Is Chemical Free Water Distribution a Possibility in the UK?

Understanding the interactions between drinking water distribution system chemical residuals, water quality and biological stability

Natalie S. T. Lamb

February 2021

Department of Civil and Structural Engineering

The University of Sheffield

Thesis submitted for the degree of Doctor of Philosophy

Abstract

Most developed world drinking water systems are chemically intensive, but distribution residuals can have unintended consequences. Finite resources, environmental issues, rising costs and other countries successfully using fewer chemicals, raises questions about the long-term viability of traditional strategies. However, the impact of reducing or removing chemicals are unknown. This work investigates these questions and if chemical free water is a possibility for the UK.

A review of the best practices from countries using minimal chemicals identified that biological stability and maintaining this during distribution, is central to achieving safe drinking water with less chemicals. This PhD investigated the possibility of chemical free water in 3 key ways. Firstly, the concept of biological stability was defined, assessing how the notion applies to UK systems. Next, chlorine and phosphate were investigated, examining how they are applied at water treatment works, how they affect water quality and how their effectiveness is influenced by biological stability. Finally, multiple full scale and idealised pipe test loops were designed and constructed specifically for this project, which investigated different chemical regimes and varying biological stabilities at multiple sites.

A novel output was the formation of a risk score matrix for biological stability which considers microorganisms, nutrients, wider water quality parameters and aesthetic parameters, tested at 6 case study sites and experimental facilities. Results showed biological stability was the driving factor for water quality differences between dosing regimes, supporting its use as a key metric. Contrast between UK chlorine and phosphate dose management raised questions regarding the value of these traditional methods. Experimental trial results found little impact when chemicals were removed, particularly in more biologically stable water. Further research is needed to assess the effect of reducing or removing chemicals in real-world systems, yet this first step indicates chemical free water is a prospect for the UK.

Acknowledgements

To the people who served as my rock, my anchor, thank you for being my extra life.

To my teachers, my mentors, both official and informal, thank you for helping me grow and develop.

For everyone who stuck with me, thank you for giving me the faith to persevere.

And finally, to those little pleasures in life that kept me smiling throughout the marathon. The metal music, video games and cups of tea of the world. Often forgotten but forever present.

Table of Contents

Thesis Structure

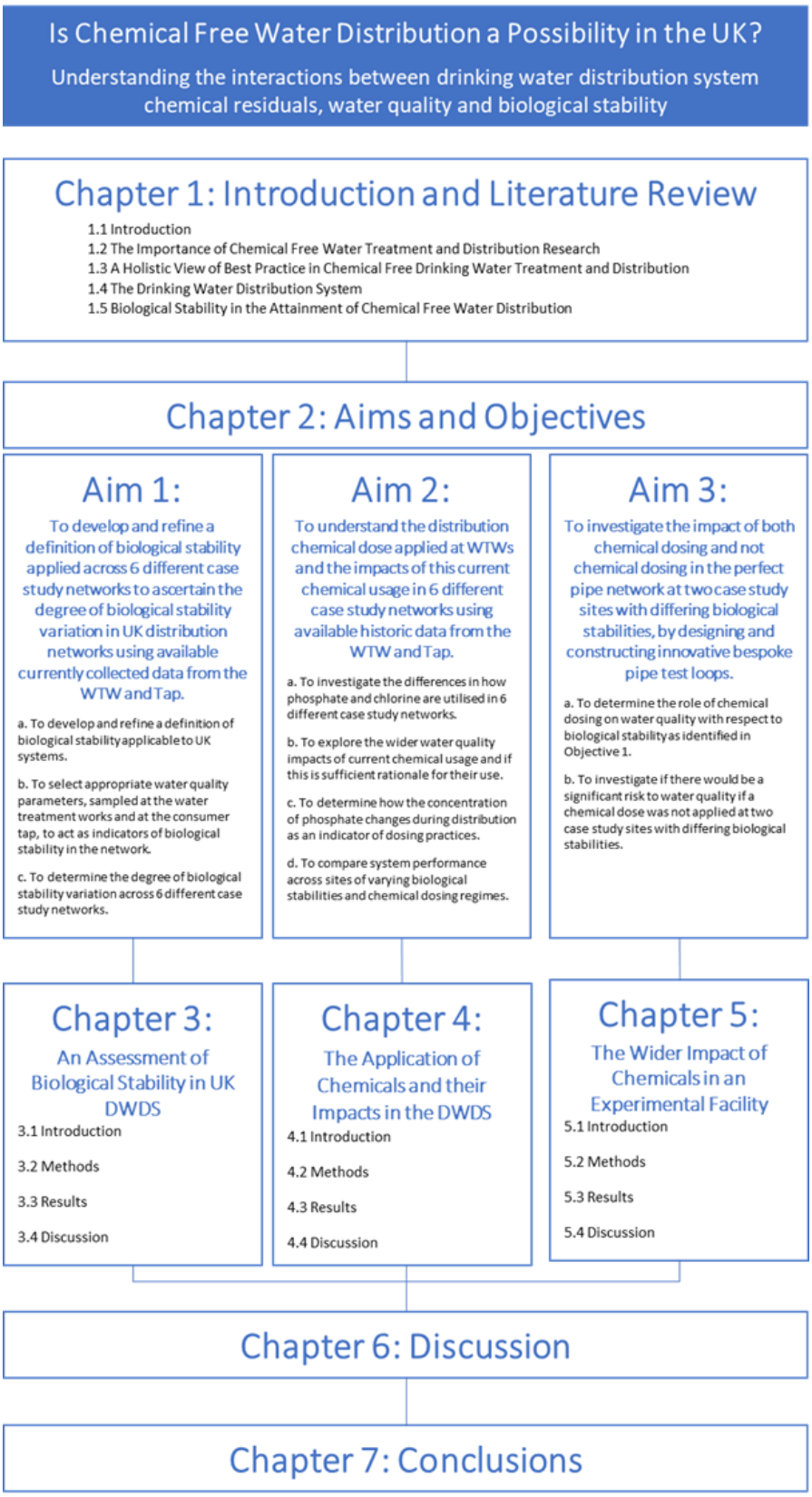


Figure 1 Contents: A visual representation of the thesis structure

Nomenclature

AMP	Asset Management Plan
AOC	Assimilable Organic Carbon
ATP	Adenosine Triphosphate
BDOC	Biodegradable Dissolved Organic Carbon
CFU	Coliform Forming Units
COMA	Control of Major Accident Hazards Regulations
cPVC	Chlorinated Polyvinyl Chloride
DAPI	4’,6-diamidino-2-phenylindole
DEPC	Diethyl Pyrocarbonate
DBP	Disinfection By-Products
DOC	Dissolved Organic Carbon
DMA	District Meter Areas
DMSO	Dimethyl Sulphoxide
DWDS	Drinking Water Distribution Systems
DWI	Drinking Water Inspectorate
DZ	Distribution Zones
EPS	Extracellular Polymeric Substances
GAC	Granular Activated Carbon
GIS	Geographic Information System
GW / SW	Groundwater / Surface Water
HAA	Haloacetic Acid
HDPE	High-Density Polyethylene
HPC	Heterotrophic Plate Count
ICC	Intact Cell Count
IMS-FCM	Immune-Magnetic Separation Flow Cytometry
LSI	Langelier Saturation Index

MDPE	Medium Density Polyethylene
NDMA	N-Nitrosodimethylamine
NFVS	Non-Flush Variable Standing
NOM	Natural Organic Matter
NTU	Nephelometric Turbidity Units
PBS	Phosphate Buffer Solution
PCV	Prescribed Concentration or Value
PODDS	Prediction and Of Discolouration in Distribution Systems
PVC	Polyvinyl Chloride
PWSZ	Public Water Supply Zones
R1	Rig 1, Chemically Treated Experimental Facility
R2	Rig 2, Chemical Free Experimental Facility
RGF	Rapid Gravity Filter
RO	Reverse Osmosis
SDR	Standard Dimensional Ratio
SEM	Scanning Electron Microscope
TCC	Total Cell Count
TCM	Turbidity Causing Material
TCP	1,2,3-Trichloropropane
THAA	Trihaloacetic Acid
THM	Trihalomethanes
TOC	Total Organic Carbon
UF	Ultrafiltration
UV	Ultraviolet
WHO	World Health Organization
WRZ	Water Resource Zones
WTW	Water Treatment Works

List of Figures

List of Tables

1 Chapter 1: Introduction and Literature Review

1.1 Introduction

Water is essential for life. Access to safe drinking water is vital for human health and is considered to be a basic human right, an essential step towards improving living standards worldwide (WHO, 2017) (United Nations General Assembly, 2010). Yet in 2015, only 71% of the global population, 5.2 billion people, used a safely managed drinking-water service, which can be described as located on the premises, available when needed and free from contamination (WHO and UNICEF, 2017). The implications of drinking microbially contaminated water on public health can range from self-limiting diarrhoeal disease (such as noroviruses and cryptosporidiosis) to severe and life-threatening diseases (including typhoid, cholera and infectious hepatitis) (WHO, 2017). In 2012, 502,000 diarrhoea deaths were estimated to be caused by inadequate drinking water (Prüss-Ustün, et al., 2014). The microbial contamination of water sources and its degradation of water quality clearly has a large impact on human health.

This water quality challenge is not just something that developing countries face. The Pacific Institute (2007) stated "according to the United Nations, if present consumption patterns continue, two-thirds of the world's population will live in water-stressed conditions by the year 2025" (World Water Assessment Programme, 2012). Climate change, as well as other large challenges to the global water sector, such as increasing water demand, population growth and demographic changes, will require the use of innovation and development to better manage water resources to ensure adequate provision and quality of drinking water (World Water Assessment Programme, 2012).

One way in which the sustainability of safe drinking water for future generations is being driven is with long-term thinking, by investigating current practices and utilising innovation to improve them. The use of chemicals to treat drinking water is a practice which has been used since water treatment was developed in the 19th century, with one of the first recorded uses, a chlorine solution, used in 1897 to disinfect drinking water pipes in the British town of Maidstone after a typhoid fever outbreak (Special Sanitary Commissioner, 1897). Today, chemicals are used at many different points during the journey of drinking water from source to tap, such as pH correction, softening, coagulation/flocculation, adsorption, dechlorination, manganese removal, disinfection, fluoridation, plumbosolvency prevention and cleaning. Chemicals, such as chlorine, are a highly successful way of improving water quality however there are now alternatives in place which may be preferable to the traditional methods of chemical dosage (Cozzolino, et al., 2005).

The use of chemicals to treat drinking water has numerous disadvantages, including health concerns, environmental damage, the cost required to purchase them and poor or unknown long-term availability. One example is the use of chemical disinfectants (e.g., chlorine, chlorine dioxide, chloramines and ozone) which form disinfection by-products that are known to have carcinogenic properties, when they react with natural organic matter and/or inorganic matter (Cozzolino, et al., 2005). A further example of this is the use of phosphate, a finite resource which is dosed at great cost to protect against lead pipes in supply, however the lead pipes could be replaced with an alternative material instead (Knowledge Transfer Network, 2008). To mitigate for the disadvantages of chemical use, there is interest in producing the same good quality water but without the

application of chemicals in the treatment and distribution processes, known as ‘chemical free drinking water’.

Water utility companies in different developed countries are at various stages of progression to adopt chemical free drinking water treatment. For example, VCS in Denmark has never used primary disinfection employing chlorine, whereas Stadtwerke Düsseldorf in Germany went chlorine free in 2015 (Isle Utilities, 2015). Countries such as the UK, who are attempting to start this journey towards chemical free drinking water treatment, are trying to learn from the best practices of companies in other countries who currently use fewer chemicals than in the UK to improve the understanding of the system characteristics, monitoring and management in these countries.

While there is no internationally agreed practice for the provision of chemical free water, there is consensus among many of the countries with a more optimised chemical dose, that biological stability of the distribution network should be closely monitored (Isle Utilities, 2015). This focus on the distribution network stems from the universal problem of microbial re-growth within the network. Although the water quality exiting water treatment works (WTW) may be very high, it can then have a long residence period, as long as 10 days in the UK, in the pipe network environment which can impact the water quality (Blokker, et al., 2016) (Seker, et al., 2012). Machell and Boxall (2012) found that the longer drinking water is in contact with the distribution network material, the higher the propensity for water quality to be impacted, influencing parameters including: taste and odour, corrosion rates, material precipitation, discolouration, disinfection-by-product formation and biological activity (American Water Works Association, 2002). The importance of the distribution network for the provision of safe drinking water should not be underestimated, even though it has been studied less than other areas of water provision, such as drinking water treatment (UKWIR, 2017). As Douterelo et al. (2018) said, the optimal drinking water distribution systems (DWDS) is key to the delivery of high-quality safe drinking water.

Despite the consensus to monitor biological stability and its role in achieving chemical free drinking water treatment, there is no internationally agreed definition, interpretation or practice for monitoring it. The World Health Organization (2011) stated that “Water entering the distribution system must be microbiologically safe and ideally should also be biologically stable." Nevertheless, the document does not define what is meant by biological stability, which is potentially why there is a lack of clarity. Regardless, there is general agreement that the term ‘biological stability’ refers to the concept of maintaining microbial water quality from the point of drinking water production up to the point of consumption.

Microbial water quality in the UK has traditionally focused on the cultivation of coliform bacteria and faecal indicator bacteria, such as *Escherichia coli,* however only a small proportion of metabolically active microorganisms in the water sample are able to be grown and so detected, depending on the environmental conditions (Lautenschlager, et al., 2013). This means that as Liu et al. (2013) said, less than 1% of the microorganisms present in drinking water can be detected using this method. This technique also focuses solely on the microorganisms present in water within the pipe networks (known as ‘bulk water’), excluding the microorganisms attached to infrastructure surfaces in the distribution system, which persist in the form of biofilms. Biofilms can be described as assemblages of microbial cells embedded with a complex matrix of extracellular polymeric substances which primarily comprises of carbohydrates and proteins but can also contain inorganics, including metals

(Fish, et al., 2017). Most microorganisms in these biofilms are harmless (e.g., Mycobacterium *gordonae*, strains of *E. coli* and heterotrophic bacteria) but they can also contain pathogens (e.g., *Helicobacter pylori, Pseudomonas aeruginosa* and *Cryptosporidium*) (Boyd & Chakrabart, 1995). Different microorganisms with their own specific health risks can be found within the bulk water and in biofilms, with the research of Liu et al. (2014) reporting that ~12% of the total bacteria found in biofilms were not shared with the bulk water, although this figure can be higher. To better understand and assess the risk that drinking water poses to human health, both the microorganisms within the bulk water and attached in the biofilm need to be assessed and monitored.

Thus, the provision of safe drinking water is of paramount importance. To keep up with increasing demand and the challenges faced by the industry in future, innovation, evolution of best practice and collaboration will be increasingly significant. To ensure water quality for future generations, practices, such as the use of chemicals to treat drinking water, will have to be revised. To achieve this aim, the unknown factors, the required steps that water companies must take to reduce chemical usage in distribution, while also maintaining compliance to drinking water standards, have to be identified. These unknowns, such as how to promote and control biological stability in a chemical free water context to ultimately protect public health, will be discussed in detail in this document, particularly in the literature review. To address this challenge, it is important to understand the wider impact of case study chemicals, such as phosphate and chlorine, their dosage within the distribution network and the role of microbially mediated processes.

1.2 The Importance of Chemical Free Water Treatment and Distribution Research

This section will discuss the many implications for the use of chemicals has which make researching the feasibility of chemical free drinking water highly important.

1.2.1 Health Concerns

One of the main reasons why chemical free water should be explored is because there are many health concerns associated with chemicals. The consumers of chemically treated drinking water are at risk from contamination, incorrect dosing and potential long-term exposure but also the staff who dose the chemicals and work on the sites of water utility companies are at risk, from inappropriate transportation, handling and storage (World Health Organization, 2006).

The risk of contamination to human health has been identified in the past. In 1988, a contractor discharged 20 tonnes of aluminium sulphate into the wrong tank at South West Water's Lowermoor Treatment Works in Cornwall, UK. This problem was not identified for two days, while the public were supplied with acidic water (pH 3.9-5) for three days (Knowledge Transfer Network, 2008) (Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment, 2013). Aluminium concentrations of 109 mg/l were detected on 7 July 1988, while the World Health Organization (WHO) Guidelines Value for Drinking Water Quality at the time was 0.2 mg/l (Lowermoor Incident Health Advisory Group, 1989) (World Health Organization, 1984). The public in the area reported a variety of symptoms immediately after drinking the contaminated water. The Lowermoor Incident Health Advisory Group report (1989) stated that "Early symptoms, which were mostly gastrointestinal disturbances, rashes and mouth ulcers, can most probably be attributed to the incident."

The impacts of the Lowermoor Incident were evidentially terrible for the consumers of the drinking water, although, it could be said that time has passed since this incident occurred and so it no longer has relevance on health concerns associated with drinking water treatment chemicals today. However, the investigation is still on-going, with the most recent report in 2013 identifying the long-term symptoms that the event has caused, including "neuropsychological effects, joint pains and/or swelling, nail problems, cancer and thyroid disease" (Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment, 2013). Even if members of the public were not directly involved with incidents such as this, the media can play a large part in damaging the trust of many members of the public in their water utility company and discourage them from drinking their tap water for years to come (Water Research Commission, 2012).

The health of the public is not only affected when accidents occur, but they can also be affected by chemicals which have been correctly added. Disinfection by-products (DBPs) (such as trihalomethanes, otherwise known as THMs and bromates) are formed when chemical disinfectants (e.g., chlorine, chlorine dioxide, chloramines and ozone) react with natural organic matter and/or inorganic matter (bromide) (World Health Organization, 2000). The law states that water companies in the UK must "design, operate and maintain the disinfection process so as to keep disinfection by-products as low as possible…" (Drinking Water Inspectorate, 2012). However, this is a challenge as new DBPs are identified on a regular basis, with their own characteristics and complications and already over 600 DBPs have been characterised (Li & Mitch, 2018). Sun et al. (2019) for instance, referred to a new class of DBPs, halobenzoquinones as well as a new group of DBPs, chlorinated

benzoquinones, both with strong genotoxicity. Currently, THMs are the DBP focused on by UK legislation, as they were characterised in 1974, so the adverse health outcomes from THMs are better studied than those DBPs more recently identified (Li & Mitch, 2018). The maximum permissible level of 100 µg/l total THMs at the consumer tap (Drinking Water Inspectorate, 2012). This legislation is in place to acknowledge studies which have determined that people who are exposed to chlorinated water over the duration of their life have an increased likelihood of developing cancer, especially of the urinary bladder and colorectum, due to the presence of disinfection by-products (International Agency for Research on Cancer, 1991) (Richardson, et al., 2007).

Regardless of the concerns regarding DBPs, chlorine is still used because, as World Health Organization guidelines say, the risks DBPs pose to human health are much smaller when compared with the risks of inadequate disinfection (World Health Organization, 2011). A study which is often mentioned in this context was completed by Anderson (1991), who reported that local water officials stopped chlorinating well water in Lima, Peru because of the fear of DBPs and cancer, a decision which later resulted in 3500 fatalities and 300,000 cases of cholera. It could be said that it was this study which influenced the World Health Organization's decision to add that it is highly important that disinfection is not compromised in an attempt to control DBPs (World Health Organization, 2011). However, the research of Tickner and Gouveia-Vigeant (2005) now challenges Anderson's (1991) study and this point of view. This research analysed the investigations and interviews conducted at the time, concluding that there was no evidence that a decision was made to stop chlorinating based on DBP concerns. In actuality, chlorination at the time of the epidemic was usually limited at best and that the cholera epidemic was in fact caused by inadequate public health infrastructure. Again, the validity of this study can also be questioned because of the difficulties associated with investigating epidemics after they have passed. Whichever study found the correct source of the cholera epidemic, it must still be argued that unless an effective alternative method of disinfection is in place, the proper and effective use of chlorine should help protect the consumer from contamination, but the DBP concerns will still present themselves and will continue to pose a potential threat to the health of the public.

Consumer safety is not the only health concern associated with chemical usage for the treatment of drinking water. The use of chemicals has added health and safety implications for the people who work on the sites of WTW, with these chemicals. The chemicals need to be secure, potentially with bunding (a containment wall), to stop leakages and inappropriate handling (Anglian Water, 2020). Gas alarms are required to notify staff of leakages and proper evacuation procedures need to be practised and followed if a leak is found, to ensure they do not get harmed (Anglian Water, 2020). Another important part of chemical storage on a treatment work site is that of proper labelling and staff knowledge of the chemicals and site. In 2013, 6000 l of sodium bisulphite, a highly reactive chemical, was delivered into a sodium hypochlorite storage facility at Testwood WTW in Southampton (Southern Water, 2016). The white fumes which were produced from the reaction were fortunately detected quickly, meaning that the issue was resolved before a full explosion occurred, but a disaster could easily have transpired. Since this incident, chemical distribution nozzle shapes have been altered so chemicals are unable to be delivered into the incorrect storage tanks. There could have been dire consequences for this mistake, but it is fortunate that the industry learned from it and used innovation to ensure it will be unlikely to happen again in future. Staff safety is something which will always be at risk when people must handle chemicals as part of their

job role, but it is something which is continuously being minimised through health and safety legislation.

A further health concern related to the use of chemicals is the potential for terrorism. Since there are few suppliers of the chemicals required in the water industry and there is sometimes only a single supply chain, there are concerns about the availability of chemicals and the raw materials required to produce them. This causes water companies to store potentially hazardous chemicals on site, sometimes for up to 30 days, to ensure that there are adequate reserves to use if necessary. Although strict health and safety measures are used to provide protection from leakage and human error, the storage of hazardous chemicals could be of interest for terrorism. As Jones, Wills and Kang (2010) said, the intentional use of chlorine as an unconventional weapon is something which has and is occurring. Furthermore, if there are fewer suppliers, it makes it more likely that chemicals are travelling greater distances to reach their destinations. Transportation is one of the riskiest parts of using chemicals. Not only are the chemicals at risk of being used for terrorism but also of normal traffic accidents, both of which could cause great damage to people and the environment.

1.2.2 Consumer Expectations

Drinking water quality is of upmost importance at the customer tap and, as The Consumer Council for Water (2015) stated “Quality drinking water is a priority for water customers”. Not only is it highly important for customers to be able to drink their supplied water without illness, but aesthetic qualities, the colour, taste and the odour of the drinking water, are also monitored because they are how consumers determine whether water is safe to drink. World Health Organization (2011) guidelines say that “water should be free of tastes and odours that would be objectionable to the majority of consumers”. The Drinking Water Inspectorate (DWI) (2016), a UK regulator of drinking water quality, interprets these guidelines by saying that taste and odours must be acceptable to consumers, with no abnormal change. The Consumer Council for Water (2015) completes yearly reports from audited data from UK water companies. In 2015 it was found that 93% of customers surveyed said they felt satisfied with the taste and colour of their water and 87% were satisfied with the taste and smell. Thus, consumers generally feel as though their drinking water is good quality, although problems do occur.

The use of chemicals to treat drinking water can result in an impact to the aesthetics of water. An example of this is that chlorine is added as a residual in the network to help protect against microorganism growth in the distribution system but can result in taste and odour complaints from the consumer, especially when chlorine concentrations are altered. This response still occurs even though customers have been advised to place jugs of tap water into the fridge to remove the taste from the residual chlorine. The American Water Works Association (1995) identified that 60% of the taste and odour problems reported by US water utilities were due to the disinfectants used. However, the US use much higher levels of chlorine than in the UK (4 mg/l compared with 0.5 mg/l) (Chlorine Chemistry Association, 2003) (Drinking Water Inspectorate, 2010). Regardless, The Water Research Foundation (2014) found that although consumers had a negative opinion of chlorinous tastes and odours, they had a much greater dislike of other taste and odours, including: earthy, musty, metallic and plastic. Thus, the use of chemicals to treat drinking water does at times have an impact on its aesthetics but this is only a small contribution to the wider water quality perception changes consumers have.

A further unknown in chemical free treatment research is how consumers perceive chemicals in drinking water treatment. It is uncertain if consumers are aware of the chemicals used and if they would be willing for chemicals to be reduced or removed, even if it meant consequences such as more frequent boil notices, where consumers must boil their water within the home before drinking it, or increased cost of bills. In other areas, such as PWN (a water utility in the Netherlands who do not use chlorination), boil notices are common practice but in the UK they are seen as highly negative, especially by regulators (Isle Utilities, 2015). Chambers and Hitchmough (1992) reported that customers were unhappy with the idea of adding more chemicals to the water, regardless of the benefits, but it would be useful if this study was repeated to understand customer views today. Customer safety and security is at the forefront of research into water and water companies should actively engage with their consumers to determine what they want from their water.

1.2.3 Environmental Concerns

The possibility of chemical free potable water should be investigated because the chemicals currently used to treat and distribute water can have negative implications for the environment.

One concern resulting from the use of chemicals is that of leakages, which can be damaging for the environment and the organisms within it. Chlorine leakages, for example, can cause damage to the biota, including death, deformities and reproductive damage (Department of the Environment, 2014). As Govier and Coulson (2018) said, chlorine readily forms a true gas which has significant potential for accidental or deliberate release, as can be seen by conflicts such as the First World War, where chlorine gas was weaponised. But it is not only humans who are at risk from chlorine leakage, for instance just one acute exposure of rats to chlorine caused airway lesions and persistent airway hyper responsiveness (Demnati, et al., 1995). Plant life can be damaged too, as shown by Harger (2010), when chlorine gas (~2-30 US tons or 1.8-27.2 metric tonnes) was released from a Chlor-Alkali plant and one mile downwind the emergent vegetation in the area had 30-90% foliage damage. The damage to vegetation due to the presence of the chlorine gas was detected up to 11 miles downwind from the release point (Harger, 2010). Although some people might argue that the chlorine residual applied at the tap is unlikely to be concentrated enough to result in such widespread damage, each WTW in the UK can store enough chlorine on site for up to 30 days of supply at any given time (Control of Major Accident Hazards Regulations, 2015). For instance, at a site with a maximum flow of 360 MLD, it would be permissible for a maximum 80 tonnes of chlorine to be stored. At a WTW level is where the environmental risk for chlorine lies.

The application of a phosphate dose, too, can have a damaging impact on the environment. Phosphate is a non-renewable resource that is mined from phosphate rock reserves, of which there are minimal reserves remaining (potentially enough for the next 50-100 years) (Jasinski, 2006) (Cordell, et al., 2009). Phosphate leakages too have a damaging impact on the environment. Anthropogenic inputs of phosphate into aquatic ecosystems result in widespread enrichment of these environments (Gooddy, et al., 2017). This eutrophication impairs water quality and encourages toxic algal blooms (CIWEM, 2005). Phosphate can also result in moderate acute and chronic toxicity to aquatic life by lowering the pH of aquatic environments (Department of the Environment, 2014). This damage to the environment is why, under The Urban Wastewater Treatment Directive (European Commission, 2016), water utility companies must remove phosphate at a sewage treatment works level, rather than discharging it into the environment. This shows that not only do

chemicals have to be well contained to prevent leakages but also that they must be properly disposed of to ensure there will be no environmental impact.

Another environmental concern is the embodied carbon of the chemicals used to treat drinking water, the carbon dioxide emitted during the chemical manufacture, transport, use and end of life. As Santana et al. (2014) said, chemicals are one of the primary contributors to the embodied energy of water treatment and supply. An example of the source of embodied carbon from the use of chemicals is the source and use of chlorine. The chlorine used for drinking water treatment in the UK is sourced from a single site, INEOS' Runcorn Site in Cheshire (INEOS, 2018) (Knowledge Transfer Network, 2008). This means that to reach the 1,433 WTW in the UK, the chlorine has to be transported throughout the country, adding to the carbon footprint of those companies (for every litre of fuel used, 2.304 kg of carbon dioxide is produced) (Gov.UK, 2015) (ComCar, 2018). Water companies have been trying to reduce their embodied carbon to mitigate the global and local risks of climate change, improve the sustainability of their drinking water by reducing the use of finite resources and as a source of cost savings (Anglian Water, 2018). One example of a water utility attempting to do this aims to deliver a 70% reduction in capital (embodied) carbon by 2030 from a 2010 baseline (Anglian Water, 2020). However, despite water companies having goals such as these to reduce their carbon usage, the embodied carbon of chemicals has not yet been quantified. The use of holistic carbon accounting methods will be needed in the water industry to achieve these ambitious goals (Knowledge Transfer Network, 2008).

1.2.4 Cost

A further negative surrounding the use of chemicals is that their use can be expensive. Although this is not as vital as the health of the public, the safety of the use of chemicals and the impact of this chemical usage on the environment, it is still an important factor to consider when assessing the implications of chemical use in future.

The Knowledge Transfer Network (2008) identified that chemicals are ~10% of operating costs. Table 1 shows the estimated chemical costs in 2008 in the UK water industry. These costs are for purchasing the chemicals themselves and not for any extra associated costs, such as transportation. These costs are estimated because it is difficult to know exactly what chemicals have been used. Not only does the amount of chemicals used vary yearly but it also varies depending on the reserves in storage, the climate and load variation.

Table 1 UK Water Industry chemical usage 2008

Chemical	Estimated Usage (tonnes)	Estimated Cost (£M)
Salt	83,270	5.28
Polymer	4,194	6.23
Phosphoric Acid	12,243	12.24
Lime	47,872	3.91
Ferric Sulphate/ Aluminium Salts	37,299	2.38
Chlorine	9,190	6.23

The figures in Table 1 are likely to be even greater today, with the amount of chemicals used and the cost for the chemicals escalating yearly. There are also uncertainties surrounding the long-term availability and affordability of some chemicals. These supply and demand pressures, too, cause rising costs. For example, as the Food and Agriculture Organization of the United Nations (2015) reported, global phosphate demand is currently growing at 2.1% every year. This problem is made more acute due to the poor optimisation of the use of these chemicals, leading to waste. One example is that Smitt and Russell (2013) said that approximately 60% of the UK population were receiving water dosed with phosphate to protect against lead pipes but did not have lead pipes in their household. When phosphoric acid is wasted, it increases the likelihood it will accumulate in the environment. Furthermore, discharging phosphate into the environment means sewage treatment works are likely to contain it in their influent. Under The Urban Wastewater Treatment Directive (European Commission, 2016), phosphate must be removed at sewage treatment works to help protect the environment, using ferrous chloride. Therefore, water companies may be paying money to dose phosphorus at WTW, which they are then paying money to remove at sewage treatment works. Lenntech (2016) summarised that phosphate removal is expensive and increases waste (in the form of sludge volume) by 40%, which are then more costly to remove. One example is that, as the Environment Agency (2012) said, one method of removing phosphorus from the final effluent of sewage treatment works, chemical precipitation, would cost £2 million to initiate (capex cost) and £0.3 million a year to operate (opex costs) for a population equivalent of 1 million people and a concentration of 1 mg/l phosphorus.

Chemicals also result in additional costs because of working practice implications. A site which is normally unmanned may need someone on site when there is a chemical delivery, for example. This is particularly expensive because to be able to properly control the chemicals, either a skilled workforce or sophisticated control and application systems are needed. Relatively inexpensive health and safety requirements such as alarms, site security and regular evacuation practices, can be costly when multiplied by the number of sites which much meet these requirements.

There are additional financial repercussions during accidents, too. When chemical-related events happen, regulatory bodies are required by legislation to begin an investigation into the incident and can also become involved financially. For example, one case from the UK water utility Severn Trent Water resulted in £350,000 of fines issued in 2018, plus court fees, following a hazardous chemical leak of 20% sodium hydroxide which subsequently affected 5 km of the River Amber and killed approximately 30,000 fish and damaged other wildlife in 2015 (Gov.UK, 2018).

1.2.5 Summary: The Importance of Chemical Free Water Treatment and Distribution Research

This section has explored that using chemicals to treat and distribute drinking water can have unintended consequences, which can impact the public, the environment and the water treatment industry itself. Although chemicals have successfully improved water quality over many years, to mitigate future challenges the sustainability of treatment and distribution processes require scrutiny. Chemical free water treatment and distribution has the potential to minimise or even remove the serious risks associated with the use of chemicals to the consumers of drinking water and the staff who produce it. However, it is unknown what the UK needs to do to take the first steps towards chemical free water, no point of reference for how the water industry should adapt or if it is possible in this country.

1.3 A Holistic View of Best Practice in Chemical Free Water Treatment and Distribution

The vast majority of developed world water systems are chemically intensive. Water utility companies in different developed countries now recognise the need to change practices but are at various stages of progression to adopt chemical free drinking water treatment and distribution. An investigation of the best practices of companies in other countries who currently use fewer chemicals than in the UK has been completed to better understand the system characteristics, monitoring and management in these countries. This comparison was completed with an Isle Utilities (2015) report as a primary source, combined with personal communications and a review of wider literature.

The case study companies for this research were chosen due to their various stages of progression to adopt chemical free drinking water treatment and distribution. They included: VCS in Denmark who have never used phosphate or primary or secondary disinfection using chlorine; Stadtwerke Düsseldorf in Germany who stopped using all chlorine in 2015 but dose 1 mg/l phosphate; PWN in the Netherlands who do not use phosphate and stopped using primary disinfection in 2005 but still use a secondary chlorine residual and Anglian Water in the UK who presently and historically used phosphate and primary and secondary disinfection using chlorine. These case study companies were carefully selected to allow comparison and best possible identification of the influence of different factors.

1.3.1 Source Water

The World Health Organization Protecting Groundwater for Health Guidelines (2006) defines groundwater as "the water contained beneath the surface in rocks and soil…", whereas surface water is the water that collects above this, on the Earth's surface, including oceans, seas, lakes, rivers and wetlands. Surface water and groundwater differ in water quality because they are exposed to different substances and contaminants. An example is surface waters are the most likely to contain contaminants such as pesticides and groundwaters are the most likely to contain dissolved minerals such as calcium.

When practising chlorine free water treatment and distribution in other countries, case study companies confirmed that the preferred source water type was groundwater. The company VCS in Denmark, for instance, uses 100% groundwater (Isle Utilities, 2015). This preference for groundwater stemmed from water utilities arguing that surface waters are more vulnerable to higher microbiological presence than groundwaters, due to a combination of the absence of soil filtration and the likelihood of heavy rainfall increasing the microbial load of running waters which quickly reach reservoirs (Kistemann, et al., 2001). Some studies do agree with this. Reynolds et al. (2008) investigated the frequency of infections and found that surface water systems were more likely to cause more infections in the USA (26 million per year) than groundwater systems (13 million per year). Although, numbers of infection caused by different types of source waters could be the result of different asset management and maintenance rather than differences in microbial load. Prest et al. (2016) stated that deep groundwater (~10^3-10^4 cells/ml) characteristically has a lower concentration of bacterial cells than surface water (~10^5-10^6 cells/ml). The use of the word "deep" when describing the groundwaters is pivotal, as the depth of the borehole's unsaturated zone can determine its vulnerability to contamination, with those that have shallow or unconfirmed water tables being more high risk (Environment Agency, 2017). That is not to say that deeper groundwater

cannot be contaminated, as a study of 110 groundwater outbreaks in the USA 1971-2008 found vulnerability in sources with a multitude of characteristics (Craun, 2012). Several other parameters also impact groundwater quality, including: the physical, chemical and biological properties of the underlying soil and rocks, the soil depth and quality and the presence of other materials (Environment Agency, 2017). Furthermore, if high microbial loads do occur in groundwater, they can be hidden, making them more difficult to characterise and the water quality issue more challenging to rectify. So, the preference for case study companies to use groundwater rather than surface water appears to be justified, however it is not a guarantee of better water quality.

Despite this preference for groundwater, other companies can achieve chlorine free water while using a combination of water sources. Specifically, PWN in the Netherlands use 22% groundwater and 78% surface water while Stadtwerke Düsseldorf in Germany use a similar combination of 25% groundwater and 75% surface water (Isle Utilities, 2015). When surface water is used by these companies, impounding reservoirs with riverbank or dune filtration with a retention time of several weeks is utilised, in an attempt to reduce biological activity and improve biological stability. A year-long study at three full-scale riverbank filtration facilities in the USA found that this method provided substantial reductions in microorganism concentrations, for example total coliforms had an average reduction of 5.5 and 6.1 log (Weiss, et al., 2005). In comparison, Anglian Water in the UK has a more even split of 40% groundwater to 60% surface water. This split in source waters, however, should not be a barrier for future reduction in the use of chemicals. Seemingly the more significant differences between Anglian Water and the case study companies is that there is no consideration about the microbiological activity of the water type in the UK. There has been a movement in recent years towards source water protection and management, to improve water quality and reduce treatment costs. There is also interest within UK utilities to continue investing in catchment management in future, to use source waters as an early warning system for potential contaminants.

1.3.2 Water Treatment Works

Much attention of drinking water treatment and distribution has been on treatment processes as water companies have the responsibility to ensure water supplied from the treatment works is 100% compliant with water quality standards. The Drinking Water Inspectorate (2016), for instance, requires water companies to sample for *E. coli*, coliform bacteria, colony counts at 22°C, nitrite, residual disinfectant and turbidity at the point at which water leaves each treatment works in the UK. New regulatory requirements, such as the addition of DBPs and emerging contaminants, are encouraging more research into the capabilities of different assets and the development of new technologies to better treat water, ensuring it remains complaint with stricter standards. One area that is of interest is the use of interstage monitoring of biological stability to provide a background of information so it can easily be detected when treatment technologies need maintenance or replacement.

The use of different treatment processes is highly important in the production of water that is compliant to regulatory standards, as the microorganisms removed by these processes have different physical, chemical and biological properties. For example, smaller organisms, such as viruses, are less likely to be removed using physical treatment methods like microfiltration (Sinclair, et al., 2018). A further example is that protozoa, such as *Cryptosporidium*, can be more resistant to disinfection by chlorine than viruses (Hill, 2014). Different species can have variation within these classifications, too, because of structural differences such as the presence of single or double-

stranded DNA or RNA (Westrell, 2004). Because of all the variation between these different microorganisms, it means a multi-barrier approach is the preferred method to use, to make removal of all organisms more likely. In the UK multi-barrier treatment is currently used but chlorine is the dominant method of disinfection. More advanced treatment methods such as Advanced Oxidation Processes are used at some sites within Anglian Water, but this is a relatively new development, and many treatment works are very much reliant on chlorine for disinfection.

The use of multi-barrier systems and extended treatment processes are also used by companies who use fewer chemicals than the UK, as well as consideration and monitoring of the biological stability of their drinking water at the treatment works. For instance, PWN in the Netherlands may pre-treat water with micro sieves, coagulation, lamella separators, rapid sand filtration and granular activated carbon (GAC) treatment, followed by treatment using ultrafiltration (UF) or reverse osmosis (RO) and UV (ultraviolet) light or hydrogen peroxide, dune passage, aeration, rapid sand filtration and UV light. Specifically, Andijk WTW in North Holland uses "advanced treatment", involving micro sieves, suspended ion exchange, CeraMac filtration, GAC and UV light (Isle Utilities, 2015). The disinfection practices differ between the different case study companies. PWN (Netherlands) uses ultrafiltration with reverse osmosis or UV light with hydrogen peroxide and chlorine dioxide, Stadtwerke Düsseldorf (Germany) uses ozone and VCS (Denmark) uses UV light disinfection (Isle Utilities, 2015). PWN (Netherlands) and Stadtwerke Düsseldorf (Germany) are due to switch to UV light disinfection in the near future and are currently completing preliminary experiments into this.

1.3.3 Distribution Network

Even after careful source water catchment management and the use of novel drinking water treatment methods, water quality degrades during its transportation to the customer tap, through the distribution network, sometimes to the point of water quality failure, despite water leaving the works being wholesome. Williamsona et al. (2014), for instance, reported that "30-60% of water quality incidents are related to events in the water distribution network". This water quality degradation occurs due to the physical, chemical and biological reactions which occur during distribution and can be impacted upon may different factors, such as pipe materials, contamination, pipe leakage and inadequate or infrequent maintenance (National Research Council, 2006).

Pipe leakage was found to vary between those companies who use fewer chemicals and the UK. Leakage as a percentage of input volume was 5%, 4% and 6% for VCS (Denmark), PWN (Netherlands) and Stadtwerke Düsseldorf (Germany), respectively (Isle Utilities, 2015). This is much higher in the UK, with 15.5% at a national level and 12% at Anglian Water (Isle Utilities, 2015). Leakage reduces water efficiency and presents an opportunity for contamination of treated water to occur. The ratio of distribution system intrusions to other sources of drinking water contamination could be as high as 1:1, ensuing approximately 20% of gastroenteritis cases in the USA being the result of pathogen intrusion into water pipes (McInnis, 2010). Thus, pipe leakage is a water quality risk but is itself interconnected to old, poor condition pipes that degrade.

This relatively higher leakage rate in the UK is linked to the age of the infrastructure (on average ~75-80 years). This, in turn, is linked to how long the network is. The Anglian Water network is ~380,000 km and supplies 6 million people, both of which figures are much greater than the case studies companies being used for comparison (VCS in Denmark using ~1026 km pipework to supply >165,000 people, PWN in the Netherlands using ~9995 km pipework to supply >1.5 million people

and Stadtwerke Düsseldorf in Germany using ~1800 km pipework to supply >600,000 people). The approximate number of pipes per person equates to 0.0633 for Anglian Water, which contrasts with 0.00622 for VCS (Denmark), 0.00666 for PWN (Netherlands) and 0.00300 for Stadtwerke Düsseldorf (Germany), orders of magnitude lower. The much larger network that Anglian Water have mean it is likely to be more expensive and time consuming to replace it (Speight, 2015) (Water UK, 2018). This explains how the Anglian Water pipe replacement rate of 0.27% or 103 km/year varies from the similar figures of the case study companies of 0.4% (40 km/year), 0.4% (7.2 km/year) and 1.1% (20.5 km/year) for PWN (Netherlands), Stadtwerke Düsseldorf (Germany) and VCS (Denmark), respectively.

Due to the UK having an older pipe system, lead, which was a pipe material used in houses built before 1970 is found in ~40% of UK homes, meaning that ~17 million people are still supplied by lead pipes (European Institution for Testing, Inspection and Certification, 1998). Since EU Guidelines say lead should be monitored at customer taps to ensure levels do not exceed 10µg/l, treatment is required to prevent this, which typically consists of the formation of a protective coating built up by dosing orthophosphate at 0.5-1 mg/l (European Commission, 2016). Orthophosphate dosing is a successful method of reducing lead consumption, as Kirmeyer et al. (2000) said, the treatment was "effective in reducing lead levels significantly". In the case study companies, there are no lead pipes, so phosphate is not dosed. Stadtwerke Düsseldorf in Germany, however, does dose phosphate silica at a low concentration of 1 mg/l to prevent corrosion in iron pipes.

To stop public health risks at the customer tap due to network leaks and other sources of contamination, chlorine residuals are used in the UK, typically 0.5 mg/l or less of free or combined chlorine (Drinking Water Inspectorate, 2010). The extent to which disinfection is effective is dependent on: the disinfection method used, the maintenance of the residual in the network, the resistance of the microorganisms to disinfection, the variety and concentration of nutrients present in the water, the pipe material and the temperature of the water (Momba, et al., 2000). However, even when dose and conditions appear to be efficient, there is much research, for example that by Pederson (1990) and Stewart et al. (1995), that provides evidence of persistent microorganisms even though chlorine was being used as a disinfectant. For instance, Venkobachar et al. (1977) found that exposures of laboratory-cultured *E. coli* to increasing chlorine doses for 15 minutes at 20°C resulted in a decline in survival rates (0 mg/l chlorine dose resulted in a 100% *E. coli* survival rate, 0.5 mg/l 84%, 1.0 mg/l 66%, 1.5 mg/l 50%, 2.0 mg/l 20%, 2.5 mg/l 15%) but never reached a chlorine dose high enough to get a complete kill effect, although it could be argued that if a greater contact time than 15 minutes was used, this may have been observed. Bertelli et al. (2018) did a similar trial in the field, so with naturally higher contact times, and found BACMON bacterial counts had a steady but slow decrease in mean total bacterial counts (with a p-value $<2.2x10^{-16}$) between low (<0.1 mg/l), intermediate (0.1-0.2 mg/l) and high (>0.2 mg/l) free chlorine concentration. Pederson (1990) investigated this greater contact time to the extreme with a 0.1 mg/l chlorine residual but found that even after 167 days, microbiology (4.9×106 cells/cm^2) was still present. Though, again it could be argued that the 0.1 mg/l chlorine residual present in this study is not representative of the higher concentration of 0.5 mg/l (or less) in UK networks. Thus, the UK method of using a chlorine residual does not fully deactivate microorganisms as intended but it could be argued that public health is at risk if chlorine is not used, as it has been evidenced that a higher chlorine concentration resulted in a decreased number of organisms (Venkobachar, et al., 1977) (Bertelli, et al., 2018). However, bulk water samples taken at the treatment works and the customer taps from unchlorinated distribution

networks in the Netherlands and were found to have a similar “microbiota structure”, despite the water being transported 0.4-35 km to reach the tap (Roeselers, et al., 2015). Linden et al. (2019) detailed that the Netherlands had fewer breaches of compliance (30 per year when scaled to the Netherlands population), when compared with England (40) and the USA (429), which both chlorinate. This indicates that the absence of a disinfectant residual did not result in increased microbial growth, microbial regrowth or severe pathogen proliferation within these distribution systems, indicating that a disinfection residual is not necessarily always required to safeguard public health.

There is one particular ecosystem that resides within the distribution system that chlorine residuals are notoriously difficult to penetrate, one in which a vast majority of microorganisms reside (10^6-10^{11} cells cm^2, which is hundreds or thousands of times greater than the concentrations free-living in the bulk water, 10^3- 10^4 cells/ml) (Prest, et al., 2016). A biofilm is when microorganisms, organic matter and inorganic matter are attached to the inside of pipes using extracellular polymer substances. The structure of this polymeric matrix means that microorganisms within the structure are protected by an extracellular coating, rendering disinfection residuals less effective, especially compared with planktonic cells. Behnke et al. (2011) found that cells and clusters that had been grown planktonically were more susceptible to disinfection that cells and clusters that had detached from biofilm. Stewart et al. (1995) instigated that chlorine was unable to penetrate more than 20% of the biofilm. However, the current practice of the use of a disinfection residual in the UK is based upon its action against planktonic cells, as this is what is regulated, *E. coli* and coliform colonies in bulk water samples from the final treatment works and the customer tap (Fish & Boxall, 2018). In an operational environment it would be very difficult to sample the biofilm from the pipe wall so instead the bulk water is focused on. This is despite the variety and numbers of microorganisms present within these biofilms, meaning that the impact of chlorine on the biofilm microbiome are largely unknown.

Despite case study companies claiming to be chlorine free, it was found that they did actually use deportable chlorine booster systems when compliance failures occur. UK companies have the ability to do similar. Anglian Water increase chlorination at the WTW initially in the instance of compliance failures and are also able to deploy deportable chlorine systems for localised chlorination in emergencies. The difference between these two strategies is the idea of a chemical balance. UK water utilities do not have a system in place for rehabilitation of the biofilm after doses decrease, while the case study companies have a prolonged time of gradually reducing the chlorine dose to allow for slow adjustment of the system and rehabilitation of the biofilm. This is one example of how other countries take a different approach to managing public health risks by monitoring and attempting to improve biological stability through improved physical integrity of the distribution system and careful management of distribution system operations. A further example of this is to utilise high velocities to limit sedimentation (Abraham, et al., 2018). PWN (Denmark) and Stadtwerke Düsseldorf’s (Germany) approach is to manage pressure and flow by moving to smaller diameter pipes. PWN in the Netherlands carries out regular flushing, particularly in areas that they categorise as being high risk for biofilm regrowth (Isle Utilities, 2015).

1.3.4 Customer Tap

Drinking water quality at the consumer tap must be of the best possible quality, wholesome and safe to drink, of the same quality as that leaving the treatment works, so biologically, chemically and physically stable. In the UK to monitor water quality at the customer tap, the water utilities are legally obliged to collect samples from random addresses in each water supply zone. These samples are analysed for numerous water quality parameters detailed in Table 2, the frequency of which are determined by parameter and population size (Drinking Water Inspectorate, 2016) (Northern Ireland Water, 2015). These results are closely monitored by the UK regulators, the Drinking Water Inspectorate, to ensure the water quality at the customer tap is fit for human consumption as per UK and EU guidelines (European Commission, 2016).

Table 2 UK regulatory frequency of customer tap sampling

Parameter	Estimated Population of Water Supply Zone						
	<100	100-4999	5000-9999	10,000-29,999	30,000-49,999	50,000-79,999	80,000-100,000
	Frequency of Required Samples/Year						
Aluminium, ammonium, colony counts 22°C, colour, conductivity, hydrogen ion, iron, manganese, nitrate, nitrite, odour, taste, turbidity	2	4	12	24	36	52	76
Antimony, arsenic, benzene, benzo(a)pyrene, boron, bromate, cadmium, chloride, chromium, *Clostridium perfringens*, copper, cyanide, 1, 2 dichloroethane, enterococci, fluoride, gross alpha, gross beta, lead, mercury, nickel, pesticides and related products, polycyclic aromatic hydrocarbon, radon, selenium, sodium, sulphate, tetrachloroethene, tetrachloromethane, total organic carbon, trichloroethene, trihalomethanes total, tritium	1	4	8	8	8	8	8
Coliform bacteria, *E. coli*, chlorine residual	4	4	12-24	24-72	72-120	120-192	192-240

In 2018 it was found that compliance with the Drinking Water Directive at the customer tap was 99.95% (Drinking Water Inspectorate, 2018). However, there is variation within the UK water industry, with a range of 99.81-100% compliance in 2015 (Consumer Council for Water, 2015). Furthermore, there is the argument that compliance can easily be achieved if standards are relatively low. UK standards currently use the presence of coliform bacteria such as *E. coli* to determine whether water at the consumer tap is safe to drink, rather than using methods capable of enumeration of all microorganisms rather than a specific one, such as the use of flow cytometry (Hassard & Whitton, 2019).

A further example of regulatory standards being considered as less stringent is that sample frequency could be considered as relatively low, as can be seen by Table 2. For instance, for a population of 90,000 people only 8 regulatory lead samples are required to be taken per year, which equates to 0.00889% of the population being tested. As water quality failures are spatially and

temporally dispersed, it is highly unlikely that failures will be detected with these sampling frequencies, this one discrete sample snapshot of all the water customers consume. The proportion of the population being tested will vary by parameter, as can be seen by Table 2, as well as the stringency of the acceptable parameter range (e.g., nitrite has an upper allowed limit of 0.5 mg/l whereas nitrate has an upper allowed limit of 50 mg/l) and the frequency compliance of a specific parameter (Drinking Water Inspectorate, 2016). Therefore, water quality compliance at the customer tap seems to be relatively high but is still not perfect everywhere in the UK yet and more stringent compliance could be a possibility in future using emerging technologies.

In the case study companies boil notices are common (Isle Utilities, 2015). PWN (Netherlands) have a contract with an external company to deliver bottled water within two hours when these boil notices occur. Boil notices are not common practice and are seen as highly negative in the UK, as described in Section 1.2.2. It is unknown if customers in the UK would be prepared to accept more frequent boil notices. It is also unknown how customers perceive chemicals in drinking water supply, if they are aware of them and if they would be willing for chemicals to be reduced or removed, even if it meant consequences such as more frequent boil notices or increased cost of bills. Chambers and Hitchmough (1992) reported that customers were unhappy with the idea of adding more chemicals to the water, regardless of the benefits, but it would be useful if this study was repeated to understand customer views today. If chemical free water was to be a possibility in the UK today, customers would have to be educated about boil notices to make them appear more common place. It would also be important to change the viewpoint of regulation, who also portray them in a negative light. Further education of the customers would be beneficial to ensure customer taps are properly cleaned, helping regulatory samples to be compliant and improve their own drinking water quality.

1.3.5 Summary: A Holistic View of Best Practice in Chemical Free Drinking Water Treatment and Distribution

When comparing the UK and those companies in other countries using fewer chemicals than the UK, the biggest area of discrepancy and so the area where the greatest amount of development is required is that of the network [Table 3]. This is the area where substantial water quality changes occur, the greatest amount of development is required, where there is relatively less research (particularly around attached rather than planktonic microorganisms) and where the maintenance of biological stability is of upmost importance to ensure the water at the customer tap is safeguarded. For these reasons, the focus of the present research into chemical free drinking water will be on those chemicals dosed specifically for the distribution network.

Biological stability was mentioned throughout the source to tap comparison, but it was not defined, and it is unclear what it specifically meant in the individual countries. It is not known if or how this concept applies to UK systems. If the UK had a network similar to those of the case study countries, with good biological stability, it is unknown if it would be possible for the UK to go chemical free, with its current source waters, WTWs and Consumer Taps or if there would be a substantial impact on water quality.

Table 3 A summary of case study utilities at different stages of chemical free water treatment and distribution

Case Study Utility	Chemical Application -Typical Chlorine Use -Last Chlorine Use -Phosphate Dosing	Source Water Type	Approach to Treatment	Leakage	Network Length -In km -People Supplied -No Pipes Per Person	Pipe Replacement Rate	Dominant Pipe Materials and Lead Presence	Consideration of Biological Stability?
VCS, Denmark	-None -Never -None	100% groundwater	Aeration, RGF, sand filtration, UV	5%	-~1026 km ->165,000 people -0.00622 pp	1.1% 20.5 km/year	PVC 0%	Yes
Stadtwerke Düsseldorf, Germany	-None -2015 -1 mg/l phosphate silica	25% groundwater 75% surface water	Ozonation, Aeration, GAC, Phosphate Dosing, Silica Dosing	6%	-~1800 km ->600,000 people -0.00300 pp	0.4% 7.2 km/year	Cement 0%	Yes
PWN, The Netherlands	-Residual chlorination -2005 -None	22% groundwater 78% surface water	Lamella Separators, RSF and GAC, UF/ Reverse Osmosis or UV/Hydrogen Peroxide, Dune Passage, Aeration	4%	-~9995 km ->1.5 million people -0.00666 pp	0.4% 40 km/year	Cement 0%	Yes
Anglian Water, UK	-Primary and secondary disinfection using chlorine-based disinfectant -0.5-2 mg/l phosphate	40% groundwater 60% surface water	Ozonation, RGF, DAF/ Lamella Separators, Carbon Filters, Chlorine, Some UV, Some Membranes	12%	-~380,000 km -6 million people -0.0633 pp	0.27% 103 km/year	Cast Iron ~15%	No

1.4 The Drinking Water Distribution System

Section 1.3 identified the distribution network as an area where there are many knowledge gaps for the attainment of chemical free water. The purpose of the present section is to deliver a brief discussion of the DWDS, including it as an environment, the microorganisms within it, the chemicals dosed to maintain the water within it and the possibilities and impacts of stopping the dose of these chemicals.

1.4.1 The Drinking Water Distribution System Environment

Drinking water distribution systems are a complex network of pipes and ancillaries that convey drinking water from a centralised treatment plant to consumers' taps. The environment is described as complex due to the numbers of pipes involved, the differing ages of the pipes, the range of materials the pipes are made from and a multitude of other sources of variance in the characteristics between the individual pipes, the water it is transporting, and the pressure and flow control devices used during this transportation. The physical and chemical properties of water and the chemical contaminants within it can all also have an impact on the network and on the water quality within it. All these factors can result in substantial changes to the biological stability within these systems. Figure 2 provides an indicator of the interconnectedness of this dynamic distribution system environment.

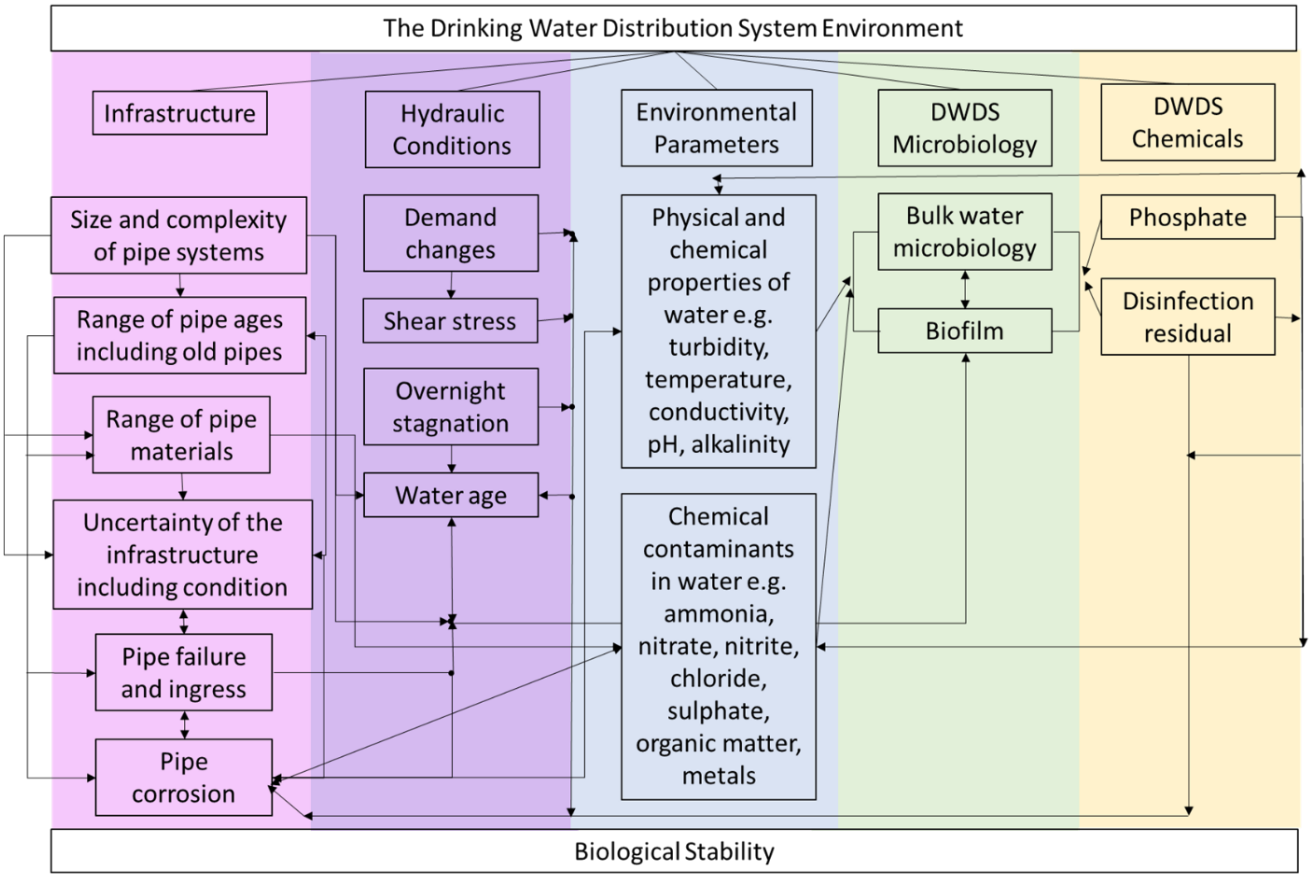


Figure 2 The interconnected varied components of the drinking water distribution system environment which interact to have an impact on biological stability

1.4.2 The Drinking Water Distribution System Microbiology

Microorganisms exist both planktonically within the distribution network and attached to the pipe surface in the form of a biofilm. These sources of organisms interact, change state and vary in number and community. This section will detail the microorganisms in their various forms that reside in the distribution network.

1.4.2.1 Bulk Water Microbiology

Microbial water quality is in flux due to the interactions between various organisms, including prokaryotes (bacteria and archaea), eukaryotes (fungi and protozoa) and viruses that exist within the bulk water of the distribution network, of which there are approximately 10^3-10^4 cells/ml (Prest, et al., 2016). These microorganisms are in turn impacted by the concentrations of different available nutrients and environmental conditions that may be favourable for select organisms. For instance, a greater concentration of iron and manganese in the distribution network supports the growth and replication of autotrophs, who then outcompete ammonia-oxidising archaea, decreasing their abundance (Hoefel, et al., 2003).

A multitude of research has found bacteria to be the most common microorganisms in potable water, chiefly *Pseudomonas spp*., *Nocardia spp*. and *Sphingomonas spp*. (LeChevallier, et al., 1987). The bacteria within the network can be harmful for human health, including the pathogens *Helicobacter pylori*, *Pseudomonas aeruginosa, Campylobacter spp*. and *Legionella pneumophila*, and can degrade water quality aesthetics and affect DWDS operation, particularly *Mycobacteria gordonae*, non-pathogenic *E. coli* and heterotrophic bacteria (Vaerewijck, et al., 2005) (Fass, et al., 1996). Despite this, most bacteria persisting in the distribution network pose no threat to public health or network operation. Bacteria are also the most intensely monitored drinking water microorganisms monitored worldwide, especially gram-negative bacteria, including coliforms such as *E. coli* (European Commission, 1998) (Drinking Water Inspectorate, 2000). Coliforms are frequently used as an indicator for bulk water quality because they are easy to culture, they can be detected in low numbers and have an abundance that corresponds to the degree of faecal pollution (Pepper, et al., 2015).

Microorganisms can enter the network by being present in source waters and then surviving treatment processes. An example of this is through disinfection with filtration, using filters designed to remove bacteria (sized 1-10 µm), but which other smaller microorganisms, such as protozoan cysts (4-6 µm) and viruses (100 times smaller at 20-400 nm) can pass through (Vreeburg & Boxall, 2007). A further speculated way in which microorganisms enter the network is by contamination from treatment systems, although the following of best practice and proper cleaning should minimise this source (Polychronopolous, et al., 2003). More commonly noted is external contamination of pipe networks due to changes in pressure that cause a flow reversal resulting in contamination of the bulk water known as pipe ingress (Kirmeyer, 2000).

1.4.2.2 Biofilm

Microorganisms can be found not only in a free-living planktonic state within the water body, but also attached to the interior of drinking water pipes in the form of a biofilm network (Fish, et al., 2017). A biofilm is the result of the adhesion and growth of microorganisms, which form a matrix of extracellular polymeric substances (EPS), composed of organic matter and inorganic matter including: polysaccharides, proteins, lipids, glycolipids, nucleic acids and the microbial cells

themselves (Flemming, et al., 2002). These components are intertwined and adhered to the pipe wall by the EPS in a sporadic manner that is described as “thin” and “patchy” (Flemming, et al., 2002).

Biofilms can be described as a reservoir of cells within the network, with cell concentrations of 10^6-10^{11} cells/cm, which is hundreds of thousands of times greater than the concentrations in the bulk water (10^3- 10^4 cells/ml) (Fish, 2013). Multiple studies report that >99% of microorganisms on Earth live in biofilms, although the direct original reference for this statistic cannot be found (Costerton, et al., 1999) (Flemming, et al., 2002) (Adetunji & Isola, 2011). Biofilms within DWDS are generally dominated by gram-negative bacteria but not exclusively so (Percival, et al., 1997). The microorganisms present in biofilms can include pathogens, such as *Helicobacter pylori, Pseudomonas aeruginosa, Cryptosporidium.* The majority of microorganisms present are harmless within biofilms, e.g., *Mycobacterium gordonae*, strains of *E. coli* and heterotrophic bacteria (Flemming, et al., 2002). These microorganisms originate from raw water, with numbers strongly decreasing through the treatment process and multiplying once more within the network at a relatively slow rate, although they can also originate from pipe ingress (Flemming, et al., 2002).

Biofilm can be mobilised if the external shear forces overcome the internal cohesive forces. A theory called the cohesive layer concept, which has been tested both with a Prediction and Of Discolouration in Distribution Systems (PODDS) model and globally validated by experiences within actual networks, describes how layers of organic and inorganic attached material develops on, and is removed from, the pipe surface (Husband, et al., 2016). This means that mature biofilms are stratified with different layers with a defined shear strength profile, including a soft top layer that is easily detached during flushing and a basal layer that is more resilient to it (Paul, et al., 2012). At every increase in applied shear stress there is a further release of accumulated material into bulk water, causing a deterioration of water quality and an increased discolouration risk, if this shear stress is occurring in an unmanaged environment (Husband, et al., 2016). During flushing, some (yet not all) biofilm can be removed so it is a method capable of “cleaning” the DWDS, to an extent. But studies have shown that recolonisation only takes a few hours, meaning that current removal methods may not be effective in the long term (Szewzyk, et al., 2000) (Fish & Boxall, 2018).

1.4.3 The Drinking Water Distribution Chemicals

One way in which water is safeguarded on its journey in the pipes is by the application of distribution network chemicals that additionally have an impact on the distribution system environment and the microorganisms within it. This section will describe these chemicals and their interactions with these parameters.

1.4.3.1 Secondary Disinfectants

Disinfectants are used in water treatment and distribution to reduce the viability of microorganisms in the water. Studies such as that completed by Kärrman et al. (2004) found a 3.5 log (99.95%) bacteria reduction, 2 log (99%) virus reduction and a 0.4 log (60%) protozoa reduction after the introduction of chlorination in bulk water.

Even though disinfectants have a series of disadvantages, as summarised in Table 4, and the choice of which specific residual disinfectant to use depends on a multitude of factors, such as water quality, pipe condition, storage, availability and cost, their use persists.

Table 4 A summary of the use of chlorination and chloramination

Residual	Advantages	Disadvantages
Chlorination	-Effective method of disinfection -Reduced the incidences of worldwide waterborne disease -Cost effective -Been used for many years so is familiar and well understood	-Produces harmful DBPs -Less effective for protozoa -Less effective for biofilm -Is a hazardous chemical to use, store and transport -Taste and odour complaints -Potential for chlorine resistance
Chloramination	-Long residence time -Decreased formation of DBPs -Minimised taste and odours -More effective for biofilm disinfection	-Not as effective as a disinfectant -Slower disinfection -Potential for nitrification -Formation of other less studied DBPs

For the most part, chlorination is a process that has had success over many years to deactivate microorganisms. Although not all organisms are deactivated, it is particularly ineffective for protozoa and biofilms. Studies such as that completed by Kärrman et al. (2004) found a 3.5 log (99.95%) bacteria reduction, 2 log (99%) virus reduction and a 0.4 log (60%) protozoa reduction after the introduction of chlorination in bulk water.

One of the main motivators for investigating disinfection practices is the formation of DBPs. Multiple studies have determined that people who are exposed to chlorinated water over the duration of their life have an increased likelihood of developing cancer, due to the presence of DBPs (International Agency for Research on Cancer, 1991) (Richardson, et al., 2007) (Moghadam & Dore, 2012). Over 600 DBPs have been identified in literature so far, with their own characteristics and complications and new DBPs are being identified on a regular basis.

There is also an emerging concern, although with mixed findings, that chlorine resistance could occur. For example, Sun et al. (2013) isolated, identified and characterised *Sphingomonas* TS001, a newly isolated highly chlorine-resistant bacteria from a model DWDS in Beijing, China. However, preliminary research conducted by Wray (n.d.) stated that with the chlorine doses used at WTW, genetic tolerance to chlorine likely has minimal contribution to coliform survival. It has been found that the application of a chlorine residual may apply selective pressure that favours certain microorganisms over others. For example, a study by Fish and Boxall (2018) found that the abundance of some bacterial classes varied by chlorine strength. Chlorine resistance can also be a factor as to why it is not an effective disinfection method for pipe biofilms. Biofilms that contain high species diversity are more likely to have chlorine resistance and biofilms in distribution networks do have a high species diversity (Morton & Surman, 1994).

Chloramination (generally using monochloramine) is a residual disinfection method that is often selected rather than chlorine. It is formed when chlorine is combined with ammonia in a 4:1 ratio (World Health Organization, 2004). Mainly due to DBP concerns, chloramines are having increased usage in the UK water industry because, when compared to chlorine use, chloramines have been found to reduce the formation of THMs by 40-60% (Kirmeyer, 2004). It can also be favoured for its increased residence time and minimised taste and odour concerns, including those that are

chlorinous (Westbrook & Digiano, 2009) (Donnermair & Blatchley, 2003). Although it is not as effective at disinfecting water as free chlorine, the majority of studies agree that the persistence of chloramine makes it better suited than chlorine for the disinfection of biofilms (Fulton & Budd, 1992) (World Health Organization, 2004) (Van der Wende & Characklis, 1990) (Momba, 1997) (Kirmeyer, 2004).

Chloramine does pose some additional disadvantages. Of particular concern with chloramine use is the increased likelihood of nitrification, the conversion of ammonia to nitrite and then nitrate. When free chlorine (HOCl) is combined with ammonia (NH_3) in a 4:1 ration, the following reactions occur:

$$NH_3 + HOCI \rightarrow NH_2Cl + H_2O$$

$$NH_2Cl + HOCl \rightarrow NHCl_2 + H_2O$$

$$NHCl_2 + HOCl \rightarrow NCl_3 + H_2O$$

When chloramine decays, ammonia is released once more, which can trigger nitrification. Nitrification impacts water quality in multiple different ways. It can reduce the pH, alkalinity and dissolved oxygen concentration of the water within the distribution network, increasing the corrosion of the surrounding pipes, particularly of lead, copper and iron (Zhang, et al., 2009) (UKWIR, 2017). Douglas et al. (2004) as an example, found that the distribution network in the City of Ottawa, Canada had high lead leaching due to nitrification.

One of the reasons why nitrification is negatively perceived is because it can increase the presence of nitrate, which can be a health risk at the customer tap. When found in high enough concentrations, nitrate in drinking water can cause methaemoglobinaemia (blue baby syndrome), a potentially fatal illness in very young children where nitrate is converted to nitrite in the gut and interferes with the absorption of oxygen by the blood (Drinking Water Inspectorate, 2019). This condition was linked with nitrate-rich shallow private wells by Comly (1945) and led to further case studies presenting themselves until 1970 when nitrate concentration limits were put in place by World Health Organization (2011), after which methaemoglobinaemia due to drinking water cases dramatically dropped.

1.4.3.2 Orthophosphoric Acid

To prevent the public ingesting toxic lead and to adhere to regulations, an orthophosphoric acid dose is currently used at ~95% of all WTW in the UK (International Water Association, 2016). Although it was banned to lay fresh lead pipes in 1969 in the UK, old lead pipes are still present, especially in houses built before this date. Approximately 40% of UK homes or ~17 million people are still supplied by lead pipes (European Institution for Testing, Inspection and Certification , 1998).

The lead can be “picked up” in the DWDS in the form of particulate lead due to lead flaking from old pipes or galvanic corrosion products as well as dissolved lead from lead carbonates on the pipe wall. Without treatment, lead would often be present in tap water because of the corrosive effects of water on household plumbing systems which contain lead pipes, solder or fittings. To monitor this, strict regulations of lead concentrations of no higher than 10 µg/l are enforced at the customer tap (World Health Organization, 2011) (European Commission, 2016).

Orthophosphoric acid or a sodium phosphate (e.g., monosodium phosphate) can be dosed to form an insoluble layer of lead phosphate over the soluble carbonate deposits on the pipe, creating a protective layer around the pipes to prevent lead leaching (European Commission, 2016). The advantages and limitations of lead risk reduction methods are summarised in Table 5 (Lamb, 2020).

Table 5 A summary of the use of orthophosphate and lead pipe replacement

Lead Risk Reduction	Advantages	Disadvantages
Lead Pipe Replacement	-A permanent solution to remove the lead from DWDS	-Difficult to find the lead pipes -Lead pipes can be removed but lead can still be present in fittings, fixtures and solder -Cost - Pipe replacement may not be the entire network because it is not all industry owned -Consumers are not incentivised or may not understand why replacement is needed
Orthophosphoric Acid	-A successful method of reducing lead consumption -Aids iron corrosion -Helps prevent discolouration - Currently no alternative treatment method to phosphate dosing for controlling lead as successfully	-Lead failures still occur despite dose -Dose is poorly understood -A continuous dose is required -Diminishing phosphate reserves -Will run out in future -Poor dose optimisation -Poor security of supply -Unknown unintended consequences for public health during consumption over a lifetime -Environmental harm and eutrophication -Cost

Particularly of note within Table 5 is phosphate optimisation, a challenge which aggravates the difficulties surrounding poor security of supply, increasing cost and finite supply. Problems with dose optimisation can occur because phosphate is normally dosed at the treatment works for a large area, meaning that the dose cannot be adjusted for specific water conditions or materials. Smitt and Russell (2013), for instance, said that ~60% of the UK population received phosphate dosed water but did not have lead pipes. Even if lead pipes are present within an area, the concentration of phosphate required to prevent lead leaching is poorly understood. Different conditions can alter the dose required to maintain the corrosion barrier, including temperature and pipe age, condition and history (Bitton, 2014). Due to this, water utilities generally overdose phosphate to ensure they remain compliant with legislation and to prevent customers consuming lead (Lamb, 2020).

Lead pipe replacement is the only long-term solution for ensuring a lead-free DWDS. Despite the cost concerns regarding lead replacement, the main argument for not replacing lead pipes, the whole-life-cost saving from stopping phosphate dosing for a particular geographical area outweighs the cost of replacing communication and supply pipes (Philp, 2019). One potential way of encouraging this discussion is for ownership of household pipes to transfer from the homeowner to the water utility. A change in ownership would allow water utilities to take sole responsibility for the whole network and so make network improvements as a whole. Yet, even under those circumstances, companies require a comprehensive knowledge at a property level, including fittings and fixtures that contain lead, before they can have the confidence required to turn off the orthophosphate dose- a knowledge that is currently lacking.

1.4.4 The Possibilities and Impacts of Chemical Free Drinking Water Distribution Systems

1.4.4.1 Is a Secondary Disinfectant Needed?

There are a number of practitioner arguments as to why a secondary disinfectant is required. This section will examine if it is possible to provide good quality, safe, biologically stable water without the use of a disinfectant.

It could be reasoned that secondary disinfectants are required by the UK (typically 0.5 mg/l or less of free or combined chlorine), to prevent public health risks at the customer tap due to regrowth, pipe ingress and other sources of contamination (Drinking Water Inspectorate, 2010). The UK currently supplies consumers with wholesome safe drinking water, with 99.95% compliance in 2018 with stringent Drinking Water Directive regulations at the customer tap, which includes the application of chlorine-based disinfectants (Drinking Water Inspectorate, 2018). Venkobachar et al. (1977) for instance, evidenced that a higher chlorine concentration resulted in fewer organisms. Bertelli et al. (2018) found similar results, noting that BACMON bacterial counts had a steady but slow decrease in mean total bacterial counts (with a p-value $<2.2 \times 10^{-16}$) between low (<0.1 mg/l), intermediate (0.1-0.2 mg/l) and high (>0.2 mg/l) free chlorine concentration.

Despite this, there is much research, for example that by Pederson (1990) and Stewart et al. (1995), that provides evidence of persistent microorganisms even though chlorine was being used as a disinfectant. For example, Pederson (1990) applied a 0.1 mg/l chlorine residual but found that even after 167 days, microbiology (4.9×106 cells/cm^2) was still present. The biofilm, especially, is an ecosystem that is notoriously difficult to penetrate using chlorine residuals, due to the structure of their polymeric matrix and protective extracellular coating (Prest, et al., 2016). Stewart et al. (1995) found that chlorine was unable to penetrate more than 20% of the biofilm. Behnke et al. (2011) agreed, learning that cells and clusters that had been grown planktonically were more susceptible to disinfection than cells and clusters that had detached from biofilm. So, even though UK drinking water supplied with chlorine is safe to drink, microorganisms within the distribution network persist.

A further reason why a residual disinfectant is used is for water utility reassurance, as an indicator for control of microorganisms. It is used to ensure that enough disinfectant was added at a WTW to provide a residual at the customer tap, suggesting that water is protected from microbial regrowth within the network (Tsitsifli & Kanakoudis, 2018). However, even if chlorine is present and plate count results show 0 CFU, microorganisms can still have a presence. As Liu et al. (2013) said less than 1% of the microorganisms in drinking water can be detected using HPC. It could additionally be argued that the presence of chlorine at the customer tap does not necessarily indicate that the optimum operational conditions are present within that network. The extent to which disinfection is effective is dependent on the distribution network infrastructure, hydrodynamics and components, as well as the characteristics of the water within the network. This includes: the disinfection method used, the maintenance of the residual in the network, the resistance of the microorganisms to disinfection, the variety and concentration of nutrients present in the water, the pipe material and the temperature of the water (Momba, et al., 2000). But this complex environment is difficult to predict and monitor. Further, the current practice of the use of a disinfection residual in the UK is based upon its action against planktonic cells, as only bulk water is regulated because in an operational environment it would be very difficult to sample the pipe wall and so the biofilm (Fish &

Boxall, 2018). This means that although chlorine is well studied, particularly its chemical impacts, the optimum operational conditions for the use of chlorine to impact the biofilm microbiome are largely unknown. Thus, chlorine presence and lack of plate count presence at the consumer tap does not necessarily indicate that microorganisms are not present and operational conditions are optimal.

UK water utilities could argue that it is not possible for them to provide safe drinking water without the use of chlorine. Regardless, a multitude of literature suggests otherwise, that it is possible to provide good quality biologically stable drinking water without the use of a secondary dose of chlorine. Roeselers et al. (2015), for instance, found that bulk water samples taken from both the treatment works and the customer tap across unchlorinated distribution networks in the Netherlands had a similar "microbiota structure", despite this water being transported 0.4-35 km to reach the tap. Linden et al. (2019) actually found the Netherlands, with its lack of a disinfectant had fewer breaches of compliance when compared with England and the USA, which both chlorinated. This equated to breaches of 30 per year when scaled to the Netherlands population, 40 for England and 429 for the USA. Thus, it is possible to supply chlorine free water that is safe to drink.

Possibly the most difficult barrier to overcome is that the provision of chlorinated water should continue because water utilities have used it for disinfection for many years. In as early as the year 1897, the use of a chlorine solution in the British town of Maidstone was used to provide protection after a typhoid fever outbreak (Special Sanitary Commissioner, 1897). Due to this longevity, there are many studies in real networks, as well as industry reports, on the successes of chlorine-based disinfectants and how to best optimise this practice and process (Kärrman, et al., 2004) (Hill, 2014) (Drinking Water Inspectorate, 2010) (World Health Organzation, 2019). If the UK stopped dosing chlorine-based disinfectants today, the impacts would be uncertain. Despite the research presented that argues chlorine free distribution is possible in other countries and in laboratory environments, this is something that has never been done in UK systems before. Other countries manage public health risks by monitoring and attempting to improve biological stability, the indicator of a healthy distribution network environment, through improved physical integrity of the distribution system and careful management of distribution system operations. If this "idealised" network was achieved by the UK and if the active monitoring of biological stability, using the definition outlined in this research, was used, it is still an unknown if safe drinking would be produced using the source waters and treatment methods utilised around the UK today.

One reason why some people say a secondary disinfectant is required, particularly operators of WTW, is because they say it is a legal requirement. One source stated that "Even treatment plants that use non-chlorine disinfection agents are required by law to add small traces of chlorine before distribution" (WaterLogic, 2015). However, a review of legislation and guidance on a worldwide, European and UK level did not find this. WHO guidelines only recommend that a free chlorine residual is required in two specific circumstances (if bulk supplies in tankers are used) but they do not give a recommendation for normal operation. The EU Drinking Water Directive does not mention residual disinfection usage. UK regulations state that all water supplied to premises must be "disinfected", not contain a disinfection residual.

1.4.4.2 The Wider Impact of Discontinuing Orthophosphoric Acid Dose

It could be that the use of orthophosphoric acid over many years has resulted in numerous unanticipated changes in the wider DWDS environment. This section will examine the possible impact of the removal of this chemical on the network.

1.4.4.2.1 Scaling

A further use of orthophosphoric acid is that it can be used as a sequestering agent to "chemically tie up" scale forming ions such as calcium and magnesium. The $P0_4^{3-}$ substitutes for CO_3^2, blocking calcium crystal growth (Knowledge Transfer Network, 2008). This could potentially mean that if orthophosphoric acid was no longer dosed without correct pH control, there could be a build-up of scale in distribution networks. This area, however, is poorly studied.

Scale is formed when magnesium and calcium combine with other minerals dissolved in water, forming a precipitate. The most commonly formed scale is calcium carbonate ($CaCO_3$) but magnesium carbonate ($MgCO_3$), calcium sulphate ($CaSO_4$) and magnesium chloride ($MgCl_2$) can also form scale (Knowledge Transfer Network, 2008). It can lead to problems in the water industry associated with decreased operating efficiency, shortened equipment life, higher cleaning and maintenance cost and increased energy consumption (MacAdam & Parsons, 2004). The development of scale is affected by several factors, including pH, temperature, supersaturation, total dissolved solids and flow velocity (MacAdam & Parsons, 2004).

1.4.4.2.2 Microbiology

If orthophosphoric acid was not dosed in DWDS, it could potentially mean that fewer nutrients would be available for microorganisms which reside in this environment. Several studies show the impact of increased phosphate on the distribution network environment. Juhna et al. (2007) researched the effect of phosphorus addition on the survival of *E. coli* in an experimental DWDS. It was found that the higher phosphorus concentrations of 20 µg/l prolonged the survival of culturable *E. coli* in water and biofilms. A further study found that phosphate also increased the survival and growth of other organisms. Lehtola et al. (2002) found that the number of planktonic heterotrophic bacteria increased 2-4 fold between bulk water containing 0.19 µg/l phosphate compared with bulk water with an added 1 µg/l. The same trial had similar results within biofilm samples, finding that at 0, 1, 2 and 5 µg/l PO_4^{3-}-P (orthophosphate as phosphorus), the numbers of cells found were 3.0 x 10^5, 1.2 x 10^6, 1.3 x 10^6 and 1.5 x 10^6 CFU/cm^2 respectively, meaning that number of cells rose with phosphate increase. Keinänen et al. (2002) found similar but varying results with a change in community due to the addition of phosphate being detected but an increase in overall microbial biomass was not found. This study identified that in biofilms grown for 11 weeks on glass plates, the addition of microbially available phosphate dosages of 0, 1, 2 and 5 µg/l seemed to increase the proportion of gram-negative bacteria, identified by the fatty acids detected and suggested that the number of other microbes might have slightly decreased. Thus, increased levels of phosphate have been shown to prolong the survival of bacteria and change communities in networks by selecting for heterotrophic bacterial growth in bulk water and selecting for gram-negative bacteria growth in biofilms but the link between phosphate and microbial biomass is unclear.

One thing that must be mentioned about the quoted studies that investigate phosphate dose and its impact on microbiology in the DWDS is that these studies have been conducted in countries, such as Latvia and Finland, where phosphate dosing for lead control is not an established practice. This is

why when Juhna et al. (2007) were investigating “high” concentrations of phosphate they selected a value of 20 µg/l (0.02 mg/l), which, for the UK, would be considered low (as an average UK phosphate dose is 0.5 mg/l) (Hayes, et al., 2014). It could be that different findings would be apparent from much higher concentration, an area relatively understudied. Despite this, it must additionally be mentioned that the orthophosphoric acid dose is not a direct measurement of the concentration of microbially available phosphorus, the fraction of total phosphorus (P) which supports microbial growth. So even though water utilities use an average dose of 0.5 mg/l of orthophosphoric acid (H_3PO_4) or orthophosphate (PO_4^{3-}) in the UK, it does not mean that the full phosphate concentration is available for use by the microorganisms present. For instance, in a study conducted by Keinänen et al. (2002), drinking water samples had a total phosphorus content of 2 µg/l but the concentration of microbially available phosphorus was 0.14 µg/l. So, the discrepancies in phosphate concentration used in different countries may not be as great as initially identified (0-5 µg/l or 0-0.005 mg/l, compared with 0.5 mg/l or 0.0005 µg/l) but is still likely to be a vast distinction.

The impact of differing phosphate concentration on the microbiology of the network, although poorly studied in the phosphate concentration used by the UK water industry, has shown the impact and influence of this chemical addition. However, if orthophosphoric acid dosing is stopped in DWDS, it is unknown what the implications would be in the transition period of going from a phosphate dosed system to a phosphate free environment. It is known that heterotrophic bacteria can store polyphosphates in granules, so in periods of external phosphorus limitation they are not limited as they can still utilise polyphosphate kinase for the synthesis of nucleic acids and phospholipids (Lehtola, 2002). But in the longer term it is not known how microorganisms would adapt to this change in phosphate concentrations and the wider impacts of this transition period are also not understood.

1.4.5 Summary: The Drinking Water Distribution System

To ensure water is biologically stable, that it remains the same on its journey from the WTW to the consumer tap, the drinking water distribution system is an environment that must be well understood, monitored and maintained, despite the variety and complexity of this environment, the components and characteristics of the water within it and the microorganisms that exist both planktonically and those attached to the pipe surface in the form of a biofilm.

Water utilities in the UK currently safeguard water on its journey in the pipe system by applying distribution network chemicals. This section argued that secondary disinfectants are not needed by the water industry. It was also identified that the wider impact of discontinuing the dose of orthophosphate is poorly understood. These chemicals are dosed with the intention of impacting their surrounding distribution system environment. As such, without the application of these chemicals, it can be postulated that the DWDS environment will change, although it is not currently understood how, nor can it be said that the present environment is currently understood or well monitored.

1.5 Biological Stability in the Attainment of Chemical Free Water Distribution

Section 1.3 found that one reoccurring parameter that all the case study water utilities agreed as being of upmost importance for maintaining the quality of water at the consumer tap while supplying water with fewer chemicals, was biological stability. There is a possibility that if UK water utilities monitored this concept of biological stability, it could be used to monitor the safety and success of chemical free DWDS. This section will further detail what the term "biological stability" means and how it could potentially be monitored within UK systems.

1.5.1 What is Biological Stability?

The World Health Organization (2011) stated that "Water entering the distribution system must be microbiologically safe and ideally should also be biologically stable." However, the document does not define what is meant by biological stability, which has resulted in different definitions of the term being used by different researchers. There is general consensus that the term "biological stability" in this context refers to the concept of maintaining water quality from the point of drinking water production up to the point of consumption, however, even this is a point of debate in some research.

There are also studies which discuss biological stability but do not state which definition of biological stability is being used. One example is a study by Grefte et al. (2011), which investigates the effect of NOM removal by ion exchange on the biological stability of drinking water. Although biological stability is frequently mentioned and the paper states that earlier definitions of biologically stable water have to be extended, no definition for the term is given for that particular study.

1.5.1.1 Microorganisms as a Direct Indicator of Biological Stability

Rittmann and Snoeyink (1984) first defined biological stability as water that does not promote the growth of microorganisms during its distribution. Lautenschlager et al. (2013) also concentrated solely on the microorganisms present within the distribution network, saying that biological stability means "the concentration of bacterial cells and composition of the microbial community should not change during distribution". This definition takes the composition of the microbial community into consideration, which may not have been done by Rittmann and Snoeyink (1984) due to the lack of sensitivity of methods used to determine biological stability at the time.

A change in bacterial abundance, viability and community composition is a direct indicator of a change of biological stability and monitoring methods for this are rapidly growing more sophisticated. An increase in bacterial abundance can be measured as a change in a specific number of microorganisms (such as but not limited to *E. coli,* total coliforms, *Legionella* and *Aeromonas*) or in the total bacterial community (e.g., by using plate counts, qPCR or flow cytometry). A further direct indicator of a change of biological stability, the viability of organisms, can also be monitored, for example using ATP, epifluorescence microscopy or flow cytometry. To assess the composition of the bacterial community as a direct indicator of change, methods can be based on fingerprinting methods (e.g., DGGE, T-RFLP and flow cytometry) or high-throughput sequencing methods (e.g., 454-pyrosequencing, Illumina, or Ion-torrent). Overall, the sophistication of methods used to detect changes in bacterial abundance, viability and community composition have significantly improved.

A problem with definitions of biological stability solely focusing on the microorganisms present within the distribution network, such as, Rittmann and Snoeyink's (1984) definition and others, including Nescerecka et al. (2014), who described biological instability as "uncontrolled bacterial growth", is that this method can be seen as over simplified. Although these definitions highlight the importance of microorganism growth during distribution for biological stability, they do not take other water quality parameters into consideration, such as the distribution conditions, ingress, pipe material etc. In the case of the definition by Lautenschlager et al. (2013), bacterial cells specifically are mentioned, which does not encompass other microorganisms, such as fungi. Also, it could be said that the composition and concentration of cells will change during distribution, when perhaps the emphasis of this definition should have been on significant changes.

1.5.1.2 Predictive Indicators of Biological Stability

Another way of assessing biological stability in a drinking water system is by using predictive methods, to complete analysis before distribution to predict the extent of growth during distribution.

One predictive method for assessing biological stability is the evaluation of the growth-promoting properties of drinking water, for example by monitoring AOC, BDOC or other forms of organic carbon as the growth-limiting substrate in drinking water (Prest, et al., 2016). An example of a study which described biological stability in this way was completed by Zlatanović et al. (2017) who defined biologically stable water as being nutrient limited, with low concentrations of AOC (<10 µg/l). This definition is different from others in that it states what is an acceptable concentration of AOC, however it focuses too heavily on organic carbon and not on other factors, such as bacterial regrowth. Furthermore, other compounds also control microbial growth, such as phosphate, ammonium, manganese and iron and these compounds are not being considered by these methods of assessing biological stability.

There are other predictive methods for assessing biological stability, for example the use of an assay to determine the growth-promoting properties of materials in contact with drinking water. This can be done in a variety of methods e.g., measuring the dissolved oxygen consumed, calculating the biofilm formation potential using ATP or assessing the biomass production potential using AOC. Van der Kooij (2000) defined biological stability as the inability of water, or a material in contact with water, to support microbial growth in the absence of a disinfectant. In this definition, both the water itself and the materials in contact with water (e.g., the pipe wall) are considered to have an impact on biological stability. However, also added is "in the absence of a disinfectant", which potentially implies that if a disinfectant is used, biological stability will not be achieved. A further problem with this definition is that it focuses on an absence of microbial growth, rather than minimal microbial growth, which is unlikely to be achievable.

Another predictive indicator for biological stability in a drinking water system is that of customer complaints and the aesthetics parameters of drinking water. Prest et al. (2016) stated that the aim of biological stability is to have minimum change in water quality during distribution to not affect consumer safety, consumer aesthetic perception or cause technical failure. This is a unique definition, with more of an industry focus, which does indicate that biologically unstable water can have negative consequences for customers. However, there are water quality changes that customers are unable to identify, which this definition does not account for.

1.5.2 Biological Stability Assessment

Section 1.5.1 described that there is much debate about the "correct" definition of biological stability but that a multi-level approach for the monitoring of biological stability is preferable in the very best definitions of the phrase. This section will further explore different methods of assessing and monitoring biological stability for use in the UK.

1.5.2.1 Heterotrophic Plate Count

Microbial water quality in the UK has traditionally focused on the cultivation of coliform and faecal indicator bacteria, such as *E. coli,* from samples leaving the treatment works and at the customer tap. Historically, enumerations of the heterotrophic bacteria present in water supplies has been completed by incubating these bacteria on a nutrient-rich media at 37°C for two days (2 day colony count) and 22°C for three days (3 day colony count). The incubation at greater temperature was done to encourage the growth of bacteria that thrive at body temperature, rather than bacteria present at normal environmental temperature, in an attempt to use the 2 day colony count as a more sensitive indicator of ingress. In 1998, the Drinking Water Directive (1998) was amended in that the requirement to enumerate colony counts at 37°C was no longer prescribed, as was the 2016 amendment to Schedule 2 in the Water Supply (Water Quality) Regulations (Drinking Water Inspectorate, 2016).

Today, regulations require the monitoring of *Enterococci* and *E. coli* at the consumer tap and coliform bacterial and *E. coli* at service reservoirs and WTW. Heterotrophic plate counts are currently used worldwide as an indication of pathogen removal but can also be used as a way of monitoring biological stability. The World Health Organization's (2011) accepted guideline for HPC is 20 CFU/ml for treated water and 300 CFU/ml for distributed water.

By focusing on the cultivation of coliform and faecal indicator bacteria, such as *E. coli*, this assessment method centres around the risk of infection as the main health concern. Reeslev et al. (2011) stated that risk of infection is not only dependent on infections from pathogenic bacteria, but also that a high number of bacteria in general can cause other health effects. For example, endotoxins, part of the outer cell membrane of gram-negative bacteria, can be present in drinking water and can cause other conditions such as hypersensitivity pneumonitis, allergy and inflammation (Reeslev, et al., 2011). Despite this, other research by Anderson et al. (2002) discussed that there was not sufficient information to quantify the potential health risks from the consumption of endotoxins in tap water, although a risk to consumers could be identified from the inhalation of these endotoxins (Reeslev, et al., 2011). Endotoxins in tap water is just one example of how other organisms not necessarily categorised as pathogenic can still pose a risk of infection. As such, although plate counts is an important method to assess public health risk, it is important to take a broader approach and enumerate other microorganisms beyond *E. coli.*

One problem with using plate counts for determining biological stability is that only a small proportion of metabolically active microorganisms in a water sample can be grown and so detected, depending on the environmental conditions (Lautenschlager, et al., 2013). The species successfully grown will vary depending on environmental conditions, for example the location the sample was taken from and the season (Lautenschlager, 2011). One example is that waterborne pathogens of concern such as *E. coli*, *Helicobacter pylori* and *Vibrio cholerae* can exist in a viable but non-cultivable state, meaning that culture-based methods may yield false negatives (Hassard & Whitton, 2019). Liu

et al. (2013) said less than 1% of the microorganisms in drinking water can be detected using this method, which is a problem in assessing biofilms because they are complex environments containing many microorganisms. Whereas, using a method such as flow cytometry ensures that all planktonic microbial cells can be detected, irrespective of their cultivability, making it a useful alternative to heterotrophic plate counts.

There are also practical limitations with plate counts. It is a time-consuming method which takes 48-72 hours to get a result (Corfitzen, et al., 2006). Corfitzen et al. (2006) stated that before the HPC results are available, elevated bacteria levels would be likely to have reached the customer tap.

1.5.2.2 Assimilable Organic Carbon

Organic carbon is a key contaminant in a variety of chemical and microbiological reactions within pipe networks yet is not well studied outside of water sources and treatment works. Total organic carbon (TOC) consists of: dissolved organic carbon (DOC), particulate total organic carbon, biodegradable dissolved organic carbon (BDOC) and assimilable organic carbon (AOC) (Pick, et al., 2018).

AOC is a sub fraction of TOC that can be used as a measurement of the nutrients available for heterotrophic bacterial growth and so is an indirect measure of biofilm growth and detachment (Tsai, 2005). AOC concentration can be monitored to better understand bacterial growth and regrowth throughout a system. PWN in the Netherlands have actively used AOC for the past 20 years and so the variability in levels is now well understood in that country but due to it being easily contaminated, they are now moving away from it to focus more on ATP and flow cytometry (Isle Utilities, 2015).

AOC is measured using a bioassay proposed by Van der Kooij in 1978, although modifications to this technique have been made over the years in an attempt to improve it (e.g., to increase the speed of this technique and to reduce the risk of cross-contamination) (Van der Kooij, et al., 1982). The method involves the inoculation of a water sample with a variety of microorganisms. The organism growth is monitored and expressed as an equivalent yield of the same bacteria grown in a known concentration of acetate carbon. So, it is based on a bioassay where two types of bacteria are used to measure the growth yield on different substrates.

Van der Kooij (2000) found that AOC values below 10 µg/l were indicative for drinking water with a limited regrowth potential for bacteria contributing to HPC values. Due to this research, biologically stable water is said to have an AOC concentration of <10 µg/l (Isle Utilities, 2015). However, even if AOC values below 10 µg/l may limit regrowth in the network, it does not prevent regrowth and there are some conflicting results regarding the "<10 µg/l" value and, specifically, *Legionella* presence. Some studies such as the work of Van der Kooij et al. (2005), have found that *Legionella* was able to grow even with very low AOC levels (2-6 µg/l). Similar findings were reported by Evides (Isle Utilities, 2015) a water utility in Rotterdam in the Netherlands, who detected that *Aeromonas* levels increased without a change in AOC. These studies conflict with the work of Van der Kooij and Van der Wielen (2013), who reported a trend that linked lower levels of AOC to greater *Legionella* control. Further research provides a middle ground between those that suggest AOC levels do not relate to all microbial growth (Van der Kooij, et al., 2005) and those that do (Van der Wielen & Van der Kooij, 2013). Vital et al. (2010) remarked that AOC concentrations were a useful measurement of net growth, that the quality and composition of AOC is critical for pathogen growth, but only to

some extent. Limiting AOC may reduce regrowth but does not prevent it, so monitoring AOC concentrations is a good indicator of regrowth but should be used with other biological monitoring methods.

A problem with AOC monitoring for assessment of biological stability is that samples are easily contaminated by common substances (e.g., the deodorant of the sampler or analyser), so all equipment associated with the sampling and analysis needs to be extremely clean or there is limited reliability and reproducibility in the results. All equipment used must be free of carbon, meaning that either a muffle furnace that can heat to 500^{o}C is required to remove the carbon from or glassware or carbon free glassware must be purchased specifically, adding to the costs and practical difficulties.

A further difficulty with the use of AOC analysis is that the sample turnaround time is 8 days. Even though incubation of the *Pseudomonas* and *Spirillium* species takes most of this time, enumeration is required on days 6, 7 and 8, meaning it is a relatively time intensive process for analysers. Therefore, even though there have been many adjustments and improvements to the original AOC method, it is still time consuming and requires multiple days of laboratory analysis per sample (Pick, et al., 2018).

1.5.2.3 ATP

ATP is a nucleotide contained within each cell which transports and stores energy required by organisms to function. ATP can be measured using bioluminescence and this bioluminescence measurement can provide an indicator of bacterial activity.

Liu et al. (2013) described the increase in the popularity of detection methods not reliant on cultivation, such as ATP and flow cytometry, due to them being: “accurate”, “rapid”, “easy to perform” as well as their ability to quantitatively detect both culturable and unculturable microorganisms. Prest et al. (2016) also found ATP to be a useful method of evaluating the viability of bulk water bacteria. In this study the temporal dynamics in bacterial community characteristics were investigated in 363 samples taken biweekly over two years at a Dutch WTW final water and in the distribution network. It was found that there was a good correlation between ATP and flow cytometry, displaying clear seasonal variation, but not with HPC and *Aeromonas* counts. This weak relationship could be due to ATP being an indirect biomass quantification method or could be a result of the lack of sensitivity in HPC.

Lautenschlager et al. (2013), too, found that ATP correlated well with flow cytometry and that ATP was a good indicator of viable biomass. The study did note that ATP could be affected by viability, activity, cell type and cell size. ATP measurements are especially ineffective with small cells, as they have low nucleic acid content and produce low amounts of ATP. Instead, Lautenschlager et al. (2013) suggested a multi-level approach was the best method of assessing the risk of pathogen growth in water. Studies, as well as practice by PWN (Netherlands), have shown that the ATP method of assessing biological stability does correlate well with flow cytometry, although it does have a weak relationship with HPC, potentially because ATP is an indirect biomass quantification method or because of the lack of sensitivity in HPC (Liu, et al., 2013).

A further problem with ATP is that, since ATP is present in all animal, plant, bacterial, yeast and mould cells, there is the potential that these sources could contaminate ATP measurements when quantifying bacterial presence (Prest, et al., 2016).

1.5.2.4 Flow Cytometry

An alternative method for the assessment of biological stability is flow cytometry. A flow cytometer is as "an instrument that measures characteristics of cells or particles as they pass through a light source" as such it is a method capable of the enumeration of total cell concentrations (Wilkerson, 2012). The cell components are fluorescently labelled and then excited by a laser to emit light at varying wavelengths, which is then measured and quantified. It has many different applications but can be used to measure the change in time of bacteria count or biofilm presence. It is a technique capable of detecting all planktonic microbial cells irrespective of their cultivability and can also be used to determine cell viability to differentiate between live and dead cells (Hoefel, et al., 2003).

Flow cytometry is a valuable tool for detection, enumeration and characterisation of waterborne microbial populations (Hassard & Whitton, 2019). This technique is faster (at ~10,000-50,000 cells/second) than plate counts, microscopy analysis and AOC, is more sensitive than culture-based methods, yields a wealth of information about microbial characteristics and viability that techniques such as ATP and HPC do not provide and is a relatively simple analysis type that is not at risk of contamination like ATP and AOC are. An advantage of flow cytometry is that it ensures that all planktonic microbial cells can be detected, irrespective of their cultivability, making it a useful alternative to heterotrophic plate counts, which are unable to do this. It is also able to determine cell viability, differentiating between live and dead cells, but good training is required to be able to determine which cells are intact and which are damaged (Prest, et al., 2016).

There has recently been an increase in popularity of cultivation independent detection methods, such as flow cytometry due to it being accurate, quick, easy to use and quantitative (Liu, et al., 2013). In Switzerland, flow cytometry is accepted as a tool for compliance assessments of drinking water and the Netherlands are now moving away from AOC to focus more on ATP and flow cytometry (Isle Utilities, 2015). A further example of the use of flow cytometry is that the Swiss government recommends the Swiss Gas and Water Association's published official methods (based on a revision of the Switzerland's Federal Office of Public Health, named Schweizerisches Lebensmittelbuch) of using flow cytometry to obtain total microbial cell counts to analyse water in drinking WTW, drinking water distribution systems and household plumbing (Hassard & Whitton, 2019). In the UK there has also been increased interest in this technique, with universities and water companies expressing an interest in organising and attending flow cytometry working group meetings to work collaboratively to share their knowledge and best practices for use of this technology type (Hassard & Whitton, 2019). Specifically, the use of total cell counting (TCC) and intact cell counting (ICC) using flow cytometry is a technology being increasingly used in water utilities. There is interest to increase its usage further in future, with potential to use it at the consumer tap for regulatory samples (Gillespie, et al., 2014).

Liu et al. (2013) completed a year-long study into two distribution systems in The Netherlands, evaluating the application of flow cytometry to assess microbial growth in DWDS. The study found that flow cytometry was "a valuable parameter to assess the drinking water biological quality and regrowth; it can directly and sensitively quantify biomass, detect small changes and can be used to determine the subgroup of active HNA bacteria that are related to ATP" (Liu, et al., 2013). Prest et al. (2016) also praised the technique, describing flow cytometry as "an alternative microbial monitoring method for rapid enumeration of the total number of bacterial cells, evaluation of cell viability and fingerprinting of bacterial communities in water samples".

1.5.2.5 Water Age

It could be argued that water age could be an indicator for the biological stability of a given network. The DWDS environment comprises of a variety of infrastructure, hydraulic conditions, environmental parameters, microbiology and chemicals, all of which interact to have an impact on the biological stability within these systems. Thus, it could be said that the longer water is in contact with the distribution network material, as well as the biofilm residing on it, the higher the propensity for water quality to be impacted (Machell & Boxall, 2012).

A number of studies have shown the impact water age has on water quality. The American Water Works Association (2002) said that water age is a major factor in water quality deterioration as it influences taste and odour, corrosion rates, material precipitation, discolouration, disinfection-by-product formation and biological activity. Seker et al. (2012) found water age a feasible and arguably potentially useful indicative surrogate for water quality. And Burllingame (1995) found older water was more corrosive to iron pipes.

Research has found that water age also has an impact on distribution microbiology. Keinänen et al. (2004) completed a trial into biofilms as a function of water residence time and development time. They found that viable microbial biomass, estimated using phospholipid fatty acid methyl esters profiles, increased but microbial communities were stable with increasing water residence times of 10-141 h. An experiment completed by Srinivasan et al. (2008) agreed with this, with conclusions suggesting that as the residence times increased to 8.2 h, 12 h, 24 h and 48 h, the median percentage of bacteria present in bulk water increased to 7%, 37%, 58% and 88% respectively. Keinänen et al. (2004), too, stated that as the average daily consumption of drinking water had continuously decreased in Finland, the distribution system water residence time has increased, further noting an impact of which was enhanced microbial growth. Sfynia (2017), in a study of three distribution systems in the Anglian Water network, found there was a higher level of haloacetic acids (HAAs, a DBP) biodegradation at higher water ages, which could either have been due to bacterial activity in the water, biofilm growth in the pipes or chemical dehalogenation due to the water contact with the unlined cast iron pipes of the distribution network.

However, not all research agrees that water age has an impact on the microbiology within a distribution network. Kerneïs et al. (1995) found that water residence times from 0-107 h did not have a significant influence on bacterial heterotrophic plate count densities within a water distribution system. A study by Seker et al. (2012), too, did not reveal a significant relationship between total bacterial numbers or community composition and mean or maximum water age. However, the sampling location chosen during this study had high residence times and a short pipe length resulting in low flow conditions which may have influenced microbial abundance. There was variance due to hydraulic conditions on weekly and daily cycles. This suggests that water age would not have been an adequate measure of water quality in these instances.

One reason some research suggests more biological growth may be present with increasing water age is because of reduced disinfection residual, rather than due to the water age itself. Older water may have less of a disinfectant residual because of substance decay and reactions with network materials (Rossman, et al., 1994). Experiments completed by Machell and Boxall (2014) found that free chlorine and total chlorine decreased as water age increased. This reduction in biocide efficiency could promote biological activity that results in unpleasant taste and odours (Machell &

Boxall, 2012). Sfynia (2017) said that the total THM concentration increased, and chlorine residual decreased as water age increased.

Further investigations state that decreased disinfectant residual due to water age does play a part in reduced water quality, along with other factors. Machell and Boxall (2012) found that decreased disinfectant residual, combined with the transportation of particulate matter and an increase in water temperature present in locations with long residence times, results in the network in these areas being vulnerable to bacteriological regrowth. The research summarised by saying there was a relationship between water age and the associated water quality, however neither the mean water age nor the maximum water age fully explained the water quality changes identified during the study, suggesting that other factors were at play. Machell and Boxall (2014) investigated the relationship between water age and water quality. The results found some relationship with water quality and mean water age, with pH, temperature, 7-day plate counts and iron increasing as water age increased (although there were unexplained deviations). In this study the actual condition of individual pipes, the interaction of plastic and metallic pipes, water age and treatment legacy, combined, influenced overall water quality behaviour more than water age alone.

It is important to have a good understanding of water age in DWDS because it does have an impact on water quality. Nevertheless, water age does not appear to be a sufficient guide to general water quality or biological stability alone because of the importance of other factors, such as: treatment legacy, mixing effects, reduced disinfection residual, maximum age volume contributions, hydraulic conditions on weekly and daily cycles, water chemistry, temperature, the transportation of sediments, pipe material, the interaction of different pipe materials and pipe geometry.

1.5.3 Summary: Biological Stability in the Attainment of Chemical Free Water Distribution

Chemical free water may well be possible in an idealised well monitored system but what is essential is doing it in such a way that the water produced is also safe. In countries such as the Netherlands, consumer safety is interpretated with the concept of biological stability. This section has shown there are lots of different definitions of biological stability. This concept can also be assessed with numerous different methods, as summarised by Table 6. Of these techniques, flow cytometry appears to be a successful instrument for the detection, enumeration and characterisation of waterborne microbial populations (Hassard & Whitton, 2019). This technique is fast (faster than plate counting, microscopy analysis and AOC), quantifies many microorganisms (more than plate counts and ATP), provides an indicator of viability (that plate counts and ATP do not) and is robust in that sample contamination is unlikely (unlike ATP and AOC).

But it is unknown if this concept applies to UK systems, if biologically stable water currently exists within the UK or if the existing application of a chemical dose disrupts this. It is not known if it would be possible to use one of the innovative assessment methods discussed to supplement regulatory samples. Doing this in the idealised pipe system could help to better understand microorganisms in DWDS in a way that encompasses both the well-studied bulk water and the biofilm.

Table 6 A summary of biological stability monitoring methods

Method	Description	Speed	Limits	Advantages	Disadvantages
Adenosine Triphosphate (ATP)	Measurement of ATP in a cell through reaction with a bioluminescent complex.	Minutes	N/A	-Fast. -Simple. -Cost-effective.	-No viability information at the single-cell level. -Samples can easily be contaminated.
Assimilable Organic Carbon (AOC)	Assay that provides an indication of the capability of water to support microbial growth.	Days	N/A	-Method is being improved. -Includes a choice of technologies.	-Time-consuming. - Samples can easily be contaminated.
Enzyme-Linked Measurement	Measurement of fluorescence based on the reaction between bacteria present and an enzyme substrate.	Hours	1 CFU/ 100ml	-Useful for the quantification of water quality.	-Few reliable studies.
Epifluorescence Microscopy	Illumination of a sample with fluorescent light to do a visual inspection of particle characteristics.	Hours	N/A	-Provides additional information about cellular morphology, cellular damage and staining efficacy.	-Time-consuming and labour-intensive. -Highly subject to human error.
Flow Cytometry	Measurement of the characteristics of cells or particles as they pass through a light source to enumerate total cell concentrations.	Minutes	116.4 cells/ml	-Can provide rapid and accurate measurements of TCC and ICC in water. -Can differentiate between live and dead cells.	-No legislative standard exists.
Heterotrophic Plate Count (HPC)	Measurement of the heterotrophic microorganism population in a water sample.	Days	N/A	-Has been used widely as well as historically. -Confirms the presence of viable bacteria.	-Takes time. -High variability. -Only about 1% of bacteria in drinking water are detectable.

Adapted from Hassard and Whitton (2019) and Safford and Bischel (2019)

1.6 Summary: Literature Review

This literature review assessed the current and best practices of the provision of drinking water to identify the various knowledge gaps, the areas where future research is required before chemical free drinking water treatment and distribution is a possibility. A key finding was the importance of microbiology within distribution networks for the provision of safe drinking water. The distribution network was chosen as the ideal research area because it is relatively poorly understood in the water industry, there is the greatest discrepancy between countries using and not using chemicals for distribution, there is large room for improvement and the chemicals being dosed in this environment have their own specific challenges. There are a multitude of factors that currently impact water quality in the distribution network, many of which are understood, the easiest of which are well monitored and some of which are maintained. A thorough understanding, regular whole-system monitoring and network maintenance of both the DWDS environment and the microbiology within it are required to manage the chemical dose of the network in such a way that public safety and regulatory compliance is possible.

One area of the distribution network that is of the upmost importance to public safety but is also relatively not well understood is that of the microbial water quality, the interactions between various microorganisms that exist both planktonically and attached to the pipe surface in the form of a biofilm. This is because the current method of monitoring microorganisms in drinking water is by using heterotrophic plate counts, which are based on bulk water samples. These methods do not account for the complex and numerous organisms present, focusing only on the heterotrophic bacteria they are designed to capture. This means that they are a poor indicator of how the biology is changing between the treatment works and the customer tap, how biologically stable the system is.

A chemical that is added to this environment is the use of a disinfectant residual to remove or reduce the viability of microorganisms in the transported water. Despite the current popularity of chlorine and chlorine-based disinfectants, a series of disadvantages, predominantly the emerging understanding surrounding the formation of harmful disinfection by-products, are causing water utilities to reconsider their traditional practices. Chlorine was first added to networks in a time when only bulk water was understood, meaning that although it is a relatively effective disinfectant for bulk water, its impact on the biofilm is comparatively unknown. Microorganisms can persist in networks, despite the addition of different forms of residuals, particularly in the form of biofilms. Instead, the application of a disinfection residual may apply selective pressure that favours certain organisms over others, changing community structure in the distribution network, rather than impacting abundance as is desired from its application. Further, it could be argued that secondary disinfectants are not needed by the water industry as microorganisms persist in this environment, the water utilities of other countries can produce safe drinking water without the use of a residual chlorine dose.

Another chemical that is used by the UK water industry to maintain water quality in the pipe network is that of orthophosphoric acid. Lead is present in the homes of ~17 million people and difficulties regarding pipe ownership and the financial responsibilities that come with this make it difficult to remove. Orthophosphate dosing is a successful method of reducing lead content at the customer tap but there are many problems with its use, chief of which is that phosphate is a finite

resource that will run out at some point in the near future. Even if water utilities had the ownership of the distribution network to be able to remove the lead, comprehensive knowledge would be needed at a property level, including fittings and fixtures within the household, to be confident all lead was gone, and the dose of orthophosphate could be discontinued. If this were possible, the wider impact on the distribution network is unknown. One effect the addition of orthophosphate has on the network is the provision of a nutrient source for microorganisms, influencing microbial community changes and enhancing organism survival rate. If phosphate dosing were to stop, it is uncertain what impact there would be on the network and how biological stability would be affected.

These chemicals that are added to an already complex and under-studied environment are clearly having unintended impacts on the distribution network, particularly on a microbial level. The understanding of the interactions of these distribution chemicals, chlorine and phosphate and their impact on biological stability is limited. If the UK water industry wishes to achieve a chemical free drinking water system that is safe for consumers, the wider impact on the network, including the impact on water quality, of these distribution chemicals would have to be studied.

2 Chapter 2: Aims and Objectives

The overall aim of this PhD is to improve the understanding of the wider impact of the chemicals, phosphate and chlorine, on the drinking water within the distribution network in the UK, using case study live areas. This will be explored across the largely unknown but central underpinning role of microbially mediated processes and the concept of biologically stable water. This research will assess the biological stability of case study areas, establish how chemicals are being used in the current system and take the first steps in determining if a water quality impact is likely if these distribution chemicals are not used with the current systems in place.

This wider aim has been explored in this thesis through three key aims and their associated objectives. Figure 1 details how these aims and objectives are structured within the thesis.

1. To develop and refine a definition of biological stability applied across 6 different case study networks to ascertain the degree of biological stability variation in UK distribution networks using available currently collected data from the WTW and Tap.
    a. To develop and refine a definition of biological stability applicable to UK systems.
    b. To select appropriate water quality parameters, sampled at the WTW and at the consumer tap, to act as indicators of biological stability in the network.
    c. To determine the degree of biological stability variation across 6 different case study networks.

2. To understand the distribution chemical dose applied at WTWs and the impacts of this current chemical usage in 6 different case study networks using available historic data from the WTW and Tap.
    a. To investigate the differences in how phosphate and chlorine are utilised in 6 different case study networks.
    b. To explore the wider water quality impacts of current chemical usage and if this is sufficient rationale for their use.
    c. To determine how the concentration of phosphate changes during distribution as an indicator of dosing practices.
    d. To compare system performance across sites of varying biological stabilities and chemical dosing regimes.

3. To investigate the impact of both chemical dosing and not chemical dosing in the perfect pipe network at two case study sites with differing biological stabilities, by designing and constructing innovative bespoke pipe test loops.
    a. To determine the role of chemical dosing on water quality with respect to biological stability as identified in Aim 1.
    b. To investigate if there would be a significant risk to water quality if a chemical dose was not applied at two case study sites with differing biological stabilities.

3 Chapter 3: An Assessment of Biological Stability in UK DWDS

This chapter addresses Aim 1, Objective a, b and c outlined in Chapter 2. This will be completed through data analysis of historic regulatory samples at the two live network sampling points, the Final Water (the treated water leaving the WTW and entering the distribution network) and the Customer Tap (samples obtained from randomised consumer taps), as well as a novel Final Water three month sampling campaign.

3.1 Introduction: An Assessment of Biological Stability in UK DWDS

Section 1.3 detailed a comparison from source water to the consumer tap of case study companies which use fewer chemicals than the UK, finding that biological stability was of upmost importance to supply water with fewer chemicals. Section 1.5 further detailed the many different definitions of the term "biological stability" and how it could potentially be monitored within UK systems.

To maintain biological stability within UK systems it is first necessary to define biological stability to ensure it can be appropriately monitored. As there is such a variety of definitions, using and combining the advantages of previous definitions, a new definition of biological stability was necessary for the purpose of the PhD, as well as the testing of this definition to determine the best method to measure and monitor biological stability in the UK today.

The literature identified key parameters for assessing biological stability, including the monitoring of: the change in microbial abundance, viability or community composition; the concentration of chemical contaminants to estimate microbial growth and the physical and chemical properties of water to asses for water quality changes over time. If biological stability is key for the attainment of chemical free water, these parameters and their causative relationships within drinking water distribution systems, outlined in Figure 3, will require investigation.

To test the novel proposed definition of biological stability, water quality samples that water utilities obtain from Final Water and at the Customer Tap were used. These samples comprise of highly valuable data that provide an insight into realistic network conditions and increased understanding regarding the variability of different systems. Not only does this data have a multitude of samples temporally, as water utilities have a historic backlog of data that can be attained but also spatially, as the whole breadth of a water utility area is sampled, rather than one small case study. It is a well-established practice within the water industry that the Final Water and Customer Tap are used to infer network condition and quality rather than this being directly perceived, due to the contained nature of pipes.

Using this historic data supplemented with a flow cytometry analysis (following Section 1.5 and its discussion of flow cytometry as an emerging technologies for monitoring and measuring biological stability), this chapter aims to investigate these parameters and their causative relationships by providing a preliminary definition of biological stability, testing this definition on a series of 6 case study sites and then providing a refined definition of biological stability informed by the obtained results.

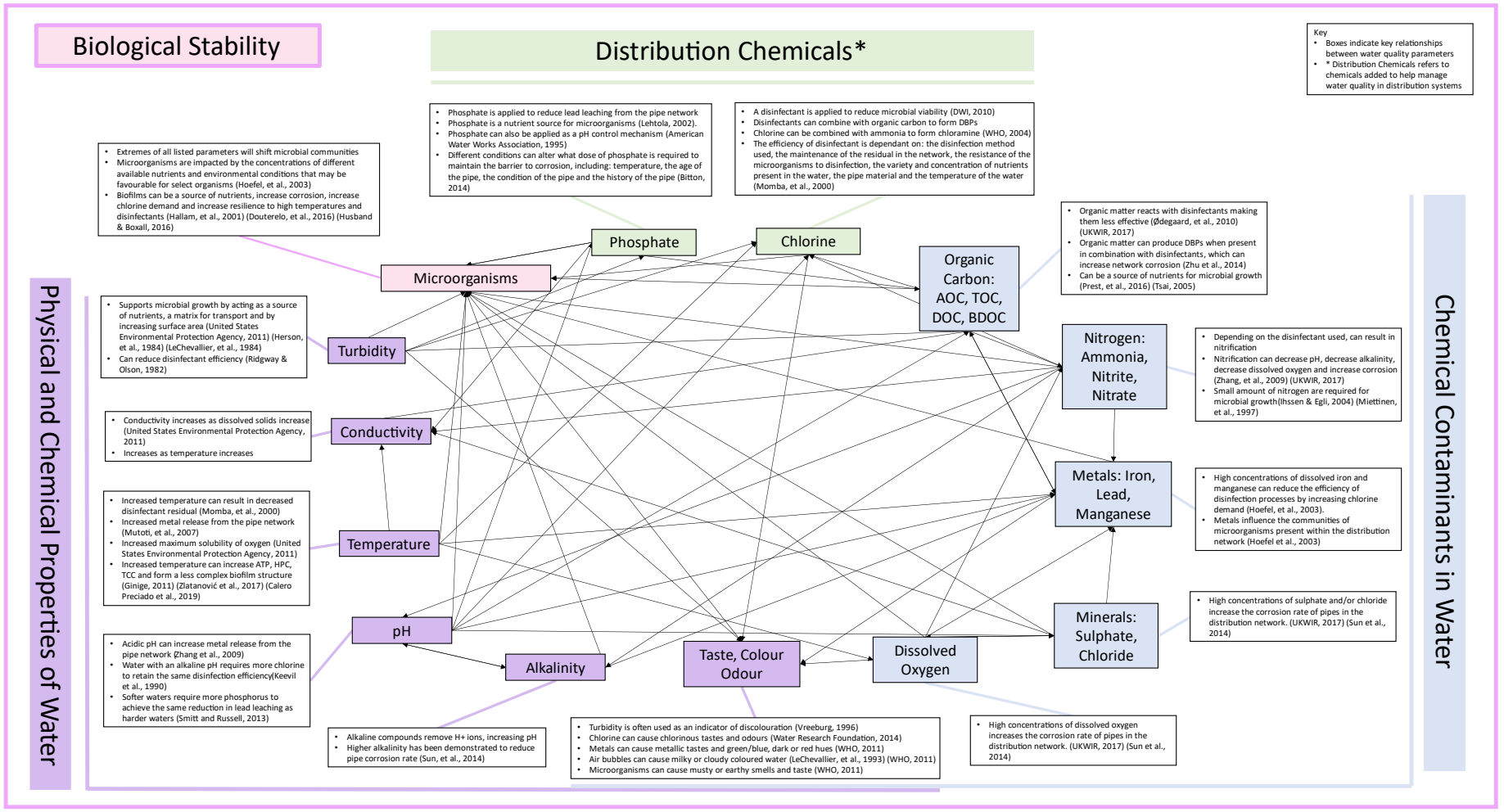


Figure 3 Expected causative relationships between the parameters the literature review found to be of importance for the attainment of chemical free water

3.2 Methods: An Assessment of Biological Stability in UK DWDS

3.2.1 The Formation of a First Proposed Novel Definition of Biological Stability

Using and combining the advantages of previous definitions, with particular attention being paid to research that suggests a multilevel approach helps to coalesce the different advantages of assessment methods (Lautenschlager, et al., 2013), a new definition of biological stability has been proposed in the below section. This definition was composed to not only learn from the best practice of previous literature but was also adapted to ensure it was appropriate for current practice in the UK water industry today.

3.2.2 First Proposed Novel Definition of Biological Stability

Biological stability can be defined as the preservation of water quality from the WTW to the consumer tap, with good biological stability being characterised by lower values and/or consistent values in a variety of key indicators including: both the attached and planktonic microorganisms of the distribution network; available nutrients; wider environmental water quality parameters and the aesthetics of the water.

The analysis of water quality should be performed with a combination of methods to analyse microbial and environmental parameters. The following combination of methods is suggested:

- Microbial parameters: TCC, ICC, 3 day colony count, 2 day colony count, coliforms, *E. coli, Enterococci.*
- Nutrient concentration: total organic carbon, phosphate, nitrate, nitrite, ammonia.
- Wider water quality parameters: total chlorine, free chlorine, turbidity, water temperature, water chemistry properties (pH, hardness, alkalinity, conductivity), metals (iron, manganese, lead).
- Aesthetic parameters: taste, odour, colour.

It is also acknowledged that other factors can affect the biological stability during distribution, including: pipe material, hydraulic conditions, residence time, residual disinfectant decay, construction, operation and maintenance practices and premise plumbing conditions.

3.2.3 Regulatory Sampling

3.2.3.1 Regulatory Sampling Protocol

Historic samples obtained for data analysis were taken as regulatory samples, the daily Final Water samples from every WTW and Customer Tap samples collected from random addresses in each water supply zone of 4-240 a year per zone depending on population size and parameter [Table 2] required by regulation (Drinking Water Inspectorate, 2016). Customer Tap sampling and Final Water sampling is an established method of inferring the drinking water quality in the network.

Customer Tap samples were taken according to Anglian Water regulation PSW-PRO-8.13 (Anglian Water, 2020). When access was gained at customer properties, a suitable kitchen tap for sampling was identified (one that is mains fed and not a private supply, is not softened or filtered and is the tap most commonly used for drinking purposes by the customer), the sampling could begin.

Samples for the analysis of trace organics (500 ml), chemicals (500 ml and 330 ml), metals (125 ml) and nutrients (125 ml) were taken in this order, both as spot samples (no flush) and as post flush samples (following a 2 minute flush). All sample bottles were filled to the brim to prevent trapped air, except metals samples and bacteriological samples, where the bottle was filled to the neck, leaving a small air gap. All bottles were rinsed prior to fill, except for organics and bacteriological samples. After a minimum 2 minutes of tap flushing, chlorine meters and turbidity meters were used to obtain a free chlorine, total chlorine and turbidity measurement.

Following a 2 minute flush, pre-disinfection bacteriological samples (500 ml) were taken. The customer tap was then disinfected using a 1% sodium hypochlorite solution. The solution was left on the tap surface for 2 minutes. Following this, a 2 minute tap flush was completed. After this flush, the tap surface was re-soaked using a 1% sodium hypochlorite solution for a further 2 minutes. Finally, a last 2 minute tap flush was completed and the post-disinfection bacteriological sample (500 ml) was taken.

Anglian Water Final Water sample taps were also taken according to Anglian Water regulation PSW-PRO-8.13 (Anglian Water, 2020). Before any samples are taken, it was checked that the tap used was representative, the sample point code and description match the desired sample location and the tap was in a satisfactory condition.

Sample collection at this sample point were similar to that at the customer tap except this location additionally has a spot bacteriological sample (500 ml). Also, the flushing time was not 2 minutes but was instead determined by the sample point label and certificate for timings, which were defined by the length of the pipe network leading to the sample tap. A final difference was that at Final Water sample points, the sample tap was flamed using a blowtorch until the water in the tap boiled before the post flush bacteriological sample (500 ml) was taken.

3.2.3.2 Regulatory Analysis Protocol

When the regulatory sampling protocol was completed, as described in Section 3.2.3.1, samples were transported to the Anglian Water Central Laboratories in refrigerated containers. Microbiological analysis occurred within 24 h of collection. The regulatory prescribed samples were analysed using the methods described in Table 7.

Table 7 Regulatory prescribed samples and the analysis methods used by water utilities

Parameter	Maximum Prescribed Concentration	Sample Location	Analysis Method	Precision % of Prescribed Concentration
1, 2 dichloroethane/µg/l	3.0	CT		25 (25)
Acrylamide/µg/l	0.1	-		-
Aluminium/µg/l	200	CT	Inductively Coupled Plasma with Optical Emission	10 (10)
Ammonium/mg/l	0.50	CT	Colorimetric methodology using discrete analysers	10 (10)
Antimony/µg/l	5.0	CT		25 (25)
Arsenic/µg/l	10	CT		10 (10)
Benzene/µg/l	1.0	CT		25 (25)
Benzo(a)pyrene/µg/l	0.010	CT		25 (25)
Boron/mg/l	1.0	CT		10 (10)
Bromate/µg/l	10	CT	Ion Chromatography	25 (25)
Cadmium/µg/l	5.0	CT	Mass Spectrometric detection	10 (10)
Chloride/mg/l	-	SP	Colorimetric methodology using discrete analysers	10 (10)
Chromium/µg/l	50	CT	Mass Spectrometric detection	10 (10)
Clostridium perfringens/no/100ml	0	SP		-
Coliform bacteria/no/100ml	0	SR, WTW	ISO 9308-1	-
Colony Counts/no/1ml at 22°C	NAC	CT, SR, WTW	PrEN ISO 6222	-
Colour/mg/l Pt/Co	20	CT		10 (10)
Conductivity/µS/cm at 20°C	2500	SP		10 (10)
Copper/mg/l	2.0	CT	Inductively Coupled Plasma with Optical Emission	10 (10)
Cyanide/µg/l	50	CT		10 (10)
E. coli/no/100ml	0	CT, SR, WTW	ISO 9308-1	-
Enterococci/no/100ml	0	CT	ISO 7899-2	-
Epichlorohydrin/µg/l	0.10			-
Fluoride/mg/l	1.5	CT	Ion selective electrode analyser	10 (10)
Hydrogen ion/pH value	6.5 - -9.5	CT		-
Indicative dose/mSv	0.10	SP		-
Iron/µg/l	200	CT	Inductively Coupled Plasma with Optical Emission	10 (10)
Lead/µg/l	10	CT	Inductively Coupled Plasma with Optical Emission	10 (10)
Manganese/µg/l	50	CT	Inductively Coupled Plasma with Optical Emission	10 (10)
Mercury/µg/l	1.0	CT	Inductively Coupled Plasma with Optical Emission	10 (10)
Nickel/µg/l	20	CT	Inductively Coupled Plasma with Optical Emission	10 (10)
Nitrate/mg/l	50	CT	Colorimetric methodology using discrete analysers	10 (10)
Nitrite/mg/l	-0.5 -0.10	CT, WTW	Colorimetric methodology using discrete analysers	10 (10)
Odour	ATC, NAC	CT	Qualitative and quantitative panel organoleptic testing	-
Other Pesticides/µg/l	0.10	CT	Gas/liquid chromatographs coupled to mass spectrometers	25 (25)
Pesticides (Aldrin Dieldrin, Heptachlor, Heptachlor Epoxide/µg/l)	0.030	CT	Gas/liquid chromatographs coupled to mass spectrometers	25 (25)
Polycyclic Aromatic Hydrocarbon/µg/l	0.10	CT	Gas/liquid chromatographs coupled to mass spectrometers	25 (25)
Radon/Bq/l	100	SP		-
Selenium/µg/l	10	CT		10 (10)
Sodium/mg/l	200	CT	Inductively Coupled Plasma with Optical Emission	10 (10)
Sulphate/mg/l	250	SP		10 (10)
Taste	ATC, NAC	CT	Qualitative and quantitative panel organoleptic testing	-
Tetrachloroethene/µg/l	10	CT		25 (25)
Trichloroethene/µg/l	10	CT		25 (25)
Tetrachloromethane/µg/l	3	CT		20 (20)
Total Organic Carbon/mg/l	NAC	SP	Carbon analyser	-
Total Pesticides/µg/l	0.50	CT	Gas/liquid chromatographs coupled to mass spectrometers	25 (25)
Total Trihalomethanes/µg/l	100	CT	Gas/liquid chromatographs coupled to mass spectrometers	25 (10)
Tritium (Radioactivity)/Bq/l	100	SP		-
Turbidity/NTU	-4 -1	CT, WTW		25 (25)
Vinyl chloride/µg/l	0.50	-		-

Adapted from the Drinking Water Inspectorate (2016) and Anglian Water (2020). CT denotes customer tap, SP supply point, WTW water treatment works, SR service reservoir, ATC acceptable to consumers, NAC no abnormal change.

3.2.3.2.1 Heterotrophic Plate Count Protocol

Plate counts were tested for on Yeast Extract Agar or Nutrient Agar. These 48 hr or 72 hr tests were mainly for non-specific organisms and yeasts and moulds.

Total coliforms and *E. coli* were enumerated using protocol ISO 9308-1 on membrane lactose glucuronide agar. After the incubation period, the number of colonies were counted and recorded as colony forming units 100 ml/l.

Enterococci were enumerated using protocol ISO 7899-2 on membrane *Enterococcus* agar, following which the number of colonies was counted and recorded as colony forming units 100 ml/l.

Clostridium perfringens were enumerated on membrane tryptose sulphite cycloserine agar. After membrane filtration, anaerobic incubation occurred at 44°C and 1°C for 21 hours and 3 hours. The opaque yellow colonies that turn pink or red after exposure to ammonium hydroxide vapours for 20 to 30 seconds were counted (Drinking Water Inspectorate, 2016) (Anglian Water, 2020).

3.2.4 Flow Cytometry Protocol

There is no official standardised, formal or regulated protocol for water quality analysis by flow cytometry. As such the staining and flow cytometry analysis methods were designed to reflect the best published and practised analysis completed to date. This method was produced based on that described by Proctor et al. (2018), who used a refined method used by Prest et al. (2013), based on the guideline method for drinking water analysis in Switzerland (SLMB, 2012). This method has previously been used by the University of Sheffield, such as the research described in Fish et al (2017).

Flow cytometry samples were taken as flush bacteriological samples, following the protocol detailed in Section 3.2.3.1. Once collected, samples were transported in refrigerated containers to the laboratory with analysis occurring within 12 h of sample collection. On arrival at the laboratory, two 500 µl aliquots were pipetted from each bulk water sample into two 1.5 ml sterile micro-centrifuge tubes and rested to allow sample temperature to reach ambient room level (~21^{o}C).

For enumeration of TCC, a working stock solution of 100x SYBR Green I was produced by adding 10 µl of SYBR Green I 10,000 X stock solution (500 µl, cat. no. S-7563; Invitrogen Ltd, Paisley, UK) to 990 µl of dimethyl sulphoxide (DMSO, 100ml, cat. No BP231-100, Fisher Scientific UK Ltd., UK), diluting at a rate of 1:100. 5 µl of 100x SYBR Green I was added to one of the prepared 500 µl samples. This solution was vortexed for 10 seconds before incubation at 37^{o}C for 13 minutes in a dark incubation unit. 50 µl of this sample was analysed using the ThermoFisher Attune NxT flow cytometer.

ICC were determined by creating a working solution of Propidium Iodide/ SYBR Green I by adding 1 part Propidium Iodide (1.0 mg/ml solution in water, cat. no. P3566, Invitrogen Ltd, Paisley, UK) (1.0 mg/ml for one sample) to 5 parts SYBR Green I (5.0 mg/ml for one sample). 6 µl of this working solution was added to the second 500 µl sample and vortexed for 10 seconds before incubation at 37^{o}C for 13 minutes in a dark incubation unit. 50 µl of this sample was analysed using the ThermoFisher Attune NxT flow cytometer.

No target cell characterisation was utilised, though an understanding of the gating process was required to ensure the validity of the data, that all cells were included in the TCC. Each scatter plot was analysed manually to ensure gating was suitable, rather than using the quicker although less

accurate method of predetermined gating using an algorithm. To reduce the background noise in a cell count, removing debris from the produced scatter plot, a standardised internal Anglian Water threshold template was applied

3.2.5 GIS Analysis Protocol

Network characteristics were investigated to get an insight into static asset data. To explore this, water utility GIS software and billing records were calibrated against telemetry data and field pressure logging to build a representative simulation of the distribution network. The modelling software package SynerGEE v4.6.0 was used to predict water age by continuously running simulations until stable water age values were calculated by the model, generating maps with coloured elements according to the water ages. These programmes were used to facilitate the calculation of the total lengths, diameters and materials of the pipes within the DMA, as a percentage, as well as the water age within them.

The models were calibrated to the UK standard using main pressure data. Age was set as zero at the outlet of the works. The case study water utility usually run simulations from zero to >36 hours but were modified to run from zero to > 144 hours to ensure stable age representation even at the extremes of networks. Dead-end pipes with water age simulation equal to simulation duration were excluded.

3.2.6 Data Analysis

Throughout this thesis, data analysis and figures were plotted using the statistical package R v3.5.3 (including the packages ggplot2, stargazer and corrplot) and Excel v2101 in Office 365.

Descriptive statistics including the range, median, mean and standard deviation were calculated for each parameter measured, which can be found in Appendix 2 and 3.

Assessments of significance were completed in R. Firstly, samples were assessed for normality using Shapiro-Wilks tests. Tests in the form of a T-Test or a Wilcoxon Test were then used depending on whether samples were normally or non-normally distributed. A significance level of <0.05 was used but p values that were not significant were not excluded and were also stated throughout.

Correlation matrices were assessed using the CORREL function, which returns the correlation coefficient of two cell ranges to determine the relationship between the two parameters.

3.2.7 Site Selection

Site selection was of upmost importance to draw conclusions between different sites of varying levels of biological stabilities. A selection of the 6 most appropriate sites to draw relevant and meaningful comparisons was utilised rather than all Anglian Water sites as the present research is driven by understanding rather than company-wide machine learning. The selected number of sites sufficiently provided the balance of a rich quality of data while also providing valuable insights into different networks.

As such, a programme of work was completed to determine the best sites to use for data analysis over the last regulatory AMP year as well as the most updated information available, running from 2014-2020. This timeframe was selected due to the importance of operational stability and planned programmes of work that vary between different AMP years to allow the actual network characteristics to be assessed, rather than the impact of a short-term flux.

The first step in the programme of work to determine site selection was the use of flow cytometry. To determine if flow cytometry, the biological stability monitoring method chosen, acts as an indicator for water quality and current compliance, a 3 month company-wide analysis of flow cytometry data at Final Water was completed at 125 Anglian Water sampling locations. The results were plotted in a similar way as in Figure 4, although this has been anonymised for confidentiality.

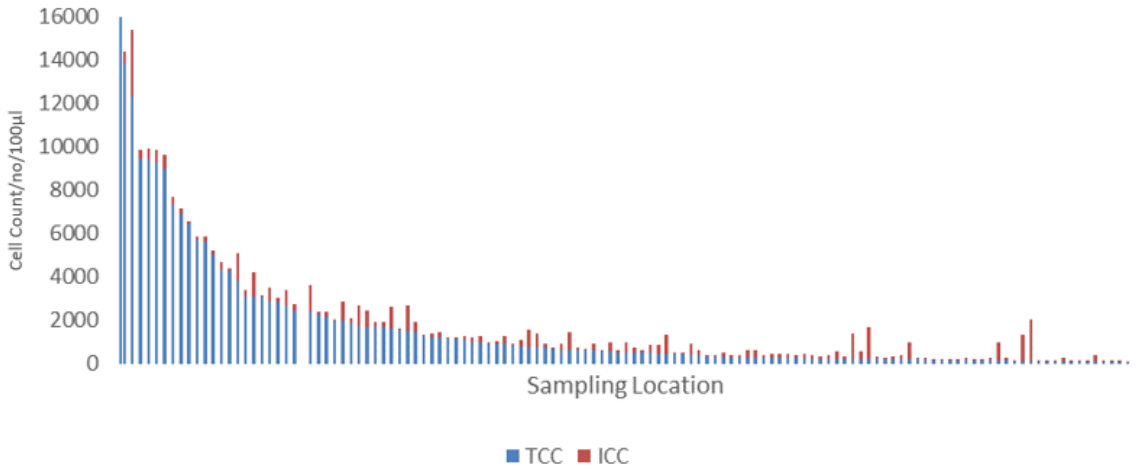


Figure 4 Average TCC and ICC at 125 of AW sites

The flow cytometry results were used to narrow down sites of interest to test the success of flow cytometry as an indicator of biological stability. Firstly, the 10 sites with the highest average TCC and the 10 sites with the lowest average TCC were selected, with adjustments to select only Final Water samples and not others such as reservoirs and towers. The shortlisted sites were then used for final site selection based on specific Final Water characteristics and specifications of the water distribution network. The criteria by which the water supply systems were selected were the following:

- Sufficient historic data availability (e.g., sites that are new or are not currently operational would have poor data availability for comparison with other sites)
- Stability of the programme of work over the most recent AMP year (e.g., if sites have moved from chlorine to chloramine in the last year the network may still be in a state of flux which could incorrectly be interpreted as poor biological stability)
- Absence of Final Water blending in the distribution network with other treatment works or sources of different quality
- Minimised network blending with storage assets to ensure high confidence in comparisons with Final Water (i.e., to ensure the water quality of the network is assessed and not the storage asset)
- Sufficient parallels and comparisons can be drawn between the different selected sites:
    - Different source waters: surface water, blended surface, blended groundwater/surface, groundwater
    - Different disinfection practices: chlormaination or a residual free chlorine dose

3.2.8 Selected WTW and Network Characteristics

The six selected case study WTW and their associated networks were assets of Anglian Water. To preserve confidentiality, they are not named in this study but are referred to as Sites A-F. Table 8 provides a detailed description of each WTW and their network characteristics.

Table 8 Specification of selected WTW and distribution networks

Site	Source Water Type	Abstraction License /m^3/day	Water Treatment Process	Network Chemicals	PWSZ Pop. Fed	Water Age /days	PWSZ Network Length /m	Predominant Network Material
A	SW	16,164	GAC, slow sand filters	Chloramine, phosphate	38,082	≥6	285,000	52% plastic 41% iron 6% cement
B	SW	63,643	Ozone, RGF, GAC	Free chlorine, phosphate	18,087	4	860,000	53% concrete 30% plastic 16% iron
C	SW Blended	763,000	Ozone, RGF, GAC	Chloramine, phosphate	16,539	≥6	280,000	60% plastic 24% iron
D	SW/GW Blended	SW 13,636 GW 21,160	Ozone, GAC	Free chlorine, phosphate	18,390	≥6	716,000	40% plastic 27% iron 15% steel
E	GW	1000	RGF	Free chlorine, phosphate	19,685	3	158,000	49% plastic 36% cement 14% iron
F	GW	3500	-	Free chlorine, phosphate	8,592	2	115,000	56% plastic 25% iron 19% cement

SW denotes surface water, GW groundwater, Pop. Population, PWSZ public water supply zone, GAC granular activated carbon, RGF rapid gravity filter.

Table 8 provides information at a WTW level as well as within the network. The different terms used to define the hierarchies of the network are presented in Figure 5. Each case study WTW feeds one PWSZ, a large area of the network used for operational planning. Within each PWSZ, two DZs were selected as smaller case study network areas. Specific DZs were chosen based on how directly they were fed from the case study WTWs, with minimal blending.

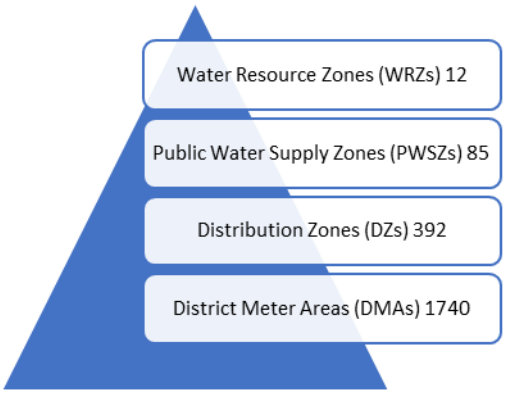


Figure 5 Hierarchy of water resources zones and the number of each within Anglian Water

3.2.8.1 Further Site Details

Site A was the largest case study site by WTW design capacity (17,971 m^3/day) and in PWSZ population (38,082), although not being used at full capacity with a relatively low abstraction licence (16,164 m^3/day). The site is chloraminated, with a fairly standard dose for the case study sites (an average of 1.01 mg/l total chlorine at the WTW and 0.540 mg/l at the tap). Both phosphate dose (average 0.775 mg/l) and lead presence at the tap (average 0.651 µg/l) were relatively low.

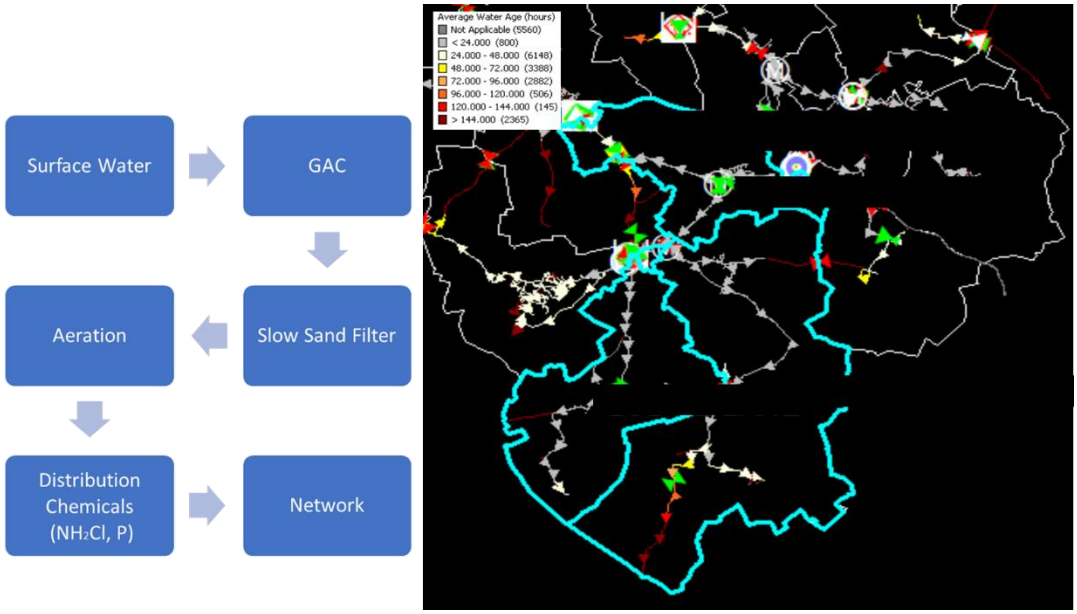


Figure 6 Site A WTW processes and water age network map

Site B had a network consisting of a lot of concrete (53%), something not often seen, as well as some plastic (30%) and iron (16%). The site has a typical chlorine dose for a surface water site (average 1.01 mg/l total chlorine) but has the lowest chlorine presence at the tap of the case study sites (0.406 mg/l). Phosphate had the highest dose (average 1.52 mg/l) and the highest phosphate presence at the tap (1.53 mg/l) but lead samples were also the highest (average 0.923 µg/l).

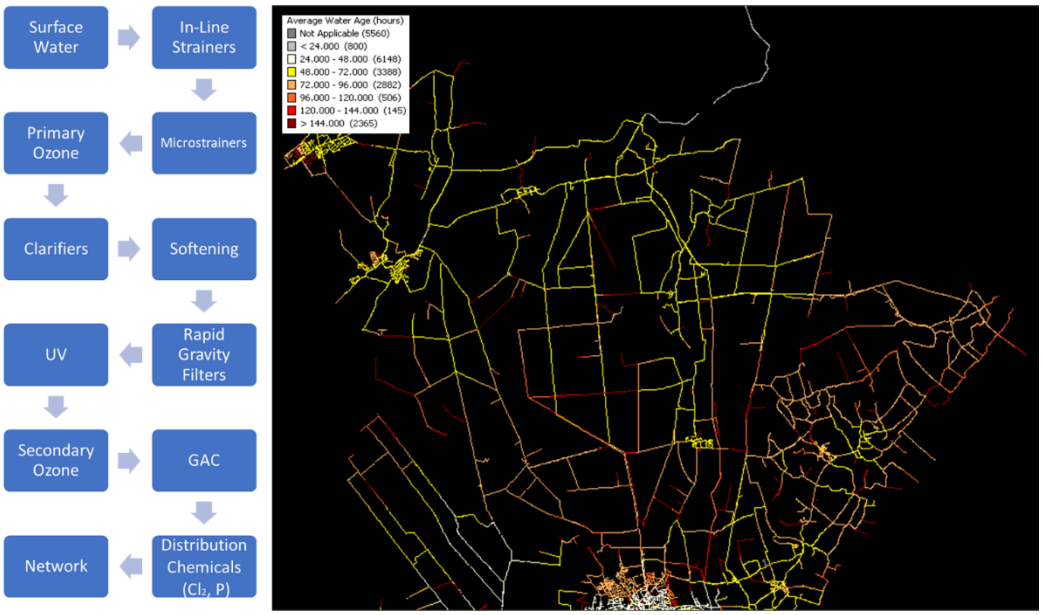


Figure 7 Site B WTW processes and water age network map

Site C is a WTW fed by a blend of two different surface waters, as such the abstraction licence for this site is very large (763,000 m^3/day) to account for the blending. Chloramine is used, with total chlorine marginally the highest of the case study sites (average total chlorine 1.11 mg/l), although middling at the tap (average total chlorine 0.436 mg/l). This site had a normal phosphate dose for a surface water site (average 1.38 mg/l) and low lead presence at the tap (average 0.668 µg/l).

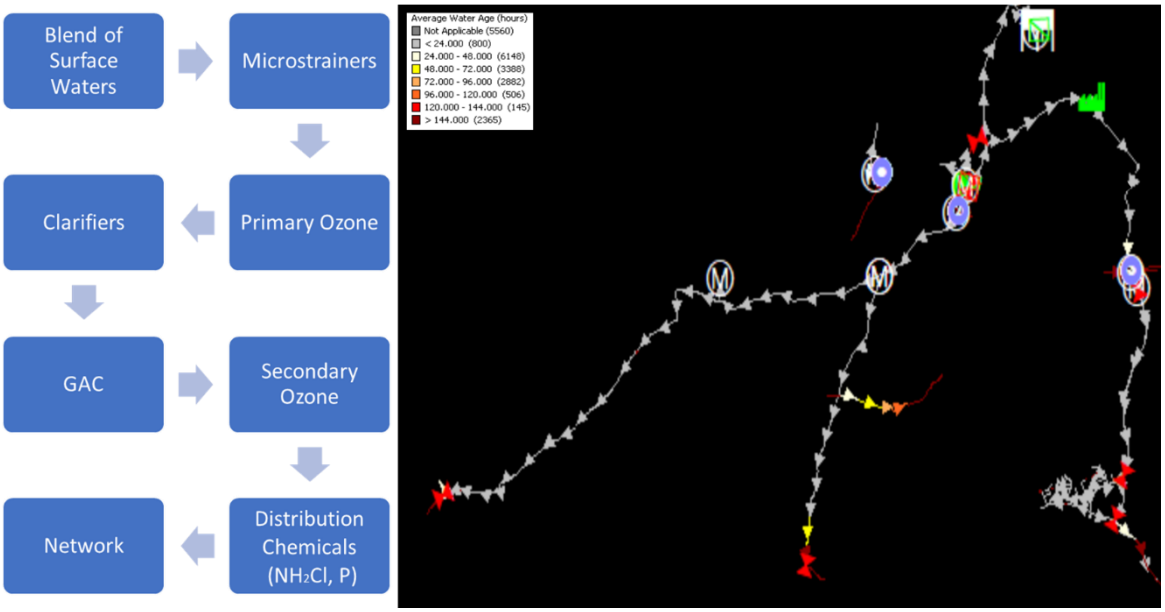


Figure 8 Site C WTW processes and water age network map

Site D is a blend of groundwater and surface water, with a network consisting of a relatively large variation of pipe materials (40% plastic, 27% iron, 15% steel and 10% cement). Despite having a normal chlorine dose for a site fed by surface water (average total chlorine 1.02 mg/l), chlorine presence at the tap was highest at this site (0.652 mg/l). A normal dose of phosphate is dosed (average 1.29 mg/l) and a middling lead presence at the tap (an average 0.881 µg/l) was found.

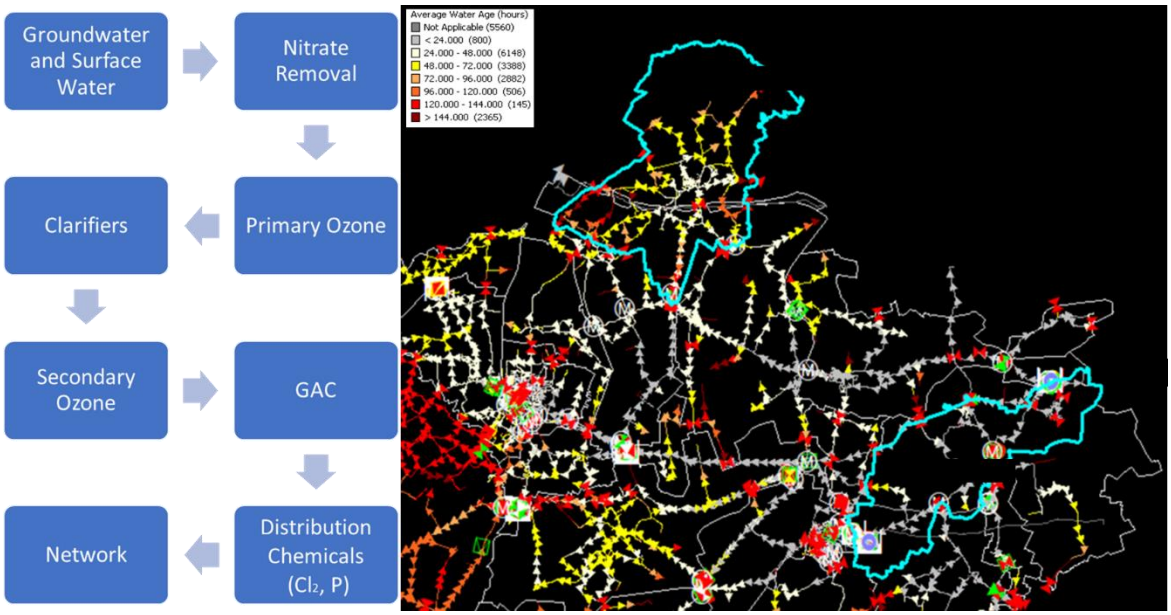


Figure 9 Site D WTW processes and water age network map

Site E had the smallest abstraction licence of the case study sites (1000 m^3/day) but has been designed for larger capacities (4100 m^3/day). The population fed by the PWSZ is similar to the larger sites (19,685) but the network feeding this PWSZ was shorter (158,000 m). Both the chlorine and phosphate dose (average 0.744 mg/l, 0.473 mg/l) were much lower than Sites A, B, C and D. Despite this lower chlorine dose at the WTW, the chlorine concentrations at the tap are similar to those sites with higher doses (average total chlorine 0.469 mg/l), although the lowest phosphate presence at the tap is seen (0.519 mg/l). It had the highest lead presence (average 1.34 µg/l).

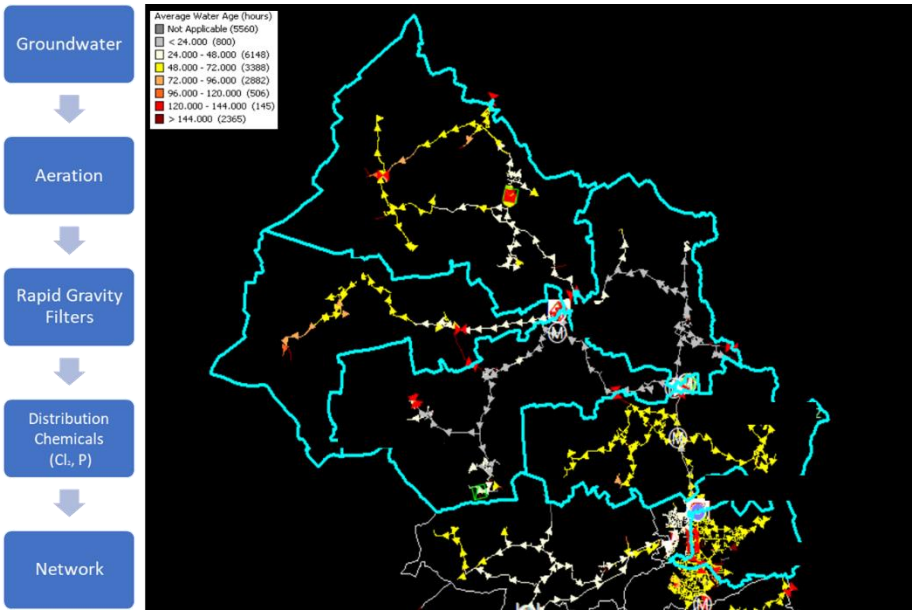


Figure 10 Site E WTW processes and water age network map

Site F had minimal treatment. It had a small design capacity (2592 m^3/day), the smallest PSWZ (8592) and the smallest network (115,000 m). Site F had the lowest dose of chlorine (average 0.440 mg/l) and phosphate (average 0.385 mg/l) and the lowest lead at the tap (average 0.214 µg/l).

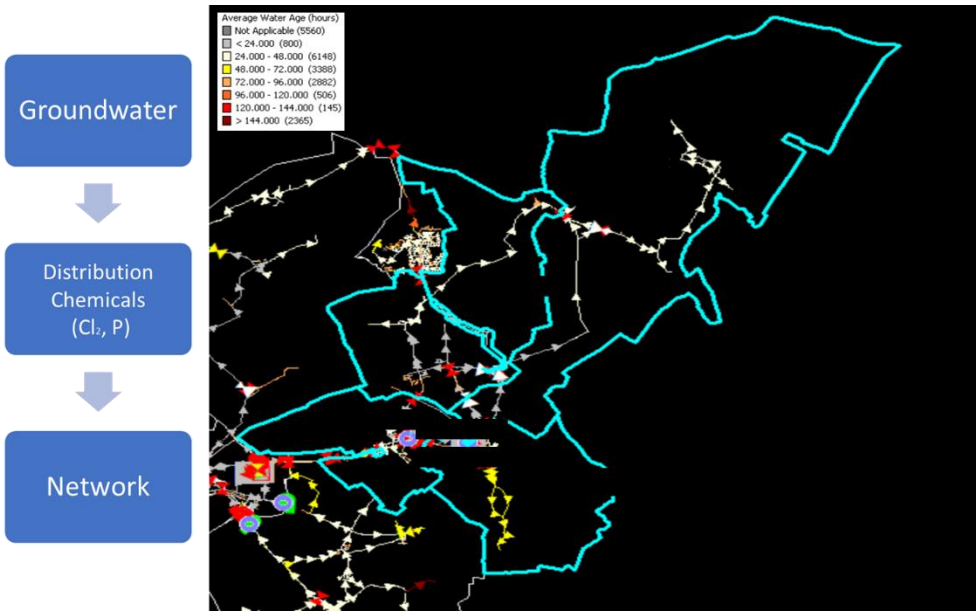


Figure 11 Site F WTW processes and water age network map

3.3 Results: An Assessment of Biological Stability in UK DWDS

Section 3.1 discussed the concept of biological stability, that case study companies that use fewer chemicals than the UK found that biological stability was of upmost importance to supply water with fewer chemicals. It went on to discuss the different definitions of the term "biological stability" and the varying monitoring methods, with a definition of biological stability proposed in Section 3.2.2.

The present section aims to use this definition, through a combination of historic regulatory samples supplemented with flow cytometry samples at a series of 6 case study sites to assess if these sites were of good or less good biological stability, determining their potential for chemical free water.

3.3.1 A Comparison and Contrast of Key Indicators of Biological Stability at Different Case Study Sites

3.3.1.1 Dosed Chemicals

The chemicals currently applied for the maintenance of water quality within the distribution network, phosphate and chlorine, are of particular interest with the focus on chemical free water in future. While Chapter 3 covers these chemicals in great detail, the present section, including Figure 12, will compare and contrast the concentrations of each at the 6 different case study sites.

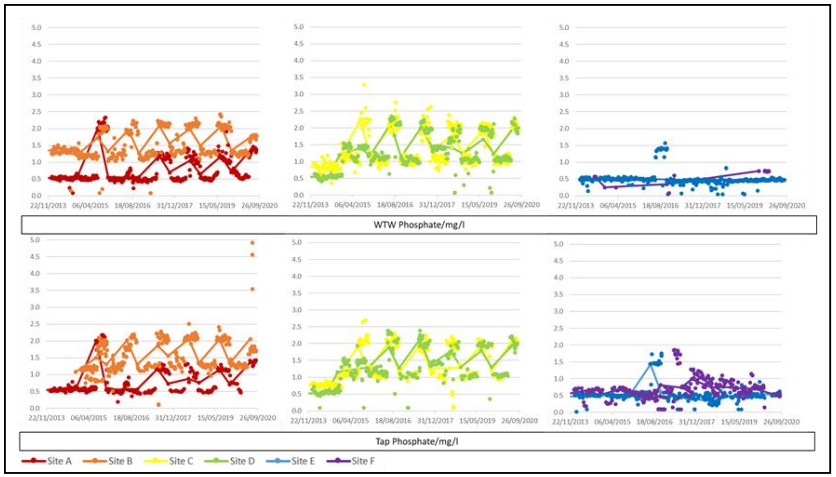
There was evidence of chlorine decay at all case study locations, with more decay occurring at the Surface Water Sies. With phosphate, however, there was limited evidence of any decay within the network. It was noted that the Tap had high phosphate concentrations, despite the long residency times in the pipe networks.

The Groundwater Sites (Sites E and F) had a lower chlorine dose and phosphate dose at the WTW than the Surface Water Sites (Sites A-D). In particular it was noted that phosphate dose varied seasonally at Sites A-D, a finding not observed in chlorine concentrations. Customer Tap samples in Surface Water areas had a lot more chlorine and phosphate variation than at Groundwater Taps, although for phosphate this was mostly likely driven by the seasonal WTW dosage.

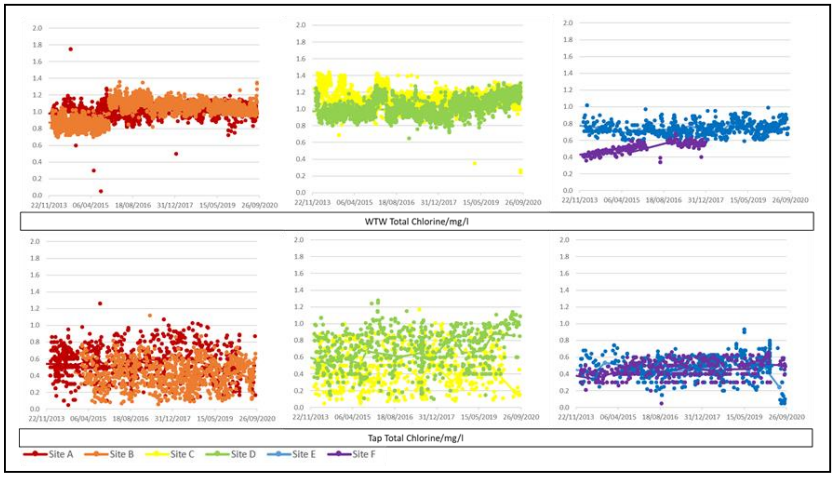
All case study areas had greater chlorine concentration variation at the Tap than at the WTW. This was likely because sampling at the Tap was taken from across the breadth of the network, from households close to the WTW to households further from the WTW. As Chapter 1 detailed, chlorine is well known to decay within the pipe network. Section 3.2.8 showed that the case study areas had differently sized networks with different water ages. Sites E and F had smaller networks (158,000 m and 115,000 m) with lower water ages (3 days, 2 days). Sites A-D had much larger networks (280,000-860,000 m) and higher water ages (4 days-≥6 days). As such, it was unsurprising to find greater chorine decay in the network at Sites A-D as well as less Tap variation. But it was notable that dose at the WTW had been adjusted to account for this decay, resulting in similar residuals at Customer Taps at all case study sites.

In phosphate, WTW and Tap concentrations were similar. The seasonal dose application at the WTW very much drove the dose observed at the Tap.

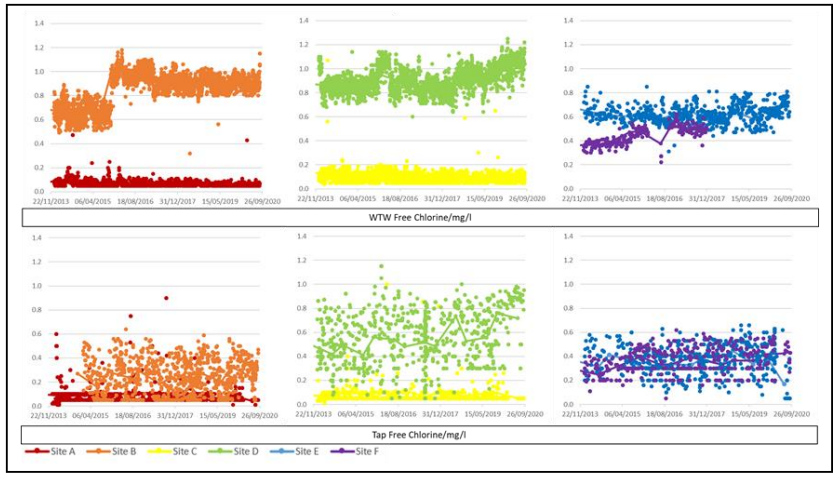
It was found that free chlorine at the Chloraminated Sites (Sites A and C) was relatively low at both the WTW and the Tap. This was to be expected with this disinfection method, as it is an indicator that combined chlorine was likely the greater of the chlorine fractions within the samples.



Phosphate



Total Chlorine



Free Chlorine

Figure 12 A comparison of the dosed chemicals at case study WTWs and Taps

3.3.1.2 Microbial Abundance

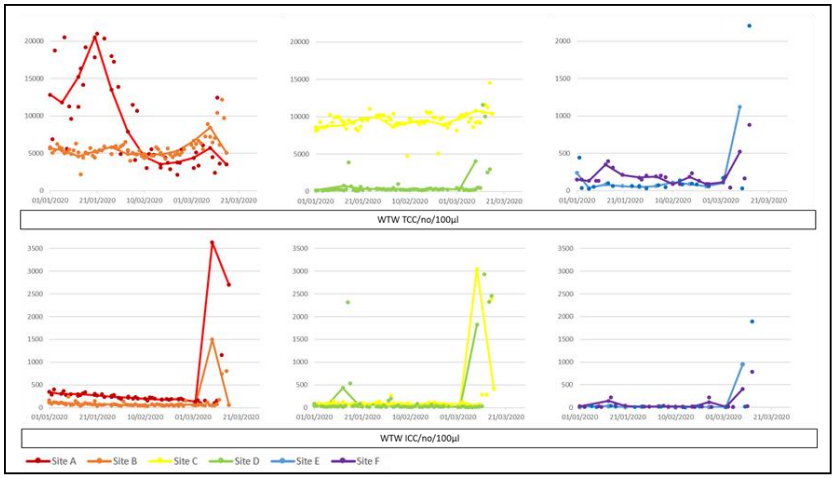
A comparison of the microorganisms within the case study sites assessed the parameters TCC, ICC, 3 day colony count, 2 day colony count, coliform count, *E. coli* count and *Enterococci* count, as shown in Figure 13. This was used as a direct measurement of the bulk water microbial concentration at the different case study locations as both the WTW and at the Tap (excluding TCC and ICC, which were not available at the Customer Tap).

Those microbial parameters sampled at both the WTW and the Tap generally had an increase in abundance within the network. All case study locations had an increase in 2 day colony counts. Most sites had an increase in coliforms (excluding Site E and Site F, which were 0 no/100ml throughout) and 3 day colony counts (excluding Sites A and C, which had a decrease in the network)

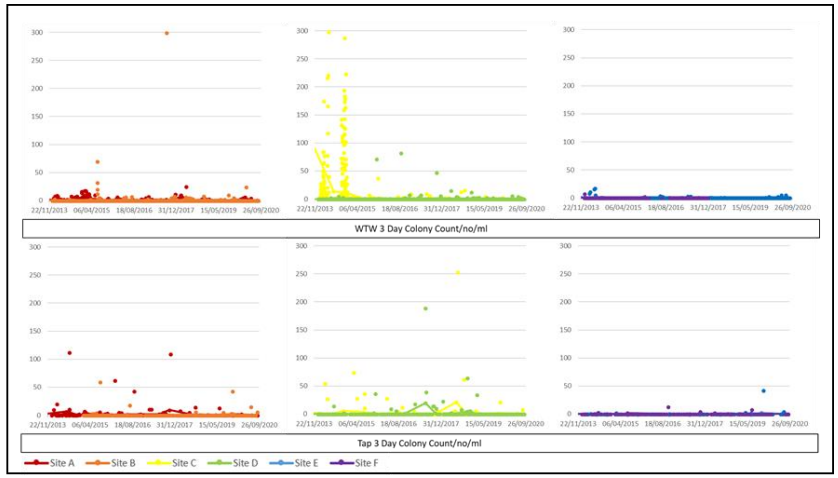
All case study sites at both the WTW and the Tap found no presence of either *E. coli* or *Enterococci,* with all sample results being 0 no/100ml.

The Groundwater Sites, Sites E and F, had lower TCC and ICC at the WTW than at the other case study locations. They also had the lowest values at both the WTW and Tap in 3 day colony counts, 2 day colony counts and coliforms.

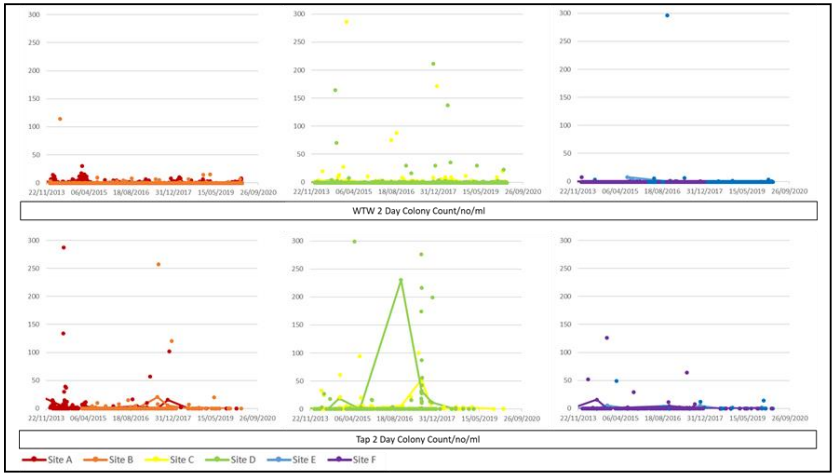
A further finding was that sites disinfected with chloramine (Sites A and C) had the highest TCC and ICC counts at the WTW. These sites also had the aforementioned decrease in 3 day colony counts within the network.



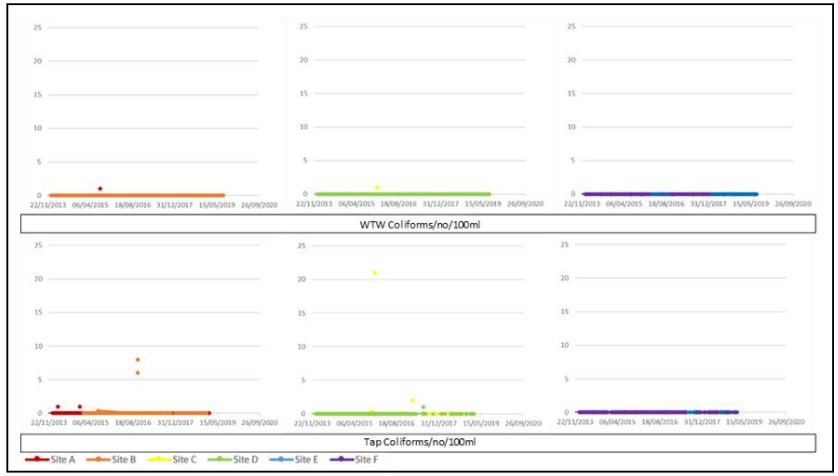
TCC and ICC



3 Day Colony Count



2 Day Colony Count



Coliforms

Figure 13 A comparison of the microbial abundance at case study WTWs and Taps

3.3.1.3 Nutrient Concentration

As discussed in Chapter 1, literature suggested that nutrients such as carbon, nitrogen and phosphate influence distribution system water quality by providing a source of nutrients that contribute to bacterial regrowth and biofilm development. Figure 14 assessed nitrogen (nitrate, nitrite and ammonia) at the WTW and Tap as well as carbon (total organic carbon) at the WTW (this parameter was not available at the Customer Tap).

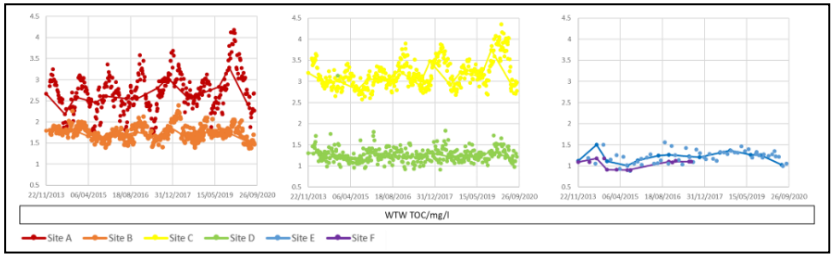
Nutrients and secondary disinfectant method were found to be highly related (although cause and effect are difficult to distinguish), with chloraminated sites showing variation and high values in certain nutrients. For instance, TOC, nitrate and ammonia at Sites A and C all had particularly higher and more variable concentrations. It must be noted that this could be the reason why chloramine was used at these sites, rather than an impact of the addition of chloramine.

Ammonia samples at both the WTW and the Tap were similar and stable at the sites treated with free chlorine (Sites B, D, E and F). While at the Chloraminated Sites (Sites A and C) there was higher values with greater variation at both the WTW and the Tap.

At the WTW, nitrite was similar in value at all case study locations. At the Tap, however, Sites A and C (and D to a lesser extent), were much greater and more variable, according to Figure 14, likely due to the impact of the addition of chloramine.

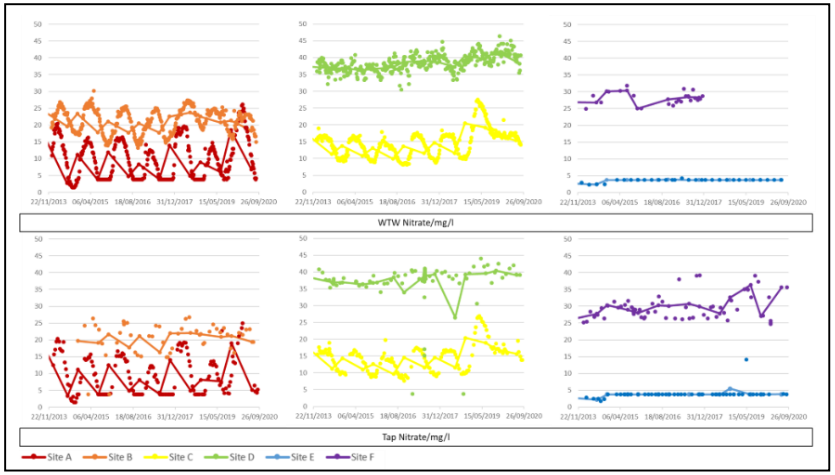
Unlike ammonia and nitrite, nitrate results were not very consistent by case study location, with Site D being highest and Site F being surprisingly high at both the WTW and the Tap.

TOC and phosphate were found to be lower in value and more consistent at the Groundwater Sites (Site E and Site F).



TOC

Nitrite



Nitrate

Ammonia

Figure 14 A comparison of the nutrient concentration at case study WTWs and Taps

3.3.1.4 Wider Water Quality Parameters

This section provides an assessment of wider water quality parameters which are necessary to get a richer picture of the complex interactions occurring within drinking water distribution systems. This includes a consideration of turbidity, water chemistry properties (including conductivity but touching on pH, hardness and alkalinity), water temperature and metals (primarily consisting of lead but also mentioning iron and manganese), as displayed in Figure 15.

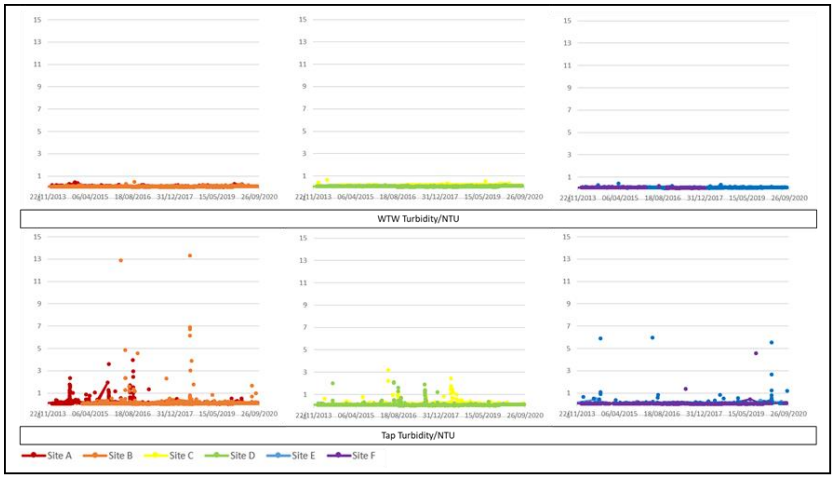
One finding was that the network had an influence over some water quality parameters, for example total lead and turbidity were found to increase from the WTW to the Tap at all case study locations. It was noted, however there was often a lack of lead samples at the WTW (e.g. an average of 14 samples at case study WTW compared to 141 samples at the Tap).

A further observation was that the Groundwater Sites (Sites E and F) could be particularly stable compared with other case study sites (e.g., in conductivity) or lower in value (e.g., in lead at the Tap). However, hardness and alkalinity were highest at the Groundwater Sites.

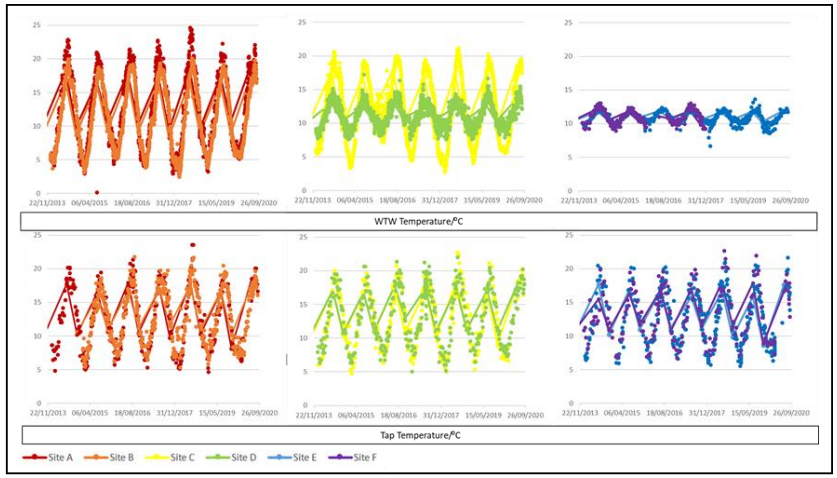
Some parameters were found to be fairly similar across all sites, including in the parameters pH, total iron and total manganese

Water temperature was also found to be greatly related with source water type at the WTW (with sites influenced by surface water having a higher temperature and those influenced by groundwater having a lower temperature) but these differences converged at the Tap, making all samples similar at this point regardless of case study location.

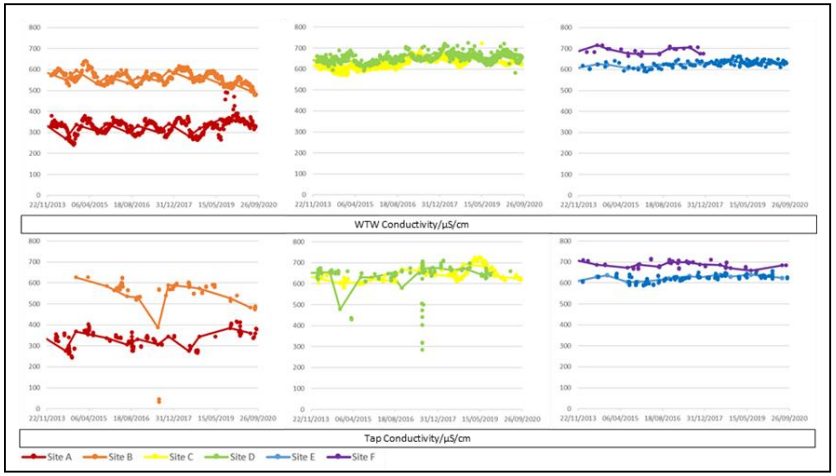
Blending of source water types was also found to influence water quality. For example, lead at the Tap was high at Surface Water Sites (Sites A and B) medium at Blended Sites (Sites C and D) and low at Groundwater Sites (Sites E and F). It must be said that, as Section 1.4.3.2 discussed, lead concentrations can be varied due to lead pipes, fittings, fixtures and solder within customer properties so can vary on a household level.



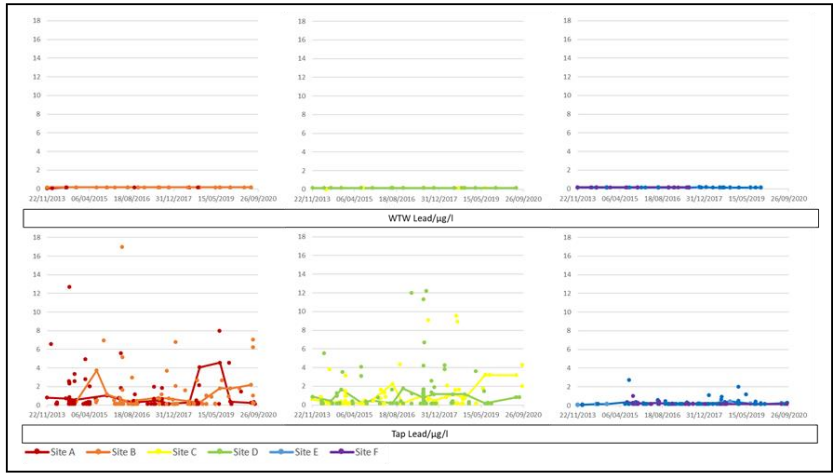
Turbidity



Temperature



Conductivity



Lead

Figure 15 A comparison of the nutrient wider water quality parameters at case study WTWs and Taps

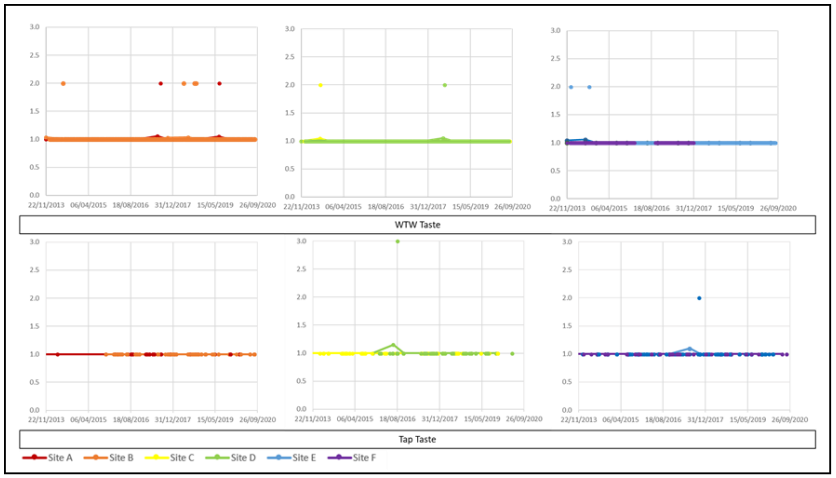
3.3.1.5 Aesthetic Parameters

Customers assess whether their drinking water is suitable for consumption based on the aesthetics, the taste, odour and look (or colour) of their tap water. As such, these were regarded as important parameters to consider when determining biological stability. Figure 16 provides a visual for these parameters which is discussed in the present section.

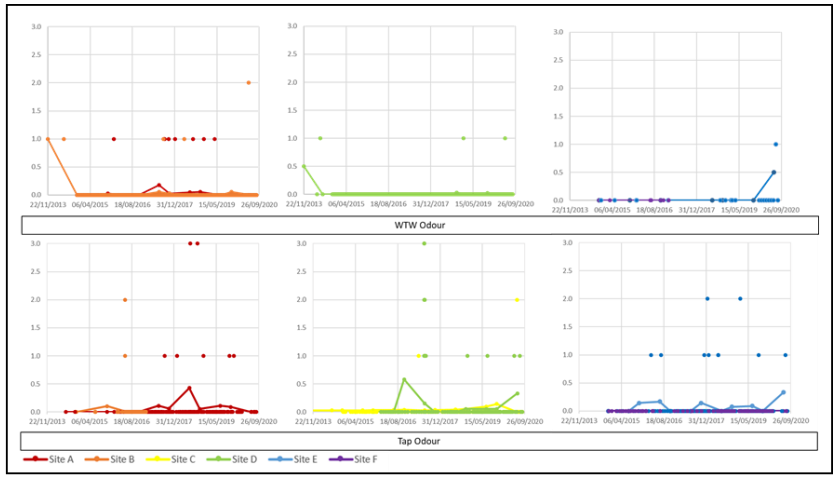
Taste and odour were found to be reasonably similar at the different case study locations, with taste in particular not being very impacted by the network.

Although the site location did not influence odour much, transportation in the distribution network resulted in an increase in odour at the majority of sites.

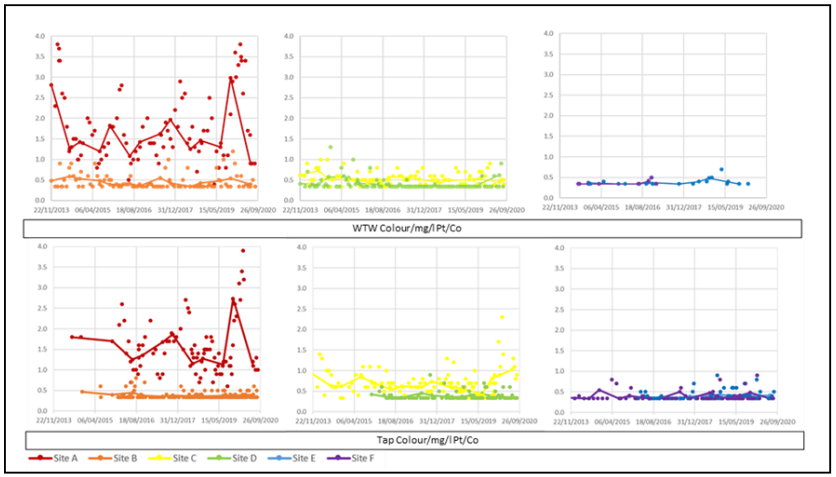
Colour had more variance by sampling location, with the chloraminated sites (Sites A and C) having comparatively high values than other sampling locations



Taste



Odour



Colour

Figure 16 A comparison of the aesthetic parameters at case study WTWs and Taps

3.3.2 The Change in Key Indicators of Biological Stability from the WTW to the Tap at Different Case Study Sites

Table 9 outlines the statistical difference from the WTW to the Tap in the parameters used to assess biological stability. Highlighted values indicate those which do not have a statistically significant difference (i.e., values are the same or statistically similar).

While it was found that the Groundwater Sites (Site E and F) did generally have fewer parameters having a statistically significant change from the WTW to the Tap, it was not substantially more than other sites.

Those parameters that had a statistically significant difference from the WTW to the Tap at the majority of case study sites appeared to be 2 day colony count, temperature, free chlorine, turbidity, conductivity and total lead.

Parameters which did not have a statistically significant difference from the WTW to the Tap at all case study sites were *E. coli*, *Enterococci*, nitrate and taste. Additionally, 3 day colony counts, phosphate, ammonia, odour and total chlorine were statistically similar at the majority of case study sites.

Table 9 Parameters with and without a statistically significant difference from the WTW to the Tap

Tap vs WTW	Microbial Abundance					Nutrient Concentration				Wider Water Quality Parameters						Customer Perception			No Parameters Without a Statistically Significant Difference
	3 Day Colony Count	2 Day Colony Count	Coliforms	*E. coli*	*Enterococci*	Phosphate	Nitrate	Nitrite	Ammonia	Total Chlorine	Free Chlorine	Turbidity	Temperature	Conductivity	Total Lead	Taste	Odour	Colour	
Site A	0.351	0.00361	0.247	NA	NA	0.445	0.416	2.2e-16	1.75e-10	0.0841	0.0375	2.2e-16	0.170	2.69e-05	0.00219	0.471	0.130	0.0413	10/18
Site B	0.0147	7.06e-05	-	NA	NA	0.174	0.774	1.00	0.344	0.0685	0.0926	2.2e-16	1.82e-11	0.00659	0.00421	0.314	0.964	0.000326	10/17
Site C	0.132	0.0507	0.0106	NA	NA	0.00183	0.624	2.2e-16	2.2e-16	0.125	0.0179	0.199	0.000819	0.0188	0.00403	NA	0.0158	2.36e-05	8/18
Site D	1.41e-06	1.11e-09	-	NA	NA	0.289	0.466	0.000493	0.240	0.0146	0.0312	1.02e-10	2.2e-16	0.199	0.00208	0.275	7.81e-07	0.932	8/17
Site E	0.699	0.00624	NA	NA	NA	0.483	0.668	-	0.305	0.0041	0.00872	0.00154	0.00322	0.0393	0.119	NA	0.762	NA	11/17
Site F	0.0691	0.000564	NA	NA	NA	0.0414	0.254	0.217	0.162	0.337	0.330	0.870	1.22e-06	0.673	0.0913	NA	NA	0.544	15/18

P values determined using a Wilcoxon Test (as described in Section 3.2.6) due to data being non-normally distributed. NA denotes no difference between values. Further information including descriptive statistics and sample numbers can be found in Appendix 2.

3.3.3 An Exploration of the Classification of "Poor" Biological Stability assessment

As assessment of longitudinal water quality and compliance with current regulations over time was completed in the case study areas to determine if any case study sites could be classified as having "poor" biological stability.

When considering current compliance, the majority of samples observed during the course of the study were compliant to regulatory standards, as shown in Table 10. This was true even though regulations were often stricter at the WTW than at the Tap, for instance, the maximum prescribed value of turbidity at the WTW is 1 NTU whereas at the tap it is 4 NTU. Regardless of the reasoning behind the prescribed value, when comparing the experimental data WTW values, they were often compliant. There were only two parameters across the six case study sites that breached compliance at a WTW level from samples across 2014-2020, Site A and Site C for coliform levels. Despite the high compliance in WTWs, all case study sites experienced some samples that breached compliance at the Consumer Tap. In these samples it was noted that this only occurred in one parameter for each of the Groundwater Sites, while the Surface Water Sites and the Blended Sites had multiple parameter failures.

Table 10 Percentage compliance of regulatory maximum prescribed values at WTWs and Tap

Parameter	Regulatory Maximum Value		Site A SW		Site B SW		Site C SW/SW Blend		Site D SW/GW Blend		Site E GW		Site F GW	
	WTW	Tap	WTW	Tap	WTW	Tap	WTW	Tap	WTW	Tap	WTW	Tap	WTW	Tap
Ammonia[1]	-	0.5	-	100%	-	100%	-	100%	-	100%	-	100%	-	100%
Coliform[5]	0	0	99.91	99.65	100%	99.43	99.95	99.44	100%	99.75	100%	100%	100%	100%
Conduct[4]	2500	-	100%	-	100%	-	100%	-	100%	-	100%	-	100%	100%
Copper[1]	-	2	-	100%	-	100%	-	100%	-	100%	-	100%	-	100%
E. coli[5]	0	0	100%	100%	100%	100%	100%	99%	100%	100%	100%	100%	100%	100%
Entero[1]	0	0	100%	100%	100%	100%	100%	99%	100%	100%	100%	100%	100%	100%
Iron[3]	-	200	-	100%	-	100%	-	100%	-	100%	-	100%	-	100%
Lead[1]	-	10	-	99.36	-	99.98	-	100%	-	97.84	-	100%	-	100%
Mangane[3]	-	50	-	100%	-	100%	-	100%	-	100%	-	100%	-	100%
Nitrate[1]	-	50	-	100%	-	100%	-	100%	-	100%	-	100%	-	100%
Nitrite[1]	0.1	0.5	100%	100%	100%	100%	100%	100%	100%	98.06	100%	100%	100%	100%
pH[2]	-	6.5-9.5	-	100%	-	100%	-	100%	-	100%	-	100%	-	100%
Sodium[2]	-	200	-	100%	-	100%	-	100%	-	100%	-	100%	-	100%
Turbidity[5]	1	4	100%	99.85	100%	98.98	100%	100%	100%	100%	100%	99.41	100%	99.58

Superscript denotes frequency of parameter sampling. [1] n=40-200, [2] n=100-300, [3] n=150-650, [4] n=100-1500, [5] n=150-2500.

In terms of variance over time, no evidence was found of water quality decrease or deterioration over time at any case study site, as shown by Figure 17 and Figure 18. It was not found, for example, that the Surface Water Sites (Sites A and B) or the Blended Water Sites (Sites C and D) had lower water quality than the Groundwater Sites (Sites E and F).

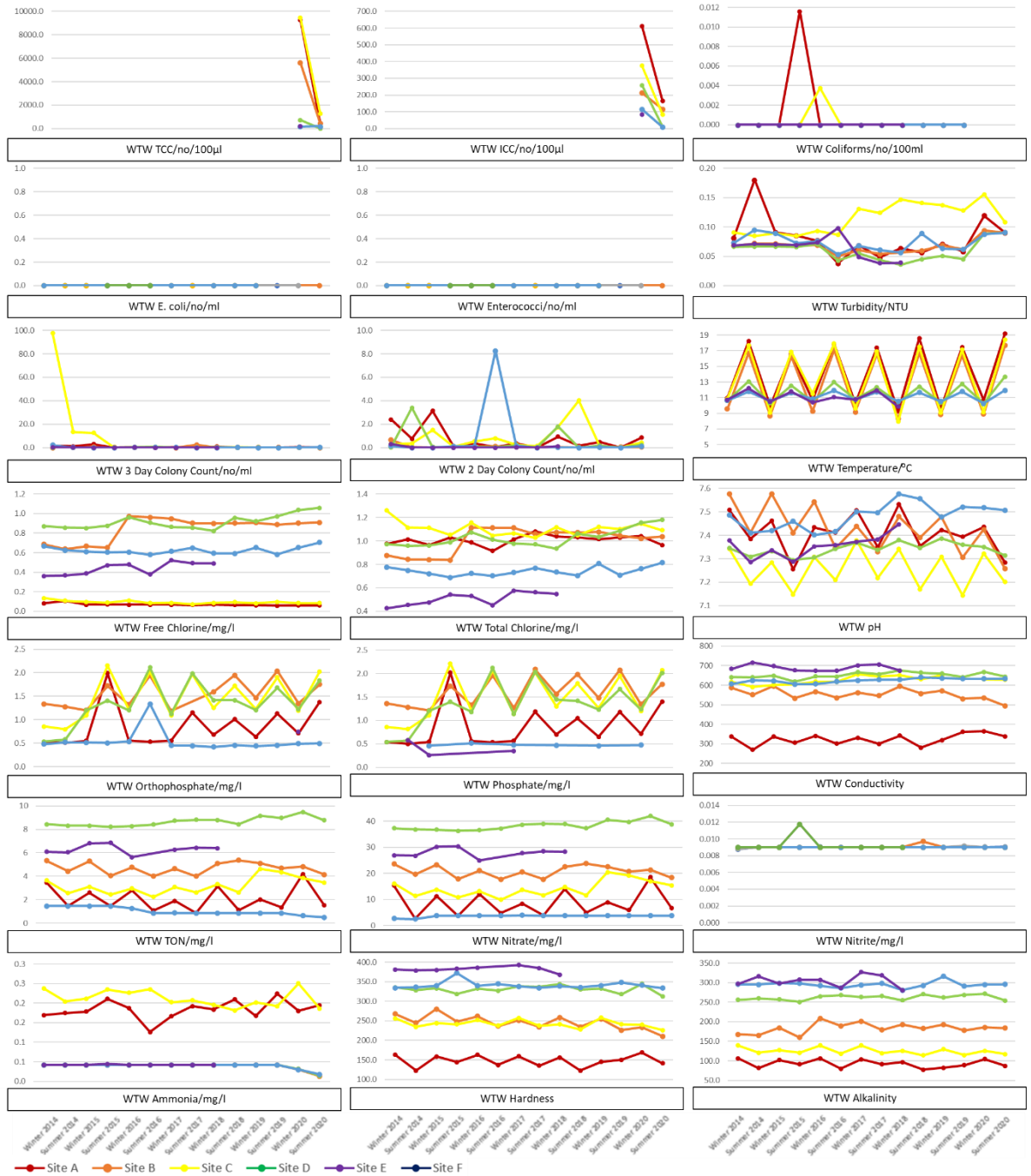


Figure 17 Temporal analysis of water quality variance at case study WTWs

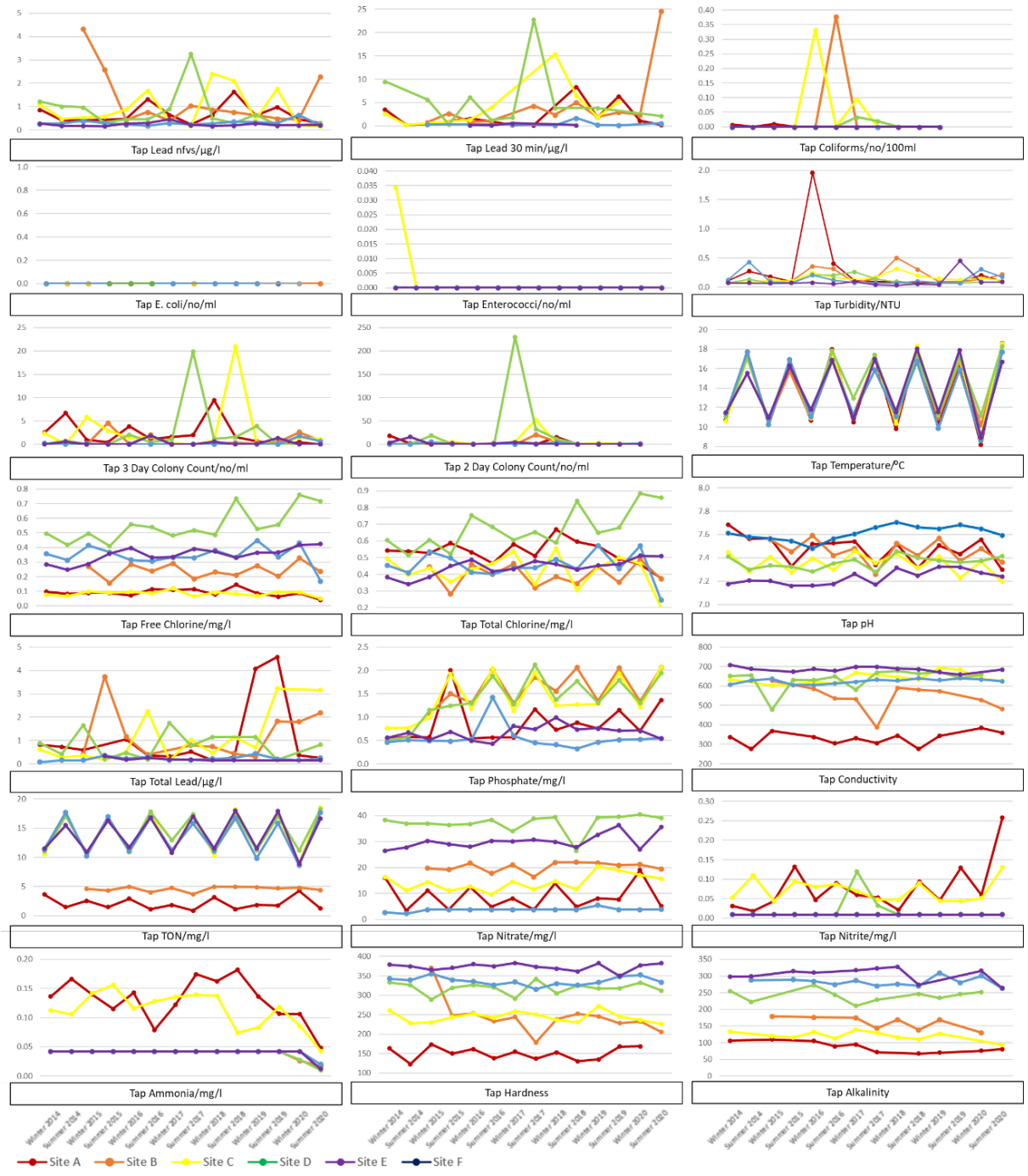


Figure 18 Temporal analysis of water quality variance at case study Consumer Taps

3.3.4 To what Extent did TCC and ICC Act as an Indicator for Biological Stability?

To determine if TCC and ICC act as an indicator for biological stability, a variety of analyses were completed to assess the relationships between these values and other water samples.

TCC correlated well with a large number of water quality parameters. Water quality parameters that had the strongest relationship with TCC were: ICC (Sites D (p=0.906), E (p=0.928) and F (p=0.958)), temperature (Sites A (p=-0.536), B (p=-0.840) and C (p=-0.934)), conductivity (Sites A (p=0.923) and B (p=0.878)) and 3 day and 2 day colony count (Site F (p=0.920)) [Figure 19]. Additionally, weaker relationships were observed between TCC and turbidity, free chlorine, total chlorine, nitrite (Site A and Site C only), orthophosphate (Site F only) and pH (Site F only). As such, it did appear that TCC acted as an indicator for water quality for a number of parameters.

Despite this, a number of water quality parameters had no relationship with TCC, regardless of site location, including: coliforms, *Enterococci*, ammonia, total nitrate, TON, hardness, alkalinity, total sodium, total potassium, total phosphorus, total calcium, total magnesium, total copper, total manganese, total iron and total lead. Therefore, while TCC may act as an indicator for water quality, it is not necessarily correlate with all WQ parameters of interest, or consistently at all sites, reflecting the complexity of processes and factors occurring in WTW.

When considering the trend between TCC and water quality parameters, it did not appear that this relationship was influenced with source water type. For instance, it was not found that TCC was a better indicator of water quality at surface water sites than other source water types. As such, Sites A and C were often similar, for example in the parameters turbidity, temperature and free chlorine as were Sites D and E in turbidity and conductivity.

TCC no/100µl WTW CORR	Intact Cell Count no/100µl	Coliforms no/100ml	*Entero cocci* no/100ml	3 Day Colony Count no/ml	2 Day Colony Count no/ml	Free Chlorine mg/l	Total Chlorine mg/l	Total P mg/l	Ortho phosphate mg/l	Total Lead µg/l	Temperature °C	Turbidity NTU	Conductivity µS/cm	pH pH units	TON mg/l	Nitrite mg/l
A	0.107	<2.2e-16	<2.2e-16	-0.0196	-0.103	-0.107	-0.147	<2.2e-16	<2.2e-16	<2.2e-16	-0.536	0.325	0.923	<2.2e-16	<2.2e-16	0.250
B	0.348	<2.2e-16	<2.2e-16	0.00123	<2.2e-16	-0.0418	-0.124	<2.2e-16	<2.2e-16	<2.2e-16	-0.840	0.102	0.878	<2.2e-16	<2.2e-16	<2.2e-16
C	0.167	<2.2e-16	<2.2e-16	-0.232	0.0302	-0.313	0.156	<2.2e-16	<2.2e-16	<2.2e-16	-0.934	0.625	<2.2e-16	<2.2e-16	<2.2e-16	0.0259
D	0.906	<2.2e-16	<2.2e-16	<2.2e-16	-0.0310	0.0189	0.0631	<2.2e-16	<2.2e-16	<2.2e-16	-0.128	-0.0469	0.126	<2.2e-16	<2.2e-16	<2.2e-16
E	0.928	<2.2e-16	<2.2e-16	<2.2e-16	-0.0816	0.561	0.547	<2.2e-16	<2.2e-16	<2.2e-16	0.110	-0.0601	0.338	<2.2e-16	<2.2e-16	<2.2e-16
F	0.958	<2.2e-16	<2.2e-16	0.920	0.920	0.0377	-0.342	<2.2e-16	-0.584	<2.2e-16	0.0638	-0.177	<2.2e-16	-0.0901	<2.2e-16	<2.2e-16

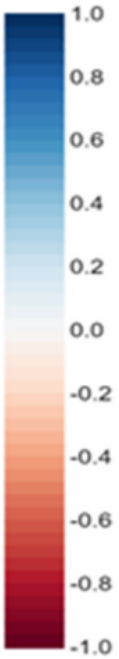


Figure 19 Correlation matrix of TCC and other water quality parameters at WTWs

The analysis was repeated for ICC, exploring its value as an indicator or WTW final water quality. The water quality parameter with the strongest relationship with ICC was TCC (Sites D, (p=0.906), E (p=0.928) and F (p=0.958)), as shown in Figure 19. There were also weaker relationships with 3 day colony count (Site F (p=0.652)), 2 day colony count (Sites A (p=0.702) and F (p=0.652)), total chlorine (Site F (p=-0.714)), total phosphorus (Site F (p=0.626)) and conductivity (Site A (p=0.780)).

For the most part, ICC had a weaker link with water quality parameters than TCC, for example in temperature, turbidity, conductivity, nitrite and 3 day colony count. These correlations, using Site A as an example can be seen in these parameters: temperature (TCC p=-0.536, ICC p=0.0525), turbidity (TCC p=0.325, ICC p=0.0336), conductivity (TCC p=0.923, ICC p=0.780), nitrite (TCC p=0.250, ICC p=0.0237) and 3 day (TCC p=-0.0196, ICC p=-0.0856). The exception to this was in free chlorine and total chlorine at Site F. Therefore, ICC was not as successful an indicator for water quality as TCC but did show some minor trends. For instance, it was expected that 2 day colony counts and 3 day colony counts would have had a stronger relationship with ICC as they are both indicators of 'viable' microbial abundance to some extent.

Again, a number of water quality parameters had no relationship with ICC, regardless of site location, including: coliforms, *Enterococci*, ammonia, total nitrate, TON, hardness, alkalinity, total sodium, total potassium, total phosphorus, total calcium, total magnesium, total copper, total manganese, total iron and total lead.

Sufficient evidence was not found to indicate that the relationship between ICC and water quality parameters were influenced by source water type.

ICC no/100µl WTW CORR	Total Cell Count no/100µl	Coliforms no/100ml	*Entero cocci* no/100ml	3 Day Colony Count no/ml	2 Day Colony Count no/ml	Free Chlorine mg/l	Total Chlorine mg/l	Total P mg/l	Ortho phosphate mg/l	Total Lead µg/l	Temperature °C	Turbidity NTU	Conductivity µS/cm	pH pH units	TON mg/l	Nitrite mg/l
A	0.107	<2.2e-16	<2.2e-16	-0.0856	0.702	-0.0425	0.110	<2.2e-16	<2.2e-16	<2.2e-16	0.0525	0.0336	0.780	<2.2e-16	<2.2e-16	0.0237
B	0.348	<2.2e-16	<2.2e-16	-0.0161	<2.2e-16	-0.154	-0.127	<2.2e-16	<2.2e-16	<2.2e-16	0.0281	0.0406	0.0575	<2.2e-16	<2.2e-16	<2.2e-16
C	0.167	<2.2e-16	<2.2e-16	-0.0220	-0.0341	-0.00702	-0.0603	<2.2e-16	<2.2e-16	<2.2e-16	0.0557	0.105	<2.2e-16	<2.2e-16	<2.2e-16	0.0188
D	0.906	<2.2e-16	<2.2e-16	<2.2e-16	-0.0390	0.0545	0.0745	<2.2e-16	<2.2e-16	<2.2e-16	0.0928	0.0748	0.0847	<2.2e-16	<2.2e-16	<2.2e-16
E	0.928	<2.2e-16	<2.2e-16	<2.2e-16	-0.0671	0.465	0.435	<2.2e-16	<2.2e-16	<2.2e-16	0.0103	0.0757	0.102	<2.2e-16	<2.2e-16	<2.2e-16
F	0.958	<2.2e-16	<2.2e-16	0.652	0.652	-0.457	-0.714	<2.2e-16	0.626	<2.2e-16	0.00804	0.254	<2.2e-16	0.102	<2.2e-16	<2.2e-16

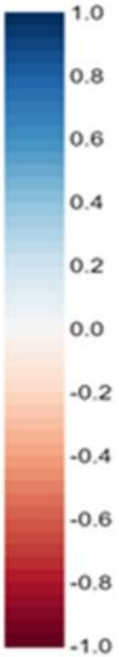


Figure 20 Correlation matrix of ICC and other water quality parameters at WTWs

3.3.5 Results Summary: An Assessment of Biological Stability in UK DWDS

This section has explored historic regulatory samples supplemented with TCC and ICC samples at a series of 6 case study sites to improve the understanding of biological stability in the current state of the system and determine their potential for chemical free drinking water treatment and distribution.

The comparison and contrast of different key indicators of water quality including the microorganisms of the distribution network, available nutrients, wider environmental water quality parameters and the aesthetics of the water as stipulated in the definition of biological stability outlined in Section 3.2.2 determined the following:

- There were no case study sites regarded as "poor" biological stability.
    - No evidence was found of water quality deterioration, temporal or otherwise, at any case study site.
    - The majority of samples observed during the course of the study were compliant to regulatory standards.
- Sites E and F were categorised as sites of "good" biological stability.
    - Sites E and F had particularly good water quality, better than that of Sites A-D, with consistently lower values and at times stability in how parameters varied at a sampling site over time and within the network. Sites E and F were also found to have had a higher level of compliance than other case study areas.
- Sites A-D were categorised as sites of "less good" biological stability.
    - Sites A-D consistently had higher parameter values than at Sites E and F, with incidences of a greater degree of variation at one sample point or within the network.

There were also multiple findings regarding indicators of biological stability assessment and how water quality was influenced at case study locations, such as if the network had an impact, disinfection method used, source water type. The wider findings evidenced during this section included:

- TCC and, to a lesser extent ICC, seemed to be successful indicators for biological stability at the WTW. TCC correlated with a number of (although not all) water quality parameters. In this way, TCC and ICC were found to have promise as a tool to provide a rich source of information, as well as a monitoring method that correlates with a number of other water quality parameters. It would have been highly valuable to also have this data at a Customer Tap level.
- All case study sites at both the WTW and the Tap found no presence of either *E. coli* or *Enterococci* but other methods of enumerating microorganisms, including TCC, ICC, 2 day colony counts, 3 day colony counts and in coliforms, did record positive values.
- There was evidence of the network having an influence over multiple water quality parameters. Some parameters increased in the network at all sites (2 day colony counts, turbidity, temperature and total lead) or at most sites (3 day colony counts, coliforms, phosphate and odour). Some parameters also decreased in the network at all sites (total chlorine) or most sites (free chlorine, nitrate and conductivity).

- Water quality at chloraminated sites could be different to those using chlorination, including:
    - Higher values in the parameters: TCC at the WTW, ICC at the WTW, TOC at the WTW, nitrite at the Tap, ammonia at the WTW and the Tap and colour at the WTW and the Tap.
    - Greater variation in the parameters: TOC at the WTW, nitrite in the network and ammonia.
- The impact of blended source waters did not appear to influence Site C (a blend of surface waters), which consistently acted like a surface water site. However, the blend of both surface water and groundwater at Site D meant that it could react unpredictably. At times, Site D behaved more like a Surface Water Site (Sites A-C), when at others, Site D behaved more like a Groundwater Site (Sites E-F).
- Not all parameters varied by the different case study locations (for instance in iron, manganese, pH, alkalinity, hardness and taste).

3.4 Discussion: An Assessment of Biological Stability in UK DWDS

This section has explored historic regulatory samples supplemented with TCC and ICC samples at a series of 6 case study sites to improve the understanding of biological stability in the current state of the system and determine their potential for chemical free drinking water treatment and distribution.

3.4.1 The Formation of a Second Refined Novel Definition of Biological Stability

The initially proposed definition of biological stability was formulated using and combining the advantages of previous definitions from literature and considering the feasibility for application to current practice in the UK water industry today.

Figure 3 assessed literature to coalesce all of the expected links between parameters that should be considered when forming a new definition of biological stability.

A thorough examination of these parameters, using 6 case study areas and historical regulatory data, has been used in Chapter 3.3 to form a counterpart, Figure 21. This figure details the parameters the experimental work has found (and has not found) to be of importance for the attainment of chemical free water.

This primary definition was explored and evaluated at 6 case study sites to test and assess it, forming the new refined definition of biological stability presented below.

The values used in Figure 22 to classify the biological stability of case study sites were extrapolated from the 6 case study sites explored in Chapter 3. The source of information for each value, the tables and figures from Section 3.3, can also be seen in Figure 22.

Appendix 4 shows this risk score matrix applied to the 6 case study sites for their classification of degree of biological stability.

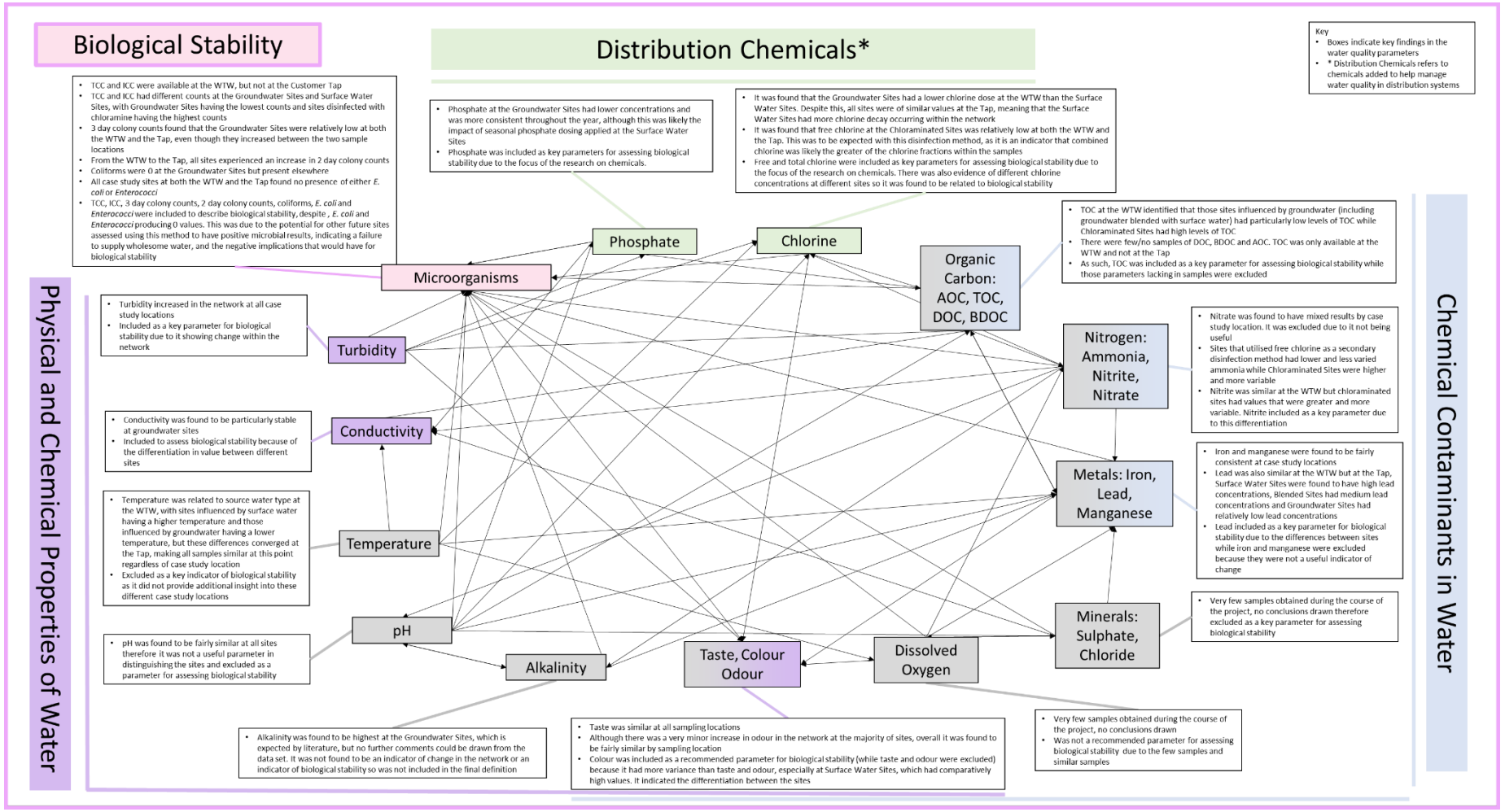


Figure 21 Parameters the experimental work has found to be of importance for the attainment of chemical free water

3.4.2 Second Refined Novel Definition of Biological Stability

Biological stability can be defined as the preservation of water quality from the WTW to the consumer tap.

The analysis of water quality should be performed with a combination of methods to analyse microbial and environmental parameters. The following combination of methods is suggested:

- Microbial parameters: TCC, ICC, 3 day colony count, 2 day colony count, coliforms, *E. coli, Enterococci.*
- Nutrient concentration: total organic carbon, phosphate, nitrate.
- Wider water quality parameters: total chlorine, free chlorine, turbidity, conductivity.
- Aesthetic parameters: colour.

Figure 22 depicts the quantifiable descriptors for each parameter to enable a classification of each to one of the three biological stability segments, “Good Biological Stability”, “Less Good Biological Stability” or “Poor Biological Stability”.

For each of these 24 key parameters, a point system is utilised, where good = 1 point, less good = 2 points and poor = 3 points. The points for each parameter should be totalled and the site classified by their level of biological stability: ≤35 is good, 36-64 is less good and ≥ 65 is poor.

It is also acknowledged that other factors can affect the biological stability during distribution, including: pipe material, hydraulic conditions, residence time, residual disinfectant decay, construction, operation and maintenance practices and premise plumbing conditions.

Figure 22 Risk score matrix for classifying biological stability as "Good", "Less Good" or "Poor"

3.4.3 As Assessment of the Definitions of Biological Stability

It could be proposed that in actuality the stability being assessed is a water quality stability metric. As Figure 21 details there are a range of biological, chemical and physical parameters and interactions that are important to consider due to the complexity of distribution networks (as shown in

Figure 2). This research has used the phrase "biological stability" because chemical free water may well be possible in an idealised well monitored system but what is essential is achieving this aim in such a way that the water produced poses no threat to public health. One particular area of the distribution network that is of the upmost importance to public safety but is also relatively not well understood is that of the microbial water quality, the interactions between various microorganisms that exist both planktonically and attached to the pipe surface in the form of a biofilm. As such, it was determined that biological stability would be the focus of investigation, rather than for instance a metric of chemical stability or physical stability.

There have been incidences of previous studies that discuss biological stability but do not state which definition of biological stability is being used, for instance the work of Grefte et al. (2011), which is problematic as different definitions of the term are used by different researchers. Both the primary and secondary novel definitions of biological stability provided helped to clarify how biological stability was assessed and how case study sites were categorised using this.

One factor that the novel definition of biological stability considered was the sampling points to where this stability or variation occurred. There is general consensus that the term "biological stability" refers to the concept of maintaining water quality within the network, for example, in the World Health Organization (2011) statement that "Water entering the distribution system must be microbiologically safe and ideally should also be biologically stable.". Also the network was discussed in multiple definitions of the term, such as those by Rittmann and Snoeyink (1984), Lautenschlager et al. (2013), Prest et al. (2016) and Sibille (1998). As such, this was included in the novel definition, "the preservation of water quality from the water treatment works to the consumer tap".

In the novel definitions of biological stability, stability was characterised by "lower values and/or consistent values in a variety of key indicators". This is because when assessing the historical data in the current research, it was often found that while parameters did not change in value between the WTW and the Tap, they could be variable in other ways. One way in which parameters were found to express variation was in high sporadic values at a sample location compared to the norm for that location. For example, in Figure 13 while TCC at the WTW of Site A had an average value of 513 no/100µl, a large spike was observed with a maximum value of 13124 no/100 µl. A further way there could be variance is when a parameter had a large range at some case study sites but not at others. For instance, Figure 14 depicted ammonia at the WTW. Sites A and C had a high range of values (~0.08-0.34 mg/l a range of 0.26 mg/l) while other sites such as Site B had lower values and a lower range (0.008-0.042 mg/l, a range of 0.034 mg/l). Therefore, there was not as much deterioration within the network itself as expected, rather greater temporal variation or more sporadic values or persistently higher values.

There was some discussion as to whether specific values should be used in the definition of biological stability. In literature, there were a few definitions of biological stability focused on nutrients, especially carbon in the form of AOC. Zlatanović et al. (2017), for instance, defined

biologically stable water as being nutrient limited, with low concentrations of AOC (<10 µg/l). One problem with the exclusive use of nutrients as a categorisation method is that, as previous studies have shown, AOC values of <10 µg/l do not always have good water quality, while sample results of >10 µg/l do not always have poor water quality (Van der Kooij, et al., 2005) (Vital, et al., 2010). Also, a specific parameter value such as >10 µg/l AOC may be normal for that specific area, and it may be variations in parameter at the same sample location or area over time that may be indicative of a change in biological stability. It is very difficult to give an absolute value of measurements because different areas have varying properties.

When definitions of biological stability focus on a small number of parameters, such as nutrient concentration and microbial presence, other impacts of water quality may be neglected. One example is that in the Netherlands a strategy was being used to maintain biological stability by limiting the growth promoting nutrients using treatment to minimise the growth of microorganisms in the distribution (Prest & Martijn, 2020). However, one area was initially categorised as biologically unstable due to customer complaints, discoloured water, taste and odour problems and the presence of invertebrates at the customer tap. This classification led to an in-depth study of the area, which found that 30% (compared to 3% in biologically stable areas) of *Aeromonas* samples exceeded regulatory guidelines every summer and that there was the presence of *Legionella* growth. As such, in this study, although the initial biological stability strategy was reliant solely on nutrients and microorganisms, it was actually the aesthetic parameters of drinking water quality at the tap e.g., how high the values were in taste, colour and odour, that led to categorisation and further investigation. If the study in question had followed only the strategy detailed, the larger water quality trends may not have been investigated.

Rather than relying on a value for a sole parameter, a system was developed to consider many different parameters with multiple different values [Figure 22]. Regardless, a caveat is that it is recommended that these values be verified before application to wider use, however they are intended to be used as an indicator for future work in the area.

Also, it was not to say that all parameters were helpful in the classification. When applying the proposed novel definition of biological stability, it was found that not all of the initially selected parameters were successful in providing an enhanced understanding of varying degrees of biological stability. This included the parameters: nitrate, ammonia, water temperature, pH, hardness, alkalinity, iron, manganese, taste and odour. This was often due to no or minimal difference in this parameter between the sites (e.g. pH, iron, manganese, taste, odour). Therefore, the historic regulatory parameters used to assess biological stability were streamlined between the primary and secondary definitions, as indicated by the transition from Figure 3 to Figure 21.

There was additionally some debate as to whether the parameters *E. coli* and *Enterococci* should be included in the second definition. There were parameters such as TCC, ICC, 2 day colony counts, 3 day colony counts, Tap turbidity and WTW TOC, which provided much more insight into the differentiation between sites, while *E. coli* and *Enterococci* remained negative throughout the historical data analysis [Figure 13]. It was mused that *E. coli* and *Enterococci* were more informative regarding point of failure, rather than a lead early indicator of biological stability. However, it was decided that if positive values were present, it would be an important factor to consider when categorising site status. For instance, if *E. coli* and *Enterococci* were 0 no/100ml at the WTW but

present at the Tap, it could be an indicator of poor biological stability and a signpost that wider investigation is required.

There was also discussion as to whether phosphate should have been included in the second definition. This is because higher concentrations of phosphate were dosed at the WTWs that had been perceived to have a higher risk for lead failure (with the categories for risk displayed in Table 11) so there could be a confusion regarding cause and effect. For example, is higher concentrations of phosphate present indicative of poorer performing sites or is it that higher phosphate concentrations are dosed at poorer performing sites? However, as phosphate does influence the network, higher concentrations could have more of a distribution network impact, which could then in turn impact the biological stability of these environments. For instance, Section 1.4.4.2 posed the question of phosphate dosing in networks be acting as a food source for microorganisms. As such, phosphate was included in the newer of the two definitions.

Hence, the most comprehensive definitions of biological stability in previous research suggested a multilevel approach. This approach helps to coalesce the different advantages of assessment methods and address factors such as microorganisms and nutrients, as well as wider parameters including aesthetic parameters and technical failure (Lautenschlager, et al., 2013). The primary novel definition of biological stability provided incorporated the following: the specification of a definition; the sampling locations to which the definition could be assessed; a consideration of the different types of variation that can be assessed for and a broader measurement, going beyond a single measurement such as microbial presence or nutrient concentration. The secondary novel definition was able to improve upon the first proposed definition to form a more comprehensive overview of what biological stability means in UK systems, derived from the findings of 6 case study sites. In this manner, this research has used and combined the advantages of previous definitions, incorporating the main factors of multiple definitions of biological stability used in previous studies. The application altered and improved the definition, validating it, and showing it works as intended.

3.4.4 The Use of Historic Regulatory Data

When discussing the novel definition of biological stability proposed, it is also important to assess if the data used was effective for this application. For this purpose, historic regulatory samples were found to be a source of highly valuable data that worked well to provide an increased understanding regarding the variability of different systems. Samples were plentiful and in a broad variety of water quality parameters at the WTW and at the Tap.

Despite this successful utilisation, the use of regulatory samples could have been improved. One way in which historic data could have been supplemented is through the use of network samples. It would have been preferable to have samples from within the network itself, rather than using WTW and Customer Tap samples to infer network condition. This information would have been particularly valuable when considering how water quality varied at different points of the network, such as at the extremes of the network. Supplementary network details would also have helped to build a richer picture of the network, with factors such as hydraulic conditions, residence time, maintenance practices and premise plumbing conditions.

Future research could further benefit from an assessment of both planktonic and attached microorganisms, which although was considered in the novel definition provided, was not possible to gauge using historic data. For this, in situ pipe sections comparable to those presented in the

work of Douterelo et al. (2015) would have allowed for the contemplation of attached microbiology. This would have been especially interesting, to compare and contrast between the attached microorganisms of the different sites with their varying biological stabilities. For example, the significant change in 3 day colony counts observed at Site B and Site D when comparing data from the WTW and the Tap, shown in Table 9. This finding could actually be because the bulk microorganisms assessed at the WTW were then attached within the network, rather than also being present in the bulk water at the Tap.

A further way in which regulatory samples could have been supplemented is with additional parameters. Although parameters sampled for by water utilities were found to go beyond the requirements stipulated by regulations, it would have been desirable to have an even more diverse parameter pool, including the relatively new and advanced techniques discussed in Section 1.5.2, such as TCC/ICC, ATP, AOC and DNA analysis. As multiple studies have outlined, in the last decade, cultivation-independent detection methods of assessing microbial presence have gained interest due to their speed, accuracy, quantitative measurement and ability to detect both cultivable and uncultivable microorganisms (Adomat, et al., 2020) (Liu, et al., 2013).

It could also be argued that historic data may have had increased validity if samples had been taken as triplicate grab samples or as samples composited over a time period such as 3 h (Cornman, et al., 2018). However, for the quantity of samples involved (~350,000 drinking water samples tested a year for 49 different parameters in the Anglian Water area), doing these samples in triplicate would exponentially increase staff time for sampling and analysis, as well as the cost associated with the sample number increase (Anglian Water, 2020). As such, the use of triplicate samples would likely only be possible for targeted specific campaigns rather than for all regulatory samples.

Overall, historic regulatory samples were found to be a source of highly valuable data with a broad number of WTW and Tap water quality parameters that worked well to provide an increased understanding regarding the variability of different systems. Nevertheless, there were a number of ways in which these samples could have been improved or supplemented to further build understanding and form a richer picture of the network, through the use of the following: samples from the network itself; further information about the network environment; an assessment of the attached microorganisms within the network; a more diverse parameter pool including new advanced techniques and potentially through the use of triplicate samples.

3.4.5 Monitoring Measurements for Biological Stability

Throughout the historic data analysis, all 6 case study sites at both the WTW and the Tap found no presence of either *E. coli* or *Enterococci*. Despite this, other methods of enumerating microorganisms, including TCC, ICC, 3 day colony counts, 2 day colony counts and coliforms, did record positive values [Figure 13]. This finding corresponds well with previous research. As Section 1.5.2.1 detailed, HPC are limited by the way that only a small proportion of metabolically active microorganisms in a water sample are able to be grown and so detected, depending on the environmental conditions (Lautenschlager, et al., 2013) (Hassard & Whitton, 2019) (Liu, et al., 2013). Much research has maintained that microbial abundance is several orders of magnitude higher when measured with absolute counting methods, such as flow cytometry, compared with culture-based quantification methods (Nocker, et al., 2017) (Hassard & Whitton, 2019). Therefore, even though

there was an abundance of *E. coli* and *Enterococci* samples from the historic data, as an indicator of biological stability, *E. coli* and *Enterococci* plate counts were not found to provide a valuable insight.

Flow cytometry was utilised as a biological stability assessment technique to supplement regulatory samples due to its ease of use, speed (~10,000-50,000 cells counted per second), its quantitative measurement method, the way it can detect microbial cells irrespective of their cultivability and its ability to determine cell viability (Wilkerson, 2012). Universities and water companies have expressed interest in organising and attending flow cytometry working group meetings to work collaboratively to share their knowledge and best practices for use of this technology type (Hassard & Whitton, 2019). As such, the water utility partners in the current research were keen to find an opportunity to test this technology, after seeing it being successfully utilised in other organisations.

Many studies have shown the use of TCC and ICC to be a valuable tool for the detection, enumeration and characterisation of waterborne microbial populations (Hassard & Whitton, 2019) (Adomat, et al., 2020) (Liu, et al., 2013). This PhD research found that the use of TCC and ICC [Figure 13] provided a more detailed and quantitative insight than that of plate counting such as *E. coli* and *Enterococci*. As Figure 19 and Figure 20 showed, there was a link between TCC, ICC, 2 day colony counts and 3 day colony counts but not with *E. coli* and *Enterococci*. As such, TCC and, to a lesser extent ICC, were successful indicators for biological stability at the WTW. TCC correlated with a number of water quality parameters including: ICC, temperature, conductivity, 3 day colony count, turbidity, free chlorine, total chlorine and at some sites 2 day colony count, nitrite, orthophosphate and pH [Figure 19]. ICC had a weaker link with water quality parameters than TCC, for example in temperature, turbidity, conductivity, nitrite and 3 day colony count [Figure 20]. Although data availability was limited to the WTW and it would have been highly valuable to also have this data at a Customer Tap level.

That is not to say that TCC and ICC were a universal predictor of environmental conditions within the distribution network. TCC did not correlate with all water quality parameters, such as: coliforms, *Enterococci*, ammonia, total nitrate, TON, hardness, alkalinity, total sodium, total potassium, total phosphorus, total calcium, total magnesium, total copper, total manganese, total iron and total lead [Figure 19]. As such, it would not be recommended that TCC and ICC be used as a sole indicator of biological stability. Although, flow cytometry should not be solely relied upon as the different methods of assessing biological stability have different advantages that could complement each other when used together in a multilevel approach (Lautenschlager, et al., 2013).

Other ways in which flow cytometry samples could have been further supplemented is through the use of other technologies used in conjunction, for instance the analysis of DNA, AOC or the use of ATP. As Prest et al. (2016) found, ATP in particular has been found to have a good correlation between ATP and flow cytometry, displaying clear seasonal variation. Another desirable additional parameter was that of AOC monitoring, which would have provided a good indication of the presence and formation of microbiology. This technique was not completed due to the ease of sample contamination (requiring a 500^{o}C furnace, a resource not available at the time of experimentation) and long sample turnaround times (even though Pick (2018) identified many adjustments and improvements to the original AOC method) but would be invaluable for future research. The use of TOC at the WTW certainly provided a clear distinction between different case study sites, with the chloraminated sites having much greater concentrations than the chlorinated

sites, as shown in Figure 14, which would have been interesting to have seen if Tap TOC had the same trends.

Future research could benefit from a greater sample pool to solidify findings further. Flow cytometry samples in this study were used to supplement historic regulatory samples and so there was only TCC and ICC data available for a three month period [Figure 13]. It would be desirable to have samples over a longer period of time to enable a detailed assessment of case study sites to be formed, to better allow deviations from the norm for that specific site to be identified. Further, the TCC and ICC were solely from bulk water samples and not from samples within the network itself, as it is in the work of Douterelo et al. (2015), which would have been a valuable step towards understanding the microbiome of distribution networks. It would additionally have been beneficial to have TCC and ICC data at the Tap to determine how the network influenced counts and if these counts varied by different areas. There was reluctance with the water utility working in collaboration to conduct this research with regard to taking TCC and ICC samples at the Tap. In this case, there was concern that high values could potentially reach the media and customers could interpret this data as their water being unsafe for consumption, even though it is well understood that the majority of organisms persisting in the distribution network pose no threat to public health.

A factor currently acting as a barrier for more widespread flow cytometry monitoring is a lack of standardisation, for instance through the use of a formal consistent method across the water industry, the use of accreditation (like other water quality parameters have) or the use of indicative prescribed concentration or value. For example, it is not known how many cells result in safe water and how many in unsafe water. It is known that microorganisms are ubiquitous; the majority of organisms persisting in the distribution network pose no threat to public health or network operation, but they can support the growth of microorganisms that are. Research to date has not found a direct link between flow cytometry result and risk to public health. Although, the same can also be said of HPC. Thus, standardisation in approach, in the form of individualised, utility specific and site-specific limits, could be used to support decision making regarding the categorisation of sites by their biological stabilities. For this to be a possibility, it is likely that several years of TCC and ICC measurements would be required, taken in parallel with existing water quality parameters, to build a solid database for comparison.

Flow cytometry analysis has the potential for further development in future. For example, the use of flow cytometry to identify specific reference pathogens using IMS-FCM (immune-magnetic separation flow cytometry). As Hassard and Whitton (2019) detailed, multiple studies have found that flow cytometry has successfully quantified *Legionella spp*., *Cryptosporidium spp*., *Campylobacter spp*. and *Vibrio spp.,* with the main limit for further organisms being the specificity of antibodies. Another such improvement is the use of imaging flow cytometry. This technique combines the use of conventional flow cytometry with high-resolution multispectral cell image analysis to measure microbial concentration, species and growth stage (Phanse, et al., 2012). As such, it is likely that flow cytometry will continue to develop as an analysis tool, incorporating further assessment techniques, including but not limited to those of imaging, sorting, fingerprinting and sequencing (Hassard & Whitton, 2019).

Thus, it was found that parameters such as *E. coli* and *Enterococci* counts were abundant but did not provide a valuable insight into biological stability while TCC and, to a lesser extent ICC, were

successful indicators for biological stability at a WTW level which correlated well with a number of other water quality parameters. As a technique, flow cytometry was easy to use, quick and provided a helpful quantification of biology even while *E. coli* and *Enterococci* plate counts found no presence. It would have been highly valuable to also have this data at a Customer Tap level as TCC and ICC to further test its effectivity as a supplement to regulatory samples for the determination of biological stability. Future research could also be further improved in a number of ways, including: samples over a longer period of time, biofilm analysis as well as bulk water, Tap water samples and further supplementation with other biomonitoring methods such as AOC. In wider water utility use, standardisation would likely aid widespread flow cytometry monitoring to provide a rich source of information. In future, the technique will likely continue to develop as an analysis tool in this sector, incorporating further assessment techniques, including but not limited to those of imaging, sorting, fingerprinting and sequencing.

3.4.6 Case Study Site Biological Stability Classification

When discussing the novel definition of biological stability, it is also important to assess the effectiveness of its application to categorise case study sites as "good" to "poor" biological stability.

Sites E and F were categorised as sites of "good" biological stability. These sites had particularly good water quality (better than that of Sites A-D), with consistently lower values, some temporal stability in parameters, consistency within the network and a higher level of compliance [Figure 12, Figure 13, Figure 14, Figure 15, Figure 16]. The case study sites that were of good biological stability were found to be groundwater sites. This was similar to observations from case study companies in countries who treat and distribute water with fewer chemicals than in the UK. In these areas, as discussed in Section 1.3, the preferred source water type was groundwater, with the reasoning that surface waters do not have soil filtration and are more vulnerable to the influences of precipitation, leading to higher microbial presence and more seasonal variability (Isle Utilities, 2015) (Kistemann, et al., 2001). Similarly, countries who currently assess biological stability did not find source water type to be a barrier for the production of chlorine free drinking water treatment and distribution, that surface water could still be used. Regardless, these companies use processes with long residence times, such as dune filtration and bank filtration, to reduce the biological activity of surface water, a practice not utilised in the UK (Isle Utilities, 2015). This method provided substantial reductions in microorganism concentrations, for example total coliforms had an average reduction of 5.5 and 6.1 log (Weiss, et al., 2005). Yet, these processes were not used in the case study systems in this study and these systems were found to not have "bad" biological stability, but to just not be as good as the groundwater sites.

It was also evidenced that there were no case study sites regarded as "poor" biological stability. It could be argued that no sites were categorised as poor biological stability and that there was minimal variance in compliance [Table 10] due to poor site selection. However, there was adequate variation between different sites and temporal variation to classify both "good" and "less good" biological stability, implying that site selection was indeed robust. Further, in previous research, there were few studies that clearly classified the biological stability of sites and gave the reasonings for these classifications and even fewer studies that stated sites were of bad stability. In that way, it could be said that similar findings were detected in other studies. There are a multitude of studies that classify source waters, for example The European Water Framework Directive (WFD; 2000/60/EC), which assesses biological, hydro-morphological and physio-chemical quality elements

to assign systems as 'High", ''Good", ''Moderate", ''Poor" and "Bad" (Borja, et al., 2009). However, there are minimal studies that classify final water from WTW as well as water from consumer taps as being regarded as "poor". This could potentially be because most research assessing these sampling points is done in collaboration with water utility companies, which might object to such negative connotations, especially if such research is published. Equally, however, an assessment of poorer stability may operate as a tool to indicate to water utilities where to focus their investments to best improve drinking water quality. Regardless, the research of Prest and Martijn (2020) was one example of a study which categorised an area as being biologically unstable, due to the following: customer complaints; discoloured water; taste and odour problems; the presence of invertebrates; *Aeromonas* samples exceeding regulatory guidelines seasonally and the presence of *Legionella* growth. In contrast, in this PhD, no evidence was found of water quality deterioration, temporal or otherwise, at any case study site (including acceptable values in colour, taste and odour at the Tap), as shown in Figure 17 and Figure 18. As detailed in Table 10, case study sites were 99.91-100% compliant with regulations at the WTW and 97.84-100% compliant at the Tap.

Thus, the combination of the novel definition of biological stability and the available historic data successfully categorised case study sites as having varied biological stabilities, as such in this manner, it was a success. These classifications did not always agree with previous literature, for example in the case of surface water requiring high retention times to have reduced microbial loads and to be biologically stable. For the most part they did, for instance, that groundwater sites had particularly good biological stability while surface water sites were less good than the groundwater sites and a lack of sites that could be identified as poor biological stability.

3.4.7 Other Factors that Influenced Biological Stability

3.4.7.1 Source Water Blending

Another factor that influenced biological stability was blended source waters. The impact of blended source waters did not appear to influence Site C (a blend of surface waters), which consistently acted like a surface water site. However, the blend of both surface water and groundwater at Site D meant that it could react unpredictably. At times, Site D behaved more like a Surface Water Site (Sites A-C) in: Tap 3 day colony count, 2 day colony count, phosphate, total chlorine, free chlorine and lead [Figure 13, Figure 12, Figure 15]. At other times, Site D behaved more like a Groundwater Site (Sites E-F) in: TCC, TOC, conductivity and colour [Figure 13, Figure 14, Figure 15, Figure 16]. These results were not always consistent with previous research, although the majority of studies, particularly those in the area of biological stability, are either on groundwater sites or surface water sites, rather than on sites with a blend of sources.

Those studies that assessed mixed water sources, have shown it can increase metal release from the distribution network, with Lintereur (2006) and Duranceau et al. (2010) studied different blends of groundwater, surface water and desalinated seawater, while Stone (2006) investigated blends of two different surface waters and groundwaters. But Peng and Mayorga (2018) had further claims, additionally noting that blending water sources altered the balance within the drinking water distribution environment, leading to pipe corrosion, decreasing disinfectant residual and microbiological growth with blends of groundwater, surface water and desalination water. Nescerecka et al. (2014), also noted that where different water sources were mixed (groundwater and surface water), there were differences in primary growth limiting nutrients, which could

contribute to biological instability in the network (in this study, biological instability was described as "uncontrolled bacterial growth").

Regardless of these past studies, in the current research, iron and manganese concentrations were fairly consistent at all case study sites, while lead was higher at blended sites than at groundwater sites but even higher at surface water sites [Figure 15]. Chlorine decay within the network was found to be similar at blended sites and surface water sites, both of which were less than groundwater sites [Figure 12]. Microbial growth could have mixed results, with Site D at times being very low (WTW TCC), other times middling (WTW ICC), others high (3 day and 2 day colony count at the Tap) and with variation (2 day colony count at the WTW) [Figure 13]. Site C was often middling (WTW TCC, WTW ICC and WTW 3 day colony count), variable (Tap 3 day colony count, WTW 2 day colony count and in coliforms) and at times high (Tap 2 day colony count) [Figure 13]. With nutrient concentration, the presence of disinfection method (chlorination or chloramination) appeared to have more of an influence than site blending [Figure 14].

Therefore, the current research found different impacts when different source waters were blended. These findings were not consistent with those of previous research, although there were few studies regarding source water blending.

3.4.7.2 The Drinking Water Distribution Network

It was evidenced that the network influenced multiple water quality parameters [Table 9]. Some parameters increased in the network at all sites (2 day colony counts, turbidity, temperature and total lead) [Figure 13, Figure 15] or at most sites (3 day colony counts, coliforms, phosphate and odour) [Figure 13, Figure 12, Figure 16]. Some parameters also decreased in the network at all sites (total chlorine) [Figure 12] or most sites (nitrate, free chlorine and conductivity) [Figure 14, Figure 12, Figure 15].

Previous research agrees with this, stating that water quality degradation occurs in the network due to the physical, chemical and biological reactions which occur during distribution and can be impacted upon many different factors, such as pipe materials, contamination, pipe leakage and inadequate or infrequent maintenance (National Research Council, 2006). Williamsona et al. (2014), for instance, reported that "30-60% of water quality incidents are related to events in the water distribution network". It also appears that current UK regulations acknowledge that water quality will change within the network and that customer tap samples are more likely to be variable than WTW samples. This is insinuated because regulations were often stricter at the WTW than at the Tap, for example, the maximum prescribed value of turbidity at the WTW is 1 NTU whereas at the tap it is 4 NTU (Drinking Water Inspectorate, 2016) [Table 10]. As such, it appears that both current regulation and previous research expect drinking water quality to be degraded by the distribution network.

Although the network did influence water quality, there were a multitude of parameters that did not change within the network. It was additionally thought that the surface water sites may have had more network degradation than at groundwater sites but in actuality they behaved in a fairly similar manner.

Therefore, the network was seen to influence water quality between the WTW and the Tap, this was not observed in all parameters and other factors could also influence water quality.

3.4.7.3 Secondary Disinfection Method

The secondary disinfection method used also impacted drinking water quality. Case study sites that utilised chloramination were different to those that used chlorination, with higher values in some parameters (TCC at the WTW, ICC at the WTW, TOC at the WTW, nitrite at the Tap, ammonia at the WTW and the Tap and colour at the WTW and the Tap) [Figure 13, Figure 14, Figure 16] and greater variation in several parameters (TOC at the WTW, nitrite in the network and ammonia) [Figure 14].

The nitrite levels being relatively low at the WTW while high at the Tap shown in Figure 14 suggests that nitrification may have been occurring within the network. Nitrification is the oxidation of ammonia (added in combination with free chlorine to produce chloramines) into nitrite (an intermediate in the oxidation of ammonia to nitrate) and nitrate (the most oxidised and stable form of nitrogen in a water body) (United States Environmental Protection Agency, 2011). This process can impact water quality by depleting the chloramination residual present, reducing pH, alkalinity and dissolved oxygen concentrations and by increasing the corrosion of the surrounding pipes, particularly of lead, copper and iron (Zhang, et al., 2009) (UKWIR, 2017). In extreme cases, nitrification can also lead to taste and odour problems, the formation of carcinogens and potentially even causing methemoglobinemia (blue baby syndrome) (Kirmeyer, 2004) (World Health Organization, 2003) (Xie, et al., 2016). Thus, it is highly important that the case study sites using chloramination are effectively monitored to reduce the occurrence of nitrification. Despite this nitrite change identified in the network, pH, alkalinity, total iron, taste and odour (excluding Site C) at chloraminated sites were not found to have substantial change within the network [Section 3.3.1.4, Table 9]. Total lead did have a significant increase in the network but at Sites A-D, not just chloraminated sites [Table 9]. As such, any apparent nitrification effects were not causing concern for these case study areas at this time.

Research widely regards chloramine to be less effective at disinfecting water than free chlorine, with a moderate biocidal activity against bacteria and a low biocidal activity against viruses and protozoan cysts, due to a lower oxidation potential (Fulton & Budd, 1992) (World Health Organization, 2004). For example, compared with chlorine, monochloramine is ~2000 times less effective for the inactivation of *E. coli* (World Health Organzation, 2019). It was found that the chloraminated sites did have the greatest microbial abundance in TCC and ICC but this was not detected in other methods of microbial assessment, such as 3 day colony counts, 2 day colony counts, coliforms, *E. coli* and *Enterococci* [Figure 13].

One potential reason why TCC and ICC could have detected more microorganisms than *E. coli* and *Enterococci* plate counts in this instance was because of the cultivability of species present. A further impact of chloramination is that it can influence the microbial community composition. High levels of nitrogen act as a source of nutrients for nitrifying bacteria in the water, specifically ammonia-oxidising bacteria (oxidise ammonia to nitrite) and nitrite-oxidising bacteria (oxidise nitrite to nitrate). Even a small amount of ammonia or nitrate has the potential to support high numbers of nitrifying bacteria (Zhang, 2008). In the present example, as Figure 14 found, nitrate levels were relatively low and similar at all case study sites. This could potentially indicate that the environmental conditions were selecting for the growth and reproduction of ammonia-oxidising bacteria, giving them a competitive advantage for survival with the abundance of ammonia available as a food source when compared to chlorinated sites. To investigate this further, it would have been

desirable to examine the DNA of microorganisms from the different case study networks to see if there was a difference in strains found under the different conditions.

Future research could be supplemented with which DBPs were being formed, the quantities of DBPs being formed and if these factors varied between both sites that are and are not biologically stable, as well as those with different disinfection methods. As Section 1.2.1 stated, multiple studies have determined that people who are exposed to DBPs (including THMs, one of the most well-studied DBPs) in water over the duration of their life have an increased likelihood of developing cancer, especially of the urinary bladder and colorectum, although other types of cancer too such as chronic myeloid leukaemia (International Agency for Research on Cancer, 1991) (Richardson, et al., 2007) (Moghadam & Dore, 2012). As such, water utility operators are often encouraged to switch to chloramines as a means of controlling disinfection by-product formation. For example, Kirmeyer (2004) found that, when compared to chlorine use, chloramines reduced the formation of THMs by 40-60%. Despite this, multiple studies have shown that even though chloramines produce fewer THMs there are other DBPs which are formed that should be of equal concern, including NDMA (N-nitrosodimethylamine), cyanogen chloride and bromide (Bull & Kopfler, 1991) (World Health Organization, 2011). Future work in the area could monitor the DBP formation because the case study sites that used chloramination additionally had the highest levels of TOC, when DBPs are formed when a chemical disinfectant (e.g., chlorine, chlorine dioxide, chloramines and ozone) reacts with natural organic matter and/or inorganic matter (World Health Organization, 2000).

Thus, the secondary disinfection method used at case study sites was found to impact drinking water quality, particularly in the abundance of specific nutrients, which in turn could have altered the microbial communities found within the network. To supplement regulatory data, an indicator of community such as DNA analysis as well as an assessment of DBP presence would have been beneficial.

3.4.8 Discussion Summary: An Assessment of Biological Stability in UK DWDS

This section has explored historic regulatory samples supplemented with TCC and ICC samples at a series of 6 case study sites to improve the understanding of biological stability in the current state of the system and determine their potential for chemical free drinking water treatment and distribution. A summary of this discussion will now be presented.

The following were seen as successes:

- The novel definition of biological stability provided successfully collated and coalesced the different advantages of assessment methods discussed in previous literature.
- Historic regulatory samples were a source of highly valuable data with a broad number of WTW and Tap water quality parameters that worked well to provide an increased understanding regarding the variability of different systems.
- The supplementary biological stability monitoring method used in this study (flow cytometry) provided a valuable insight into water quality, which correlated well with a number of other water quality parameters and was found to be a successful addition to regulatory samples at the WTW. The technique was found to be easy to use, quick and provided a helpful quantification of biology even while *E. coli* and *Enterococci* plate counts found no presence, as suggested in previous studies.

- The combination of the novel definition of biological stability, the available historic data and the supplementary flow cytometry data successfully categorised case study sites as having varied biological stabilities, as such in this manner, it was a success.
- For the most part, the classifications of biological stability agreed with previous literature, for instance that groundwater sites had particularly good biological stability, but other factors were also found to have influenced biological stability.
    - There were different water quality impacts when certain types of source waters were blended (of which there is little research).
    - That the network influenced water quality between the WTW and the Tap but not to the same extent as described in literature.
    - That the secondary disinfection method used at case study sites had an impact on microbial presence and the abundance of specific nutrients (agreeing with previous research).

4 Chapter 4: The Application of Chemicals and their Impacts in the DWDS

This chapter will utilise data analysis of historic regulatory samples at the WTW, Final Water and the customer tap to investigate Aim 2, Objectives a, b, c and d as described in Chapter 2.

4.1 Introduction: The Application of Chemicals and their Impacts in the DWDS

4.1.1 Chlorine

Many studies that specifically focus on biological stability in the network have taken place in live distribution networks that do not receive a chlorine dose, for example the work of Lautenschlager et al. (2013), Prest et al. (2016) and Liu et al. (2013). However, disinfected WTW can behave differently to those without a residual. Prest et al. (2016) regarded the question of applying a disinfectant residual in water as central to the context of biological stability.

Of the studies to date that focus on disinfected networks, there is an emphasis on monthly or seasonal variations in the numbers of microorganisms. This is because one reason why the application of a chlorine residual is likely to impact the biological stability of the network is chlorine dosage at a WTW is not necessarily constant over time. An example of this occurrence is that water utilities may choose to increase chlorine dose seasonally because chlorine decays quicker at higher temperatures. Pinto et al. (2014), for instance, found that microbial richness had a strong seasonal trend with warmer temperatures increasing the observed number of operational taxonomic units, during a monthly sampling campaign of a chlorinated treatment works and distribution network in Ann Arbor, Michigan, USA. Despite studies such as this, El-Chakhtoura et al. (2015) stated that research investigating spatial and temporal variations in the network microbial water quality were "…mainly determined based on sampling a few locations in the network…" and that this data was "…rarely compared to the original treatment plant samples".

Another reason the application of a chlorine residual likely has an impact on biological stability is because chlorine dose within the network is not homogenous at all points of the network in its entirety. The application of a chlorine residual is a balance between protecting the public from microorganisms while also protecting them against disinfection by-products. The distribution network is a complex environment. As such the optimum chlorine dose is difficult to determine because the amount required is governed by the composition of the water, the condition of the distribution network, the length of time the water is within this network and environment parameters such as temperature and pH. For example, networks with more corrosion have a higher chlorine decay rate and in the UK some pipes still in use today have been in use for over 100 years (UKWIR, 2003). As Al-Jasser (2011) noted, a change in the chlorine wall decay from 8-531% when comparing freshly laid pipes (0 years old) and old pipes (55 years old). Because of this variation, even if the dose of secondary disinfection is the same at a WTW, the residual chlorine dose at the consumer tap may not be homogeneously distributed across all network points.

When a residual disinfectant has been depleted or partially depleted in distribution networks, increased bacterial abundance has been identified because of the multitude of reactions occurring between the bacterial cells, NOM, particles, sediments and biofilms (Servais, et al., 1995).

LeChevallier et al. (1996) reported high numbers of coliform with a free chlorine concentration of <0.2 mg/l and a monochloramine concentration of <0.5 mg/l. Similar results were found by Gillespie et al. (2014) who showed that free chlorine concentrations <0.5 mg/l were related to higher intact bacterial cell concentrations in bulk water. Gibbs et al. (1990) found a reduction in the microbiology present in the distribution network immediately after booster chlorination (0.5 mg/1 of free chlorine) was applied but regrowth rapidly occurred after the decrease of residual chlorine through the network. Therefore, it appears that when chlorine residual decreases, microbial presence in the network increases because of a change in the distribution network environment.

Due to the relationship between chlorine concentration and microbial presence, it could be possible that chlorination has a different impact in networks with differing biological stabilities, as the various network conditions impact the chlorine decay. Pieri et al. (2014), for example, investigated two DMAs supplied by the same WTW in Nicosia, Cyprus, that were identified as high-risk and low risk because of their pipe leakage and average night water flow. These two areas displayed contrasting behaviour with respect to the interplay between added chlorine, formed DBPs and measurements of microbial indicators at ~150 households.

Overall, chlorination is a practice that is thoroughly studied in live networks, especially regarding the optimum chlorine dose to use, but less so linking chlorination and biological stability. Many studies into biological stability are based on networks that do not have a chlorine residual. The research into chlorinated networks have mostly focused on the impact of temporal changes, the effect of varied dose at WTW and ensuring that despite different network characteristics, a chlorine residual is universal. In-depth studies of chlorine utilisation in different networks (such as those which are biologically stable and those which are not) can help to establish the trend characteristics of a particular drinking water distribution system to allow for deviations from that trend to be recognised and investigated.

4.1.2 Phosphate

The vast majority of research exploring the use of phosphate is focused specifically on lead because of the serious consequences lead can have on human health and the strict regulations stating that the lead concentration at customer taps should be no higher than 10 µg/l (World Health Organization, 2018) (European Commission, 2016). For example, the work of Hayes et al. (2008) described the process of optimising the orthophosphate dose for controlling lead at the tap in Dwr Cymru Welsh Water, which relied on laboratory-based plumbosolvency testing and monitoring of live network lead levels by random daytime sampling at the consumer tap. In this study and many others, the phosphate dose at the WTW entering the network was considered but the phosphate residual at the customer tap was not.

Experiments with greater emphasis on phosphate rather than lead have generally been laboratory experiments, for instance the work of McNeill and Edwards (2000) who investigated a phosphate dose of 1 mg/l in 36 cm long sections of 1.4 cm diameter cast iron pipes or Butt's (2016) study of 0-2 mg/l phosphate with 12 short lead and copper pipe sections harvested from live distribution networks. In laboratory experiments exploring phosphate such as these, there is a high level of control that could potentially oversimplify the nuances found in live water distribution systems, meaning that they cannot effectively replicate such a system. One example is that these studies generally focus on stagnation because it is well documented that stagnation results in the increase

of lead concentrations, but this trend is not observed in a real system as there is instead variance due to hydraulic conditions on weekly and daily cycles (Butt, 2016). Further, although there are laboratory experiments that have greater emphasis on phosphate rather than lead, phosphate residual is not explored in the form of a residual at the customer tap.

The exploration of phosphate use throughout a network by analysing phosphate concentrations at the WTW and at the Customer Tap is not, by any means, commonplace. The use of historic phosphate samples were found to be an invaluable tool because the real network environment of chosen case study areas have received a dose of orthophosphate for many years, meaning a sufficient equilibrate has been reached within these systems. Laboratory investigations into this area could have proven problematic in the timeframes of a PhD as it can take a prolonged time (ranging from a few months to several years) for lead pipes in a zone to equilibrate with the orthophosphate dose applied (Hayes, et al., 2008). As such, it was determined that to further explore how phosphate is being used in the drinking water distribution environment, the study of historic data from real case study networks would be highly desirable.

4.2 Methods: The Application of Chemicals and their Impacts in the DWDS

Regulatory samples were collected and analysed using the methods detailed in Section 3.2.3.1 and Section 3.2.3.2. Data analysis (Section 3.2.6) of the regulatory samples was then completed at the sites specified in Section 3.2.8, determined using the site selection process in Section 3.2.7. For this data analysis, there was a specific focus on the understanding of chemical usage in the current state of the system.

4.2.1 Regulatory Analysis Protocol

4.2.1.1 Phosphate Analysis Protocol

The 125 ml nutrient samples (collected using the method detailed in Section 3.2.3.1) were analysed for phosphate concentrations using colorimetric methodology and a discrete analyser (Anglian Water, 2020). A colorimetric test works by adding a reagent that reacts specifically with the component of interest (in this case, phosphate), colouring it. As the variation of colour changes with the concentration of the desired parameter, a spectrophotometer is able to detect the differences in shade (Abdelgader, 2014).

To measure total (dissolved and suspended) orthophosphate, the ascorbic acid method was used. In this method, ascorbic acid and ammonium molybdate react with orthophosphate in the sample to form a blue compound. For the measurement of total phosphorus (orthophosphate, condensed phosphate and organic phosphate), the sample was digested by heating and acidifying it to convert all forms of phosphorus to orthophosphate (dissolved and suspended), which was then measured using the ascorbic acid method. For measurements of filtered phosphate, samples were ran through a 0.45 µm filter, then heated and acidified and finally the ascorbic acid method was used (United States Environmental Protection Agency, 2012).

4.2.1.2 Chlorine Analysis Protocol

As regulatory samples were being collected in accordance with Section 3.2.3.1, chlorine concentration was determined on site at the customer tap using a Hach DR300 Pocket Colorimeter. This instrumentation has a precision (95% confidence interval) of 5.0 ± 0.2 mg/l and measurement range of 0.02-2.00 mg/l and 0.1-8.0 mg/l (Hach, 2020).

Free chlorine was measured in a solution as hypochlorous acid or hypochlorite ion while combined chlorine measurements included monochloramine, dichloramine, nitrogen trichloride and other chloro-derivatives. Combined chlorine oxidised iodide to iodine. Free chlorine and iodine react with DPD (N,N-diethyl-p-phenylenediamine) to form a red solution. The greater the chlorine concentration, the greater the intensity of the solution, which is then measured using the colorimeter (Hach, 2018).

To zero the device, a 10 ml sample cell was filled to the 5 ml mark with sample. The same cell was cleaned and dried, inserted into the device and then the instrument cap added. The zero button was pressed to initiate the zero. A second 10 ml sample cell was then filled to the 5 ml mark with sample. One 25 ml DPD Free Chlorine Reagent Powder Pillow was added to the first sample cell while 25 ml DPD Total Chlorine Reagent Powder Pillow was added to the second sample cell. The samples were shaken for 20 seconds to dissolve the reagent. Each sample cell was then cleaned and inserted into the device (within a 1 minute time frame). The instrument cap was added, and the device displayed the chlorine concentration in the sample in mg/l (Hach, 2018).

4.3 Results: The Application of Chemicals and their Impacts in the DWDS

Section 4.1 introduced the chemicals used to preserve the quality of drinking water through distribution networks, chlorine and phosphate, and how their study to date has primarily been focused on dose optimisation rather than on the wider implication of their dosage. The present section aims to explore this dose further, to build on the understanding of the wider impact this chemical usage is having.

4.3.1 Improving the Understanding of Chlorine Use in the Current State of the System

As Section 4.1.1 identified, secondary disinfection has been thoroughly studied with the driver of selecting the optimum dose for operational purposes but an area with poorer understanding is the link between chlorination and biological stability.

This section will explore the link between chlorination and biological stability to improve the understanding of chlorine use in the current state of the system.

4.3.1.1 Is Chlorine Being Used and Consumed Differently in Networks that have been Classified as Having "Good" or "Less Good" Biological Stability?

The concentration of the secondary disinfectant at the WTW and at the Tap was compared to determine how chlorine was being used in the distribution network and to see how this varied within the different case study networks, with their varying levels of biological stability.

Figure 23 compared total chlorine at the Final Water and Customer Tap of 6 case study sites with varying levels of biological stability. It was found that the Surface Water and Surface Water Blended Sites (Sites A-D) had the highest total chlorine dose (~1 mg/l at the WTW). The Groundwater Sites (Sites E and F) had lower chlorine doses at the WTW, with Site F in particular having the lowest average total chlorine concentration of 0.5 mg/l, half of the dose applied at the Surface Water Sites.

Across all sites, total chlorine values were lower at the Tap, meaning that chlorine consumption was occurring within the distribution networks, regardless of biological stability. At the Tap, total chlorine concentrations were fairly similar, ranging from 0.406-0.652 mg/l. Therefore, even though the Groundwater Sites received lower total chlorine doses, it was not consumed as much by the network, resulting in similar Tap values to Surface Water Sites.

The same analysis was also completed with the parameter free chlorine [Figure 23]. A similar finding to total chlorine values was identified, with Sites B and D having higher free chlorine concentrations at the WTW than Groundwater Sites (Sites E and F) but with all of these sites having similar concentrations at the Consumer Tap.

One further difference to total chlorine that was observed was at Sites A and C. These sites utilise chloramination as a disinfectant, meaning that ammonium sulphate is added to produce chloramines. Total chlorine is a measurement of the sum of free chlorine concentration and the combined chlorine concentration. As such, the high total chlorine presence at these sites and the low free chlorine indicates a high concentration of combined chlorine, or chloramine.

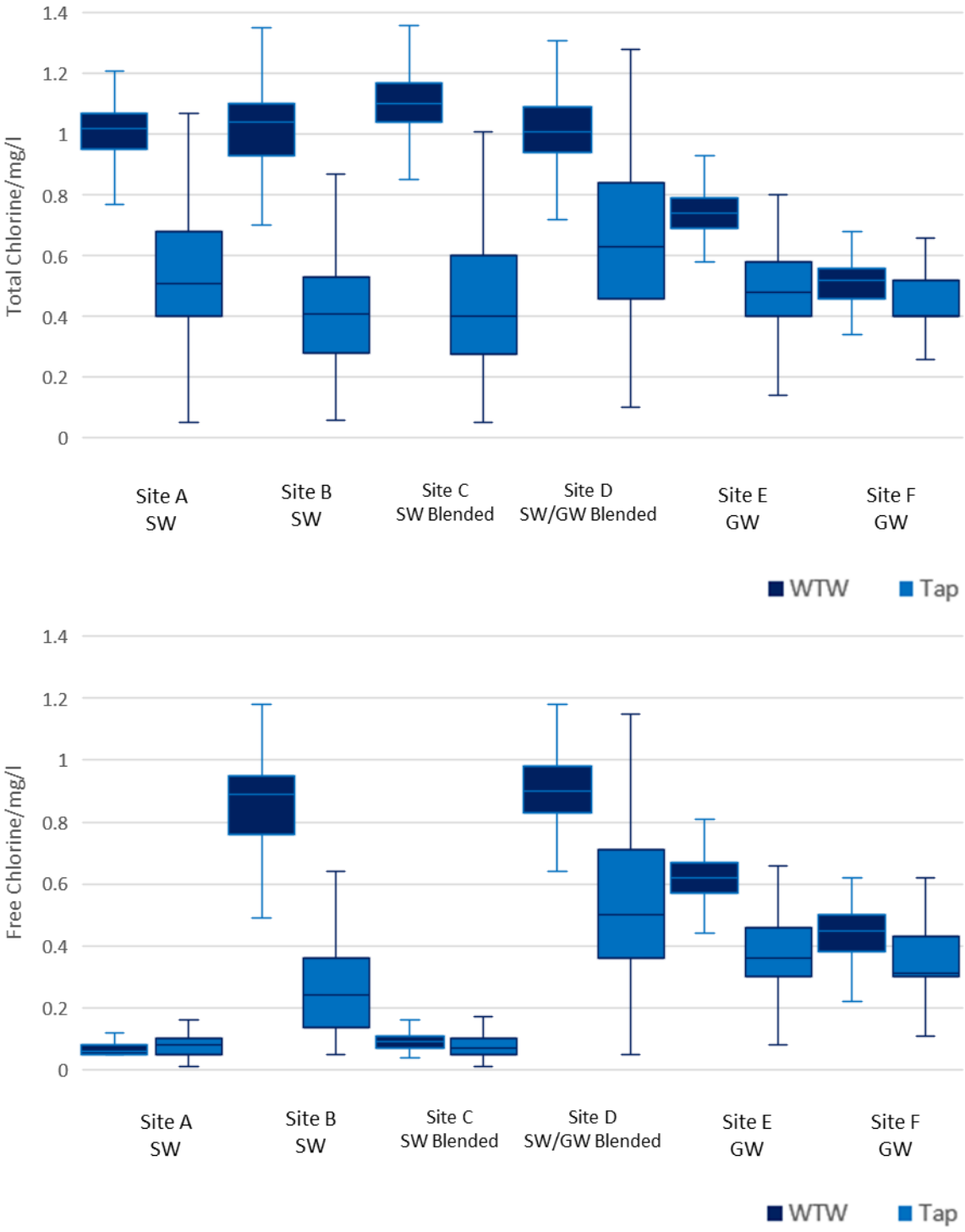


Figure 23 Total chlorine and free chlorine at the Final Water and the Tap (whole PWSZ)

4.3.1.2 Did Biological Stability Result in More of a Seasonal Difference in Chlorination?

One potential reason why the network consumed chlorine differently at the Surface Water Sites (identified in Section 4.3.1.1), could be because of the impact of seasonality. The present section explored chlorine dose temporally and determined the impact of differing levels of biological stability on seasonal consumption of chlorine.

Figure 24 shows there was no seasonal difference in chlorine dose at the different case study WTWs or at the Tap. This means that any temporal difference in chlorine concentration at the Tap was present due to network interactions, rather than dose or due to seasonal temperature changes.

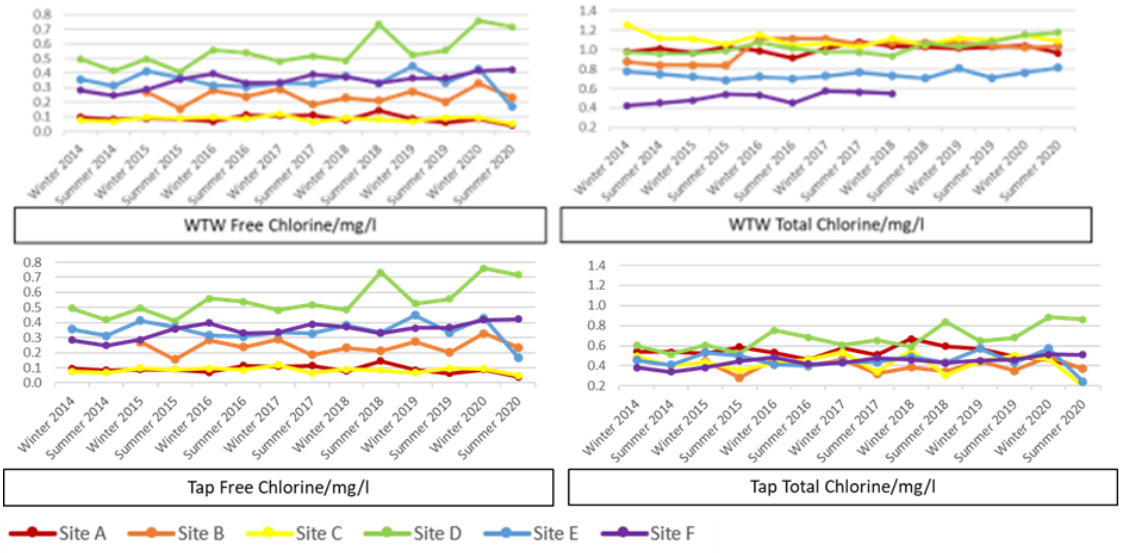


Figure 24 Temporal analysis of case study WTW and Customer Tap chlorine concentrations

4.3.1.3 Was Chlorine Having a Different Impact on Water Quality at those Sites with "Good" or "Less Good" Biological Stability?

Free and total chlorine at the WTW and at the Tap were compared with a number of water quality parameters to determine how chlorine concentration was impacting the water before and after distribution and to see if this water quality impact varied based on the biological stability of the case study networks.

Figure 25 showed that the parameters with the most consistent relationships with both free chlorine and total chlorine across the majority of case study area WTWs included free/total chlorine temperature, pH, conductivity, turbidity and total iron.

The parameters with the strongest relationships with both free chlorine and total chlorine, although not necessarily found among all case study area WTWs, were free/total chlorine, hardness, sodium, total copper, total manganese and calcium. When comparing the water quality impact of chlorine at the WTW across the different case study sites with their varying levels of biological stability, no clear trend was identified.

This analysis was also completed at the Customer Tap. The parameters with the most consistent relationships with both free chlorine and total chlorine across the majority of case study area taps included free/total chlorine, temperature, hardness, calcium, alkalinity, sodium, lead nfvs (non-flush

variable standing), lead 30 mins stagnation, conductivity, total manganese, magnesium, pH, total copper, 2 day colony count, nitrite and total phosphorus.

The parameters with the strongest relationships with both free chlorine and total chlorine, although not necessarily found among all case study area taps, were free/total chlorine, hardness and calcium. The Chloraminated Sites (Site A and C) additionally had strong relationships with total chlorine, ammonia and nitrite. When comparing the water quality impact of chlorine at the Customer Tap across the different case study sites with their varying levels of biological stability, no clear trend was identified.

A comparison of both the chlorine WTW and Tap results showed similar findings to other studies, with chlorine having consistent relationships with temperature, pH and conductivity, with hardness and calcium also expressing an influence at times. In terms of wider water quality parameters, chlorine had more of an influence over water quality at the WTW than at the Customer Tap. As such, the sample location (WTW or Customer Tap) impacted water quality more than the biological stability of the site.

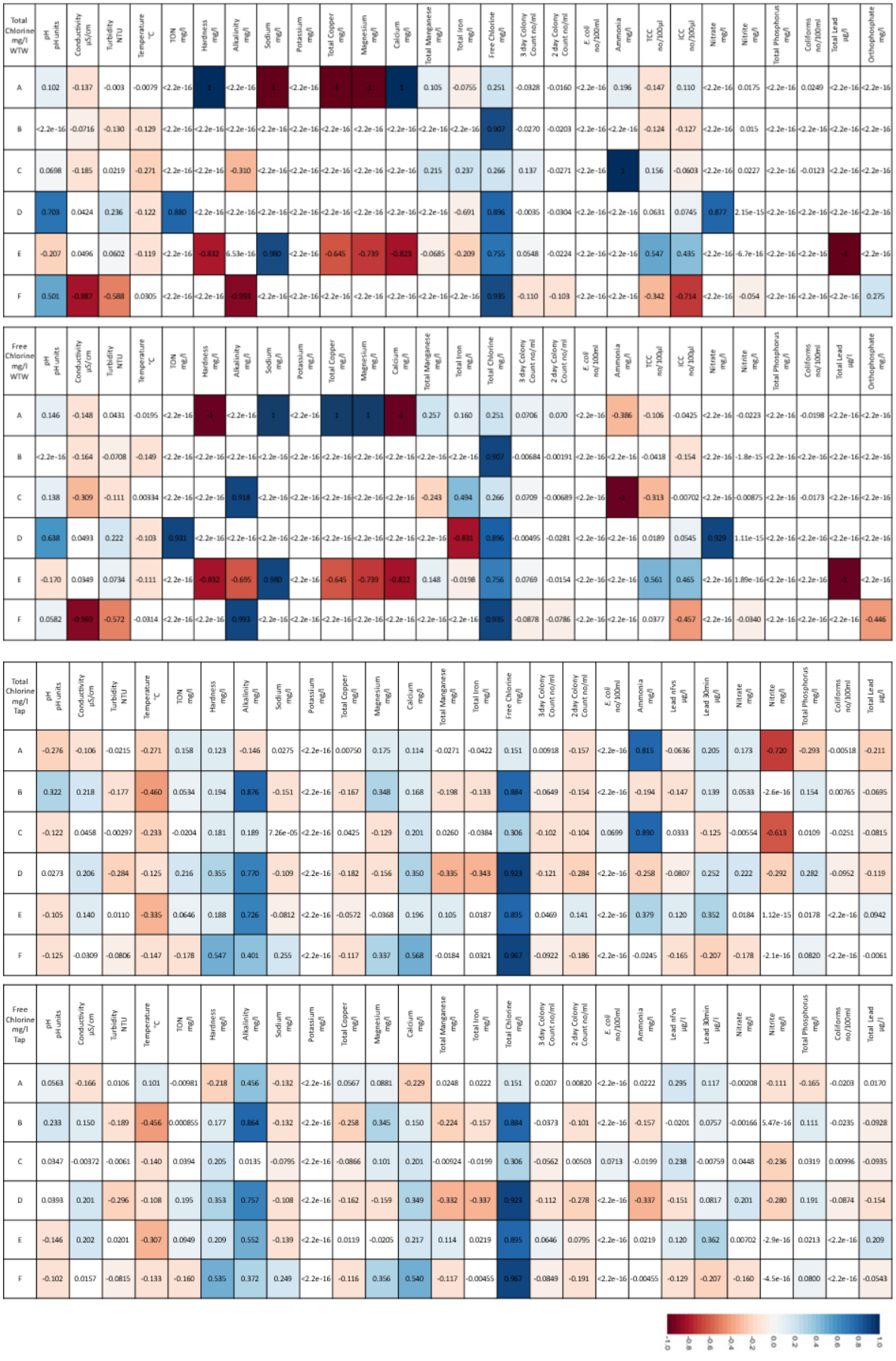


Figure 25 Correlation matrix of chlorine and water quality parameters at the WTW and Tap

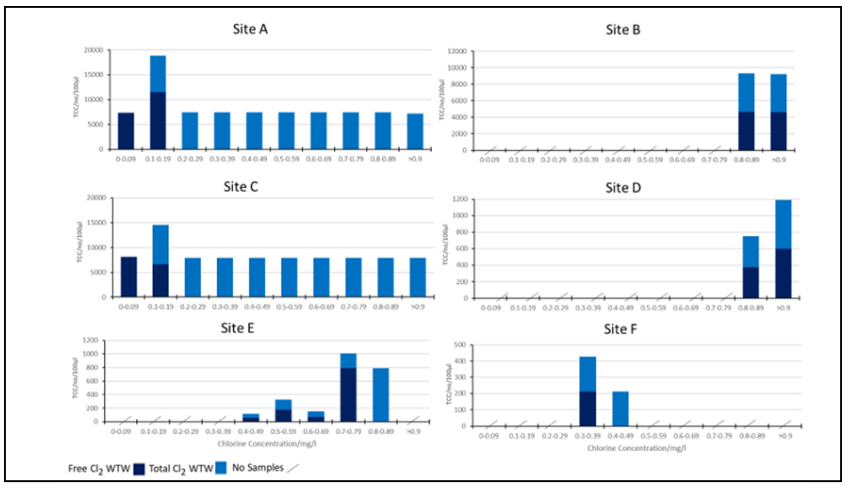
4.3.1.4 Did Chlorine Concentration Influence the Impact the Secondary Disinfectant had on Microbial Abundance?

A comparison of chlorine concentration and microbial abundance was completed to further investigate the relationship between chlorine and microorganisms, to see what impact biological stability had on this and how low chlorine concentrations impacted microbial abundance.

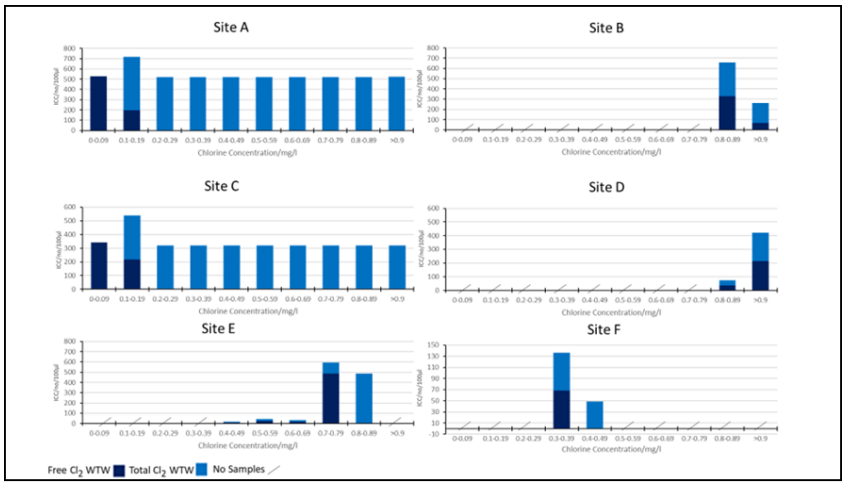
TCC and ICC were fairly consistent, despite the chlorine concentration present [Figure 26]. It was noted that there were incidences where there was little chlorine variance in the time period that TCC and ICC was sampled for (January-March 2020 while other samples were assessed 2014-2020).

Figure 27 found very limited evidence that specific chlorine concentrations at the WTW or the Tap impacted coliforms, 2 day colony counts, 3 day colony counts or *E. coli.* For example, an optimal chlorine dose was not detected, below which microbial abundance was high and above which it was low. As an example, Site D actually had a greater number of 3 day colony counts when the Tap free chlorine residual was 0.1-0.19 mg/l than at either 0-0.09 mg/l or 0.7-0.79 mg/l.

There were, however some exceptions. For instance, at Site C when the total chlorine residual at the Tap was ≤0.7 mg/l, *E. coli* presence appeared to be more likely in drinking water samples [Figure 27].

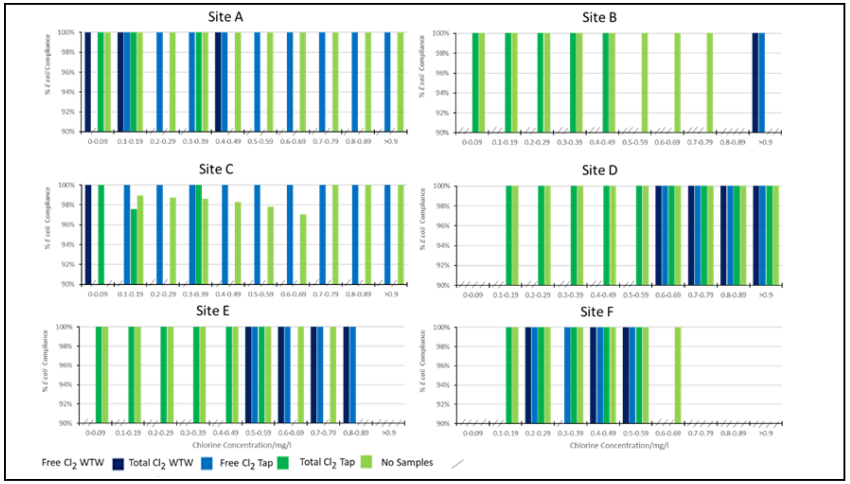


TCC

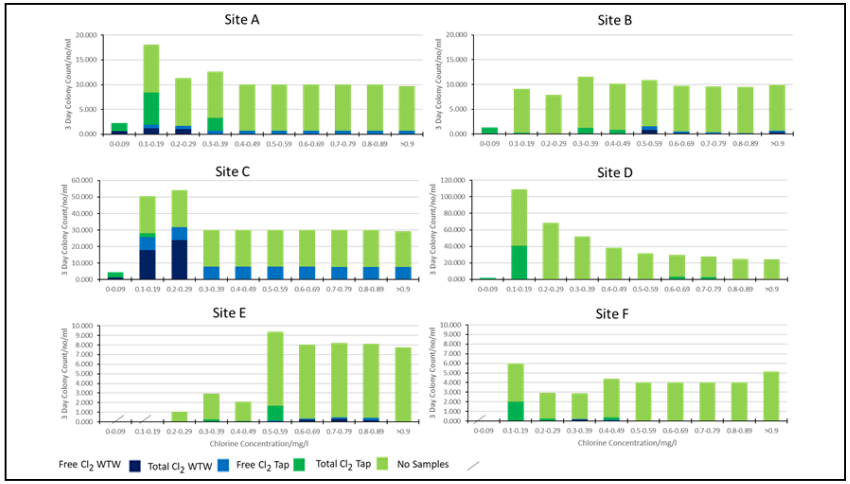


ICC

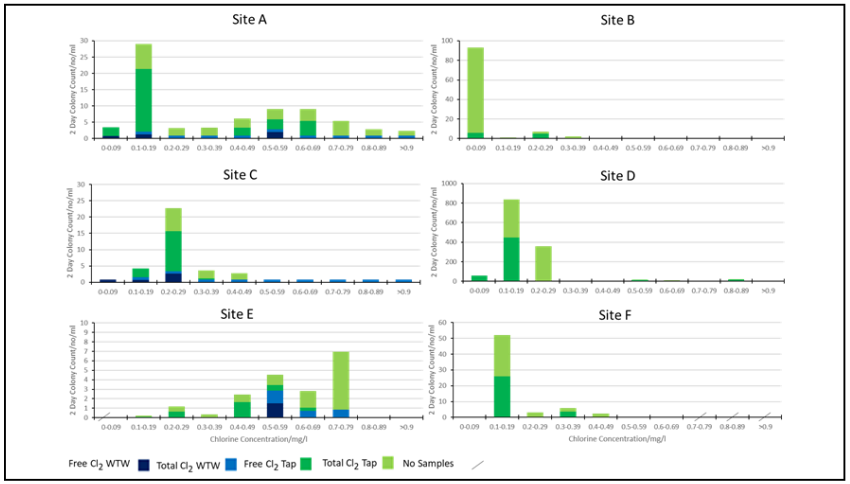
Figure 26 A comparison of TCC and ICC and chlorine concentrations at the WTW



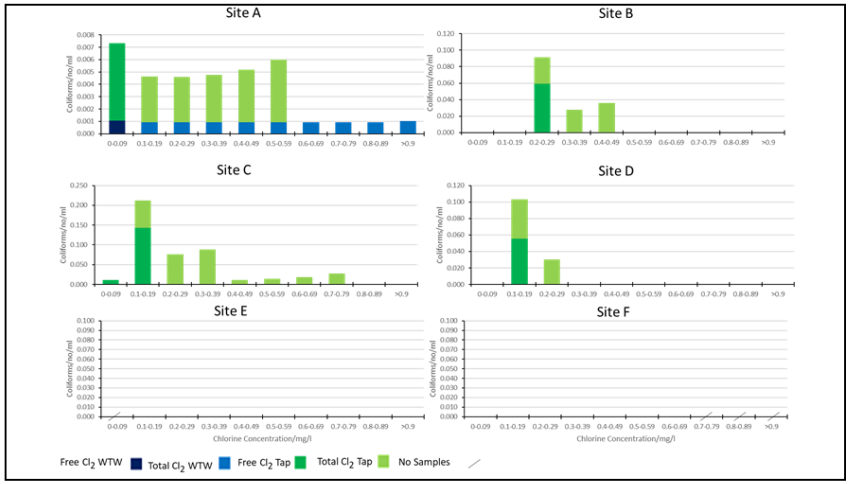
E. coli



3 Day Colony Count



2 Day Colony Count



Coliforms

Figure 27 A comparison of microbial abundance and chlorine concentrations at the WTW and Tap

4.3.1.4.1 The Relationship between Chlorine Concentration and Microbial Abundance

The correlation matrix, Figure 28, summarises the relationships that chlorine has with the different parameters for assessing microbial abundance presented in this section.

The relationship between microbial abundance and chlorine concentration was not as clear as suggested by other research. Previous studies found negative relationships as well as no correlations, while the present study found no relationships, negative relationships and also positive relationships.

Consistent relationships between parameters were not often detected. For example, while it was observed that TCC and ICC often had stronger relationships with chlorine at the WTW than other microbial assessment methods, it was not always a negative or a positive relationship across all case study sites.

At the Tap, 2 day colony counts had one of the most consistent relationships with chlorine, with mostly negative relationships. It would have been interesting to have also had these results for TCC and ICC.

The biological stability of the case study site did not appear to influence the relationship between chlorine and microbial abundance.

Case Study Site	Total Chlorine/mg/l WTW						Free Chlorine/mg/l WTW						Total Chlorine/mg/l Tap				Free Chlorine/mg/l Tap			
	3 day Colony Count no/ml	2 day Colony Count no/ml	*E. coli* no/100ml	TCC no/100µl	ICC no/100µl	Coliforms no/100ml	3 day Colony Count no/ml	2 day Colony Count no/ml	*E. coli* no/100ml	TCC no/100µl	ICC no/100µl	Coliforms no/100ml	3 day Colony Count no/ml	2 day Colony Count no/ml	*E. coli* no/100ml	Coliforms no/100ml	3 day Colony Count no/ml	2 day Colony Count no/ml	*E. coli* no/100ml	Coliforms no/100ml
A	-0.0328	-0.0160	<2.2e-16	-0.147	0.110	0.0249	0.0706	0.070	<2.2e-16	-0.106	-0.0425	-0.0198	0.00918	-0.157	<2.2e-16	-0.00518	0.0207	0.00820	<2.2e-16	-0.0203
B	-0.0270	-0.0203	<2.2e-16	-0.124	-0.127	<2.2e-16	-0.00684	-0.00191	<2.2e-16	-0.0418	-0.154	<2.2e-16	-0.0649	-0.154	<2.2e-16	0.00765	-0.0373	-0.101	<2.2e-16	-0.0235
C	0.137	-0.0271	<2.2e-16	0.156	-0.0603	-0.0123	0.0709	-0.00689	<2.2e-16	-0.313	-0.00702	-0.0173	-0.102	-0.104	0.0699	-0.0251	-0.0562	0.00503	0.0713	0.00996
D	-0.0035	-0.0304	<2.2e-16	0.0631	0.0745	<2.2e-16	-0.00495	-0.0281	<2.2e-16	0.0189	0.0545	<2.2e-16	-0.121	-0.284	<2.2e-16	-0.0952	-0.112	-0.278	<2.2e-16	-0.0874
E	0.0548	-0.0224	<2.2e-16	0.547	0.435	<2.2e-16	0.0769	-0.0154	<2.2e-16	0.561	0.465	<2.2e-16	0.0469	0.141	<2.2e-16	<2.2e-16	0.0646	0.0795	<2.2e-16	<2.2e-16
F	-0.110	-0.103	<2.2e-16	-0.342	-0.714	<2.2e-16	-0.0878	-0.0786	<2.2e-16	0.0377	-0.457	<2.2e-16	-0.0922	-0.186	<2.2e-16	<2.2e-16	-0.0849	-0.191	<2.2e-16	<2.2e-16

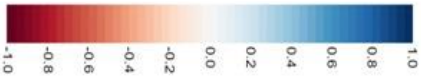


Figure 28 A correlation matrix of chlorine concentration and microbial abundance

4.3.1.5 Summary: Improving the Understanding of Chlorine Use in the Current State of the System

Therefore, this section explored the link between chlorination and biological stability to improve the understanding of chlorine use in the current state of the system.

This section found that the use of chlorine impacted the current state of the system in the subsequent ways:

- Chlorine was consumed at all sites between the WTW and the Tap. This consumption remained constant throughout the year.
- There was no seasonal chlorine dose applied at the WTW, chlorine dose was consistent throughout the year.
- Chlorine had consistent relationships with temperature, pH and conductivity, with hardness and calcium also expressing an influence at times.
- Chlorine had more of an influence over water quality at the WTW than at the Customer Tap.
- 2 day colony counts, 3 day colony counts, TCC and ICC were more consistent measures for quantifying microbial abundance than *E. coli* and coliforms.
- At times (particularly at the Customer Tap), low chlorine concentrations did correlate with high microbial abundance (especially in colony counts but not as clearly in TCC and ICC) but at times there were positive correlations (especially at the WTW) and no relationship, suggesting that the relationship between microbial abundance and chlorine concentration is not as clear as suggested by other research.

When comparing how biological stability influenced how chlorine impacted the current state of the system, the following was found:

- Sites that were less biologically stability received higher chlorine doses at the WTW.
- Chlorine consumption occurred universally in the network regardless of the level of biological stability of the site.
- The degree of chlorine consumption varied based on the biological stability of sites, as those sites influenced by surface water (either directly or blended) had more chlorine consumption in the network.
- The biological stability of case study sites did not influence any seasonal differences in chlorine dose at WTW or chlorine consumption within the network.
- Sample location (WTW or Customer Tap) impacted how chlorine influenced water quality more than the biological stability of the site.
- The biological stability of the sites did not change how chlorine impacted water quality.
- The biological stability of the sites did not appear to influence the relationship between chlorine and microbial abundance.

4.3.2 Improving the Understanding of Phosphate Use in the Current State of the System

As Section 4.1.2 identified, phosphate has been studied with the driver of selecting the optimum dose for protection against lead pipes within consumer homes. The study of the chemical phosphate itself however and in particular the phosphate residual at the Tap, is understudied. The link between the application of an orthophosphate dose and biological stability is also an unknown.

This section will explore the phosphate residual at the tap, as well as the link between phosphate and biological stability, to improve the understanding of phosphate use in the current state of the system.

4.3.2.1 How was Phosphate Consumed from Treatment to the Tap?

The concentration of total phosphorus at the WTW and at the Tap were compared to: improve the understanding of how orthophosphate was being used within the different networks; to see if it was being dosed efficiently (i.e., identifying if there was a phosphorus residual at the Customer Tap) and to determine if phosphate use and residual was influenced by the biological stability of the selected sites.

A comparison of the total phosphorus at the WTW and at the Tap of case study areas found that across all of the case study sites, regardless of their varying levels of biological stability, there was always a substantial phosphorus residual at the Customer Tap and minimal consumption within the network [Figure 29]. In some sites, average phosphorus at the Tap was even higher than at the WTW. This could potentially suggest that the dose of orthophosphate being used is not efficient because it appears to not be being consumed by the network.

The same results were found with both data from the whole PWSZ and from two DZs within each network directly fed from the case study WTWs. The DZ analysis was completed because, while random daytime samples from PWSZ data can have a wealth of sample numbers, they can be influenced by a number of different types of blending, which can also increase the residency time. These DZs were selected to determine if the trends detected to date were influenced by blending or remain the same, even when looking in depth at a smaller area. For instance, Customer Tap sampling of total phosphorus in the selected DZs could be very low and not evenly distributed at times. From 2014-2020, Site A had 188 total phosphorus Tap samples, Site B had 318, Site C had 5, Site D had 4, Site E had 61 and Site F had 2. Nevertheless, sample numbers for the PWSZ and WTW data were adequate (288-433 samples).

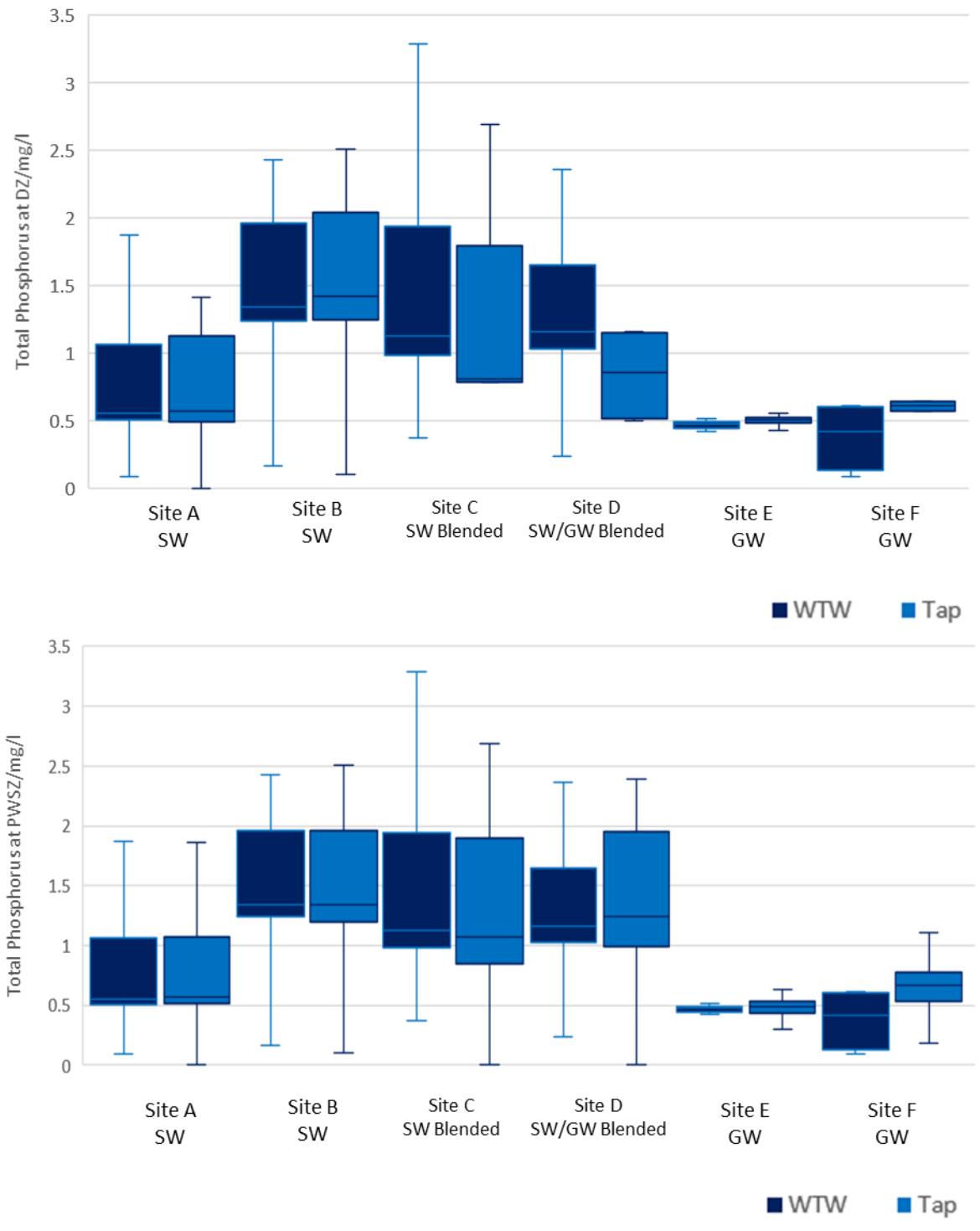


Figure 29 Total phosphorus at the Final Water and the Tap (selected DZs and whole PWSZ)

4.3.2.1.1 What are the Factors that Influenced Phosphate Consumption from Treatment to the Tap?

Section 4.3.2.1 found that case study areas, regardless of their varying biological stabilities, all had residual phosphorus at the Tap, with an increase in concentration between the WTW and the Tap at times. This section aims to compare this occurrence in the different case study areas, while also providing some explanation of why this consumption occurred.

4.3.2.1.1.1 Pipe Material

An assessment of the distribution network materials in the different case study areas was completed to determine if pipe material influenced phosphorus consumption between the WTW and the Tap and to establish if there was any variance based on biological stability.

There was no relationship with pipe material and total phosphorus, regardless of site biological stability. As Figure 30 shows, most of the case study networks were similar, consisting mostly of a blend of plastics, iron and cement. For instance, it was not found that networks with plastic pipes, as an example, have more phosphorus at the Tap than in networks without. The pipe material variation was also similar, with a blend of 13 different pipe materials in the PWSZs of Sites A and B, 12 in Sites C, D, E, F and 10 in Site F.

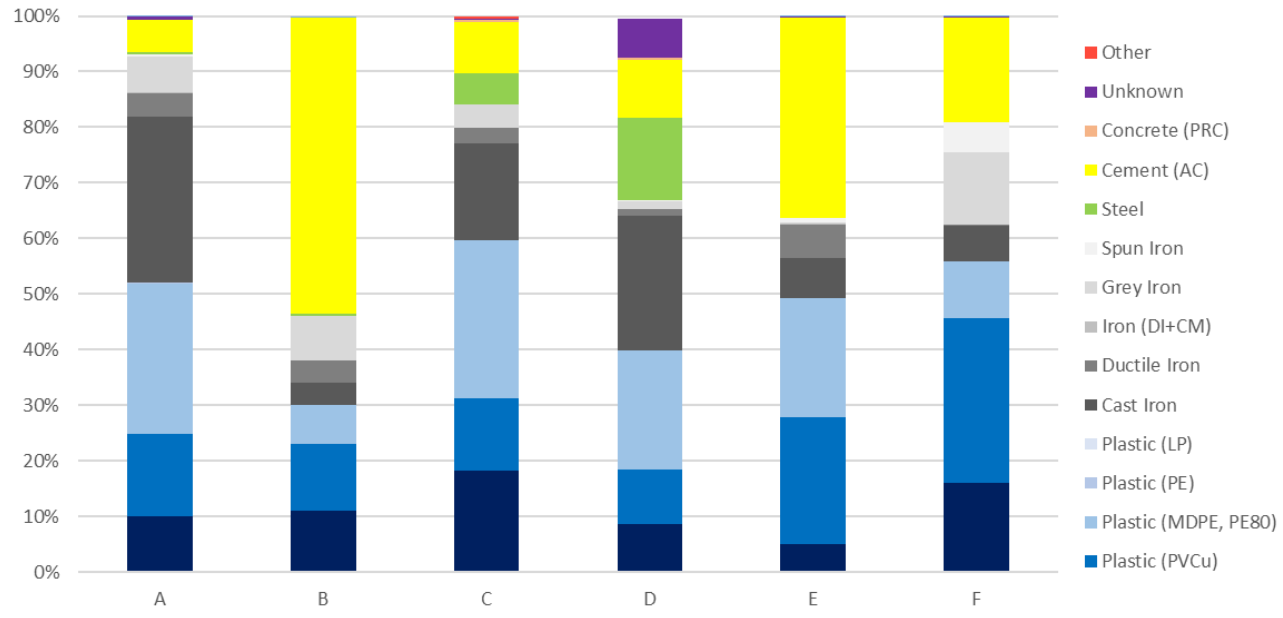


Figure 30 Pipe material in the different case study networks at a PWSZ level

To determine if there was a correlation between pipe material and total phosphorus on a DZ level, pipe material at two DZs per case study area was assessed, as Figure 31 shows. Like on a PWSZ level, there was no relationship with pipe material and total phosphorus at a DZ level, regardless of case study site. There was also no link with pipe material variation, as Sites B, D and E were the most variable (with 11-12, 10-11 and 11-12 different pipe materials respectively in the different DZs within the PWSZ) and Sites A, C and F were the least variable (with 7-8, 6-8 and 6-10). Thus, although other research has shown pipe material can influence phosphate presence in the bulk water, in the case study networks studied it seems unlikely to be the main driver for phosphate consumption at the Customer Tap.

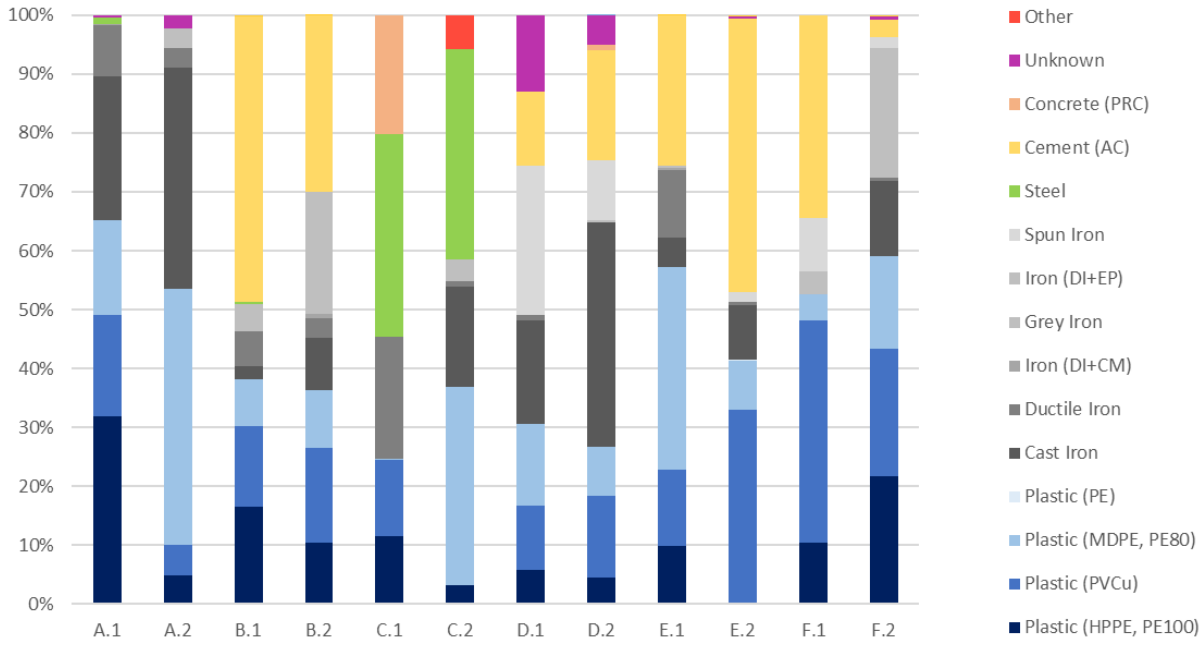


Figure 31 Pipe material in the different case study networks at a DZ level

It should be observed that the company data obtained to produce Figure 30 and Figure 31 were from company owned distribution networks, excluding customer pipework, fittings and fixtures as water utilities do not have access to this information. Despite utilities owning these assets, the categories "Unknown" and "Other" were present. It was also noted that lead was not one of the pipe material categories identified in case study areas, although lead could be present in the "Unknown" and "Other" categories or at a household level.

Therefore, although previous studies have found mixed results regarding phosphate and pipe material, in the present research, pipe material did not appear to influence phosphate levels at the Tap at either a larger PWSZ level or over a smaller DZ level. No link with biological stability was detected. Pipe material did not seem to have a dominant trend and did not appear to be biasing the phosphate residual at the Consumer Tap.

4.3.2.1.1.2 Orthophosphate Dosing Technique

To better explore the dose of orthophosphoric acid dosing at all case study WTWs, the dosing technique was studied to see if there had been any problems during the dosing process, to see if dosing targets were met and to explore further operational changes in dose. For example, the high levels of phosphate residual found at the Tap could potentially be present due to dosing errors.

It was found that the case study water utility began seasonal dosing of WTW for PWSZs deemed to be high or medium risk for lead presence at the Customer Tap (including all surface water sites) on 01/07/15, with the risk categories displayed in Table 11. Since this date, every year a higher concentration of orthophosphate was dosed on 1st July, which then drops down to its baseline on 1st November. This increased seasonal dosing was completed by the company to account for higher lead dissolution in higher water temperatures seasonally that is well documented in literature and the water industry (Masters, et al., 2016). For instance, the International Water Association (2010) stated that lead concentrations doubled when temperatures increased from 12°C to 25°C.

Table 11 Seasonal dosing at the WTW of the case study sites

Site	Normal Target Dose /mg/l	Seasonal Target Dose/mg/l	Average Normal Achieved Dose/mg/l	Average Seasonal Achieved Dose/mg/l	Lead Risk	Lead Risk Meaning
Site A	0.5	1	0.599	1.10	Medium	Any PWSZ with >2% and <5% samples >5 µg/l
Site B	1.25	2	1.35	1.79	Medium	Any PWSZ with >2% and <5% samples >5 µg/l
Site C	1	2	1.13	1.8	High	Any PWSZ with >5% samples >5 µg/l
Site D	1	2	1.13	1.57	Medium	Any PWSZ with >2% and <5% samples >5 µg/l
Site E	0.5	0.5	0.475	0.60	Very Low	No samples with >5 µg/l lead
Site F	0.75	0.75	0.742	NA	Very Low	No samples with >5 µg/l lead

"Normal Dose" refers to phosphate dosing from 2nd November-30th June and "Seasonal Dose" refers to 1st July-1st November.

As Figure 32 shows, the introduction of the seasonal dose at the case study WTWs increased the target orthophosphoric acid dose of multiple sites. For example, Site C had a target dose of 0.75 mg/l from 2009-2014, that was increased to 2 mg/l in the seasonal dosing in 2015, after which was cycled between 1 mg/l and 2 mg/l ever year. This use of seasonal dosing was only applied at Sites A, B, C and D, those sites with a surface water influence.

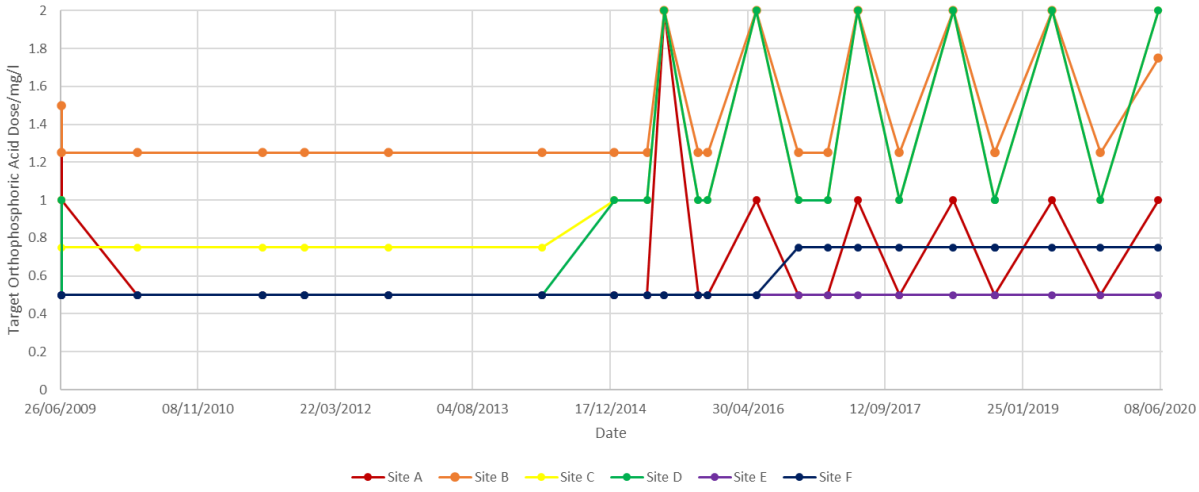


Figure 32 Target orthophosphoric acid dose at case study sites before and during seasonal dosing

A potential reason why residual phosphate presence at the Tap occurred to such an extent could be because of dosing problems, such as the application of an incorrect orthophosphoric acid dose. However, the target dose and average achieved dose for the case study sites, both the normal dosing and the seasonally increased dosing can be found in Table 11. As can be seen, the target dose of orthophosphoric acid and the achieved dose were fairly similar for all case study areas.

The difference in phosphate concentration between seasonal dose and normal dose was also detected at the Customer Tap in the case study sites, as shown in Figure 33. This suggests that the phosphate was having minimal consumption by the network, from the WTW to the Tap.

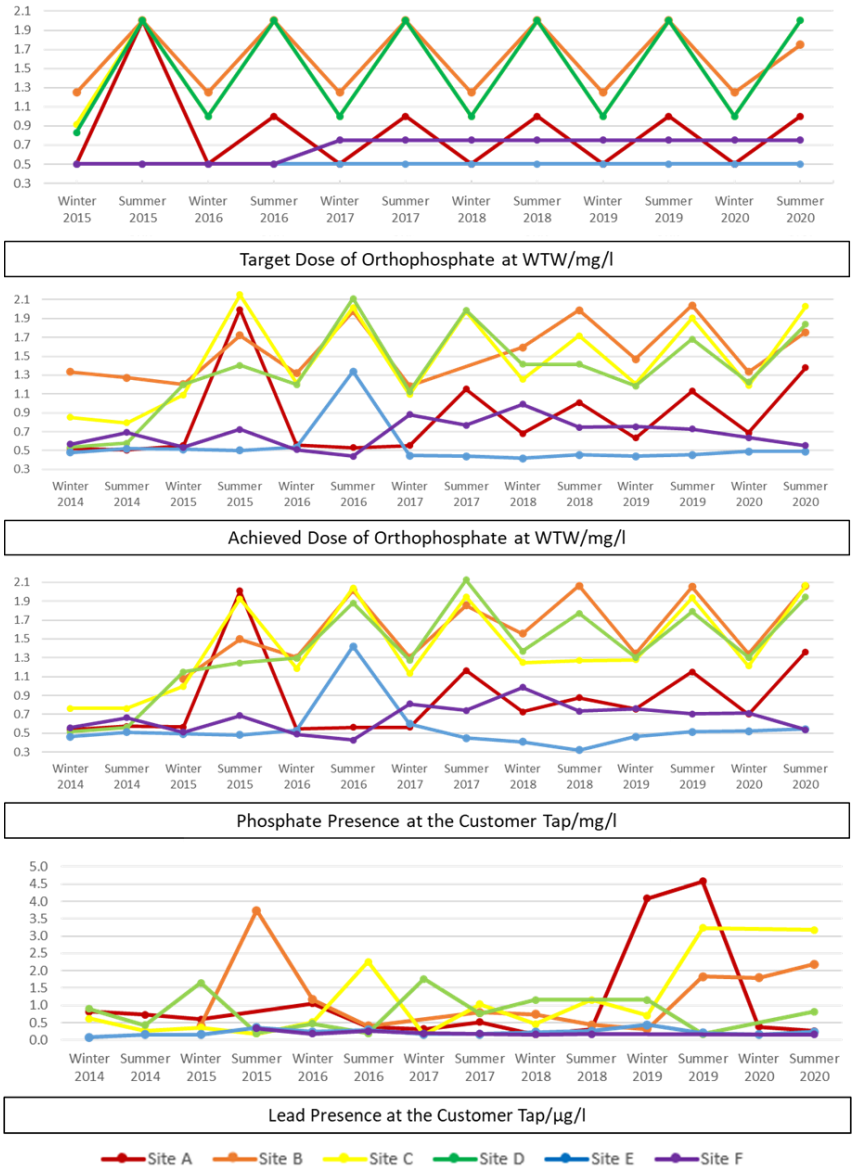


Figure 33 Phosphate concentration at the WTW and the Customer Tap

An assessment of this seasonal dosing found that at Sites B and D, total lead was found to have a positive relationship with water temperature (p=0.518 and p=0.198, respectively), while no other sites had a correlation between these two parameters [Figure 34]. Similarly, mixed results were found in lead nfvs, and no relationships were found between 30 minute stagnation lead samples and temperature at any case study companies. This analysis was additionally completed in the time period before seasonal dosing was implemented (01/01/14-01/07/15) and similar results were found, with lead nfvs still having mixed results and no relationships at 30 minute samples, although there were also no relationships at a total lead level too.

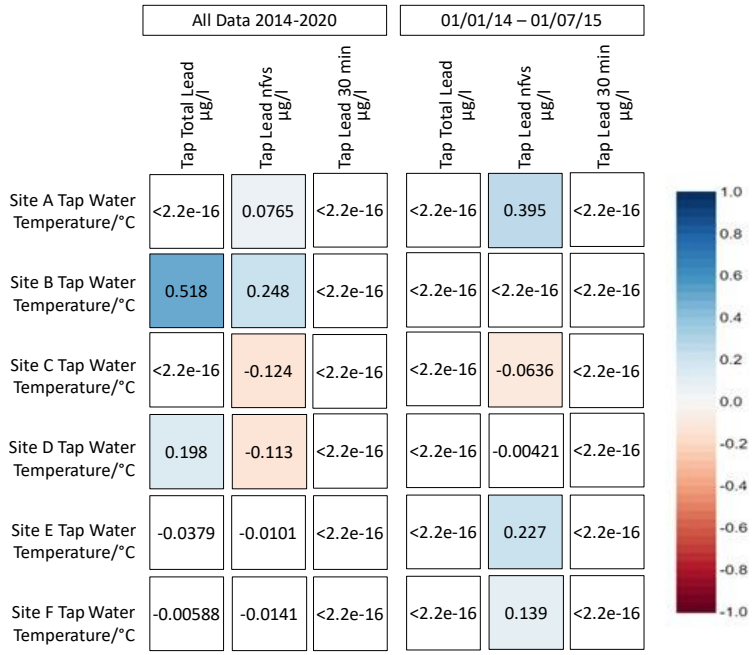


Figure 34 A correlation matrix of lead concentration and temperature

4.3.2.1.1.3 Microbial Influence

It could also be argued that phosphate consumption in the distribution network can be influenced by microorganisms, particularly when considering the varying biological stabilities of the different sites. As Section 1.4.4.2.2 stated, virtually all of the orthophosphoric acid (H_3PO_4) added to drinking water is biologically available for microorganisms, especially once passivation (a full layer of phosphate on pipe walls) has been completed. There have been numerous studies, including that of Del Olmo et al. (2020), who found the presence of phosphate promoted the presence of microorganisms that carry genes associated with the solubilisation and transport of PO_4^{3-}, including *Sphingomonas*, *Bradyrhizobium* and *Acidovorax spp*. This phosphorus is retained in microbial cells to produce phospholipids, nucleic acids and other cellular components. When the cell dies and lyses, the phosphate ion contained within is rapidly released by hydrolysis and becomes available for incorporation into metabolically active cells.

It may possibly be that the phosphorus was being accumulated within microbial cells and then was being released at once, resulting in an increase in total phosphorus at the Customer Tap, when the cells were dying. If this was occurring, however, it would likely be expected that the minimum values of total phosphorus in the Customer Tap would be much lower, to account for those periods when the phosphate was being “tied up” by the microorganisms present within the drinking water distribution system. Further, this source and sink behaviour would also be apparent in cycling of total phosphorus levels over time at the Customer Tap. Figure 35 shows that while Customer Tap samples are more variable than Final Water samples, there does not appear to be a clear nutrient cycling effect. Also, microorganisms are unable to produce phosphate, only utilising that which is present in the environment.

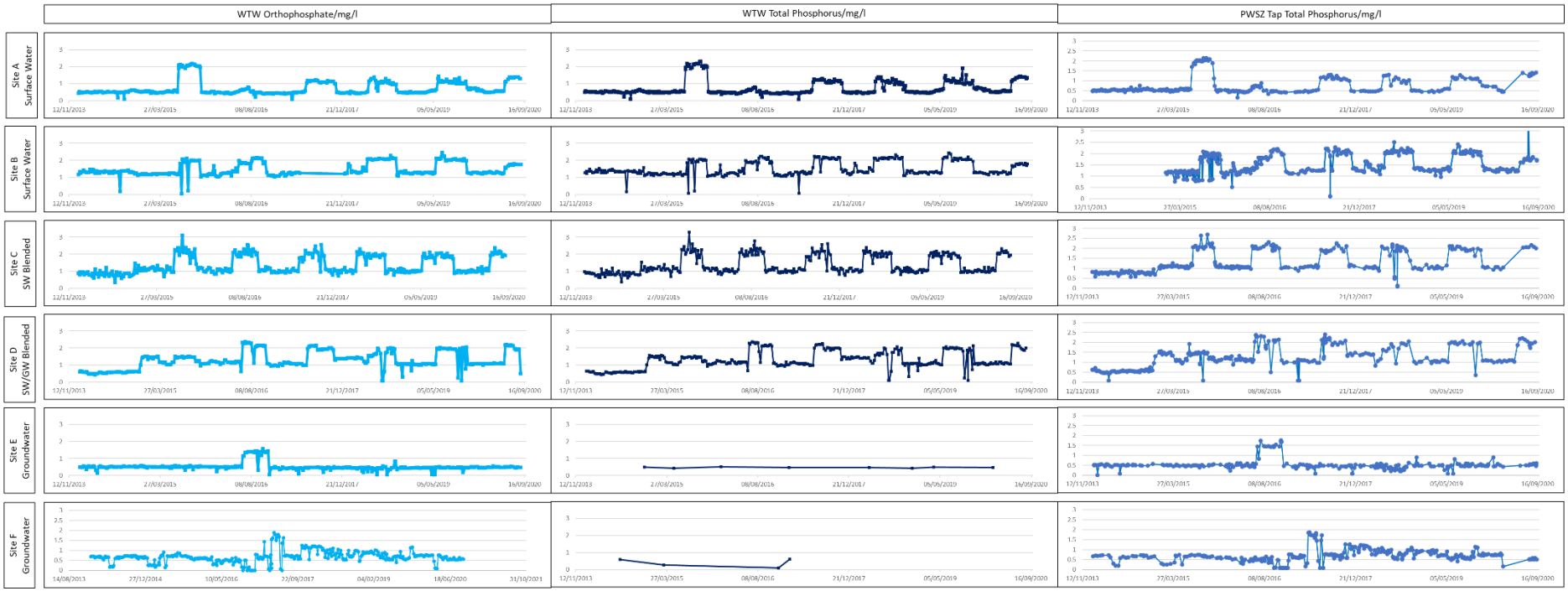


Figure 35 A comparison of phosphate analyses at the Final Water and Tap

It may well be said that phosphate consumption between the WTW and the Tap could have been influenced by phosphate in the network coming loose and entering the Tap. As stated in Section 3.2, an orthophosphoric acid dose of 1 mg/l is required to maintain the lead phosphate or calcium phosphate precipitates on the pipe wall and if the phosphate dose is stopped, its impacts are reversed, with the phosphorus in this form re-entering the bulk water. This could be because of microorganisms consuming and so destabilising it, by corroding it or by flushing events in the network. However, Figure 35 additionally details that orthophosphate dose at the WTW rarely drops to 0 mg/l and if this value is reported, it is a single isolated sample. As such, phosphate is being continuously added so it is unlikely that scales are leaching phosphorus.

Figure 36 compared the relationship between total phosphorus and microbial abundance. No relationships were found at the WTW. At the Tap, there were several incidences of strong correlations, but these were not consistently found, either across all sites or across the different indicators of microbial abundance. There was also no observed link with biological stability.

Case Study Site	Total Phosphorus/mg/l WTW						Total Phosphorus/mg/l Tap			
	3 day Colony Count no/ml	2 day Colony Count no/ml	*E. coli* no/100ml	TCC no/100µl	ICC no/100µl	Coliforms no/100ml	3 day Colony Count no/ml	2 day Colony Count no/ml	*E. coli* no/100ml	Coliforms no/100ml
A	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	0.232	<2.2e-16	<2.2e-16	<2.2e-16
B	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16
C	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	-0.409	<2.2e-16	<2.2e-16	<2.2e-16
D	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	-0.778	<2.2e-16	-0.619
E	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16
F	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	-0.0767	0.112	<2.2e-16	<2.2e-16

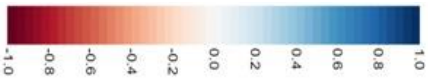


Figure 36 A correlation matrix of phosphate concentration and microbial abundance

Therefore, although microorganisms were present in the distribution networks, they were not seen to directly influence phosphate consumption so as to result in the phosphate residual seen at the Tap. Phosphate is also unable to be produced by microorganisms. There was no evidence of phosphate acting in a source and sink manner as microbes were using it as a food source. Instead, phosphate dose was continuous, also meaning that it was unlikely that scales were leaching phosphorus. Overall, there were no consistent relationships between microbial abundance and phosphorus, with no link found by the differing biological stabilities of case study sites.

4.3.2.2 Was Phosphate Having a Different Impact on Water Quality at those Sites with "Good" or "Less Good" Biological Stability?

A comparison of total phosphorus with water quality parameters at the WTW and Customer Tap was completed to determine how phosphate concentration was impacting the water before and following distribution. This was done to see how the network was influencing water quality and how this relationship varied at the different case study sites, with their differing biological stabilities.

As Figure 37 denoted, across both the WTW and at the Customer Tap, the parameters with most consistent relationship with total phosphorus were pH, hardness, total copper, total iron, total manganese, calcium, conductivity, alkalinity and magnesium. While the strongest relationship across both sampling locations with phosphate was pH.

A comparison of the WTW and the Tap found that total phosphorus could often have stronger relationships with water quality parameters at the Tap, for instance with alkalinity and sodium (e.g., Site A WTW alkalinity p=-0.297 and at the Tap p=-0.880). This means that after the retention time within the distribution network, the concentration of phosphate was either having the same impact or more of an impact on water quality than it was at the WTW.

In a comparison of the case study sites, it was noted that at the WTW relationships between phosphate and other water quality parameters were often weaker at Groundwater Sites (Sites E and F) rather than in Surface Water and Blended Sites. However, at a Customer Tap level, the same was not found and all case study sites were relatively similar.

One potential argument is that the stronger phosphate/water quality relationships at the WTW at sites with less good biological stability could be due to seasonal phosphate dose rather than biological stability. This is because it is Sites A-D that had less good biological stability and that received a seasonal phosphate dose, the dosing described in Section 4.3.2.1.1.2.

Nevertheless, the impact of this seasonal dosing on water quality did not appear to influence the majority of parameters. The only parameter with evidence of a seasonal total phosphorus dose impacting it was pH, where it was found that when phosphate levels increased seasonally, pH decreased (although pH did have other influences), as shown in Figure 38.

P mg/l WTW	pH pH units	Conductivity µS/cm	Turbidity NTU	Temperature °C	TON mg/l	Hardness mg/l	Alkalinity mg/l	Sodium mg/l	Potassium mg/l	Total Copper mg/l	Magnesium mg/l	Calcium mg/l	Total Manganese mg/l	Total Iron mg/l	Free Chlorine mg/l	3 day Colony Count no/ml	2 day Colony Count no/ml	*E. coli* no/100ml	Ammonia mg/l	TCC no/100µl	ICC no/100µl	Nitrate mg/l	Nitrite mg/l	pH pH units	Coliforms no/100ml	Total Lead µg/l	Orthophosphate mg/l
A	-0.647	-0.179	<2.2e-16	<2.2e-16	-0.350	-0.153	-0.297	0.467	0.389	-0.102	0.489	-0.212	0.128	0.145	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	0.468	<2.2e-16	<2.2e-16	-0.377	0.0338	<2.2e-16	0.0994	0.993
B	-0.661	<2.2e-16	<2.2e-16	<2.2e-16	-0.0717	-0.356	-0.131	0.0325	-0.00556	0.316	-0.126	-0.372	-0.183	-0.146	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	-0.0641	<2.2e-16	<2.2e-16	-0.0717	0.0859	<2.2e-16	-3.8e-16	0.979
C	-0.639	0.456	<2.2e-16	<2.2e-16	-0.113	-0.124	-0.406	0.306	0.267	-0.167	0.310	-0.168	0.0454	0.0977	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	-0.0595	<2.2e-16	<2.2e-16	-0.113	0.0609	<2.2e-16	2.55e-16	0.995
D	-0.146	0.183	<2.2e-16	<2.2e-16	0.0658	-0.275	-0.0111	-0.0167	-0.008	-0.234	0.0117	-0.285	2.66e-16	0.370	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	-0.157	<2.2e-16	<2.2e-16	0.0657	8.88e-17	<2.2e-16	-3.6e-16	0.997
E	-0.502	<2.2e-16	-1	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	0.326	0.368	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	-1	0.538
F	-0.890	<2.2e-16	1	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16

P mg/l Tap	pH pH units	Conductivity µS/cm	Turbidity NTU	Temperature °C	TON mg/l	Hardness mg/l	Alkalinity mg/l	Sodium mg/l	Potassium mg/l	Total Copper mg/l	Magnesium mg/l	Calcium mg/l	Total Manganese mg/l	Total Iron mg/l	Free Chlorine mg/l	3 day Colony Count no/ml	2 day Colony Count no/ml	*E. coli* no/100ml	Ammonia mg/l	pH pH units	Lead ntvs µg/l	Lead 30min µg/l	Nitrate mg/l	Nitrite mg/l	Coliforms no/100ml	Total Lead µg/l
A	-0.835	-0.680	-0.0250	<2.2e-16	<2.2e-16	-0.486	-0.880	0.766	-0.971	0.161	0.207	-0.503	0.455	0.129	-0.165	-0.293	0.232	<2.2e-16	<2.2e-16	<2.2e-16	-0.0534	-0.178	<2.2e-16	<2.2e-16	<2.2e-16	-0.0298
B	0.500	0.675	0.178	-0.482	<2.2e-16	-0.0789	-0.791	0.825	0.463	0.270	0.533	-0.142	0.254	0.205	0.111	0.154	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	-0.0692	0.624	<2.2e-16	<2.2e-16	<2.2e-16	0.280
C	-0.566	0.152	0.0584	<2.2e-16	<2.2e-16	0.0522	-0.318	0.790	0.325	-0.0439	0.721	-0.0252	0.148	0.170	0.0319	0.0109	-0.409	<2.2e-16	<2.2e-16	<2.2e-16	-0.0589	-0.221	<2.2e-16	<2.2e-16	<2.2e-16	-0.260
D	-0.631	0.462	0.109	0.367	0.601	0.474	-0.0257	-0.371	-0.472	-0.179	-0.367	0.485	0.0600	0.0738	0.191	0.282	<2.2e-16	-0.778	<2.2e-16	1.08e-15	-0.00377	0.334	0.596	-0.255	-0.619	-0.285
E	0.0155	-0.295	-0.0585	0.745	<2.2e-16	0.0366	0.147	-0.138	0.415	-0.0884	0.202	0.0228	0.0279	-0.0437	0.0213	0.0178	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	-0.0816	-0.166	<2.2e-16	<2.2e-16	<2.2e-16	0.0337
F	-0.145	-0.324	0.189	-0.830	-0.841	-0.151	0.0311	0.808	-0.874	0.0369	-0.101	-0.133	0.0495	0.117	0.0800	0.0820	-0.0767	0.112	<2.2e-16	2.32e-15	-0.249	0.963	-0.845	<2.2e-16	<2.2e-16	-0.0266

Figure 37 Correlation matrix of phosphate and water quality parameters at the WTWs and Taps

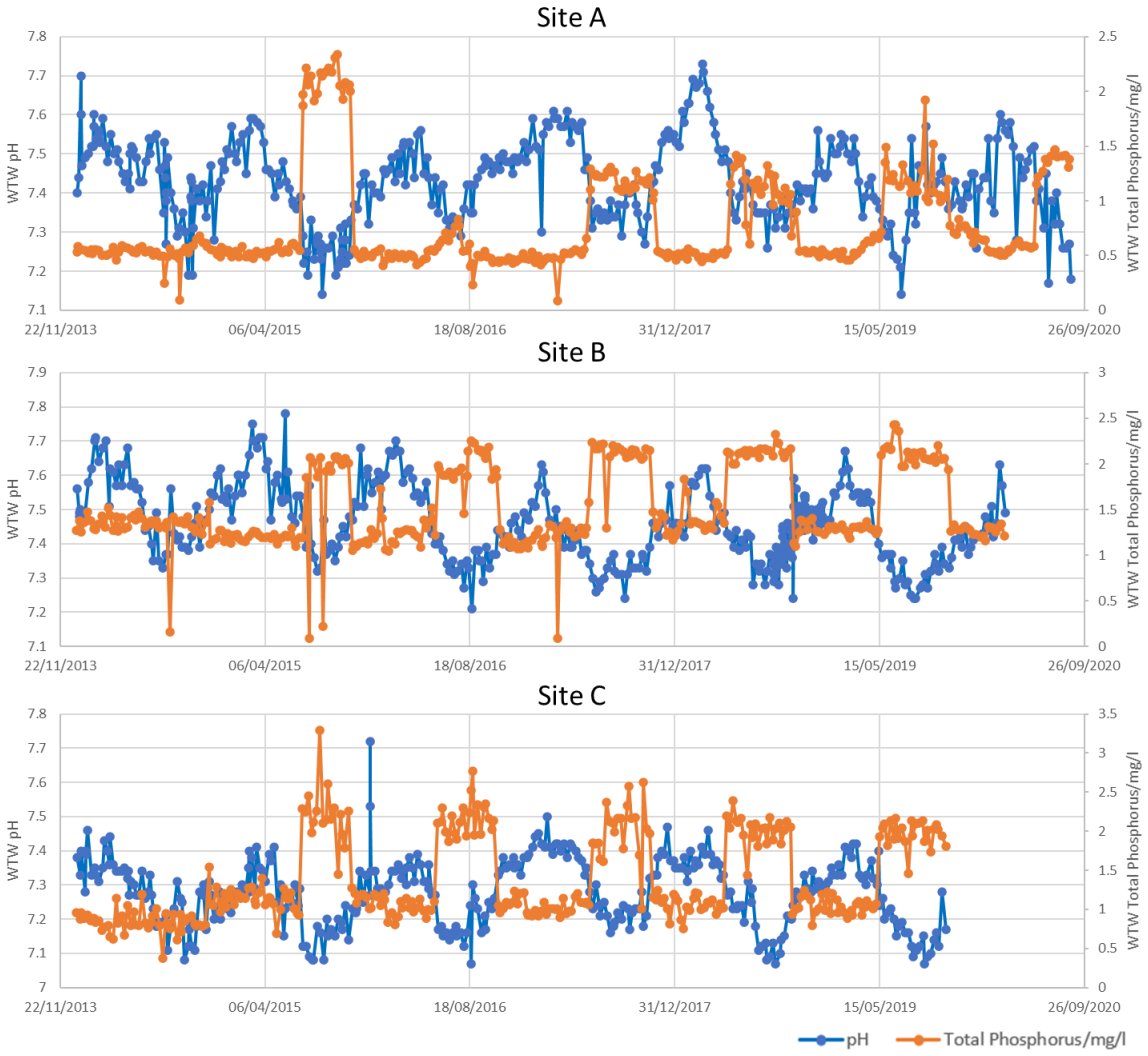


Figure 38 Temporal pH and total phosphorus at the WTW of Case Study Sites A-C

It could be argued that this trend (phosphate levels increased seasonally, pH decreased) was due to seasonally mediated processes rather than phosphate dose, however as shown in Figure 39 an incidence of a large spike in phosphate concentration coincided with a decrease in pH.

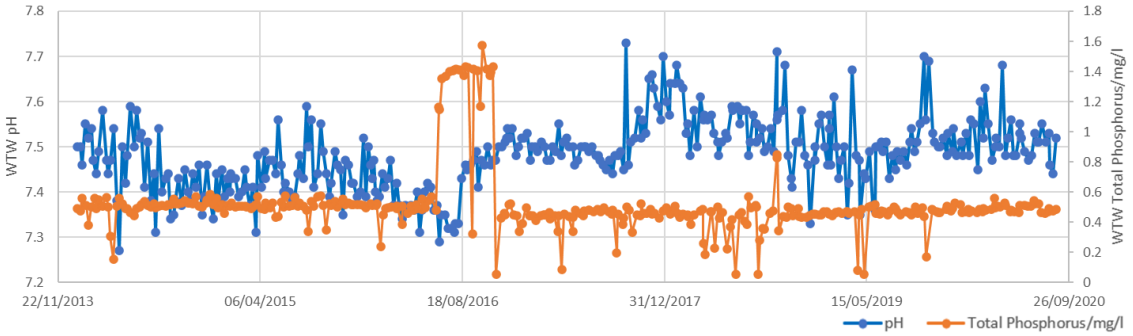


Figure 39 Temporal pH and total phosphorus at the WTW of Case Study Site E

Thus, apart from potentially pH at the WTW, the trend of seasonal phosphate dose was not found to influence any other water quality parameters, including those at the Tap.

4.3.2.3 Summary: Improving the Understanding of Phosphate Use in the Current State of the System

This section explored the phosphate residual at the Tap, as well as the link between phosphate and biological stability, to improve the understanding of current phosphate use.

The use of phosphate impacted the current state of the system in the subsequent ways:

- Compared with chlorine, there was minimal consumption of phosphate in the network, with high concentrations of phosphate residual at the Tap at all sites and at times, an increase in the means of phosphorus from the WTW to the Tap.
- PWSZ and DZ samples had similar phosphate consumption findings.
- Pipe material did not influence phosphate concentration at the Tap.
- Orthophosphoric acid dosing at the WTW had no observed errors, with company dosing targets achieved during the experimental analysis.
- Seasonal orthophosphoric acid dosing at the WTW occurred, with dose increasing every summer at sites considered to be of high or medium risk for lead presence at the Customer Tap, which consisted of those sites categorised as less good biological stability in the present study. This summer increase was also observed at the Tap.
- Although the seasonal dosing was implemented due to the relationship between lead and temperature, the links between these parameters were tremulous.
- The seasonal orthophosphoric acid dosing at the WTW appeared to influence pH at the WTW but no further parameters or any parameters at the Customer Tap.
- Phosphate consumption did not appear to be a microbially mediated process and there were no consistent relationships between microbial abundance and phosphorus.
- Phosphate dose at the WTW was a continuous process so it was unlikely that scales were leaching phosphorus within the network.
- Phosphate had more of a relationship with water quality parameters and microbial abundance at the Customer Tap than at the WTW.
- Phosphate had consistent relationships with pH, hardness, total copper, total iron, total manganese, calcium, conductivity, alkalinity and magnesium. The strongest relationship across both sampling locations with phosphate was pH.

When comparing how biological stability influenced how chlorine impacted the current state of the system, the following was found:

- Compared with chlorine there was minimal consumption of phosphate in the network, with high concentrations of phosphate residual at the Tap at all sites, regardless of the level of biological stability of the site.
- The relationship between pipe material of the network and phosphate residual at the Tap was not influenced by the biological stability of the case study areas.
- The biological stability of different case study sites did not appear to influence the relationship between phosphate consumption and microbial abundance between the WTW and Tap.
- At the WTW, sites with “good” biological stability were found to have a weaker relationship between phosphate and water quality parameters. At the Tap, the different case study sites and their varying biological stabilities did not have different impacts.

4.3.3 Improving the Understanding of the Interactions between Chlorine and Phosphate

The relationship between chlorine and phosphate was assessed to see the interplay between the two distribution chemicals.

Chlorine and phosphate were found to have a weak relationship with each other [Figure 40]. The strong relationships observed in literature were not found but the correlations observed were persistent, if not always strong.

The correlations chlorine and phosphate had were not impacted by the biological stability of the different case study sites.

Parameter	Site A			Site B			Site C			Site D			Site E			Site F		
	WTW Phosphate	WTW Ortho phosphate	Tap Phosphate	WTW Phosphate	WTW Ortho phosphate	Tap Phosphate	WTW Phosphate	WTW Ortho phosphate	Tap Phosphate	WTW Phosphate	WTW Ortho phosphate	Tap Phosphate	WTW Phosphate	WTW Ortho phosphate	Tap Phosphate	WTW Phosphate	WTW Ortho phosphate	Tap Phosphate
WTW Free Chlorine	<2.2e-16	<2.2e-16	0.0282	<2.2e-16	<2.2e-16	0.103	<2.2e-16	<2.2e-16	-0.185	<2.2e-16	<2.2e-16	0.0483	<2.2e-16	<2.2e-16	-0.262	<2.2e-16	-0.446	0.178
WTW Total Chlorine	<2.2e-16	<2.2e-16	0.202	<2.2e-16	<2.2e-16	0.0896	<2.2e-16	<2.2e-16	-0.227	<2.2e-16	<2.2e-16	0.0500	<2.2e-16	<2.2e-16	-0.174	<2.2e-16	0.275	0.185
Tap Free Chlorine	0.174	0.169	-0.165	-0.0445	-0.0662	0.111	-0.0729	-0.0706	0.0319	0.0459	0.0530	0.191	0.130	-0.0621	0.0213	-0.997	-0.168	0.0800
Tap Total Chlorine	0.193	0.192	-0.293	0.00190	-0.0177	0.154	-0.0878	-0.0806	0.0109	0.0656	0.0695	0.282	0.184	-0.0845	0.0178	-0.997	-0.177	0.0820

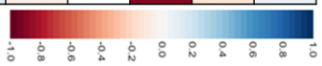


Figure 40 Correlation matrix of phosphate/mg/l and chlorine/mg/l at the WTW and Tap

4.3.4 Results Summary: The Application of Chemicals and their Impacts in the DWDS

This section has explored the link between chlorination, phosphate and biological stability to improve the understanding of distribution chemical use in the current state of the system.

An assessment of the relationship between chlorine and phosphate found that there was a weak relationship with each other, not influenced by the biological stability of the case study site.

There were also multiple findings regarding how chlorine and phosphate impacted the current system. A comparison of these findings for chlorine and phosphate can be found below:

- Chlorine was consumed at all sites between the WTW and the Tap, while there was minimal consumption of phosphate in the network in comparison, with high concentrations of phosphate residual.
- There was no seasonal chlorine dose applied at the WTW but phosphate dose was seasonally applied at Sites A-D and was observed at both the WTW and the Tap.
- Chlorine had consistent relationships with temperature, pH and conductivity, with hardness and calcium also expressing an influence at times. Phosphate had consistent relationships with pH, hardness, total copper, total iron, total manganese, calcium, conductivity, alkalinity and magnesium. The strongest relationship across both sampling locations with phosphate was pH.
- Chlorine had more of an influence over water quality at the WTW, while phosphate had more of an influence at the Customer Tap. But the relationship between chlorine and water quality parameters were quite different at the WTW and the Tap whereas with phosphate, the WTW and Tap were relatively similar.
- Chlorine had some incidences of relationships with microbial abundance, with consistent relationships found with TCC and ICC at the WTW and to a lesser extent with 3 day and 2 day colony counts across sampling locations. Microbial abundance had no relationships with phosphate at the WTW. At the Customer Tap, there were some strong microbial relationships, but they were not consistent across parameters or sample locations.

Specifically in the area of biological stability, a comparison of the influence of chlorine and phosphate found:

- Chlorine consumption occurred universally in the network regardless of the level of biological stability of the site and there was a high concentration of phosphate residual at the Tap everywhere in the network regardless of the level of biological stability of the site.
- The degree of chlorine consumption varied based on the biological stability of sites, with those sites influenced by surface water (either directly or blended) having more chlorine consumption in the network. The degree of phosphate consumption did not vary based on the biological stability of the sites.
- Sites that were less biologically stable received higher chlorine doses and higher, as well as seasonal, dosing of phosphate at the WTW.
- For the most part, the biological stability of the sites did not change how chlorine or phosphate impacted water quality or microbial abundance. Although, there were some incidences at the WTW of sites with good biological stability having a weaker relationship

between water quality parameters and phosphate. The same trend was not observed with chlorine.

- Sample location (WTW or Customer Tap) impacted how chlorine influenced water quality more than the biological stability of the site. In phosphate, the sample locations were very similar and biological stability did appear to have some influence at the WTW.

4.4 Discussion: The Application of Chemicals and their Impacts in the DWDS

4.4.1 Phosphate

4.4.1.1 The Phosphate Residual Presence and Likely Impacts

One of the key findings from this chapter was regarding phosphate use in the case study systems. This is an area which is not often studied, with previous research instead focusing on lead because of the serious consequences lead can have on human health and the strict regulations in place to safeguard against this (World Health Organization, 2018) (European Commission, 2016) (Hayes, et al., 2008). It was found that there was a substantial and persistent phosphate residual presence at the customer tap, as shown in Figure 29. Prior to this analysis, it was predicted that phosphate would have been consumed by the network, with phosphate being used up to make lead phosphate or calcium phosphate precipitates on the pipe wall in the network (International Water Association, 2016) (Hayes, et al., 2014). As stated in Section 1.4.3.2, a commonly used practice in the UK water utility companies is to apply an orthophosphate dose of 1 mg/l for one year, followed by a continuous lower dose (0.5-2 mg/l) to indefinitely maintain the layer. It is thought that if this lower dose ceases, the benefit of the treatment stops, the pipe wall precipitates disperse and the phosphorus in this form re-enters the bulk water. Section 1.4.4.1 provided a critique into this practice in the UK, concluding that the water industry can, at times, rely on information that has been known to be "common knowledge", passed on by water operatives, rather than trying to find the exact source of this information and questioning it. As such, although Section 1.4.4.2 commented that phosphate was likely overdosed by water utilities, the findings in the present chapter have gone beyond this initial analysis, determining that the extent to which phosphate is overdosed is vastly underestimated. Phosphate concentrations at the WTW were found to be very similar to at the Tap (an average of 1.24 mg/l at the WTW and 1.07 mg/l at the Tap) [Figure 29]. At sites with seasonally increased phosphate dosing at the WTW, the customer tap phosphate levels duplicated this seasonal trend [Figure 32] [further discussed in Section 4.4.1.3]. According to these results, it appears that phosphate dose is not being utilised efficiently through the network and is instead overdosed. Therefore, despite the lack of research in the area of phosphate residual at the consumer tap, it is abundant and so the question of efficiency in dose persists, it is influencing water quality and it is clearly a factor that needs consideration to further optimise networks.

Section 1.4.3.2 discussed the problems with phosphate overdose. Phosphate is a non-renewable resource that is mined from phosphate rock reserves. There are an estimated 67,700 million tonnes of global phosphate rock remaining, which is enough for the next 50-100 years (Jasinski, 2006) (Cordell, et al., 2009). Thus, poor dose optimisation worsens this situation of diminishing phosphate reserves for future use. Substantial phosphate residual at the tap also results in substantial phosphate presence entering sewerage water systems. UKWIR (2012) estimated that drinking WTW dosing phosphate typically results in 10-20% of the phosphorus load entering wastewater treatment works, which needs costly removal (~£2 million capex and £0.3 million opex for 1 mg/l phosphate for 1 million people) to ensure excess phosphate does not enter the environment, where it can cause eutrophication of water bodies (Environment Agency, 2012). The estimates provided by UKWIR (2012) on drinking water contribution to sewage load could be much greater. Thus, the phosphate overdose identified is a concern for the wider water industry, both with diminishing phosphate

reserves for future drinking water orthophosphate dose and to protect the environment from phosphate influx from sewage treatment.

Therefore, phosphate use in the network, an understudied area, was found to have a substantial and persistent phosphate residual presence at the customer tap. Section 1.4.3.2 assessed with literature that phosphate was likely overdosed by utilities, but the present body of work has determined that the extent to which phosphate is overdosed has been vastly underestimated. Such overdose is of concern for the wider water industry, as diminishing phosphate reserves grow scarcer and there is still no viable alternative for plumbosolvency.

4.4.1.2 The Extent to which Phosphate Influenced Lead at the Customer Tap

As discussed, the application of a phosphate dose is intended to influence lead presence at the customer tap (Kirmeyer, et al., 2000) (McNeill & Edwards, 2001). At the tap, phosphate was found to have a relationship with lead in Figure 37, as suggested by past research into the area, although this relationship was not as clear as suggested in other research and varied by the parameter used to assess lead. Total lead had a patchy relationship with phosphate, with a positive correlation at Site B (p=0.280), negative relationships at Sites C (p=-0.260) and D (p=-0.285) and no relationship at Sites A, E and F. Relationships were found at all case study sites with lead samples after a 30 minute stagnation, but again with a mixture of positive (Sites B, D and F) and negative (Sites A, C and E) correlations. Lead nfvs samples had consistent weak negative relationships with phosphate at all case study sites. This clearly shows that the different parameters to assess lead did have varying relationships with phosphate concentrations.

Further, it was also noted that at this sample point, a multitude of other water quality parameters had a strong relationship with phosphate, beyond lead, including pH, conductivity, temperature, hardness, alkalinity, sodium, potassium, magnesium and calcium, to name but a few [Figure 37]. In particular, pH was found to have a very strong (and often negative) relationship with phosphate, which was present both at the WTW and the Tap, further proved in Figure 38 and Figure 39.

The relatively poor correlation between lead and phosphate was not unexpected. One reason for this was due to the way lead is sampled. For instance, for a population of 90,000 people only 8 regulatory lead samples are required to be taken per year, which equates to 0.00889% of the population being tested. As the lead concentration in tap water is spatially and temporally dispersed, it is highly unlikely that lead presence will be detected with these sampling frequencies, this one discrete sample snapshot of all the water customers consume [Table 2] (Drinking Water Inspectorate, 2016).

A further reason why a strong correlation was not detected could be because of properties without lead presence in the case study distribution networks. In the present research, lead was not one of the 12 predominant pipe materials in the network, either at a PWSZ or at a DZ level, as shown in Figure 30 and Figure 31. This does not seem unusual, however, as Smitt and Russell (2013) said, approximately 60% of the UK population receive water dosed with phosphate but do not have lead pipes. Nevertheless, this assessment did not include quantification of lead presence at the household level of the premises where lead samples were obtained or consider the lead concentrations found outside of lead pipes, such as that found within solder or fittings and fixtures.

It was not a surprise that lead and phosphate was poorly correlated due to the way that lead is sampled (e.g., many properties have no lead, lead concentration is highly variable in tap water) and the low utilisation of phosphate is also well known by the sector. This should be acknowledged more in the thesis.

One factor that could potentially account for the mixed relationship between phosphate and lead, especially across the different sampling parameters, could be due to sampling technique, as shown in Table 12.

Table 12 Lead sampling technique and result variation

Lead Sampling Parameter	Description of Sampling Method	Average Lead Result
Total Lead	Sample taken after a 2 minute flush	0.633 µg/l
Lead nfvs (Non-Flush Variable Standing)	A first-draw sample taken where it is unknown how long it was since the tap was last ran	0.758 µg/l
30 Minute [Stagnation] Lead	Sample taken following a 2 minute flush and then a 30 minute stagnation	3.66 µg/

As Table 12 showed, total lead and lead nfvs were fairly similar to each other in value, while 30 minute stagnation samples had a pronounced difference, and this finding agrees with previous research. For example, Hayes and Croft (2012) noted how the different methods for assessing lead presence can give different results, including random daytime sampling, stagnation sampling after 30 minutes or overnight standing and sampling after full flushing. Despite the differences in the different parameters, previous research has specifically supported the use of the total lead sampling method for assessing lead concentration at a tap level, agreeing that it provides a more representative picture of lead concentrations that a consumer would experience within the household (Van den Hoven, et al., 1999) (Triantafyllidou, et al., 2017). Thus, even though the different sampling method produced different results, all sampling methods followed both regulatory and internal water utility (Anglian Water regulation PSW-PRO-8.13) standards and “total lead” samples in particular were commended by previous research (Drinking Water Inspectorate, 2016) (Anglian Water, 2020).

Another factor that could potentially account for the mixed relationship between phosphate and lead observed in Figure 37 could be due to the extent to which phosphate is overdosed. It could be that at no point did the phosphate dose at the WTW drop below the threshold needed to significantly impact lead leaching in the network, that the phosphate within the network was saturated.

Overall, phosphate was found to have a relationship with lead, as suggested by past research into the area. Although, this correlation was not as clear as suggested in other research as it varied by the parameter used to assess lead and there were a number of other water quality parameters that had a stronger relationship with phosphate. There were several potential reasons why this occurred, including the sporadic sampling of lead, that lead concentration in tap water is highly variable, that many properties have no lead, differences in lead sampling method and phosphate overdose. To further research this area, stopping the dose of phosphate in case study areas would be recommended to see if the phosphate/lead relationship then strengthens.

4.4.1.3 The Impact of Seasonal Dosing

Section 4.4.1.2 mentioned that seasonal dosing of phosphate at the WTW was mirrored with phosphate concentration at the Tap, indicating that phosphate was being vastly overdosed. But the wider impact of seasonal dosing was not, at this point, discussed.

Seasonal phosphate dosing began on 01/07/15 by the case study utility company at WTWs which had PWSZs deemed to be high or medium risk for lead presence at the Customer Tap (including all surface water sites), as shown in Figure 32. Since this date, every year a higher concentration of orthophosphate was dosed on 1st July and then this concentration drops down to its baseline on 1st November. At the case study sites, this seasonal dosing was often found to double the dose of orthophosphate applied. For example, Site C had a target dose of 0.75 mg/l from 2009-2014, that was increased to 2 mg/l in the seasonal dosing in 2015, after which was cycled between 1 mg/l and 2 mg/l ever year.

The reasoning behind this increased seasonal dosing was to try to account for higher lead dissolution in higher water temperatures seasonally that is well documented in literature and the water industry (Masters, et al., 2016) (International Water Association, 2010). This matter was investigated in Figure 34. While at the Customer Taps of Sites B and D, total lead was found to have a positive relationship with water temperature ($p=0.518$ and $p=0.198$, respectively), no other sites had a relationship between these two parameters. Similarly, mixed results were found in lead nfvs, and no relationships were found between 30 minute stagnation lead samples and temperature at any case study sites. Although it could be argued that the lack of a strong relationship between lead and temperature was due to the seasonal dosing, that this operational technique was successfully preventing lead dissolution at higher water temperatures. However, additional research analysing data specifically from before seasonal dosing was implemented (01/01/2014-01/07/15) found similar results to post seasonal dosing with, if anything, less of a relationship between temperature and lead in this time period. Lead nfvs still had mixed results and there were still no relationships in 30 minute samples, but there were also no relationships at a total lead level too. Further, Section 4.4.1.2 further discussed that phosphate and lead did not have as clear a relationship in the present research as suggested in other works. In this way, the seasonal dose of phosphate or, indeed, the doses of phosphate applied outside of seasonality, did not appear to strongly influence lead concentrations and so did not seem to have the desired effect.

It is known that the seasonal application of a higher phosphate dose did not decrease lead presence at higher water temperatures, as desired, but it could be said that such great increases in phosphate could have had wider unintended impacts on the drinking water networks, leading to further investigation. One specific concern was that the seasonal phosphate dose would have provided a greater source of nutrients for microorganisms, resulting in a seasonal microbial boom or community shift. As Section 1.4.4.2.2 said, most microorganisms utilise inorganic phosphate (PO_4^{3-}) for growth, with even low concentrations of 1 µg/l of phosphate able to increase and support intense levels of biological activity (Miettinen, et al., 1997). As Del Olmo et al. (2020) found, microbial community structure changes due to treatment with orthophosphoric acid can promote the presence of microorganisms that carry genes associated with the solubilisation and transport of PO_4^{3-}, including *Sphingomonas*, *Bradyrhizobium* and *Acidovorax spp*. Chapter 3 found little clear evidence of 2 day colony counts or 3 day colony counts changing seasonally at either the WTW or the Tap [Figure 13]. In the present chapter, total phosphorus was found to have no relationship with

3 day colony counts, 2 day colony counts, *E. coli,* TCC, ICC and coliforms [Figure 36]. At the Tap, however, there were some incidents of strong but sporadic and mixed relationships between phosphate and microbial abundance. For example, as Figure 36 portrayed, 3 day colony counts had a positive correlation with phosphate at the Tap at Site A (p=0.232), Site C and F have a negative correlation (p=-0.409, p=-0.0767) and Sites B, D and E had no correlation. Another argument is that phosphate increases in this manner may not have resulted in shifts in microbial abundance because sufficient concentrations of phosphate were always present for microbial growth, an average of 1.07 mg/l at the Tap, rather than the levels of 1 µg/l assessed in the study completed by Miettinen et al. (1997).

A recommendation for future research would be to assess the microbial communities present to determine if community was shifting due to the phosphate concentrations, in a way similar to the work of Del Olmo et al. (2020). Although *E. coli* and *Enterococci* were not found in any samples at the WTW or at the Tap. The use of DNA analysis, for example, would provide a valuable source of information regarding community and potential microbiome community changes when the seasonal dosing was occurring at those case study sites. Therefore, evidence was not found that the seasonal orthophosphate dose supported a greater abundance of microorganisms, but an assessment of community would be recommended for future similar work. This is a particularly encouraging finding because it could insinuate that if phosphate concentrations drop in an effort to become chemical free, it is not likely to influence microbial growth, according to the results of this study.

In an assessment of other, wider water quality parameters, it was found that seasonal orthophosphoric acid dosing at the WTW had the greatest influence on pH at both the WTW and, to a lesser extent, at the Customer Tap, with limited evidence of further parameters being impacted [Figure 37]. This finding is consistent with previous research, as orthophosphate can additionally be used as a sequestering agent to “chemically tie up” scale forming ions such as calcium and magnesium, decreasing the pH of the water (American Water Works Association, 1995). Although this means that there does not appear to be a great extent of wider water quality changes due to the addition of a seasonal phosphate dose, pH is an important factor to be discussed when investigating water quality as it impacts a multitude of other processes. For example, acidic soft waters increase the corrosion of pipes and the dissolution of metals, while hard alkaline waters can cause the formation of scale (Smitt & Russell, 2013) (Zhang, et al., 2009). This means that seasonal pH changes on this scale could be challenging for operational management practices. Further, pH change in this magnitude would likely be a big consideration underpinning the feasibility of chemical free drinking water treatment and distribution. If the case study water utility were to cease their chemical dose, they would have to apply some means of other pH correction.

Thus, seasonal dosing of orthophosphate did not appear to strongly influence lead concentrations and so did not seem to have the desired effect. However, it was also not evidenced to impact microbial growth (although an insight into community would have had added value). Rather, the main effect seen by this process was a seasonal pH value, which could potentially mean pH would be the biggest risk to water quality if phosphate was removed from the current system, without further mitigation strategies in place.

4.4.1.4 How Phosphate Influenced Water Quality

In-depth analysis of the wider impact of orthophosphoric acid dosing found that phosphate had consistent and/or strong relationships with a number of water quality parameters, including pH, hardness, total copper, total iron, total manganese, calcium, conductivity, alkalinity, water temperature, sodium, potassium and magnesium [Figure 37]. Of the few previous experiments that have an emphasis on phosphate (rather than a lead-based focus), they have generally been laboratory experiments, for instance the work of McNeill and Edwards (2000) and Butt (2016). In laboratory experiments exploring phosphate such as these, there is a high level of control that could potentially oversimplify the nuances found in live water distribution systems, meaning that they cannot effectively replicate such a system. The way in which phosphate had a relationship with multiple water quality and microbial abundance parameters emphasises the importance of fieldwork in situations such as these, when considering the complexity of drinking water distribution systems. In this instance, historic regulatory samples were able to provide an indicator of the application of a chemical can influence many aspects of this environment. This does mean that care should be taken were lowering or removing the dose of orthophosphate because it is likely that a number of parameters will be impacted. Thus, historic data analysis of real distribution networks went beyond the observations of laboratory work in this area.

Section 4.4.1.3 discussed how seasonal phosphate was found to have a strong correlation with pH. The same was true of general phosphate dose without consideration to seasonal dosing. pH was found to have a very strong (and often negative) relationship with phosphate, which was present both at the WTW and the Tap, although with a stronger relationship at the Tap [Figure 37]. All of the water quality correlations with phosphate were also found to be stronger at the Tap, including with microbial abundance (microorganisms are further commented on in Section 4.4.1.3). These relationships could have been stronger at the Tap than at the WTW because of the residency time of orthophosphate within the network, with its impact on the network being greater the longer it was present. Machell and Boxall (2012) noted that the longer water is in contact with the distribution network material, the higher the propensity for water quality to be affected. The current research postulates that the same is also true of orthophosphate as a distribution chemical, that the longer water is in contact with distribution network chemicals, the higher the propensity for water quality to be impacted. To further investigate this, it would be interesting to sample throughout the network, including at the furthest reaches of the network to see if the correlations with phosphate shifted at varying points throughout the network.

Overall, phosphate was found to correlate with a number of water quality parameters and especially pH. These correlations were stronger at the Tap than at the WTW, likely due to the residency time of orthophosphate within the network, an area that could benefit through network sampling at various points. The impact phosphate had on a large variety of parameters, reinstated the value of fieldwork sampling to build a fuller, richer picture of the interplay within drinking water distribution environments.

4.4.1.5 Phosphate and Biological Stability

An area for investigation was how phosphate was used in different networks, including those of good biologically stability and those which were less biologically stable. This was a complex question to answer because further examination of the case study sites found management practices were different between the sites, with the phosphate doses of Sites A-D increasing seasonally every year to double the normal dose [Figure 33].

The varying biological stabilities of case study sites were not found to influence how phosphate was consumed within the network, how phosphate and pipe material interacted nor did it influence the relationship between phosphate consumption and microbial abundance, as shown in Figure 29, Figure 30 and Figure 36. The only difference that was detected by case study site was that at the WTW, sites with "good" biological stability were found to have a weaker relationship between phosphate and water quality parameters [Figure 37]. However, when sampled at the Tap, the different case study sites and their varying biological stabilities did not have different impacts. Therefore, this potentially suggests that biological stability was not a driving factor in phosphate impact and thus, by extension, that to achieve a future phosphate-free distribution network, site biological stability could be seen as not a high priority consideration.

It is difficult to say if these results compared well to past research because the area of biological stability and its link with orthophosphate is relatively understudied. One of the reasons for this, as Section 1.4.4.2.2 noted, is that numerous studies that investigated phosphate dose and its impact on the drinking water distribution system have been conducted in countries where phosphate dosing for lead control is not an established practice, for example in the Netherlands. In these studies, such as that of Juhna et al. (2007), an investigation of "high" concentrations of phosphate equated to 20 µg/l (0.02 mg/l), which, for the UK, would be considered low (as an average UK phosphate dose is 0.5 mg/l) (Hayes, et al., 2014).

Even if microbiology within the network and phosphate are both studied, the concept of biological stability may not be referred to in past research. One example of this is the work of Douterelo et al. (2020), who compared drinking water samples in a live UK network with (~1.2 mg/l) and without (~0.03 mg/l) the addition of a phosphate dose. It was found that with a phosphate dose, the relative abundance, diversity and richness was higher in fungi but lower in bacteria, compared to without a dose. Although as the purpose of this research was to investigate the impact of phosphate dosing on the microbial ecology of drinking water distribution systems, no comment was made regarding the biological stability of these networks.

Despite this, there are areas where phosphate is present, and utilities have categorised their drinking water as biologically stable. One example where this was found was in Stadtwerke Düsseldorf in Germany, one of the three case study companies in countries using fewer chemicals discussed earlier in this research. This company, although chlorine free, doses ~1 mg/l phosphate silica to prevent corrosion in iron pipes (rather than lead as in the UK) (Isle Utilities, 2015). Regardless of this dose, Stadtwerke Düsseldorf consider their systems to be biologically stable, assessed using specific levels of stability for AOC (<10 µg/l), TOC (0.3-0.5 mg/l) and network pressure (~6 bar), as well as a focus on having a continuous steady flow rate in the network. Nevertheless, it is still noted that orthophosphoric acid is not utilised by Stadtwerke Düsseldorf, but rather a different

chemical, a phosphate silicate. Even though it is not a direct comparison, it appears that it is possible for a utility to have good biological stability with the addition of a phosphate corrosion inhibitor.

However, there are also studies with conflicting findings. Jang et al. (2012) added 5 mg/l of phosphate to an annual reactor system, finding that the chemical addition impaired the biological stability of the network system. Although biological stability was never explicitly defined in this study, it was commented that the addition of the phosphate "promoted the growth of biofilm" and "the species diversity in biofilm… significantly increased". Regardless, this study used much greater concentrations of phosphate than in UK systems and may not have as fully captured the effects of phosphate dose as studies on a live network would have.

Overall, there is little research into how orthophosphate impacts biological stability, particularly with similar doses used as in the UK and of the research that has been conducted, there are conflicting results. Biological stability and phosphate were not found to have a strong link. The varying biological stabilities of different case studies did not impact how phosphate was being used or how phosphate itself was impacting the network environment. As such, these results suggest that in the area of phosphate removal, biological stability was not found to be a driving factor, that to achieve a future phosphate-free distribution network, site biological stability was not key.

4.4.2 Chlorine

4.4.2.1 How Chlorine Influenced Water Quality

In comparison with phosphate, which had substantial residual concentrations at the Tap [Figure 29], chlorine was consumed at all sites between the WTW and the Tap, as shown in Figure 23. This agrees with a multitude of past research that showed chlorine decay across the network, as well as that which supports the use of booster chlorination to replenish chlorine dosage between the treatment works and customer tap (Machell & Boxall, 2012) (Blokker, et al., 2016) (Kirmeyer, 2004). Indeed, it was found that sample location (WTW or Customer Tap) impacted how chlorine influenced water quality more than site biological stability [Figure 25] It was likely that chlorine had more of an influence over water quality at the WTW than at the Tap, because at this point chlorine concentrations were stronger and had not yet had time to decay. Conversely, phosphate had more of an influence over water quality at the Tap than at the WTW because it did not decay throughout the network and instead had a longer retention time within which to impact water.

One finding regarding how chlorine influenced water quality was that chlorine had consistent relationships with temperature, pH and conductivity, with hardness and calcium also expressing an influence at times, as shown in Figure 25. These findings build on a wealth of existing evidence that the success of chlorination as a disinfectant is strongly influenced by pH and water temperature (American Water Works Association, 1995) (Momba, et al., 2000) (Keevil, et al., 1990) (Kirmeyer, 2004).

One way in which the present research differed from previous work into the impact of chlorine on drinking water quality, was there was not always as clear and consistent a relationship between TOC or turbidity and chlorine as demonstrated in past research. In some previous investigations, equations have been used to describe chlorine demand kinetics as a function of TOC and turbidity as a rough indicator for both chlorine demand and TOC (Black & Veatch Corporation, 2010) (Lantagne, 2008). However, other findings were discovered within these parameters. For instance, as Section

3.4 noted, the case study sites that used chloramination rather than free chlorine had the highest levels of TOC at the WTW and that turbidity was fairly low and stable across all of the case study WTWs but then increased in the network at every site [Figure 14, Figure 15]. It would have been valuable to have an assessment of TOC and turbidity at the case study sites before the addition of chlorine to get a better understanding of the baseline conditions. As Section 3.4 outlined, it also would have been interesting to sample TOC at the Tap to determine how TOC levels varied throughout the network, as well as monitoring DBP formation at both the WTW and the Tap, since DBPs are formed when a chemical disinfectant (e.g., chlorine, chlorine dioxide, chloramines and ozone) reacts with natural organic matter and/or inorganic matter (World Health Organization, 2000).

Therefore, how chlorine influenced water quality was quite similar to findings from past research, including the extent to which chlorine decayed within case study networks and the propensity of chlorine to be strongly related to that of pH and temperature. The link between chlorine, turbidity and TOC, however, was not as clear and consistent as previous research has suggested. While the strong relationships noted with chlorine and conductivity, as well as with hardness and calcium at times, was not commented on in earlier works and could benefit as an area of further research.

4.4.2.2 The Extent to which Chlorine Influenced the Microbiome

Regarding how chlorine impacted the microbiome, chlorine had some incidences of relationships with microbial abundance [Figure 28] and was found to have more of a relationship with microbial abundance than phosphate [Figure 36]. Chlorine had relationships with TCC and ICC at the WTW and to a lesser extent with 3 day and 2 day colony counts across sampling locations and sites. However, these relationships were not consistent, with some positive correlations and some negative. These relationships did not appear to be influenced by the biological stability of the case study site or by disinfectant choice (chlorination or chloramination). There was little evidence that low chlorine concentrations resulted in high microbial abundance [Figure 26, Figure 27]. This suggests that the relationship between microbial abundance and chlorine concentration is not as clear as suggested by other research, including that of Servais et al. (1995), LeChevallier et al. (1996) and Gillespie et al. (2014), all of whom found evidence of increased bacterial abundance where there was depletion of the residual disinfection.

One interpretation of the microbial findings is they could indicate how chlorination impacts the microorganisms of different systems in different ways. An assessment of species or community would be helpful to explore this further, especially considering all *E. coli* and *Enterococci* samples throughout the sampling period were negative, yet TCC and ICC, 3 day colony counts and 2 day colony counts persisted [Figure 13]. Clearly, microorganisms were present that were not being captured by plate counting methods such as *E. coli* and *Enterococci*, as other researchers have postulated (Lautenschlager, et al., 2013) (Hassard & Whitton, 2019) (Liu, et al., 2013). The application of a disinfection residual may apply selective pressure that favours certain organisms over others, changing community structure in the distribution network, rather than affecting abundance as is desired from its application. One of the reasons why a residual disinfectant is applied is for water utility reassurance, as an indicator for control of microorganisms. It is used to ensure that enough disinfectant was added at a WTW to provide a residual at the customer tap, suggesting that water is protected from microbial regrowth within the network (Tsitsifli & Kanakoudis, 2018). However, the results from this PhD imply that the presence of a chlorine residual

at the Tap as well as a negative *E. coli* and *Enterococci* plate counting does not indicate that microorganisms are not present, even though operational conditions may be optimal.

An alternative explanation could be that different relationships were observed because the methods used to assess microbial abundance did not include an assessment of the biofilm. It is well known that the current practice of the use of a disinfection residual in the UK is based upon its action against planktonic cells, as only bulk water is regulated because in an operational environment it would be very difficult to sample the pipe wall and the attached biofilm (Fish & Boxall, 2018). Thus, although chlorination has been well established to be an effective disinfectant for bulk water, its impact on the biofilm is comparatively unknown. For example, the mixed relationships observed between microbial abundance and chlorination observed in Figure 28 could have been the result of different hydraulic regimes within the network causing more disruption of the biofilm. Any apparent decreases in bulk water microorganisms from the WTW to the Tap could be due to biofilm formation, while any substantial microbial network increases could be due to increased biofilm mobilisation. A study conducted by Husband and Boxall (2011) monitored 67 pipe sections from 15 locations across the UK found that the mobilisation of material was universal to every flushing operation monitored. Further, it has been found that with every increase in applied shear stress, there is an increased release of accumulated material into bulk water, causing a deterioration of water quality and an increased discolouration risk (Husband, et al., 2016). However, it is difficult to assess the extent to which this phenomenon occurred at the case study sites, without further access to hydraulic and operational regimes, a factor which has the potential to be rectified in future works.

Different relationships may also have been observed at the different sites because of differing levels of chlorine resistance. Some of the case study areas could have had greater species diversity. Biofilms that have a high species diversity are more likely to have greater resilience towards chlorine (Morton & Surman, 1994). A further potential possibility is that some of the networks could have had the presence of chlorine-resistant microorganisms. Although this appears to be an emerging risk that is becoming increasingly studied, presently research in this area is conflicting (Sun, et al., 2013) (Farkas-Himsley, 1964) (Wray, n.d.).

Overall, chlorine had some incidences of relationships with microbial abundance, however these relationships were not consistent, with some positive correlations and some negative and did not appear to be impacted by the biological stability of the case study site or the choice of chlorination or chloramination. Previous research indicated that low chlorine residual would favour microbial growth but little evidence for this was found. It is unknown exactly the reason why chlorine impacted different systems in different ways, particularly the positive correlations identified. This could potentially indicate that chlorine could be aiding microbial growth or acting as a food source, however unlikely. Future research could explore this further with an assessment of species or community as well as access to hydraulic and operational regimes. Regardless of the reason, it is clear that the application of a chlorine dose was not universally reducing microbial abundance as intended, which could potentially mean that the transition to chemical free water would not cause as much microbial disruption as previously thought.

4.4.2.3 Chlorine and Biological Stability

One way in which the application of a chlorine residual can impact biological stability is because chlorine dose within the network is not homogenous at all points of the network. It was found that unlike the seasonal dosing observed in orthophosphate [Figure 33], chlorine dose at the WTW remained the same throughout the study period [Figure 24]. However, because of the variation of different distribution networks, even if the chlorine dose at the WTW remains stable, the residual chlorine dose at the consumer tap may not be homogeneously distributed across all network points [Figure 12]. An assessment of chlorine decay at various points of the network, however, was not completed and would have potential for future work.

In the area of biological stability, a comparison of the influence of chlorine found that unlike in phosphate, where the degree of phosphate consumption did not vary based on the biological stability of the sites [Figure 29], chlorine consumption did [Figure 23]. Case study sites of less good biological stability were found to have more chlorine consumption in the network [Figure 23]. It is well understood that more chlorine consumption occurs where water quality and/or network conditions are poorer (Kirmeyer, 2004). It is also often considered that sites of poorer biological stability require more treatment steps, for instance the reasoning behind the case study companies using fewer chemicals than in the UK (Isle Utilities, 2015). However, chlorine consumption and biological stability, together, are rarely discussed. This is likely because many of the countries who consider the concept of biological stability, such as the Netherlands, do not chlorinate.

The greater chlorine decay in sites of less good biological stability occurred even though these were the sites that received the highest doses of chlorine at the WTW, as shown in Figure 12. Sites A-D had an average WTW dose of 1.04 mg/l total chlorine, while Sites E-F had 0.627 mg/l. At the tap, however, values were similar, with an average of 0.509 mg/l for Sites A-D and 0.454 mg/l at Sites E-F. It is likely that WTW operators were aware of chlorine decay being greater in these specific networks because of the high numbers of chlorine samples at the Tap and so adjusted dosed concentrations for all sites to have similar residuals at the Tap. Section 1.4.4.1 remarked that chlorine samples at the customer tap could at times be used as a crude indicator of microbial presence or absence (Tsitsifli & Kanakoudis, 2018). But the results of the present section dispute this, instead proposing that chlorine dose is being strongly considered at the tap by operators to better understand effective dosage. Unlike phosphate with its high tap residual, the dose of chlorine used was found to be effectively managed. This shows that chlorine is well understood. The wealth of research regarding chlorine use and then the application of this knowledge is commendable, especially when compared with the limited research that has been conducted in the area of phosphate. The presence of the chlorine knowledge emphasises the lack of phosphate understanding, especially when seasonal phosphate dosing and its impacts are considered.

Even though sites of less good biological stability had greater chlorine decay, demonstrating a greater link between biological stability and chorine than there was with biological stability and phosphate, wider effects were not detected. The biological stability of the sites did not change how chlorine impacted water quality and it did not influence the relationship between chlorine and microbial abundance [Figure 25, Figure 28]. Rather, sample location (WTW or Customer Tap) impacted how chlorine influenced water quality more than the biological stability of the site. This could potentially be because the chlorine dose was effectively managed and consistent, resulting in minimal drinking water distribution changes. In this way, it could be that the transition from a

chlorinated network to a non-chlorinated network may not pose as great a threat to biological stability as initially thought.

Thus, chemical consumption was influenced by biological stability in the application of a chlorine dose, even though it was not with phosphate, with sites of less good biological stability having greater decay. It could be argued that the chlorine decay within the network is indicative of chlorine dose not being homogenous at all points of the network and so creating a different impact in differing areas, meaning it is not possible to have a biologically stable chlorinated network. However, as Section 3.4.7.3 showed, two of the chlorinated case study sites were identified as having good biological stability. Further, the present section found biological stability of the sites did not change how chlorine impacted water quality and it did not influence the relationship between chlorine and microbial abundance.

4.4.3 Discussion Summary: The Application of Chemicals and their Impacts in the DWDS

Although both chlorine and phosphate are similar in that they are both chemicals added to maintain drinking water quality from the point of production to the point of consumption, the current chapter found them to be very different in the way they were used and how they influenced differing networks.

The main purpose of phosphate application is to reduce the dissolution of lead in the network, but phosphate was actually found to have a relationship with a wide range of water quality parameters, (including pH, hardness, total copper, total iron, total manganese, calcium, conductivity, alkalinity, water temperature, sodium, potassium and magnesium), especially pH. Although phosphate was found to have a relationship with lead, there were a number of other water quality parameters that had a stronger relationship with phosphate. The relatively poor correlation between lead and phosphate was not unexpected due to the sporadic sampling of lead, that lead concentration in tap water is highly variable and that many properties have no lead. The link between phosphate and biological stability was tenuous, with the varying biological stabilities of different case studies not impacting how phosphate was being used or how phosphate itself was impacting the network environment. As such biological stability would be unlikely to be a driving factor for the future removal of phosphate. If phosphate were to be removed however, it is highly likely a large number of water quality parameters will be impacted and further research would need to be done to fully understand the measures needed to prepare for this eventuality, for instance supplementary pH control techniques. Phosphate is clearly an understudied area, especially when compared with the wealth of understanding surrounding chlorine. This is especially apparent as new operational strategies, such as the seasonal application of a phosphate dose, are being introduced but the desired effect, an impact on lead concentrations, has not been achieved. The overdosing of phosphate is not surprising but there is very limited published evidence to confirm this. The residuals detected in case study networks, compounded by the diminishing phosphate reserves and the risk of lead to consumers, indicate it is of great need for further work.

Chlorine influenced water quality in a similar way to that of other research (especially in the area of strong relationships with pH and temperature), although TOC and turbidity were found to be less pivotal while conductivity, hardness and calcium were found to be more so. Inconsistent and often weak relationships between chlorine and microbial abundance posed the question if chlorination was fulfilling its purpose. It was not universally reducing microbial abundance as intended, which

could potentially mean that the transition to chemical free water would not cause as much microbial disruption as previously thought. Section 1.4.4.1 posed the question “Is a Secondary Disinfectant Needed?”. The identified arguments as to why a secondary disinfectant is required, included: to prevent regrowth and microbial contamination; to ensure microorganisms are not present at the customer tap; as an indicator of operational conditions; it is not certain if UK water utilities could provide the same safe drinking water without the use of chlorine; for UK legislation and for taste and odour purposes. The literature review concluded that a chlorine residual is not a requirement of WHO, EU or UK regulation (World Health Organization, 2011) (European Commission, 2016) (Drinking Water Inspectorate, 2016). It was also noted that multiple taste and odour complaints are due to chlorination, rather than eased by its application (Department of the Environment, 2014) (Knowledge Transfer Network, 2008). The present section has proven that even though a chlorine residual is present, microorganisms are still present. The question remains, therefore, if it is possible for UK water utilities to provide the same safe drinking water without the use of chlorine as other countries can or if the specific conditions and challenges posed within the UK (e.g., ageing infrastructure, population growth etc.) are too great a barrier to overcome. Biological stability was found to influence how chlorine was consumed in the different networks, with sites of less good biological stability having a greater rate of decay. But this biological stability did not shape how chlorine impacted water quality or the relationship between chlorine and microbial abundance. It is clear that the concept of biological stability plays a part in chlorination, but it is uncertain what the impact would be on the network were chlorine to be removed, particularly if sites previously of good biological stability would then become less good or vice versa.

5 Chapter 5: The Wider Impact of Chemicals in an Experimental Facility

This chapter consists of the design, construction and operation of a bespoke pipe rig testing facility intended to fulfil Aim 3, Objective a and b, as outlined in Chapter 2.

5.1 Introduction: The Wider Impact of Chemicals in an Experimental Facility

Much of the research into the biological stability of drinking water systems has taken place through sampling at WTW, such as the work of Lautenschlager et al. (2013). In research such as this, sampling points for the study are generally at the end of the WTW (at the Final Water sampling point) so no conclusions can be drawn regarding the interactions between chemical presence and biological stability. Whereas the research into the chemicals used in drinking water distribution systems is generally based on pilot rig systems, including the research of Fish and Boxall (2018), so the chemical doses can be adequately controlled with fine-scale control of variables and replications.

Chapter 3 and Chapter 4 demonstrated how water quality samples that water utilities obtain from the Final Water and Customer Tap comprise of highly valuable data that provide insight into realistic network conditions. Regardless, this type of analysis does come with challenges. With Customer Tap samples, the safety and satisfaction of the customer is of upmost concern. This means that experiments that could impact their water quality, such as reducing the chemical dosage used to treat their water, is not permissible. Additionally, as sampling parameters obtained are those that are required by current regulation, varying sampling parameters or sampling frequency for experimentation purposes can be difficult. For instance, DNA sampling at the Customer Tap may cause concern to a water utility if pathogenic strains were detected or repeat sampling of a particular customer's tap could cause too much disruption to the consumer and the risk of a lack of repeat future access. It also must be noted that true random regulatory sampling is difficult to achieve as access to a customer household is required; instead, samplers are given a select area to choose from and choose a property based on ease of access and limited disruption to consumers.

There are additional concerns regarding live network samples. Normally, sampling within the network itself is minimal because of the contained nature of pipes so samples from the Final Water and Customer Tap are used to infer network condition and quality rather than this being directly perceived. This is especially true when attempting to study biofilm as pipe networks are sealed, working systems, meaning that the removal of biofilms from the internal pipe surface can be highly difficult, expensive and poses a risk to customer water if done improperly.

With laboratory experiments, there is much greater control of sample type, sample frequency and experimental parameters, such as the ability to adjust the chemical dose entering a system. It could be said, however, that this high level of control results in oversimplifying the nuances that fieldwork has, that laboratory experiments cannot effectively replicate the realistic conditions found in live water distribution systems. These studies conducted in a laboratory are often in a different location from the WTW, with no control over how the distribution network is impacting water quality on its journey from the WTW to the location of the pilot. Section 1.4.3.2 described how the longer water is in contact with the distribution network material, the higher the propensity for water quality to be impacted (Machell & Boxall, 2012). Moreover, as laboratory studies utilise water that is already

chemically treated, those chemicals would have to removed for chemical free investigation, in a way which does not have any additional impacts on water quality. A study by Fish and Boxall (2018) was found to have some success at removing chlorine from 0.45 to 0.05 mg/l for incoming pilot drinking water, without any other known impact on water quality, but total removal of chlorine was not achieved. Similarly, two trials were conducted in the present study to see if pellets used to remove phosphate from sewage could remove phosphate from incoming water but success was not found.

To coalesce the advantages of both laboratory studies and experimentation within the field a series of novel purpose-built re-circulating test loop facilities were designed and constructed, to achieve Aim 3 Objective a and b, outlined in Chapter 2. These facilities allowed for the wider impact of the presence and absence of the distribution chemicals chlorine and phosphate to be studied without any risk of altering public health. The facilities were designed in such a way as to allow for the bulk water quality and chemistry to be investigated as well as the exchange mechanisms between the bulk water and the pipe wall, as can be seen in Figure 41. Differing levels of biological stability were also able to be studied due to the production of two identical pipe testing facilities at two WTW with different sources of water, one a surface fed WTW and one fed by groundwater. As the facilities were located directly at the WTW, there was not the concern of heightened water age that test facilities based in a laboratory face.

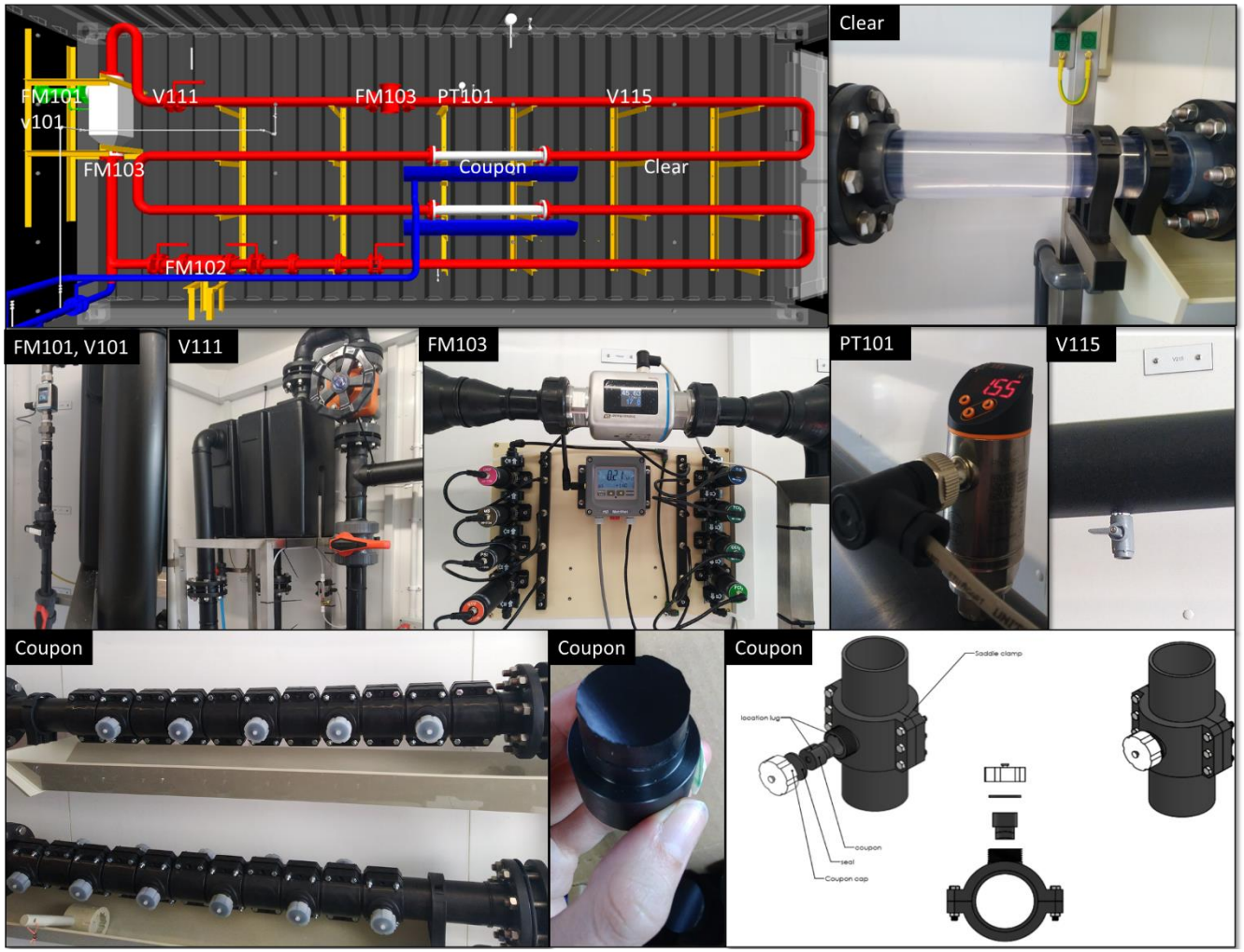


Figure 41 Drinking water distribution simulation pipe rig

Experimental test facility, including coupon pipe sections containing 22 coupons per rig, clear pipe section, flow meters on the tank Inlet (FM101), pump (FM102) and main pipe (FM103), main pipe pressure transmitter (PT101), sampling taps on the rig Inlet (V101) and Outlet (V115) and ATi MetriNet.

5.2 Methods: The Wider Impact of Chemicals in an Experimental Facility

5.2.1 Experimental Facility

Two sites were selected to house the experimental facilities, a Surface Water Site and a Groundwater Site. Chapter 3 and 4 described these sites as Site D and Site F. It was desirable to have a mixture of source water types because, as discussed in Section 1.3.1, case studies and research agree that groundwater sources are more suitable than surface water for the adoption of chemical free drinking water, because they are less vulnerable to microbiological presence and have a greater biologically stability (Isle Utilities, 2015) (Kistemann, et al., 2001) (Prest, et al., 2016). Thus, a site of good biological stability (the Groundwater Site) and a site of less good biological stability (the Surface Water Site) were preferred. Chapter 3 and 4 found that a comparison of the Surface Water and Groundwater Sites showed both the Final Water and the Customer Tap data within the two areas had a multitude of parameters that differed between the areas, which cumulated in different risk scores for biological stability [Figure 71, Figure 73]. This, in combination with practicalities, for instance their proximity to each other for ease of sampling, the availability of room on site to contain the pipe testing facilities and the treatment processes used, confirmed that appropriate site locations had been selected for experimental facilities.

The series of novel bespoke purpose-built re-circulating test loop facilities designed and constructed, consisted of two identical 20 ft shipping containers. Each container housed two 14 m long 90 mm external diameter (73.05 mm internal diameter) butt-fusion welded pipe loop systems with a pipe volume of 170 l, including a tank volume of 80 l, made from grade PE100 SDR17 High-Density Polyethylene (HDPE), as depicted in Figure 42.

The water feed to the drinking water test facility was supplied directly from two WTW. One of the independent pipe systems per site received a feed from before the distribution chemicals chlorine and phosphate were dosed, the Chemical Free Rig, and one from after chlorine and phosphate were dosed, the Chemically Fed Rig. Each of the four independent pipe facilities had their own enclosed reservoir tank, the water within which was refilled using a float and a ball valve. Fed from this tank, drinking water was re-circulated around the system, the retention time within which was determined using an adjustable trickle feed drain controlled by a flow meter. For the study duration, a target water age of 3 days was chosen to mimic the average water ages within the actual distribution network. Flow rate, pressure and so velocity and shear stress, could be controlled using the pump and the valves within the pipe loop system.

HDPE pipe was the chosen pipe material for the production of the bespoke pipe testing facilities due to its prevalence as a next generation pipe material. Section 1.4.3.2 discussed the importance of pipe material used as it has the potential to impact the quality of the water residing within it. A “next generation” of pipe materials are steadily being introduced in UK water utilities, including PVC (polyvinyl chloride), HDPE (high density polyethylene) and MDPE (medium density polyethylene) (UKWIR, 2003) (Husband & Boxall, 2016). Of the next generation pipe materials, HDPE has been found to be one of the most commonly used drinking water distribution system materials as it is flexible, inexpensive, resists corrosion and has a long expected service life (Heim & Dietrich, 2007) (Whelton, et al., 2010) (Davis, et al., 2006).

Grade PE100 SDR17 pipe was utilised for the purpose of the experiment to ensure quality and resilience for the project duration. Pipe material of grade PE100 has a high minimum required strength value, meaning that it is more resistant to rapid crack propagation, long-term stress cracking and is capable of having a thinner pipe wall for the same operating pressure, compared with, for instance grade PE80 (International Organization for Standardization, 2012). Similarly, a Standard Dimensional Ratio (SDR) of 17 means the pipe can withstand higher pressures, compared to SDR35 for example.

The chosen dimensions of the pipe loops, 14 m long and 90 mm external diameter, have a larger pipe surface area than laboratory-based experiments meaning that it will build a more realistic picture of the accumulation process and nutrient exchanges between the bulk water and the pipe wall. By simulating the correct pipe wall to bulk water ratio and the correct gradient of nutrients to the pipe wall, the test facility means that the replication of realistic boundary layer flows is possible, which are likely to impact accumulation and mobilisation processes and nutrient exchanges. The presence of coupons within the pipe loop system, of which there are 22 per system, allowed for investigations to occur on both a bulk water and a pipe surface level.

The experimental facility was in operation for one 3 month “growth” phase, where biofilm development was monitored as it built over time, deteriorating the freshly laid “idealised” pipe system. Previous studies investigating biofilm within the distribution networks have varied from days (Deines, et al., 2010) to years (Martiny, et al., 2003). Deines et al. (2010), for instance found that after only 7 days of exposure, clean coupons in a nonsterile pilot water distribution system formed a complex, structurally mature biofilm. The 3 month duration in the current project was chosen to get a balance between a great enough growth phase to see the impacts of different regimes in each loop on both the biofilm and the bulk water, the fouling of the “idealised” pipe, as well as a time frame that fit within the confines of a PhD.

The original experimental programme consisted of a 6 month “growth” phase, where biofilm development was monitored and a “mobilisation” phase, to analyse the biofilm response to increasing shear stress under controlled increases in flow rate. The mobilisation phase of the experiment was not completed due to a worldwide pandemic. Details of this proposed phase can be found in Appendix 1.

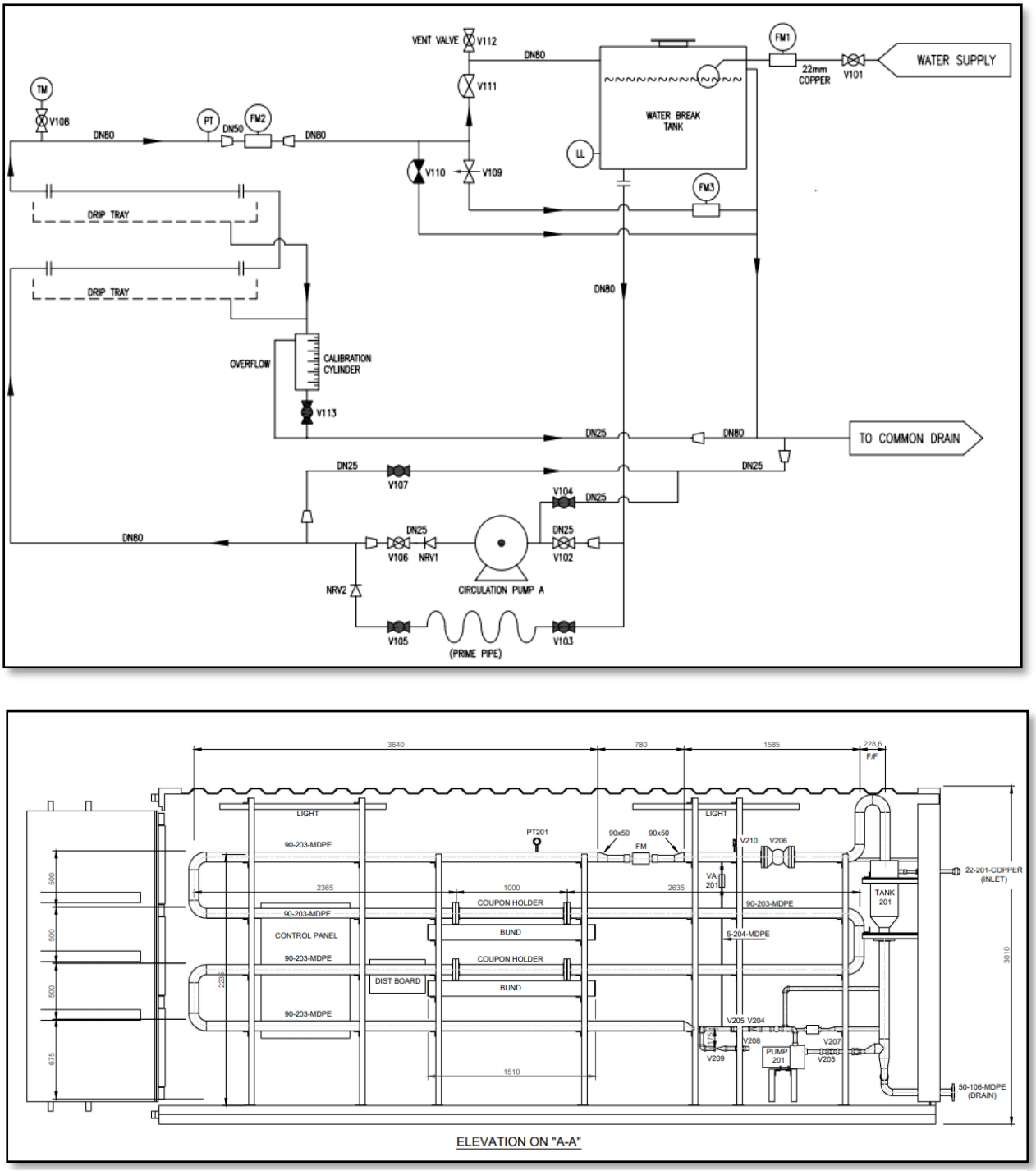


Figure 42 Schematic diagram of the experimental facility

Experimental test facility, including coupon pipe sections containing 22 coupons per rig, clear pipe section, flow meters on the tank Inlet (FM101), pump (FM102) and main pipe (FM103), main pipe pressure transmitter (PT101), sampling taps on the rig Inlet (V101) and Outlet (V115) and ATi MetriNet.

5.2.1.1 Coupons

Previous studies that investigated biofilm formation in drinking water distribution systems have used a number of different methods including: the use of glass slides for biofilm growth in a pipe with glass slides jutting out into the water column known as a Robbins device (Manz, et al., 1993); annular or Propella reactors that consists of concentric cylinders, propellers and glass beads for biofilm growth (Bauman, et al., 2009); flow through cells that mimic the network conditions with small sections of stainless steel an PVC for biofilm growth (Simões, et al., 2006) and HDPE Pennine Water Group coupons that are small circular sections of pipe that fit flush with a replicate pipe system for biofilm growth coupon (Deines, et al., 2010). Some of these listed options can distort or inaccurately replicate the boundary layer hydraulics of an actual distribution network. As the boundary layer dynamics drive the shear stress, nutrient gradients and interactions at the bulk water-pipe wall interface, if these are inaccurately replicated so too could the biofilm formation. However, the Pennine Water Group coupons were designed to fit into the apertures of a pipe, following the internal pipe curvature with a maximum deviation from curvature of 0.064 mm, in the order of magnitude of the surface roughness coefficient used in hydraulic models (Deines, et al., 2010). In this way, the Pennine Water Group coupon allows direct insertion and close alignment with the internal pipe surface, minimising the distortion of boundary layer conditions that influence biofilm formation, such as boundary shear stress and turbulent driven exchange with the bulk water body (Douterelo, et al., 2013). Thus, removable coupons, such as the Pennine Water Group coupons, allow a more efficient monitoring of biofilm formation in pilot and real systems. Gomes et al. (2014), who delivered a review on the reactors used to study drinking water biofilms, affirmed that coupons are “the best devices for biofilm monitoring”. The use of appropriate coupons, though, is an important choice as the size, shape and material of the coupon can impact the heterogenous distribution of the biofilm over the surface area (Okabe, et al., 1995).

To allow for the quantitative and qualitative compositional characterisation of in situ biofilms on a pipe surface level, a sampling device that could be inserted directly into the pipe loop system was designed [Figure 43]. These devices, known as a coupon, were a disk of HDPE comprised of two parts, an “outer coupon” and an “insert”. The two-part system, although complicating the coupon design, allowed for each coupon to have multiple analyses, getting more data from each sample, a design component used advantageously in the Pennine Water Group coupon (Deines, et al., 2010) (Sharpe, 2012). Whereas the Pennine Water Group coupon fixed in place using flanged pipe sections and a gasket, the coupons used in this PhD research utilised a saddle clamp, location lug, seal and coupon cap to decreases the likelihood of leakage from this coupon section. The outer coupon retains the curvature of the pipe and fits precisely into a hole made in a saddle clamp, as can be seen in Figure 43. The location lug on the coupon and the saddle clamp aligns and then the coupon is fixed in place using a seal and a coupon cap to prevent leakage. The insert fits inside of the outer coupon in a way to allow the outer surface to be in direct contact with the water.

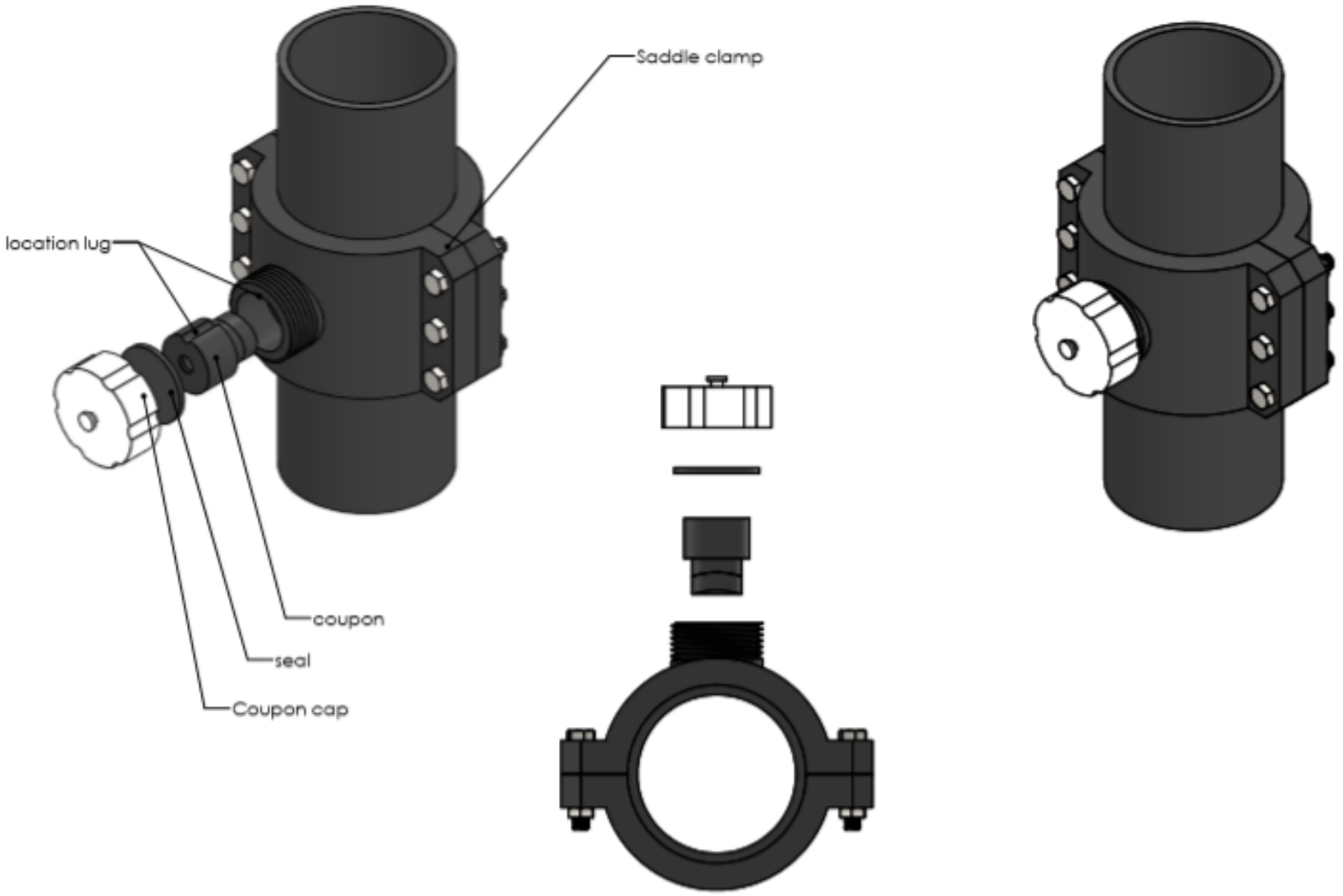


Figure 43 Coupon and saddle clamp fitting

5.2.1.1.1 Investigations into Coupon Surface Roughness and Insert Fit

Section 5.2.1.1 described the importance of biofilm collection devices being directly inserted and closely aligned with the internal pipe surface. The purpose of this alignment is to minimise the distortion of boundary layer conditions that influence biofilm formation, such as boundary shear stress and turbulent driven exchange with the bulk water body, so as to ensure biofilm formation is accurate to the formation in real drinking water networks (Douterelo, et al., 2013). The smoothness of the coupon surface is also of great importance to ensure they are as flat as possible to allow for microscopic analysis.

To achieve an acceptable level of smoothness and surface fit and finish to match the internal pipe wall, the coupons took several redesigns, as can be seen by Figure 44. In these images, Coupon Sample 1 was machine finished, which resulted in deep grooves and a much greater surface roughness than Pipe Section Sample 1 and 2. If the coupons had been used with these grooves it would have impacted the accumulation of particles and biofilm development, as Alnnasouri et al. (2011) found, surface irregularities introduced on a biofilm bed improved adhesion. In an attempt to remove these grooves, heat treatment was applied to produce Coupon Sample 2. Although Coupon Sample 2 had fewer clear grooves, they were still present, unlike in Coupon Sample 3, where the desired surface roughness was achieved.

These investigations into different materials and cutting methods were undertaken using light microscopy, a Nikon SMZ1500 stereoscopic zoom microscope, a Nikon DXM1200F Digital Camera and Eclipsenet Software with the settings: Shutter Speed- 1/7s, Sharpness- 3, Live Resolution- 640x480, Exposure- Focus AE and Compensation- 0.3EV. The light microscopy allowed for a comparison of the surfaces between the coupons and the pipe with a quick turnaround time for decision making purposes to drive the next step of the coupon design. These images were then complimented at a later date with SEM (Scanning Electron Microscope) images that had a longer turnaround time but offered a greater depth of detail, as it has a much wider range of magnification from 100×-100,000×, compared to the 3.75x-540x magnification of the light microscope used. The SEM used was a Tescan Vega 3 LMU Scanning Electron Microscope, following the standardised method of adding the sample to the vacuum chamber of the microscope and using a fine beam of high energy electrons to conduct topographic examination. The samples were additionally coated using Edwards S150B Gold Sputter Coater to improve the imaging of the samples, creating a conductive layer of metal on the sample to inhibit charging, reduce thermal damage and improve the secondary electron signal.

After the ideal coupon surface was achieved, which matched the surrounding pipe surface finish, further test coupons were produced to ensure this quality remained the same with the addition of the insert cut into the coupon. It was found, as can be seen in Figure 44, that the surface of Coupon Sample 4 still had the desired smoothness, but the insert did not fit snuggly enough and had excess movement, meaning that there could have potentially been additional microbiological growth around the inserts, and they could have dropped off within the pipe loops. In Coupon Sample 5, the fit was a lot more snug and the gap between the insert and the coupon reduced to an acceptable amount.

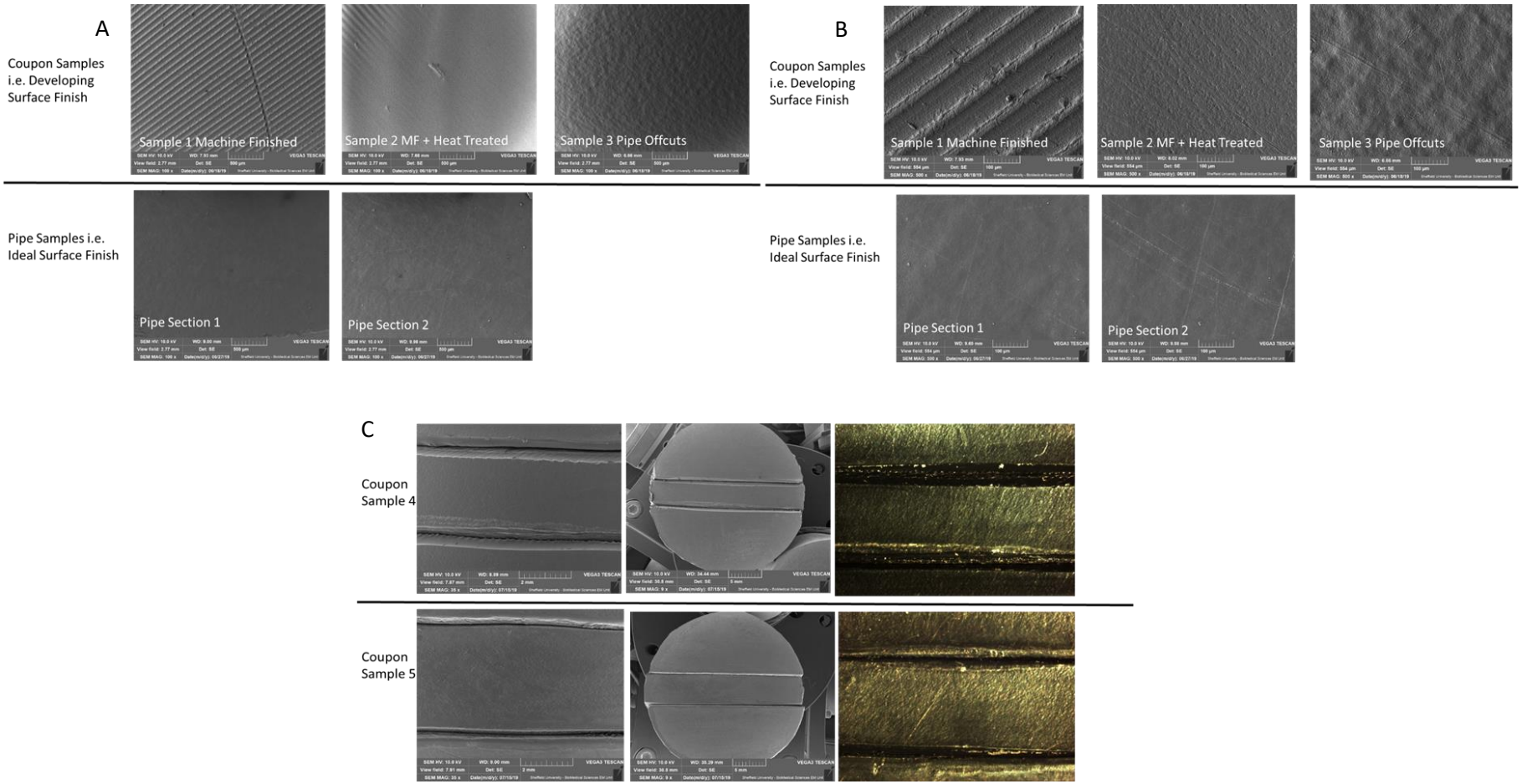


Figure 44 Coupon redesign for smoothness and fit

SEM imagery showing refinements in coupon design (Coupon Sample 1-5) to achieve better smoothness and fit as compared with internal pipe sections (Pipe section 1 and Pipe Section 2). Image A: Coupon Surface Finish Comparison with SEM 100x mag. Image B: Coupon Surface Finish Comparison with SEM 300x mag. Image C Insert Fit Comparison with SEM 35x and 8x mag.

5.2.1.2 Experimental Operation

5.2.1.2.1 Initial Disinfection

All components of the experimental pipe testing facility were disinfected prior to use to best imitate the "idealised" freshly laid pipe.

The coupons were autoclaved for 15 minutes in an Avidity Science System DX200 at a temperature of 120^{o}C, with an actual chamber temperature of 123.7^{o}C and a pressure of 217 KPa/~2.1 bar. These settings were determined to ensure there was a balance between disinfecting the coupons while also not distorting or melting the plastic, as the melting point of HDPE is ~130.8°C and it can only withstand high temperatures of 120°C for short periods (Thakare, et al., 2015). A laboratory study by Oyawale and Olaoye (2007) assessed the effectivity of autoclaves with different settings, finding that operating conditions of 120^{o}C and 1.25 bar for 12 minutes resulted in the total deactivation of all microorganisms present in a mixed culture.

After the autoclaved coupons were inserted into the experimental test facility, the pipe loops themselves were disinfected using a method successfully demonstrated in a number of experiments for the study of microbially mediated processes (Douterelo, et al., 2013) (Sharpe, 2012). 20 mg/l Rodolite-H (RODOL Ltd, Liverpool, UK) and a sodium hypochlorite solution (<16% free chlorine) were the disinfectants added. This solution was circulated through each of the closed loop environments of the testing facilities for a 1 hour period using the flushing pump, a Grundfos CRIE15-03 N-CA-A-E-HQQE. After this flushing period, the solution was circulated within the systems for 24 hours at ~0.7 l/s, the flow for normal growth phase operation, using the normal growth phase operational pump, the Grundfos CRIE1-6. Following the 24 hour period, the rigs were again flushed for a 1 hour period using the flushing pump and then were drained and refilled with fresh water entering the Inlet until a baseline chlorine residual was met.

5.2.1.2.2 Growth Phase Operation

For the normal growth phase operation of the pipe facilities, each of the four independent pipe systems recirculated the water within them for a target water age of 3 days before water left via a trickle feed drain. The outgoing water was compensated for utilising a float and a ball valve within enclosed reservoir tanks, which allowed fresh water from the WTW to enter at the Inlet.

Under normal growth phase operation, the flow in the pipe testing facilities was ~43 l/m or 0.7 l/s using a Grundfos CRIE1-6 0.55 kW duty booster pump, as can be seen by Figure 45. This flow was maintained throughout the 3 month growth phase period. The only exception to this was the instances of coupon removal, every 1.5 months, where coupons were removed (in triplicate) by stopping the pump and closing the valves, to limit the water loss from the rig during the removal.

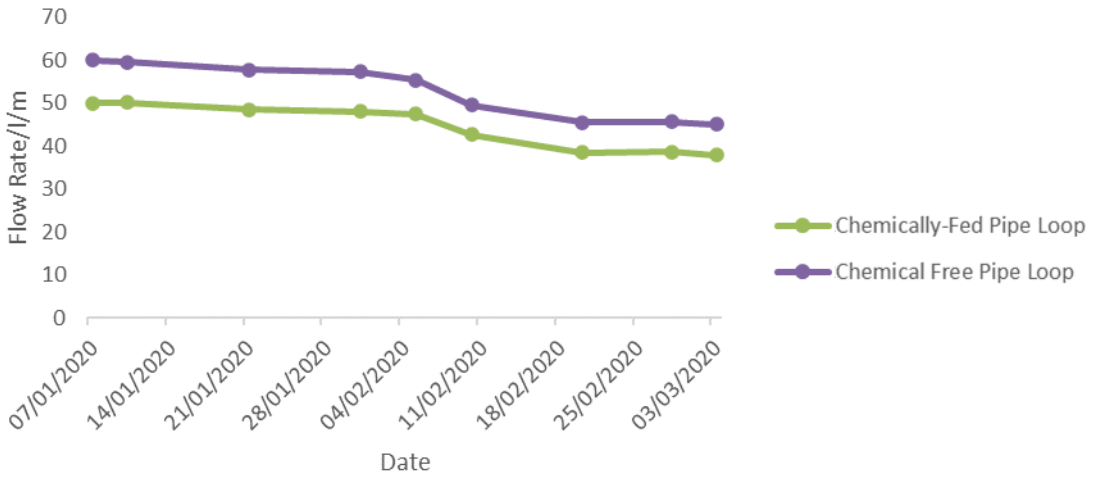


Figure 45 Growth phase flow rate/l/m at the Groundwater Site

Online instrumentation was used to ensure the testing facility received an adequate feed of water and to allow the desired flow rate to be set. This included flow meters, an Endress & Hauser Turbimax CUE21 Online Turbidity Meter, an Endress & Hauser Picomag Electromagnetic Flow Meter and a IFM SM4000 Magnetic-Inductive Bleed Flow Meter, on the tank Inlet, pump and main pipe, as well as a pressure transmitter, an IFM PT201 PN 2093 Pressure Transmitter, on the main pipe, as can be seen in Table 13.

Table 13 Online instrumentation to ensure adequate pipe facility function

Instrument	Parameter	Measurement Range	Resolution
Endress & Hauser Turbimax CUE21 Online Turbidity Meter	Turbidity	0-1000 NTU	0.0001 NTU
Endress & Hauser Picomag Electromagnetic Flow Meter	Volume Flow	0-400 l/m	±2%
	Totaliser (sum of positive and negative flow values to date)	$-9.9 \cdot 10^9$ $-9.9 \cdot 10^9$	±2%
	Temperature	–10 to +60°C	±2.5°C
IFM SM4000 Magnetic-Inductive Bleed Flow Meter	Flow	0-3 l/s	± (2% MW + 0.5% MEW)
IFM PT201 PN 2093 Pressure Transmitter	Pressure	0-5 bar	±0.4

An additional component of the pipe testing facility was a clear section of pipe to visualise the material building up over time. Though not a quantitative measure during the growth phase, this pipe section provided useful imagery for stakeholder engagement.

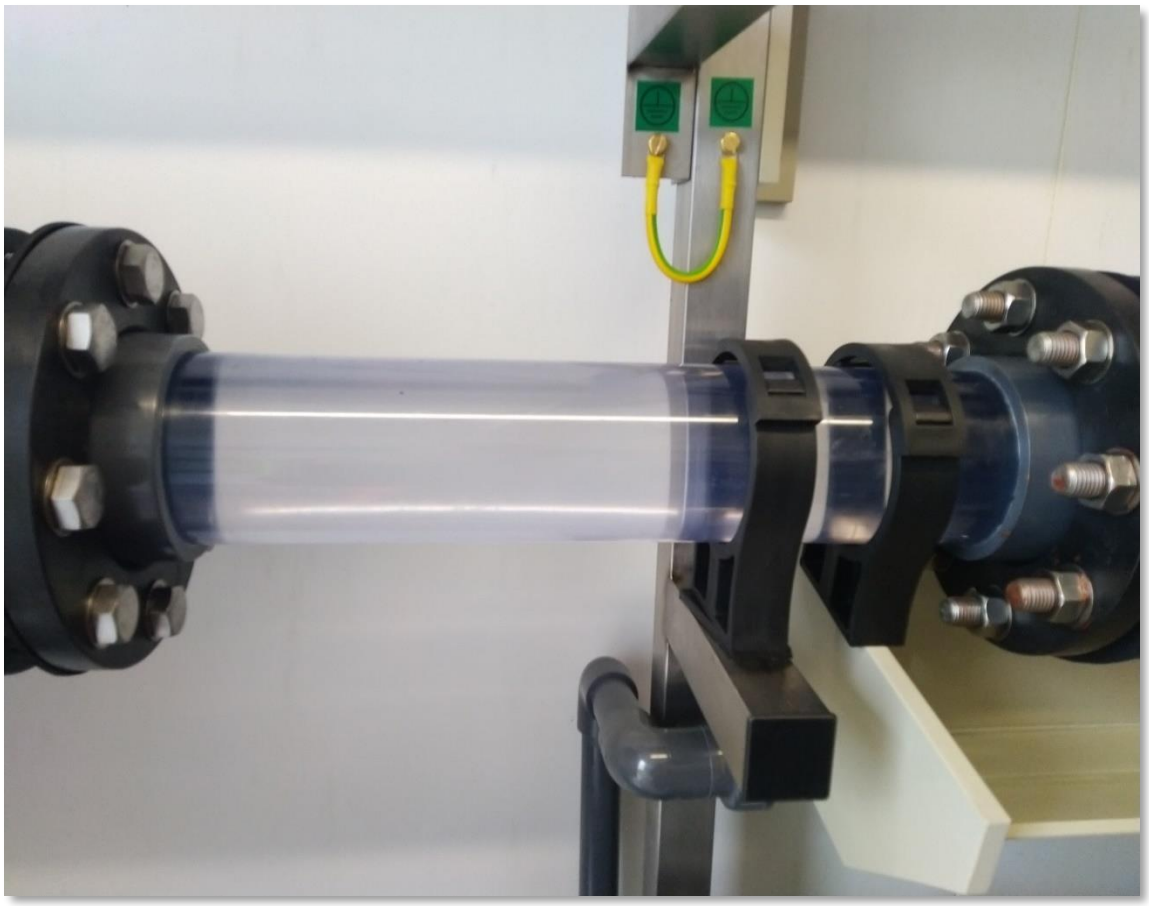


Figure 46 Pipe experimental facility clear pipe section

5.2.2 Water Quality Analysis and Sampling

Water quality samples were taken throughout the growth phase. Section 1.4 described how the physical and chemical properties of water, such as: turbidity, temperature, conductivity and pH and the chemical contaminants within it, including ammonia, nitrate, nitrite, chloride, sulphate, natural organic matter and metals, can all have an impact on the network and on the water quality within it. As such, the water quality analysis of the experimental rig facility included these parameters to get an understanding of how they interact with each other and vary temporally and spatially throughout a network and between systems, such as those with and without chemicals or those with and without good biological stability. These parameters were also utilised to determine the differences in the bulk water during the growth phase over time to see how the "idealised" length of pipe fouls. These water quality samples also allowed for a comparison between samples taken within the experimental rigs, at the WTW and at the Customer Tap to determine if the testing facilities were representative of the real network.

The water quality analysis during the experiment consisted of in-series bulk water analysis using online instrumentation, bulk water samples that were manually taken in triplicate and analysed in a laboratory and biofilm samples using coupons that were manually taken in triplicate and analysed in a laboratory. Analysis was completed in the manner described in Section 3.2.6.

5.2.2.1 In-Series Bulk Water Analysis

Throughout the experimentation the test loop facility was fitted with online instrumentation to provide a real time continuous measurement of important water quality parameters. All equipment was calibrated and maintained in accordance with the manufacturers' guidelines. The different online instruments installed are summarised in Table 14.

In-series water quality analysis was completed with an ATI MetriNet, a water quality monitor for potable water distribution monitoring. The unit consists of a controller (Q52) and 8 sensing nodes (M-Node or Q32) installed in modular flow chambers, with each node measuring a different parameter [Figure 47].

Table 14 Online instrumentation within the experimental test facility

Instrument	Parameter	Measurement Range	Resolution
ATi MetriNet 00-1733 Free Chlorine M-Node	Free Chlorine	0-4.00 ppm	0.01 ppm
ATi MetriNet 00-1758 Combined Chlorine M-Node	Combined Chlorine	0-4.00 ppm	0.01 ppm
ATi MetriNet 00-1780 Total Chlorine M-Node	Total Chlorine	0-4.00 ppm	0.01 ppm
ATi MetriNet 00-1739 Turbidity M-Node	Turbidity	0-40.00 NTU	0.01 NTU
ATi MetriNet 00-1736 ORP M-Node	ORP	0-1000 mv	1 mv
ATi MetriNet 00-1737 Dissolved Oxygen M-Node	Dissolved Oxygen	0-20.00 ppm	0.01 ppm
ATi MetriNet Pressure M-Node	Pressure	0-150 PSI	1 PSI G
Endress & Hauser Turbimax CUE21 Online Turbidity Meter	Turbidity	0-1000 NTU	0.0001 NTU
Endress & Hauser Picomag Electromagnetic Flow Meter	Volume Flow	0 to 400 l/m	±2 %
	Totaliser	$-9.9 \cdot 10^9$ - $9.9 \cdot 10^9$	±2 %
	Temperature	–10 to +60°C	±2.5°C
IFM SM4000 Magnetic-Inductive Bleed Flow Meter	Flow	0-3l/s	± (2 % MW + 0,5 % MEW)
IFM PT201 PN 2093 Pressure Transmitter	Pressure	0-5 bar	± 0,4

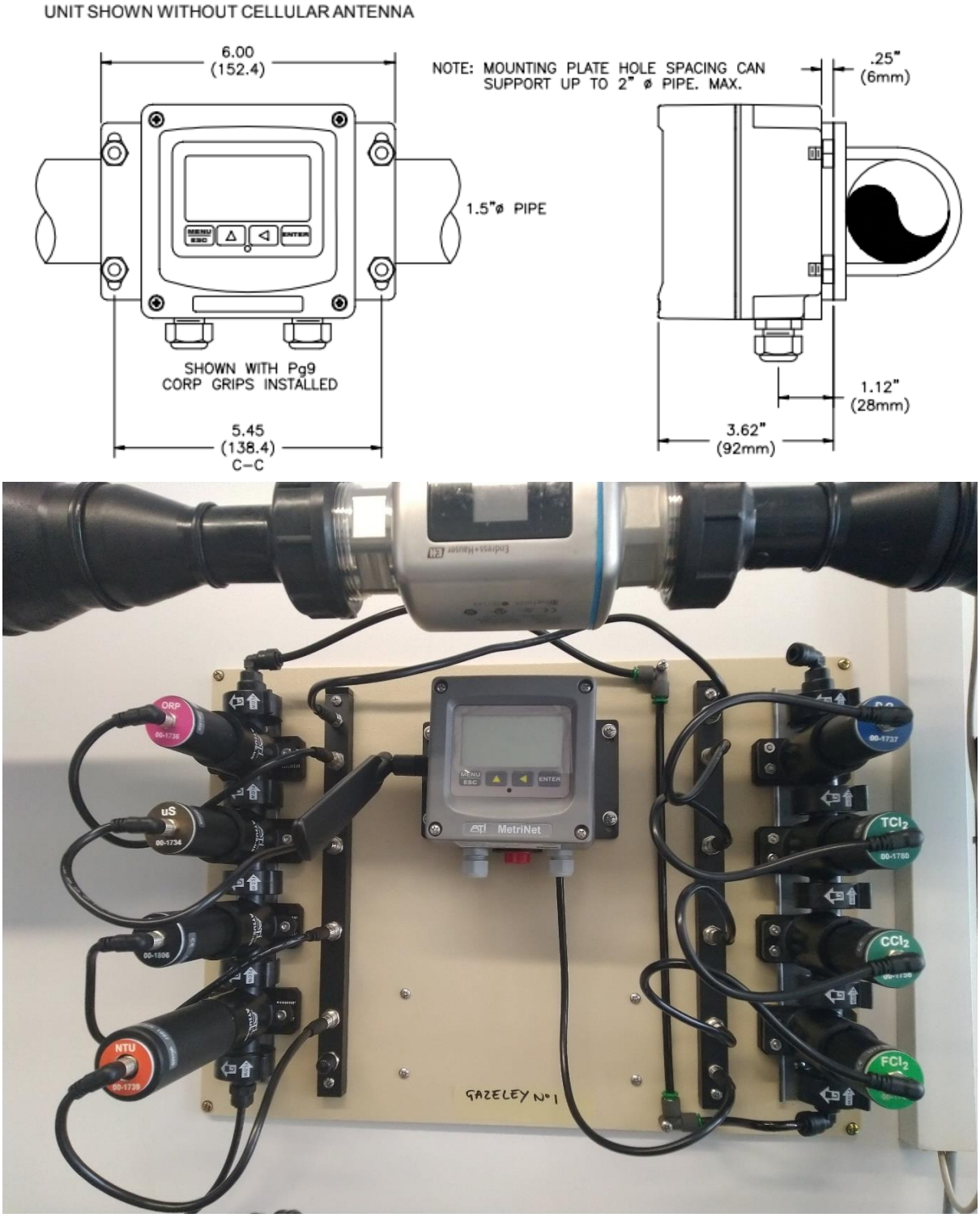


Figure 47 ATI MetriNet for in-series water quality monitoring within the experimental test facility

5.2.2.2 Bulk Water Samples

During the Growth Phase, bulk water quality samples were taken every two weeks from Rig 1 Outlet and Rig 2 Outlet of the experimental test facilities of each of the two sites, as Figure 48 depicts. These samples were complimented with monthly samples from the Rig 1 Inlet and the Rig 2 Inlet of each of the facilities, the Final Water of the respective WTW and at the Customer Tap within the two networks fed by the studied WTW. All samples were taken in triplicate to improve the robustness of the data.

The water quality parameters used for bulk water sampling, as well as the methods of their analysis and the resolution of these methods can be seen in Table 15. It was also intended to utilise DNA analysis, as outlined in the protocols in Appendix 1 but this was not completed as part of the present experiment.

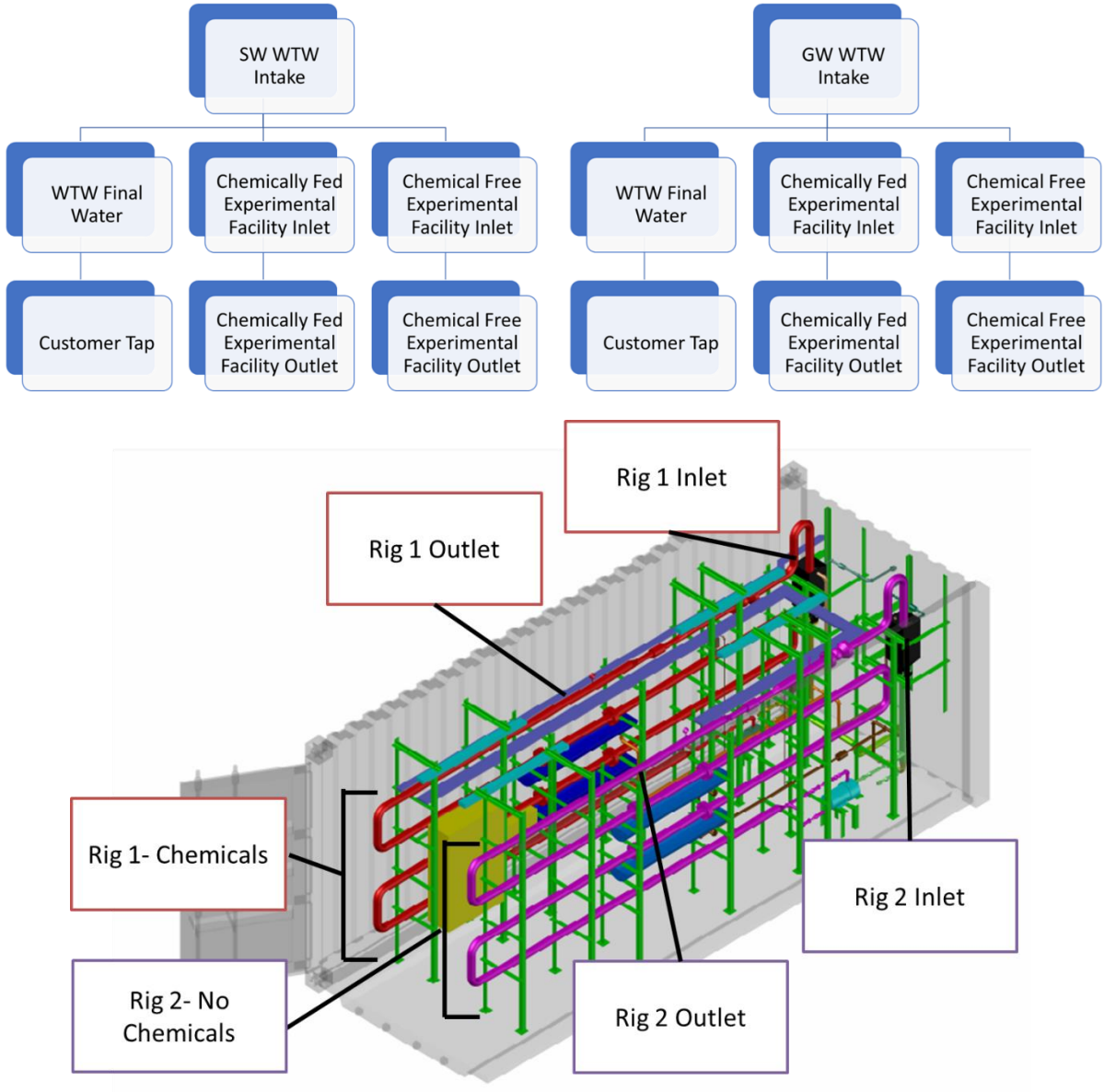


Figure 48 Pipe testing facility sampling locations

Table 15 Bulk water quality samples during the operation of the experimental testing facility

Parameter	Unit	Sampling Method	Analysis Method / Instrumentation Used	Measurement Range / Limit of Detection
2 Day Colony Count (37°c), 3 Day Colony Count (22°c)	no/ml	FL		
Alkalinity	mg/l	FL		
Ammonia	mg/l	FL		
Biological Dissolved Organic Carbon	mg/l	FL		
Chloride	mg/l	FL		
Clostridium perfringens, Enterococci, E. coli, coliforms	no/100ml	FL		
Combined Chlorine	mg/l	OI	ATi MetriNet 00-1758 Combined Chlorine M-Node	0-4.00 ppm
Conductivity	µS/cm	OI		
Dissolved Organic Carbon	mg/l	FL		
Dissolved Oxygen	Ppm	OI	ATi MetriNet 00-1737 Dissolved Oxygen M-Node	0-20.00 ppm
Magnesium	mg/l	FL		
Nitrite, Nitrate	mg/l	FL		
Oxidation-Reduction Potential	mV	OI	ATi MetriNet 00-1736 ORP M-Node	0-1000 mv
pH	pH	FL		
Pressure	PSI, bar	OI	ATi MetriNet Pressure M-Node, IFM PT201 PN 2093 Transmitter	0-150 PSI, 0-5 bar
Sodium	mg/l	FL		
Sulphate	mg/l	FL		
Temperature	°C	OI	Endress & Hauser Picomag Electromagnetic Flow Meter	–10 to +60°C
Total Cell Count, Intact Cell Count	no/100µl	FL		
Total Chlorine, Free Chlorine	mg/l	HH, OI	ATi MetriNet 00-1780/00-1733 Total/Free Chlorine M-Node	0-4.00 ppm
Total Iron, Filtered Iron	mg/l	FL		
Total Lead, Filtered Lead	µg/l	FL		
Total Manganese, Filtered Manganese	mg/l	FL		
Total Organic Carbon	mg/l	FL		
Total Organic Nitrogen	mg/l	FL		
Total Phosphorus	mg/l	FL		
Turbidity	NTU	OI, FL	ATi MetriNet 00-1739 Turbidity M-Node, Endress & Hauser Turbimax CUE21 Online Turbidity Meter	0-40.00 NTU

HH denotes handheld equipment, OI online instrumentation, FL sample taken in field an analysed in the laboratory.

5.2.2.3 Biofilm Samples

Coupon inserts from each of the four test pipe facilities were sampled at month 0 and 1.5 so that n=2 for TCC and ICC using flow cytometry (the initial proposed sample numbers can be found in Appendix 1.3). Although Deines et al. (2010) found that there was no significant difference in biofilm growth between coupons sampled in triplicate, triplicate samples were still taken to improve the robustness of the cell count data.

In preparation for coupon removal, a mobile laboratory was set up to provide as sterile an environment as possible within the pipe testing facility, consisting of a table, a surgical tray and coupon removal tools, all disinfected with a sodium hypochlorite solution. Coupon removal tools included a tweezer-like implement disinfected with a sodium hypochlorite solution, a biofilm brush that was a toothbrush-like tool that had been autoclaved for 15 minutes in an Avidity Science System DX200 at 120^{o}C and 217 KPa and phosphate buffer solution that had also been autoclaved for 15 minutes in an Avidity Science System DX200 at 120^{o}C and 217 KPa.

To remove the coupons from the facility, the rig was powered off, temporarily stopping flow. The coupon cap was removed and the coupon within the facility was exchanged immediately with a fresh autoclaved coupon, after which the coupon cap was replaced to prevent further losses from the system. The coupon insert was then removed using a coupon removal tool, a tweezer-like implement. The biofilm was extracted from the coupon insert by brushing the biofilm from the insert surface and into 30 ml of sterile phosphate buffer solution using the sterile biofilm brush. To do this brushing, a standardised motion and number of strokes were used (30 horizontal and 30 vertical, rinsing the brush in the solution after every 10 strokes) (Fish, 2013). This biofilm solution was then deposited into a sample bottle containing 0.35 ml of 1.8% sodium thiosulphate and placed into a sample fridge (a Campos 25 l 12 V Electric Coolbox capable of lowering temperature to 10-15^{o}C below ambient temperature) until arrival at the laboratory.

Biofilm analysis consisted of TCC and ICC using flow cytometry. It was also intended to utilise SEM Microscopy and DNA analysis, as outlined in the protocols in Appendix 1 but this was not completed as part of the present experiment.

5.2.3 Methods Summary: The Wider Impact of Chemicals in an Experimental Facility

The below figure summarises the bulk water and biofilm samples analysed from January to March 2020, as explained throughout this chapter.

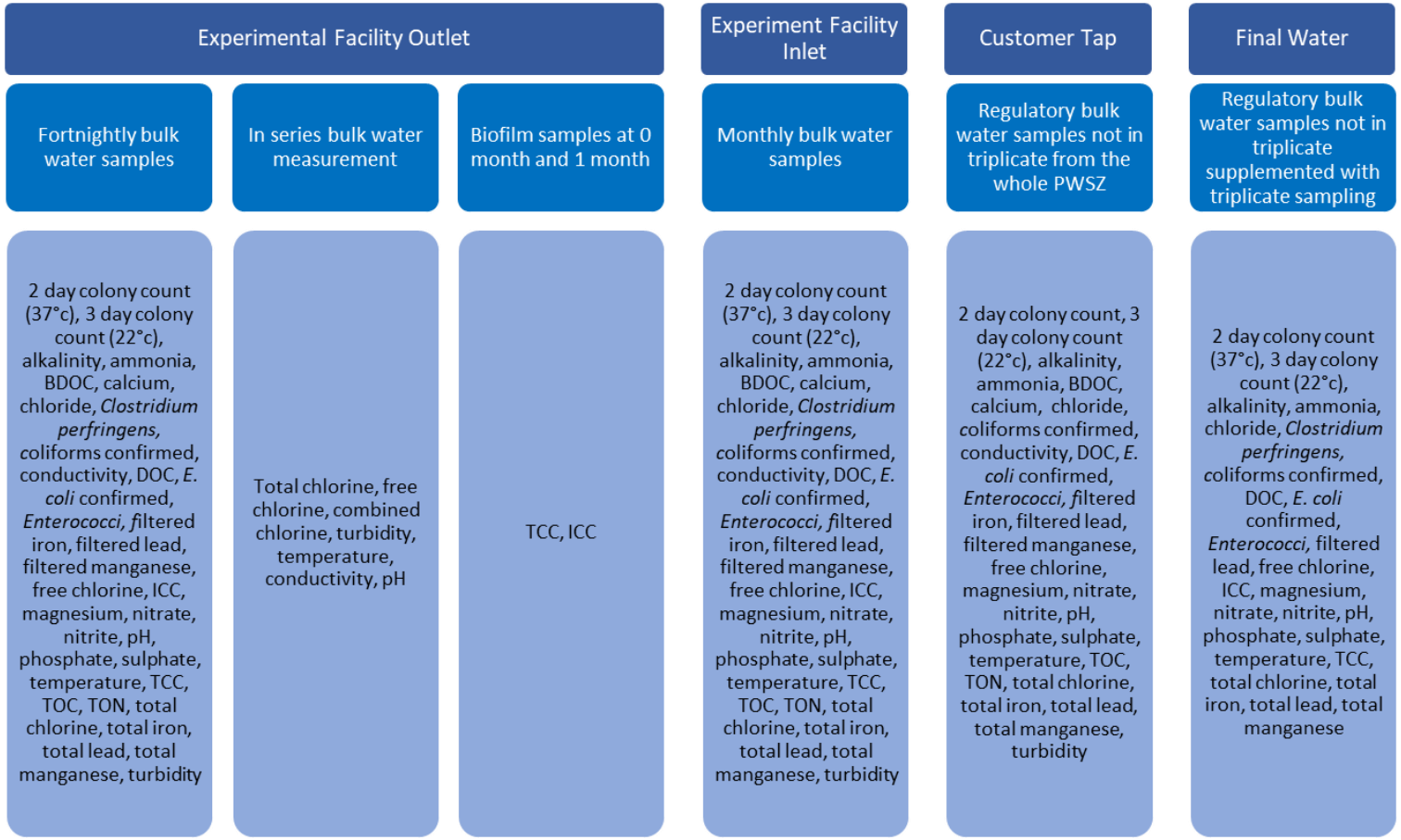


Figure 49 Summary of experimental facility samples

5.3 Results: The Wider Impact of Chemicals in an Experimental Facility

The present section compares the sampling locations detailed in Figure 48 to explore how biological stability and chemicals influenced water quality within a series of innovative bespoke pipe test loops.

5.3.1 Were the Experimental Facilities Representative of the Live Network and Functioned as Anticipated?

A comparison of the sampling locations described in Figure 48 found the experimental facilities were relatively representative of the live network.

Table 16 details specific parameters which did or did not have a statistical difference between the two sampling locations. For example, while 3 day colony count, free chlorine, ICC, TCC and total chlorine did have a statistically significant difference from the Experimental Inlet to the Final Water, more parameters did not (including ammonia, *C. perfringens*, DOC, *E. coli*, *Enterococci*, nitrate, sulphate, TOC and TON). Figure 50 details examples of the live Final Water and the Experimental Facility Inlets having similar water quality.

Table 16 Parameters without a statistically significant difference between the Final Water and Experimental Inlets

Parameter	2 Day Colony Count	3 Day Colony Count	Alkalinity	Ammonia	*C. perfringens*	Chloride	DOC	*E. coli*	*Enterococci*	Free Chlorine	ICC	Magnesium	Nitrate	Nitrite	Orthophosphate	pH	Sulphate	TCC	TOC	TON	Total Chlorine	Total Iron	Total Manganese	Number of Parameters without a Statistically Significant Difference
SW R1 Inlet vs SW Final	5.73E-04	8.83E-15	3.06E-02	NA	NA	0.9354	2.34E-01	NA	NA	5.12E-05	3.00E-05	5.48E-01	0.8771	0.00E+00	3.27E-01	0.01894	4.22E-01	1.19E-06	3.62E-01	8.63E-01	< 2.2e-16	3.51E-03	NA	14/23
SW R2 Inlet vs SW Final	1.23E-01	8.82E-15	7.72E-03	NA	NA	0.6064	0.9709	NA	NA	5.10E-05	< 2.2e-16	0.6973	0.7572	0.00E+00	1.22E-04	0.003394	5.73E-01	1.19E-06	0.146	0.587	5.04E-05	NA	NA	15/23
GW R1 Inlet vs SW Final	0.07724	0.005317	1.41E-01	NA	NA	0.000551	0.1013	NA	NA	4.27E-02	4.11E-05	1.08E-05	8.41E-02	NA	0.7484	3.75E-01	0.4127	9.59E-10	0.8437	8.49E-02	4.46E-02	NA	NA	16/23
GW R2 Inlet vs SW Final	0.07724	0.000161	3.41E-05	NA	NA	9.93E-06	0.05659	NA	NA	4.27E-02	4.11E-05	1.11E-05	6.90E-01	7.69E-02	1.60E-04	9.29E-01	2.56E-01	4.11E-05	0.1081	0.6585	4.42E-02	0.001836	2.96E-03	12/23

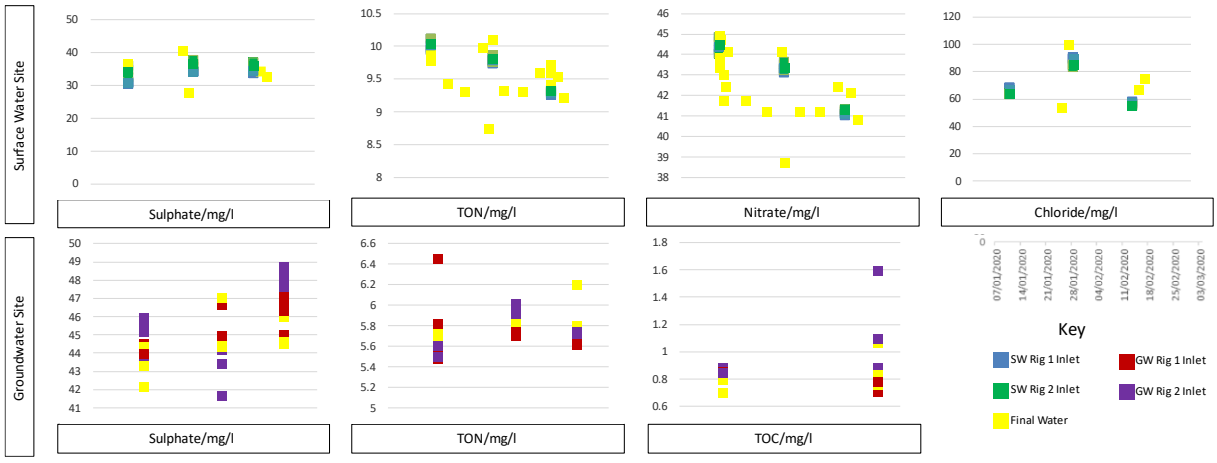


Figure 50 Final Water samples with similar water quality values to Experimental Facility Inlets

SW denotes Surface Water, GW Groundwater, R1 Chemically Fed Rig, R2 Chemical Free Rig.

A further example of where experimental facilities were relatively representative of the live network could be found when comparing the live Customer Tap and the Experimental Facility Outlet, as shown in Figure 51. Table 17 details specific parameters which did or did not have a statistical difference between the two sampling locations.

Table 17 Parameters without a statistically significant difference between the Customer Tap and Experimental Outlets

Parameter	3 Day Colony Count	Ammonia	Chloride	*E. coli*	*Enterococci*	Free Chlorine	ICC	Magnesium	Nitrate	Nitrite	Orthophosphate	pH	Sulphate	TCC	TOC	TON	Total Chlorine	Total Iron	Total Manganese	Turbitity	Total Lead	Filtered Lead	Calcium	Number of Parameters without a Statistically Significant Difference
SW R1 Outlet vs SW Tap	0.001143	0.009643		NA	NA	7.10E-02			0.02689	4.72E-01	7.28E-03	1.21E-03				2.49E-03	2.96E-06	1.93E-01	0.253	1.32E-04	0.000145			6/15
SW R2 Outlet vs SW Tap	0.00389			NA	NA	1.74E-07		0.2311	0.027	3.92E-01	4.35E-06	1.20E-03				2.74E-02	1.73E-07	7.13E-01		NA			5.00E-01	7/14
GW R1 Outlet vs SW Tap	0.00389	2.56E-04	0.172	NA	NA	9.33E-01	1.50E-03	1.28E-01		4.33E-01	6.02E-01	1.21E-03	0.4154	7.85E-02	0.01125	0.3271	0.9731	3.67E-01		NA	2.10E-06	8.69E-03		13/20
GW R2 Outlet vs SW Tap	0.000583	2.56E-04	0.007697	NA	NA	< 2.2e-16	1.50E-03	1.93E-01		1.64E-01	3.33E-07	2.90E-02	0.6504	1.05E-02	4.31E-02	0.7333	< 2.2e-16	1.22E-02		9.98E-07	9.74E-05	7.00E-03		6/20

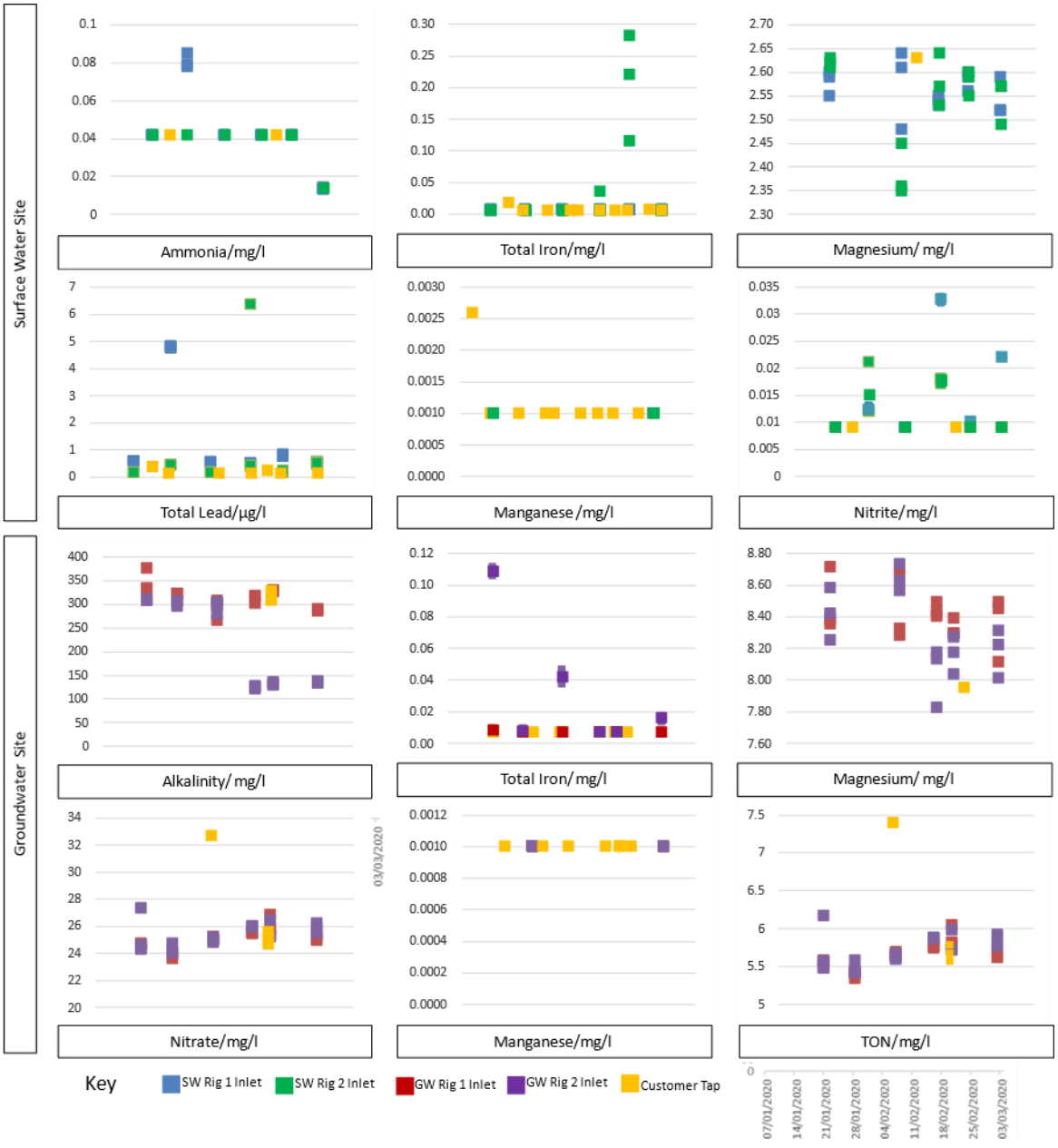


Figure 51 Customer Tap samples with similar water quality values to Experimental Facility Outlets

In fact, throughout the experimental period, there were a number of parameters that did not change over time or by sample location, as shown in Table 18.

Table 18 Parameters that did not change during the experimental period

Parameter	Value	Sample Location Without Parameter Change											
		SW Final	GW Final	SW Tap	GW Tap	SW Inlet R1	SW Inlet R2	GW Inlet R1	GW Inlet R2	SW Outlet R1	SW Outlet R2	GW Outlet R1	GW Outlet R2
2 Day CC[2]	0		X	NA	X								
3 Day CC[2]	0	X	X	X	X								
Ammonia	0.042	X	X		X	X	X	X					
C. perfringens[1]	0	X	X	X	X	X	X	X	X	X	X	X	X
E. coli[1]	0	X	X	X	X	X	X	X	X	X	X	X	X
Enterococci[1]	0	X	X	X	X	X	X	X	X	X	X	X	X
F. Iron	0.001	NA	NA	NA	NA	X	X	X		X	X	X	
F. Manganese	0.001	NA	NA	NA	NA	X	X	X		X		X	X
Free Chlorine	0.05						X		X		X		X
Nitrite	0.009	X	X	X	X	X	X						
Orthophosphate	0.055						X		X		X		X
T. Iron	0.007	X	X		X		X	X		X		X	
T. Lead[3]	0.16	NA	X				X						
T. Manganese	0.001	X	X		X	X	X	X	X	X	X	X	
Total Chlorine	0.05						X		X		X		X

Units are mg/l except: [1] no/100ml, [2] no/ml, [3] µg/l. CC denotes colony count, T. total, F. filtered, SW Surface Water, GW Groundwater, R1 Chemically Fed Rig, R2 Chemical Free Rig, X sample did not change over sampling period, NA no samples.

The experimental facilities were found to function as anticipated. For example, it was noted that the desired differences in chemical dose was achieved, both at the Experimental Facility Inlet and at the Experimental Facility Outlet, as shown in Figure 52.

Therefore, the experimental rig facility Inlets and Outlets were found to have relatively comparable water quality to the Final Water and Customer Tap respectively. They also achieved the desired differences in chemical dose and as such, functioned as anticipated.

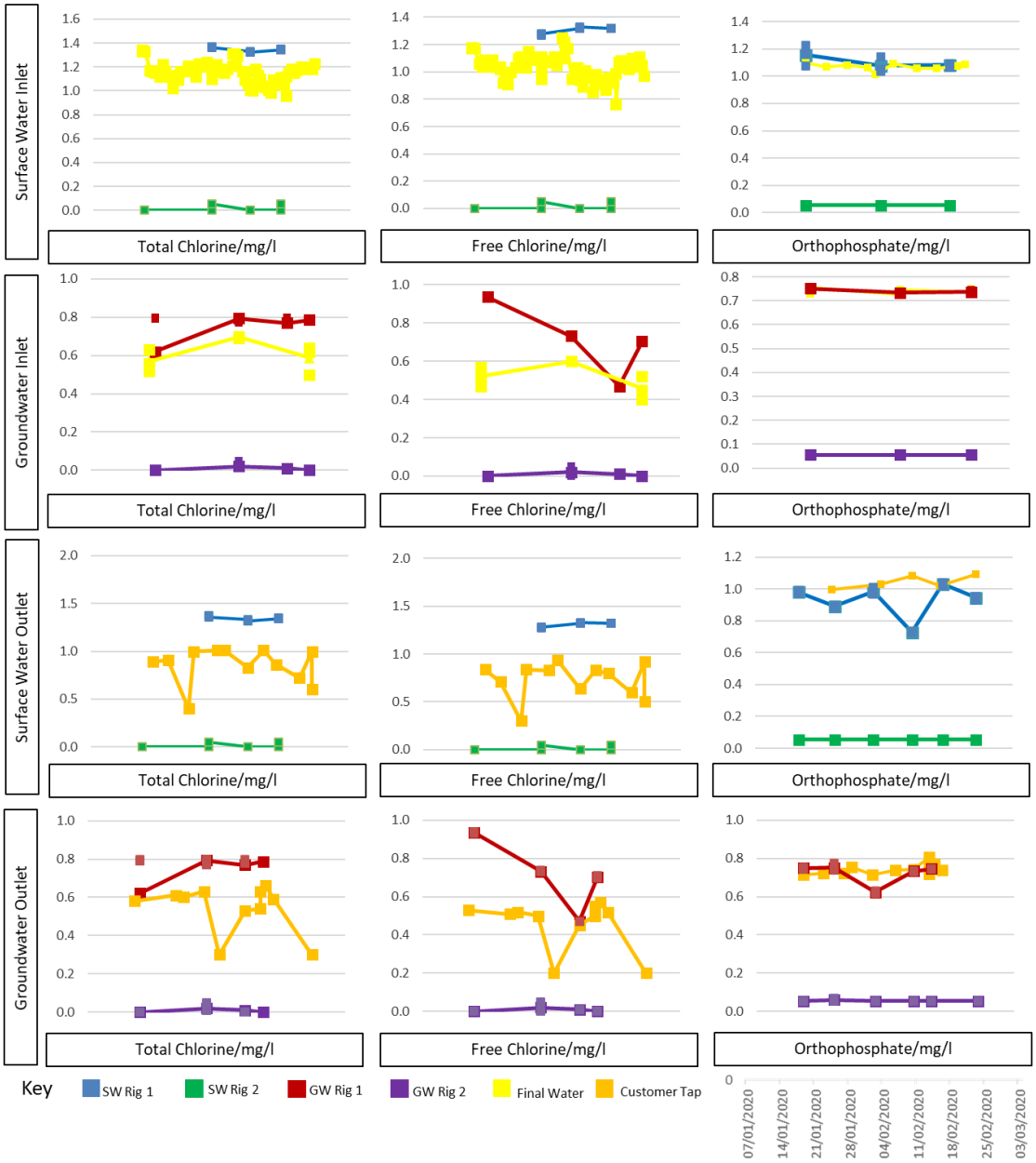


Figure 52 Chemical presence in Rig 1 and chemical absence in Rig 2

SW Surface Water, GW Groundwater, R1 Chemically Fed Rig, R2 Chemical Free Rig.

5.3.2 Did the Experimental Pipe Network Influence Water Quality?

Experimental facility Inlets were compared with experiment facility Outlets to determine if and how water quality changed within the facilities, under different experimental conditions and locations.

The universal changes that occurred in all experimental conditions from Inlet to Outlet were that the pH increased, alkalinity decreased, 2 day colony count increased, TCC increased and ICC increased, as shown in Figure 53. Although, as Table 19 notes, these changes were not always significant, with the exception of pH.

Table 19 Parameters without a statistically significant difference between the Experimental Inlets and Outlets

Parameter	2 Day Colony Count	3 Day Colony Count	Alkalinity	Ammonia	*C. perfringens*	Chloride	DOC	*E. coli*	*Enterococci*	Filtered Iron	Filtered Lead	Free Chlorine	ICC	Magnesium	Manganese	Nitrate	Nitrite	Orthophosphate	pH	Sulphate	TCC	TOC	TON	Total Chlorine	Total Iron	Total Lead	Number of Parameters without a Statistically Significant Difference
SW R1 Inlet vs SW R1 Outlet	0.02442	0.376	1.23E-02	8.14E-01	NA	0.01019	0.1482	NA	NA	NA	3.95E-02	1.15E-01	2.53E-01	6.96E-01	NA	9.79E-01	1.34E-01	1.62E-14	6.23E-05	7.38E-01	1	0.1696	0.2467	0.203	6.57E-02	7.49E-04	6/15
SW R2 Inlet vs SW R2 Outlet	0.544	1	2.84E-02	2.20E-01	NA	0.4245	0.5765	NA	NA	NA	1.30E-02	6.94E-03	7.05E-01	7.89E-01	2.20E-01	8.77E-01	4.66E-07	4.24E-07	2.47E-07	0.8485	1.00E+00	0.01984	9.38E-01	0.00021	0.3358	7.57E-02	7/14
GW R1 Inlet vs GW R1 Outlet	0.005544	0.0356	2.69E-02	NA	NA	0.625	0.1657	NA	NA	NA	4.56E-01	1.89E-01	2.08E-03	1.98E-01	2.20E-01	5.03E-01	6.10E-02	NA	6.07E-05	0.1419	4.66E-04	0.1005	0.4707	1.89E-01	0.1301	0.102	13/20
GW R2 Inlet vs GW R2 Outlet	0.4542	0.07174	8.17E-01	2.20E-01	NA	0.9376	0.008102	NA	NA	2.25E-02	9.33E-03	1.06E-11	5.96E-01	3.21E-02	5.48E-01	7.99E-01	7.44E-01	0.5297	0.000744	5.03E-01	6.31E-01	7.43E-01	0.7678	2.65E-09	2.15E-01	0.02877	6/20

In chemically dosed facilities, there was also a decrease in phosphate and chlorine concentration from the Inlet to the Outlet, as can be seen in Table 20.

Table 20 Average chemical reduction from Inlet to Outlet

Parameter	Facility	Inlet	Outlet	% Change
Free Chlorine /mg/l	SW R1	1.30	0.368	-71.7%
	GW R1	0.73	0.61	-16.4%
Total Chlorine /mg/l	SW R1	1.36	0.404	-70.3%
	GW R1	0.80	0.67	-16.3%
Phosphate /mg/l	SW R1	1.1	0.927	-15.7%
	GW R1	0.741	0.61	-17.7%

Additional parameters that changed within the experimental facilities consisted of TON, nitrate, chloride and DOC [Figure 54]. These parameters appeared to change differently in the network depending on whether the experimental facilities were Surface Water or Groundwater fed. However, again these changes were not statistically significant [Table 19].

It was noted that microbial abundance was often a parameter that frequently increased within the experimental facilities, as shown in Figure 55. This figure also showed that even though *Enterococci*, *E. coli* and *C. perfringens* had a value of 0 no/100ml throughout the study, ICC and TCC and often 3 day and 2 day colony counts were identified. This was a finding repeatedly observed in the study period, within the experimental facilities and in the live network, including at a customer tap level.

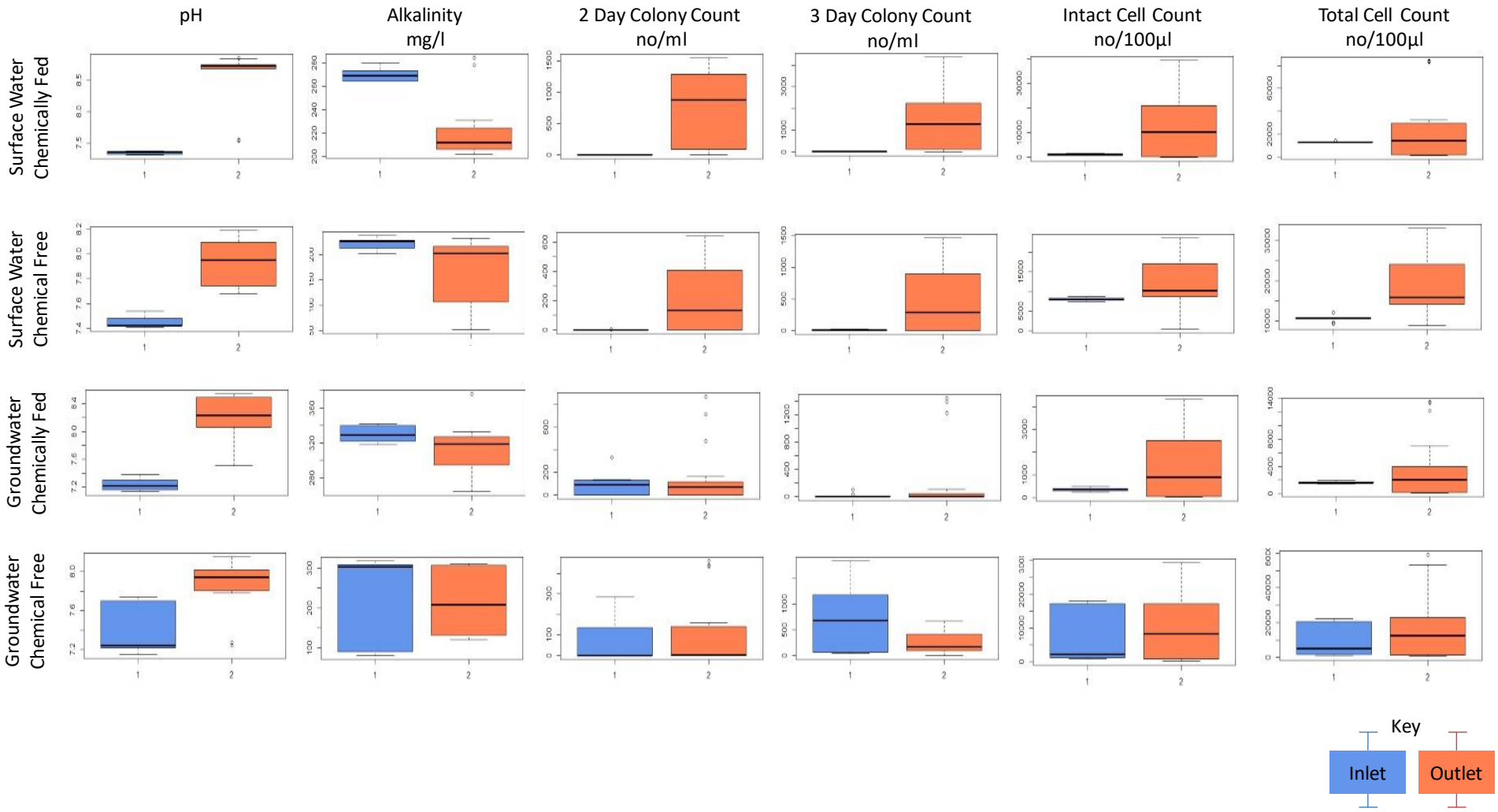


Figure 53 A comparison of experimental facility Inlet and Outlet water quality

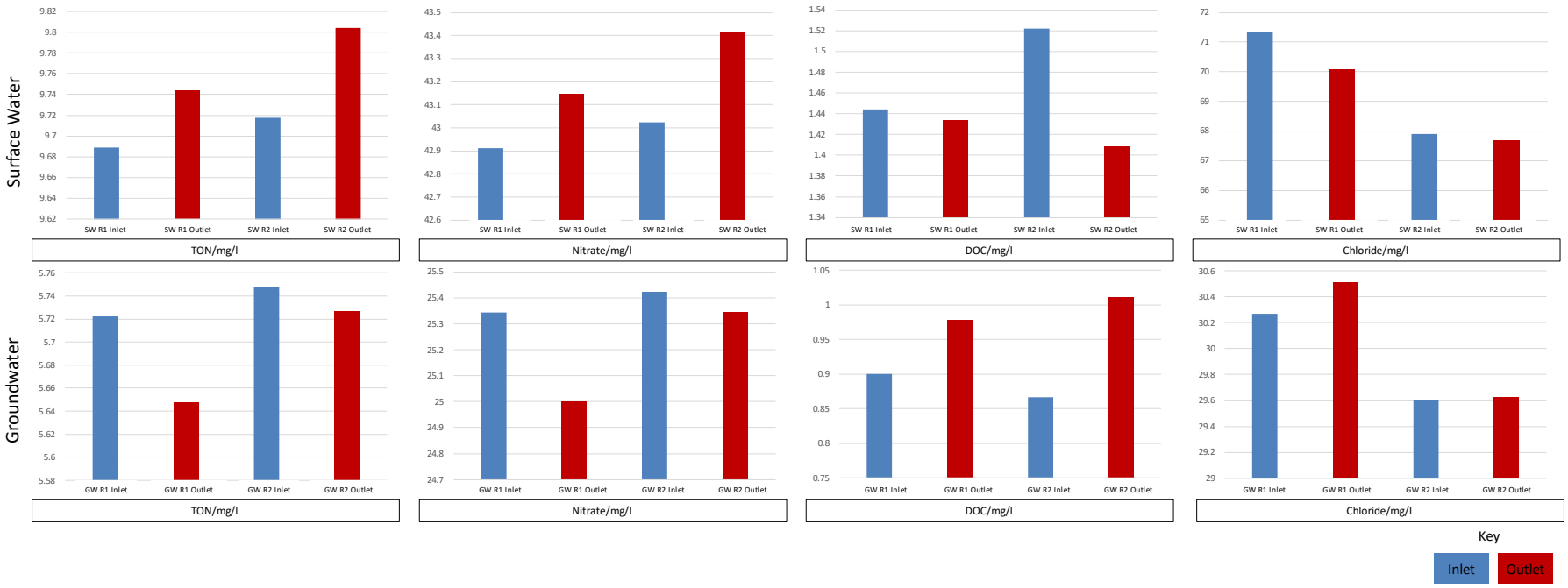


Figure 54 Parameters with different experimental facility Inlet to Outlet changes

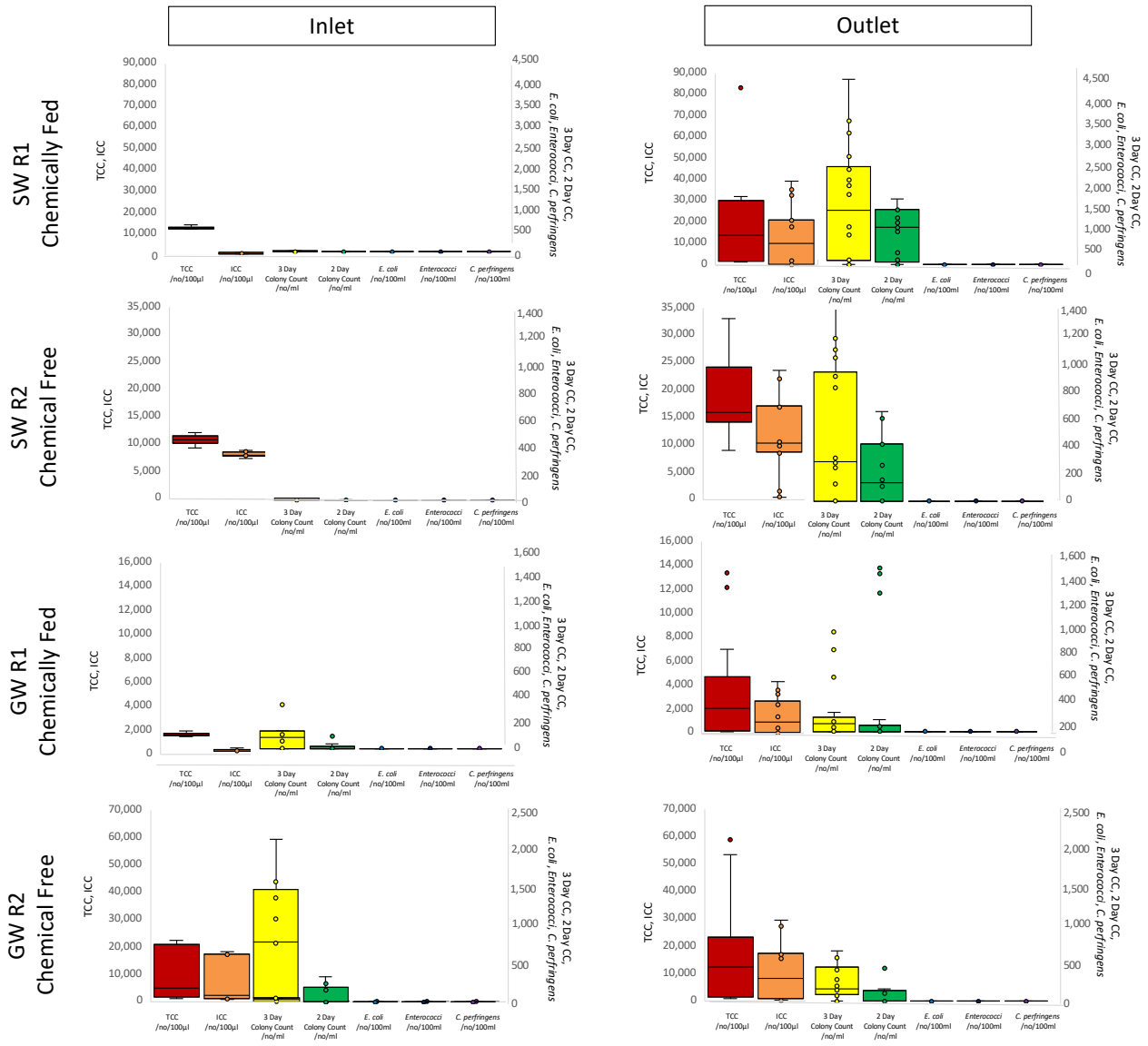


Figure 55 Microbial abundance at experimental Inlet and Outlet

CC denotes Colony Count, SW Surface Water, GW Groundwater, R1 Chemically Fed Rig, R2 Chemical Free Rig.

Another observed change in the difference between the Inlet water entering the experimental rig facilities and the Outlets to those systems was the formation of a fine scale. A dulling of the clear pipe sections [Figure 56] as well as some small visible traces of scale in Outlet samples were observed at the start of February 2020. It was observed that there was no scale in the Inlets of both experimental conditions, some traces of visible scale were captured in the Chemically Fed Rigs and large amounts of visible scale in the Chemical Free Rigs.

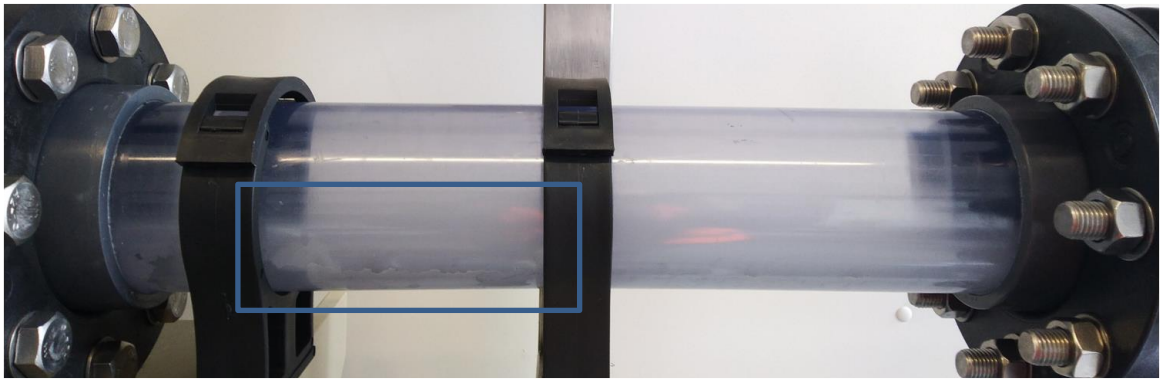


Figure 56 Photo showing the dulling of the Surface Water Chemical Free Rig clear pipe section 05/02/20

When comparing the WTW to the Customer Tap in the live network, it was found both networks had an increase in magnesium and a decrease in phosphate, free chlorine and total chlorine. As such, there were a number of parameters that had a change in the experimental network but not in the live network, as can be seen from Table 21.

Table 21 Parameters that did not change in the network during the experimental period

Parameter	Sample Location with Network Change					
	SW R1 Inlet-Outlet	SW R2 Inlet-Outlet	GW R1 Inlet-Outlet	GW R2 Inlet-Outlet	Live SW Final-Tap	Live GW Final-Tap
2 Day CC	+	+	+	+	NA	=
Alkalinity	-	-	-	-	NA	-
Chloride	-	-	+	+	NA	+
DOC	-	-	+	+	NA	NA
Free Chlorine	-	NA	-	NA	-	-
ICC	+	+	+	+	NA	-
Magnesium	+	-	+	-	+	+
Nitrate	+	+	-	-	-	+
Phosphate	-	NA	-	NA	-	-
pH	+	+	+	+	+	-
TCC	+	+	+	+	NA	+
TON	+	+	-	-	-	+
Total Chlorine	-	NA	-	NA	-	-

CC denotes colony count, SW Surface Water, GW Groundwater, R1 Chemically Fed Rig, R2 Chemical Free Rig, + increase in network, - decrease in network, NA no samples, = no change in network.

To investigate the network change further, one incidence of triplicate sample was obtained from the GW Customer Tap. Although further samples were planned from both the GW and SW Tap (further details can be found in Appendix 1.4), this sampling was sufficient to evidence the variation in sampling, even at the consumer tap [Table 22].

Table 22 The extent to which a triplicate GW Customer Tap varied

pH[1]	Nitrate	TOC	Alkalinity	Chloride	Phosphate	Sulphate	TCC[2]	ICC[2]	Free Chlorine	Total Chlorine	Total Lead[3]	Temperature[4]
7.3-7.33	24.7-25.6	1.04-1.06	309-328	35.2-36.7	0.72-0.806	42.2-45.3	72-1102	13-25	0.5-0.55	0.54-0.63	0.16-0.297	8.9-9.1
Parameters without variation: turbidity[5] (0.09), manganese (0.001), total iron (0.007), 3[6] and 2 day colony count[6] (0), *Enterococci*[7] (0), ammonia (0.042), nitrite (0.009), *E. coli*[7] (0), *C. perfingens*[7] (0)												

Units are mg/l except: [1] pH units, [2] no/100µl, [3] µg/l, [4]°C, [5] NTU, [6] no/ml, [7] no/100ml.

Overall, a comparison of experimental facility Inlets and experiment facility Outlets determined that water quality changed within the facilities, regardless of the different experimental conditions and locations. These experimental network changes, however were not often mirrored in their live network counterparts. There were also some that appeared to change differently in the network depending on whether the experimental facilities were Surface Water or Groundwater fed.

5.3.3 To What Extent Did Biological Stability Influence Water Quality Within the Experimental Test Facilities?

A comparison of the Experimental Facility Outlets identified that the greatest difference between the four facilities was depending on if the experimental facility was at the Surface Water Site or at the Groundwater Site, as evidenced by parameters in Figure 57.

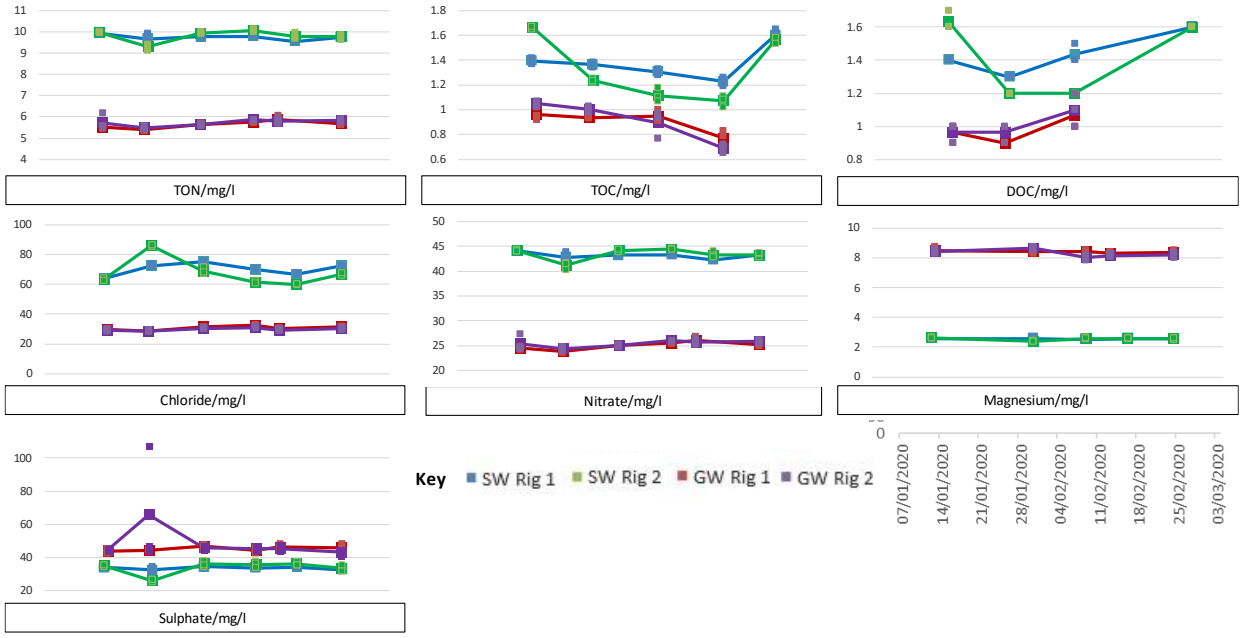


Figure 57 Experimental facility Outlet parameters with similar values depending on the site

SW denotes Surface Water, GW Groundwater, R1 Chemically Fed Rig, R2 Chemical Free Rig.

Where the Surface Water Rigs and Groundwater Rigs differed, the Surface Water Site often had higher values than the Groundwater Site.

Surface Water Site Rig 1 (the Chemically Fed Rig), in particular, had higher values, as well as some of the highest values detected at all sampling points during experimentation. This included the parameters: pH, TOC, alkalinity, chloride, orthophosphate, filtered lead, free chlorine, total chlorine, magnesium, DOC, 3 day colony count, 2 day colony count, total lead, ammonia, nitrite, TCC and ICC. The only exceptions to this, where Rig 1 did not have the highest values of the Outlets, were in: TON, sulphate, filtered manganese, total iron and nitrate.

The same was not true, however at the Groundwater Site. This site had a more even split of Rig 1 having higher values at times (e.g., pH, alkalinity, chloride, orthophosphate, filtered lead, free chlorine, total chlorine, magnesium and total lead) and Rig 2 having higher values on other occasions (e.g., TON, sulphate, DOC, filtered manganese, total iron, 2 day colony count, nitrate, nitrite, TCC and ICC).

When considering microbial abundance, Surface Water Site Rigs had consistently higher values than the Groundwater Rigs, as shown by Table 23 and Figure 58. This occurred in TCC, ICC and 3 day colony count in the bulk water as well as in the biofilm ICC. It must be noted, however that the biofilm samples, particularly the ICC, had low values and were very similar.

Table 23 Average enumeration of microorganisms within the experimental facility Outlets

Location	Bulk Water				Biofilm	
	TCC/ no/100µl	ICC/ no/100µl	3 Day Colony Count/ no/ml	2 Day Colony Count/ no/ml	TCC/ no/100µl	ICC/ no/100µl
SW Rig 1 Chemically Fed	24047	12769	1436	719	541	65
SW Rig 2 Chemical Free	19143	11608	454	207	714	75
GW Rig 1 Chemically Fed	3760	1396	157	233	594	28
GW Rig 2 Chemical Free	17180	10571	252	111	418	38

SW denotes Surface Water, GW Groundwater, R1 Chemically Fed Rig, R2 Chemical Free Rig.

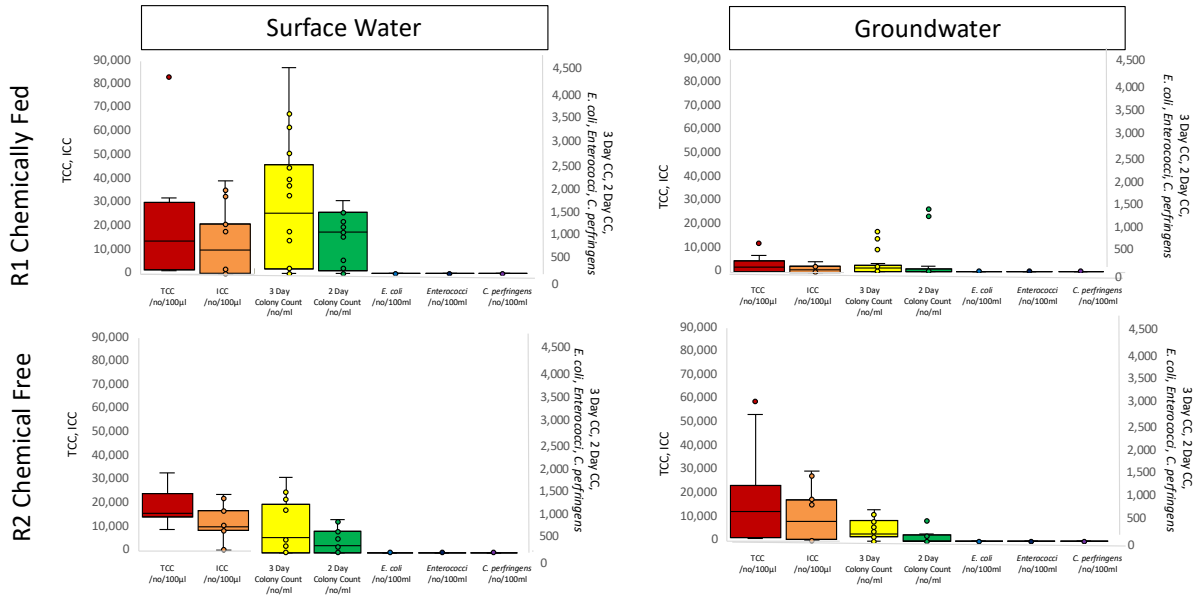


Figure 58 Microbial abundance at experimental Outlets

CC denotes Colony Count, SW Surface Water, GW Groundwater, R1 Chemically Fed Rig, R2 Chemical Free Rig.

These findings mirrored analysis completed at the Final Water and at the Customer Tap, which showed that parameters differed between the two case study areas and, when they did, it was often the Surface Water Site which had higher values than the Groundwater Site [Figure 59]. For example, all Groundwater WTW 2 day colony counts samples were 0 no/ml while Surface Water WTW samples exhibited spikes of up to 23 no/ml [Figure 60].

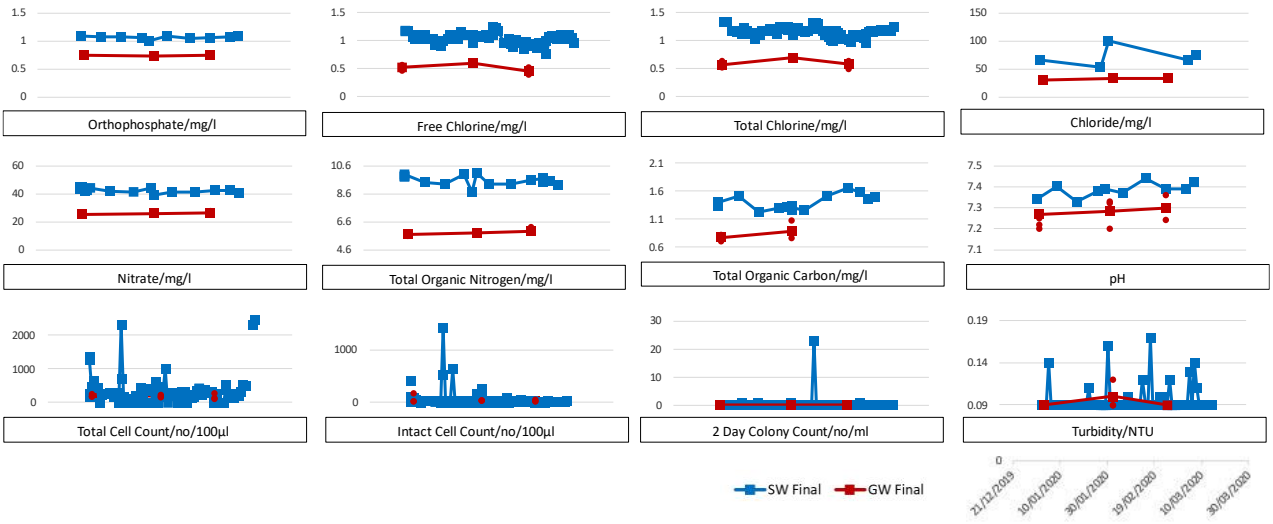


Figure 59 Final Water samples with higher values at the SW Site than at the GW Site

SW denotes Surface Water, GW Groundwater.

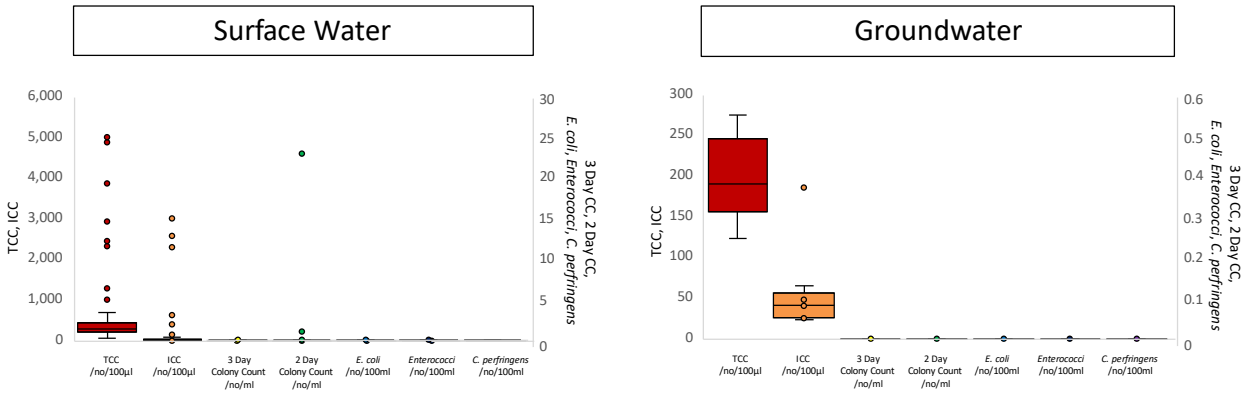


Figure 60 Bulk water microbial abundance at GW and SW WTW

CC denotes Colony Count, SW Surface Water, GW Groundwater.

When the differences between the facilities at the Surface Water Site and the Groundwater Site were considered, it was found that the Surface Water Outlets were more different to each other than the Groundwater Outlets. Some examples of this can be found in Figure 61. Although, as Table 24 shows, these differences were not always statistically significant.

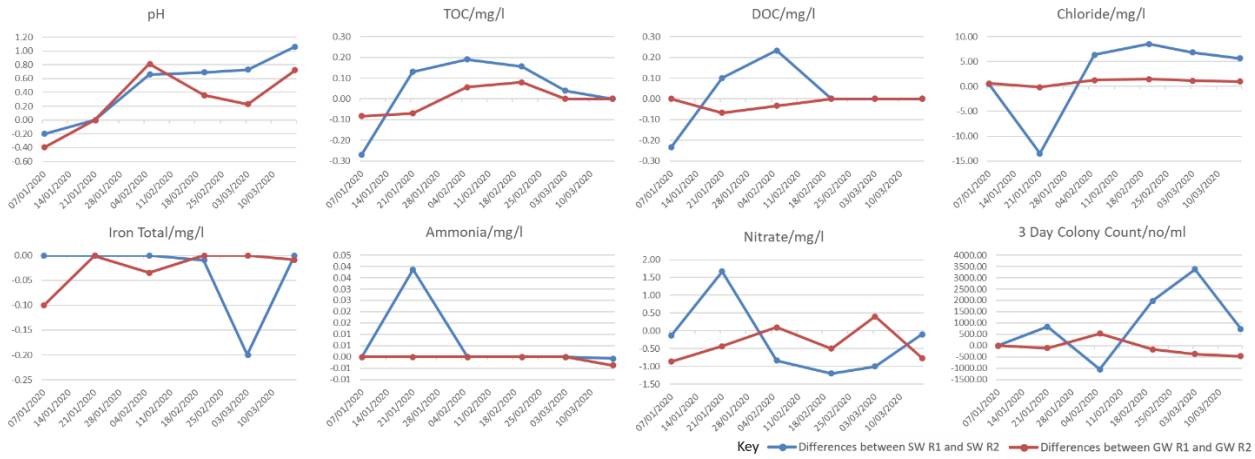


Figure 61 A comparison of the difference between Surface Water and Groundwater Outlets

SW denotes Surface Water, GW Groundwater, R1 Chemically Fed Rig, R2 Chemical Free Rig.

Table 24 Parameters without a statistically significant difference between the Experimental Outlets

Parameter	2 Day Colony Count	3 Day Colony Count	Alkalinity	Ammonia	*C. perfingens*	Chloride	DOC	*E. coli*	*Enterococci*	Free Chlorine	ICC	Magnesium	Manganese	Nitrate	Nitrite	Orthophosphate	pH	Sulphate	TCC	TOC	TON	Total Chlorine	Total Iron	Total Lead	Number of Parameters without a Statistically Significant Difference
SW R1 Outlet vs SW R2 Outlet	0.02457	0.05836	2.78E-02	6.85E-01	NA	2.00E-02	1	NA	NA	2.19E-08	0.7666	1	1.11E-01	8.68E-02	4.67E-01	4.72E-08	5.39E-03	4.12E-02	1.03E-02	0.2995	9.65E-02	2.17E-08	1.45E-01	2.77E-07	14/24
GW R1 Outlet vs GW R2 Outlet	0.5093	0.04719	3.48E-04	8.45E-01	NA	0.03184	3.60E-01	NA	NA	< 2.2e-16	0.0129	1.36E-01	1	1.02E-12	3.16E-01	3.78E-06	5.41E-03	0.6807	0.01086	3.70E-01	< 2.2e-16	< 2.2e-16	0.002251	0.005107	12/24
SW R1 Outlet vs GW R1 Outlet	0.01807	0.002509	5.28E-07	0.3595	NA	< 2.2e-16	0.000112	NA	NA	0.6215	0.01063	< 2.2e-16	NA	< 2.2e-16	0.05149	4.72E-08	0.00539	< 2.2e-16	0.006202	6.77E-11	< 2.2e-16	0.6214	0.03958	1.87E-05	8/24
SW R2 Outlet vs GW R2 Outlet	0.1838	0.4181	0.09046	0.8645	NA	3.22E-07	0.000209	NA	NA	0.197	0.6058	3.35E-06	NA	3.17E-07	0.5314	0.3449	0.6931	3.20E-07	0.2649	2.99E-05	3.21E-07	0.01632	0.1549	0.004207	15/24

SW denotes Surface Water, GW Groundwater, R1 Chemically Fed Rig, R2 Chemical Free Rig.

Overall, a comparison of the water quality of the experimental facility Outlets by site found that site location was a major driving factor for water quality differences. Site location had the greatest influence over: TOC, TON, chloride, sulphate, filtered lead, total lead, 2D, 3D, DOC, magnesium, nitrate, nitrite, TCC and ICC. Where water quality differed between the different sites, the Surface Water Site was frequently found to have higher values, for example in microbial abundance. Surface Water Rig 1 and Rig 2 were also found to be more different than Groundwater Rig 1 and Rig 2.

5.3.4 To What Extent Did Chemical Presence or Absence Influence Water Quality Within the Experimental Test Facilities?

Figure 57 evidenced that the greatest difference between the four facilities was depending on if the experimental facility was at the Surface Water Site or at the Groundwater Site, not depending on whether the rigs were Chemically Fed or Chemical Free.

However, there was still some evidence of parameters where this was not the case and instead differences between the facilities appeared to be more due to if chemicals were or were not used. Namely, in the parameters pH, alkalinity and total iron [Figure 62].

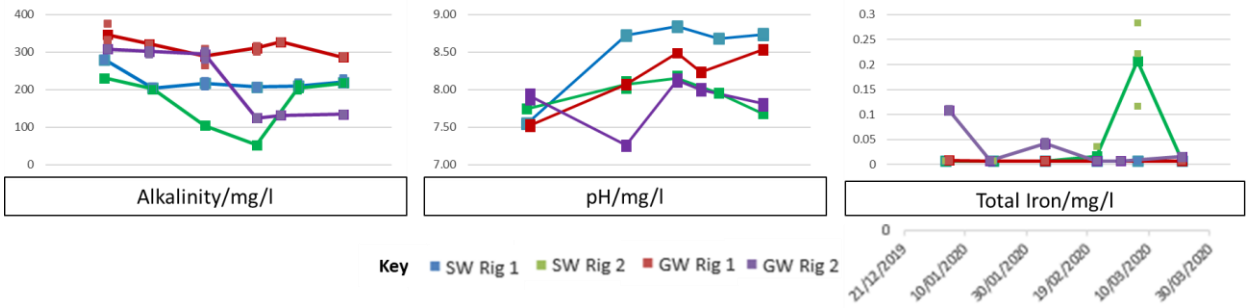


Figure 62 Parameters with differences when chemicals were and were not used

SW denotes Surface Water, GW Groundwater, R1 Chemically Fed Rig, R2 Chemical Free Rig.

It was also noted that the Chemically Fed Rigs at both the Surface Water and Groundwater Sites often showed some degree of greater temporal stability than the Chemical Free Rigs, as illustrated by Figure 63.

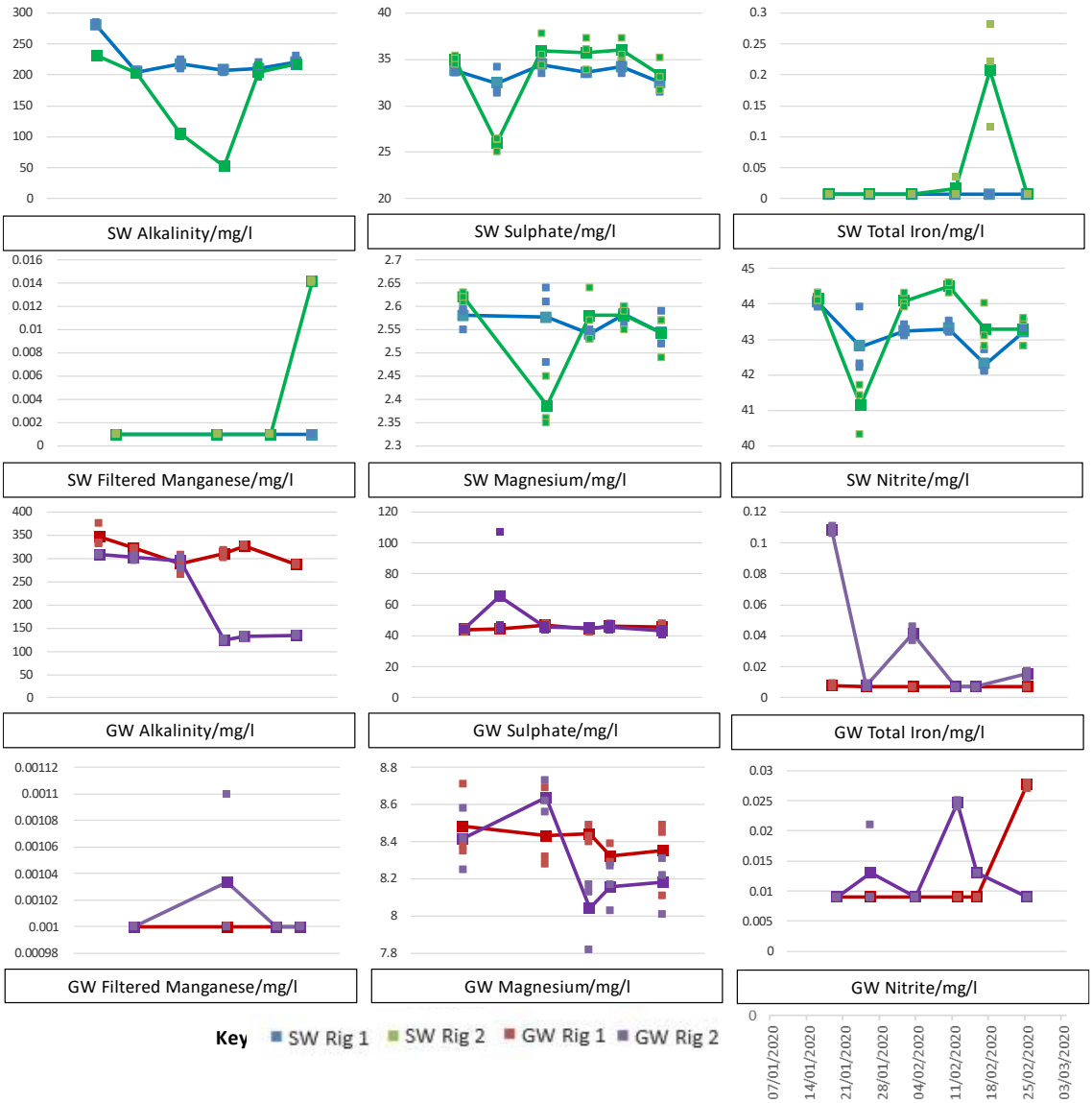


Figure 63 A demonstration of the variability of Rig 1 compared to Rig 2

SW denotes Surface Water, GW Groundwater, R1 Chemically Fed Rig, R2 Chemical Free Rig.

For the most part, under all experimental conditions, the pipe loops did not express signs of water quality degradation over time. There was one sampling location where a potential trend was observed. Rig 2 (the Chemical Free Rig) on the Groundwater Site could possibly have had an increasing trend in 3 day colony counts, TCC and ICC, as shown in Figure 64.

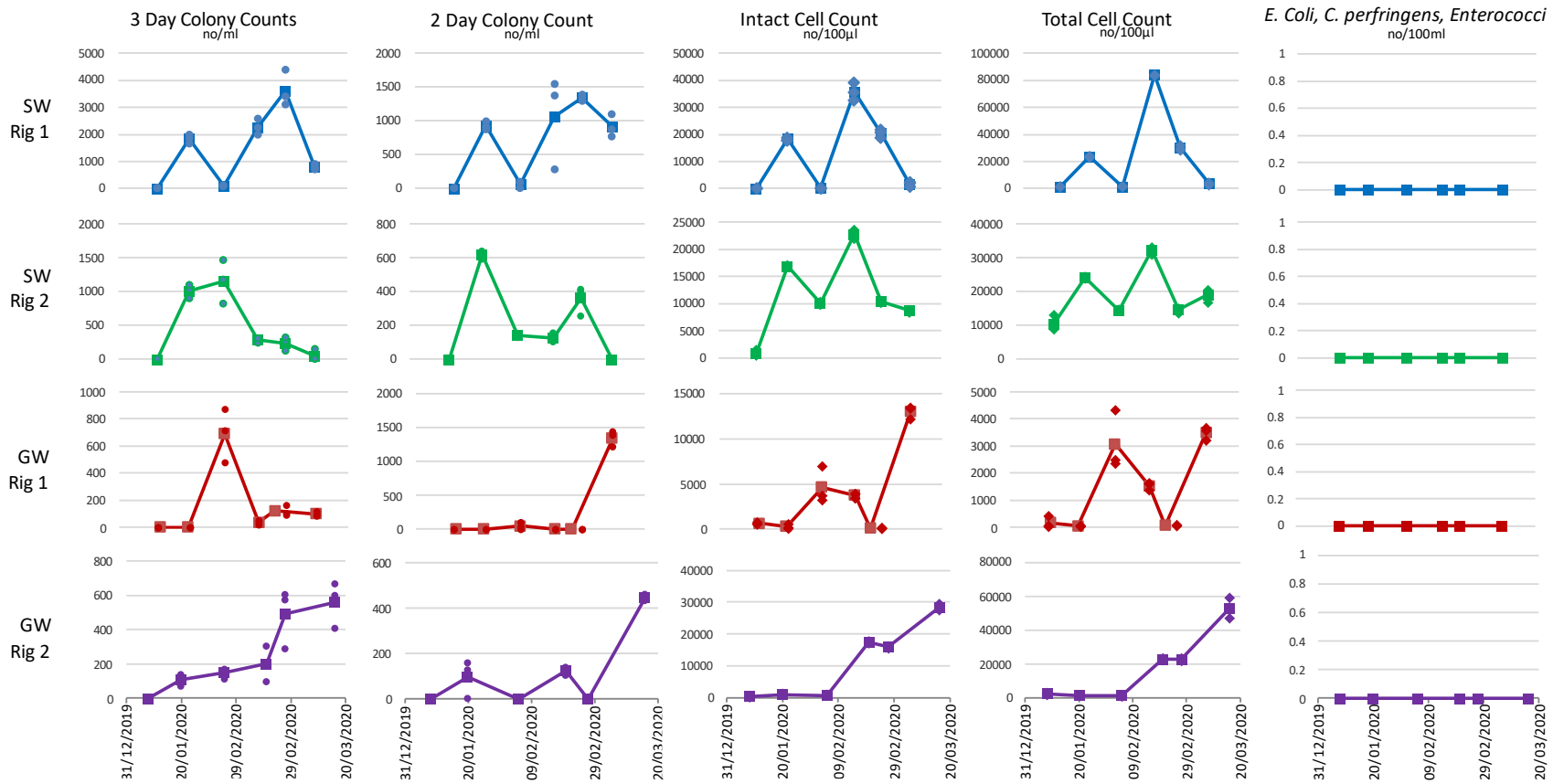


Figure 64 Experimental facility Outlet bulk water microbiological abundance

SW denotes Surface Water, GW Groundwater, R1 Chemically Fed Rig, R2 Chemical Free Rig.

Some evidence of this was found in the limited biofilm samples obtained. Despite only two samples of biofilm (in triplicate) being taken over the course of the experiment per experimental rig facility (at day 0 and after one month of operation), the TCC and ICC from the coupon surface increased over that one month growth period [Figure 65].

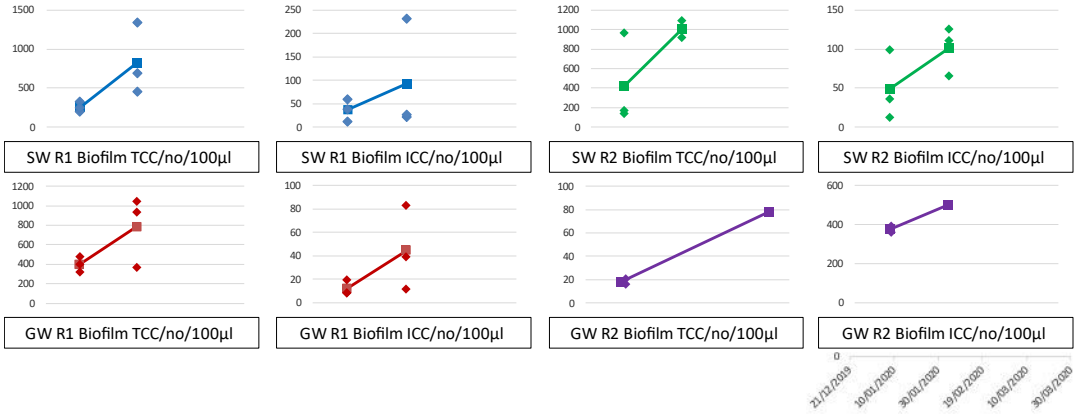


Figure 65 Biofilm TCC and ICC increases after a one month growth period

SW denotes Surface Water, GW Groundwater, R1 Chemically Fed Rig, R2 Chemical Free Rig.

Overall, a comparison of the water quality from the chemically fed and chemical free experimental facility Outlets found that there was some evidence of a difference in water quality when chemicals were and were not used. Water quality of the chemical free experimental conditions were found to have the greatest amount of variation over time.

5.3.5 Results Summary: The Wider Impact of Chemicals in an Experimental Facility

This section has explored the findings of a series of novel purpose-built re-circulating test loop facilities which were designed and constructed during the course of this study to explore how biological stability and chemicals influenced water quality within a series of innovative bespoke pipe test loops.

The comparison and contrast of different key indicators of water quality determined the following:

- Even though the experimental conditions produced water that had variations, the drinking water quality was within the realm of the reasonable minimum requirements of safe drinking water, for instance all Outlet samples were comparable to live network samples.
    - Even though water quality differences occurred between the different experimental conditions, an increased risk to public health and current compliance when chemicals were not used at both site locations was not identified, for instance all *E. coli*, *Enterococci* and *C. perfringens* samples throughout were 0 no/100ml.
    - None of the experimental facility Outlets (including the Chemical Free Rigs) had a strong decrease in water quality. Despite this, biofilm TCC and ICC at all sites increased from time point 0 to 1 months.
    - The experimental rig facility Inlets and Outlets were found to have relatively comparable water quality to the Final Water and Customer Tap respectively.
    - Parameters that were similar at all Outlets despite experimental conditions included: total iron, filtered iron, total manganese, filtered manganese, *E. coli, Enterococci* and ammonia.
    - The experimental facilities achieved the desired differences in chemical dose and as such, functioned as anticipated.

- Water quality changes occurred within the experimental facilities, from the Inlets to the Outlets, regardless of the different experimental conditions and locations.
    - A comparison of the experimental facility Inlets with the Outlets found differences, indicating that water quality changes occurred within the facilities.
    - Between the Inlet and Outlet of all sites, pH increased, alkalinity decreased, TCC increased, ICC increased, and scale formed. The chemically dosed rigs additionally had a decrease in phosphate and chlorine concentration from the Inlet to the Outlet.
    - There were some parameters (TON, nitrate, chloride and DOC) changed differently within the experimental facilities depending on whether the experimental facilities were Surface Water or Groundwater fed.
    - There were a number of parameters that had a change in the experimental network but not in the live network and vice versa.

- There were some incidences of water quality differences observed depending on if experimental rigs were treated with or without chemicals.
    - In particular, water quality of the Chemical Free experimental conditions were found to have the greatest amount of variation over time.
    - The Chemically Fed Rigs were more similar to each other than the Chemical Free rigs
    - The Chemically Fed Surface Water Rig had the highest values, as well as some of the highest values detected at all sampling points during experimentation, for instance in the microbiology samples, in TCC, ICC, 3 day colony count and 2 day colony count.
    - Chemical presence and absence had the greatest influence over: pH and alkalinity (as well as free chlorine, total chlorine and phosphate).

- Substantial water quality differences occurred depending on the site location. This was found to be a major driving factor, more so than that of chemical presence or absence.
    - Where water quality differed between the different sites, the Surface Water Site was frequently found to have higher values. For example, both Surface Water Rigs (containing chemicals and not containing chemicals) consistently had more microorganisms than both Groundwater Rigs.
    - The Groundwater experimental facility Outlets (Chemically Fed and Chemical Free) were more similar to each other than the Surface Water Outlets.
    - Site location had the greatest influence over: TOC, TON, chloride, sulphate, filtered lead, total lead, 2D, 3D, DOC, magnesium, nitrate, nitrite, TCC and ICC.
    - If the site was Groundwater or Surface Water had more of an influence on Outlet Water Quality than chemical presence or absence.

5.4 Discussion: The Wider Impact of Chemicals in an Experimental Facility

5.4.1 The Extent to which the Experimental Rig Facilities were Representative of the Live Network

The experimental pipe loop systems were of a bespoke design and purpose built, as discussed in Section 5.2. Nevertheless, a series of factors could have potentially influenced the observed results. The present section assesses the extent to which the pipe facilities were representative of the live network.

The pipe loop systems had dimensional constraints. The chosen dimensions of the pipe loops, 14 m long and 90 mm external diameter, had a larger pipe surface area than laboratory-based experiments meaning that they were built to form a more realistic picture of the accumulation process and nutrient exchanges between the bulk water and the pipe wall. They were also designed to allow for the number of pipe loops desired for the different experimental conditions, while also fitting within the constraints of a shipping container for transportation to live WTW sites [Figure 42]. However, due to the target water age of 3 days (to mimic the average water ages within the actual distribution network), the pipe facilities had to recirculate the drinking water within. One problem this design could have caused is that it may not have been the pipe loop system having an impact on water quality but, rather, the water tank used as part of the circulation mechanism. Nevertheless, the facilities were designed to prevent this being cause for concern, ensuring the pipe loop system (170 l) had a greater volume than that of the tank network (80 l). A further way in which this recirculation effect could have changed the system is that of increased temperature observed within the rigs but not observed within the live network, also compounded by the use of a shipping container to house the facilities. Blokker at al. (2016) noted that the water temperature range of the actual network is ~4-14°C. During experimental operation there was an average water temperature of 16.5°C, which was higher than that of the actual network. The live network during the experiment operation had an average temperature of 10.5°C at the Final Water and 8.5°C at the Tap. This temperature increase could have potentially affected water within the systems through the formation of the scale (discussed in Section 1.4.4.2.1). In future works, temperature could be better controlled through the use of vents and air conditioning units. The focus had instead been on insulation and heaters to prevent freezing within the rigs. It would be recommended that both situations be prepared for in future work. Therefore, the design of the pipe loop systems could have resulted in increased temperatures which may have influenced the experiment, although all pipe rigs had the same conditions.

One way in which the experimental pipe loop could have influenced water quality was because of problems with pipe material, for instance the warping of materials, as well as the possibility of the pipe material used not being adequately representative of the idealised pipe network being simulated. Section 1.4 discussed the importance of pipe material as it has the potential to impact the quality of the water residing within it. A “next generation” of pipe materials was desirable, of which HDPE was selected. HDPE was chosen due it being commonly used in industry, flexible, inexpensive, corrosion resistant and since it has a long expected service life (Heim & Dietrich, 2007) (Whelton, et al., 2010) (Davis, et al., 2006). Grade PE100 SDR17 pipe was utilised for the purpose of the experiment to ensure quality and resilience for the project duration. It is a material that is resistant to rapid crack propagation and long-term stress cracking, is capable of having a thinner pipe wall and can withstand higher pressures (International Organization for Standardization, 2012). The only

exception to this pipe material was that an additional component of the pipe testing facility was a clear section of pipe to visualise the material build up over time [Figure 46]. The use of the clear pipe section could have impacted water quality, for instance through the formation of algae, although a covering was made to block out external light and cover the section. It could also have impacted the facilities through the different combinations of pipe materials used. Although, as Chapter 3 demonstrated, combinations of different pipe material are commonplace in the UK water industry and also occurred within the case study sites the facilities were comparable with. Thus, the pipe materials used, both the HDPE and the clear pipe section, were appropriate for use, they functioned adequately and acted sufficiently as the next generation of pipe material.

It could be that the results shown would have been different had the experiment continued for a greater time period. For instance, a deterioration effect may not have been seen in the chemical free rig systems because they were in operation for a long enough time period or because enough coupon samples had not been taken for observable biofilm changes. In a live experimental environment, pipe age is debatable, but estimates have been made that the average pipe age in the UK is 75-80 years old (Speight, 2015). The experimental facility was in operation for one 3 month "growth" phase, where biofilm development was monitored as it built over time, deteriorating the freshly laid "idealised" pipe system. Previous studies investigating biofilm within the distribution networks have varied from days (Deines, et al., 2010) to years (Martiny, et al., 2003). However, research from Deines et al. (2010) found that after only 7 days of exposure, clean coupons in a nonsterile pilot water distribution system formed a complex, structurally mature biofilm. The 3 month duration in the current project was found to be sufficient for an increase in microbial abundance at the pipe wall to be observed. In the biofilm, an average TCC increase was found, from 363 no/100µl to 835 no/100µl and ICC from 30 no/100µl to 80 no/100µl [Table 23]. Further, during the study period it was evidenced that the facilities did impact water quality, both when chemicals were or were not present and differences depended on the biological stability of the case study site. Therefore, although the experimental operation could have been longer and for future work it would have been interesting to observe how the facilities deteriorated over a longer period of time and to also consider seasonality, the operational period should have been sufficient for observational water quality changes to be made.

The experimental design and, in particular, the presence of the removable pipe sections (coupons), could have impacted the flow of water within the facilities or caused leaks that resulted in pipe ingress. Section 5.2.1.1 described the importance of biofilm collection devices being directly inserted and closely aligned with the internal pipe surface. The purpose of this alignment was to minimise the distortion of boundary layer conditions that influence biofilm formation, such as boundary shear stress and turbulent driven exchange with the bulk water body, so as to ensure biofilm formation is accurate to the formation in real drinking water networks (Douterelo, et al., 2013). As Section 5.2.1.1.1 described, the coupons were redesigned several times until an acceptable level of smoothness and surface fit was achieved which matched the internal pipe wall [Figure 44]. This was shown using light microscopy and SEM imagery. Also, the coupons were redesigned from the Pennine Water Group coupons to use a saddle clamp, location lug, seal and coupon cap as shown in Figure 43 (rather than coupons fixed in place using flanged pipe sections and a gasket), which prevented pipe leakage (Deines, et al., 2010) (Sharpe, 2012). This new technique, however, did make coupons very difficult to remove. It was also found that although operation leakage was removed, when coupons were removed, leakage persisted. Potentially, in future, further redesigned facilities

could incorporate the use of covers to prevent this. Therefore, the use of multiple test coupons as manufacturing techniques developed, allowed the coupons to be highly flush with the pipe wall with no evidence of pipe leakage. Although, the reduction in pipe leakage did result in coupon caps being difficult to remove and leakage did occur when coupons were removed.

Extensive measures were taken to compare the experimental rig facilities with the live networks they were designed to replicate. This included the comparison of the Final Water with the facility Inlet and the Customer Tap samples with the facility Outlet [Figure 50, Figure 51]. Both of these comparisons found that the bulk water within the rigs had comparable water quality with their respective sample points within the live network. This research was conducted to confirm that the water quality changes within the rig systems occurred because of the retention within the experimental pipe facilities and not because of differences in the incoming water. It was remarked that the Inlets were stable, with no evidence of deterioration over time. It was also noted that if the site was Groundwater or Surface Water had more of an influence on Inlet water quality than chemical presence or absence. This same trend was noted at the Outlet and discussed further in Section 5.4.3 and Section 5.4.4.

An example of how representative the rig facilities were to the live network can be seen in a comparison of phosphate concentrations from the Inlets to the Outlets. It was interesting that the phosphate was found to decrease in the chemically treated experimental pipe systems because Chapter 3 noted the substantial phosphate residual presence. It was expected that phosphate would have more of a consumption effect in the live network than in the experimental rig facilities because of the difference in pipe material. In the live network, with its presence of a mix of pipe materials including those which are metalloid, it was assumed that more phosphate would have been consumed by the network to make lead phosphate or calcium phosphate precipitates on the pipe wall (International Water Association, 2016) (Hayes, et al., 2014). In the experimental rig systems, with the consistent use of plastics, it was expected that phosphate would have little change. Instead, comparable values, as well as a slight consumption of phosphate in both locations, were noted [Table 25]. Therefore, the experimental facilities had similar substantial phosphate residual presence at the Tap or Outlet, with only a small amount of consumption observed.

Table 25 A comparison of phosphate concentrations before and after distribution

Sample Location	Average P/mg/l Before Network	Average P/mg/l After Network	Difference
WTW - Tap 6 Case Study Areas 2012-2020	1.24	1.07	-0.17
WTW - Tap 2 Case Study Areas 2020	0.922	0.869	-0.053
Inlet - Outlet Experimental Pipe Rig 2020	0.921	0.769	-0.152

Overall, the experimental facilities were purpose-built and took every possible attention to detail to ensure they were as representative as possible to the live network. The experimental design and operation; pipe materials used; the duration of the experimental operation; the coupon design, construction and application were appropriate for use and functioned adequately to provide confidence in the research findings. Extensive measures were taken to compare the experimental rig facilities with the live networks they were designed to replicate.

5.4.2 The Extent to which the Experimental Rig Facilities Impacted Water Quality

A comparison of the water entering the experimental facilities at the Inlets and the water leaving the experimental facility through the Outlets found small variations in water quality, evidencing that both microbiological and chemical processes were occurring within the experimental rig facilities. Under all experimental conditions, pH increased, alkalinity decreased, 2 day colony count increased, TCC increased, ICC increased and scale formed [Figure 53]. Where chemicals were applied, phosphate, total chlorine and free chlorine all additionally decreased in the experimental network [Table 20].

There is substantial research that supports the experimental work, which shows how water quality degrades during its transportation to the customer tap, through the live distribution network, sometimes to the point of water quality failure, despite water leaving the WTWs being wholesome. Williamsona et al. (2014), for instance, reported that 30-60% of water quality incidents are related to events in the distribution network. This water quality degradation occurs due to the physical, chemical and biological reactions which occur during distribution and can be impacted by many different factors, such as pipe materials, contamination, pipe leakage and inadequate or infrequent maintenance (National Research Council, 2006). However, the network used for the experimental rigs was designed to minimise the impacts of these factors and instead focus on the differences between the chosen experimental conditions. One explanation could be that the pipe system was not as robust as it was designed to be, although Section 5.4.1 discussed this at length and found this not to be the case. One alternative explanation is that even in the idealised pipe system, water quality degradation occurs, that it cannot be avoided and instead must be accounted for, similar to the flaws of the live network today.

When changes within the network were compared from the WTW to the Customer Tap of the live network, it was found that there were a number of parameters that had a change in the experimental network but not in the live network. To further examine this, the comparison displayed in Table 21 has been updated in Table 26 to also consider data from Chapter 3 and Chapter 4. The additional data proved helpful. A comparison of the data presented in both Chapter 3, Chapter 4 and Chapter 5 found that 2 day colony counts increased, pH increased and alkalinity decreased at either all or most of both the rig facilities and also across all Sites A-F. As such, the rig facility water quality network changes that occurred bore a resemblance to water quality changes that occur within the live network.

Table 26 Parameters that did not change during the experimental period

Parameter	Sample Location with Network Change											
	SW R1 Inlet-Outlet	SW R2 Inlet-Outlet	GW R1 Inlet-Outlet	GW R2 Inlet-Outlet	SW Final-Tap	GW Final-Tap	Site A WTW-Tap	Site B WTW-Tap	Site C WTW-Tap	Site D WTW-Tap	Site E WTW-Tap	Site F WTW-Tap
2 Day CC	+	+	+	+	NA	=	+	+	+	+	+	+
3 Day CC	+	+	+	-	=	=	+	+	-	+	+	+
Alkalinity	-	-	-	-	NA	-	-	-	-	-	-	+
Ammonia	+	-	-	-	=	=	-	+	-	+	+	-
Calcium	NA	NA	NA	NA	-	NA	-	-	-	-	-	-
Chloride	-	-	+	+	NA	+	NA	NA	NA	NA	NA	NA
Coliforms	NA	NA	NA	NA	NA	NA	+	+	+	+	+	+
Conductivity	NA	NA	NA	NA	NA	NA	-	-	+	-	-	-
DOC	-	-	+	+	NA	NA	NA	NA	NA	NA	NA	NA
Free Chlorine	-	NA	-	NA	-	-	-	-	-	-	-	-
F. Lead	+	+	+	-	NA	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	-	-	-	-	-	-
ICC	+	+	+	+	NA	-	NA	NA	NA	NA	NA	NA
Magnesium	+	-	+	-	+	+	-	-	-	+	+	-
Nitrate	+	+	-	-	-	+	-	-	-	-	+	+
Nitrite	+	+	+	-	=	=	+	-	+	-	-	-
Orthophosphate	-	NA	-	NA	-	-	-	+	-	+	+	+
pH	+	+	+	+	+	-	+	+	+	+	+	-
Sulphate	+	-	+	+	NA	-	NA	NA	NA	NA	NA	NA
T. Iron	-	+	+	-	+	=	+	+	-	+	+	+
T. Lead	-	+	+	-	NA	+	+	+	+	+	+	+
T. Manganese	=	=	=	-	+	NA	+	+	+	+	+	+
TCC	+	+	+	+	NA	+	NA	NA	NA	NA	NA	NA
TOC	-	-	+	-	NA	+	NA	NA	NA	NA	NA	NA
TON	+	+	-	-	-	+	-	-	+	+	+	+
Total Chlorine	-	NA	-	NA	-	-	-	-	-	-	-	-
Turbidity	-	+	-	+	+	-	+	+	+	+	+	+

CC denotes colony count, T. total, F. filtered, SW Surface Water, GW Groundwater, R1 Chemically Fed Rig, R2 Chemical Free Rig.

These findings strongly indicate that the pipe facilities had an impact on water quality. The facilities were designed to represent the idealised network, for example with: next generation pipe material, consistency of pipe material, brand new in age, freshly disinfected pipes, consistent residency times, consistent flows, no diurnal variation, no pipe ingress, no leakage, no areas of stagnation and no influence of household fittings, fixtures or pipes. This means that, although the experimental pipe system was designed to be the idealised distribution network, even this network impacted water quality.

Further, the water quality changes did not only occur in a system that was designed to be the idealised pipe network but also occurred in an area with good biological stability, as classified by Chapter 3 (Site F). Therefore, this research showed, even if water quality is from a site of good biological stability, that does not mean it is immune to water quality deterioration. As such, even if future research includes the classification of sites by biological stability as an indicator of readiness

for chemical free water, the regular monitoring of numerous water quality parameters is still incredibly important. This includes microbiological, physical, chemical and aesthetic parameters at the customer tap, rather than solely at the point of water production (Northern Ireland Water, 2015) (European Commission, 2016).

The concern that water quality was impacted by the pipe system, even though it was designed to be the idealised pipe network and also as the WTW supplying it was an area categorised as good biological stability, could lead people to believe that chemical free water is not a possibility in this country. However, although the experimental conditions produced water that had variations, the drinking water quality at the Outlets was similar to that of live networks. For example, all of the experimental Outlets had no statistically significant difference to live Customer Tap samples in numerous parameters, including: *Enterococci*, *E. coli* and nitrite, and at many sites in ammonia, manganese, nitrate, sulphate, TON and total iron [Figure 51, Table 17]. As such, even those experimental facilities not treated with chemicals and those in areas of less good biological stability, had comparable water quality with Customer Taps in live networks, with no strong difference or decrease in water quality between the two.

Plate counting techniques such as those for *E. coli* and *Enterococci* have been evidenced to produce a negative result when microbial abundance is detected using other measures throughout this research. This occurred both in Chapter 3, Chapter 4 and now in the experimental results in Chapter 5 at the Final Water, Customer Tap, experimental Inlets and experimental Outlets [Figure 64]. Even though plate counts have a number of limitations, it is the current indicator for if drinking water is microbially safe for consumption. The World Health Organization (2011) accepted guideline for HPC is 20 CFU/ml for treated water and 300 CFU/ml for distributed water. All Outlet samples, even those not treated with chemicals and those in areas of less good biological stability, had *E. coli*, *Enterococci* and *C. perfringens* samples of 0 no/100ml throughout [Figure 64].

Therefore, even though experimental pipe facilities were found to have different water qualities between the Inlets and the Outlets, so too did a comparison of the WTW and the Customer Tap. Both the live network and the experimental network impacted water quality, despite whether the area was categorised as good or less good biological stability, though not in a way that made water quality unsuitable for consumption. In this way, even if investment is made to improve the UK distribution network, regular monitoring of numerous water quality parameters is still required, similar to the best practices of case study companies in countries presently using fewer chemicals.

5.4.3 The Impact of Chemical Presence or Absence on the Experimental Rig Facilities

The experimental rig facilities found that there was some evidence of a difference in water quality when chemicals were and were not used [Figure 62]. Chemical presence and absence had the greatest influence over pH and alkalinity (as well as free chlorine, total chlorine and phosphate). It is likely that the driving chemical that caused this change was phosphate. Historic data analysis of 6 case study live networks in Chapter 4 found that chlorine had consistent relationships with temperature, pH and conductivity, while phosphate had relationships with a wide range of water quality parameters, (including pH, hardness, total copper, total iron, total manganese, calcium, conductivity, alkalinity, water temperature, sodium, potassium and magnesium) [Figure 37]. Of the two chemicals, phosphate had particularly strong correlations with pH.

Chapter 1 discussed that phosphate would likely impact the pH of systems, due to the way in which it has been applied as a pH control mechanism by water utility operators previously (American Water Works Association, 1995) (MacAdam & Parsons, 2004). The literature also considered that phosphate has an influence on microbial growth, especially since virtually all of the orthophosphoric acid added to drinking water is biologically available for microorganisms [Section 1.4.3.2]. A number of studies demonstrated this, including Del Olmo et al. (2020), who provided evidence of microbial community structure change due to treatment with orthophosphoric acid. In this PhD study, however, it was noted that neither historic data analysis nor experimental facility research found that phosphate had a substantial impact on microbial growth [Figure 36]. This finding shows promise for future use of water treated without a phosphate dose. Although it is still unknown how microorganisms would adapt to this change in phosphate concentrations in the longer term, especially in the transition period, the present work speculates the impact would not be so great as to disrupt microbial abundance, though community was not studied and cannot be commented on.

Although the experimental facilities show promise for chemical free removal, with pH and alkalinity only being the main parameters influenced, variation of these factors can still cause system-wide impacts, such as the formation of scale, which should not be taken lightly [Figure 62, Figure 56]. The formation of scale can decrease operating efficiency, shorten equipment life, increase cleaning and maintenance costs and increase energy consumption (MacAdam & Parsons, 2004). Also, the relationships phosphate was observed to have with a wide number of parameters in the earlier historic data analysis in Chapter 4 could be a cause for concern [Figure 37].

Section 1.4.4.2, discussed the potential impacts of phosphate removal. It is thought that the use of orthophosphoric acid over many years has resulted in numerous unanticipated changes in the wider live drinking water distribution environment. Literature suggests (although this was scrutinised and criticised, especially in Section 1.4.4.1) that UK best practice for plumbosolvency is the application of a phosphate dose of 1 mg/l to build up the insoluble layer of lead phosphate over the course of a year, after which a continuous lower dose (which is on average 0.5 mg/l in the UK) is used indefinitely to maintain the layer; otherwise it is thought the benefit of the treatment stops as soon as the dosing stops (Hayes, et al., 2014). However, in the experimental facilities, an impact on the system (i.e. the pH increase and alkalinity decrease) was detected after only 3 months of operation (with an average phosphate concentration of 0.921 mg/l) [Figure 62]. This influence could have caused more drastic changes as the system continued to be in operation. This means that care should be taken when lowering or removing the dose of orthophosphate and measures would need to be taken to limit impact on the system, for instance the use of supplementary pH control techniques.

It has briefly been discussed that the impact of chemical presence or absence was likely due to the absence of a phosphate dose, rather than the absence of a chlorine dose, but there could be an additional explanation [Figure 62]. In the experimental facility, both phosphate and chlorine were removed together. Thus, the impacts when a chemical dose was not applied could have been due to the way they were both removed together. Chapter 4 studied the relationship between chlorine and phosphate with historic regulatory samples at 6 case study sites to study the interplay between the two distribution chemicals [Figure 40]. Although multiple weak relationships were found between phosphate and chlorine, it did not appear that the concentration of either chemical was influencing the other very much. Literature found evidence that the presence of phosphate reduces the

effectivity of chlorine (Brown, et al., 2011) (Del Olmo, et al., 2020) (LeChevallier, et al., 1993). However, the strong relationships observed in literature were not found in this research [Figure 40].

A further impact of the use of chemicals was that the Chemically Fed Rigs were found to have less temporal variation than the Chemical Free Rigs, as shown in Figure 63. It is interesting that the chemical application increased the stability of the experimental facilities. Neither of the distribution chemicals applied in the live network is done with the primary aim of stabilising the chemical balance within these systems. Rather, the network chemical doses are designed to impact systems, not cause stability within them. For instance, chlorine is applied to form hypochlorous acid and dissociate into hydrogen and hypochlorite ions, all of which inactivate microorganisms (Bitton, 2014). Meanwhile, phosphate is dosed to form an insoluble layer of lead phosphate over the soluble carbonate deposits on the pipe wall to prevent the lead from leaching (European Commission, 2016). This is compounded by the way that neither chemical influences the distribution network in a homogenous manner. For example, phosphate was found to be dosed seasonally at multiple sites [Figure 33], while chlorine has been shown in literature to be consumed and so decays at different rates throughout the network [Figure 13] (Gibbs, et al., 1990) (Al-Jasser, 2011). Despite this, it could be argued that a phosphate dose is omnipresent in chemically dosed rigs, as the research within Chapter 4 and Chapter 5 both note the substantial phosphate presence at the Tap [Figure 29]. It was additionally observed in Chapter 3 that it is possible to have sites of good biological stability in a network with the use of chemicals. Overall, it is unknown exactly why the Chemically Fed Rigs were found to have less temporal variation than the Chemical Free Rigs, but it did result in the Chemically Fed Rigs being more similar to each other than the Chemical Free Rigs were.

Section 5.4.3 discussed the impact biological stability had on the experimental rig facilities and that this impact was more substantial than the influence of chemical presence or absence discussed in the present section [Figure 57]. However, the way in which the Chemically Fed Rigs were more similar to each other than the Chemical Free Rigs [Figure 63] could provide evidence for a theory that the water quality differences between the Surface Water Site and Groundwater Site would become more pronounced when chemicals were not used over a greater period of time [Figure 61]. As such, the categorisation of sites as being of good or bad biological stability may be seen as highly important to achieve chemical free water. In particular, that compliant and safe chemical free drinking water may pose more of a challenge at Surface Water Sites. For example, the Chemically Fed Surface Water Rig had the highest values, as well as some of the highest values detected at all sampling points, during experimentation, including in the microbiology samples, in TCC, ICC, 3 day colony count and 2 day colony count [Figure 58].

Therefore, there was an impact on water quality if chemicals were or were not present, although it was a relatively small impact, especially when considering that these chemicals are specifically dosed to cause changes within the network. In this way, the presence or absence of chemicals had less of an effect on the network than previously expected. Although it was proposed that chemical free drinking water without an impact on drinking water quality may pose more of a challenge at Surface Water Sites, supporting the idea of categorisation by biological stability.

5.4.4 The Impact of Biological Stability on the Experimental Rig Facilities

The experimental phase of the research involved the study of two case study locations in detail, named the Groundwater Site (a site of good biological stability) and Surface Water Site (a site of less good biological stability) for confidentiality. In each of these two locations, water quality samples were taken from the WTW, Customer Tap, experimental Inlets and experimental Outlets.

At the WTW sample point, different water quality was observed between the two Final Water sampling locations during the course of the study and of this difference, the Groundwater quality was deemed to be higher and more stable [Figure 59, Figure 60]. This occurred even though Chapter 3 found compliance at both sites to be 100% at the WTW (denoted as Site D and Site F in Table 10). This difference could be due to the increased frequency of sampling at the Surface Water Site detecting minor changes over time. The number of samples at the Final Water varied, with there being a high number of samples at the Surface Water Site and fewer samples taken in triplicate. The majority of samples at the Groundwater Site were taken at 3 time points but all of these samples were taken in triplicate. This was because the Surface Water Final sample location was frequently used during the course of the study for regulatory purposes, whereas any samples taken at the Groundwater location were purely taken for the purposes of this experiment.

At the Customer Tap, samples at both locations had minimal variation over time as was expected because of the excellent compliance with strict regulatory standards in the UK. This compliance is not only important so customers can drink their supplied water without illness, but also so there is minimal change in the aesthetic qualities that consumers use to determine whether water is safe to drink (Drinking Water Inspectorate, 2018). Although the UK water industry often quotes how there was 99.95% compliance with the Drinking Water Directive at the Customer Tap in 2018, in Chapter 3 Customer Tap compliance was found to be 97.84-100% at the Surface Water Site and 100% at the Groundwater Site (denoted as Site D and Site F in Table 10). Even in the Groundwater area with these lower values, one triplicate sample taken at the Customer Tap did show evidence of variation between these triplicate samples, as shown in Table 22, meaning that the discrepancy between the two sites could in actuality be even greater. At the Customer Tap there was also evidence that there were parameters with a difference between the two locations, with Surface Water values being higher than Groundwater. Due to this and since the same pattern was noticed at the Final Water supported the theory that this observed difference could be because of differing biological stabilities in the two areas [Figure 59].

The same trend was also noted in the experimental facilities. A comparison of the Inlets at the two sites found the Surface Water Inlets had higher values than their Groundwater counterparts for the most part. Meanwhile, a comparison of the experimental facility Outlets found that substantial water quality differences occurred depending on the site location (e.g., differences were noted in the parameters: TOC, TON, chloride, sulphate, filtered lead, total lead, 2D, 3D, DOC, magnesium, nitrate, nitrite, TCC and ICC) [Figure 57]. This was found to be a major driving factor, more so than that of chemical presence or absence [Figure 62]. Indeed, the two Groundwater experimental facility Outlets (Chemically Fed and Chemical Free) were more similar to each other than the two Surface Water Outlets [Figure 61]. This likely means that the application of a chemical dose was not impacting water quality very much at the Groundwater Site. One potential explanation for this could be because at this location, the quality of water was already very good, and the chemicals could do little to improve it.

Across all sampling points, at the WTW, Customer Tap, experimental Inlet and experimental Outlet there was evidence of water quality variation driven by site location, if the site sampled was of good biological stability or less good biological stability [Figure 59, Figure 60, Figure 57, Figure 58]. Samples consistently indicated that Surface Water samples were slightly higher than Groundwater samples, a finding other practice supports [Figure 59, Figure 60, Figure 57, Figure 58]. The practices of case study companies in other countries using fewer chemicals than the UK have a preference for groundwater when treating water without chlorine and state that surface waters are more vulnerable to higher microbiological presence than groundwaters (Isle Utilities, 2015). Literature suggests that surface water has less biological stability due to a combination of the absence of soil filtration and the likelihood of heavy rainfall that could increase the microbial load (Kistemann, et al., 2001). For instance, the work of Prest et al. (2016) found that groundwater sources had a lower concentration of bacterial cells with ~10^3-10^4 cells/ml than surface water, which has ~10^5-10^6 cells/ml. In the present research, microbiological abundance was one of the parameters noted as being higher at the Surface Water than in the Groundwater, particularly in TCC, ICC, 2 day colony counts and at times in 3 day colony counts. For example, at the Groundwater facility Outlets, TCC and ICC were orders of magnitude lower than The Surface Water Outlets, as shown in Figure 58. This was despite the Surface Water Site having more treatment steps as well as more advanced water treatment methods. The Surface Water treatment steps consisted of primary ozone, clarifiers, secondary ozone, GAC, chlorine and orthophosphate before storage and distribution while the Groundwater Site had only chlorine and orthophosphate before storage and distribution, as shown in Figure 9 and Figure 11 (Site D and Site F).

It could be that the clear differences observed between the two sites, which continued even when a chemical dose was not applied, did so because they were not comparable. Not only did these two locations have different source waters (surface water blend compared with groundwater) but they also had different treatment stages (ozone with GAC compared with no major treatment), different retention time in the network (≥6 days compared with 2 days), different network complexities (716,000 m compared with 115,000 m) and served different populations (18,390 compared with 8,592) [Sites D and F in Table 8]. It appears that at all points, the Surface Water Site was predisposed to have less good water quality than the Groundwater Site. Nevertheless, regulations stipulate that these two WTW and their respective Customer Taps are comparable. Guidelines require that all sites in the UK, even those as different as the two presently examined, are compliant with the same drinking water quality permissible levels. Further, any decisions water utilities make if they decide to go chemical free in future would be a choice between sites similar to this. It is therefore recommended that water utilities investigate the biological stability of their WTWs to assess and categorise their readiness or preparedness to go chemical free in future.

The biological stability risk score matrix presented in Chapter 3 was applied to the experimental rig facilities (where the Rig Inlet values were used as the WTW and Rig Outlets as the Tap), as presented in Table 27, with the breakdown of matrix results in Figure 74, Figure 75, Figure 76 and Figure 77. As can be seen, all rig facilities regardless of experimental condition, if facilities were Surface Water fed or Groundwater Fed, if facilities received a chemical dose or not, were found to have the same classification, Less Good Biological Stability. The facilities had the same water age, the same pipe material and the same biological stability rating. None of the rig facilities were found to be of Poor Biological Stability, despite Rig 2 of each not having received a chemical dose, potentially indicating that chemical free drinking water would be a possibility in the UK. This coincides with the idea that

there was not a deterioration in water quality when distribution chemicals were not used to treat drinking water within the facilities, that the only exception to this was pH and alkalinity [Figure 62].

The fact that both the Surface Water and Groundwater facilities were classified as Less Good Biological Stability was surprising, as the Groundwater Sites (Site E and F) in Chapter 3 were found to be of a different biological stability to the Surface Water and Surface Water Blended Sites (Sites A-D). However, it was noted that the Surface Water facilities scored consistently lower in stability than their Groundwater counterparts [Table 27].

Table 27 Biological stability risk score matrix results for experimental rigs

Biological Stability Values	Sample Location											
	SW R1 Inlet-Outlet	SW R2 Inlet-Outlet	GW R1 Inlet-Outlet	GW R2 Inlet-Outlet	SW Final-Tap	GW Final-Tap	Site A WTW-Tap	Site B WTW-Tap	Site C WTW-Tap	Site D WTW-Tap	Site E WTW-Tap	Site F WTW-Tap
Risk Score	60	52	49	53	48	37	51	48	49	45	27	25
Classification	LG	LG	LG	LG	LG	LG	LG	LG	LG	LG	G	G

Where “Good Biological Stability” (G) is ≤35, “Less Good Biological Stability” (LG) is 35-65 and “Poor Biological Stability” (P) is ≥65. SW denotes Surface Water, GW Groundwater, R1 Chemically Fed Rig, R2 Chemical Free Rig.

The biological stability risk score matrix was also applied to the live networks during the experimental period. The live networks of both the Surface Water Site and the Groundwater Site were classified as Less Good Biological Stability (January-March 2020 at the WTW and Tap) [Table 27]. This is surprising because the same two sites had also been assessed as Sites D and F in Chapter 3 (2012-2020), where they were classified as “Less Good” and “Good” respectively. It is unclear exactly why the same sites could have had different results from the matrix. It could be because the matrix does not accurately classify biological stability. It could be because the matrix does not accurately classify biological stability over a shorter time frame. Or it could genuinely be because the biological stability was less good from January-March 2020 than it had been from 2012-2020, perhaps that biological stability varies throughout the course of a year.

Therefore, across all sampling points (WTW, Customer Tap, experimental Inlet and experimental Outlet) there was evidence of water quality variation driven by site location, if the site sampled was of good biological stability or less good biological stability. Even though the application of the biological stability matrix proposed in Chapter 3 found rig facilities to have the same overall classification (Less Good Biological Stability), Surface Water Facilities consistently scored as having lower stability than their Groundwater counterparts. This finding agrees with best practices of companies in countries using fewer chemicals than in the UK, as well as findings in literature. Should utilities in future wish to go chemical free, it is recommended that a thorough consideration of location for this practice be considered. Ideally, sites across the utility would be categorised using biological stability to assess each location’s readiness to adapt for this approach with minimal water quality changes.

5.4.5 Discussion Summary: The Wider Impact of Chemicals in an Experimental Facility

This section has explored the findings of a series of novel purpose-built re-circulating test loop facilities which were designed and constructed during the course of this study. This research was completed to study the wider impact of the presence and absence of the distribution chemicals chlorine and phosphate in two different fieldwork environments.

It was found that the experimental facilities were carefully designed to ensure they were as representative as possible to the live network. This included details such as: experimental design and operation; pipe materials used; the duration of the experimental operation and the coupon design, construction and application. The facilities functioned adequately, providing confidence in the research findings, which was backed up by monitoring of the live network for comparison.

Even though these facilities were designed to be the idealised pipe network and were located in an area of good biological stability (as classified in Chapter 3 as Site F), water quality changed within the networks. Despite this, the water quality changes were not to the extent to which would make the water within unsuitable for consumption for the most part. Rather, the deterioration observed in the present chapter was comparable to the water quality deterioration in the live network between the WTW and Tap investigated in Chapter 3. The pipe facilities were designed to represent systems such as this. Therefore, even if investment is made to improve the UK distribution network, regular monitoring of numerous water quality parameters is still required, similar to the best practices of case study companies in countries using fewer chemicals than in the UK.

It was observed that there was an impact on water quality if chemicals were or were not present, although it had less of an impact than originally expected, when assessing current literature into the matter. The chemical addition seemed to mostly influence only the parameters pH and alkalinity, factors which could have caused the scale formation problems discussed earlier. Even though the impacts of the chemical presence or absence were relatively minor, the water quality differences between the Surface Water Site and Groundwater Site became more pronounced when chemicals were not used. This provided weight to the idea that the categorisation of sites as being of good or poor biological stability may be seen as highly important to achieve chemical free water in future.

Across all sampling points, the greatest difference in water quality observed was that of site location, if the site sampled was of good or less good biological stability. The site of good biological stability had consistently lower water quality parameters and greater temporal stability. Equally, the two rig facilities at the site of good biological stability were more similar to each other than the two rig facilities at the site of less good biological stability. Equally, the application of the biological stability risk score matrix presented in Chapter 3 to the experimental rig facilities found rigs of all experimental conditions to have the same overall classification (Less Good Biological Stability), although Surface Water Facilities consistently scored as having lower stability than their Groundwater counterparts. This additionally provided a further opportunity to validate the definition of biological stability. These findings agree with best practices of companies in countries using fewer chemicals than in the UK, as well as findings in literature. Should utilities in future wish to go chemical free, it is recommended that a thorough contemplation of location be considered. Ideally, sites across the utility would be categorised by biological stability to assess the readiness of each location to adapt for this approach with minimal water quality changes. The results indicated that chemical free water will likely be easier in areas of better biological stability.

6 Chapter 6: Discussion

6.1 Research Summary

The vast majority of developed world water systems are chemically intensive. Despite the current popularity of chlorine and chlorine-based disinfectants, a series of disadvantages, predominantly the emerging understanding about the formation of harmful disinfection by-products are causing water utilities to question their use [Section 1.4.4.1]. Orthophosphate dosing is another widely used distribution network chemical that has many problems with its use, chief of which is that phosphate is a finite resource that will run out at some point in the near future [Section 1.4.3.2]. This additionally drives its cost for water utilities, but there is currently no known alternative for the control of toxic lead release at the customer tap. It is for these reasons and other motivations for water utilities to reconsider their traditional chemically intensive practices have been discussed as part of this research.

Water utility companies in different developed countries now recognise the need to change practices but are at various stages of progression to adopt chemical free drinking water treatment and distribution. An investigation of the best practices of companies in other countries which currently use fewer chemicals than in the UK, include VCS in Denmark, Stadtwerke Düsseldorf in Germany and PWN in the Netherlands [Section 1.3]. These case study areas were used in comparison with the case study company Anglian Water in the UK. The comparison has been completed to better understand the system characteristics, monitoring and management in these countries. It was identified that the biggest area of discrepancy between the UK case study company and the other case study companies and so the area with the greatest amount of potential for development, was that of the network. One recurring factor that all the case study water utilities agreed as being of the upmost importance for maintaining the quality of water at the consumer tap while supplying water with fewer chemicals, was that of biological stability.

Biological stability is a concept with many different definitions, with different researchers providing their own interpretations in a multitude of studies [Section 1.5.1]. As there is such a variety of definitions, using and combining the advantages of previous definitions, a new definition of biological stability has been formed for the purpose of this PhD, which was devised, tested and refined [Section 3.2.2, Section 3.4.2].

> *"Biological stability can be defined as the preservation of water quality from the water treatment works to the consumer tap, with good biological stability being characterised by lower values and/or consistent values in a variety of key indicators including: both the attached and planktonic microorganisms of the distribution network; available nutrients; wider environmental water quality parameters and the aesthetics of the water."*

Although these are the fundamentals of the definition, the full version also encompasses a combination of methods to analyse microbial and environmental parameters, as well as the acknowledgement that a number of other factors can also affect the biological stability during distribution and a risk score matrix for the classification of "Good Biological Stability, "Less Good Biological Stability" and "Poor Biological Stability" [Section 3.4.2, Figure 22]. The definition used in the current research was provided alongside a summary of the different approaches to assess biological stability, to provide an indicator of how it could potentially be monitored within UK

systems. Of these techniques, flow cytometry was selected as a monitoring technique which acts as an appropriate indicator for biological stability in the UK, as a successful medium for the detection, enumeration and characterisation of waterborne microbial populations [Section 1.5.2.4]. The practicability of this assessment method for UK water companies was tested during a 3 month water utility-wide flow cytometry campaign of the Final Water of 125 WTWs, confirming it as a quick, repeatable and practical option for further use [Section 3.2.7, Figure 4].

Chapter 3 explored historic regulatory samples supplemented with flow cytometry (TCC and ICC) samples at a series of 6 case study sites to improve the understanding of biological stability in the current state of the system and to determine the potential for chemical free drinking water treatment and distribution. It was found that TCC and, to a lesser extent ICC, correlated well with a number of other water quality parameters at the WTW [Figure 19, Figure 20]. The current monitoring methods used by the UK, such as *E. coli* and *Enterococci* plate counting were not a valuable source of information and were mostly negative values while flow cytometry values persisted. This was evidenced in Chapter 3 [Figure 13] and Chapter 5 [Figure 64]. As such, flow cytometry showed promise as a tool to provide a rich source of information, as well as acting as a biological stability monitoring method. It would have been highly valuable to also have this data at a Customer Tap level. Despite this, flow cytometry alone is not recommended to provide a standalone measurement of biological stability. It is instead expressed that flow cytometry is a useful indicator of biological stability at the WTW when used in conjunction with a number of different key indicators of water quality including the microorganisms of the distribution network, available nutrients, wider environmental water quality parameters and the aesthetics of the water, as stipulated in the definition of biological stability for this PhD.

The definition provided in the current PhD in conjunction with the use of flow cytometry samples and historic regulatory data successfully categorised the 6 case study sites as having varied biological stabilities, in the manner suggested by case study companies in countries using fewer chemicals than in the UK [Section 3.3.1]. Sites A-D were categorised as sites of “less good” biological stability, while Sites E and F were categorised as sites of “good” biological stability [Appendix 4]. Sites E and F had particularly good water quality, better than that of Sites A-D, with consistently lower values and at times stability in how parameters varied at a sampling site over time and within the network. Sites E and F were also found to have had a higher level of compliance than other case study areas. It is also noted that there were no case study sites regarded as “poor” biological stability [Section 3.3.3]. No evidence was found of water quality deterioration, temporal or otherwise, at any case study site and the majority of samples observed during the course of the study were compliant to regulatory standards [Table 10, Figure 17, Figure 18]. Following the application of the proposed definition of biological stability [Section 3.2.2] to 6 case study sites which acted as verification of this risk scoring matrix, this definition was then refined, as described in Section 3.4.2.

Although the case study companies in countries using fewer chemicals than in the UK discussed the importance of biological stability at length, these companies did not have the addition of distribution network chemicals to potentially alter this stability [Section 1.3]. Chapter 4 explored the link between chlorine, phosphate and biological stability at 6 case study sites to improve the understanding of distribution chemical use in the current state of the system and to discuss the likely system impacts if the dose of the chemicals were stopped in future.

The main purpose of phosphate application is to reduce the dissolution of lead in the network [Section 1.4.3.2]. It is thought that the phosphate dose applied at the WTW is consumed throughout the network to prevent plumbosolvency. However, there was a highly substantial and very persistent phosphate residual presence at the customer tap [Figure 29]. According to these results, it appeared that the phosphate dose was not being utilised efficiently through the network and was instead being overdosed. The overdosing of phosphate is not surprising (as noted in Section 1.4.3.2) but previously there has been very limited published evidence to confirm this. Further, even though the main purpose of phosphate is for plumbosolvency, it was found to have relationships with a wide range of water quality parameters (including pH, hardness, total copper, total iron, total manganese, calcium, conductivity, alkalinity, water temperature, sodium, potassium and magnesium), many of which were stronger than that of lead [Figure 37]. The relatively poor correlation between lead and phosphate was not unexpected due to the sporadic sampling of lead, that lead concentration in tap water is highly variable and that many properties have no lead. The link between phosphate and biological stability was tenuous, with the varying biological stabilities of different case studies not impacting how phosphate was being used or how phosphate itself was affecting the network environment. As such biological stability would be unlikely to be a driving factor for the future removal of phosphate. The findings from Chapter 4 suggested that if phosphate were to be removed, it is highly likely that a large number of water quality parameters will be impacted. Further research would need to be done to fully understand the measures needed to prepare for this potential eventuality, for instance supplementary pH control techniques.

Meanwhile, the application of a chlorine dose was found to influence water quality in a similar way to other research (especially in the area of strong relationships with pH and temperature) [Figure 25]. Chlorine was found to have consistent relationships with temperature, pH and conductivity, with hardness and calcium also expressing an influence at times, with more of a relationship at the WTW than at the Tap. Inconsistent and often weak relationships between chlorine and microbial abundance posed the question as to whether chlorination was fulfilling its purpose [Figure 28]. Chlorination was not universally reducing microbial abundance as intended, which could potentially mean that the transition to chemical free water would not cause as much microbial disruption as previously thought [Figure 26, Figure 27, Figure 28]. Biological stability was found to influence how chlorine was consumed in the different networks, with sites of less good biological stability having a greater rate of chlorine decay [Figure 23]. But this biological stability did not shape how chlorine impacted water quality or the relationship between chlorine and microbial abundance [Section 4.3.1.4]. It is clear that the concept of biological stability plays a part in chlorination but it is uncertain what the impact would be on the network were chlorine to be removed, particularly if sites previously of good biological stability would then become less good or vice versa.

Although both chlorine and phosphate are similar in that they are both chemicals added to maintain drinking water quality from the point of production to the point of consumption, Chapter 4 found them to be very different in the way they were used and how they influenced different networks. Despite this, while biological stability was found to influence how chlorine was consumed in the different networks, it did not shape how either distribution network chemical impacted water quality. This work potentially suggests that biological stability was not as important to achieve chemical free water as case study countries suggested, however it is difficult to theorise the possible impacts of not having a chemical dose in the UK, when all UK networks have a dose of chlorine and phosphate applied. This thinking forged the investigation explored in Chapter 5.

Chapter 4 considered the potential implications of stopping the dose of chlorine and phosphate in UK distribution networks, drawing on historic regulatory samples. Chapter 5 took this further, as a series of novel purpose-built re-circulating test loop facilities were designed and constructed, purpose built for this PhD research. These facilities allowed for the wider impact of the presence and absence of chlorine and phosphate to be studied within the distribution network without any risk of altering public health. The facilities were designed in such a way as to allow for the bulk water quality and chemistry to be investigated as well as the exchange mechanisms between the bulk water and the pipe wall [Figure 41]. Differing levels of biological stability were also able to be studied due to the production of four identical pipe testing facilities, two (chemically fed and chemical free) at a case study site categorised as having good biological stability and two (chemically fed and chemical free) at a case study site categorised as less good biological stability [Figure 48].

All of the outlet samples of the experimental rig facilities, including those of the chemical free rig and those at sites of less good biological stability (as scored in Chapter 3), contained drinking water which was within the realm of the reasonable minimum requirements of safe drinking water with no strong decrease in water quality during the facility operation [Figure 50, Figure 51]. These samples were similar to that of live actual WTW and Customer Tap samples, for instance all *E. coli*, *Enterococci* and *C. perfringens* samples throughout were 0 no/100ml [Figure 64]. Despite this finding, there was some variation in water quality between the different experimental conditions, in a way similar to the 6 initial case study sites not being categorised as having bad biological stability but still having varying biological stabilities as observed in Chapter 3.

The biological stability matrix presented in Chapter 3 [Figure 22] was applied to the experimental facilities in Chapter 4 [Appendix 5], providing a further opportunity to validate the definition of biological stability. None of the rig facilities were found to be of Poor Biological Stability, despite Rig 2 of each not having received a chemical dose, potentially indicating that chemical free drinking water would be a possibility in the UK. Rather, all experimental conditions had the same overall classification (Less Good Biological Stability), although Surface Water Facilities consistently scored as having lower stability than their Groundwater counterparts.

There were some incidences of water quality differences observed depending on if experimental rigs were treated with or without chemicals [Figure 62]. This supports the work of Chapter 4, that chemicals impact and influence the drinking water quality within the network [Figure 25, Figure 37]. The application of distribution chemicals in the chemically fed rigs were found to result in more temporally stable drinking water quality, particularly in the parameters pH and alkalinity [Figure 62]. However, of the four experimental facilities with their differing conditions, the chemically fed facility at the sites with less good biological stability was found to have the highest values, as well as some of the highest values detected at all sampling points during experimentation. For instance, the microbiology samples TCC, ICC, 3 day colony count and 2 day colony count were highest at this location [Figure 58]. This implies that despite the application of chemicals resulting in more stable chemistry, the network microbiology may not be as serene.

The most substantial water quality differences occurred in the experimental facilities depending on the site location [Figure 57]. This was found to be a major driving factor, more so than that of chemical presence or absence. Where water quality differed between the different sites, the site with less good biological stability (as assessed in Chapter 3) was frequently found to have higher

values. For example, both of the facilities on this site, those containing chemicals and those that did not, consistently had more microorganisms than both of the facilities on the site with good biological stability [Figure 58]. This finding was consistent with literature, the practices of case study companies using fewer chemicals than in the UK and with the findings of initial research into biological stability presented in Chapter 3. The experimental facilities at the site with good biological stability (both the chemically fed facility and the chemical free facility) were also found to be more similar to each other than those at the site of less good biological stability [Figure 61]. This potentially means that chemicals have more of an influence at sites of less good biological stability and so removing them from these sites has a greater water quality impact than removing them from a site of good biological stability, where initial water quality is good and chemicals do little to improve it. This therefore suggests that safe chemical free drinking water treatment and distribution would be a more attainable goal if it were completed at a location with good biological stability.

Thus, in the area of chemical free drinking water treatment and distribution, a number of findings were identified. Chapter 5 postulated that some difference in water quality is to be expected when removing chemicals in the UK and Chapter 6 found evidence that this is true. Although, surprisingly, the removal of phosphate appears as though it would be more likely to have a greater impact on measured water quality parameters than that of chlorine [Figure 25, Figure 37]. Phosphate is clearly an understudied area, especially when compared with the wealth of understanding surrounding chlorine. This is especially apparent as new operational strategies, such as the seasonal application of a phosphate dose, are being introduced but the desired effect, an impact on lead concentrations, has not been achieved [Figure 37]. The residuals detected in case study networks, compounded by the diminishing phosphate reserves and the risk of lead to consumers, indicate that it is of great need for further work. It is also maintained that the attached microbial community within the network and the impact chemical removal would have on this is still vastly unknown.

When considering the concept of biological stability, a series of theories were explored. Chapter 5 studied how the idealised pipe network would react if it received a chemical free dose. This research evidenced that safe chemical free drinking water was not impossible in the UK, even though the formation of scale posed a novel concern that would need to be addressed in future [Figure 56]. It was found that the idealised pipe network did not have a deterioration in water quality when chemicals were not used to treat drinking water, although biological stability was very much an influencing factor into this feasibility. However, Chapter 4 did not find substantial evidence that the biological stability of sites influenced how chemicals impacted water quality in historic data analysis. Chapter 4 and Chapter 5 had different findings about how chemicals influenced water quality because of the differences between the live network and the experimental facilities. The facilities represented the idealised network, for example with: next generation pipe material, consistency of pipe material, brand new in age, freshly disinfected pipes, consistent residency times, consistent flows, no diurnal variation, no pipe ingress, no leakage, no areas of stagnation and no influence of household fittings, fixtures or pipes. The experimental rig facility investigation suggested that for chemical free water to be possible, sites of better biological stability are required. The network fed from preliminary selected sites then needs to be reviewed, renewed and monitored, in a way similar to case study countries currently using fewer chemicals than in the UK. Overall, this study has found that the provision of safe and chemical free water in the UK is possible, but it would not be easy and would require a great deal of investment if it is still a desirable aim in future.

6.2 Further Recommendations for Future Work

Overall, it is likely that the future of biological stability for the UK is the adoption of emerging technologies to better and more accurately detect failures in water quality, particularly using flow cytometry. Universities and water companies have expressed interest in organising and attending flow cytometry working group meetings to work collaboratively to share their knowledge and best practices for use of this technology. Specifically, the use of TCC and ICC is being increasingly used in water utilities interstage at WTW to enable companies to determine deviations in asset performance. There is interest to increase its usage further in future, with potential to use it at the consumer tap for regulatory samples (Gillespie, et al., 2014). TCC or ICC is not currently required by regulation but there is potential that it may be utilised henceforth, especially due to the drawbacks of the current use of plate counting, including how only a small proportion of metabolically active microorganisms can be detected. The use of regular interstage and consumer tap flow cytometry could help achieve this future aim, as well as having an increased understanding of baseline biological stability in these systems. Although, flow cytometry should not be solely relied upon as the methods of assessing biological stability have different advantages that could complement each other when used together in a multilevel approach (Lautenschlager, et al., 2013).

Going forward, biological stability and enhanced monitoring of this biology is likely to become more important for the maintenance of water quality because continuing to supply safe drinking water in future is likely to become more challenging. Climate change, for instance, has many likely impacts on the planet, including higher average air temperatures, increased precipitation intensity, sea level rises, hot extremes and increased frequency of extremes of the hydrologic cycle, floods and droughts, all of which will alter the amount, distribution, timing and quality of available drinking water (Patz, et al., 2008) (Coffey, et al., 2013) (IPCC, 2018) (Johnson, et al., 2009). A further challenge is that population growth is thought to increase over the next 25 years, increasing the demand for water. According to the United Nations, if present consumption patterns continue, two-thirds of the world's population will live in water-stressed conditions by the year 2025 (World Water Assessment Programme, 2012). The increased demand is going to be difficult to cater for with the current aging infrastructure in the UK, on average ~75-80 years and the replacement rates not keeping up (Speight, 2015). Indeed, these challenges, such as climate change and population growth, will become even more difficult when considered in combination with the difficulties posed to enable chemical free drinking water treatment and distribution to be a possibility. To be able to supply the quantities of water required as well as the excellent quality supplied today, long term research is needed and should be prioritised, such as that of chemical free drinking water treatment.

Recommendations for what this future potential experimentation into chemical free water could encapsulate are presented below, including trials in the live network, future research using the experimental rig facilities and the wider desirable data and supplementary samples that would have added to the present body of research.

- Future potential live network experimentation:
  - It would be desirable to have water utilities continue to monitor TCC and ICC using flow cytometry over a longer period of time to help establish a historic backlog of data similar to that of the present regulatory samples. This could be used to act more proactively than reactively, as a more immediate indicator of asset condition.
  - Ideally water utility companies will categorise further sites by their varying biological stabilities using the matrix presented in this research and building on this matrix. Doing so would enable them to detect potential areas where chemical free water is a possibility in future and also where further investment is required.
  - Future work should focus on the importance of chemical reactions and their relationship to biological stability. It would be recommended to have more sampling locations outside of this very hardwater area, to see if this occurrence is a localised one or if it occurs elsewhere in the country. Hardness is a poorly studied area, particularly the impact of very hard waters (such as those concentrations detected in the case study sites) on wider water quality or the microbiome.
  - There is a clear lack of research in the area of phosphate residual at the consumer tap. The phosphate residual identified in this study is abundant and so the question of efficiency in dose persists, it is influencing water quality and it is clearly a factor that needs consideration to further optimise networks. A determination if this phosphate residual is a UK wide occurrence would be of aid. An assessment of the microbial communities present, for example with the use of DNA analysis, would provide a valuable source of information regarding community and potential microbiome community changes when the seasonal dosing was occurring at case study sites.
  - The next stage when assessing chemical free water would be to identify an area of the live network with the most similarities to that of the experimental facility. This area would be used as a case study to trial if chemical free water is a possibility within the live network.

- Future potential experimental rig facility work:
  - To study the use of a “mobilisation” phase, to analyse the biofilm response to increasing shear stress under controlled increases in flow rate. This could be done to determine if biofilm shearing responded differently under the differing experimental conditions.
  - Operating the rig facilities for a greater period of time, for instance over the course of a year, to see the extent to which the facilities fouled over time and to gauge any impacts from seasonality, especially when considering a comparison of sites of good and less good biological stability.
  - Further observations of the scale formation to better understand the reactions occurring within the model distribution system, including capturing scale samples and completing further analysis for chemical construction as well as quantification by sample location. This could be completed with powder X-ray diffraction, scanning electron microscopy and atomic absorption spectroscopy. These samples should be used in conjunction with further and more frequent samples surrounding pH, hardness and similar parameters to better calculate indices of scale-forming calculations at a wider number of sampling locations.

- If scale was not the purpose of future research but a persistent by-product, mechanisms could be put in place to reduce the scale formation within the experimental facilities. There are a number of alternative non-chemical treatment options available, among these are the use of magnetic, electronic and electrolytic treatment devices, as well as the use of physical removal methods such as the filtration of Inlet water.
- Stopping the dose of phosphate in case study areas to see if the phosphate/lead relationship then strengthens.
- It would also be interesting to study the transition, the movement of chemicals to no chemicals, within the experimental rig facility.
- To fit the experimental pipe facility with older pipe materials to see if the findings obtained remained the same outside of the "idealised" pipe system.

- Desirable data and supplementary samples for future research:
  - The use of samples in triplicate, rather than one off grab samples typically used by water utilities for regulatory samples.
  - Taking samples from the network itself, rather than an assessment at the WTW and at the Tap, which were used to assume case study network conditions. This could additionally include sampling at the furthest reaches of the network to see if the correlations with chlorine and phosphate identified shifted at varying points throughout the network.
  - Further information about the network environment to build a richer picture of the network, with factors such as hydraulic conditions, residence time, maintenance practices and premise plumbing conditions.
  - An assessment of chlorine decay at various points of the network would have been helpful in combination with an awareness of biological stability, as these factors together, are rarely discussed. This is likely because many of the countries who consider the concept of biological stability, such as the Netherlands, do not chlorinate.
  - An assessment of the attached microorganisms within the network, rather than solely bulk water analysis, both of the live network and within experimental facilities. Attached microbial evaluation would ideally consist of TCC and ICC analysis as well as DNA analysis and SEM microscopy.
  - A more diverse parameter pool including new advanced techniques and novel combinations of these techniques. For example, the use of flow cytometry with other biomonitoring methods such as AOC, the application of a combination of imaging, sorting, fingerprinting and sequencing and an assessment of DBP presence and concentrations.

7 Chapter 7: Conclusions

This chapter shows the key conclusions investigated in the three main chapters presented in this thesis, an investigation of biological stability in Chapter 3, an examination of the chemicals used to distribute water in Chapter 4 and the exploration of how these biological stability and chemicals influenced water quality within a series of innovative bespoke pipe test loops in Chapter 5. In this work, the understanding of the interactions between chemicals, water quality and biological stability in drinking water distribution systems have been improved to determine if chemical free water is a possibility in the UK.

7.1 Improving the Understanding of Biological Stability

- A definition of biological stability was provided, verified and refined in the current PhD research.
- Flow cytometry acted as an effective indicator of biological stability at the WTW when used in conjunction with other indicators of water quality, nutrient availability and water aesthetics.
- The definition of biological stability used in conjunction with the biological stability monitoring methods and a biological stability risk scoring matrix successfully categorised 6 case study sites as having “good” or “less good” biological stability.

7.2 Improving the Understanding of Current Chemical Usage

- Chlorine was found to have consistent relationships with temperature, pH and conductivity, with hardness and calcium also expressing an influence at times. There were inconsistent and often weak relationships between chlorine and microbial abundance.
- Although phosphate was found to have a relationship with lead, as intended by water utilities, there were a number of other water quality parameters that had a stronger relationship with phosphate.
- The wider water quality impacts of current chemical usage did not vary based on the biological stability of case study sites.
- The biological stability of different case study locations had an impact on how chlorine was consumed throughout the network, with more decay in sites of less good biological stability.
- Phosphate was found to have a highly substantial and very persistent phosphate residual at the customer tap which did not vary by case study biological stability.
- Dosing practices varied between chlorine and phosphate. Operators were found to understand chlorine dose well, with chlorine residual at the customer tap informing dose of chlorine at the WTW. The same was not found with phosphate, as phosphate concentration at the tap was not being used to inform dosing practices. The impact of chlorine was better understood than that of phosphate.

7.3 Investigating Chemical Dose at Different Sites using Innovative Bespoke Pipe Loops

- Innovative bespoke pipe test loops were successfully designed and constructed. Two identical full scale and idealised pipe test loops housed in one container enabled direct comparison of chemical presence and absence. Two identical containers allowed varying biological stabilities at multiple sites to be assessed.

- A risk scoring matrix was applied to successfully categorise experimental facilities by biological stability. The matrix scored all facilities the same, as “Less Good” biological stability, including those without a chemical dose, although Surface Water Facilities consistently scored as having lower stability than their Groundwater counterparts.

- The experimental facilities did have some incidences of water quality differences depending on if experimental rigs were treated with or without chemicals, specifically in the area of chemical stability (pH and alkalinity), but the driving factor for water quality differences between experimental conditions was the incoming biological stability of the water.

- The better the biological stability, the less valuable the chemicals were found to be.

- There was not a deterioration in water quality when distribution chemicals were not used to treat drinking water, especially in the biologically stable area, suggesting that the provision of safe and chemical free water in the UK is possible.

References

Abdelgader, G., 2014. Characterisation of Phosphate Rocks at Kurun Mountain, Sudan, Khartoum: University of Khartoum.

Abraham, E., Blokker, M. & Stoianov, I., 2018. Decreasing the Discoloration Risk of Drinking Water Distribution Systems through Optimized Topological Changes and Optimal Flow Velocity Control. Journal of Water Resources Planning and Management, 144 (2). doi: https://doi.org/10.1061/%28ASCE%29WR.1943-5452.0000878

Adetunji, V. & Isola, T., 2011. Crystal Violet Binding Assay for Assessment of Biofilm Formation by Listeria monocytogenes and *Listeria spp* on Wood, Steel and Glass Surfaces. Global Veterinaria, 6(1), pp. 1208-1212.

Adomat, Y. et al., 2020. New Methods for Microbiological Monitoring at Riverbank Filtration Sites. Water, 12(584). doi: https://doi.org/10.3390/w12020584

Al-Jasser, A., 2011. Pipe Service Age Effect on Chlorine Decay in Drinking-Water Transmission and Distribution Systems. Clean Soil Air Water, 39(9), pp. 827-832.

Alnnasouri, M. et al., 2011. Influence of Surface Topography on Biofilm Development: Experiment and Modelling. Biochemical Engineering Journal, 57, pp. 38-45.

American Water Works Association, 1995. Advances in Taste-and-Odour Treatment and Control. Denver: American Water Works Association.

American Water Works Association, 1995. Water Treatment: Principles and Practices of Water Supply Operations. 2nd ed. Denver: AWWA.

American Water Works Association, 2002. Effect of Water Age on Distribution System Water Quality, Washington: Office of Groundwater and Drinking Water.

Anderson, C., 1991. Cholera Epidemic Traced to Risk Miscalculation. Nature, 354, p. 255.

Anderson, WB., Slawson, RM. & Mayfield, CI., 2002. A Review of Drinking-Water-Associated Endotoxin, Including Potential Routes of Human Exposure. Canadian Journal of Microbiology, 48(7), pp. 567-587.

Anglian Water, 2018. Carbon Goals. [Online] Available at: http://newhawk/AboutUs/Business-guide-AMP-6/Pages/Carbon-goals.aspx [Accessed 10 July 2018].

Anglian Water, 2020. Fast Facts: A Quick Guide to your Water Company. [Online] Available at: https://www.anglianwater.co.uk/about-us/media/fast-facts/#:~:text=Each%20year%20we%20carry%20out,to%20the%20highest%20quality%20possible. [Accessed 9 December 2020].

Anglian Water, 2020. Hardness. [Online] Available at: https://www.anglianwater.co.uk/help-and-advice/drinking-water-advice/hard-water/ [Accessed 29 December 2020].

Anglian Water, 2020. Love Every Drop. [Online] Available at: http://newhawk/AboutUs/LoveEveryDrop/Pages/Love-Every-Drop.aspx [Accessed 26 January 2020].

Anglian Water, 2020. Management of Hazardous Substances. [Online] Available at: http://newhawk/SafeandWell/Pages/Hazardous-Substances.aspx [Accessed 25 January 2020].

Anglian Water, 2020. PSW-PRO-8.13: Collecting and Labelling Operational Water Quality Samples. Peterborough: Anglian Water.

Bauman, W., Nocker, A., Jones, W. & Camper, A., 2009. Retention of a Model Pathogen in a Porous Media Biofilm. Biofouling, 25(3), pp. 229-240.

Behnke, S., Parker, A., Woodall, D. & Camper, A., 2011. Comparing the Chlorine Disinfection of Detached Biofilm Clusters with Those of Sessile Biofilms and Planktonic Cells in Single- and Dual-Species Cultures. Applied and Environmental Microbiology, 77(20), pp. 7176-7184.

Bertelli, C. et al., 2018. Reduced Chlorine in Drinking Water Distribution Systems Impacts Bacterial Biodiversity in Biofilms. Frontiers in Microbiology, 9, p. 2520.

Bitton, G., 2014. Microbiology of Drinking Water Production and Distribution. 1st ed. Singapore: Wiley Blackwell.

Black & Veatch Corporation, 2010. White's Handbook of Chlorination and Alternative Disinfectants. Hoboken: John Wiley & Sons.

Blokker, E. et al., 2016. Relating Water Quality and Age in Drinking Water Distribution Systems Using Self-Organising Maps. Environments, 3(2), p. 10.

Borja, A., Muxika, I. & Rodríguez, J., 2009. Paradigmatic Responses of Marine Benthic Communities to Different Anthropogenic Pressures, using M-AMBI, within the European Water Framework Directive. Marine Ecology, 30(2), pp. 214-227.

Boyd, A. & Chakrabart, YA., 1995. Pseudomonas-aeruginosa Biofilms - Role of the Alginate Exopolysaccharide. Journal of Industrial Microbiology & Biotechnology. 15(3), pp. 162-168.

Brown, D., Bridgeman, J. & West, J. R., 2011. Predicting Chlorine Decay and THM Formation in Water Supply Systems. Reviews in Environmental Science and Biotechnology, 10, pp. 79-99.

Bull, R. & Kopfler, F., 1991. Health Effects of Disinfectants and Disinfection By-Products, Denver: American Water Works Association.

Burllingame, GA., Korntreger, G. & Lahann, C., 1995. Configuration of Standpipes in Distribution. Journal of the New England Water Works Association, 95(12), pp. 281-289.

Butt, S., 2016. Understanding Phosphate for Controlling Metals Release and Biofilm Formation in Drinking Water Distribution Systems. Halifax: Dalhousie University. doi: https://doi.org/10222/72608

Chambers, V. & Hitchmough, M., 1992. Economics of Lead Pipe Replacement, Swindon: WRc.

Chlorine Chemistry Association, 2003. Drinking Water Chlorination: A Review of Disinfection Practices and Issues, s.l.: s.n.

Coffey, R. et al., 2013. Assessing the Effects of Climate Change on Waterborne Microorganisms: Implications for EU and U.S. Water Policy. Human and Ecological Risk Assessment: An International Journal, 20, pp. 724-742.

ComCar, 2018. Kg CO2 per Litre of Petrol. [Online] Available at: https://comcar.co.uk/newcar/companycar/poolresults/co2litre.cfm?clk=a [Accessed 7 August 2018].

Comly, H., 1945. Cyanosis in Infants Caused by Nitrate in Well Water. Journal of the American Medical Association, 129, p. 112–116.

Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment, 2013. Subgroup Report on the Lowermoor Water Pollution Incident, s.l.: Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment.

Consumer Council for Water, 2015. Water Matters: Household Customers' Views on their Water and Sewerage Services 2015, Birmingham: Consumer Council for Water.

Control of Major Accident Hazards Regulations, 2015. Understanding COMAH: A guide for new entrants, London: COMAH.

Cordell, D., Drangert, J. & White, S., 2009. The Story of Phosphorus: Global Food Security and Food for Thought. Global Environmental Change, 19(2), pp. 292-305.

Corfitzen, C. et al., 2006. Rapid Methods for Detection of Bacteria. Icelandic Water and Wastewater Association, Reykjavik, pp. 139-143, 5.

Cornman, R. et al., 2018. An Experimental Comparison of Composite and Grab Sampling of Stream Water for Metagenetic Analysis of Environmental DNA. PeerJ, Issue 6. doi: https://doi.org/10.7717/peerj.5871

Costerton, J. W., Stewart, O. & Greenberg, E., 1999. Bacterial Biofilms: A Common Cause of Persistent Infections. Science, 284(5418), pp. 1318-1322.

Cozzolino, L., Pianese, D. & Pirozzi, F., 2005. Control of DBPs in Water Distribution Systems Through Optimal Chlorine Dosage and Disinfection Station Allocation. Desalination, 176(1-3), pp. 113-125.

Craun, G., 2012. The Importance of Waterborne Disease Outbreak Surveillance in the United States. Annali dell'Istituto Superiore di Sanità, 48(4), pp. 447-459.

Davis, P. et al., 2006. Final Report: Long Term Performance Prediction for PE Pipes, Denver: AWWA.

Deines, P. et al., 2010. A New Coupon Design for Simultaneous Analysis of In-Situ Microbial Biofilm Formation and Community Structure in Drinking Water Distribution Systems. Applied Microbiology and Biotechnology, 87(2), pp. 749-56.

Del Olmo, G. et al., 2020. Influence of Phosphate Dosing on Biofilms Development on Lead in Chlorinated Drinking Water Bioreactors. Biofilms and Microbiomes, 6 (1). doi: https://doi.org/10.1038/s41522-020-00152-w

Demnati, R., Fraser, R., Plaa, G. & Malo, J., 1995. Histopathological effects of acute exposure to chlorine gas on Sprague-Dawley rat lungs. Journal of Environmental Pathology, Toxicology and Oncology, 14(1), p. 15–19.

Department of the Environment, 2014. National Pollutant Inventory: Chlorine and Compounds. [Online] Available at: http://www.npi.gov.au/resource/chlorine-and-compounds [Accessed 07 06 2016].

Department of the Environment, 2014. National Pollutant Inventory: Phosphoric Acid. [Online] Available at: http://www.npi.gov.au/resource/phosphoric-acid [Accessed 07 06 2016].

Donnermair, M. & Blatchley, E., 2003. Disinfection Efficacy of Organic Chloramines. Water Research, 37(7), pp. 1557-1570.

Douglas, I., Guthmann, J., Muylwyk, Q. & Snoeyink, V., 2004. Corrosion Control in the City of Ottawa-Comparison of Alternatives and Case Study for Lead Reduction in Drinking Water. Eleventh Canadian National Conference and Second Policy Forum on Drinking Water, s.n.

Douterelo, I. et al., 2020. Impact of Phosphate Dosing on the Microbial Ecology of Drinking Water Distribution Systems: Fieldwork Studies in Chlorinated Networks. Water Research, 187(15). doi: https://doi.org/10.1016/j.watres.2020.116416

Douterelo, I., Fish, K. & Boxall, J., 2018. Succession of Bacterial and Fungal Communities Within Biofilms of a Chlorinated Drinking Water Distribution System. Water Research, 141, pp. 74-85.

Douterelo, I., Jackson, M., Soloman, C. & Boxall, J., 2016. Microbial Analysis of In Situ Biofilm Formation in Drinking Water Distribution Systems: Implications for Monitoring and Control of Drinking Water Quality. Applied Microbiology and Biotechnology, 100(7), pp. 3301-3311.

Douterelo, I., Sharpe, R. & Boxall, J., 2013. Influence of Hydraulic Regimes on Bacterial Community Structure and Composition in an Experimental Drinking Water Distribution System. Water Research, 47(2), pp. 503-516.

Drinking Water Inspectorate, 2000. Guidance on the Implementation of the Water Supply (Water Quality) Regulations 2000 (as amended) in England, London: Drinking Water Inspectorate.

Drinking Water Inspectorate, 2010. Chlorine, London: Drinking Water Inspectorate.

Drinking Water Inspectorate, 2010. Discoloured Water, London: Drinking Water Inspectorate.

Drinking Water Inspectorate, 2012. DWI PR14 Guidance – Disinfection By-products, s.l.: s.n.

Drinking Water Inspectorate, 2016. The Water Supply (Water Quality) Regulations, London: Drinking Water Inspectorate.

Drinking Water Inspectorate, 2018. Drinking Water 2018: A Report by the Chief Inspector of Drinking Water, London: Drinking Water Inspectorate.

Drinking Water Inspectorate, 2019. Nitrate, London: Drinking Water Inspectorate.

Duranceau, S., Lintereur, P. & Taylor, J., 2010. Effects of Orthophosphate Corrosion Inhibitor on Lead in Blended Water Quality Environments. Desalination and Water Treatment, 13(1-3), pp. 348-355.

El-Chakhtoura, J. et al., 2015. Dynamics of Bacterial Communities Before and After Distribution in a Full-Scale Drinking Water Network. Water Research, 74(1), pp. 180-190.

Environment Agency, 2012. Review of Best Practice in Treatment and Reuse/Recycling of Phosphorus at Wastewater Treatment Works, London: Environment Agency.

Environment Agency, 2017. Protect Groundwater and Prevent Groundwater Pollution, London: Environment Agency.

European Commission, 1998. Council Directive 98/83/EC of 3 November 1998 on the Quality of Water Intended for Human Consumption. s.l.: European Commission.

European Commission, 2016. Legislation: The Directive Overview. [Online] Available at: http://ec.europa.eu/environment/water/water-drink/legislation_en.html [Accessed 20 September 2016].

European Commission, 2016. Urban Wastewater Directive Overview. [Online] Available at: http://ec.europa.eu/environment/water/water-urbanwaste/index_en.html [Accessed 21 November 2016].

European Institution for Testing, Inspection and Certification, 1998. Developing a New Protocol for the Monitoring of Lead in Drinking Water, Amsterdam: KIWA.

Farkas-Himsley, H., 1964. Killing of Chlorine-Resistant Bacteria by Chlorine-Bromine Solutions. Applied Microbiology, 12(1), pp. 1-6.

Fass, S. et al., 1996. Fate of Escherichia coli Experimentally Injected in a Drinking Water Distribution Pilot System. Water Research, 30, pp. 2215-2221.

Fish, K., 2013. The Impact of Hydraulic Regime upon Biofilms in Drinking Water Distribution Systems, Sheffield: University of Sheffield.

Fish, K. & Boxall, J., 2018. Biofilm Microbiome (Re)Growth Dynamics in Drinking Water Distribution Systems Are Impacted by Chlorine Concentration. Frontiers in Microbiology, 9, p. 2519.

Fish, K., Osborn, A. & Boxall, J., 2017. Biofilm Structures (EPS and Bacterial Communities) in Drinking Water Distribution Systems are Conditioned by Hydraulics and Influence Discolouration. Science of the Total Environment, 593(4), pp. 571-580.

Flemming, H., Percival, C. & JT, W., 2002. Contamination Potential of Biofilms in Water Distribution Systems. Water Science and Technology, 2(1), pp. 271-280.

Food and Agriculture Organization of the United Nations, 2015. World Fertiliser Trends and Outlook to 2018, s.l.: Food and Agriculture Organization of the United Nations.

Fulton, T. & Budd, G., 1992. Disinfection Alternatives for Safe Drinking Water. London: Van Nostrand Reinhold International.

Gibbs, R., Scutt, J. & Croll, B., 1990. Microbiological and Trihalomethane Responses to Booster Chlorination. Water and Environment Journal, 4(2), pp. 131-139.

Gillespie, S. et al., 2014. Assessing Microbiological Water Quality in Drinking Water Distribution Systems with Disinfectant Residual Using Flow Cytometry. Water Research, 65, pp. 224-234.

Gomes, I., Simõesa, M. & Simõesa, L., 2014. An Overview on the Reactors to Study Drinking Water Biofilms. Water Research, 62, pp. 63-87.

Gooddy, D. et al., 2017. Mains Water Leakage: Implications for Phosphorus Source Apportionment and Policy Responses in Catchments. Science of The Total Environment, 579, pp. 702-708.

Gov.UK, 2015. Water and Treated Water, London: Department for International Trade.

Gov.UK, 2018. Significant Fine for Water Company Following Hazardous Chemical Leak. [Online] Available at: https://www.gov.uk/government/news/significant-fine-for-water-company-following-hazardous-chemical-leak [Accessed 1 June 2020].

Govier, P. & Coulson, J., 2018. Civilian Exposure to Chlorine Gas: A Systematic Review. Toxicology Letters, 293, pp. 249-252.

Grefte, A. et al., 2011. Improving the Biological Stability of Drinking Water by Ion Exchange. Water Science & Technology: Water Supply, 11(1), pp. 107-112.

Hach, 2018. Chlorine, Free and Total, High Range, Loveland: Hach.

Hach, 2020. DR300 Pocket Colorimeter. [Online] Available at: https://www.hach.com/dr300-pocket-colorimeter-chlorine-free-total-with-box/product-details?id=55321383862 [Accessed 18 June 2020].

Harger, J., 2010. Damage to Vegetation by Chlorine Gas. International Journal of Environmental Studies, 4(1-4), pp. 93-108.

Hassard, F. & Whitton, R., 2019. Understanding the Use of Flow Cytometry for Monitoring of Drinking Water, Bedford: Drinking Water Inspectorate.

Hayes, C. & Croft, T., 2012. An Investigation into the Representativeness of Random Daytime Sampling for Lead in Drinking Water, Using Computational Modelling. Journal of Water Supply: Research and Technology, 61(3), pp. 142-152.

Hayes, C. et al., 2014. Computational Modelling Techniques in the Optimization of Corrosion Control for Reducing Lead in Canadian Drinking Water. Water Quality Research Journal of Canada, 49(1), pp. 82-93.

Hayes, C., Incledion, S. & Balch, M., 2008. Experience in Wales (UK) of the Optimisation of Orthophosphate Dosing for Controlling Lead in Drinking Water. Journal of Water and Health, 6(2), pp. 177-185.

Heim, T. & Dietrich, A., 2007. Sensory Aspects and Water Quality Impacts of Chlorinated and Chloraminated Drinking Water in Contact with HDPE and cPVC Pipe. Water Research, 41(4), pp. 757-764.

Hill, D., 2014. Basic Microbiology for Drinking Water. 3rd ed. Denver: American Water Works Association.

Hoefel, D. et al., 2003. Enumeration of Water-Borne Bacteria Using Viability Assays and Flow Cytometry: A Comparison to Culture-Based Techniques. Journal of Microbiological Methods, 55(3), pp. 585-597.

Husband, S. & Boxall, J., 2011. Asset Deterioration and Discolouration in Water Distribution Systems. Water Research, 45(1), pp. 113-124.

Husband, S. & Boxall, J., 2016. Understanding and Managing Discolouration Risk in Trunk Mains. Water Research, 107, pp. 127-140.

Husband, S., Boxall, J.B. & Saul, A.J., 2008 Laboratory Studies Investigating the Processes Leading to Discolouration in Water Distribution Networks. Water Research, 42(16), pp. 4309-4318.

Husband, S., Fish, K., Douterelo, I. & Boxall, J., 2016. Linking Discolouration Modelling and Biofilm Behaviour Within Drinking Water Distribution Systems. Water Science and Technology: Water Supply, 16(4), pp. 942-950.

International Agency for Research on Cancer, 1991. Chlorinated Drinking-Water; Chlorination By-Products; Some Other Halogenated Compounds; Cobalt and Cobalt Compounds. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, No. 52 ed. Lyon: International Agency for Research on Cancer.

International Organization for Standardization, 2012. ISO 9080:2012: Plastics Piping and Ducting Systems -- Determination of the Long-Term Hydrostatic Strength of Thermoplastics Materials in Pipe Form by Extrapolation, s.l.: International Organization for Standardization.

International Water Association, 2010. Best Practice Guide on the Control of Lead in Drinking Water, London: International Water Association.

International Water Association, 2010. Guide for Small Community Water Suppliers and Local Health Officials on Lead in Drinking Water, London: International Water Association.

International Water Association, 2016. Global Trends & Challenges in Water Science, Research and Management, London: International Water Association.

Isle Utilities, 2015. International Experience on Chlorine Free Disinfection, s.l.: s.n.

Jang, H., Choi, Y., Ro, H. & Ka, J., 2012. Effects of Phosphate Addition on Biofilm Bacterial Communities and Water Quality in Annular Reactors Equipped with Stainless Steel and Ductile Cast Iron Pipes. The Journal of Microbiology, 50(1), pp. 17-28.

Jasinski, S., 2006. Phosphate Rock, Statistics and Information, s.l.: s.n.

Johnson, A. et al., 2009. The British River of the Future: How Climate Change and Human Activity Might Affect Two Contrasting River Ecosystems in England. Science of the Total Environment, 407(17), pp. 4787-4798.

Jones, R., Wills, B. & Kang, C., 2010. Chlorine Gas: An Evolving Hazardous Material Threat and Unconventional Weapon. West Journal of Emergency Medicine, 11(2), pp. 151-156.

Juhna, T., Birzniece, D. & Rubulis, J., 2007. Effect of Phosphorus on Survival of Escherichia coli in Drinking Water Biofilms. Applied and Environmental Microbiology, 73(11), p. 3755.

Kärrman, E. et al., 2004. Systemanalys av dricksvattenförsörjning med avseende på mikrobiologiska barriärer ochmiljöpåverkan, Stockholm: VA-Forsk.

Keevil, C., Mackerness, C. & Colbourne, J., 1990. Biocide Treatment of Biofilms. International Biodeterioration and Biodegradation, 26(2-4), pp. 169-179.

Keinänen, MM. et al., 2002. The Microbial Community Structure of Drinking Water Biofilms Can Be Affected by Phosphorus Availability. Applied and Environmental Microbiology, 68(1), pp. 434-439.

Keinänen, MM., Martikainen, PJ. & Kontro, MH., 2004. Microbial Community Structure and Biomass in Developing Drinking Water Biofilms. Canadian Journal of Microbiology, 50(3), pp. 183-191.

Kerneïs, A. et at., 1995. The Effects of Water Residence Time on the Biological Quality in a Distribution Network. Water Research, 29(7), pp. 1719-1727.

Kirmeyer, G. J., 2000. Guidance Manual for Maintaining Distribution System Water Quality, s.l.: American Water Works Association.

Kirmeyer, G., 2004. Optimizing Chloramine Treatment. Denver: American Water Works Association.

Kirmeyer, G., Clement, J. & Sandvig, A., 2000. Distribution System Water Quality Changes Following Implementation of Corrosion Control Strategies, Denver: AWWA Research Foundation.

Kistemann, T., Dangendorf, F. & Exner, M., 2001. A Geographical Information System (GIS) as a Tool for Microbial Risk Assessment in Catchment Areas of Drinking Water Reservoirs. International Journal of Hygiene and Environmental Health, 203, pp. 225-233.

Knowledge Transfer Network, 2008. Towards Chemical Free Water and Wastewater Treatment, London: Knowledge Transfer Network.

Lamb, N., 2020. Taking the Lead: An Insight into Orthophosphoric Acid Treatment for Lead Control in the UK Drinking Water Industry. Perspectives in Public Health, 140(3), pp. 133-134.

Langelier, WF., 1936. The Analytical Control of Anti-Corrosion Water Treatment. Journal of the American Water Works Association, 28(10), pp. 1500-1521.

Lantagne, D., 2008. Sodium Hypochlorite Dosage for Household and Emergency Water Treatment. Journal of American Water Works Association, 100, pp. 106-119.

Lautenschlager, K. et al., 2013. A Microbiology-Based Multi-Parametric Approach Towards Assessing Biological Stability in Drinking Water Distribution Networks. Water Research, 47(9), pp. 3015-3025.

Lautenschlager, K., 2011. Origin, Function and Stability of Microbial Communities in Non-Chlorinated Public Drinking Water. Zürich: ETH Zürich. doi: https://doi.org/10.3929/ethz-a-006741838

LeChevallier, et al., 1993. Examining the Relationship Between Iron Corrosion and the Disinfection of Biofilm Bacteria. Journal of American Water Works Association, 85(7), pp. 111-123.

LeChevallier, M., Babcock, T. & Lee, R., 1987. Examination and Characterization of Distribution System Biofilms. Applied and Environmental Microbiology, 53(12), pp. 2714-2724.

LeChevallier, M., Hassenauer, T., Camper, A. & McFeters, G., 1984. Disinfection of Bacteria Attached to Granular Activated Carbon. Applied Environmental Microbiology, 48, pp. 918-923.

LeChevallier, M., Lowry, C., Lee, G. & Gibbon, D., 1993. Examining the Relationship Between Iron Corrosion and the Disinfection of Biofilm Bacteria. Journal of American Water Works Association, 85(7), pp. 111-123.

LeChevallier, M., Welch, N. & Smith, D., 1996. Full-Scale Studies of Factors Related to Coliform Regrowth in Drinking Water. Applied Environmental Microbiology, 62, pp. 2201-2211.

Lehtola, M., 2002. Microbially Available Phosphorus in Drinking Water, Helsinki: University of Kuopio.

Lehtola, M., Miettinen, I. & Martikainen, P., 2002. Biofilm Formation in Drinking Water Affected by Low Concentrations of Phosphorus. Canadian Journal of Microbiology, 48(6), pp. 494-499.

Lenntech, 2016. Phosphorous Removal from Wastewater. [Online] Available at: http://www.lenntech.com/phosphorous-removal.htm [Accessed 21 November 2016].

Li, X. & Mitch, W., 2018. Drinking Water Disinfection Byproducts (DBPs) and Human Health Effects: Multidisciplinary Challenges and Opportunities. Environmental Science and Technology, 52, pp. 1681-1689.

Linden, KG., Hull, N. & Speight, V., 2019. Thinking Outside the Treatment Plant: UV for Water Distribution System Disinfection. Accounts of Chemical Research, 25(5), pp. 1226-1233.

Lintereur, P., 2006. Effects Of Source Water Blending Following Treatment with Sodium Silicate as A Corrosion Inhibitor on Metal Release Within a Water Distribution System. Florida: B.S. University of Central Florida.

Liu, G. et al., 2014. Pyrosequencing Reveals Bacterial Communities in Unchlorinated Drinking Water Distribution System: An Integral Study of Bulk Water, Suspended Solids, Loose Deposits and Pipe Wall Biofilm. Environmental Science and Technology, 48(10), pp. 5467-5476.

Liu, G., Van der Mark, E., Verberk, J. & Van Dijk, J., 2013. Flow Cytometry Total Cell Counts: A Field Study Assessing Microbiological Water Quality and Growth in Unchlorinated Drinking Water Distribution Systems. BioMed Research International. doi: https://doi.org/10.1155/2013/595872

Lowermoor Incident Health Advisory Group, 1989. Water pollution at Lowermoor, North Cornwall, Cornwall: Lowermoor Incident Health Advisory Group.

Ma, X. et al., 2018. Biofilm Bacterial Community Transition under Water Supply Quality Changes in Drinking Water Distribution Systems. Environmental Science: Water Research & Technology, 4, pp. 644-653.

MacAdam, J. & Parsons, S., 2004. Calcium Carbonate Scale Formation and Control. Reviews in Environmental Science and Bio/Technology, 3, pp. 159-169.

Machell, J. & Boxall, J., 2012. Field Studies and Modelling Exploring Mean and Maximum Water Age Association to Water Quality in a Drinking Water Distribution Network. Journal of Water Resources Planning and Management, pp. 624-638.

Machell, J. & Boxall, J., 2014. Modelling and Fieldwork to Investigate the Relationship between the Age and the Quality of Drinking Water at Customer's Taps. Journal of Water Resources Planning and Management, 140(9). doi: http://dx.doi.org/10.1061/(ASCE)WR.1943-5452.0000383

Manz, W. et al., 1993. In Situ Identification of Bacteria in Drinking Water and Adjoining Biofilms by Hybridization with 16S and 23S rRNA-Directed Fluorescent Oligonucleotide Probes. Applied Environmental Microbiology, 59(7), pp. 2293-2298.

Martiny, A. et al., 2003. Long-Term Succession of Structure and Diversity of a Biofilm Formed in a Model Drinking Water Distribution System. Applied and Environmental Microbiology, 69(11), pp. 6899-6907.

Masters, S., Welter, G. & Edwards, M., 2016. Seasonal Variations in Lead Release to Potable Water. Environmental Science and Technology, 50(10), pp. 5269-5277.

McInnis, D., 2010. A Relative-Risk Framework for Evaluating Transient Pathogen Intrusion in Distribution Systems. Urban Water Journal, 1(2), pp. 113-127.

McNeill, L. & Edwards, M., 2000. Phosphate Inhibitors and Red Water in Stagnant Iron Pipes. Journal of Environmental Engineering, 126(12), p. 1096.

McNeill, L. & Edwards, M., 2001. Review of Iron Pipe Corrosion in Drinking Water Distribution Systems. American Water Works Association, 93(7), pp. 88-100.

Miettinen, I., Vartiainen, T. & Martikainen, P., 1997. Phosphorus and Bacterial Growth in Drinking Water. Applied and Environmental Microbiology, 63(8), p. 3242–3245.

Moghadam, A. & Dore, R., 2012. Cost and Efficacy of Water Disinfection Practices: Evidence from Canada. Review of Economic Analysis, 4, pp. 209-223.

Momba, M., 1997. The Impact of Disinfection Processes on Biofilm Formation in Potable Water Distribution Systems, Pretoria: University of Pretoria.

Momba, M., Kfir, R., Venter, S. & Loete, T., 2000. An Overview of Biofilm Formation in Distribution Systems and its Impact on the Deterioration of Water Quality. Water SA, 26(1), pp. 59-66.

Morton, L. & Surman, S., 1994. Biofilms in Biodeterioration — A Review. International Biodeterioration and Biodegradation, 34(3-4), pp. 203-221.

National Research Council, 2006. Drinking Water Distribution Systems: Assessing and Reducing Risks. Washington, DC: The National Academies Press.

Nescerecka, A. et al., 2014. Biological Instability in a Chlorinated Drinking Water Distribution Network. PLoS One 9(5), pp. 96354.

Nocker, A. et al., 2017. When are Bacteria Dead? A Step Towards Interpreting Flow Cytometry Profiles after Chlorine Disinfection and Membrane Integrity Staining. Environmental Technology, 38(7), pp. 891-900.

Northern Ireland Water, 2015. Drinking Water Quality: Annual Report 2015, Belfast: Northern Ireland Water.

Okabe, S., Hirata, K. & Watanabe, Y., 1995. Dynamic Changes in Spatial Microbial Distribution in Mixed-Population Biofilms: Experimental Results and Model Simulation. Water Science and Technology, 32(8), pp. 67-74.

Oyawale, F. & Olaoye, A., 2007. Design and Construction of an Autoclave. The Pacific Journal of Science and Technology, 8(2).

Pacific Institute referencing Food and Agriculture Organization of the United Nations, 2007. Making Every Drop Count, Rome: Food and Agriculture Organization of the United Nations.

Patz, J., Vavrus, S., Uejio, C. & McLellan, S., 2008. Climate Change and Waterborne Disease Risk in the Great Lakes Region of the U.S. American Journal of Preventive Medicine, 35(5), pp. 451-458.

Paul, E. et al., 2012. Effect of Stress and Growth Conditions on Detachment and Physical Properties of Biofilms. Water Research, 46(17), pp. 5499-5508.

Pederson, K., 1990. Biofilm Development on Stainless Steel and PVC Surfaces in Drinking Water. Water Research, 24(2), pp. 239-243.

Peng, W. & Mayorga, R., 2018. Developing a Statistical Model to Improve Drinking Water Quality for Water Distribution System by Minimizing Heavy Metal Releases. Water, 10(7), p. 939.

Pepper, I., Gerba, C. & Gentry, T., 2015. Environmental Microbiology. 3rd Edition ed. s.l.: Academic Press.

Percival, et al., 1997. Biofilm Development on Stainless Steels in a Potable Water System. Journal of International Water Management, 11, pp. 289-294.

Phanse, Y. et al., 2012. Analyzing Cellular Internalization of Nanoparticles and Bacteria by Multi-spectral Imaging Flow Cytometry. Journal of Visualized Experiments, 64(3884). doi: https://doi.org/10.3791/3884

Philp, R., 2019. Taking the Lead, Water & Wastewater Treatment Magazine, 62(4), pp. 13-15.

Pick, FC. et al., 2018. Development of Assimilable Organic Carbon Assay and Field Application within Drinking Water Treatment. Sheffield: WDSA / CCWI Joint Conference 2018.

Pieri, P. Andra, S., Charisiadis, P. & Demetriou, G., 2014. Variability of Tap Water Residual Chlorine and Microbial Counts at Spatially Resolved Points of Use. Environmental Engineering Science, 31(4), pp. 193-201.

Pinto, A. et al., 2014. Spatial-Temporal Survey and Occupancy-Abundance Modeling to Predict Bacterial Community Dynamics in the Drinking Water Microbiome. American Society for Microbiology, 5(3). doi: https://doi.org/10.1128/mBio.01135-14

Prest, E. & Martijn, B., 2020. How to Run a Disinfectant-Free Distribution System. Cranfield, 13th Conference of the UK Network on Potable Water Treatment and Supply.

Prest, E. et al., 2013. Monitoring Microbiological Changes in Drinking Water Systems Using a Fast and Reproducible Flow Cytometric Method. Water Research, 47(19), pp. 7131-7142.

Prest, E. et al., 2016. Long-Term Bacterial Dynamics in a Full-Scale Drinking Water Distribution System. PLOS ONE. doi: https://doi.org/10.1371/journal.pone.0164445

Prest, E., Hammes, F., Van Loosdrecht, M. & Vrouwenvelde, J., 2016. Biological Stability of Drinking Water: Controlling Factors, Methods and Challenges. Frontiers in Microbiology, 7(45). doi: https://doi.org/10.3389/fmicb.2016.00045

Proctor, C., Reimann, M., Vriens, B. & Frederik Hammes, F., 2018. Biofilms in Shower Hoses. Water Research, 131, pp. 274-286.

Prüss-Ustün, A. et al., 2014. Burden of Disease from Inadequate Water, Sanitation and Hygiene in Low- and Middle-Income Settings: A Retrospective Analysis of Data from 145 Countries. Tropical Medicine and International Health, 19(8), pp. 894-905.

Ratnayaka, D., Brandt, M. & Johnson, K., 2009. Twort's Water Supply. 6th ed. Butterworth-Heinemann: Butterworth-Heinemann.

Reeslev, M., Nielsen, JC. & Rogers, L., 2011. Assessment of the Bacterial Contamination and Remediation Efficacy After Flooding Using Fluorometric Detection. Journal of ASTM International, 8(10), pp. 290-296.

Reynolds, K., Mena, K. & Gerba, C., 2008. Risk of Waterborne Illness Via Drinking Water in the United States. Reviews of Environmental Contamination and Toxicology, 192, pp. 117-158.

Richardson, S. et al., 2007. Occurrence, Genotoxicity and Carcinogenicity of Regulated and Emerging Disinfection By-Products in Drinking Water: A Review and Roadmap for Research. Mutation Research/Reviews in Mutation Research, 636(1-3), pp. 178-242.

Rittmann, B. & Snoeyink, V., 1984. Achieving Biologically Stable Drinking Water. Journal of the American Water Works Association, 76, pp. 106-114.

Roeselers, G. et al., 2015. Microbial Biogeography of Drinking Water: Patterns in Phylogenetic Diversity Across Space and Time. Environmental Microbiology, 17(7), pp. 2505-2514.

Rossman, LA., Clark, RM. & Grayman, WM., 1994. Modelling Chlorine Residuals in Drinking Water Distribution Systems. Journal of the Environmental Engineering Division, 120(4), pp. 803-820.

Safford, H. & Bischel, H., 2019. Flow Cytometry Applications in Water Treatment, Distribution and Reuse: A Review. Water Research, 151, pp. 110-133.

Santana, M., Zhang, Q. & Mihelcic, J., 2014. Influence of Water Quality on the Embodied Energy of Drinking Water Treatment. Environmental Science and Technology, 48, pp. 3084-3091.

Seker, R. et al., 2012. Bacterial Water Quality and Network Hydraulic Characteristics: A Field Study of a Small, Looped Water Distribution System using Culture-Independent Molecular Methods. Journal of Applied Microbiology, 112(6), pp. 1220-1234.

Servais, P., Laurent, P. & Randon, G., 1995. Comparison of the Bacterial Dynamics in Various French Distribution Systems. Journal of Water Supply: Research and Technology-Aqua, 44, pp. 10-17.

Sfynia, C., 2017. Minimisation of Regulated and Unregulated Disinfection By-Products in Drinking Water, London: Imperial College London. doi: https://doi.org/10.25560/58879

Sharpe, R., 2012. Laboratory Investigations into Processes Causing Discoloured Potable Water, Sheffield: University of Sheffield.

Sibille, I. et al., 1998. Protozoan Bacterivory and Escherichia coli Survival in Drinking Water Distribution Systems. Environmental and Public Health Microbiology, 64(1), pp. 197-202.

Simões, L. et al., 2006. Drinking Water Biofilm Assessment of Total and Culturable Bacteria Under Different Operating Conditions. Biofouling, 22(2), pp. 91-99.

Sinclair, T. et al., 2018. Virus Reduction Through Microfiltration Membranes Modified with a Cationic Polymer for Drinking Water Applications. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 551, pp. 33-41.

SLMB (Switzerland: Federal Office of Public Health), 2012. Determining the Total Cell Count and Quantitative Ratios of High and Low Nucleic Acid Content Cells in Freshwater using Flow Cytometry, s.l.: Federal Office of Public Health, Switzerland.

Smitt, M. & Russell, A., 2013. Pipe Up- Finding a Remedy for Lead, s.l.: Water and Wastewater Treatment.

South West Water, 2020. Do You Have Hard Water?. [Online] Available at: https://www.southwestwater.co.uk/advice-and-services/your-water/water-hardness/ [Accessed 29 December 2020].

Southern Water, 2016. Preventing the Contamination of Water Treatment Chemicals. London, CIWEM.

Special Sanitary Commissioner, 1897. Typhoid Epidemic at Maidstone. Journal of the Sanitary Institute, 18, p. 388.

Speight, V., 2015. Innovation in the Water Industry: Barriers and Opportunities for US and UK Utilities. WIREs Water, 2(4), pp. 301-313.

Srinivasan, S. et al., 2008. Factors Affecting Bulk to Total Bacteria Ratio in Drinking Water Distribution Systems. Water Research, 42(13), pp. 3393-3404.

Stewart, P., Murga, R., Srinivasan, R. & De Beer, D., 1995. Biofilm Structural Heterogeneity Visualized by Three Microscopic Methods. Water Research, 29, pp. 2006-2009.

Stone, E., 2006. Effects of Orthophosphate Corrosion Inhibitor in Blended Water Quality Environments. Florida: B.S. University of Central Florida.

Sun, H., Shi, B., Bai, Y. & Wang, D., 2014. Bacterial Community of Biofilms Developed under Different Water Supply Conditions in a Distribution System. Science of the Total Environment, 472, pp. 99-107.

Sun, W. et al., 2013. Characterization and Identification of a Chlorine-Resistant Bacterium, Sphingomonas TS001, from a Model Drinking Water Distribution System. Science of The Total Environment, 458-460, pp. 169-175.

Sun, X. et al., 2019. Formation of Novel Disinfection By-Products Chlorinated Benzoquinone, Phenyl Benzoquinones and Polycyclic Aromatic Hydrocarbons During Chlorination Treatment on UV Filter 2,4-dihydroxybenzophenone in Swimming Pool Water. Journal of Hazardous Materials, 367, pp. 725-733.

Szewzyk, U., Szewzyk, R. & Manz, W. a. S. K., 2000. Microbiological Safety of Drinking Water. Annual Review of Microbiology, 54, pp. 81-127.

Thakare, K., Vishwakarma, H. & Bhave, A., 2015. "Experimental Investigation of Possible Use of HDPE as Thermal Storage Material in Thermal Storage Type Solar Cookers. International Journal of Research in Engineering and Technology, 4(12), pp. 92-99.

Tickner, J. & Gouveia-Vigeant, T., 2005. The 1991 Cholera Epidemic in Peru: Not a Case of Precaution Gone Awry. Risk Analysis, 25(3), pp. 495-502.

Triantafyllidou, S., Schock, M., Tully, J. & Cahalan, k., 2017. Sampling for Lead in Drinking Water. Hamilton, US Environment Protection Agency.

Tsai, YP., 2005. Simulation of Biofilm Formation at Different Assimilable Organic Carbon Concentrations under Lower Flow Velocity Condition. Journal of Basic Microbiology, 45(6), pp. 475-485.

Tsitsifli, S. & Kanakoudis, V., 2018. Disinfection Impacts to Drinking Water Safety- A Review. Proceedings, 2(603). doi: https://doi.org/10.3390/proceedings2110603

UKWIR (UK Water Industry Research), 2003. National Database of Mains Failures 2003 (No. 23/3G/05/7), London: UKWIR.

UKWIR (UK Water Industry Research), 2012. Alternatives to Phosphate for Plumbosolvency Control, London: UKWIR.

UKWIR (UK Water Industry Research), 2017. Drinking Water Quality Big Questions: How Can We Achieve 100% Compliance with Drinking Water Standards at Point of Use by 2050?, London: UK Water Industry Research.

United Nations General Assembly, 2010. Resolution adopted by the General Assembly on 28 July 2010: 64/292. The human right to water and sanitation, s.l.: United Nations General Assembly.

United States Environmental Protection Agency, 2011. River Water Monitoring - Description of Parameters: Appendix 7 Information of Water Quality Parameters, Monaghan: Environmental Protection Agency.

United States Environmental Protection Agency, 2012. 5.6 Phosphorus. [Online] Available at: https://archive.epa.gov/water/archive/web/html/vms56.html [Accessed 18 June 2020].

Vaerewijck, et al., 2005. Mycobacteria in Drinking Water Distribution Systems: Ecology and Significance for Human Health. FEMS Microbiology Review, 29, pp. 911-934.

Van den Hoven, T. et al., 1999. Developing a New Protocol for the Monitoring of Lead in Drinking Water, Brussels: European Commission.

Van der Kooij, D., 2000. Biological Stability: A Multidimensional Quality Aspect of Treated Water. Air and Soil Pollution, 123(1), pp. 25-34.

Van der Kooij, D., Veenendaal, H. & Scheffer, W., 2005. Biofilm Formation and Multiplication of Legionella in a Model Warm Water System with Pipes of Copper, Stainless Steel and Cross-Linked Polyethylene. Water Research, 39(13), pp. 2789-2798.

Van der Kooij, D., Visser, A. & Hijnen, W., 1982. Determining the Concentration of Easily Assimilable Organic Carbon in Drinking Water. American Water Works Association, 74(10), pp. 540-543.

Van der Wende, E. & Characklis, W., 1990. Biofilms in Potable Water Distribution Systems. Drinking Water Microbiology, pp. 249-268.

Van der Wielen, P. & Van der Kooij, D., 2013. Nontuberculous Mycobacteria, Fungi and Opportunistic Pathogens in Unchlorinated Drinking Water in the Netherlands. Applied and Environmental Microbiology, 79(3), pp. 825-834.

Venkobachar, C., Iyengar, L. & Prabhakara Rao, A., 1977. Mechanism of Disinfection: Effect of Chlorine on Cell Membrane Functions. Water Research, 11(8), pp. 727-729.

Vital, M., Stucki, D., Egli, T. & Hammes, F., 2010. Evaluating the Growth Potential of Pathogenic Bacteria in Water. Applied and Environmental Microbiology, 76(19), pp. 6477-6484.

Vreeburg, I. & Boxall, J., 2007. Discolouration in Potable Water Distribution Systems: A Review. Water Research, 41(3), pp. 519-529.

Vreeburg, J., 1996. Brown Water, Cause and Consequence: Efficiency of Cleaning with Flushing, Water/Air Scouring and Pigging, Nieuwegein: KIWA.

Water Research Commission, 2012. Chemical-Free Treatment, s.l.: WRc Open Innovation Workshop.

Water Research Foundation, 2014. Advancing the Science of Water: WRF and Research on Taste and Odour in Drinking Water, Denver: Water Research Foundation.

Water UK, 2018. England and Wales, Apr 2017 - Mar 2018, s.l.: s.n.

WaterLogic, 2015. Why does my water smell like bleach?. [Online] Available at: https://www.waterlogic.com/en-us/resources/water-problems/why-does-my-water-smell-like-bleach/ [Accessed 4 May 2020].

Weiss, W. et al., 2005. Riverbank Filtration for Control of Microorganisms: Results from Field Monitoring. Water Research, 39(10), pp. 1990-2001.

Westbrook, A. & Digiano, F., 2009. Rate of Chloramine Decay at Pipe Surfaces. Journal of American Water Works Association, 101(7), pp. 59-70.

Westrell, T., 2004. Microbial Risk Assessment and its Implications for Risk Management in Urban Water Systems. Linköping: Linköping Studies in Arts and Science.

Whelton, A., Dietrich, A. & Gallagher, D., 2010. Contaminant Diffusion, Solubility and Material Property Differences between HDPE and PEX Potable Water Pipes. Journal of Environmental Engineering, 136(2), pp. 227-237.

Wilkerson, M., 2012. Principles and Applications of Flow Cytometry and Cell Sorting in Companion Animal Medicine. The Veterinary Clinics of North America. Small Animal Practice, 42(1), pp. 53-71.

Williamsona, F. et al., 2014. Online Water Quality Monitoring in the Distribution Network. IWA Publishing, 9(4), pp. 575-585.

World Health Organization, 1984. Guidelines for Drinking Water, Geneva: World Health Organization.

World Health Organization, 2000. Disinfectants and Disinfectant By-Products, Geneva: World Health Organization.

World Health Organization, 2003. Ammonia in Drinking Water: Background document for development of WHO Guidelines for Drinking-water Quality, Geneva: World Health Organization.

World Health Organization, 2004. Monochloramine in Drinking Water, Geneva: World Health Organization.

World Health Organization, 2006. Chemical Hazards, Geneva: World Health Organization.

World Health Organization, 2010. Hardness in Drinking-Water, Geneva: World Health Organization.

World Health Organization, 2011. Chapter 3 Inactivation (Disinfection) Processes, Geneva: World Health Organization.

World Health Organization, 2011. Guidelines for Drinking Water Quality: Fourth Edition, Geneva: World Health Organization.

World Health Organization, 2018. Lead Poisoning and Health. [Online] Available at: https://www.who.int/news-room/fact-sheets/detail/lead-poisoning-and-health [Accessed 8 July 2019].

World Health Organization, 2019. Disinfectants and Disinfection By-Products. [Online] Available at: https://www.who.int/water_sanitation_health/dwq/S04.pdf [Accessed 22 November 2019].

World Water Assessment Programme, 2012. The United Nations World Water Development Report 4: Managing Water under Uncertainty and Risk, Paris: United Nations Educational, Scientific and Cultural Organization.

Wray, C., n.d.. Roots and Causes of Chlorine Resistance in Coliform and Other Indicator Organisms in Water Treatment Systems. Newcastle: Newcastle University.

Xie, L. et al., 2016. Association Between Dietary Nitrate and Nitrite Intake and Site-Specific Cancer Risk: Evidence from Observational Studies. Oncotarget, 7(35), pp. 56915-56932.

Zhang, Y. et al., 2009. Lead Contamination of Potable Water Due to Nitrification. Environmental Science and Technology, 43(6), pp. 1890-1895.

Zhang, Y., 2008. Nitrification in Premise Plumbing and its Effect on Corrosion and Water Quality Degradation, Virginia: Virginia Polytechnic Institute and State University.

Zhou, J., Bruns, M. & Tiedje, J., 1996. DNA Recovery from Soils of Diverse Composition. Applied Environmental Microbiology, 62(2), pp. 316-322.

Zlatanović, L., van der Hoek, J. & Vreeburg, J., 2017. An Experimental Study on the Influence of Water Stagnation and Temperature Change on Water Quality in a Full-Scale Domestic Drinking Water System. Water Research, 123, pp. 761-772.

Appendices

1 Outline of Proposed Experimental Phases

The original experimental programme consisted of a 6 month "growth" phase, where biofilm development was monitored and a "mobilisation" phase, to analyse the biofilm response to increasing shear stress under controlled increases in flow rate. The mobilisation phase of the experiment was not completed due to a worldwide pandemic. This section details the motivations for building the pipe rig system capable of doing a mobilisation phase as well as the proposed operation of this phase during the experiment.

1.1 Mobilisation Phase

Constant steady flow rates rarely occur over a long period of time within real live drinking water distribution networks. There can be smaller flow changes, for instance overnight stagnation and diurnal demands that differ between weekends and weekdays, as well as larger flow changes like seasonal variation as well as cleaning strategies like flushing programmes and unanticipated events such as pipe bursts (Ratnayaka, et al., 2009). A change in hydraulic conditions, including a large increase in flow, can raise the force acting tangentially on the pipe surface, the boundary wall shear stress, above the conditioned value (Husband, et al., 2008). The cohesive layer theory described in the Prediction of Discolouration in Distribution Systems model suggests that biofilm has a defined increasing strength profile which requires increasing shear stresses to be mobilised via flushing (Husband, et al., 2008). This means that mature biofilms are stratified with different layers with a defined shear strength profile, including a soft top layer that is easily detached during large flow events and a basal layer that is more resilient to it (Paul, et al., 2012). At every increase in applied shear stress there is a further release of accumulated material into bulk water, causing a deterioration of water quality (Husband, et al., 2016).

Thus, the growth phase of the experiment investigated how biofilm is formed in a steady state environment and how it builds over time, deteriorating the freshly laid "idealised" pipe system, to see what the impact of a chemical free distribution network would be on the biofilm attached to the pipe surface and in the bulk water quality. It could be that chemical free water is only possible in a compliant manner in a distribution environment where there are no increased flow events. To increase the validity of the experiment and to determine if this is the case, an increased flow event, a mobilisation phase of the experiment, was proposed to investigate what the impact of such an event would be on the biofilm retained at the pipe surface and in the bulk water quality.

Hence, it was decided that the experimental programme involved one 6 month "growth" phase, where biofilm development was monitored and a "mobilisation" phase, to analyse the biofilm response to increasing shear stress under controlled increases in flow rate.

1.1.1 Mobilisation Phase Operation

Following the 6 month growth phase, the mobilisation phase was conducted. At the beginning of the mobilisation phase, each loop was isolated with closed entry and exit valves to prevent a drain rate or trickle feed to ensure there was a consistent water quality. The series of flushing steps or shear stresses used to investigate the removal of material from the pipe wall into the bulk water, were determined based on a range of shear stresses experienced in live networks, as well as the capabilities of the pipe testing facilities to operate under different flows (Husband, et al., 2008) (Sharpe, 2012) (Water Research Commission, 2012).

Three flow increases were chosen to be used to ensure there were adequate coupons to be able to see the impact of each step on the pipe wall, to yield measurable differences in turbidity and to ensure different flow steps were used because of the precise movements required to achieve this. The selected flows used to generate the sheer stresses required were low (150 l/m), medium (275 l/m) and high (400 l/m). These target flows were achieved by using a flushing pump, the Grundfos CRIE15-03 N-CA-A-E-HQQE, moving it between the four rigs and utilising a gate valve with small precise movements to achieve the desired flow rate. Relatively small increments with a smooth transition were used to generate more detailed data, as used advantageously in a number of studies including that of Husband et al. (2008), Sharpe (2012) and Fish (2018). After each flow increase, the flow was maintained for three turnovers of water within the pipe rigs to allow enough time for the system and turbidity within it, to stabilise and for the water quality to become well mixed (Sharpe, 2012). After which time coupons and bulk water samples were taken to determine the combined impact of applied shear stress, as can be seen in Figure 66.

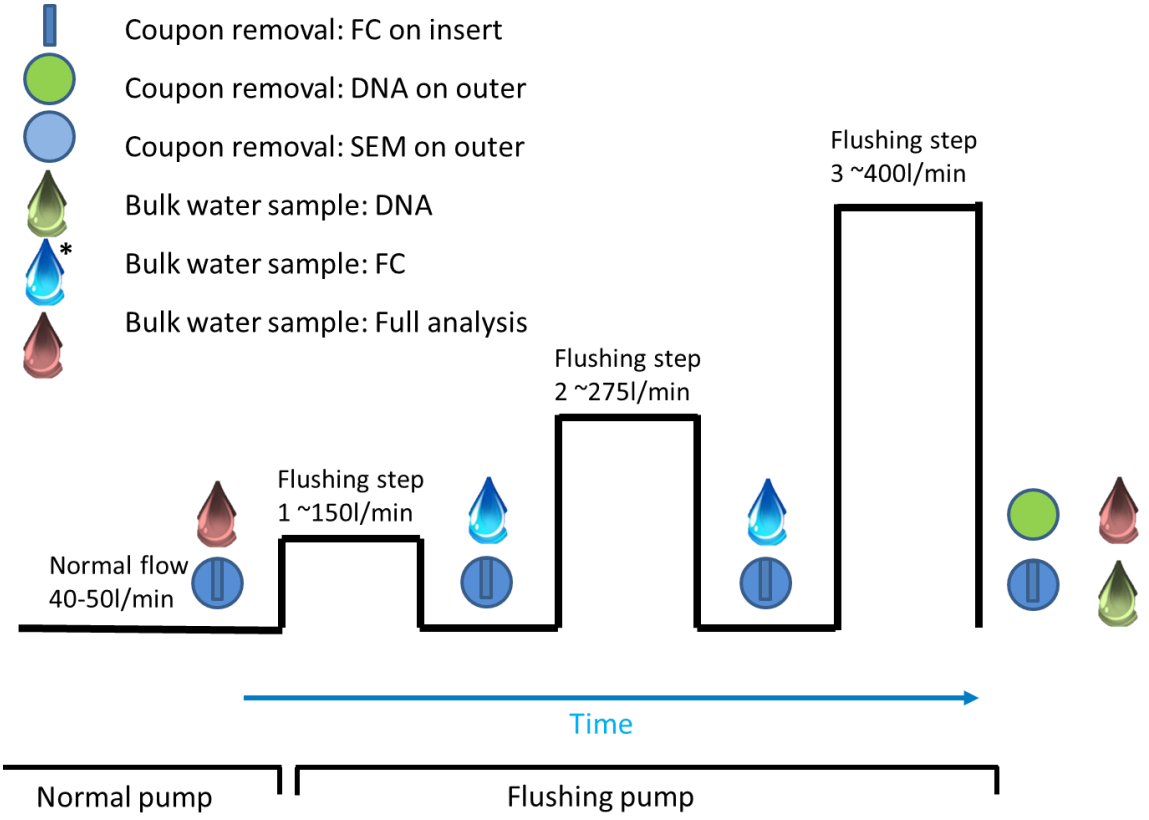


Figure 66 Mobilisation phase schematic showing the flushing steps and sampling regime

1.2 Bulk Water DNA Sequencing

Following a growth period, DNA sequencing of bulk water was due to take place to provide further insight into the microbiome of the experimental test facilities. This phase of the experiment was not completed due to a worldwide pandemic.

For bulk water DNA sequencing, a Tangential Flow Filtration System was used to concentrate 10 l of water from each experimental pipe rig Outlet in triplicate. The water was filtered through 0.22 µm nitrocellulose membrane filters (Millipore, Corp) to obtain 50-100 ng/uL of DNA. Filters were then stored in sterile bags at -80°C ready for DNA extraction.

The DNA was extracted from the filters using a CTAB (hexadecyltmethyl ammonium bromide) and Proteinase K chemical lysis protocol (Zhou, et al., 1996). This consisted of the filters being transferred into a 15 ml tube which contained 720 µl of SET buffer and 90 µl of lysozyme 10 mg/ml (Sigma Aldrich Co., UK). Following an incubation period of 30 minutes at 37°C (with rotation), 90 µl of 10% SDS (w/v) and 25 µl of Proteinase K (Applied Biosystems, Life Technologies Ltd., UK) were added to the samples. After a second incubation period of 2 hours (with rotation) at 55°C, 137 µl of 5 M NaCl and 115 µl of CTAB solution were added. A third incubation period of 65°C for 30 minutes (with rotation) was then completed, after which the top aqueous layer of the sample was removed. This supernatant was extracted and added to an equal volume of chloroform. The solution formed was centrifuged at 13,000 RPM for 5 minutes.

DNA precipitation occurred at -20°C, over a 12-14 hour period with 815 µl of 100% isopropanol alcohol. After centrifugation at 13,000 RPM for 30 minutes, the supernatant was discarded and the DNA pellet washed twice in 1 ml of 70% ethanol. After a second period of centrifugation at 13,000 RPM for 10 minutes, 30 µl of sterile nuclease free water (Ambion, Warrington, UK) was added. It was then possible to visualise the precipitated DNA using gel electrophoresis and quantify the precipitated DNA using a Nanodrop ND-1000 spectrophotometer (Nanodrop, Wilmington, USA).

1.3 Enhanced Coupon Analysis

Following a growth period, DNA sequencing and SEM of the coupons was due to take place to provide further insight into the microbiome of the experimental test facilities. Coupon inserts from each of the four test pipe facilities were intended to be sampled at month 0, 1.5, 3, 4.5, 6 and post flush. This phase of the experiment was not completed due to a worldwide pandemic.

1.3.1 SEM Microscopy

SEM was used to provide qualitative data on the biofilm physical structure and surface coverage. The coupons surface, developed biofilm, single cells and inorganics were differentiated visually from one another. In this manner, SEM provided a visual representation of how biofilm developed during the growth phase and how it was impacted by the mobilisation phase.

The SEM used was a Tescan Vega 3 LMU Scanning Electron Microscope, following the standardised method of adding the sample to the vacuum chamber of the microscope and using a fine beam of high energy electrons to conduct topographic examination. The samples were additionally coated using an Edwards S150B Gold Sputter Coater to improve the imaging of the samples, creating a conductive layer of metal on the sample to inhibit charging, reduce thermal damage and improve the secondary electron signal.

1.3.2 DNA Sequencing

Section 5.2.2.3 referred to the process of collected biofilm samples. Once the biofilm/PBS solution was formed using this method, DNA was then collected from this solution by filtering it through a 0.22 µm nitrocellulose membrane filter.

1.4 Customer Tap Sampling

To determine if the water quality of the rigs was representative of that within the network, a sampling campaign was going to be ran at the Customer Tap in the network. This phase of the experiment was not completed due to a worldwide pandemic.

1.4.1 Customer Tap Sampling Location

Customer Tap sampling was completed utilising the method detailed in Section 3.2.3.1. 3-5 random Customer Taps were sampled in a specific area in each of the two networks.

The specific areas chosen within the networks were selected due to their comparability with the pipe material (plastics, specifically HPPE PE100 and MDPE PE80) and water age (approximately 2 days) of the pipe rigs. It was difficult to find such an area large enough to complete random sampling without sampling the same property over the 3 month period, especially in the Groundwater Network, because it is relatively small with a population of ~8400, rather than Surface Water Area with ~18,000. As a compromise, the “ideal sampling area” with plastic pipe material and water age was expanded through the use of a “back up sampling area” with the ideal pipe material but not the water age, as can be seen in Figure 67. This was completed because minimal disruption to customers is of upmost importance in the water industry and repeated sampling of the same household would not be appropriate. A minimum of two samples were taken monthly from the ideal sampling areas and 1-3 samples were taken from the back up sampling areas.

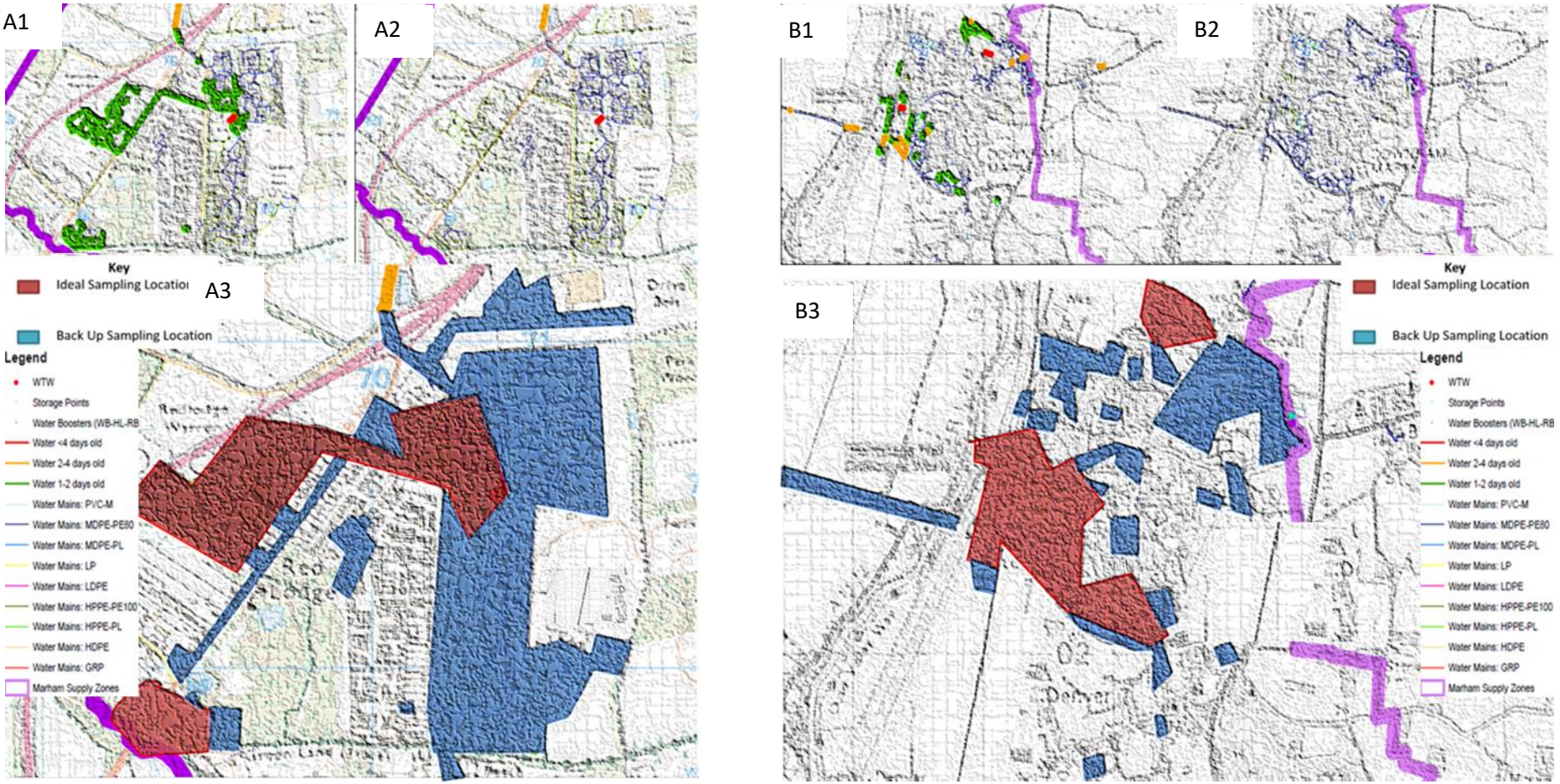


Figure 67 Ideal and back up network sampling areas

Figure A1-3 depict the surface water supplied households and Figures B1-3 the groundwater. Figure A1 and B1 desirable water age. Figure A2 and B2 desirable pipe material. Figure A3 and B3 the ideal sampling location (in red) and the back-up sampling location (in blue).

2 Tables of Data: An Overview of Water Quality Samples for Historical Data Analysis

Table 28 Site A descriptive statistics for the Final Water and Customer Tap

Parameter	Site A WTW				Site A PWSZ				Site A DZ			
	N	Min-Max	Mean	SD	N	Min-Max	Mean	SD	N	Min-Max	Mean	SD
pH	381	7.14-7.73	7.4	0.108	267	7.18-8.42	7.18	0.176	152	7.18-7.84	7.49	0.13
Cond	400	240-491	327	34	256	244-416	315	40	109	267-416	324	37.2
Turbidity	1268	0.013-0.46	0.0798	0.05	662	0.013-97.7	0.339	4	335	0.013-97.7	0.459	5.34
Temp	1207	0.2-25.8	12.6	5.7	366	0.4-23.7	13.0	4.7	260	4.7-23.6	13.0	4.8
Dissolved O2	353	7-12	10	1								
TOC	357	1.72-4.18	2.70	0.463								
TON	351	1-6	2	1	159	0.849-5.69	2	1	110	0.849-5.69	2.14	1.31
Hardness	86	104-197	151	19	198	101-418	147	30	92	107-418	150	35.4
Alkalinity	85	65-126	95	14	45	54.3-112	90	18	32	54.3-111	83.8	17
Chloride	118	20-53	26	4								
Ortho P	353	0.09-2.2	0.760	0.423								
Sulphate	118	28-74	40	6								
Sodium	86	10-30	12	3	228	9.47-42.1	12	3	111	9.82-25.3	12.6	2.78
Copper	16	0.0086-0.029	0.0155	0.00959	172	0.002-0.68	0.0209	0.0561	73	0.002-0.0953	0.016	0.018
Magnesium	86	5-8	6	0.479	198	4.15-7.28	5	1	92	4.59-7.28	5.59	0.474
Calcium	86	34-66	51	8	198	32.8-156	50	12	92	34.1-156	51.0	14.1
T Manganese	411	0.001-0.0773	0.00228	0.00534	597	0.001-0.46	0.00386	0.0195	329	0.001-0.455	0.001	0.025
Total Iron	380	0.003-0.037	0.00785	0.00413	595	0.003-7.63	0.0490	0.329	333	0.007-7.63	0.06	0.435
Free Chlorine	1307	0.05-0.55	0.0684	0.032	1183	0.01-0.9	0.0930	0.084	582	0.01-0.9	0.098	0.094
T Chlorine	1307	0.05-2	1.01	0.095	1185	0.05-1.26	0.541	0.187	582	0.1-1.07	0.545	0.204
3 Day CC	1256	0-25	7.23	2	235	0-112	2.3	11	148	0-109	2.09	11
2 Day CC	1175	0-31	0.841	2	323	0-1284	7.21	74	101	0-102	1.99	11.7
Enterococci	210	0-0	0	0	93	0-0	0	0	50	0-0	0	0
E. coli	1083	0-0	0	0	196	0-0	0	0	140	0-0	0	0
Coliforms	1083	0-1	0.00092	0.03	579	0-1	0.00345	0.0587	8	0-0	0	0
Ammonia	93	0.084-0.332	0.181	0.04	146	0.017-0.27	0.133	0.06	97	0.024-0.269	0.132	0.06
Nitrate	351	1-26	9.93	6	159	1.459-25	9.37	6	110	2.78-25	9.40	5.84
Nitrite	1144	0.005-0.011	0.009	0.002	159	0.009-0.39	0.0629	0.09	110	0.009-0.392	0.065	0.09
T Phosphorus	353	0.09-2.34	0.775	0.424	314	0.189-2.17	0.769	0.4	188	0.189-1.41	0.777	0.327
Crypto spp.	1147	0-0	0	0								
TCC	53	76-23058	7448	6820								
ICC	53	88-13124	522	1800								
Total Lead	16	0.052-0.16	0.153	0.03	157	0.16-12.7	0.651	1.5	73	0.16-8	0.593	1.26

Table 29 Site B descriptive statistics for the Final Water and Customer Tap

Parameter	Site B WTW				Site B PWSZ				Site B DZ			
	N	Min-Max	Mean	SD	N	Min-Max	Mean	SD	N	Min-Max	Mean	SD
pH	405	7.2-7.8	7.5	0.109	260	6.45-7.8	7.48	0.136	224	6.45-7.8	7.48	0.135
Conductivity	475	478-641	559	30	102	32.4-627	556	81	101	32.4-625	555	81.3
Turbidity	1929	0.013-0.49	0.0670	0.03	636	0.013-13.3	0.225	0.9	540	0.013-13	0.25	1.01
Temperature	2461	3-20	11.5	4.9	366	5.3-21.3	13	4.1	437	5.4-21.8	13.1	4.18
Dissolved O2	79	6-11	9	1								
TOC	404	1.37-2.39	1.73	0.167								
TON	330	3-7	5	1	50	1.467-6.05	5	1	42	3.35-6.05	4.80	0.77
Hardness	80	206-296	249	20	134	19-392	247	57	124	19-352	239	48.4
Alkalinity	79	144-227	188	18	37	122-188	159	22	36	122-188	159	22.1
Chloride	125	43-56	49	3					1	50.1-50.1	50.1	0
Ortho P	295	0.055-2.45	1.48	0.382								
Sulphate	125	58-85	70	6					1	70.9-70.9	70.9	0
Sodium	80	25-33	29	2	156	8.38-160	30	16	142	8.38-160	30.8	16.1
Copper	15	0.002-0.0226	0.00397	0.0053	103	0.002-0.39	0.0232	0.0531	103	0.002-0.4	0.02	0.05
Magnesium	80	8-10	9	1	134	0.787-10.8	9	2	124	0.787-11	8.89	1.65
Calcium	80	69-103	85	7	134	6.23-143	84	21	124	6.23-124	81.0	16.9
T Manganese	331	0.001-0.008	0.000408	0.0004	612	0.001-0.11	0.00259	0.0086	516	0.001-1.1	0.003	0.01
Total Iron	331	0.003-0.172	0.00744	0.0093	617	0.003-2.65	0.0398	0.206	521	0.007-2.7	0.0451	0.22
Free Chlorine	2487	0.32-1.18	0.850	0.136	962	0.05-0.64	0.249	0.137	848	0.05-0.64	0.252	0.14
Total Chlorine	2487	0.7-1.36	1.01	0.123	963	0.06-1.12	0.406	0.166	848	0.06-1.12	0.410	0.17
3 Day CC	2466	0-299	0.272	6	211	0-59	0.791	5	175	0-43	0.583	3.73
2 Day CC	2304	0-16	0.114	2	163	0-257	2.85	22	128	0-257	3.48	25.1
Enterococci	200	0-0	0	0	79	0-0	0	0	1	0-0	0	0
E. coli	2067	0-0	0	0	259	0-0	0	0	166	0-1	0.0060	0.08
Coliforms	2068	0-0	0	0	353	0-8	0.0397	0.532	259	0-8	0.0541	0.62
Ammonia	84	0.008-0.042	0.04	0.008	98	0.013-0.04	0.0409	0.005	63	0.01-0.04	0.0403	0.006
Nitrate	353	13-30	21.3	4	50	3.763-26.8	20.7	5	42	14.9-26.8	21.3	3.41
Nitrite	410	0.005-0.024	0.00903	0.0008	50	0.009-5e-18	0.009	5e-18	42	0.009	0.009	5.27e-18
T Phosphorus	350	0.09-2.43	1.52	0.413	433	0.1-4.92	1.53	0.5	318	0.1-4.92	1.62	0.493
Cryptosporidium	517	0-0	0	0								
TCC	88	271-12198	4637	2430								
ICC	88	6-8601	195	926								
Total Lead	15	0.16-0.16	0.16	0	98	0.16-12.7	0.923	2.2	0.2-13	0.16-12.7	0.923	0.16

Table 30 Site C descriptive statistics for the Final Water and Customer Tap

Parameter	Site C WTW				Site C PWSZ				Site C DZ			
	N	Min-Max	Mean	SD	N	Min-Max	Mean	SD	N	Min-Max	Mean	SD
pH	375	7.07-7.72	7.28	0.0967	166	6.99-7.67	7.37	0.114				
Conductivity	1139	570-723	627	24	184	580-726	636	33				
Turbidity	2215	0.04-0.66	0.0670	0.03	495	0.04-3.17	0.167	0.3	1	0.08-0.08	0.08	0
Temperature	2460	3-21	12.1	4.9	335	5.2-15.6	13.1	4.5	1	14.9-14.9	14.9	0
Dissolved Oxygen	86	7-11	9	1								
TOC	388	2.58-4.35	3.19	0.347								
TON	355	2-6	3	1	163	1.74-6.11	3	1				
Hardness	85	217-300	245	14	154	206-285	241	15				
Alkalinity	92	94-157	127	13	44	86.2-156	121	13				
Chloride	177	59-85	69	6								
Orthophosphate	352	0.344-3.09	1.35	0.515								
Sulphate	177	103-137	115	6								
Sodium	85	38-62	45	4	182	36.8-53.6	44	4	1	42.8-42.8	42.8	0
Copper	15	0.0029-0.0167	0.00646	0.0038	132	0.002-0.40	0.0199	0.0468	1	17.9-17.9	17.9	0
Magnesium	85	9-12	10	0.439	154	8.67-10.6	10	0.38				
Calcium	85	70-101	82	6	154	68.1-97.9	80	6				
Total Manganese	373	0.001-0.0034	0.00103	0.000201	471	0.001-0.04	0.001	0.0028	1	0.0014	0.0014	0
Total Iron	500	0.003-0.109	0.0421	0.022	484	0.003-0.47	0.0348	0.048	1	0.013	0.013	0
Free Chlorine	2561	0.04-1.07	0.0942	0.044	825	0.01-1	0.0878	0.077	6	0.07-0.1	0.0833	0.0103
Total Chlorine	2561	0.24-1.44	1.11	0.111	825	0.05-1.17	0.436	0.226	6	0.5-0.68	0.632	0.0656
3 Day CC	2503	0-3055	9.78	112	241	0-253	2.77	18	1	0-0	0	0
2 Day CC	2337	0-504	0.785	13	215	0-950	6.59	66	1	0-0	0	0
Enterococci	86	0-0	0.01	0	100	0-0	0	0	1	0-0	0	0
E. coli	590	0-0	0	0	190	0-0	0	0	1	0-0	0	0
Coliforms	2100	0-1	0.00048	0	359	0-21	0.0641	1.11	2	0-0	0	0
Ammonia	88	0.08-0.34	0.214	0.05	163	0.025-0.3	0.117	0.07				
Nitrate	355	8-28	14.6	4	163	7.68-26.9	14.5	4				
Nitrite	1670	0.005-0.035	0.00901	0.0007	167	0.009-0.33	0.0661	0.08				
Total Phosphorus	352	0.372-3.29	1.38	0.535	334	0.09-2.69	1.26	0.6	5	0.782-2.7	1.19	0.838
Cryptosporidium	377	0-0	0	0								
TCC	71	4771-14514	9481	3450								
ICC	71	42-13319	378	1520								
Total Lead	14	0.16-0.16	0.16	3e-18	134	0.16-9.55	0.668	1.5	2	0.16-15.8	7.98	0

Table 31 Site D descriptive statistics for the Final Water and Customer Tap

Parameter	Site D WTW				Site D PWSZ				Site D DZ			
	N	Min-Max	Mean	SD	N	Min-Max	Mean	SD	N	Min-Max	Mean	SD
pH	370	7.17-7.5	7.35	0.0608	281	7.15-7.93	7.35	0.11	9	7-2470	283	0.0778
Conductivity	522	582-724	655	21	205	285-711	646	64	5	200-284	20.5	12
Turbidity	1873	0.013-0.17	0.0612	0.02	577	0.013-2.1	0.120	0.2	5	0-357	71.8	0.0217
Temperature	2466	8-17	11.2	1.7	408	4.3-25.1	13.9	4.2	5	5-24	172	1.98
Dissolved Oxygen	84	8-11	9	1								
TOC	357	0.91-3.14	1.36	0.185								
TON	349	7-11	9	1	103	0.849-9.94	8	1	5	12.7-29.2	149	15
Hardness	84	291-397	334	16	184	135-396	321	41				
Alkalinity	82	238-292	263	12	76	120-281	235	27	5	69.4-120	54.0	12
Chloride	120	53-100	66	10								
Orthophosphate	348	0.055-2.35	1.28	0.508								
Sulphate	120	21-41	31	4								
Sodium	84	15-24	17	1	214	7.58-103	18	13	5	10-348	70.1	22
Copper	15	0.002-0.0103	0.00336	0.0026	155	0.002-0.542	0.027	0.068				
Magnesium	84	2-3	3	0148	184	0.991-15.7	3	2				
Calcium	84	112-154	129	6	184	45.7-154	124	18				
Total Manganese	352	0.001-0.001	0.001	6e-19	557	0.001-0.015	0.001	0.002	9	0.002-18	9.73	4.47e-05
Total Iron	353	0.003-0.009	0.006	0.00151	559	0.003-0.41	0.016	0.04	5	0-84	16.4	0.00846
Free Chlorine	2518	0.003-0.009	0.908	0.101	889	0.05-1.15	0.525	0.230	9	0.05-2150	263	2.16
Total Chlorine	2518	0.6-1.25	1.02	0.103	889	0.1-1.28	0.652	0.245	5	0.1-1	0.2	0.203
3 Day CC	2320	0-82	0.145	3	256	0-189	1.95	13	9	0-2080	232	0.447
2 Day CC	2154	0-212	0.405	7	234	0-2070	38.4	222	9	0-209	232	173
Enterococci	60	0-0	0	0	76	0-0	0	0	5	0-35	69.8	
E. coli	2084	0-0	0	0	70	0-0	0	0				
Coliforms	2086	0-0	0	0	400	0-1	0.0025	0.05				
Ammonia	84	0.008-0.094	0.0403	0.008	121	0.01-0.042	0.0410	0.005	5	0.01-0.160	0.0340	12
Nitrate	349	31-46	38.4	3	103	3.763-44	37.6	6	1	37.7-37.7	37.7	0
Nitrite	416	0.009-0.009	0.009	7e-17	103	0.009-0.998	0.0283	0.133	1	0.009-0.009	0.009	0
Total Phosphorus	347	0.09-2.36	1.29	0.514	336	0.09-2.39	1.34	0.6	4	0.496-1.2	0.841	0.353
Cryptosporidium	7	0-0	0	0								
TCC	85	6-5023	412	791								
ICC	85	1-11597	388	1710								
Total Lead	15	0.16-0.16	0.16	0	139	0.16-12.2	0.881	2	4	0.16-0.27	0.198	0

Table 32 Site E descriptive statistics for the Final Water and Customer Tap

Parameter	Site E WTW				Site E PWSZ				Site E DZ			
	N	Min-Max	Mean	SD	N	Min-Max	Mean	SD	N	Min-Max	Mean	SD
pH	359	7.27-7.73	7.48	0.078	238	7.37-8	7.62	0.11	38	7.38-7.96	7.58	0.107
Conductivity	118	590-663	629	15	179	588-658	625	15	24	589-640	608	14.7
Turbidity	1973	0.013-0.42	0.0731	0.04	506	0.013-5.9	0.140	0.5	79	0.06-5.9	0.18	0.67
Temperature	597	7-14	11	0.8	372	5.5-21.2	12.3	4.1	45	6.1-20.2	12.7	4.14
Dissolved O2	350	9-11	10	0.2								
TOC	56	0.88-1.56	1.22	0.145								
TON	30	0-1	1	0.2	49	0.425-3.2	1	0.2	10	1.47-1.47	1.47	2.34e-16
Hardness	33	322-372	340	10	211	19-385	336	31	36	324-356	340	6.17
Alkalinity	33	272-330	297	13	77	255-323	287	17	12	266-304	289	11.9
Chloride	61	29-36	33	2								
Ortho P	349	0.055-1.57	0.52	0.229								
Sulphate	61	28-55	49	5								
Sodium	33	14-18	16	1	238	13.4-165	18	15	44	14.6-17.9	16.3	0.685
Copper	16	0.0021-0.03	0.01	0.008	176	0.002-0.6	0.03	0.07	34	0.0023-0.6	0.05	0.105
Magnesium	33	5-6	5	0.3	211	0.787-6.2	5	0.4	36	4.8-5.76	5.34	0.247
Calcium	33	121-140	128	4	211	6.23-145	126	12	36	122-134	127	2.35
T Manganese	202	0.001-0.0012	0.001	0.001	491	0.001-0.2	0.003	0.01	80	0.001-0.06	0.002	0.006
Total Iron	365	0.003-0.253	0.01	0.02	497	0.003-0.8	0.02	0.05	80	0.003-0.75	0.03	0.087
Free Chlorine	635	0.31-0.85	0.621	0.076	777	0.05-0.66	0.359	0.125	109	0.2-0.6	0.37	0.117
T Chlorine	633	0.51-1.02	0.744	0.0786	777	0.05-0.93	0.469	0.136	109	0.25-0.71	0.48	0.117
3 Day CC	614	0-17	0.16	1	146	0-42	0.37	3	18	0-1	0.06	0.236
2 Day CC	568	0-297	0.579	12	123	0-49	0.691	5	27	0-0	0	0
Enterococci	72	0-0	0	0	80	0-0	0	0	19	0-0	0	0
E. coli	500	0-0	0	0	72	0-0	0	0	27	0-0	0	0
Coliforms	500	0-0	0	0	274	0-0	0	0	72	0-0	0	0
Ammonia	84	0.012-0.042	0.0401	0.007	60	0.02-0.04	0.0413	0.004	12	0.042-0.04	0.04	1.45e-17
Nitrate	30	2-4	3.62	0.4	49	1.72-14.1	3.84	2	10	1.72-3.76	3.08	0.771
Nitrite	84	0.009-0.009	0.009	5e-18	49	0.009-0.009	0.009	5e-18	10	0.009-0.009	0.009	1.83e-18
T Phosphorus	349	0.423-0.517	0.473	0.0306	288	0.016-1.8	0.519	0.3	61	0.428-0.55	0.50	0.0296
Crypto spp.	3	0-0	0	0								
TCC	25	11-2213	197	460								
ICC	24	3-1892	94	383								
Total Lead	16	0.16-0.011	0.16	0.01	134	0.04-148	0.242	0.3	24	0.038-2.74	0.27	0.53

Table 33 Site F descriptive statistics for the Final Water and Customer Tap

Parameter	Site F WTW				Site F PWSZ				Site F DZ			
	N	Min-Max	Mean	SD	N	Min-Max	Mean	SD	N	Min-Max	Mean	SD
pH	182	7.19-7.57	7.35	0.0678	104	7.04-7.46	7.23	0.08				
Conductivity	90	664-716	689	16	65	659-715	687	16				
Turbidity	182	0.013-0.23	0.0623	0.03	239	0.013-4.56	0.0850	0.3	2	0.06-0.07	0.065	0.00707
Temperature	178	9-13	11	1.0	182	7-15.2	13.2	3.9	2	11.9-15.4	13.65	2.47
Dissolved O2	15	9-10	10	0.2								
TOC	7	0.9-1.18	1.05	0.107								
TON	22	6-7	6	0.2	60	5.57-8.86	7	1				
Hardness	15	368-399	384	10	85	342-396	375	12				
Alkalinity	17	277-345	305	18	30	264-350	308	22				
Chloride	15	29-40	34	4								
Ortho P	9	0.727-0.754	0.742	0.009								
Sulphate	15	38-59	46	5								
Sodium	15	15-17	16	1	100	12.6-37	16	3				
Copper	7	0.03-0.08	0.0476	0.02	56	0.0062-0.33	0.03	0.07				
Magnesium	15	5-9	8	1	85	3.36-9.04	7	1				
Calcium	15	137-146	141	3	85	129-146	139	3				
Total Manganese	15	0.001-0.001	0.001	4e-19	221	0.001-0.0021	0.003	0.012	2	0.001-0.001	0.001	0
Total Iron	15	0.003-0.007	0.006	0.002	221	0.003-0.047	0018	0.05	2	0.003-0.007	0.005	0.00283
Free Chlorine	189	0.22-0.62	0.440	0.079	415	0.05-0.62	0.346	0.107	8	0.2-0.33	0.276	0.049
Total Chlorine	189	0.34-0.68	0.509	0.0686	415	0.05-0.66	0.439	0.102	8	0.3-0.41	0.375	0.0466
3 Day CC	185	0-7	0.0487	0.5	113	0-13	0.274	2	2	0-0	0	0
2 Day CC	185	0-8	0.0541	0.6	103	0-126	2.88	15	2	0-0	0	0
Enterococci	60	0-0	0	0	61	0-0	0	0				
E. coli	185	0-0	0	0	53	0-0	0	0	6	0-0	0	0
Coliforms	185	0-0	0	0	203	0-0	0	0	8	0-0	0	0
Ammonia	41	0.042-0.055	0.0423	0.002	51	0.012-0.042	0.0414	0.004				
Nitrate	22	25-32	28.2	2	60	24.7-39.2	29.6	4				
Nitrite	38	0.009-0.02	0.00929	0.002	60	0.009-0.009	0.009	5e-18	8	0.009-0.009	0.009	0
Total Phosphorus	288	0.09-0.611	0.385	0.253	291	0.09-1.86	0.7	0.3	2	0.57500.646	0.611	0.0502
Cryptosporidium												
TCC	18	42-879	215	185								
ICC	19	12-788	86	181								
Total Lead	7	0.16-0.16	0.16	0	44	0.16-1.02	0.214	0.1	2	0.16-0.292	0.226	0

3 Tables of Data: An Overview of Water Quality Samples in the Experimental Systems

Table 34 A comparison of Final Water quality samples

Parameter	Surface Water Final Water					Groundwater Final Water				
	N	Min	Max	Mean	SD	N	Min	Max	Mean	SD
2 Day Colony Count	58	0.00	23.00	0.47	3.02	9	0.00	0.00	0.00	0.00
3 Day Colony Count	58	0.00	0.00	0.00	0.00	9	0.00	0.00	0.00	0.00
Alkalinity	2	253.00	282.00	267.50	20.51	9	314.00	335.00	323.67	7.73
Ammonia	8	0.04	0.04	0.04	0.00	9	0.04	0.04	0.04	0.00
C. perfringens	1	0.00	0.00	0.00	0.00	9	0.00	0.00	0.00	0.00
Chloride	4	53.20	283.00	125.58	106.77	9	31.00	33.60	32.34	0.99
DOC	12	1.30	9.78	2.93	3.17	6	0.90	1.50	1.15	0.28
E. coli	67	0.00	0.00	0.00	0.00	9	0.00	0.00	0.00	0.00
Enterococci	11	0.00	0.00	0.00	0.00	9	0.00	0.00	0.00	0.00
Filtered Lead	1	0.16	0.16	0.16	0.00	9	0.16	0.16	0.16	0.00
Free Chlorine	59	0.85	1.25	1.04	0.09	6	0.60	0.86	0.73	0.14
ICC	59	10.00	2318.00	100.76	316.96	9	24.00	187.00	54.22	51.59
Magnesium	16	0.001	2.63	0.32	0.86	9	7.00	7.94	8.00	0.00
Nitrate	19	0.04	44.90	38.24	13.55	9	24.80	27.50	25.68	0.75
Nitrite	19	0.01	0.01	0.01	0.00	9	0.01	0.01	0.01	0.00
Orthophosphate	10	1.01	42.10	9.14	17.03	9	0.73	0.75	0.74	0.01
pH	11	0.01	7.44	5.37	3.44	9	7.00	7.36	7.00	0.00
Sulphate	5	0.82	40.40	21.21	19.17	9	42.10	47.00	44.50	1.41
TCC	67	80.00	4882.00	447.13	727.35	9	124.00	276.00	197.56	50.27
Total Chlorine	59	0.98	1.34	1.15	0.08	6	0.69	0.92	0.81	0.12
Total Iron	22	0.01	1.23	0.74	0.57	9	0.01	0.01	0.01	0.00
Total Lead	0	-	-	-	-	9	0.001	0.001	0.001	0.00
Total Manganese	22	0.001	0.001	0.001	0.00	9	0.001	0.001	0.001	0.00

Table 35 A comparison of Customer Tap water quality samples

Parameter	Surface Water Customer Tap					Groundwater Customer Tap				
	N	Min	Max	Mean	SD	N	Min	Max	Mean	SD
2 Day Colony Count	0	-	-	-	-	9	0.00	0.00	0.00	0.00
3 Day Colony Count	7	0.00	0.00	0.00	0.00	7	0.00	0.00	0.00	0.00
Alkalinity	0	-	-	-	-	9	314	335	324	7.73
Ammonia	2	0.04	0.04	0.04	0.00	4	0.04	0.04	0.04	0.00
Calcium	1	131	131	131	000	1	139	139	139	0
Chloride	0	-	-	-	-	9	31.00	33.60	32.34	0.99
E. coli	13	0.00	0.00	0.00	0.00	14	0.00	0.00	0.00	0.00
Enterococci	2	0.00	0.00	0.00	0.00	4	0.00	0.00	0.00	0.00
Free Chlorine	13	0.30	0.94	0.70	0.22	14	0.30	0.66	0.47	0.12
ICC	0	-	-	-	-	3	13	25	17.7	5.25
Magnesium	1	2.63	2.63	2.63	0.00	1	7.95	7.95	7.95	0.00
Nitrate	2	38.30	39.40	38.85	0.78	9	0.15	32.70	12.35	14.11
Nitrite	2	0.01	0.01	0.01	0.00	4	0.009	0.009	0.009	0
Orthophosphate	5	1.00	1.09	1.04	0.04	8	0.71	0.81	0.75	0.03
pH	5	7.39	7.43	7.41	0.02	5	7.21	7.34	7.30	0.05
Sulphate	0	-	-	-	-	9	42.10	47.00	44.50	1.41
T Manganese	10	0.001	0.001	0.001	0.00	8	0.001	0.001	0.001	0.00
TCC	0					3	72	1102	416	485
Temperature	10	7.40	9.20	8.14	0.60	10	7.4	9.4	8.82	0.531
TOC	0	-	-	-	-	6	0.70	1.07	0.83	0.13
TON	2	8.65	8.89	8.77	0.17	4	5.57	7.39	6.10	0.86
Total Chlorine	13	0.40	1.01	0.82	0.22	14	0.001	0.63	0.339	0.31
Total Iron	10	0.01	0.02	0.01	0.00	8	0.01	0.01	0.01	0.00
Total Lead	7	0.16	0.38	0.21	0.09	4	0.001	0.001	0.001	0.00
Turbidity	10	0.09	0.16	0.10	0.02	8	0.09	0.10	0.09	0.00

Table 36 A comparison of experimental facility Inlet water quality samples

Parameter	Surface Water Rig 1 Inlet					Surface Water Rig 2 Inlet					Groundwater Rig 1 Inlet					Groundwater Rig 2 Inlet				
	N	Min	Max	Mean	SD	N	Min	Max	Mean	SD	N	Min	Max	Mean	SD	N	Min	Max	Mean	SD
2 Day CC	6	0	9	3	3	9	0	4	1	1	9	0	95	15	32	9	0	285	70	111
3 Day CC	6	2	23	15	8	9	0	19	8	7	9	0	334	94	105	9	34	1846	759	643
Alkalinity	6	264	280	270	6	9	202	237	221	11	9	318	342	329	10	9	81	317	233	110
Ammonia	6	0.04	0.04	0.04	0.00	9	0.04	0.04	0.04	0.00	9	0.04	0.04	0.04	0.00	9	0.04	0.04	0.04	0.00
C. perfringens	6	0	0	0	0	9	0	0	0	0	9	0	0	0	0	9	0	0	0	0
Chloride	6	57	68	62	5	9	55	85	68	13	9	30	31	30	1	9	29	31	30	1
DOC	6	1.30	1.60	1.47	0.12	9	1.40	15.00	3.02	4.49	6	0.60	1.10	0.90	0.18	6	0.80	1.00	0.87	0.08
E. coli	6	0	0	0	0	9	0	0	0	0	9	0	0	0	0	9	0	0	0	0
Enterococcus	9	0	0	0	0	9	0	0	0	0	9	0	0	0	0	9	0	0	0	0
Filtered Iron	9	0.01	0.01	0.01	0.00	9	0.01	0.01	0.01	0.00	9	0.01	0.01	0.01	0.00	9	0.01	0.04	0.01	0.01
Filtered Lead	9	0.10	0.90	0.34	0.26	9	0.20	0.30	0.24	0.05	9	0.60	4.90	1.73	1.62	9	0.70	2.90	1.41	0.69
Free Chlorine	6	1.32	1.33	1.32	0.00	12	0.00	0.00	0.00	0.00	15	0.10	0.94	0.59	0.30	15	0.00	0.38	0.08	0.15
ICC	9	707	1567	1023	370	9	7366	8795	8070	443	9	245	490	355	79	9	775	18115	6924	8031
Magnesium	6	2.50	2.62	2.56	0.05	6	2.55	2.65	2.60	0.03	7	8.25	8.63	8.39	0.13	7	8.36	8.55	8.45	0.07
Nitrate	9	41.00	44.80	42.91	1.45	9	41.20	44.80	43.02	1.43	9	24.20	28.60	25.34	1.29	9	24.30	26.50	25.42	0.66
Nitrite	9	0.01	0.01	0.01	0.00	9	0.01	0.01	0.01	0.00	9	0.01	0.01	0.01	0.00	9	0.01	0.08	0.03	0.04
Ortho P	9	1.04	1.22	1.10	0.06	9	0.06	0.06	0.06	0.00	9	0.73	0.75	0.74	0.01	9	0.06	0.06	0.06	0.00
pH	9	7.32	7.38	7.35	0.02	9	7.41	7.54	7.45	0.05	9	7.14	7.38	7.24	0.09	9	7.15	7.74	7.39	0.26
Sulphate	9	30	35	33	2	9	32	38	36	1	9	43	47	45	1	9	42	49	46	2
T Manganese	9	0.001	0.001	0.001	0.00	9	0.001	0.001	0.001	0.00	9	0.001	0.001	0.001	0.00	9	0.001	0.001	0.001	0.00
TCC	9	12295	14269	13030	586	9	9326	12108	10767	908	9	1434	1964	1625	171	9	1105	22457	9375	9239
TOC	9	1.31	1.62	1.45	0.13	9	1.33	1.68	1.49	0.14	6	0.71	0.86	0.82	0.06	6	0.81	1.59	0.97	0.31
TON	9	9.26	10.10	9.76	0.37	9	9.30	10.10	9.72	0.32	9	5.47	6.45	5.72	0.29	9	5.49	6.01	5.75	0.16
Total Chlorine	6	1.32	1.35	1.33	0.01	12	0.00	0.00	0.00	0.00	12	0.62	0.80	0.74	0.07	12	0.00	0.57	0.14	0.25
Total Iron	9	0.001	0.05	0.02	0.02	9	0.01	0.05	0.02	0.02	9	0.01	0.01	0.01	0.00	9	0.01	0.15	0.06	0.06
Total Lead	9	13.90	72.00	31.19	19.45	9	0.16	0.36	0.21	0.08	9	0.64	4.67	2.16	1.68	9	1.67	12.10	4.14	3.32
Turbidity	3	1	2	2	0	3	0	0	0	0	2	0	0	0	0	2	0	0	0	0

Table 37 A comparison of experimental facility Outlet water quality samples

Parameter	Surface Water Rig 1					Surface Water Rig 2					Groundwater Rig 1					Groundwater Rig 2				
	N	Min	Max	Mean	SD	N	Min	Max	Mean	SD	N	Min	Max	Mean	SD	N	Min	Max	Mean	SD
2 Day CC	18	0	1548	719	575	18	0	640	207	228	18	0	1438	233	515	18	0	460	111	164
3 Day CC	18	0	4369	1436	1344	18	0	1466	454	483	18	0	872	157	258	18	0	670	252	226
Alkalinity	18	202	284	223	27	18	52	232	169	68	18	265	376	314	25	18	121	310	216	88
Ammonia	18	0.01	0.08	0.04	0.02	15	0.04	0.04	0.04	0.00	18	0.01	0.04	0.04	0.01	18	0.02	0.04	0.04	0.01
C. perfringens	18	0	0	0	0	18	0	0	0	0	18	0	0	0	0	18	0	0	0	0
Calcium	3	129	129	129	0	3	125	130	128	2	3	128	135	132	3	3	68	72	70	1
Chloride	18	64	76	70	4	18	60	86	68	9	18	28	33	31	1	18	28	31	30	1
Conductivity	3	656	658	657	1	3	671	673	672	1	3	660	663	662	3	3	446	449	448	1
DOC	9	1.30	1.50	1.38	0.07	12	1.20	1.70	1.41	0.22	9	0.90	1.20	0.98	0.10	9	0.90	1.20	1.01	0.09
E. coli, Entero	18	0	0	0	0	18	0	0	0	0	18	0	0	0	0	18	0	0	0	0
Filtered Iron	9	0.01	0.01	0.01	0.00	15	0.01	0.01	0.01	0.00	15	0.01	0.01	0.01	0.00	15	0.01	0.01	0.01	0.00
Filtered Lead	15	0.30	0.80	0.50	0.17	15	0.20	0.50	0.26	0.12	15	1.40	17.10	6.19	5.79	15	0.20	2.70	0.77	0.82
Free Chlorine	18	0.10	3.30	1.38	1.07	21	0.00	0.02	0.00	0.01	21	0.06	0.70	0.38	0.25	21	0.00	0.01	0.00	0.00
ICC	18	73	39200	12769	13734	18	454	23631	11608	7072	18	29	4320	1396	1526	18	176	29375	10571	10994
Magnesium	15	2.48	2.64	2.57	0.04	15	2.35	2.64	2.54	0.09	15	8.11	8.71	8.41	0.15	15	7.82	8.73	8.29	0.25
Nitrate	18	42.1	44.3	43.1	0.66	18	40.3	44.60	43.41	1.20	18	23.60	26.80	25.00	0.76	18	24.0	27.30	25.34	0.88
Nitrite	18	0.001	0.03	0.01	0.01	18	0.01	0.02	0.01	0.00	18	0.01	0.03	0.01	0.01	18	0.01	0.03	0.01	0.01
Ortho P	18	0.725	1.037	0.927	0.102	18	0.06	0.055	0.055	0	18	0.06	0.776	0.61	0.259	18	0.06	0.069	0.056	0.003
pH	15	7.54	8.85	8.51	0.50	15	7.68	8.19	7.921	0.19	15	7.51	8.54	8.171	0.379	15	7.24	8.15	7.825	0.313
Sulphate	18	31	35	34	1	18	25	38	34	4	18	42	48	45	2	18	41	107	48	15
T Manganese	15	0.001	0.001	0.001	0.00	18	0.001	0.01	0.001	0.01	18	0.001	0.01	0.001	0.01	18	0.001	0.01	0.001	0.01
TCC	18	1368	84150	24047	29800	18	8977	33046	19143	7517	18	99	13531	3760	4697	18	929	58919	17180	19290
TOC	15	1.19	1.65	1.38	0.13	15	1.02	1.67	1.33	0.25	12	0.70	1.03	0.91	0.09	12	0.65	1.07	0.91	0.15
TON	18	9.51	10.0	9.74	0.15	18	9.11	10.10	9.80	0.27	18	5.34	6.04	5.65	0.17	18	5.42	6.17	5.73	0.20
Total Chlorine	18	1.03	4.80	2.17	1.32	21	0.00	0.03	0.00	0.01	21	0.08	0.74	0.44	0.26	21	0.00	0.04	0.00	0.01
Total Iron	9	0.01	0.01	0.01	0.00	18	0.01	0.28	0.04	0.08	18	0.01	0.01	0.01	0.00	18	0.01	0.11	0.03	0.04
Total Lead	18	47.3	82.6	57.1	10.9	18	0.16	6.37	0.64	1.44	18	0.90	33.40	8.03	10.87	18	0.16	4.19	1.73	1.39

4 Risk Score Matrix: Classification of Site Biological Stability using Historical Data Analysis

Figure 68 Site A classification of "Less Good" biological stability

Figure 69 Site B classification of "Less Good" biological stability

Figure 70 Site C classification of "Less Good" biological stability

Figure 71 Site D classification of "Less Good" biological stability

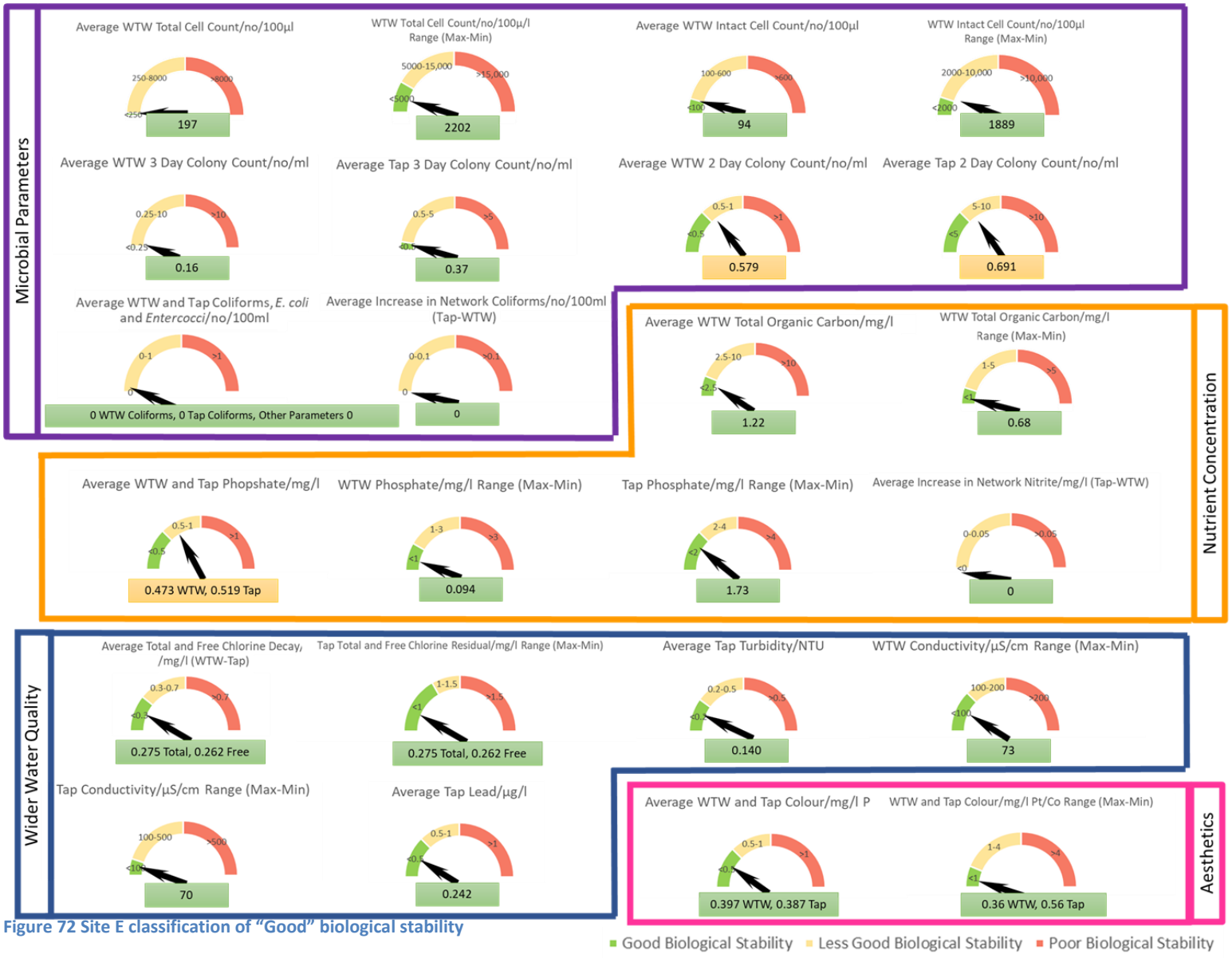


Figure 72 Site E classification of "Good" biological stability

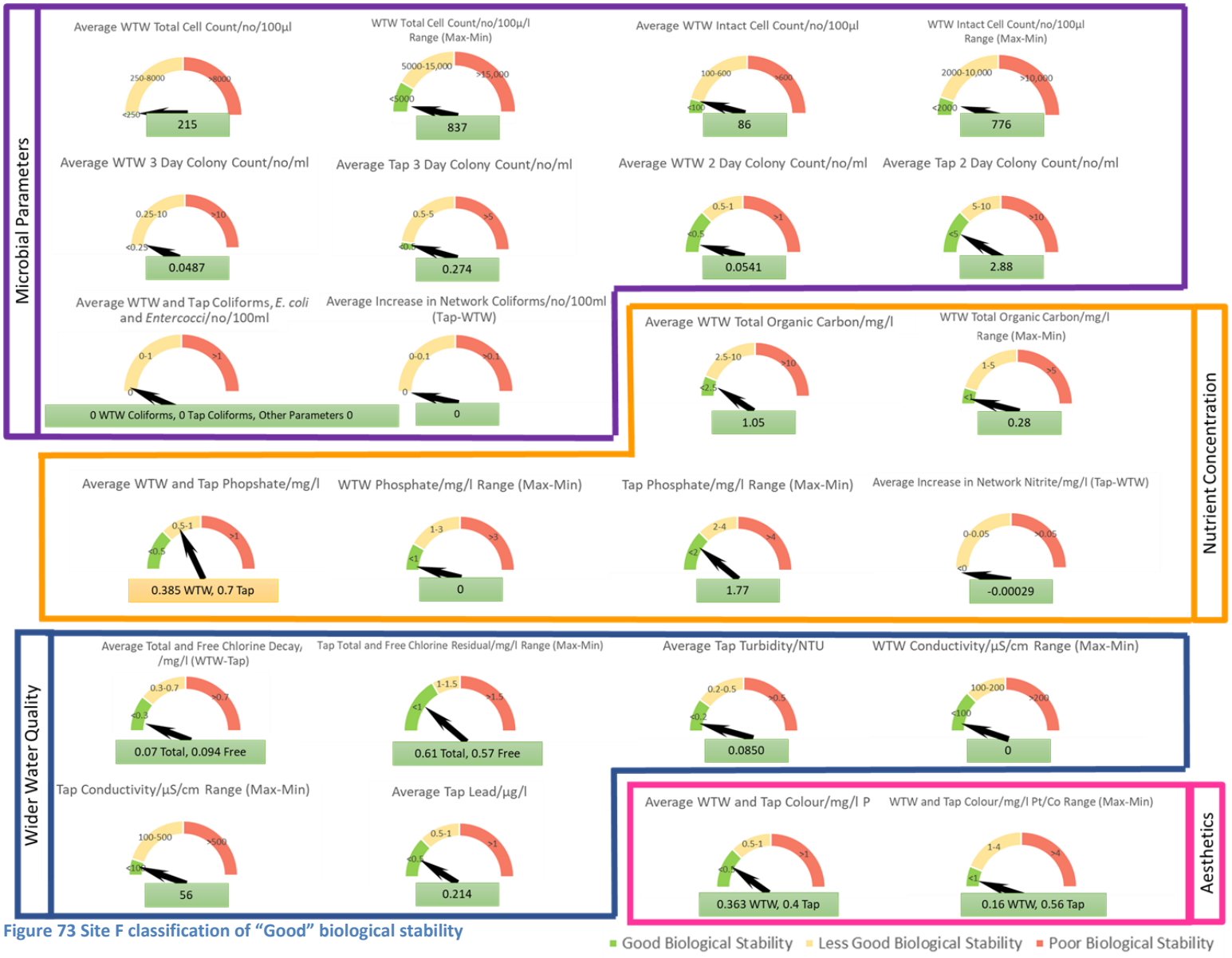


Figure 73 Site F classification of "Good" biological stability

5 Risk Score Matrix: Classification of Rig Facilities using Experimentation Data Analysis

Figure 74 Surface Water R1 Chemically Fed Rig classification of "Less Good" biological stability

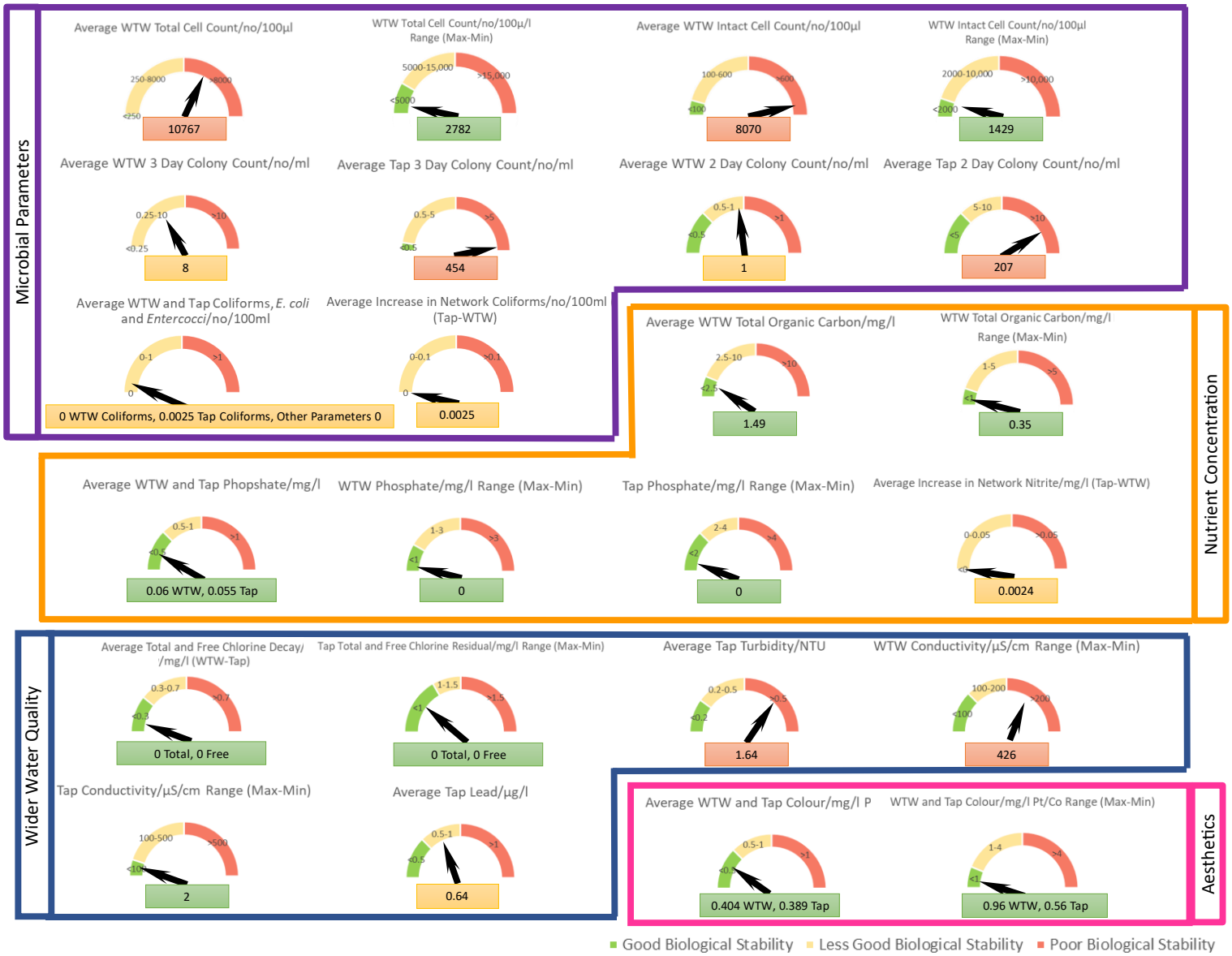


Figure 75 Surface Water R2 Chemical Free Rig classification of "Less Good" biological stability

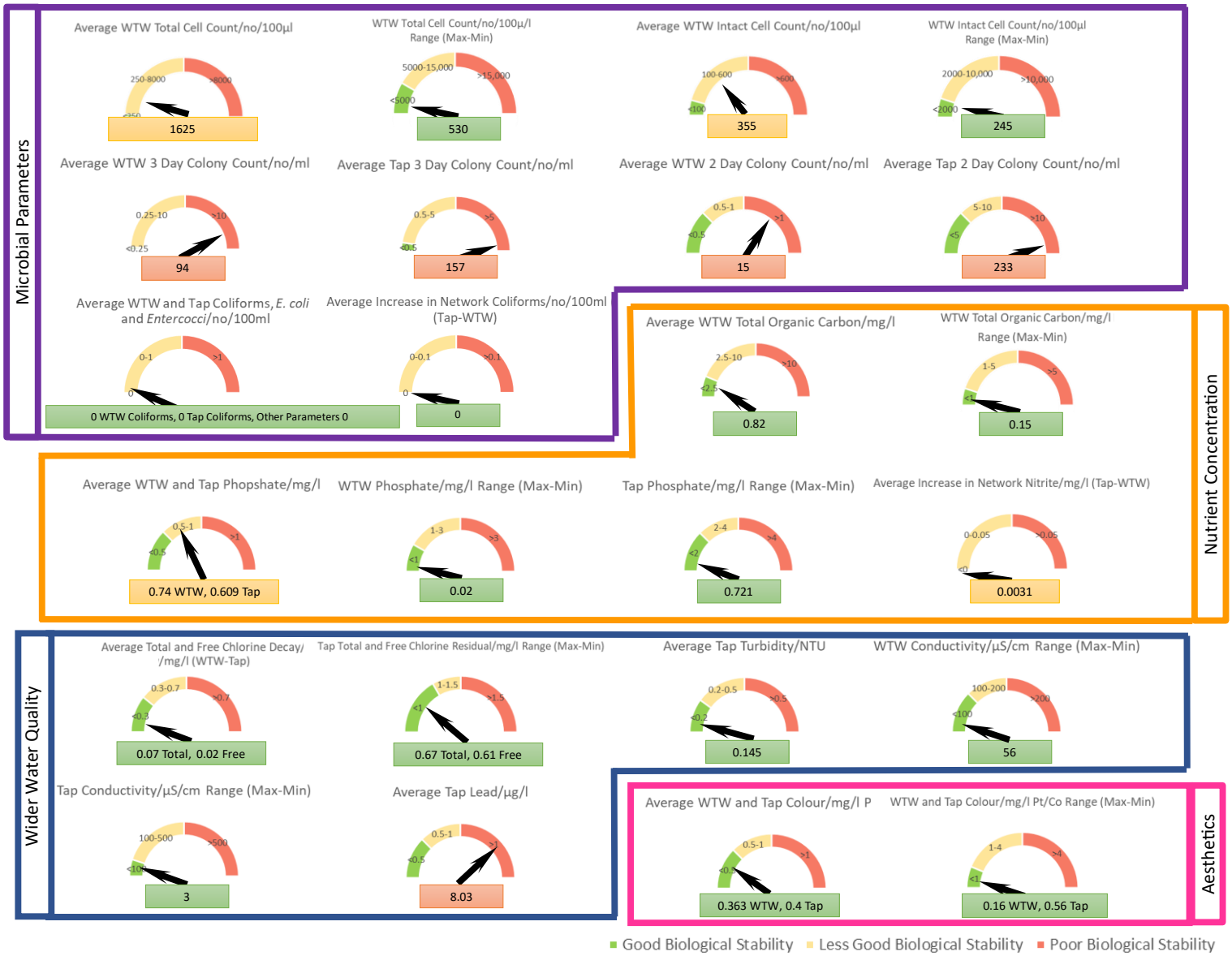


Figure 76 Groundwater R1 Chemically Fed Rig classification of "Less Good" biological stability

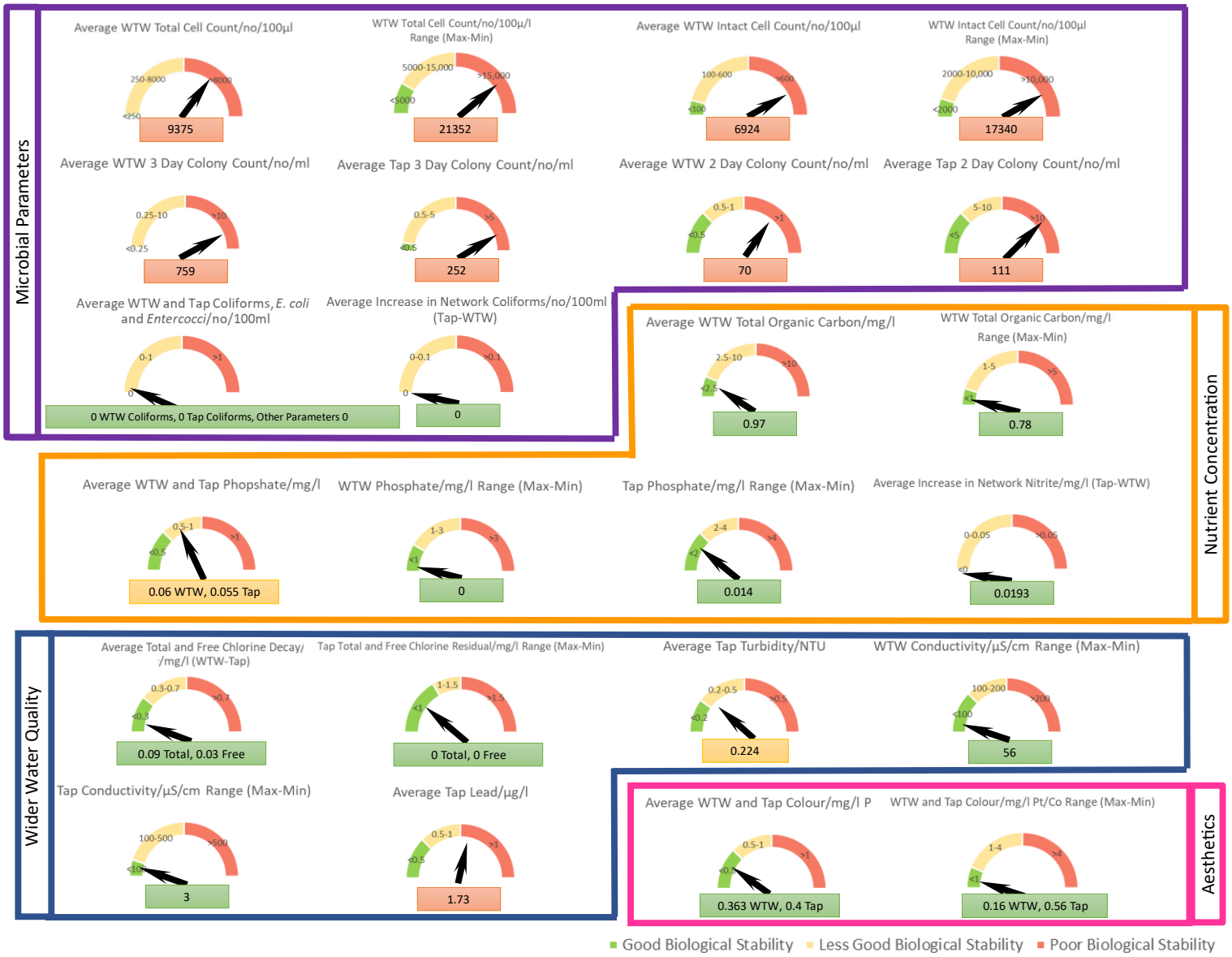


Figure 77 Groundwater R2 Chemical Free Rig classification of "Less Good" biological stability