceptual map describing the potential causes and consequences of *Legionella* colonisation, an improvement on the causality relationships from the alpha model. The output conceptual map from the bowtie representation is shown in *Figure 5.6*.

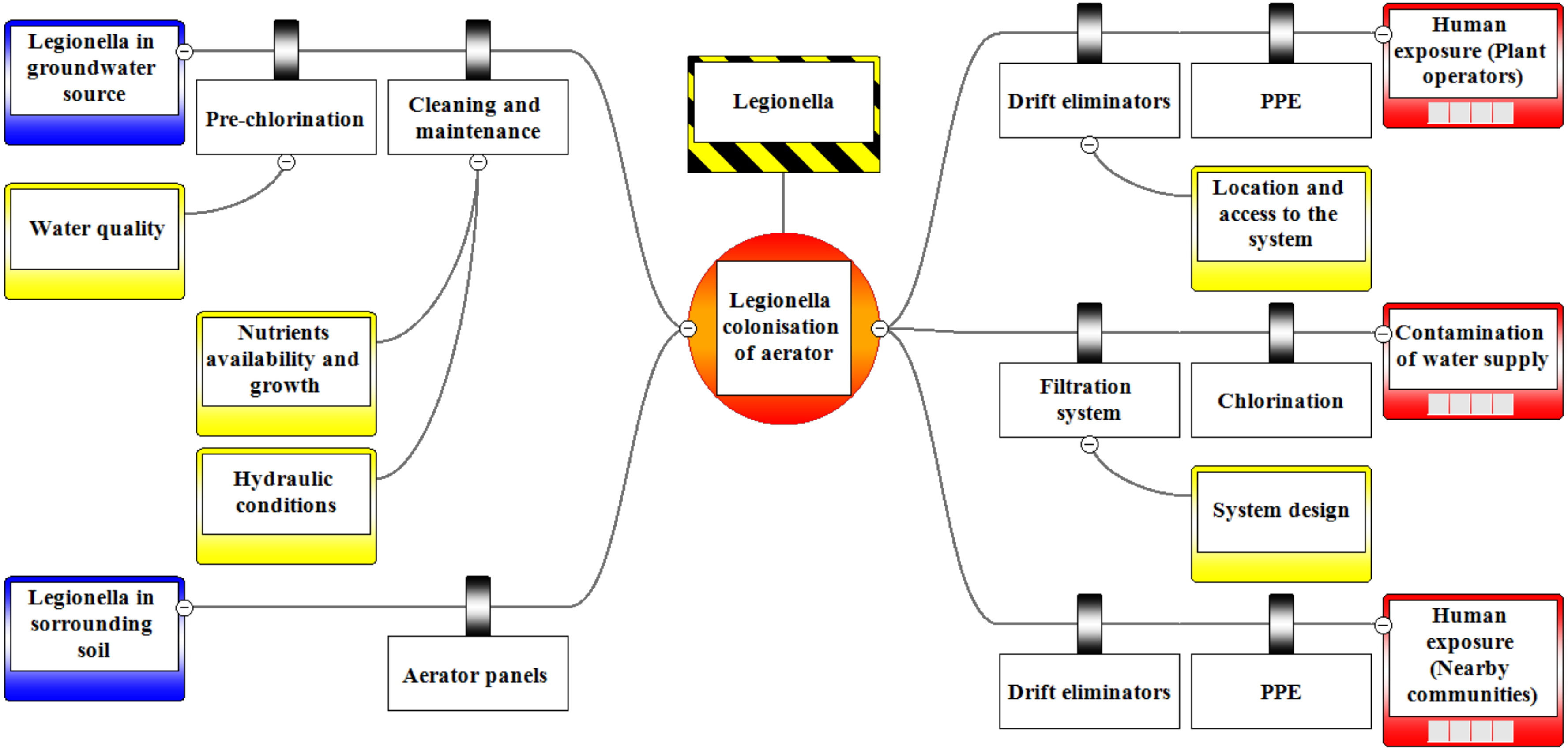

Figure 5.6: Conceptual mapping describing the potential causes and consequences of Legionella colonisation. From left to right is the proactive and reactive side of the risk of Legionella. Colour coding categorised the threat (blue), escalation factors (yellow), barrier (in white) and consequences (red).

The conceptual map reveals the consequences of *Legionella* colonisation to include public health exposure and contamination of water supply. The contamination of water supply was not modelled due to the multiple treatment barriers in the water system train that is considered sufficient to inactivate the pathogen. The improved diagram provides a simplified and better overview of the mechanistic dependencies in the model, allowing for the selection of causally linked-influence for the growth and exposure separately (Getz *et al.,* 2018). The output map becomes the foundational framework for the revised BNs model. To avoid the complexity pitfall from the initial BNs model, the divide and conquer modelling approach was adopted (McDonald *et al.,* 2016). Hence, the *Legionella* growth and transmission scenarios were modelled into two sub-models, deconstructing the problem into a more elemental component that can be modelled more easily.

5.3.3.2 Legionella growth model

Figure 5.7 shows the *Legionella* growth sub-model, (i.e., a mapping of the left-hand side of the conceptual diagram). The endpoint of this model is *Legionella* bacteria presence with modified states of detected and not-detected (cyan coloured). The modifications of the target node to the qualitative thresholds were made to represent realistic and more interpretable divisions. Three of the critically defined risk areas (orange coloured) are captured in the growth sub-model. The probability distribution on the *Legionella* bacteria nodes is 78.9% and 21.3% for detected and not detected respectively, suggesting the current weighting is skewed towards positive detection. The new endpoint variable is an observable and measurable parameter in contrast to the *Legionella* exposure in the initial model. This provides the illustration of causal links between *Legionella* bacteria and predictor variables.

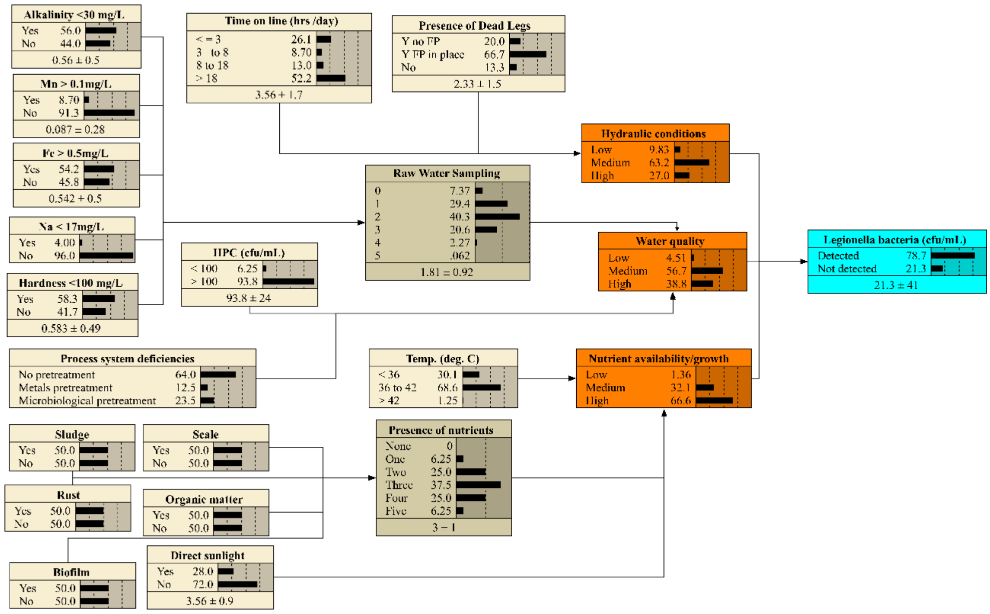

Figure 5.7: Legionella growth BN sub-model

The sensitivity analysis related to this specific growth model revealed water quality (0.205), nutrient availability and growth (0.127) and hydraulic conditions (0.126) as the three variables with the most impact on the detection of *Legionella* (*Figure 5.8*). The quantifiable and observable nature of the output *Legionella* bacteria node, allows to evaluate the model performance (Marcot, 2012).

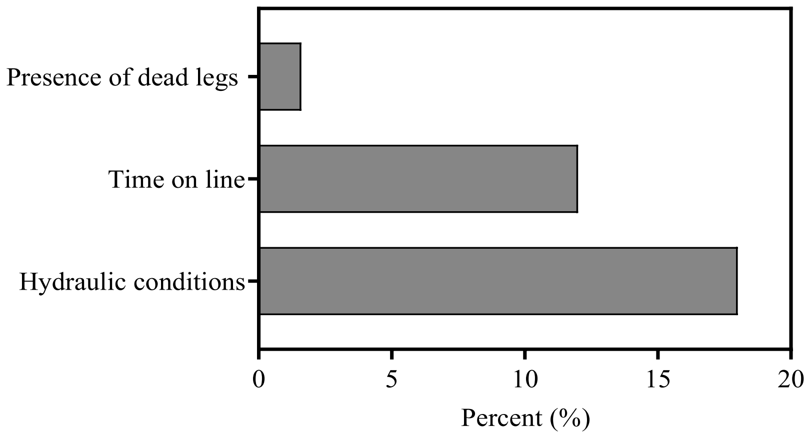

Figure 5.8: Sensitivity analysis profile for Legionella growth sub model, represented as percentage variance reduction (VR) of Legionella bacteria as target variable (only variables with VR greater than 1% shown)

5.3.3.3 Performance evaluation of the growth sub-model

Performance validation is a crucial step for evaluating the prediction and success rate of models (Marcot, 2012). The process of model validation assesses the quality of the model prediction under independent conditions using data not previously applied in the BNs construction. Ideally, when the data set is large enough, the k-fold cross-validation approach that splits the data into two folds, one for training and the other for validation is recommended (Marcot, 2012). Constrained by the sparse data considered in this study relative to the complex process (and model), the total model historical records from the LHLRA were utilised in the model learning. This validation of the model provides an assessment of the accuracy of BNs predictions, comparing the state with the highest predicted probability to the observed outcomes.

As summarised in Table 5.5, for the 23 sites, the model incorrectly predicted the status "*Legionella* not detected" as the more likely option in the three observations of *Legionella* detection (type I error) (true positive rate of 0%), but correctly predicted "non-detected" scenarios in 18 out of 20 cases (true negative rate of 88%) cumulating into a (model error rate of 21%). These results suggested a highly imbalanced dataset.

Table 5.5: Confusion matrix showing the prediction accuracy of the BN model in *Figure 5.7*.

	Predicted *Legionella* bacteria	
Actual observation	Detected	Non - detected
Detected	0	3
Not – detected	2	18

The kappa coefficient was (k) was negative, signifying an apparent lack of agreement between the predicted and observed *Legionella* occurrence outcomes. The prevalence index of 0.78 confirmed the existence of prevalence effect as evident in the different proportion of agreements for the detected and not detected classification of *Legionella* occurrence. The bias indexes of 0.7 and 0.13 disagreement for the detected and not-detected class also confirmed the very asymmetrical dataset. The significant prevalence and bias effect in the datasets can reduce the expected prediction to random and subsequently a weak agreement in the outcomes (Byrt *et al*., 1993; Sim and Wright, 2005). The certainty of the causal relationship and difficulty in species detectability have been listed as the main contributors of model error in environmental modelling (MacKenzie *et al.,* 2003).

The current system understanding, that translated into the mechanistic models, are likely to be correlational and not necessarily causal, as revealed in the error rate (Marcot *et al*., 2006). Equally, the trivial detection of *Legionella* counts in the sampling reference data can be a limitation to the reliability of the validation, complicating the distinction of randomness and the power of the model to make useful prediction for *Legionella*.

At the current stage of the iteration with no true positives predicted for *Legionella* and the highly imbalanced datasets, the model accuracy metric is expected to be a poor performance indicator (Marcot *et al*., 2006).

Given the weakness of the model accuracy indicators, the sensitivity analysis was used to interpret the ultimate behaviour of the current relationships in the model. The sensitivity analysis classification of key influential variables suggested the need for further structural revision before advancing the model towards implementation. For example, the impact of temperature on *Legionella* exposure was found to be insignificant, while expected to be of major influence. Particularly, it will be critical to examine the distinction between relationships that are causal and correlational in future studies.

Therefore, the acquisition of more empirical data through more frequent *Legionella* sampling is recommended. This will allow further structural learning and training of the model with a larger pool and more diverse input data in manner that increase certainty and prioritise causality relationship.

5.3.3.4 Legionella transmission modelling (human health exposure)

To understand the impact of *Legionella* growth, the right side of the conceptual map was modelled for the risk of human exposure leading to infection. The risk of infection is defined by the *Legionella* bacteria as well as two critically defined risk areas of location and access to the system and system design (*Figure 5.9*).

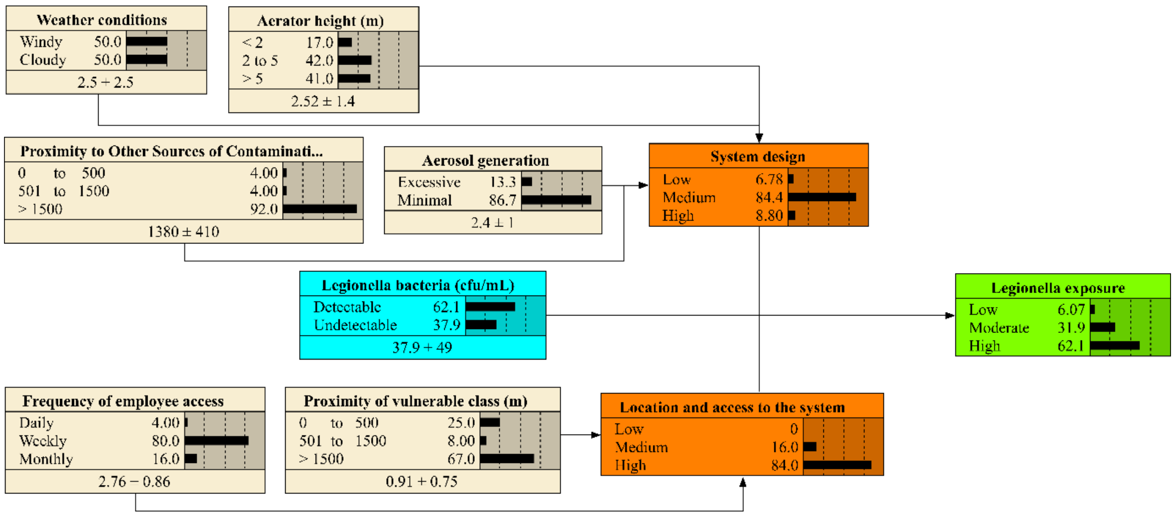

Figure 5.9: Legionella transmission BN sub model

The sub-model was evaluated through sensitivity analysis. The sensitivity analysis of the exposure model revealed two most impactful variables as *Legionella* counts as expected (0.958) and frequency of employee access (0.241). This result was expected as infection strongly depends on the concentration in the system (Pepper and Gerba, 2018).

The difference in the output format of the LHLRA and the BN model made it difficult for direct comparison, however, the two approaches can be interpreted in a complementary manner. While both models are built based on expert judgement and intended for prediction purposes, only the BNs allow for testing of the reliability and accuracy of the belief structure or theory that define the relationships through the sensitivity analysis.

Unlike the growth sub-model, the endpoint of the transmission modelling (*Legionella* exposure) is categorical ('low', 'medium', and 'high'), and with no measurable and/or observable records, making validation difficult. However, the BN probability distributions on *Legionella* exposure provided estimate of the chance of occurrence of the different risk levels. Despite the trivial count occasionally captured in the monitoring program, and no records of *Legionella* outbreak in the GWTPs, the baseline probability distributions in the refined model showed a skewness towards the high-risk scenario (with over 50% chance of high-risk level) (Yunana *et al.*, 2019).

5.3.4 Towards better risk assessment

Despite advances in standard management frameworks, managing the risk of *Legionella* colonisation of water systems still presents important challenges. As demonstrated in this study, using synergies between complementary techniques can advance mechanistic understanding and predictive performance that paves the way towards achieving improved and more comprehensible risk assessment. Particularly, iterative BNs modelling demonstrates the capability for a more transparent, structured and quantitative approach to assessing the risk of *Legionella* exposure (Uusitalo *et al.,* 2015).

To be useful for environmental management, model outputs must be aligned with stakeholders' objectives, hence, retention of the BNs structure from the causal connections and functional relationships among variables from the LHLRA. While the approach allowed simple model development, a number of limitations can be identified. Firstly, the CPTs are deterministic, providing a single point estimate of individual risk as scored in the LHLRA. In the deterministic estimate, the outputs of the variables are fully determined by the parameter and the initial values, which is not a satisfactory representation for complex systems like the risk of *Legionella* exposure. Generally, eliciting expert knowledge deterministically has been criticised as being vague, ambiguous, and susceptible to inconsistent judgements (Gregory and Keeney, 2017; Hemming *et al.*, 2018). Therefore, to better model *Legionella* interaction with multiple variables, capture uncertainties and inherent variabilities, a probabilistic model that provides the distribution of estimated risks and not a single point estimate is recommended (Chen and Pollino, 2012b; Richardson, 1996). The probabilistic approach to expert elicitation as outlined in Hemming *et al*., (2018) has the capability to improve consistency in defining the strength of relations between variables such as those presented in the LHLRA (Hemming *et al.,* 2018). Secondly, the definitions and classifications of certain variables were appropriate for the LHLRA but tend to increase the model complexity when translated into the BNs. For example, the five risk areas adapted from the LHLRA are all latent and derived from the interrelations of immediate parameters. Translating such relations into BNs can greatly reduce the classification accuracy, explanatory power, and the possibility of quantitative validation of the model (Marcot, 2012). However, the revised model better refined the endpoint node to focus on the observable and testable variable of *Legionella* presence.

Contrary to the LHLRA, the BNs, through the sensitivity analysis, scenarios assessments, and evaluation of prediction accuracy, provided systematic evaluation and complementary insights into the structure and the relative importance of input variables (Linkov *et al.*, 2009; Marcot, 2012). Based on the strength of definition in the BNs, the results of this study identified the inconsistent impact on the relationship between certain variables. For example, the BN models indicated the relatively minimal contribution from temperature contrary to expectation, suggesting the need for refinement to the current causal structure (Linkov *et al*., 2009; Marcot, 2012). The traditional approach to improving inconsistent structure in BNs is the multi-model ensembles and calibration check to determine the most appropriate version leading to a reasonable result (Carvajal *et al*., 2015). Hence, revised (beta) BN models were constructed and evaluated towards an improved and optimal structure.

By comparison, the initial (alpha) and revised (beta) BN models have some clear and significant discrepancies. The initial model, which is a direct mapping of LHLRA appears broad and generic, while the revised version, through the restructuring of the interdependence, provided a more focused and in-depth analysis of the risk of growth and exposure separately. The sub-modelling into growth and exposure successfully deconstructed the problem into more elemental components that allowed for a more systematic evaluation.

Clearly, the endpoint variable in the exposure sub-model is qualitative and not observable. On the other hand, *Legionella* growth endpoint is quantitative and observable, this allowed for data driven validation using predictive accuracy. The predictive accuracy of *Legionella* detection in the growth sub-model is an important step employed to advance the credibility and robustness of the model.

Furthermore, the revised growth sub-model with a refined endpoint of *Legionella* count (an observable and testable variable) is a step in the direction of developing useful predictor variable from the shortlisted water quality and operational parameters. Previously, BNs has been successful in developing predictor variables for *C. parvum* and *G. lamblia* in wastewater management (Carvajal *et al*., 2015). In a related way, as *Legionella* counts, corresponding water quality and operational parameters monitoring data become more readily available, there is the opportunity to develop predictor variables for *Legionella* as a conceptual alternative to direct measurement of the pathogen. These indicators have the potential to be utilised by regulators and water utilities to assess the microbial quality of the water in real-time for more informed risk-based *Legionella* management (Bargellini *et al.*, 2011d; Panidhapu *et al*., 2020b; Völker *et al.*, 2016a). This will complement the monitoring and maintenance program for improved management of *Legionella* exposure from aeration systems.

While progress is continuously been made in the application of BNs in environmental risk assessments, challenges still remain (Kaikkonen *et al.*, 2020; Landis, 2020). The findings from this study suggest the iterative updating of BN models have the potential to address the challenge of inconsistent and poor representation of interactions between multiple risk variables as demonstrated in this specific application. In addition, exhausting the many different performance metrics available in BNs (sensitivity analysis, scenario assessment, kappa coefficient, performance accuracy, etc) can provide a framework that improve the often-missing practice of model validation.

5.4 Conclusions

Given the complex measure of the interaction of *Legionella* with associated risk variables in aerator environment, the failure of microbial indicators and the limitations of traditionally employed risk modelling techniques, an improved assessment was proposed in this study, where BNs were implemented. The following main conclusions could be drawn:

- The application of BNs facilitated a more structured, transparent, and quantitatively rigorous assessment of *Legionella* growth and exposure compared to the current LHLRA method.
- Using BNs provided a robust probabilistic method of reasoning under uncertainty and appeared more suitable to represent the complex dependencies of *Legionella* growth and exposure.
- Through iterative approach, BNs characterised the complex mechanisms of *Legionella* (growth and exposure) and revealed some uncertainties associated with limited data and poor knowledge as captured in the current mechanistic relationships between the variables.
- The development and evaluation of the current BNs model provided a valuable insight into the interdependence of the variables and pointed the direction for subsequent refinement to the current considerations of cause-effect mechanisms.
- The next Chapter will focus on updating the current structure and conditional probabilities with new and larger validation data to create the final-application gamma-level model. Through the hybrid of data driven and expert learning approach, the gamma model will be explored to strengthen patterns and predictive power of different combinations of explanatory variables contributing to *Legionella* growth.

CHAPTER 6

INTEGRATING DATA-DRIVEN LEARNING AND EXPERT KNOWLEDGE TO IMPROVE BAYESIAN NETWORKS MODEL PREDICTION OF *LEGIONELLA* OCCURRENCE

6 INTEGRATING DATA-DRIVEN LEARNING AND EXPERT KNOWLEDGE TO IMPROVE BAYESIAN NETWORKS MODEL PREDICTION OF *LEGIONELLA* OCCURRENCE

6.1 Introduction

In chapter 5, a BN model that characterised *Legionella* growth and transmission in aeration systems was developed. Currently, there is a lack of historical water quality data and limited knowledge with regards to the mechanistic relationships between the key variables, resulting in modelling outputs with weak sensitivity (chapter 5). The limited interdependence between the growth sub-model's variables (mostly correlational), and deterministic conditional probabilities were unable to capture uncertainties and were identified as additional limitations of the model.

BN models developed through expert knowledge and data-driven approach have successfully established logical relationships among water quality parameters with faecal indicators and total coliform with reliable performance accuracy (Panidhapu *et al*., 2020a). Data-driven models are based on data mining algorithms and statistical techniques that analyse and identify patterns within the monitored data to create anticipating rules linked to microbial dynamics. When combined with expert knowledge, data driven learning can help identify hidden relationships between model variables to achieve a better model performance (Sousa *et al*., 2018).

Therefore, to improve the performance and sensitivity of the *Legionella* growth sub-model, a hybrid approach that incorporate expert knowledge and data-driven learning from a comprehensive historical water quality using machine learning algorithm is explored. In the context of aerators, extensive historical documentation of *Legionella* occurrence, along with associated water quality and operating parameters, was not obtainable. This is mostly due to the fact that this pathogen is not generally monitored as part of current water utilities' water safety plans. However, while aerators which are an overlooked source of *Legionella*, historical datasets are more likely in other systems already considered to favour the harbouring of *Legionella*.

One source of historical *Legionella* occurrence data is from the current body of literature that exists for drinking water treatment systems. Previous studies have reported significantly large datasets for *Legionella* occurrence, monitored alongside water quality variables. In one of such studies, water quality variables was measured across three different locations in a drinking water systems; including the water source, treatment plant effluent, and distribution systems' finished water reservoirs or storage tanks (LeChevallier, 2019a, 2019b). Despite the majority of the data being obtained from distribution systems which are not inherently representative of groundwater aeration systems, they do share similarities with respect to *Legionella* growth.

Legionella growth in engineered systems, including drinking water treatment processes, is defined by operating conditions and water quality. While operating conditions can be variable across different systems, water characteristics favourable for *Legionella* occurrence were observed to be consistent across different systems (Armero *et al.*, 2011; Serrano-Suárez *et al.*, 2013). As such, *Legionella* and water quality from other drinking water systems can be adapted to improve the BN models performance through data driven learning.

Water quality data and operating conditions from different systems have been adapted in a successful case study of data driven modelling for microbial pathogens (Carvajal *et al.,* 2015b). Adapting wide and diverse datasets has been considered to enhance BN model performance, robustness, and versatility (Rousso *et al*., 2020).

To this end, there is the opportunity to utilise datasets monitored across drinking water treatment systems to develop a model with potential for application in other industrial/municipal water systems beyond aeration systems.

This chapter explores the potential of data-driven and expert knowledge to create a more sensitive and accurate growth sub-model with improved structural dependence between variables to better predict *Legionella* occurrence from water characteristics. The study aim is achieved through the following objectives:

i. Identify the key water characteristics that drive *Legionella* occurrence,
ii. Integrate data mining of historical water quality parameters and expert understanding of the relationship between variables to create BN models for predicting *Legionella* occurrence,
iii. Determine the optimal model through performance metric analysis,
iv. Evaluate performance of the optimised *Legionella* BN model, and
v. Assess the capability of the model to predict *Legionella* occurrence in aeration systems.

6.2 Developing the Bayesian model

The stepwise modelling approach adopted is summarised in *Figure 6.1.* The approach follows the BN modelling framework and consists of four steps. The steps include data preparation, model conceptualisation, creation, and evaluation (Chen and Pollino, 2012).

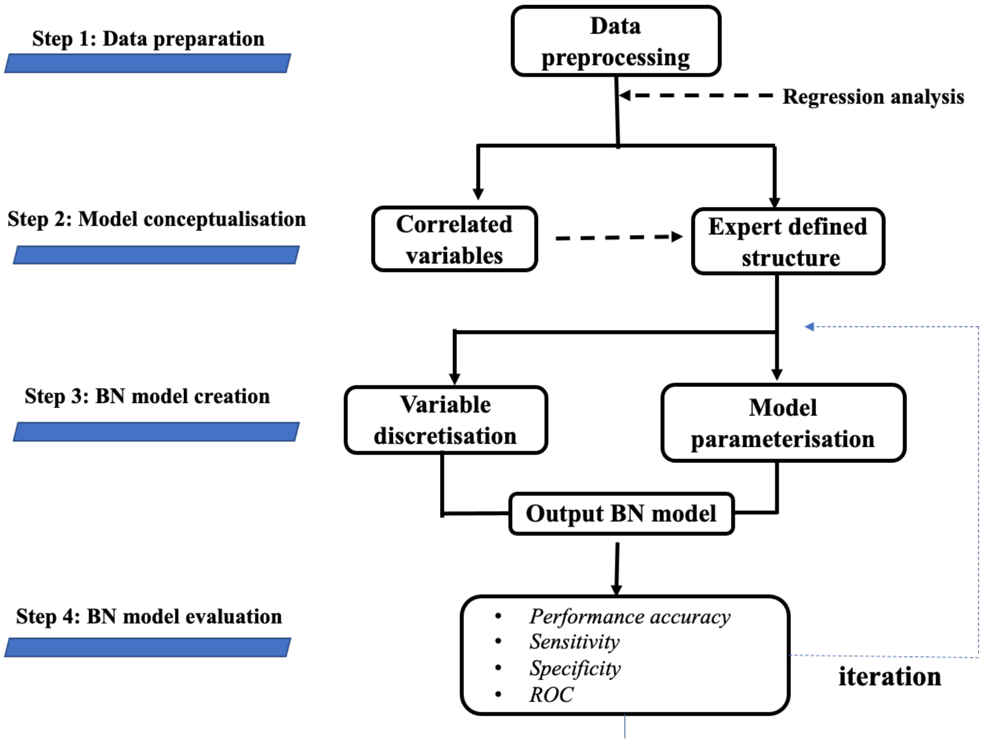

Figure 6.1: Schematic diagram showing the development and evaluation of the Bayesian model.

6.2.1 Data preparation

Water characteristics records from across 12 drinking water treatment systems, totaling 1400 datasets, consisting of nine water quality variables, were collected from the literature. The samples characterised were collected from the feedwater stream, effluent of the plants and the finished water reservoirs or storage tanks (representing the distribution system) (LeChevallier, 2019a, 2019b). *Legionella* was measured alongside eight variables related to the water quality including, HPC, total coliform, *E. coli*, total chlorine, free chlorine, temperature, pH, total organic carbon. Although season was monitored as an environmental parameter, due to its correlation to *Legionella*, it was included in the list of variables (Bentham, 1993).The preparation of data for the model included pre-processing to address missing datasets and poisson regression analysis to defined variables correlated to *Legionella.*

The nine parameters were processed in Microsoft Excel. Two data processing techniques were used to address the missing datasets (Peugh and Enders, 2004). First, data elimination was applied to remove every dataset where *Legionella* occurrence was not reported. Second, missing values in datasets with *Legionella* occurrence reported were filled up using the mean values of the total records. After eliminating the cases with missing data, there were 1373 pairs of samples with 88 samples of positive detection utilised for the data driven learning.

Poisson regression as previously applied was completed on pre-processed nine water quality parameters pre-processed to select variables associated with *Legionella*. The season values which were categorical (spring, summer, winter and autumn) were transformed into dummy variables to allow for their inclusion as predictors in the regression analysis. Only parameters with a P-value < 0.05 from the regression were considered to indicate positive association with *Legionella* and included in developing the predictive model. The pre-processed data were then used as input to the data-driven machine learning model.

6.2.2 Model creation

The model creation step consists of conceptualisation of interdependencies, discretisation of the variables, and parameterisation to establish the connection between the variables in a semi-supervised learning approach. GTT machine learning algorithm, built into GeNIe, was selected to supplement the structure of interdependence between the variables in the model. The GTT algorithm allowed specification of expert definitions of relationships before algorithm learning of the interdependence.

Expert knowledge complemented by GTT algorithm was utilised to create a conceptual model with logical and valid interdependence. Expert understanding of the interdependencies of the variables were first inputted into the GTT platform. The GTT platform have three different tiers for the arrangement of the variables. Arrangement of variables in the GTT was completed in three different iterations to allow for evaluation of the effect of the algorithm in model structure.

In the first iteration, *Legionella* was assigned in tier 3 and the remaining shortlisted variables (temperature, season, pH, free chlorine, HPC) were assigned into tier 1 without any restraint in connection. In the second iteration, *Legionella* was assigned into tier 3, an intermediate node was created by assigning HPC and temperature to tier 2 and only season and free chlorine were left in tier 1. In third iteration, the arrangement in the second iteration was retained with additional permissible and constraint to the link between variables. Following the arrangements, GTT algorithm learning was ran to supplement the model structure (*Full outline for all three different structure learning iteration is provided in the appendix 4)*.

The machine learning algorithm used in this study required discrete data. As such manual and supervised discretisation methods as described in the literature were adopted (Beuzen et al., 2018a). Manual discretisation (also referred to as expert discretisation) involves manually selecting thresholds based on physical meaningfulness, theoretical knowledge, or expert interpretation of the problem domain (Chen and Pollino, 2012c). Supervised discretisation is based on the distribution of each individual variable with an equal-width (Dougherty *et al.*, 1995; Zielosko and Stanczyk, 2021).

For each of the conceptual model structural iteration, a manual and supervised four equal width binning was applied to the variables. Season variables discretisation was maintained in all through binning and was based on the date of sample collection of winter, spring, summer, and autumn.

Manual discretisation was based on the expert established thresholds for the water quality variables defined in the literature. The supervised option discretised variables into four bins. The output node, for *Legionella* occurrence, was set to binary class of 'Yes' and 'No'. The "Yes" class represented a positive measure of *Legionella* bacteria (≥ 10 CFU/mL). The structural three iterations and combined with the two discretisation options were translated to six output BN models.

Parameterisation involved specifying the conditional probabilities between the variables. The strength of the relationships between nodes was quantified in the conditional probability tables (CPTs) attached to each node. The conditional probabilities were derived based on the combination of the different bins define for the variables. The discretisation of the variables, parametrisation, structure and performance of the BN were completed in the GenIE, a software with in-built machine learning algorithms that allow the integration of domain knowledge (Bayes Fusion LLC, Pittsburgh, PA).

6.2.3 Model evaluation

Five performance metrics were assessed to determine the optimal models from the six output models. The performance assessment metrics were derived through the 10-fold randomised cross validation using GeNIe (Marcot and Hanea, 2020).

The first metric was the performance accuracy (i.e., the proportion of correct predictions). The second and third metrics were sensitivity and specificity measures. Sensitivity is a measure of true positive rate. Specificity is a measure of true negative rates. Higher sensitivity and specificity value signify a good model performance (i.e., low false negative and false positive results).

The fourth metric was the Cohen's Kappa (k). Cohen's Kappa measures true model prediction and the outputs attributed to randomness (Ben-David, 2008). The fifth metric is area under the curve (AUC) which relates sensitivity and specificity. AUC is an indicator of the model's ability to differentiate

Legionella positive and negative classes and is used as a summary of the receiver operator characteristics (ROC) curve. Higher AUC signifies better performance.

The optimised model (i.e., the iteration) considered to have better performance form the five metrics was selected and further evaluated using the limited historical datasets from GWTPs aerator.

6.3 Results and Discussion

6.3.1 Parameters correlated to *Legionella*

The strength of relationship between the water quality and *Legionella* positivity is summarised in *Table 6.1*. Only four of the nine monitored water quality variables showed strong correlation and can be considered to provide reasonable indicator for *Legionella* positivity ($P < 0.05$). The observed relationships were expected as earlier studies have correlated *Legionella* occurrence with water quality and environmental parameters in including HPC, free chlorine, water temperature (Bargellini *et al.*, 2011d; Lasheras *et al.,* 2006b; Pierre *et al*., 2019). Elevated temperature and warmer seasons have been associated with increase viability of *Legionella* in environmental samples. Also, residual chlorine has been shown to lower *Legionella* pathogen occurrence in water systems (Paranjape *et al*., 2020).

Table 6.1: Correlation of specific water quality variables and Legionella positivity

Parameters	P-value
Seasons	**< 0.005**
Total coliform	0.2426
E. coli	0.2064
HPC	**0.0088**
Total chlorine	0.8883
Free chlorine	**0.0114**
Temperature (^{0}C)	**0.0289**
pH	0.8945
Total organic carbon	0.4792

**P values bolded indicative of variables with positive correlation with Legionella*

The lack of relations observed between *Legionella* with *E. coli* and faecal coliform were unsurprising. Given that *faecal coliform and E. coli* are reliable indicators of enteric pathogens and faecal contaminants, but not of water-based opportunistic pathogenic. Expectedly, HPC showed correlation with *Legionella*. Therefore, only HPC was included in the variables to represent microbial indicators alongside the three other physiochemical characteristics (Whiley *et al*., 2015).

Contrary, the lack of correlation between *Legionella* with TOC and pH was surprising. Earlier study has demonstrated strong relationship between TOC and *Legionella.* The lack of correlation of TOC observed in this study can be attributed to the significant missing data (43% of the original TOC values were missing). Replacement of such a large missing value is likely to have contributed to the lack of correlation observed.

The values of pH on the other hand do not have significant missing values. However, average 95% of the pH values were between 7 – 9. This represents a narrow pH range with no sensitive to *Legionella.* Therefore, the lack of correlation can be attributed to the neutrality of the wide pH distribution.

While the water quality variables used to establish the association with *Legionella* were mostly sourced from distribution systems, the same associated is expected in aerator context. Earlier studies have shown that variables that correlate to *Legionella* remain the same across shower system (Hayes-Phillips *et al.*, 2019) water treatment process and distribution system (Felföldi *et al*., 2010). Therefore, the correlational relationship from the distribution systems would be consider relevant in an aeration system.

Best BN modelling practice recommends including variables that have reasonable influence on the final output model as nodes only (in this case the occurrence of *Legionella*) (Chen and Pollino, 2012b). Addition of uncorrelated variables to BN model have been reported to reduce sensitivity and can increase the complexity of the network (Borsuk *et al*., 2004). As such only HPC, free chlorine, temperature, seasons were included in modelling the occurrence of *Legionella.*

6.3.2 GTT integrated BN models of *Legionella* occurrence

All the six learned structure using the expert knowledged supported with the GTT algorithm were converted into output BN models. The output BN models are shown for the first (*Figure 6.2*), second (*Figure 6.3*) and third iterations (*Figure 6.4*) respectively. The models were developed through a combination of data driven techniques with expert knowledge.

The model has *Legionella* occurrence as the outcome node with HPC, chlorine and pH defined as intermediate variables. In a related study, Wilmot *et. al* 2000, establish the relationship between *Legionella* and the factors that promote its growth and spread in cooling towers using expert opinion and modelled in Bayesian network (Wilmot *et. al.*, 2000). This suggest incorporating expert elicitation is an appropriate approach for modelling complex systems like *Legionella* occurrence with logical and valid interdependencies.

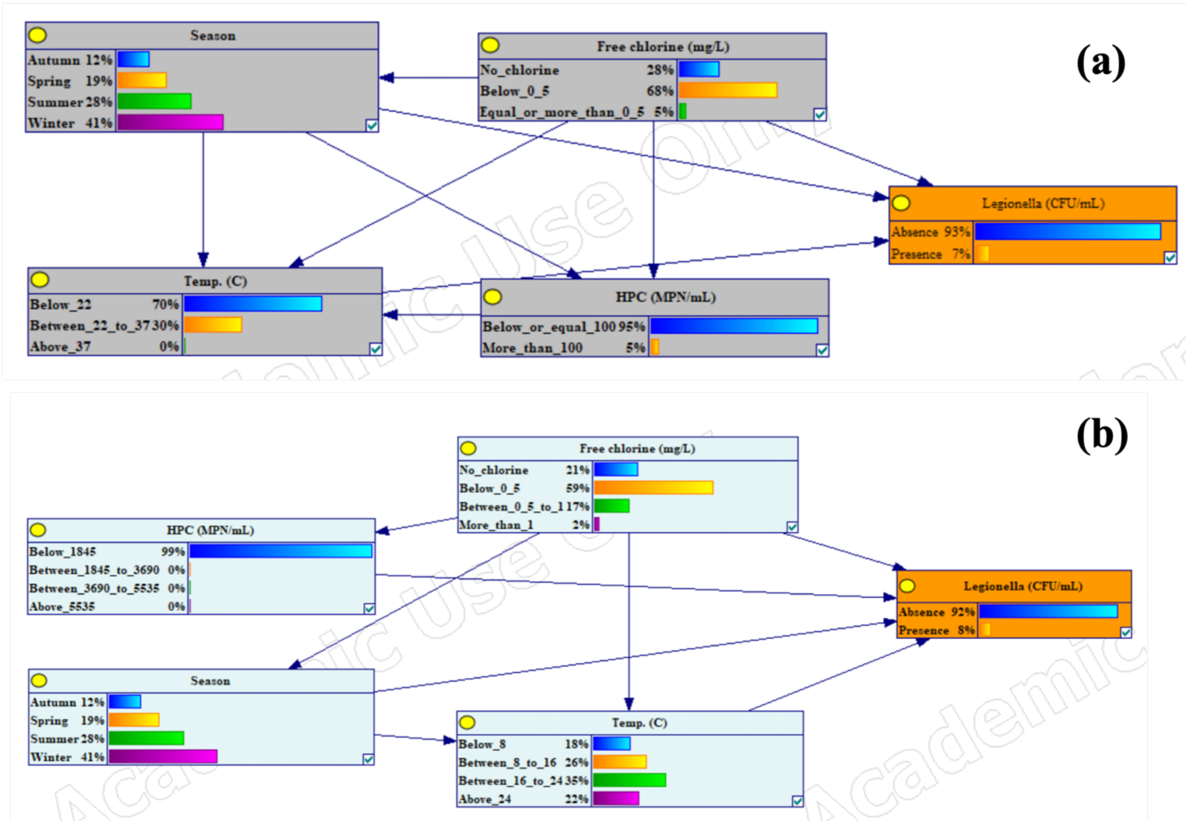

Figure 6.2: Output BN for the first tiered expert-defined interdependencies of the nodes in supported by GTT with input variables discretised using (a) expert discretisation (b) four bins

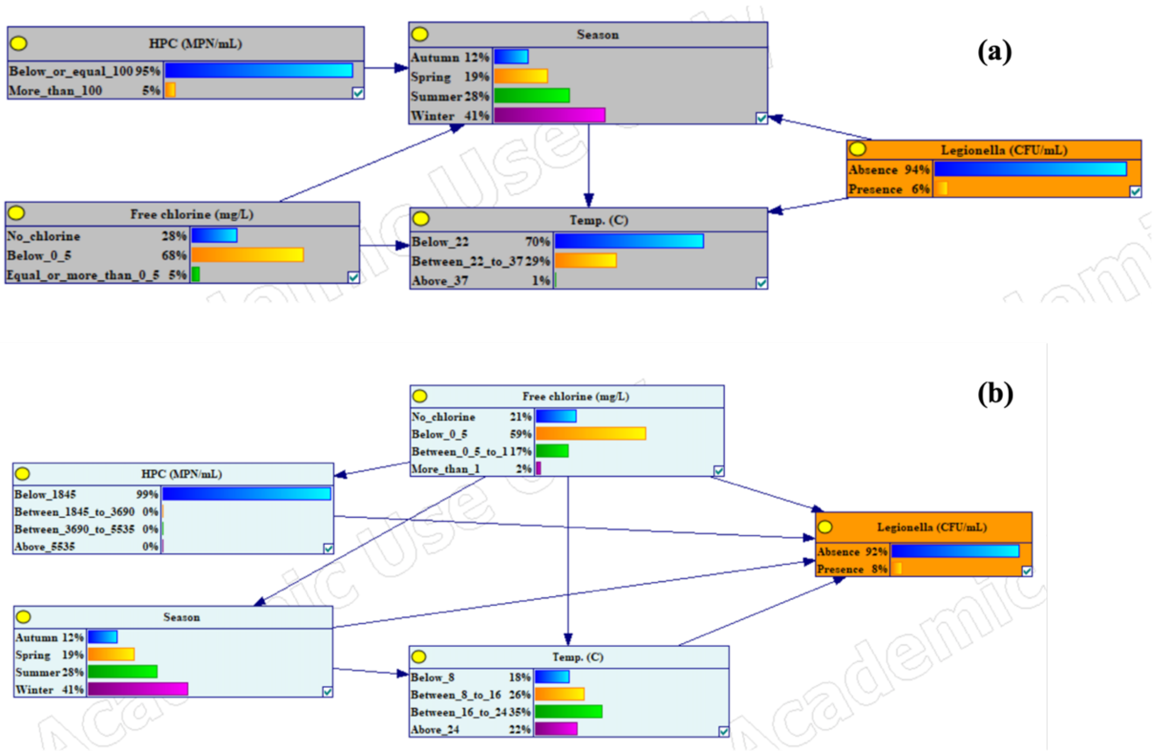

Figure 6.3: Output BN for the second tiered expert defined interdependencies of the nodes in supported by GTT with input variables discretised using (a) expert discretisation (b) four bins

The different output BN output models displayed transparently the interactions between the various variables to predict the presence or absence of *Legionella* as the endpoint. The valid and logical interrelations between all the variables were different across the three conceptual iterations. Illogical relations between variables were observed in the output models for the first (*Figure 6.2*) and second iterations (*Figure 6.3*). In the first iteration model, the connectivity link defined *Legionella* to impact on temperature and seasons. This is a reversal of our understanding that temperature and seasons are the variables considered to impact on *Legionella* occurrence and not vice versa. Similarly, an illogical connectivity was observed in the output model for the second conceptual iteration. Free chlorine was lined to season. This is inconsistent with our understanding of the interaction of theses variables.

However, output from the third iteration with established constraints and permissibility of connectivity produced the most logically valid interrelations. This suggests imputation of background knowledge into the algorithmically learning can guarantee output model with logically valid of interdependence of *Legionella* and associated variables. Logical validity of interrelation between variables is considered an important preliminary qualitative indicator for model reliability and stable (Chen and Mynett, 2003). Additionally, the improved logical validity of variables in the output model reinforced the hypothesis that hybrid learning using domain knowledge complemented with data driven can achieved better definition of variable interaction particularly in complex environment like *Legionella* when the systems understanding is limited (Panidhapu *et al*., 2020).

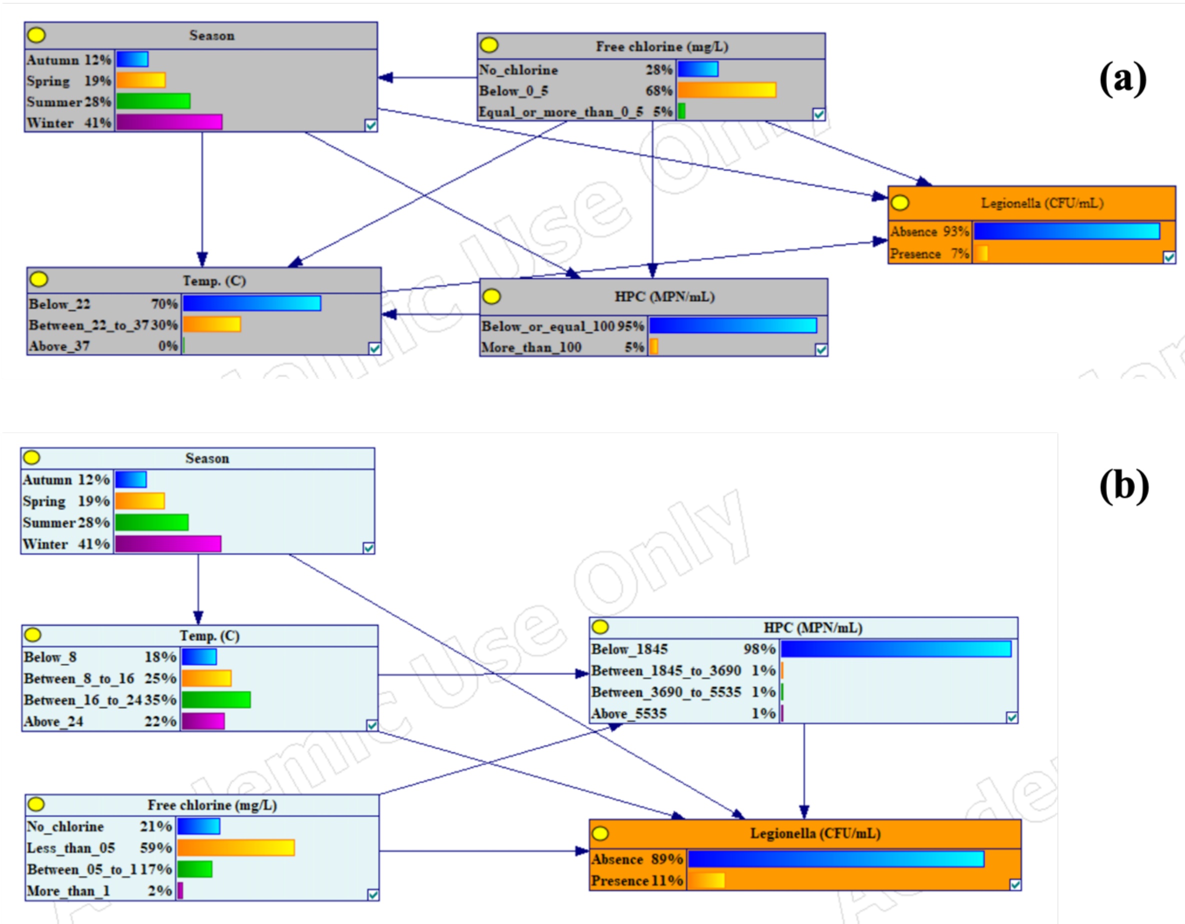

Figure 6.4: Output BN for the third tiered expert defined interdependencies of the nodes in supported by GTT with input variables discretised using (a) expert discretisation (b) four bins

6.3.3 Evaluation of model performance

The performance measures for each of the six output models are summarised in

This trend of BN model from supervised discretisation overperforming the manual method in terms of predictive capabilities observed in this study is consistent with the literature (Beuzen et al., 2018a). The strength of the supervised method includes minimising loss during discretisation and discovering new threshold particularly in systems with limited expert knowledge such as microbial occurrence in engineered water environments.

Table 6.2. This trend of BN model from supervised discretisation overperforming the manual method in terms of predictive capabilities observed in this study is consistent with the literature (Beuzen *et al.*, 2018b). The strength of the supervised method includes minimising loss during discretisation and discovering new threshold particularly in systems with limited expert knowledge such as microbial occurrence in engineered water environments.

All the six models achieved reasonable performance (over 92% prediction accuracy). The four-bins discretisation resulted in a higher k value and sensitivity (true positive results) compared to the and expert led models in all the three iterations.

This trend of BN model from supervised discretisation overperforming the manual method in terms of predictive capabilities observed in this study is consistent with the literature (Beuzen et al., 2018a). The strength of the supervised method includes minimising loss during discretisation and discovering new threshold particularly in systems with limited expert knowledge such as microbial occurrence in engineered water environments.

Table 6.2: Comparison of performance of the different models in predicting Legionella occurrence (≥ 10 CFU/ mL). Highest performance for each dataset is indicated in bold.

	First tiered		**Second tiered**		**Third tiered**	
	Expert bin	**Four bins**	**Expert bin**	**Four bins**	**Expert bins**	**Four bins**
Accuracy (%)	93.3	93.7	93.5	93.2	93.7	92.7
Sensitivity (%)	1.0	4.5	2.3	22.7	4.5	42.0
Specificity (%)	99.7	99.8	99.8	96.7	99.8	97.6
Cohen kappa (k)	0.34	0.37	0.35	0.44	0.1	0.3
ROC	0.76	0.89	0.75	0.89	0.78	0.89

Defining the appropriate bin size for discrete variables has been a challenge in the attempt for BN modelling of microbial pathogens from water systems. In the modelling of *Legionella* and water characteristics from the evaporative systems, all variables were binned into two classes to allow for an easier modelling process (Armero *et al*., 2011).

In a more recent study, Panidhapu *et al*., (2020) found varying the bin size of water quality variable to impact on BN model prediction of faecal coliform and *E. coli* in the water systems (Panidhapu *et al.,* 2020a). In the study, four bin outperformed two bin discretisation for modelling of indicator microorganisms from water characteristics.

The ROC values were between 0.7 – 0.8, a threshold considered to represent a reasonable capacity of the model to distinguish a positive and negative *Legionella* occurrence. Overall, the model defined using the four-bin sizing achieved relatively improved performance with better k and ROC values.

The third iterations of the expected supported interdependence of the variables (*Figure 6.5*) showed a superior performance compared to the first and second.

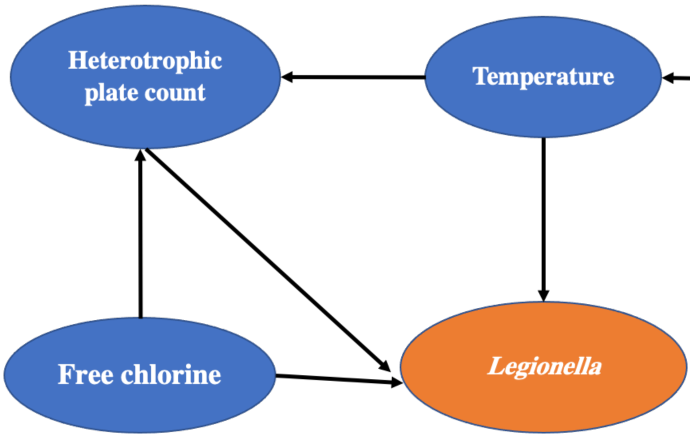

Figure 6.5: Expected interdependence between the different variables established through expert knowledge for the third conceptual iterations

In the third iteration, the four-bin discretisation of the variable presented improved performance. Of the all the six models, the third iterations with expert defined constraint and supported by the four bins discretisation is considered the optimal with consistently performance indicator metrics and valid interdependence. Therefore, the four-bin discretised model was selected for validation testing with GWTP aerator data.

6.3.4 Model testing with GWTP aerator data

The historical water quality datasets from the GWTP aerators were sorted and tested using the optimised model. Only 10 datasets from the influent records containing *Legionella* measurement alongside at least three of variables represented in the BN model. A comparison of performance results from the calibration and validation of selected four bin model is presented in

Table 6.3. The prediction using the GWTP aerator data showed a consistent decrease in performance across all metrics. The k value reduced by 60% to 0.1. This indicated a weak agreement between the BN model prediction and the observed *Legionella* occurrence in the GWTP aerator data.

Table 6.3: Performance accuracy of the calibrated model compared to the aerator tested data

Model	Cohen's kappa (k)	Accuracy (%)	Sensitivity (%)	Specificity (%)
Calibrated	0.25	92.7	22.7	97
Test with aerator data	0.1	78	8	78

The low performance in the tested dataset compared to the calibrated model's can be attributed to the small size and incomplete dataset. BN modelling of faecal coliform observed a decreased performance in the BN prediction of *E.coli* in water systems using incomplete datasets (Panidhapu *et al*., 2020). While BN have the advantage of making prediction from incomplete datasets, the accuracy seems to be limited.

The k value was observed to decrease significantly between the model calibrated using cross validation and the tested data. It is possible that with improved data availability and increasing the size of the training dataset, the model parameters will be better tuned to make more meaningful predictions with incomplete data. Further consideration should consider data from both aerator and distribution systems collectively and split for the model calibration and testing to minimise asymmetricity.

6.4 Conclusion

An attempt to conceptually develop and evaluate BN model using measured microbial and physiochemical water quality parameters from a large dataset was completed. Although the data was not measured aerator systems, the relevance of the correlations revealed from this dataset was explained in the context of aerator processes. The following conclusions can be drawn from the study:

- Regression analysis identified the complex relationships between *Legionella* occurrence and water quality characteristics.
- Useful predictors for *Legionella* occurrence in water systems included season, temperature, chlorine, and HPC.
- Integrating data driven and expert knowledge into BN learning improved the predictive performance for *Legionella* occurrence. This result highlighted the need to better understand the relationship between the *Legionella* and water characteristics.
- When fully established, BN modelling of *Legionella* using water quality measures could reduce the need for frequent *Legionella* enumeration measurement, by providing alternative prediction of their occurrence.
- BNs showed promise to assist in assessing risk of *Legionella* through prediction of occurrence of the bacteria in drinking water aerator assets in an interpretable and flexible model.
- Purposeful gathering of water quality data taking into consideration parameters correlated to the *Legionella* growth in aerators systems will allow for the development of robust and reliable BN models in the future.

CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

7 CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

With increasing incidences of legionellosis worldwide and the discovery of new sources of transmission, the importance of documenting all possible reservoirs for *Legionella* becomes critical. The research aimed to investigate factors that create a favourable environment and support the growth and subsequent transmission of *Legionella* pathogen in GWTPs aerators. The literature review (Chapter 2) revealed that *Legionella* commonly occurs in groundwater and can be harboured in drinking water treatment process including aeration system where they can grow to critical density (>1000CFU/mL), posing exposure risk and requiring introduction of preventive measures like additional monitoring and decontamination.

Critical density of *Legionella* in aerator systems can pose health risk via inhalation of contaminated aerosols. Currently, there is no industry standard for managing *Legionella* in aerators used in drinking water treatment plants. In this research, three key research directions (*Figure 7.1*) were proposed to improve our current understanding of *Legionella*-associated health risk and to enable the development of appropriate management plans. The conclusions of each study are described below.

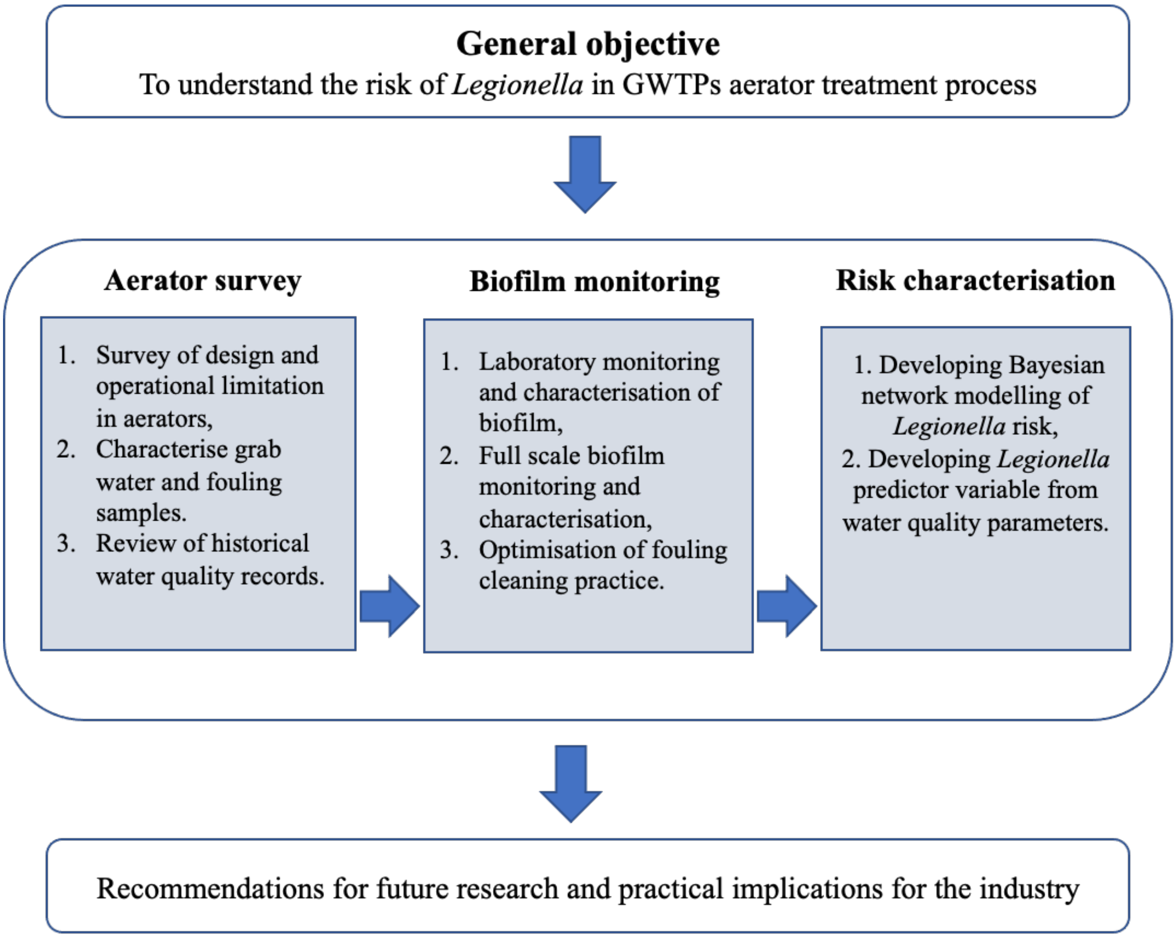

Figure 7.1: Completed work packages to achieve the project objectives. Groundwater aerators were surveyed to confirmed Legionella risk factors, temporal changes in biofilm were monitored to develop management options and growth and transmission risk were characterised using advanced risk frameworks.

7.1.1 Preliminary assessment of *Legionella* risk in aerators

The survey of 13 GWTPs aerators, including tray, open and semi-enclosed configurations, revealed design and operational constraints in current assets to favour elevated levels of nutrients, periodic water stagnation, challenging water quality, aerosolisation, and inconsistent operation and maintenance. These observations are important for outlining design considerations for the next generation of safer aerators that can overcome identified *Legionella* risks factors (further discussed in section 7.2 on practical implications for the water industry).

Aerator systems can harbour *Legionella* considering the 8%, 15% and 28% detection frequency enumerated from the influent, effluent and biofilms from historical sampling. The dispersion of aerosols from aerators further demonstrated that these assets are a potential source of legionellosis outbreaks.

Aerator fouling is a heterogenous mixture of organic and inorganic materials and can support elevated concentrations of nutrients, such as iron, that are favourable for *Legionella* growth. Characteristics of the influent to the aerators contained water quality parameters such as iron (0.06 µg/L), zinc (0.03 mg/L), manganese (0.02 mg/L), temperature (22 – 35^{0}C), hardness (180 mg/L), and alkalinity) in measures correlated to *Legionella* growth.

7.1.2 Fouling monitoring and characterisation to develop indicator parameters for biological growth on surfaces of aerators.

Temporal changes of the fouling characteristics on two different aerator surfaces showed steady behaviour after six weeks of operation with significantly higher concentration (277 ng ATP/cm^2) on the sprayed (vertically placed) coupon compared to the submerged (horizontally laid) coupons (73 ng ATP/cm^2). Concentrations of dissolved organic carbon (DOC) were statistically similar for the two tested conditions, measuring (6 - 39 µg/cm^2) and (4 - 39 µg/cm^2) on the sprayed and submerged coupons respectively, as the fouling reached steady state. Comparing fouling characteristics from the lab and full-scale coupons confirmed the significant impact of properties of wettability, surface orientation and influent on biofilm formation.

The inorganic elements measured on the coupons did not correlate strongly with microbial activity in the fouling samples. The correlation between DOC and biological activity was weak, while the biopolymer showed the strongest positive correlation with ATP density. This suggests that organic matter, and biopolymer in particular, can be considered as additional indicators for monitoring microbial activity in water and biofilm. Fouling cleaning of aged coupons with NaOCl (at concentrations ≥ 6%) for 30 min achieved 99.9% efficiency in biofilm inactivation (measured as ATP). In aerators, ATP levels can be considered a reliable indicator of cleaning and pathogen inhibition efficiency. Oxalic acid (at concentration >1%) significantly removed inorganic materials like iron and manganese. This indicated that biocides combined with antiscalants are important cleaning agents to address biofouling challenges in aerators.

7.1.3 Developing Bayesian networks for *Legionella* in aerators

In absence of relevant industry standards, WCWA proposed a semi-qualitative assessment framework that integrates five risk areas (poor water quality, nutrient availability and growth, water stagnation, system deficiencies and location and access) associated with *Legionella* to define thresholds that trigger management interventions.

A multi-tiered modelling approach (including conceptual fishbone diagram and bowtie analysis) coupled with iterative BN models addressed the limitations of the WCWA semi-qualitative framework and created a quantitative practical risk assessment tool. The initial output BN model elicited deterministically using expert weighted scoring process and discretisation approach defined relative contributions of variables and efficiently categorised *Legionella* risk thresholds. The baseline *Legionella* exposure risk from aerators assets was estimated to be low.

The revised BN model conceptually mapped and estimated the causes and consequences of *Legionella* aerosolisation separately. The sensitivity analysis of the growth model revealed water quality as the most impactful variable for *Legionella* occurrence. However, the *Legionella* growth sub-model showed weak prediction accuracy with a negative kappa coefficient, signifying inconsistency in predicted and observed *Legionella* occurrence.

Although further validation is required, the multi-tiered approach to *Legionella* risk characterisation provided a useful path to a quantitative risk assessment technique and can use to determine trigger points for appropriate management interventions.

7.1.4 Integration of data-driven learning and expert knowledge to improve Bayesian networks model prediction of *Legionella* occurrence

A hybrid of data-driven and expert knowledge was integrated to better characterise and model the interdependence between factors and further streamlined the risk of *Legionella* occurrence. The combination of historical water quality data and a knowledge-based modelling approach showed promise towards providing an additional tool for predicting *Legionella* in water systems. Data-driven learning using diverse historical water quality records was explored to strengthen the predictive power of *Legionella* occurrence models. The optimised BN model utilised GTT approach, complemented with domain knowledge achieved superior performance accuracy exceeding 90%. The kappa coefficient was 0.3, sensitivity (i.e., true positive rate) was greater than 40%, and specificity (true negative rate) above 95%.

The results indicated that season and commonly monitored water quality parameters of temperature, free chlorine, and heterotrophic plate count can be utilised for binary tracking of *Legionella* in water systems. Long-term data analysis through BNs can assist in establishing indicators in water variables for *Legionella* monitoring. The use of BNs in risk modelling can significantly improve assessment of *Legionella* occurrence and exposure for more informed management decisions.

7.2 Opportunity for future studies

From the conclusions and outputs of this work, recommendations for future work are presented below.

7.2.1 Establish the source of *Legionella* in aerators

In comparison to its concentration in groundwater, *Legionella* occurrence was more frequent and significant in the bulk water and biofilm within the aerators. *Legionella* bacteria are expected to be ubiquitous in natural soil and water environment (in both soil and water) (Schalk *et al.*, 2014). At present, it is unclear if the pathogen infiltrates aeration systems through groundwater and/or includes cross-contamination from *Legionella* growing in the surrounding soil. Therefore, a radial assessment of the distribution of *Legionella* in the surrounding environment (soil sample) and groundwater will determine the true source of the pathogen in the aerator environment. This is an important initial step to design appropriate risk mitigation strategies.

7.2.2 Improve method for sampling biofilm from aerator surfaces

Legionella bacteria are strongly associated with biofilm formation. In this study, biofilm formed on coupon were recovered using scrapping and sonication methods (Chapter 4). Scrapping like other biofilm recovering methods (including swabbing) has had limited value in decision making for microbial control in drinking water systems due to high user variability, low recovery, and poor reproducibility (Branck *et al*., 2018).

New methods for sampling biofilm are needed to improve the accuracy of biofilm enumeration in aerators systems and the wider drinking water treatment process. One of such methods with promise for improved reliability for biofilm study is the quartz crystal microbalance (QCM) (Ripa *et al*., 2020). QCMs allow for the non-destructive measurement of biofilm accumulation as a function of time and can measure very minute mass accumulations and changes to biofilm attachment on surfaces.

7.2.3 Extend *Legionella* monitoring to include in Biofilms

Current local standards (AS/NZS 3666) define critical *Legionella* concentrations that can be used as triggers for operator's intervention for maintenance or cleaning. It is important to note that only water samples are considered in current standards, ignoring the presence and characteristics of biofilm.

Preliminary modelling of *Legionella* from shower systems provided an estimate of the critical density necessary in the biofilm to cause infections (Schoen and Ashbolt, 2011). Given the ubitiquous nature of biofilm on the surfaces of aerators, research is needed to establish critical biofilm density that can be used as a trigger threshold for corrective actions in managing standards and guidelines for drinking water systems. Such guidelines can be extended to air and water handling systems susceptible to *Legionella* health risk.

7.2.4 Determine aerosols characteristics and mechanism of *Legionella* survival

The characteristics and mechanism of *Legionella* survival within aerosol dispersed from aerator treatment is currently not known. Research into characterisation and quantification of aerosols has been limited due to unavailability of enumeration techniques. Recently, Pepper and Gerba (2018), modelled aerosol characteristics and *Legionella* infection risk from sprinkler systems from irrigation practice using reclaimed wastewater. They concluded that infection risk is highly dependent on the duration of exposure, sizes of droplets and concentration of *Legionella* in water sample (Pepper and Gerba, 2018). There is the opportunity to apply numerical modelling to characterise the sizes of water dispersed from the aerators into droplets and aerosol components (Wolf *et al,* 2013).

Building on the aerosol characterisation, there will be the opportunity to assess quantitative microbial risk assessment of *Legionella* from aerators using similar approach previously implemented (Pepper and Gerba, 2018). The estimated risk of infection from GWTPs aerator treatment processes can then be expressed as infection probability targets. This will allow from modelling and planning for different event scenarios and designing appropriate risk assessment process (Signor and Ashbolt, 2009).

7.2.5 Increase the accuracy and sensitivity of risk characterisation model through improved definition of input variables

7.2.5.1 Structure elicitation to improve qualitative relationship

The limited interdependence between variables (mostly correlational), and deterministic conditional probabilities that were unable to capture uncertainties were identified as limitations of the BN risk models. The corresponding strength of the relationship between variables and *Legionella* have been estimated using expert judgement derived in an informal and largely untested protocols (Chapter 5).

It is proposed that structured expert judgment such as investigate, discuss' estimate, aggregate (IDEA) method can systematically gather qualitative and objectively weighted opinion and discretise the risk variables (Hemming *et al*., 2018a). The IDEA protocol has successful improved quantitative judgement of experts in eliciting the biotic and abiotic factors on the Great Barrier Reef in Australia (Hemming *et al.,* 2018b).

There is the opportunity to explore structured expert elicitation (using the IDEA) to strengthen the relationship between variables that are non-observable and quantifiable to better estimate the risk of *Legionella*. It is generally agreed that an elicitation protocol for quantifying uncertainty can benefit from the involvement of more than one domain expert (Hemming *et al*., 2018b). As such, individuals with expertise in *Legionella* and associated risk can be assembled to provide evidence-based elicitation of the strength of the different variables. This will allow for a fully transparent list of justifications that will be used in the production of the risk ranking tool.

7.2.5.2 Bayesian networks for data mining of Legionella interaction with risk variables

Data mining has the capability to improve the accuracy of relationship between observable and quantifiable *Legionella* risk parameters. In drinking water treatment plants, large amount of data is generated and archived every day, as part of the water safety plans, but sometimes without providing any additional value. BNs are practical tools for discovering and characterising associations between variables in a system or process. Dedicated algorithms have been developed to deal with structure and parameter learnings. In this thesis, structure learning algorithms were not fully explored, except from chapter 6 in which the GTT structure learning algorithms were tested. However, there are greater opportunities to explore all the features (structure learning and parameter discretisation) of BNs to improve our understanding of *Legionella* interaction with the different risk factors. These models could be used to improve risk management practices which would be based on data-driven approaches.

7.2.6 Opportunities and challenges for BN modelling of *Legionella* occurrence using monitoring parameters

The ability of BN to model the presence and absence of *Legionella* using monitored water quality and environmental conditions was explored as a measure for improved risk characterisation. BNs have special characteristics that make them unique. These features include inferential reasoning through the Bayesian rule, explicit representation of uncertainties, and capability to handle missing values. BNs on their own were found to be somewhat limited for the full range of applications considered. The promise from this application and the lessons learnt for the study are discussed.

This study optimised a BN model that defines the interactions of *Legionella* occurrence and water quality parameters using datasets created in a different context than studied in this research. The assessment of critical parameters and model performance were steps forward in the attempt at modelling *Legionella* occurrence and water characteristics. The first study that model water quality and *Legionella* from condenser systems made no attempt at evaluating the model performance (Armero *et al.*, 2011). However, the limitation from this study is the absence of records of the shortlisted variables used in the development of the models from aerators for further testing of the optimised model performance. Given the increasing number of engineered water systems confirmed as source of *Legionella* growth, a flexible and reliable model should consider integration of datasets from different systems for the appropriate learning, training, and validation protocols.

The ability of BN modelling to support algorithm led learning and expert knowledge integration helped to better model the limited understanding of *Legionella* in complex water systems and remains an advantage for microbial risk modelling (Panidhapu *et al.*, 2020a). The BN model created in this chapter through data-driven and domain knowledge integration significantly improved model performance in positive and negative *Legionella* prediction as demonstrated with a positive Cohen value against the negative value observed in the beta model previously modelled in *section 5.3.2.* The attempt at integrating expert knowledge and algorithm driven learning of water characteristics in exploring the probabilistic and graphical modelling capability of BNs to predict *Legionella* is showing some promise for further exploration to develop a valuable risk managing tool.

It is important to note that although BNs can deal with missing values, the data still needs to be of good quality. This characteristic conversely implies that large data gaps would probably have an influence on the model outcomes or generate misleading results. Although techniques exist for managing missing data, the process can affect the model outcomes. Water treatment systems represent a typical example of an observational study where factors such as water quality and environmental conditions are random and can contain missing data. To decrease uncertainty in the model, it is preferable when studying potential predictors for microbial occurrence to minimise the occurrence of missing values.

7.3 Practical implications for the water industry

The improved understanding of the presence and growth of *Legionella* within aerators systems guided the recommendation of practical industry applications. These implications include improved aerator design, targeted microbial and water quality monitoring and potentially overlooked assets that need to be better investigated for health risks associated with *Legionella.*

7.3.1 Improved aerator design

The next generation of safer aerators that made engineering provisions to overcome the limitations identified in the current assets as detailed in *Table 3.6* (Chapter 3) will allow for safer operation and optimised maintenance.

A new tray aerator design was proposed with adequate features for safer operation. A complete drawing (plans and sections view) for new design is provided in *appendix 5.* The new features include the following (i) sampling points located on inlet, outlet, and on each aerator tray (ii) isolation and drainage by-pass lines on aeration asset (iii) sloping aerator base for water drainage (iv) chamfered circular trays built out of fibreglass reinforced PVDF material (v) PVDF lining added to trays (vi) introduction of mist eliminators panels.

The new design has been adopted by the industry and recently implemented in Western Australia. An example of such an appropriate design for tray aerators has been piloted by WCWA (*Figure 7.2*).

Figure 7.2: Improved aerator design recently tested at GWTPS in Western Australia

The improved aerator was built to minimise the risk of *Legionella*. The design incorporates biofilm and corrosions resistant materials, aerosol limiting barriers, sloping aerator base, water quality and microbial sampling points (inlet and outlet), and adequate access for inspection and cleaning.

7.3.2 Targeted microbial and water quality monitoring to improved risk assessment

The current practice of monitoring water quality and microbial parameters independently presents a challenge in integrating the datasets for data mining and risk assessment. *Legionella* and water quality monitoring in aerators mostly has different sampling dates, frequencies, and locations.

Over the years, *Legionella* and water quality monitoring has been completed in GWTPs aerators plants for different purposes. Water quality monitoring is performed periodically (daily and weekly) to determine the efficacy of treatment processes. *Legionella* records are collected quarterly to assess the potential occurrence of opportunistic pathogens. The inconsistency in sampling date, data collection points and frequency between the historical *Legionella* and water quality records makes it difficult to implement a data-driven machine learning model (*Chapter 6*). Therefore, integrating the cycle and location of microbial and water quality parameters monitoring including *Legionella* will support the collection of consistent and complete datasets that can be used for data mining and the development of improved risk assessment tool.

7.3.3 ATP as promising indicator for assessing efficacy of cleaning practice

The synergistic use of chemical treatment (NaOCl and oxalic acid) and mechanical/physical action was observed to restore laboratory fouled coupons to a nearly pristine condition. The measure of ATP and inorganics reductions provided reasonable insight into the actual efficiency of the chemical cleaning agents in biomass removal (*Chapter 4).*

ATP testing is a proven technique in the food and biomedical communities for monitoring microbial contamination. The technique is very reliable and can be performed quickly on suspended or attached cells. As such, ATP measurements can be considered a reliable indicator of cleaning and pathogen inhibition efficiency in aerator fouling as a strategy to managing *Legionella* risk.

7.3.4 Extending learning and risk assessment approach from aerator to other systems

The experimental and numerical approach from this study has improved our current understanding and assessment of *Legionella* risks in water treatment aeration systems. The learnings and established risk assessment procedure can be extended to other often overlooked potential sources of *Legionella* growth and transmission. An example of such sources can include wastewater treatment facilities, sprinkler, and heating, ventilation, and air conditioning (HVAC).

Wastewater treatment plants (WWTPs) are an obvious source of *Legionella* outbreak, but have been, understudied. The process of risk characterisation defined for aerators can be extended to develop an assessment model which will identify WWTPs processes presenting high risk of *Legionella* growth and aerosols production capable of being inadvertently ingested by operators and site staff.

Similarly, HVAC systems can be a source of *Legionella* infections because they have abundant nutrient, elevated water temperature and can disseminate *Legionella*-contaminated aerosols (Prussin *et al*., 2017). There is an opportunity to extend the investigation of *Legionella* health risk to these systems. The understanding and most of the control strategies developed in this research can also be beneficial for these systems.

CHAPTER 8

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CHAPTER 9

9 APPENDICES

9.1 APPENDIX 1: CHAPTER 3

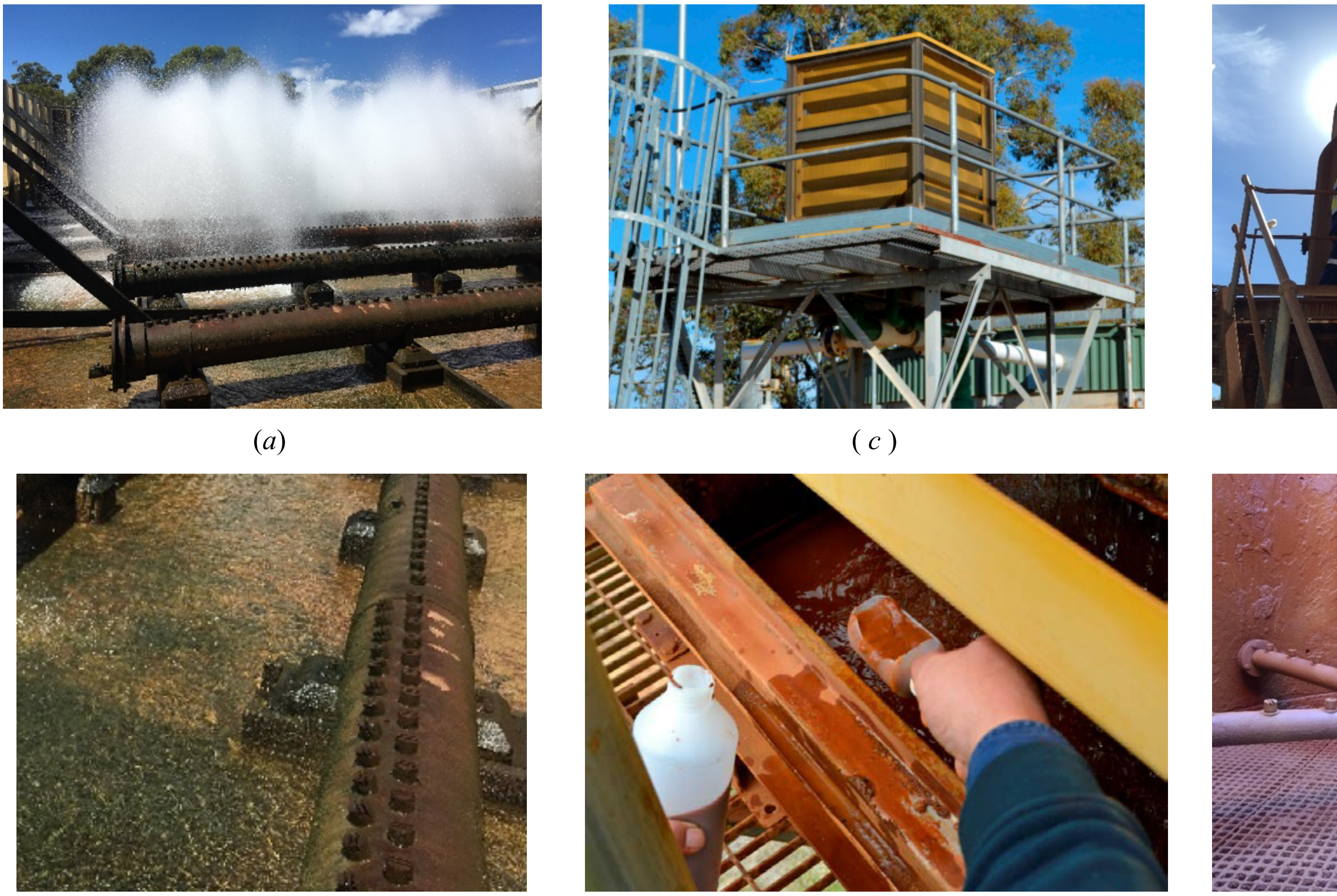

Figure A1.1: Observed changes within the aerators following prolonged operations (a & b) showing open spray aera
(e & f) semi-enclosed aeration system.

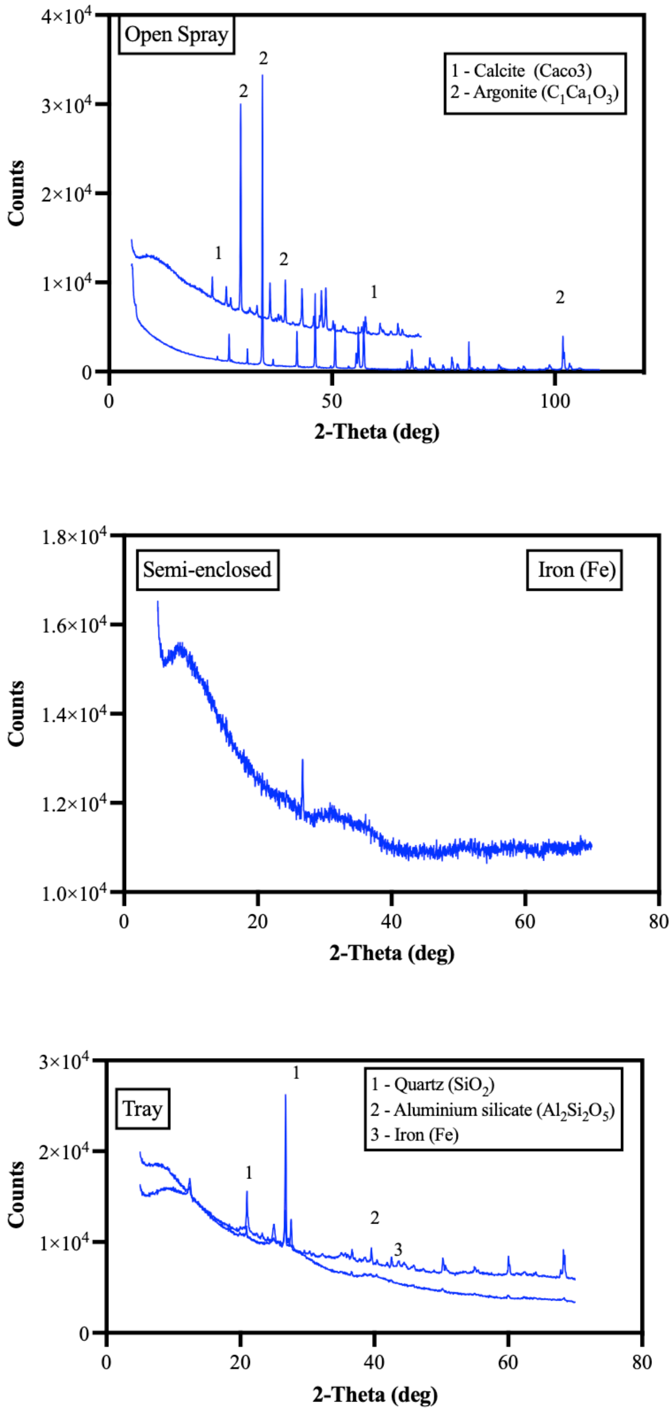

Figure A1.2: Primary crystalline phases from the sampled deposits.

Open Spray **Semi closed** **Tray system**

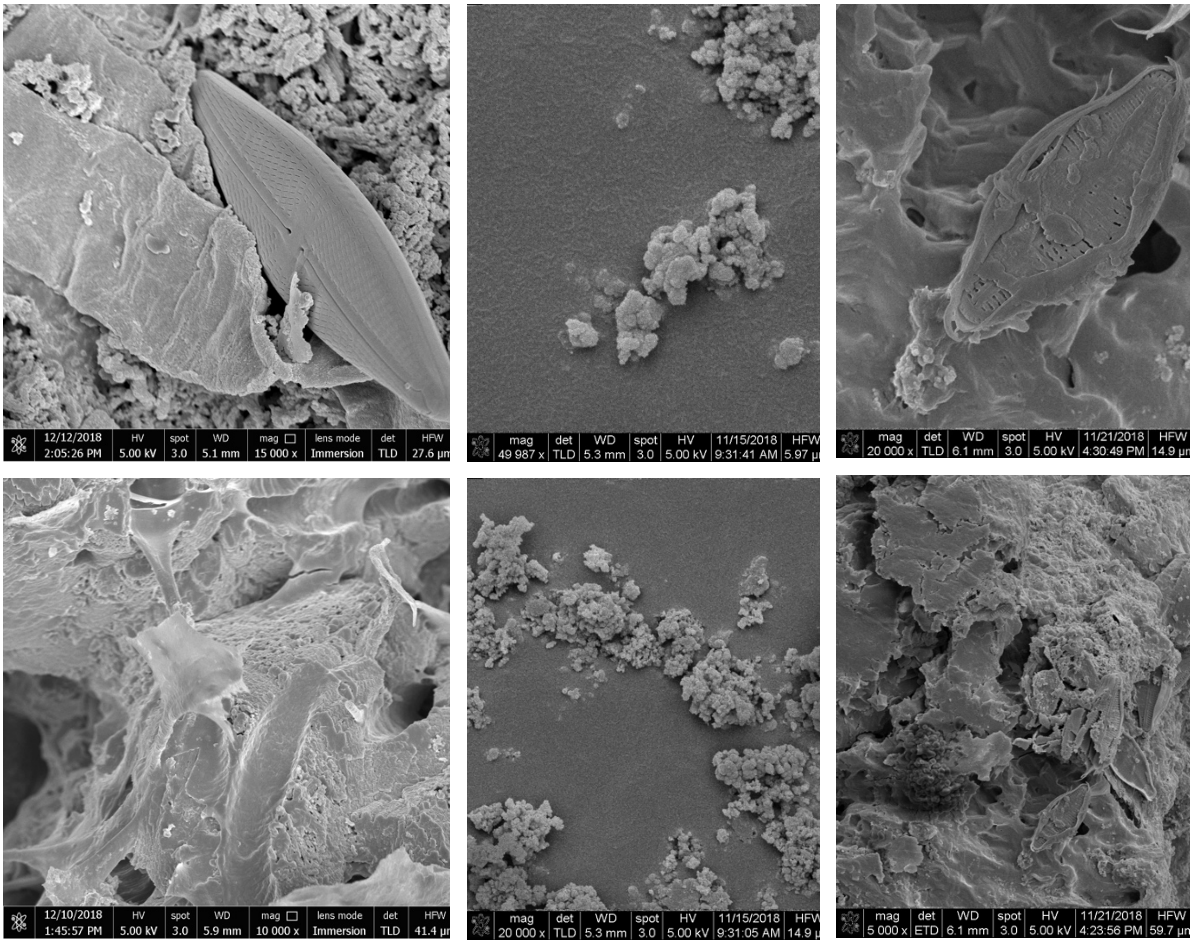

Figure A1.3: Images of suspended materials from the precipitates from the different aeration configurations

9.2 APPENDIX 2: CHAPTER 4

Supplementary figures for characterisation of samples from the laboratory and full-scale fouling monitoring studies.

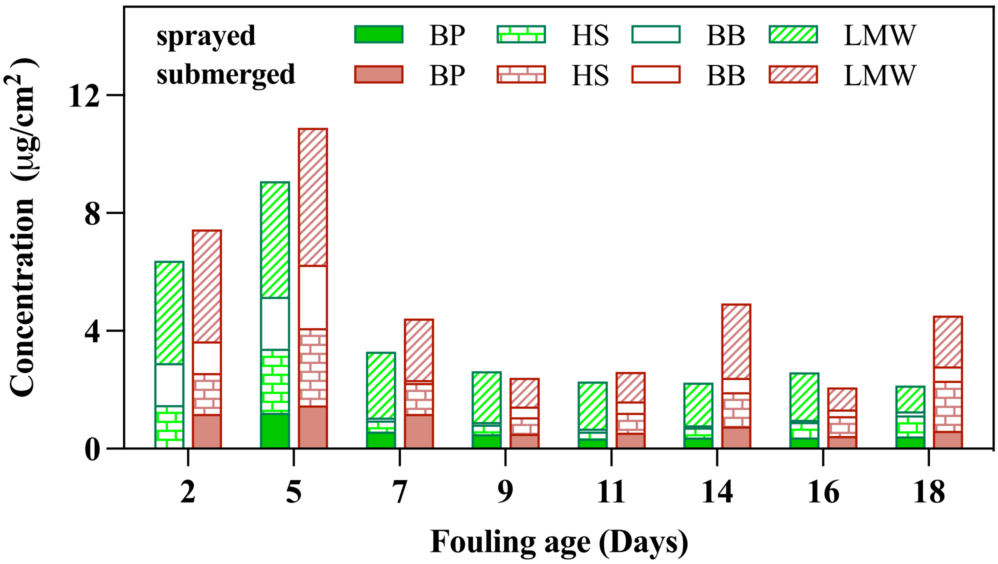

Figure A2.1: LC-OCD distribution of the fouling on the sprayed and submerged coupons in the laboratory modelled study.

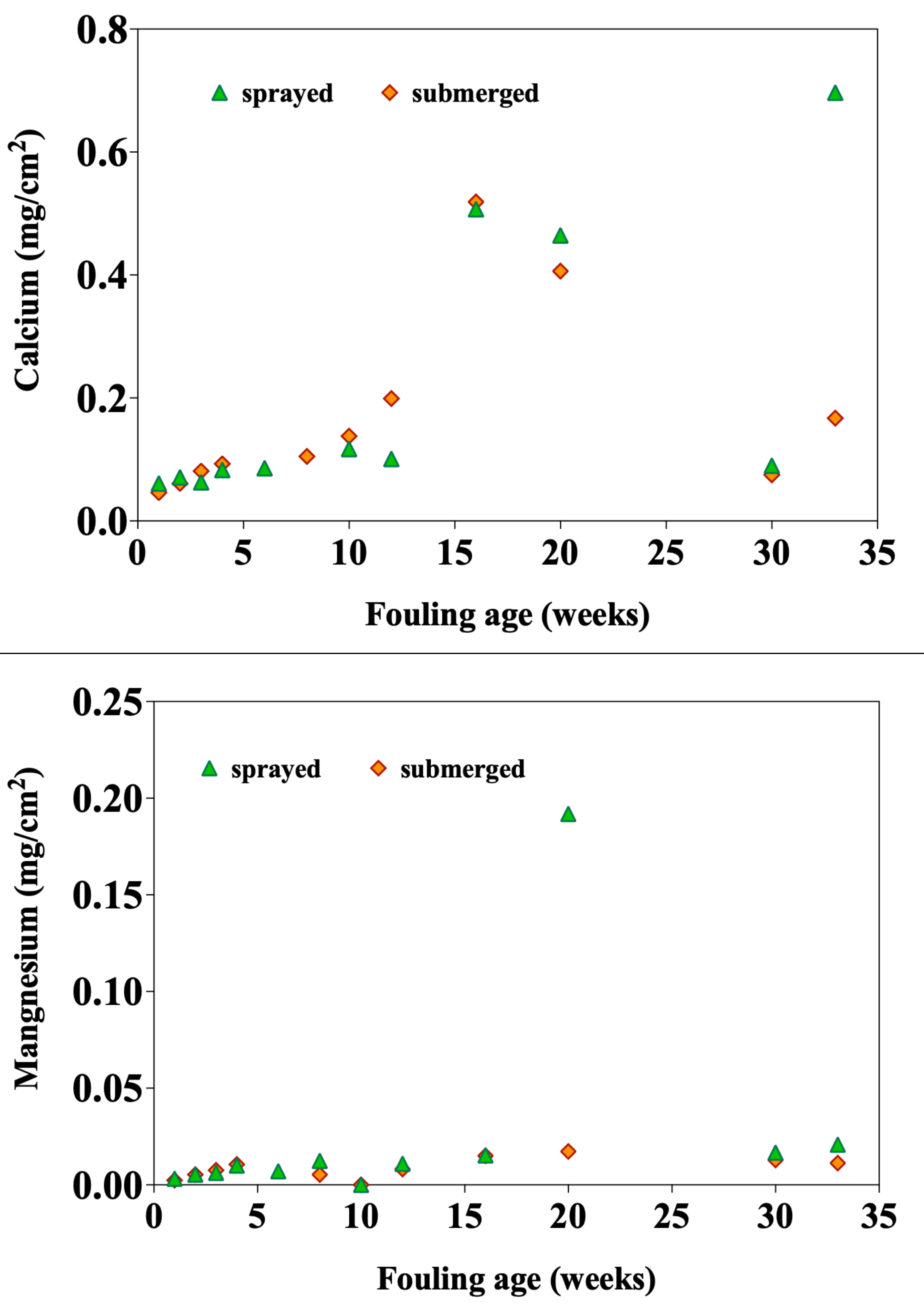

Figure A2.2: full scale coupon: Inorganic elements plotting against time for the fouling

A2.3: Algae growth on the sprinkler pipe and trickling down onto the sprayed vertically placed coupons

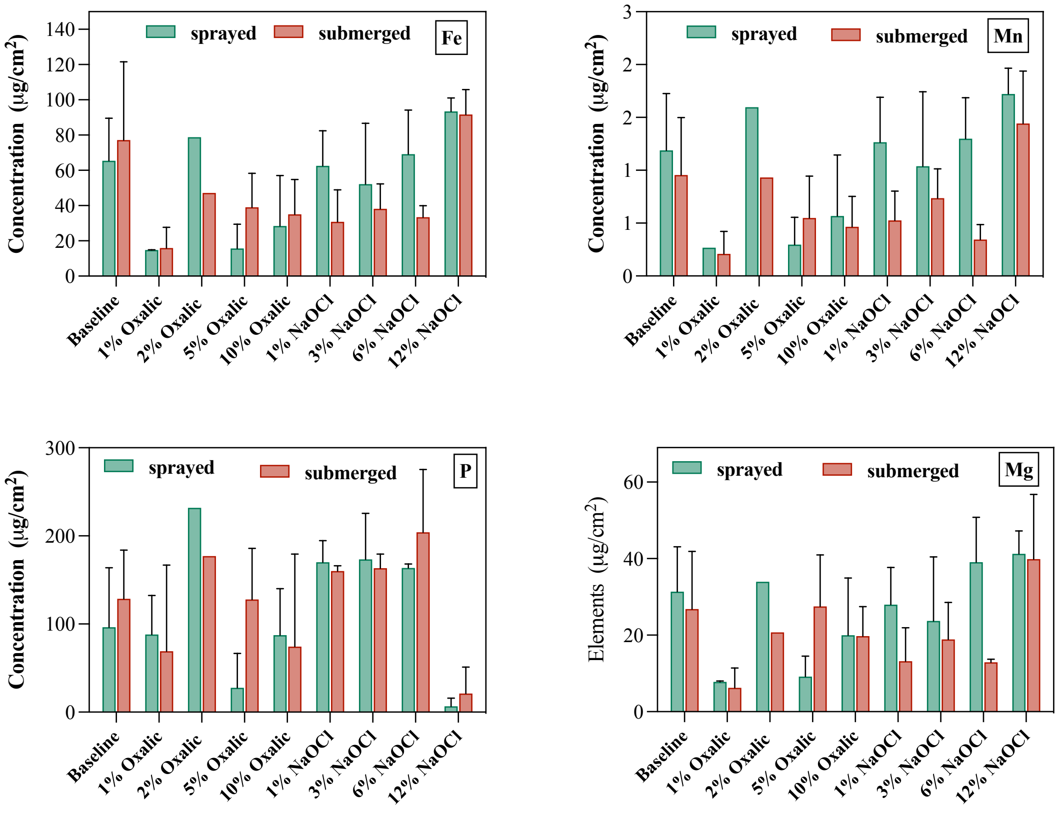

Figure A2.4: Chemical cleaners' reductions of the different of the different elements measured

Table A2.1 Elemental deposition on the sprayed and submerged coupons (The p-values are consistently above 0.1 in the steady growth phases).

Concentration ($\mu g/cm^2$)	**Sprayed**	**Submerged**	**p values**
Calcium	240 ± 210	190 ± 100	0.531
Iron	15 ± 10	10 ± 8	0.311
Magnesium	4.2 ± 2	4 ± 1	0.921
Manganese	18 ± 12	20 ± 8	0.581
Sodium	850 ± 440	690 ± 380	0.125
Phosphorus	130 ± 80	170 ± 80	0.201
Sulphur	10 ± 7	14 ± 13	0.985
Zinc	1.4 ± 2	1.2 ± 1.6	0.158

Table A2.2: Distributions of DOC and LC-OCD fractions for the sprayed and submerged coupons.

	Sprayed	**Submerged**
DOC including:		
Hydrophilic	91 %	95 %
Hydrophobic	9 %	5 %
LC- OCD fractions		
Biopolymer	30%	25%
Humic substances	23%	26%
Building blocks	5%	6%
Low molecular weight	42%	43%

Table A2.2 Correlations (r^2) between component scores and LC-OCD fraction concentrations of influents water samples

	Peak A	**Peak B**	**Peak C**	**Peak M**	**Peak T**
LC - COD					
Biopolymer	- 0.007	0.599	0.053	0.037	0.528
Humic substances	0.019	0.614	0.082	0.062	0.519
Building blocks	0.344	0.417	0.383	0.383	0.753
LMW	- 0.526	- 0.205	-0.576	-0.511	0.053

Table A2.3: Correlations (r^2) between component scores and LC-OCD fraction concentrations of effluents water samples

	Peak A	**Peak B**	**Peak C**	**Peak M**	**Peak T**
LC - COD					
Biopolymer	0.309	-0.185	0.287	0.338	0.351
Humic substances	0.569	-0.200	0.589	0.552	0.267
Building blocks	0.117	0.538	0.110	0.103	-0.017
LMW	0.567	-0.191	0.518	0.568	0.304

Table A2.4: Correlations (r^2) between component scores and LC-OCD fraction concentrations of sprayed coupon samples.

	Peak A	Peak B	Peak C	Peak M	Peak T
LC - COD					
Biopolymer	0.134	0.026	0.114	0.209	- 0.005
Humic substances	0.367	0.365	0.356	0.374	0.333
Building blocks	0.520	0.540	0.472	0.524	0.471
LMW	0.737	0.693	0.705	0.742	0.608

Table A2.5: Correlations (r^2) between component scores and LC-OCD fraction concentrations of sprayed coupon samples.

	Peak A	Peak B	Peak C	Peak M	Peak T
LC - COD					
Biopolymer	0.252	0.173	0.228	0.334	0.235
Humic substances	0.424	0.412	0.433	0.502	0.518
Building blocks	0.389	0.547	0.414	0.319	0.576
LMW	0.657	0.749	0.661	0.522	0.703

Table A2.6: p-values for the comparison of the concentration of the fractions at the different biofilm growth phases.

	Exponential growth stage	Stability growth stage
BP	0.1257	0.0673
HS	0.2445	0.0297
BB	0.9689	0.3744
LMWN	0.3915	0.7885
LMWA	0.7006	0.0451

9.3 APPENDIX 3: CHAPTER 5

Supplementary materials to the article on Developing Bayesian Networks in managing the risk of *Legionella* colonisation of groundwater aerators.

Journal: Water research

Title: Developing Bayesian Networks in managing the risk of Legionella colonisation of groundwater aerators.

Authors: Danladi Yunana, Stuart Maclaine, Keng Han Tng, Luke Zappia, Ian Bradley, David Roser, Greg Leslie, C Raina MacIntyre, Pierre Le-Clech

Number tables: 4

Number of figures: 3

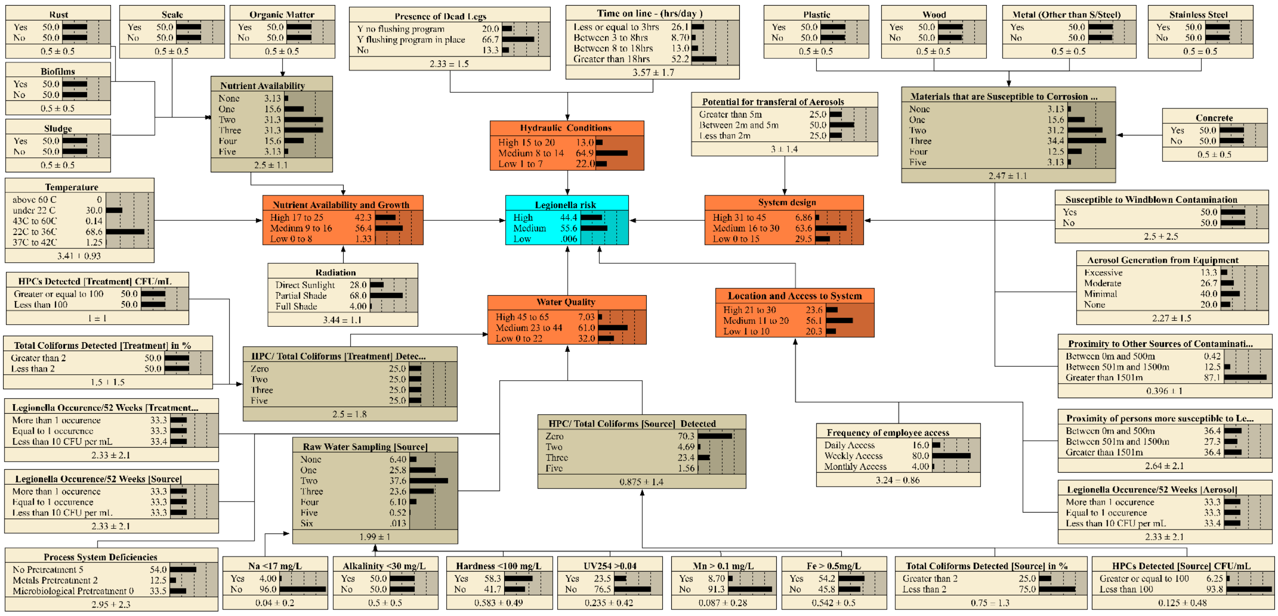

Figure A3.1: Initial output BN model in the belief bar style with the probability distributions on the variables

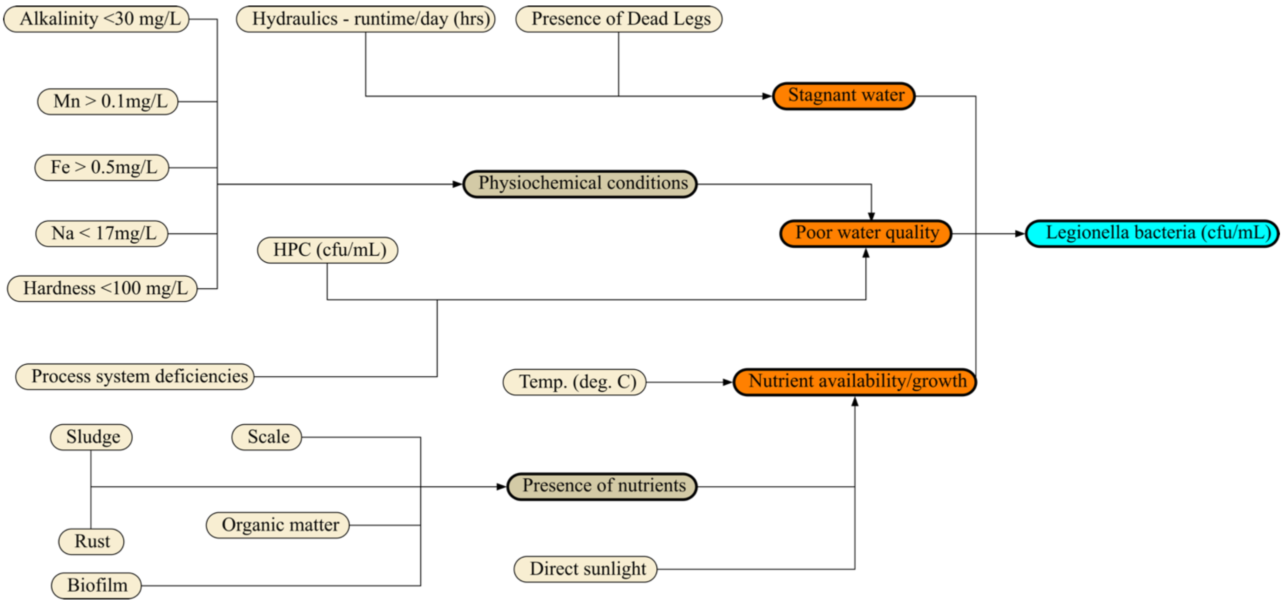

Figure A3.2: Legionella growth output sub model BN model in a labelled bar style

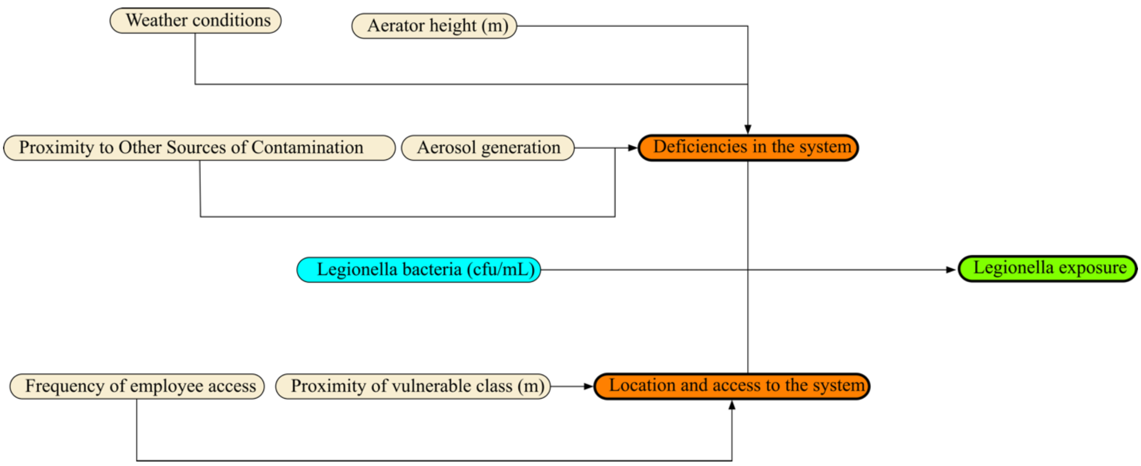

Figure A3.3: Legionella transmission sub model in a labelled bar style

Table A1.1: Characterisation of the states of the variables in the BN models

Latent variables	**Observed variables**	**Discretisation principle**	**States**	**References**
Nutrient availability and growth		Uniform distribution of the total weighted score from the risk factors in the domain.	Low Medium high	(Vital et al., 2010)
	Sources of nutrients	The presence of corrosion, biofilm, sludge, organic matter and rust can release iron product and serve as nutrient that will encourage *Legionella* growth.	Yes No	LHLRA
	Water temperature	The temperature of the water can affect nutrient growth. Warm temperature has been to *Legionella* proliferation	< 36^0C 36 – 42^0C >42^0C	(Wadowsky et al.,1985) (Proctor et al., 2017) LHLRA
	Presence of Nutrients	The presence of rust biofilm, scale, sludge, and organic matter. Expert elicitation guided by field observation definition.	Low High	Qiu et al., 2002
	Radiation	Field observation of exposure of the different aeration configuration with regards to sunlight.	Direct sunlight Partial shade Full shade	LHLRA
Water quality		Uniform distribution of the total weighted score from the risk factors in the domain.	Low Medium High	LHLRA
	Fe > 0.5mg/L	Level of iron deposition defined to promote biological activity.	Yes No	Ginige, Wylie, & Plumb, 2011 Tulkki et al., 2003
	Hardness <100mg/L	Increased hardness level in the water has been strongly correlated statistically with the presence of *Legionella*	Yes No	Lasheras et al., 2006
	Na <17mg/L	Increased Na concentration could impact on water quality leading to more biological activities.	Yes No	Ginige et. al; 2010
	Mn < 0.6mg/L	A high concentration of Mn correlated to increase biofilm activity.	Yes No	Bargellini et al., 2011
	HPC in system (source and treatment)	Increase HPC count indicates high propensity of microbial activity sometimes including *Legionella*	< 100 cfu/mL >100 cfu/mL	Höfle et al., 2015 Türetgen & Cotuk, 2007
	Legionella occurrence in the source and treated water	The values of results from the testing of *Legionella*.	< 10 cfu/mL >= 10 cfu/mL	LHLRA

Hydraulic conditions		Uniform distribution of the total weighted score from the risk factors in the domain	Low Medium High	LHLRA
	Presence of dead legs	The presence of regions with pool of water with little or no flow and the type of flow regime program in place.	Yes No	LHLRA
	Time online (hrs/day)	The period the system is operational and under constant flow	<=3 3 to 8 8 to 18 >18	LHLRA
	Process system deficiencies	The introduction of acid for pH adjustment and chlorine for has the potential to improve water quality.	No pre-treatment Pre-treatment (Metals) Pre-treatment (Microbiological	LHLRA
Location and access to the system	Location and access to the system	Uniform distribution of the total weighted score from the risk factors in the domain.	Low Medium High	LHLRA
	Frequency of access	The frequency of access to the aeration system for sampling and maintenance.	Daily Weekly Monthly	LHLRA
	The proximity of vulnerable persons	The proximity of children school, elderly home, and hospitals, etc	501 – 500m 501 – 1500m >1500m	LHLRA
System design		Uniform distribution of the total weighted score from the risk factors in the domain	Low Medium High	LHLRA
	Susceptible to wind	The presence of wind at the location of the aeration asset.	Yes No	LHLRA
	Aerator height leading to aerosols transferral potential	Field observations and measurements of existing assets.	<2m 2-5m >5m	LHLRA
	The proximity of other sources	Field observations and measurements of existing assets	0 – 500m 501 – 1500m >1500	LHLRA
	Aerosols generation	Visual observation of aerosols /mist from the aerator	Excessive Moderate Minimal None	LHLRA
Legionella exposure		Critical values defined through dose responses modelling.	Tolerable Low High	Pepper & Gerba, 2018

	Legionella bacteria	The occurrence of *Legionella* in the aeration systems sample	Detected Undetected	LHLRA

Table A2.2: Sensitivity analysis for the initial BN model with the Legionella risk as the target node.

Node	Mutual info	Percent	Variance of Beliefs
Legionella risk	0.991	100	0.247
System design	0.225	22.7	0.065
Location and access to system	0.166	16.7	0.051
Legionella occurrence in aerosol	0.097	9.8	0.032
Susceptible to windblown	0.073	7.41	0.025
Hydraulic conditions	0.069	6.96	0.022
Water Quality	0.062	6.21	0.020
Time inline	0.042	4.25	0.014
Aerosol generation	0.037	3.71	0.012
Proximity of susceptible person	0.026	2.59	0.009
Potential for transferal of aerosols	0.024	2.41	0.008
Presence of dead legs	0.020	2.04	0.007
Legionella occurrence (treatment)	0.016	1.63	0.005
Legionella occurrence (source)	0.014	1.44	0.005
Nutrient availability and growth	0.006	0.57	0.002
Materials that are susceptible to corrosion	0.005	0.461	0.001
HPC/Total coliform (Treatment)	0.004	0.436	0.001
Proximity to other sources of contamination	0.003	0.342	0.001
Total coliform detected	0.003	0.300	0.001
HPC/Total coliform (Source)	0.002	0.247	<0.001
Process system deficiencies	0.002	0.235	-
Total Coliform detected (source)	0.002	0.216	-
Frequency of employee access	0.002	0.206	-
Nutrient sources	0.002	0.202	-
Raw water sampling	0.001	0.148	-
HPCs detected [Treatment]	0.001	0.136	-
Temperature	0.001	0.118	-
Plastic	< 0.001	0.0907	-
Wood	-	0.0784	-
Metal [other than S/steel]	-	0.0784	-
Concrete	-	0.0784	-
Radiation	-	0.0735	-

Sludge	-	0.0367	-
Biofilms	-	0.0367	-
Rust	-	0.0367	-
Scale	-	0.0367	-
Organic matter	-	0.0367	-
Alkalinity < 30 mg/L	-	0.0353	-
Fe > 0.5 mg/L	-	0.0352	-
Hardness < 100 mg/L	-	0.0350	-
HPCs Detected [source]	-	0.0305	-
UV 254 > 0.004	-	0.0253	-
Mn > 0.1 mg/L	-	0.0108	-
Na < 17 mg/L	-	< 0.01	-

Table A3.3: Sensitivity analysis for the revised Legionella growth sub model with the Legionella bacteria as the target node.

Node	Mutual info	Percent	Variance of Beliefs
Legionella bacteria	0.74718	100	0.167632
Water quality	0.20498	27.4	0.0393
Nutrient availability and growth	0.12682	17	0.0307938
Hydraulic conditions	0.12560	16.8	0.0269
Temperature	0.10326	13.8	0.025598
Physiochemical conditions	0.06259	8.38	0.015073
Presence of dead leg	0.05330	7.13	0.0145474
Process system deficiencies	0.05243	7.02	0.0126174
Hydraulics -runtime	0.05542	7.42	0.0126174
HPC	0.01595	2.14	0.0044320
Hardness	0.01666	2.23	0.0039194
Alkalinity	0.01646	2.2	0.0038463
Fe > 0.5 mg/L	0.1622	2.17	0.0037719
Presence of nutrients	0.00868	1.16	0.0023059
Mn > 0.1mg/L	0.00385	0.516	0.0007963
Na	0.00183	0.24	0.0003728
Biofilm	0.00094	0.126	0.0002182
Sludge	0.00094	0.126	0.0002182

Organic matter	0.00094	0.126	0.0002182
Scale	0.00094	0.126	0.0002182

Table A3.4: Sensitivity analysis for the revised Legionella transmission sub model with the Legionella exposure as the target node.

Node	Mutual info	Percent	Variance of Beliefs
Legionella exposure	1.19815	100	0.2907778
Legionella bacteria	0.95755	79.9	0.1765138
Frequency of employee access	0.24060	20.1	0.0073347
Location and access to the system	0.24060	20.1	0.0073347
Proximity of vulnerable persons	0.000	0	0

9.4 APPENDIX 4: CHAPTER 6

The different iterations of conceptual arrangement of the variables in the GTT platform in GenIe

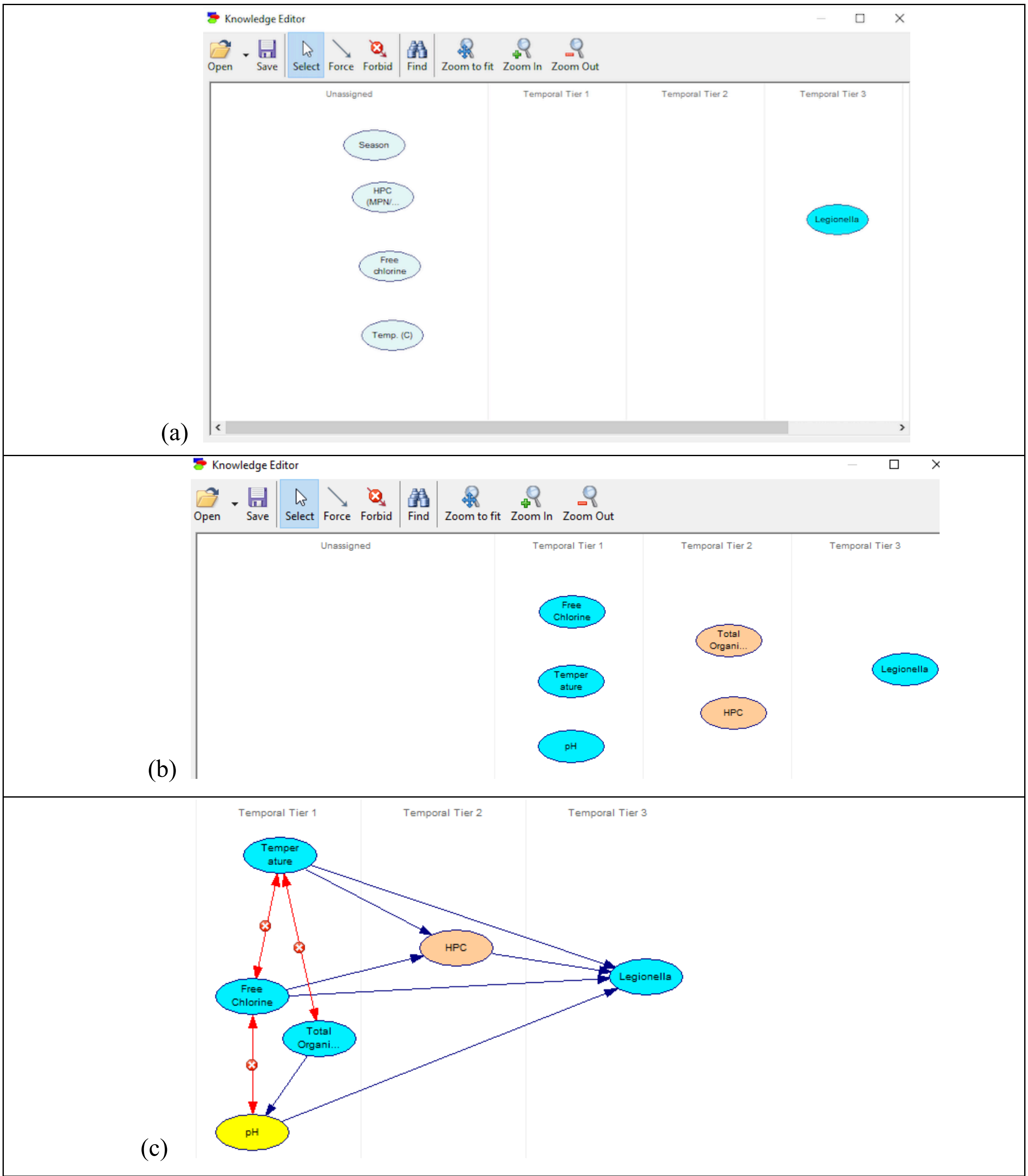

Figure A4.1: The background arrangement of the different variables in the GTT algorithm (a) 1^ST iteration (b) 2^nd iteration (c) 3^rd iteration

The different iterations of conceptual arrangement of the variables in the GTT platform in GenIe recreate for clarity.

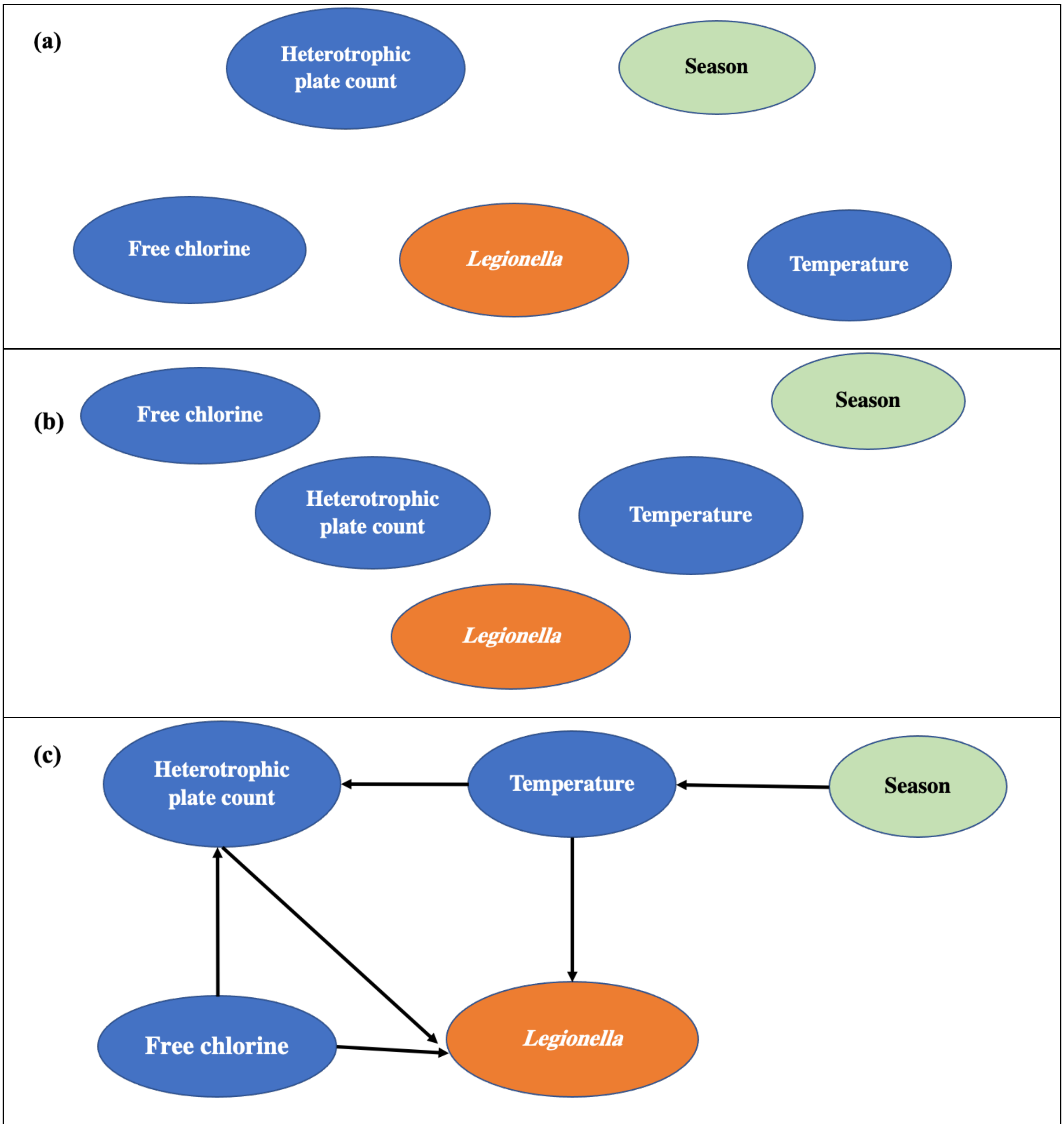

Figure A4. 2: Expected interdependence between the different variables established through expert knowledge. (a) first tiered (b) second tiered and (c) third tiered expert defined interdependencies between the variables

9.5 APPENDIX 5: CHAPTER 7

Detailed drawings for improved Aerator Design are provided below.

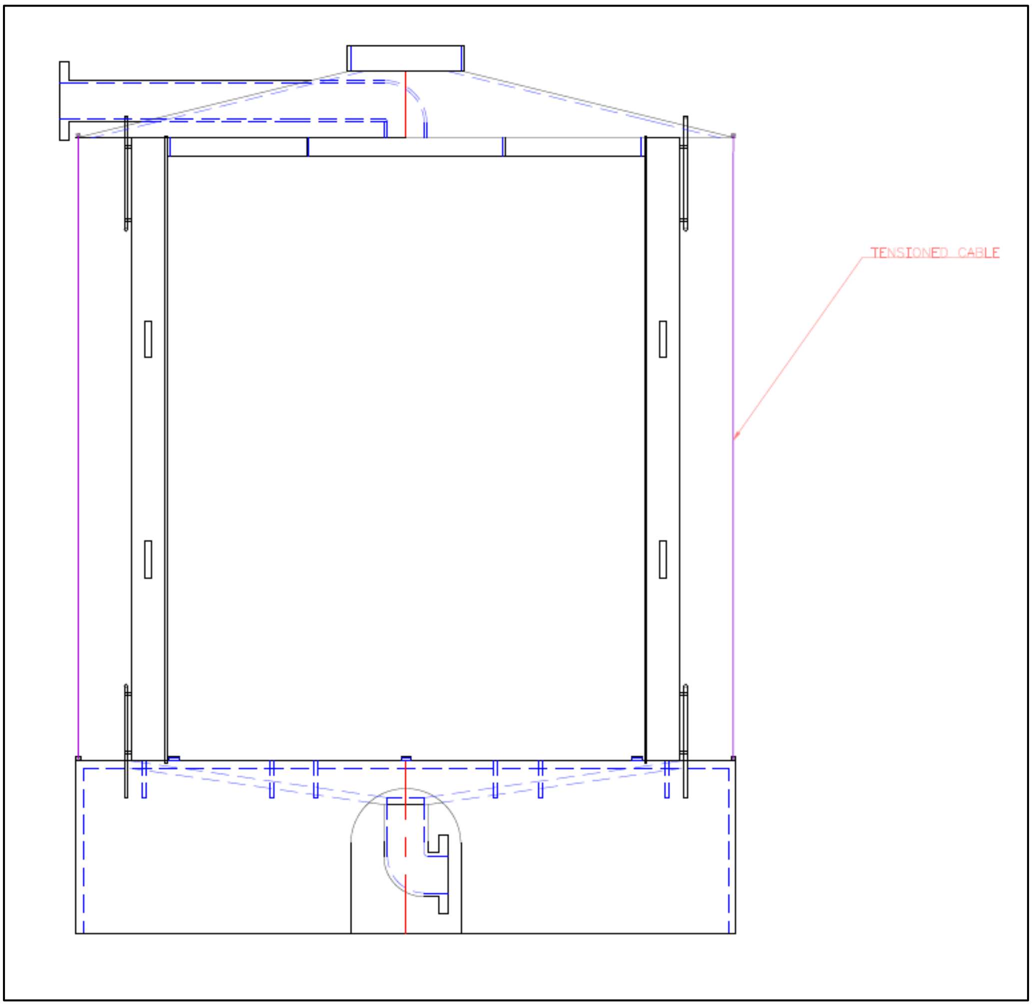

Figure A5.1: Side Profile of Pilot Aerator

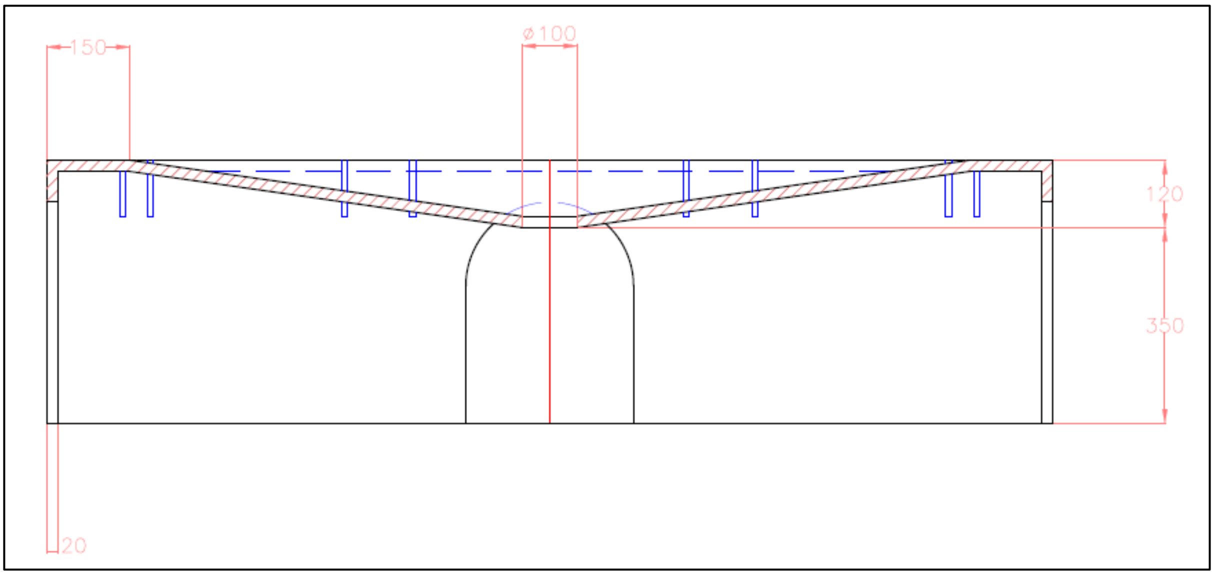

Figure A5.2: Cross-section View of Aerator Base

The diameter of the base was increased from 1500mm to 1800mm to ensure that the pilot aerator is structurally stable and provide for sufficient clearance to ensure that the panels would be resting on a flat surface for movement of panels (access to aerator's internal components). The aerator base's height was also adjusted from 300mm to 470mm to accommodate the 90-degree flanged elbow outlet pipe (*Figure A5.2).*

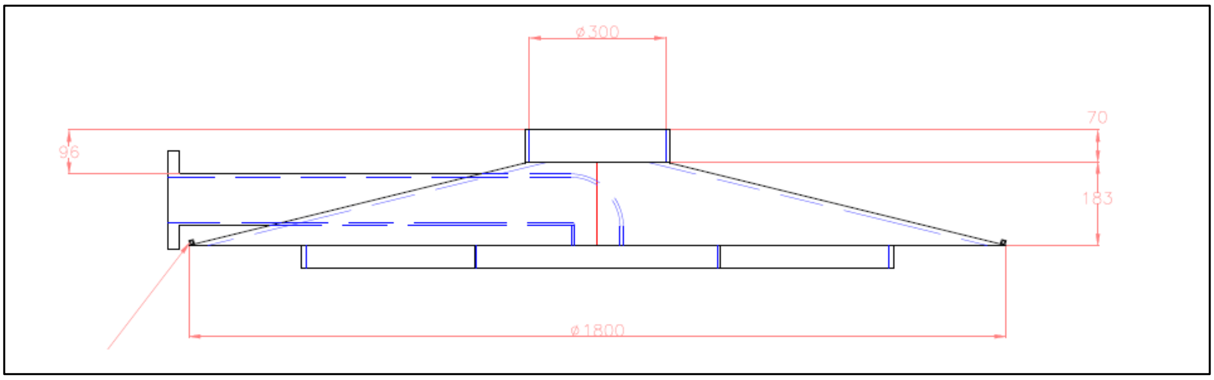

Figure A5.3: Pilot Aerator Top Cover

Although the roof of the aerator was originally designed to be flat to accommodate cut-outs for a fan blower and flanged inlet pipe, a curved top cover was decided upon for its better structural integrity whilst providing enough clearance for the 100mm flanged elbowed inlet pipe. A central 300mm (ID), 70mm high skirt at the top of the cover allows for a fan blower duct to be attached to it if a force air induction is to be considered *(Figure A5.3).*

Four tensioned turnbuckle cables with d-rings are used to secure the top cover to the base with the cables located outside the octagonal housing to allow for easy access to tighten cables. Cables and d-ring are set at locations that do not hinder opening of the aerator (Figure 1).

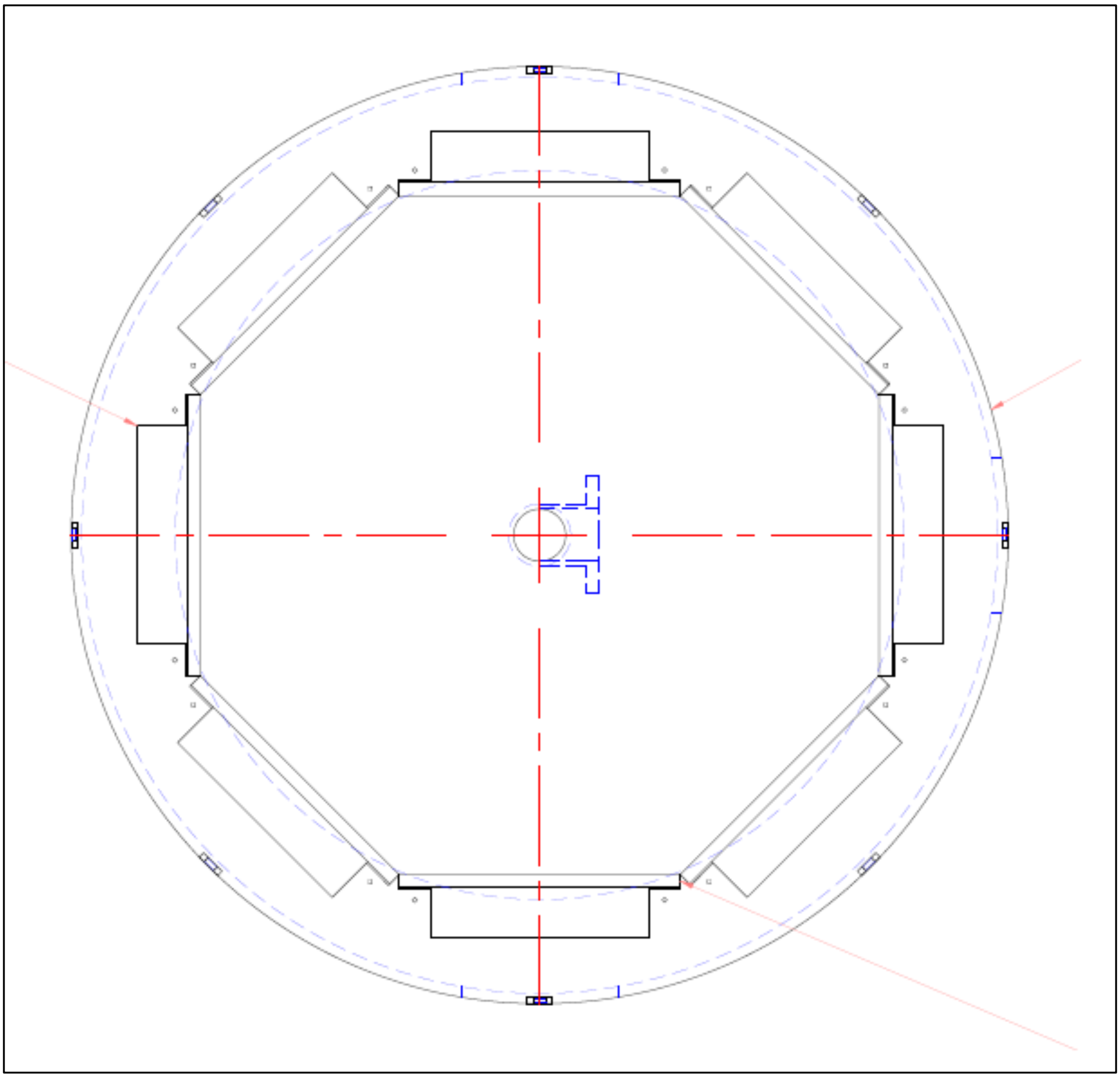

Figure A5.4: Top View of Octagonal Housing mist eliminator panels

In the original design, the octagonal housing comprised of five fixed panels and three removable panels, however, due to the overall weight of having fixed panels making it difficult to assemble on-site, it was suggested that each panel be made individually and mounted flush to each other to form the octagonal housing.

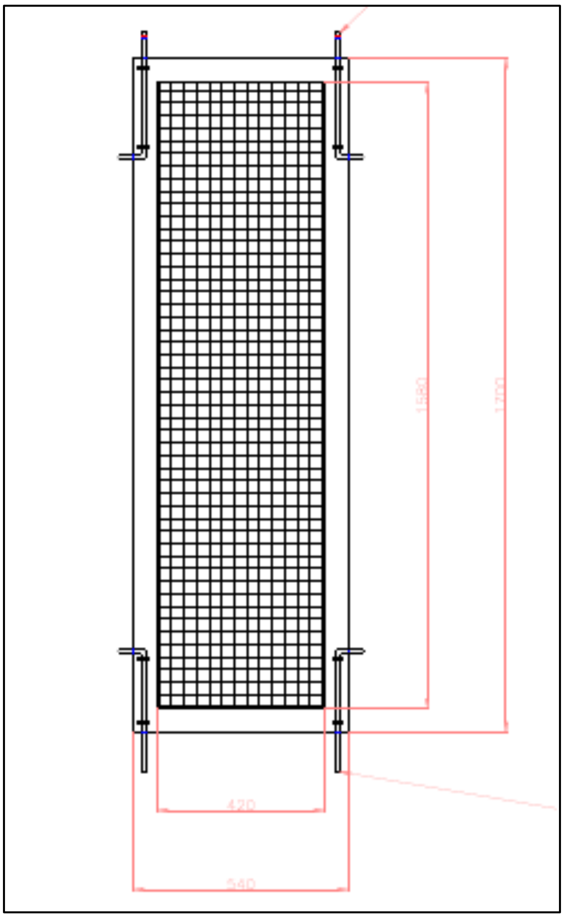

Figure A5.5: Removable Mist Eliminator Panel

Each mist eliminator will be mounted onto the panel's frame. Panels will also have rubber seals that will seal off gaps between each adjacent panels when mounted together.

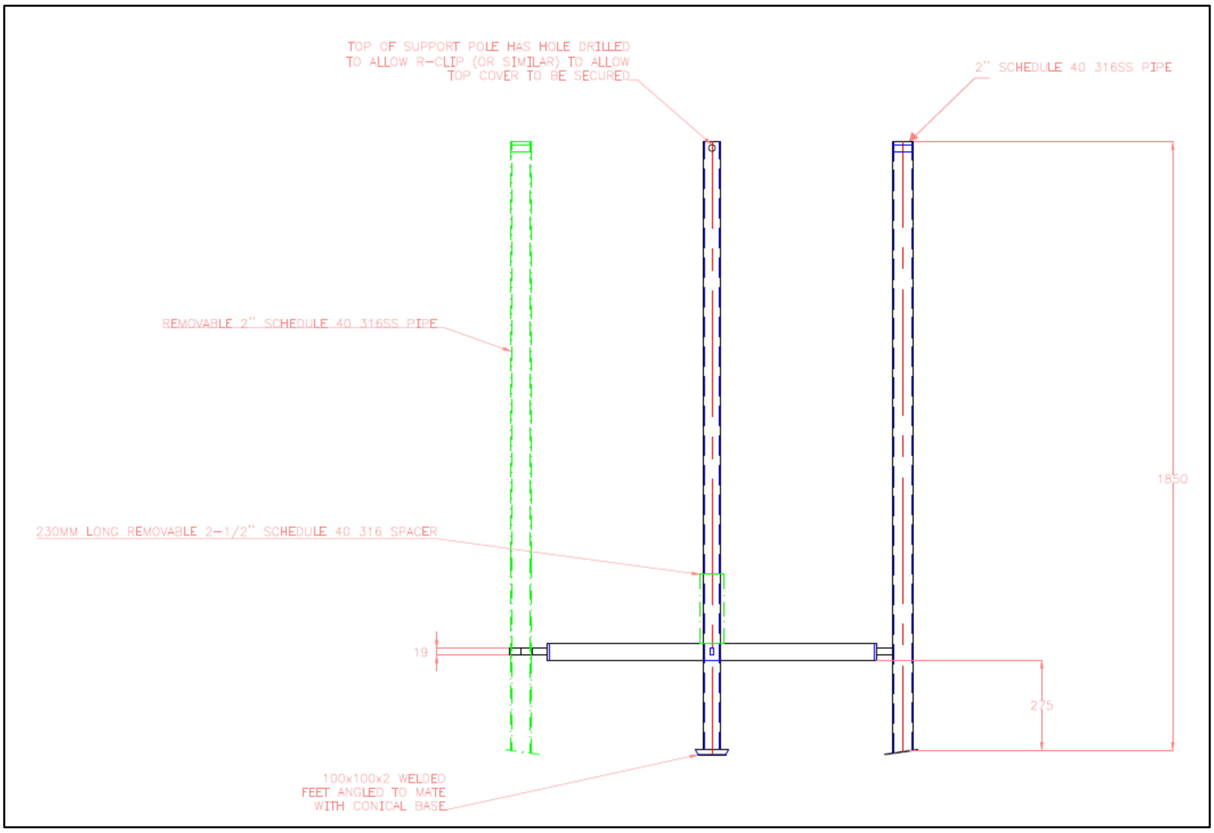

Figure A5.6: Cross-sectional View of the Tray Holder Structure

The four support poles are made out of 2" Schedule 40 316SS pipes with 1850mm heights so as to allow the poles to protrude through the top of the top cover and allow for fixing to the top cover. One of the four poles is removable to allow for trays to be removed from the tray holder rings. Top of support poles have a hole drilled to allow for R-clips for securing to the top cover. The feet of the support poles are welded to 100mm x 100mm x 2mm angled plates to allow for flush mounting onto the sloping aerator base floor. 230mm long removable spacers of Schedule 40 316SS with diameter of 2.5" are used to provide adequate spacing between trays. As the spacers are removable, the number of trays and spacing between trays can be adjusted for optimisation as part of the pilot aerator on-site testing.

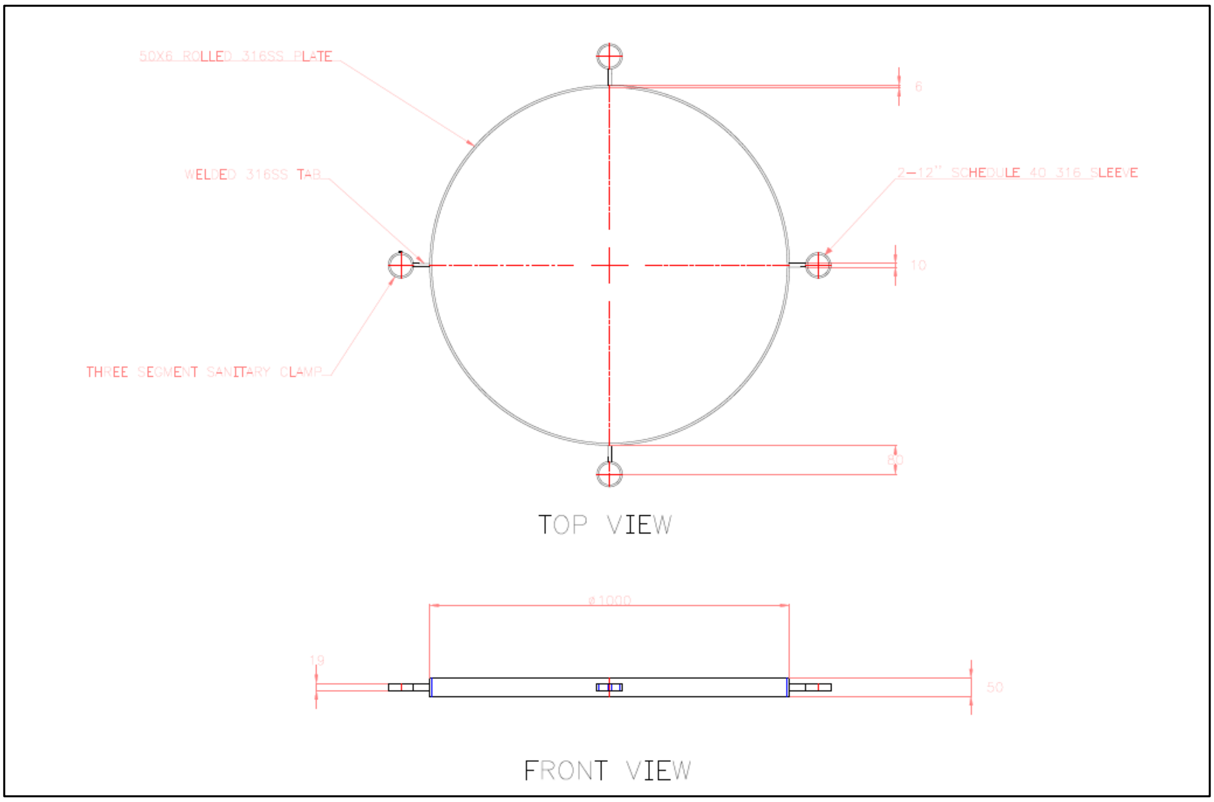

Figure A5.6: Top and Frontal Views of Each Tray Holder Ring

Each tray holder ring is made out of a 50mm x 6mm rolled steel plate to form a 1000mm diameter ring before being welded to four sanitary clamps for attachment to support poles.

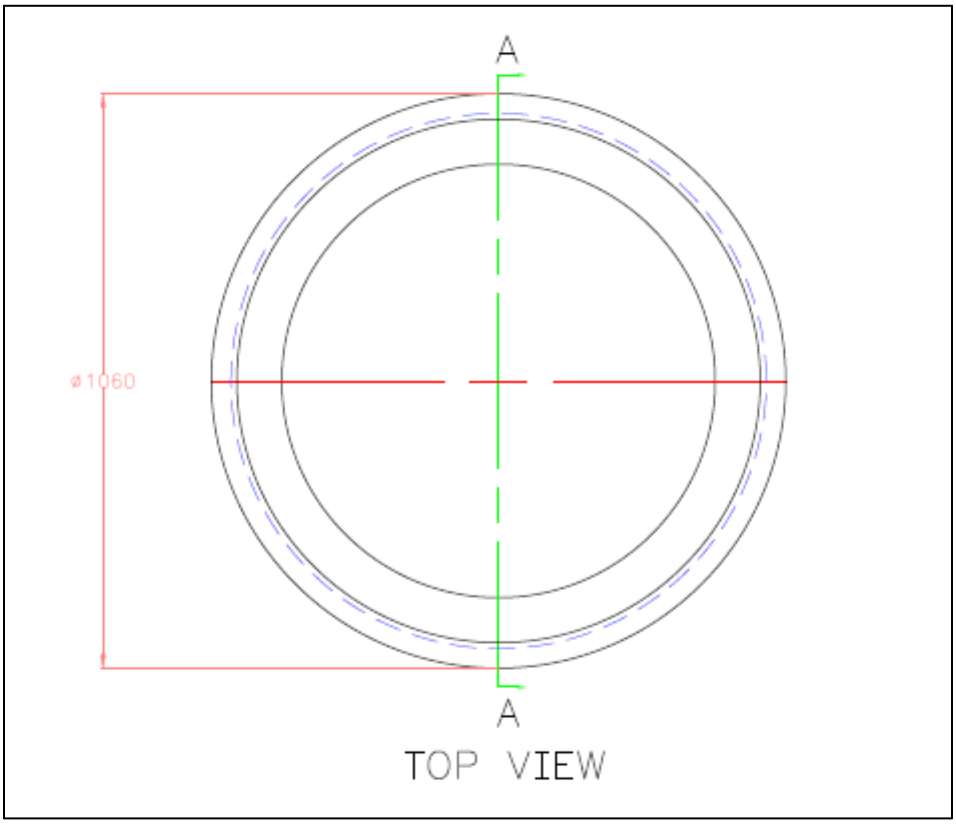

Figure A5.7: Top View of Tray without Internal Mesh

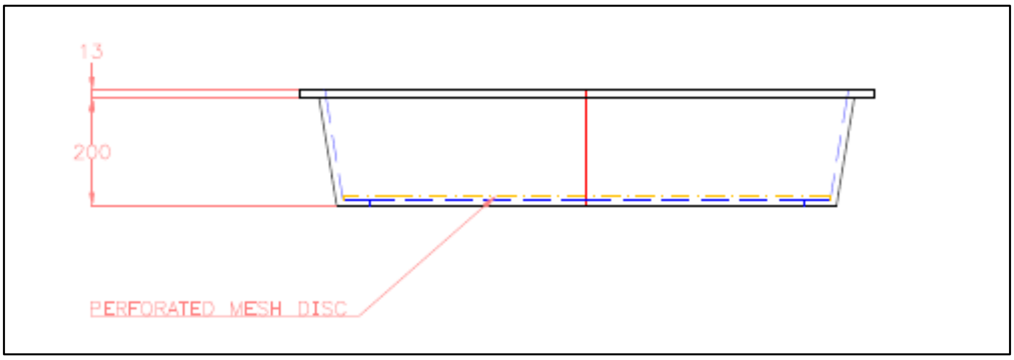

Figure A5.8: Side View of Aerator Tray

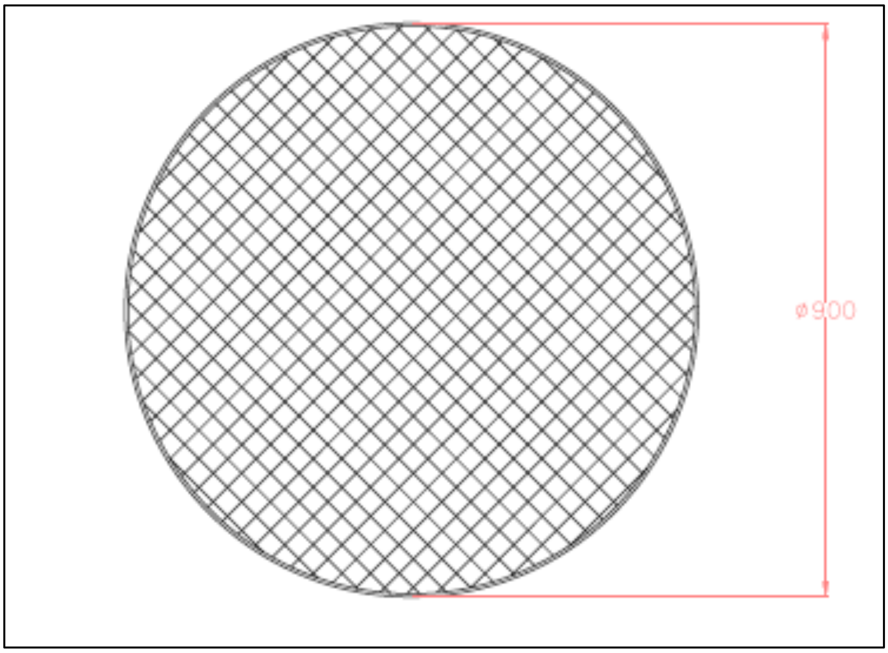

Figure A5.9: Top View of Internal Mesh

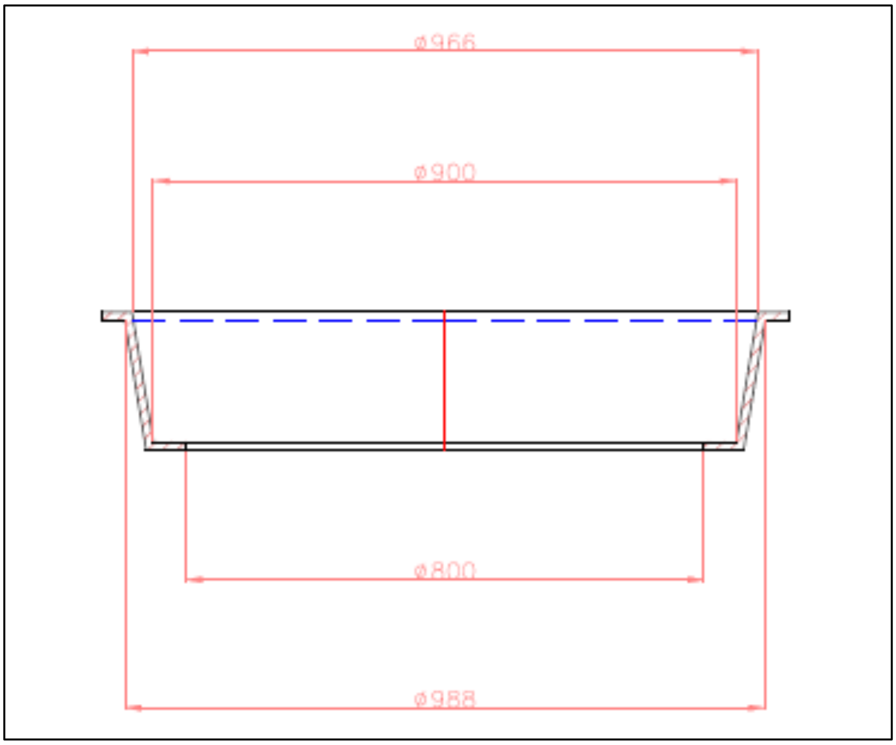

Figure A5.10: Dimensions of Aerator Tray