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MONITORING BIOSTABILITY AND BIOFILM FORMATION POTENTIAL IN DRINKING WATER DISTRIBUTION SYSTEMS

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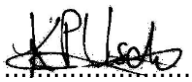
A research report submitted to the Faculty of Engineering and the Built Environment, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Science in Engineering.

August, 2017

Declaration

I, **Kowho Pearl Useh** declare that this research report is my own unaided work. It is being submitted for the Degree of Master of Science in Engineering at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at the University of the Witwatersrand or to any other University.

K.P Useh



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Abstract

The foremost aim of potable water treatment is to produce water that does not pose a health risk when consumed and/or otherwise used. Nevertheless, research has established that the quality of treated water deteriorates during distribution. The nature and extent of this deterioration varies from system to system and from time to time. The aim of this research study was to monitor the parameters that are known to significantly affect biostability and biofilm formation potential in drinking water distribution systems. Biweekly water samples were collected from thirteen sites, across a section of Johannesburg Water's network, between September 2015 and August 2016. All samples were assayed for a suite of fifteen water quality parameters using standard methods. Heightened temperature, dearth of chlorine residuals, availability of biodegradable dissolved organic carbon (BDOC), and advanced water age all engendered the loss of biostability (instability). Biostability controlling parameters varied seasonally and spatially. Samples collected during spring and summer, in general, were most likely to be characterized by instability than samples collected during winter and autumn. Samples collected from sites RW80, RW81, RW82, RW83, RW104 and RW253 were more prone to instability compared to samples from other sites. From the results, it is clear that chlorine residuals ought to be kept above 0.2 mg/l, and, BDOC below 0.3mg/l to prevent the loss of heterotrophic stability in distributed water. BDOC concentrations can be decreased by, flushing the pipes, cleaning reservoirs regularly and by further treating feed water before distributing. Booster disinfection can be relied upon to ensure that chlorine residuals are maintained throughout the network. Apart from potential health risks, biological instability and biofilm growth can result in non-compliance with regulations.

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Author

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Table of contents

Declaration ii

Abstract iii

Acknowledgments iv

List of figures vii

List of tables ix

List of abbreviations x

1. Introduction 1

1.1 Johannesburg Water 2

1.2 Problem statement 5

1.3 Research question 6

1.4 Hypothesis 6

1.5 Objective 7

1.6 Assumptions 7

1.7 Scope of research 7

1.8 Rationale for the study 8

1.9 Report structure 9

2. Literature Review 10

2.1 Introduction 10

2.2 Bacterial growth and water quality in drinking water distribution systems 10

2.2.1 Water quality 11

2.2.2 Bacterial growth and the loss of biological stability 12

2.2.3 Methods to measure biostability 19

2.2.3.1 Strategies for controlling bacterial growth and regrowth and the loss of biostability 20

2.2.4 Biofilm 21

2.2.4.1 Factors affecting biofilm formation 25

2.2.4.2 Other biofilm microorganism 27
2.3 Water quality modelling 27
2.3.1 Types of models 27
2.4 Literature review summary and conclusions 33
3. Study Design 34
3.1 Sampling procedures 34
3.1.1 Sampling protocol 38
3.1.2 Sampling plan 38
3.1.3 Sample preparation 40
3.2 Methods and instruments for data gathering 41
3.2.1 Measurement of physical water quality parameters 41
3.2.2 Measurement of chemical water quality parameters 42
3.2.3 Measurement of microbiological water quality parameters 46
3.3 Research ethics 48
3.4 Data analysis 48
4. Results and Discussion 49
4.1 Results 49
4.2 Discussion 68
5. Conclusions and Recommendations 79
5.1 Conclusions 79
5.2 Recommendations for future research 80
References 82
Appendix A – Raw data 90
Appendix B – Map of City of Johannesburg existing water districts and bulk supply system 99

List of figures

Figure 1: Water districts that are supplied by Johannesburg water. (Source: Google map, 2016) 3
Figure 2: Locations within area 10 and area 5 of Johannesburg waters districts. (Source: Google maps, 2016) 5
Figure 3: Processes involved in biofilm formation: (1) preconditioning the pipe surface by macromolecules (organic and inorganic) present in the water; (2) transport of planktonic cells from water to pipe surface; (3) adsorption of cells at the pipe surface; (4) desorption of reversibly adsorbed cells; (5) irreversible adsorption of cells; (6) production of signalling molecules; (7) transport of substrates to and within the biofilm; (8) substrate metabolism by the biofilm-bound cells and transport of products out of the biofilm, accompanied by cell growth, replication, and production of eps; (9) biofilm removal by detachment or sloughing. (Source: Simoes and Simoes, 2013) 22
Figure 4: Processes taking place within a pipe in a drinking water distribution system. (Source: Blokker *et al*., 2008) 29
Figure 5: Electron flow schematic for bacterial metabolism in the CDWQ-E model (Biyela, 2010) 31
Figure 6: Sampling points in relation to each other and the rand water treatment plant. (Source: Google maps, 2016) 36
Figure 7: Temperature over time for all 13 sites 50
Figure 8: ph over time for all 13 sites 51
Figure 9: Conductivity over time for all 13 sites 52
Figure 10: Dissolved oxygen over time for all 13 sites 53
Figure 11: Alkalinity over time for all 13 sites 54
Figure 12: Total nitrogen over time for all 13 sites 55
Figure 13: Nitrate over time for all 13 sites 56
Figure 14: Total ammonia over time for all 13 sites 57
Figure 15: Ammonium over time for all 13 sites 58
Figure 16: Free chlorine over time for all 13 sites 59
Figure 17: Monochloramine concentration over time for all 13 sites 60
Figure 18: Total chlorine over time for all 13 sites 61
Figure 19: HPC over time for all 13 sites 62

Figure 20: Faecal coliform over time for all 13 sites 62
Figure 21: Total coliform over time for all 13 sites 63
Figure 22: Dissolved organic carbon over time for all 13 sites 64
Figure 23: BDOC concentration over time for all 13 sites. 65

List of tables

Table 1: Physical, chemical and microbiological parameters (Source: SANS241, 2011) 11
Table 2: Characteristics of an ideal disinfection method. (Source: Metcalf and Eddy, 2014) 14
Table 3: Advantages and disadvantages of chlorine, chlorine dioxide, ozone and UV for disinfection of treated water. (Source: Metcalf and Eddy, 2014)..... 15
Table 4: Tower and reservoir capacities. (Source: JW, 2011) 35
Table 5: Sample location and description (Source: JW, 2015) 37
Table 6: Sampling frequency and regulation of the measured parameters (Source: SANS241, 2011) 39
Table 7: Internal and external replicates for each parameter tested 47
Table 8: Correlation coefficients between different sets of parameters for all 13 sites over one year 67

List of abbreviations

AOC	Assimilable organic carbon
APHA	American public health association
BAP	Biomass associated products
BAR	Biofilm annular reactor
BAS	Biologically active sand
BIS	Bureau of Indian standards
BOM	Biodegradable organic matter
BDOC	Biodegradable dissolved organic carbon
BRP	Bacterial regrowth potential
CDWQ	Comprehensive disinfection and water quality model
CDWQ-E	Expanded comprehensive disinfection and water quality model
CDWQ-E_2	Expanded comprehensive disinfection and Water quality model, version 2
CFU	Colony forming unit
EPA	Environmental protection agency
EPS	Extracellular polymeric substances
DBPs	Disinfection by-products
DOC	Dissolved organic carbon
DO	Dissolved oxygen
DWDS	Drinking water distribution system
DWS	Department of water and sanitation

HPC	Heterotrophic plate count
JW	Johannesburg Water
MAP	Microbial available phosphorus
MPN	Most probable number
NH_3	Total ammonia
NH_4	Ammonium
NO_3	Nitrate
NOM	Natural organic matter
OHS	Occupational health and safety
PE	Polyethylene
PVC	Polyvinylchloride
RW	Rand water
SANS	South African national standards
SMP	Soluble microbial products
TCC	Total cell concentration
TN	Total nitrogen
TOC	Total organic carbon
UAP	Utilization associated products
UV	Ultra violet radiation
WHO	World health organization

1. Introduction

In today's society, drinking water distribution systems (DWDSs) are vital for protecting public health and for fostering economic growth (Ramos *et al.*, 2010). Water distribution infrastructure is susceptible to material and technological failure of such hydraulic system components as valves and pipes. The failure of any distribution component can compromise the quality and quantity of water available to consumers. In a distribution system, water has to travel through a network of pipes. In the time it takes for the water to leave the treatment plant and reach the consumer's tap, its quality is affected by a series of physical, chemical and biological processes (Ramos *et al.*, 2010). This is why post treatment water quality monitoring and management is important.

One of the central goals of drinking water treatment is removal of microbial disease agents present in the water, as well as ensuring protection against the intrusion of microbial disease agents downstream of treatment. Protection against microbial agents is usual accomplished through disinfection. However, water treatment processes are not designed to produce sterile water, even when disinfection is employed there are always microorganisms that bypass the treatment barrier. These microorganisms can contribute towards the deterioration of water quality, as previous studies have shown (Zhang *et al.*, 2004; Moritz *et al.*, 2010; Wang *et al.*, 2014). For this reason, constant monitoring of water quality downstream of treatment is necessary. The monitoring can be in the form of field and laboratory studies or through the use of water quality models. Incorporating this in the management of potable water will assist in the identification of factors that lead to the deterioration of water quality over time in a distribution system. This will help address one of the most common problems faced by drinking water utilities (such as Johannesburg Water), which is how to continuously maintain the levels of residual disinfectants necessary to effectively control bacterial growth without resorting to using excessively high concentrations of disinfectants during treatment (Woolschlager *et al.*, 2005; Lu *et al.*, 2014).

1.1 Johannesburg Water

Johannesburg Water (JW) is a state owned water utility that serves the City of Johannesburg (population approximately 4.5 million people), and, as is the case with all water utilities in South Africa, reports to the Department of Water and Sanitation (DWS)(Johannesburg Water, 2016). The utility owns drinking water distribution infrastructure (composed of a network of approximately 11 000km distribution pipes; 89 water reservoirs; 28 water towers; 35 pump stations; and other fixtures), wastewater collection and transmission infrastructure, as well as wastewater treatment infrastructure (composed of 6 wastewater treatment works, 6 sludge treatment facilities and 37 sewer pump stations) (Johannesburg Water, 2016). Johannesburg Water buys potable water from Rand Water (RW) and the only form of treatment that the water is subjected to under the control of JW is booster chlorination that happens inside the storage tanks and at various points along the network (Johannesburg Water, 2016). Figure 1, below, is a map of the area serviced by JW.



Figure 1: Water districts that are supplied by Johannesburg Water. (Source: Google Map, 2016)

In line with the South African National Standards specification, SANS 241 for drinking water, JW has a rigid water quality monitoring programme for their distribution system. Over and above this, the utility also has water quality monitoring programmes for effluents from their wastewater treatment plants as well as surface water and groundwater sources (Johannesburg Water, 2014). In terms of drinking water quality management, JW has achieved the blue drop status for the 2009, 2010, 2011, 2012 and 2014 assessments. This equates to a blue drop for each blue drop assessment ever carried by the Department of Water and Sanitation and its predecessors (DWS, 2016). The blue drop programme is an initiative of the Department of Water and Sanitation, created to ensure that water utilities comply with regulation standards of drinking water and that they employ adequate water monitoring programmes. If blue drop status has been achieved this means that the water utility complies with (1) process control, maintenance

and management, (2) water quality monitoring programmes and analyses, (3) regular submission of water quality results to DWS, (4) compliance with SANS241 and (5) water quality failure response management, to achieve and maintain standards of best practice and excellence (DWS, 2008; Johannesburg Water, 2016). Achieving a blue drop status does not necessarily mean that a utility is producing and/or supplying water that meets the highest standards of quality, as is often portrayed by marketing campaigns. The blue drop certification programme seems to place more importance on administrative processes rather than on the water quality (Polasek, 2009). Using the blue drop grading system too much to assess the water quality in a particular district would be irresponsible as it creates a false sense of security. For example, when it comes to water quality standards the blue drop programme is fully dependant on SANS241 (Polasek, 2009). While this makes perfect sense as SANS241 is South Africa's current water quality regulatory code, SANS241 is in no way as comprehensive as it can be (Polasek, 2009). When it comes to measuring natural organic matter (NOM), for example, the code only includes total organic carbon and dissolved organic carbon and does not consider the other forms of NOM.

Historical data obtained from JW for this research, only included parameters analysed and recorded for compliance purposes, these include: conductivity, pH, turbidity, taste, colour, odour, alkalinity, disinfectant residuals, heavy metals, dissolved nitrogen species, organic matter, ions, phenols, coliforms and heterotrophic plate count (HPC). Some parameters that are important as determinants of biostability and biofilm formation potential such as temperature, biodegradable dissolved organic carbon (BDOC), dissolved oxygen (DO), ammonium and total nitrogen are not monitored. Also, and most importantly, the database had gaps even for parameters that are monitored. While the data collected by JW is enough to measure water quality trends across the network this data is not sufficient to predict biostability and biofilm formation potential. It was for this reason that in this study a more comprehensive water quality monitoring programme was undertaken. This programme spanned one of the seven regions supplied and monitored by JW, which is approximately 25% of the entire distribution network (Johannesburg Water, 2011). Within this region are area 5 (Fairland and Northcliff) and area 10 (Blairgowrie) situated within the greater Randburg area (Figure 2). These areas were chosen because of their history of

poor water quality. A total of thirteen sampling sites, two from area 10 and eleven from area 5, were monitored. The reason for the choice in sampling sites is addressed further in chapter 3 of this report.



Figure 2: Locations within area 10 and area 5 of Johannesburg Waters districts. (Source: Google Maps, 2016)

1.2 Problem statement

In this research report regrowth is defined as the process whereby bacteria that were previously injured during the treatment process start to multiply after recovering from reversible injury (Momba *et al.*, 2000; Bartram *et al.*, 2003; Srinivasan and Harrington, 2007; Lu *et al.*, 2014). Growth is defined as the growth of new bacteria fuelled by the oxidation of substrates in the water (Bartram *et al.*, 2003; Lu *et al.*, 2014). Biostability is the ability to limit new growth and/or regrowth

of microorganisms by creating a balance in the availability of substrate and residual disinfectants (Srinivasan and Harrington, 2007; Lu *et al.*, 2014). It is a concept that is poorly understood by water utilities (Lu *et al.*, 2014). The decline of drinking water quality in distribution systems can potentially lead to the proliferation of pathogens and opportunistic pathogens linked to water-borne diseases (Biyela, 2010; Hammes *et al.*, 2010; Lu *et al.*, 2014) and may also result in the breaching of regulatory standards such as coliform standards. In this study a very detailed water quality monitoring programme was carried out over a year. The programme focused on the monitoring of water quality parameters that are known to affect biological stability and biofilm formation potential. The data from the field study will enable JW to identify hotspots for such problems as extensive bacterial growth and extreme loss of disinfectant residuals within the distribution network.

1.3 Research question

The research question, which in this study was, “which water quality parameters have the most effect on the biostability of potable water during distribution?”

To be able to come up with an answer to the research question adequate knowledge and understanding of drinking water distribution systems and water quality parameters was required.

1.4 Hypothesis

In a distribution system, the water quality parameters that will have the most impact on biostability are temperature, disinfection residual and oxidizable substrates.

1.5 Objective

The objective of this research study was:
To monitor water quality parameters that are known to affect biostability and biofilm formation potential in drinking water distribution systems.

1.6 Assumptions

The following assumptions were made:

- Within the distribution network bacterial growth is primarily dependent on temperature, availability of oxidizable electron donor substrates (BDOC, ammonia and nitrite) the waters residence time inside the network and residual disinfectant(s) concentration(s)(Zhang *et al.*, 2004; Biyela, 2010; Hammes *et al.*, 2010; Lu *et al.*, 2014).

- Within the distribution network, biofilm formation and accumulation potential depends on water quality as detailed in the previous assumption and the hydraulic conditions in the network e.g. flow rate, (plays a role in detachment and attachment of microorganisms) and retention time (Lehtola, 2006; Manuel *et al.*, 2007,2010)

1.7 Scope of research

The focus of this research was monitoring water quality to determine biostability and biofilm formation potential within a segment of JW's distribution network using field and laboratory data. Only water quality parameters known to affect biostability and biofilm formation and growth were considered. This research did not account for the engineering aspects in a distribution system that could affect water quality.

1.8 Rationale for the study

This research was justified on the grounds of practical and knowledge based contributions.

Practical contributions

This research study produced field and laboratory data that can be used to deduce information about water quality, biostability, and biofilm formation potential in a distribution system. In future, data obtained from this study will be used in a separate research study to calibrate an existing model to determine its accuracy in predicting water quality in drinking water distribution systems. It will improve the management of drinking water distribution systems to ensure that the regulations are adhered to and create a model that can be adapted for other systems all over South Africa and not just for Johannesburg Water.

Knowledge contributions

This research study improves the knowledge base on water quality models and the importance of water hydraulics on water quality. It also emphasizes the importance of field and laboratory data in model calibration and testing. This is extremely important, as models cannot be accurately calibrated without sufficient, relevant and reliable field and laboratory data. There can never be one model that fits all distribution systems. Hence, the need for field data that corresponds with the chosen distribution system.

1.9 Report structure

This section is giving an overview of this report, to show how the objective mentioned in section 1.5 of chapter 1 was achieved.

Chapter 1: Chapter 1 introduces the research area and articulates the relevance of the research and the objective that it strives to meet.

Chapter 2: Chapter 2 reviews the factors that affect biostability and biofilm growth, as well as water quality models that have been used in the past. The information provided in this chapter aids in the understanding of the need for improved water quality monitoring programmes.

Chapter 3: Chapter 3 details the methodology used to carry out this research, from the planning phase all the way to the implementation phase.

Chapter 4: Chapter 4 documents and analyses the data obtained from the field and laboratory.

Chapter 5: Chapter 5 provides a summary of the findings from this study, as well as, suggestions for future research.

2. Literature Review

2.1 Introduction

While producing biologically stable water at treatment plants is an important step in ensuring that distributed water is also biologically stable, sometimes this is not enough. Chief amongst the causes of the loss of biostability in distributed water is biofilm formation within drinking water distribution systems (DWDSs) (van der Kooij, 1999; Manuel *et al.*, 2010).

Drinking water regulations have been put in place to limit bacterial growth and regrowth in distribution systems but the lack of understanding of the functioning of the system makes it difficult for utilities to adhere to these regulations. Distribution systems need to keep up to date with their methods of managing bacterial growth and biofilm formation.

This review analyses literature on biostability and biofilm formation within drinking water distribution systems. A brief background is given on various water quality models that can be used to monitor distribution systems water quality deterioration.

2.2 Bacterial growth and water quality in drinking water distribution systems

In DWDSs surfaces in contact with water are at risk of microbial contamination. The bulk of new bacterial growth occurs on the pipe surfaces compared to the flowing water (Lehtola *et al.*, 2004; Moritz *et al.*, 2010). Microbial growth in DWDSs is usually controlled by either maintenance of residual disinfectants or by achieving biological stability at the drinking water treatment plant (van der Kooij, 1999; Lu *et al.*, 2014). Control of microbial growth leads to better water quality in the distribution system and at consumer's taps.

2.2.1 Water quality

Water quality testing is necessary in determining the quality of the water source and the effects it may have if consumed and/or used in other ways. For example, dissolved oxygen (DO) is a measure of how much oxygen is in the water (Kumar and Puri, 2012). DO is an important parameter when assessing water quality because the level of DO will determine whether certain microorganisms will thrive. The DO levels can also affect the longevity of the pipe material used due to the importance of oxygen in corrosion chemistry (Sarin *et al.*, 2004). An increase in temperature leads to a decrease in DO. An increase in oxygen-respiring bacteria (and other microorganisms) will also lead to a decrease in DO levels (LeChevallier, 1999).

In South Africa the drinking water standards are detailed in SANS241 (2011). Table 1 below, show water quality parameters regulated according to the SANS241 (2011) code, the risks associated with each water quality determinant, and the upper limit of each determinant. If the SANS241 (2011) regulation is not adhered to by water utilities, it could lead to bacterial growth and the loss of biostability in the water.

Table 1: Physical, chemical and microbiological parameters (Source: SANS241, 2011)

Determinant	Risk	Unit	Standard Limits
Physical determinants			
Colour	Aesthetic	mg/l	≤15
Odour and taste	Aesthetic	N/A	Inoffensive
Turbidity	Aesthetic	NTU	≤5
	Operational	NTU	≤1
Chemical determinants			
Free chlorine	Chronic health	mg/l	≤5
monochloramine	Chronic health	mg/l	≤3
Conductivity at 25°C	Aesthetic	mS/m	≤170
pH at 25 °C	Operational	pH units	≥5 to ≤9.7
Nitrate as N	Acute health -1	mg/l	≤11
Nitrite as N	Acute health -1	mg/l	≤0.9
Ammonia as N	Aesthetic	mg/l	≤1.5

Determinant	Risk	Unit	Standard Limits
Microbiological determinants			
E. coli [a] or Faecal coliforms [b]	Acute health -1	Count per 100ml	Not detected
Total coliforms [c]	Operational	Count per 100ml	≤10
Heterotrophic plate count [d]	Operational	Count per ml	≤1000
[a] Definitive preferred indicator of faecal pollution; [b] Indicator of unacceptable microbial water quality. It provides information on treatment efficiency and after-growth in distribution networks; [c] Indicates potential faecal pollution and provides information on treatment efficiency and after growth; [d] Process indicator that provides information on treatment efficiency, after growth in distribution networks and adequacy of disinfectant residuals.			

2.2.2 Bacterial growth and the loss of biological stability

There are several factors that affect bacterial growth, and therefore fuel the loss of biological stability. Biological stability, commonly known as biostability is the ability to limit new growth and/or regrowth of microorganisms by creating a balance between the availability of substrate and residual disinfectants (Srinivasan and Harrington, 2007; Lu *et al.*, 2014). Biostability serves as an indication of water quality within the distribution system (Srinivasan and Harrington, 2007; Hammes *et al.*, 2010). In a drinking water distribution system, there will be multiple biostability curves representing the multiple species of bacteria present. This means that the water can simultaneously be biologically stable for one bacteria species while being unstable for another species (Srinivasan and Harrington, 2007). Biostability is affected by several factors, which are discussed below.

Disinfection

The treatment of drinking water often requires both primary and secondary disinfection. Primary disinfection refers to the disinfection that takes place at the treatment plant and is aimed at killing and/ or inactivating pathogens and opportunistic pathogens, as well as the reduction of pollution indicator microorganisms (van der Walt *et al.*, 2009; EPA, 2011). Secondary disinfection refers to the addition of disinfectants at the final stage of the water treatment process just before the treated water is distributed and/or the addition of

disinfectants during distribution either at booster chlorination stations or inside storage reservoirs/towers (van der Walt *et al.*, 2009; EPA, 2011).

Disinfection can be carried out by the use of chemical agents, physical mechanism (e.g. ionizing radiation and high/low temperatures) as well as mechanical action (Metcalf and Eddy, 2014). Chemical disinfecting agents include chlorine and its compounds (combined chlorine, chlorine gas, sodium hypochlorite and chlorine dioxide) and ozone (Metcalf and Eddy, 2014).

There are several factors that need to be considered when selecting a method of disinfection. The Water Treatment Manual: Disinfection (EPA, 2011) and the, South African Oxidation and Disinfection Manual (van der Walt *et al.*, 2009) provide lists of key factors to consider, these include:

- The effectiveness of the disinfectant in destroying the pathogen(s) of concern;
- The quality of the water to be disinfected;
- The potential for formation of undesirable disinfection by-products;
- The ability to easily verify the operation of the chosen disinfection system by reference to system validation, collation of monitoring data and alarm generation.
- The ease of handling, and health and safety implications of a disinfectant;
- The treatment processes that will be situated upstream of the disinfection step;
- The overall cost of treatment in general and disinfection in particular

In addition to the different factors that need to be considered when selecting a method of disinfection, the chosen method of disinfectant should have the desired response to the characteristics tabulated in Table 2.

Table 2: Characteristics of an ideal disinfection method. (Source: Metcalf and Eddy, 2014)

Characteristic	Disinfectant reaction
Availability	Should be available in large quantities and reasonably priced
Deodorizing ability	Should deodorize while disinfecting
Homogeneity	Solution must be uniform in composition
Interaction with unrelated material	Should not be absorbed by organic matter other than bacterial cells
Noncorrosive and non-staining	Should not disfigure metals or stain fabric
Nontoxic to higher forms of life	Should be toxic to microorganisms but nontoxic to humans and animals
Penetration	Should have the capacity to penetrate through particle surfaces
Safety	Should be safe to transport, store, handle and use
Solubility	Must be soluble in water or cell tissue
Stability	Should have low loss of germicidal power with time not in use
Toxicity to microorganisms	Should be effective at high dilutions
Toxicity at ambient temperatures	Should be effective in ambient temperature range
Alteration of solution characteristics	Should remain effective with minimum alterations to the characteristics of the parent solution.

Advantages and disadvantages of chlorine, chlorine dioxide, ozone and UV for disinfection of treated water are given in Table 3. This table is not exhaustive and additional information can be obtained in Metcalf and Eddy, (2014).

Table 3: Advantages and disadvantages of chlorine, chlorine dioxide, ozone and UV for disinfection of treated water. (Source: Metcalf and Eddy, 2014)

Advantages	Disadvantages
Free and combined chlorine species	
• Well established technology • Effective disinfectant • Combined chlorine (chloramines)residual can also be provided by adding ammonia • Germicidal chlorine residual can be maintained in long transmission lines • Available as calcium and sodium hypochlorite that are considered to be safer than chlorine gas • Inexpensive • Oxidizes sulfides	• hazardous chemical to come in contact with • combined chlorine is less effective in inactivating some viruses, spores and cysts at low dosages used for coliform organisms • forms trihalomethanes and other disinfection by products (DBPs) • acid generation if alkalinity of treated water is insufficient • oxidizes a variety of organic compounds (consumes disinfectant) • residual toxicity of treated water must be removed through dechlorination
Chlorine dioxide	
• effective for bacteria and viruses • More effective than chlorine in inactivating most viruses, spores, cysts and oocysts • Biocidal properties not influenced by pH • Under proper conditions some DPDs are not formed • Oxidizes sulfides • Provides residuals	• Unstable, must be produced on site • Oxidizes a variety of organic compounds • Forms DBPs • Decomposes in sunlight • Can cause odours • Operating costs can be high

Advantages	Disadvantages
Ozone	
• Effective disinfectant • More effective than chlorine in inactivating most viruses, spores, cysts and oocysts • Biocidal properties not influenced by pH • Contributes dissolved oxygen • Oxidizes sulfides • At higher doses than required for disinfection, ozone reduces the concentration of trace organic matter	• No residual effect • Forms DBPs • Oxidizes a variety of organic compounds (consumes disinfectant) • Highly corrosive and toxic • Energy intensive • Expensive •
UV	
• Effective disinfectant • No residual toxicity • No formation of DBPs at dosages used for disinfection • At higher doses than required for disinfection, UV radiation reduces the concentration of trace organic matter • Requires no hazardous chemicals • More effective than chlorine in inactivating most viruses, spores and cysts	• No immediate measure of whether disinfection was successful • No residual disinfectant • Energy intensive • Hydraulic design of UV system is critical • Large number of UV lamps required where low pressure, low-intensity systems are used • Lamp disposal is problematic due to presence of mercury • Expensive

It is clear from Table 3 that chlorine is the most common form of disinfection method for treated water as well as the cheapest (Metcalf and Eddy, 2014; Monteiro *et al.*, 2015). However, the choice of disinfectant and dosage will depend on the type of microorganism(s) targeted for inactivation as well as the pH and temperature of the water to be disinfected (van der Walt *et al.*, 2009; EPA, 2011).

Environmental factors

Temperatures above 15 degrees Celsius are known to favour bacterial growth in drinking water distribution systems (U.S.A EPA, 1992; Lautenschlager *et al.*, 2010; Henne *et al.*, 2013). There are studies that have shown that certain pathogenic species of bacteria such as, *Legionella pneumophila* adapt well in hot (25-45°C) water (Lau and Ashbolt, 20009; Moritz *et al.*, 2010). pH is another environmental factor that affects water quality, particularly the speciation of disinfectants (Simoes, 2013). Changes in pH can also affect bacterial growth. Bacteria respond to changes in internal and external pH by adjusting the activity and synthesis of proteins associated with many different cellular processes (Garrett *et al.*, 2008). A gradual increase in acidity increases the chances of cell survival in certain species of microorganisms, in comparison to a sudden increase in acidity. This suggests that some bacteria species contain mechanisms which allow the bacterial population to adapt to small changes in environmental pH (Garrett *et al.*, 2008). A change in pH results in shifts in the speciation of free chlorine residuals, which could favour bacterial growth and biofilm formation in drinking water distribution systems (Simoes, 2013).

Nutrients

Microorganisms such as coliform and heterotrophic bacteria consume nutrients from the environment to grow. These nutrients include nitrogen, phosphorus and organic carbon (U.S.A EPA, 1992; Fang *et al.*, 2009; Simoes and Simoes, 2013). It is well known that nutrient availability is an important factor for biofilm formation with organic carbon said to be the most important growth limiting nutrient in drinking water distribution systems (Lehtola *et al.*, 2006; Fang *et al.*, 2009; Lautenschlager

et al., 2010; Simoes 2013). This is because bacteria need it to grow and multiply. Some countries, such as the Netherlands, Germany and Switzerland prefer not to use disinfection to control microbial growth. Instead they control growth by limiting the influx of nutrients that are essential for growth, into distribution systems, by producing biologically stable water at treatment plants (Hammes *et al.*, 2010; Simoes, 2013).

Water age

Water age, also known as residence time, is defined as, “the amount of time water spends in the distribution system between the treatment plant and the consumer” (Tinker *et al.*, 2009). Water age is a function of flow rate, distance from the treatment plant, storage and system demand, among other things (Tinker *et al.*, 2009; Shamsaei *et al.*, 2013). So the lower the demand the longer the water will stay within the system. The longer the water stays in the system, the more likely it is to become contaminated, as disinfectant residuals will decay over time (Tinker *et al.*, 2009; Wang *et al.*, 2014). Water demand will vary depending on the time of day, season and size of the population relying on a distribution system. Water demand will be specific to each distribution system. Water quality parameters that can be affected by water age are, HPC, DO, disinfectant residual and nutrients (Kernies *et al.*, 1995; Wang *et al.*, 2014). Zhang *et al.* (2004) showed that increasing the disinfectant residual or limiting the substrate concentration was ineffective in reducing bacterial growth in areas where the residence time were long. This means that a thorough understanding of the hydraulic conditions within the distribution system would be useful in maintaining biostability. As water age is an important parameter in ensuring biostability, methods that can be used to measure biostability are discussed below.

2.2.3 Methods to measure biostability

HPC is widely used as a measure of bacterial regrowth in drinking water distribution systems (Bartram *et al.*, 2003; Allen *et al.*, 2004; Lu *et al.*, 2014). This method gives a general idea of the bacterial population; it does not provide any information on the bacterial species present (Hammes *et al.*, 2010; Lu *et al.*, 2014). An alternative or addition to the HPC method would be the flow-cytometric total cell concentration (TCC). This method provides a more detailed description of the bacterial composition than the HPC method (Hammes *et al.,* 2010).

Assimilable organic carbon (AOC) concentration has also been used to assess the biostability of drinking water (Lu *et al.*, 2014; Wang *et al.*, 2014). AOC is the portion of dissolved organic carbon (DOC) that can be converted into biomass (Huck, 1990; Frias *et al.*, 1995; Wang *et al.*, 2014). The AOC method is based on a linear relationship between AOC concentration and maximum batch bacterial growth in water from inoculation to the stationary phase (Wang *et al.*, 2014).

Microbial available phosphorus (MAP) and bacterial regrowth potential (BRP) are also methods used to determine biostability in water. Both these methods are also based on the relationship between the substrate concentration and the maximum batch bacterial growth from inoculation to the stationary phase (Wang *et al.*, 2014).

Biodegradable dissolved organic carbon (BDOC) is another method that is used to determine biostability in water. BDOC is the portion of DOC that can be mineralized by heterotrophic microorganisms (Huck, 1990; Frias *et al.*, 1995; Wang *et al.*, 2014). BDOC is the difference between the initial DOC and the final DOC in an inoculated sample after a given period of incubation (Wang *et al.*, 2014).

A method suggested by Sharp *et al.*, (2001), known as the biofilm annular reactor (BAR) is said to be more reliable than AOC and BDOC in measuring biostability. This is due to the continuous flow and dynamic nature of the system that allows it to simulate water flow through a biofilm laden pipe containing attached bacterial populations. The BAR method allows for biostability assessment using both biomass and organic carbon based methods. The biomass method involves the

measurement of the total amount of multispecies biofilm growth supported by water. The organic carbon method involves the measurement of the amount and type of natural organic matter (NOM) available to, or utilized by the biofilm bacteria within the reactors.

Once a method to measure biostability has been selected, different strategies can be adopted by the distribution system that will limit bacterial growth and regrowth, as well as limit the loss of biostability.

2.2.3.1 Strategies for controlling bacterial growth and regrowth and the loss of biostability

Bacterial growth can lead to the deterioration of water quality, biocorrosion, undesirable tastes and odours in drinking water (Hammes *et al.*, 2010; Lu *et al.*, 2014). It can also allow pathogens to grow. If this occurs it can have significant impacts on public health.

Studies have shown that the addition of a disinfectant such as chlorine and monochloramines to the treated water will help limit growth and/or regrowth and thus improve the chances of maintaining biostability (USEPA, 1992; Roeder *et al.*, 2010; Lautenschlager *et al.*, 2010; Hammes *et al.*, 2010; Lu *et al.*, 2014). Some bacteria are resistant to chlorine and chlorine has been known to produce harmful DBPs (Bowden *et al*, 2006; Monteiro *et al.*, 2015). The DBPs problem allowed for the creation of an alternative solution to reduce bacterial growth and/or regrowth and this would be to limit the nutrients necessary for bacterial growth and/or regrowth, namely organic carbon or inorganic nutrients such as phosphate (Fang *et al.*, 2009; Hammes *et al.*, 2010; Lu *et al.*, 2014). This reduction in bacterial growth, could in turn reduce the potential for biofilm growth.

2.2.4 Biofilm

Biofilms are a collection of microbial cells that accumulate at solid–liquid interfaces and are entrapped within a gelatinous matrix comprising mostly insoluble extracellular polymeric substances (EPS) that the cells secrete (Garrett *et al.*, 2008; Fang *et al.*, 2009; Flemming and Wingender, 2010).

In most biofilms the microorganisms (dead and live cells) constitute up to 10% of the dry mass while the matrix (EPS) makes up the remaining 90% (Hallam *et al.*, 2001; Flemming and Wingender, 2010; Hobley *et al*., 2015). The EPS is responsible for adhesion of the biofilm to surfaces and for cohesion of cells within the biofilm (Garrett *et al.*, 2008; Flemming and Wingender, 2010). The EPS immobilizes the biofilm cells and keeps them in close proximity, thus allowing for intense interactions, such as cell to cell communication (Garrett *et al*., 2008; Flemming and Wingender, 2010).

Biofilm growth is not only a problem to water utilities. Food factories, paper mills, oil refineries, and hospitals also deal with the negative effects of biofilm growth. These effects include product spoilage, corrosion, pipe blockages, malodours, infections and increased maintenance costs (Garrett *et al*., 2008).

On the other hand, biofilms have also proven useful in the field of bioremediation. Biofilms have also been beneficial to bacterial longevity by protecting them from disinfectants, antibiotics, dynamic environments and allowing them to survive in nutrient deficit water, all of which is detrimental to human health (Garrett *et al.*, 2008; Flemming and Wingender, 2010).

The main requirement for biofilm growth in a drinking water distribution system is the microorganisms, water and a surface on which to adhere (Garrett *et al.*, 2008; Moritz *et al.*, 2010). Figure 3 below, adapted from Simoes and Simoes, (2013) shows how biofilm develops on a pipe surface in a drinking water distribution system. Nine steps have been identified which were initially described by Characklis and Marshall, (1990), (cited in Garrett *et al.*, 2008) namely:

- preconditioning of the adhesion surfaces either by macromolecules present in the bulk liquid or intentionally coated on surfaces;

- Transport of planktonic cells from the bulk liquid onto pipe surfaces;
- Adsorption of cells onto pipe surfaces;
- Desorption of reversibly adsorbed cells;
- Irreversible adsorption of bacterial cells onto pipe surfaces;
- Production of cell to cell signalling molecules;
- Transport of substrates into and within the biofilm;
- Substrate metabolism by biofilm-bound cells and transport of products out of biofilms. These processes are accompanied by cell growth, replication, and production of EPS;
- Biofilm removal by detachment and/or sloughing.

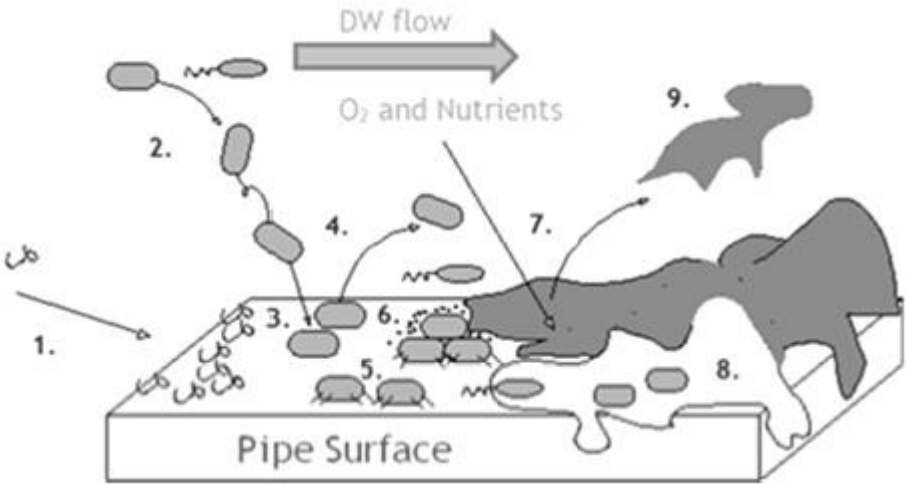

Figure 3: Processes involved in biofilm formation: (1) Preconditioning the pipe surface by macromolecules (organic and inorganic) present in the water; (2) Transport of planktonic cells from water to pipe surface; (3) Adsorption of cells at the pipe surface; (4) Desorption of reversibly adsorbed cells; (5) Irreversible adsorption of cells; (6) Production of signalling molecules; (7) Transport of substrates to and within the biofilm; (8) Substrate metabolism by the biofilm-bound cells and transport of products out of the biofilm, accompanied by cell growth, replication, and production of EPS; (9) Biofilm removal by detachment or sloughing. (Source: Simoes and Simoes, 2013)

The nine steps to biofilm growth listed above can be grouped further into four groups, namely: surface conditioning, adhesion, biofilm growth and maturation and detachment (Garrett *et al*., 2008; Simoes and Simoes, 2013).

Conditioning film

The first step in biofilm formation is preconditioning of the pipe surface also known as the adhesive surface or substratum. The conditioning film is a thin layer that covers the pipe surface and this film is made up of organic and inorganic molecules and ions. The molecules may adhere to the pipe surface by physical or chemical adsorption. Physical adsorption uses weak Van der Waals forces to keep the molecules adhered to the pipe surface; is reversible and does not require any activation energy, while chemical adsorption uses chemical bonds to keep the molecules adhered to the pipe surface; it is not a reversible process and it requires activation energy. Once adsorption has occurred the pipe is now 'conditioned' (Garrett *et al*., 2008; Simoes, 2013).

Anything that may be present within the bulk fluid can settle on the pipe surface and become part of a conditioning layer. This layer modifies the pipe surface and facilitates accessibility to bacteria. Surface charge can be altered favourably by the interactions between the conditioning layer and the pipe surface. The pipe surface provides anchorage and nutrients augmenting growth of the bacterial community (Garrett *et al*., 2008).

Adhesion

Following surface preconditioning, planktonic cells can adhere to pipe surfaces. Figure 3 shows steps 2-5, which leads to effective adhesion of microorganisms (Simoes, 2013). A fraction of the cells reaching the pipe surface reversibly adsorbs. Factors such as available energy and carbon sources, surface functionality, temperature and pressure conditions, are local environmental variables which contribute to bacterial adhesion (Garrett *et al*., 2008). If repulsive forces are greater than the attractive forces, the bacteria will detach from the surface at a later stage (Garrett *et al*., 2008). This is more likely to occur before conditioning of the pipe

surface than after conditioning. A number of the reversibly adsorbed cells remain immobilised and become irreversibly adsorbed.

Biofilm growth and maturation

After the cells adhere to the pipe surface, growth, cell replication and EPS production can occur. This process is depicted in Figure 3 from steps 6-9. The cells multiply from the point of attachment and form clusters. After an initial lag phase, the exponential phase occurs, which results in a rapid increase in the population size (Garrett, *et al*., 2008). This growth depends on the physical and chemical characteristic of the environment. At this stage the physical and chemical contributions to the initial cell attachment ends, and the biological processes begin to dominate (Garrett, *et al*., 2008). Excretion of EPS helps with adhesion, aggregation of bacterial cell, cohesion of biofilms, retention of water, serves as a protective barrier, stores excess energy, binds enzymes, is a nutrient source, provides an environment in which the exchange of genetic information is possible, acts as an electron donor in the form of biomass associated products (BAP) after hydrolysis (Laspidou and Rittmann, 2002; Garrett *et al*., 2008; Korake *et al*., 2009; Flemming and Wingender, 2010). As the biofilm thickness increases, the microorganisms within it will need nutrients from the water flowing in the pipe to stay alive. Throughout the biofilm growth phase bacterial detachment starts to occur although it occurs at a slower rate compared to the biofilm growth rate (Simoes, 2013).

Detachment

The final stage in biofilm development is comprised of the stationary phase of growth. This is the point at which the rate at which new cells are generated (i.e. new cell growth) is equal to the rate of cell death (Garrett, *et al*., 2008).

The death phase involves the breakdown of the biofilm. This occurs when enzymes within the biofilm are released breaking the EPS structure that holds the cells together (Garrett, *et al*., 2008).

Detachment occurs in step 9. This is the last step in Figure 3. Cells and other components of the biofilm detach from the pipe surface. Shear stress due to high flow velocities can lead to the detachment of bacteria and large portions of biofilm elements. This process is known as sloughing (Simoes, 2013). Detachment can be caused by sloughing, erosion (continuous loss of single cells or small portions of the biofilm) and abrasion (repeated collision between the biofilm and particles) (Simoes, 2013). Prior to detachment, the biofilms formed can be affected by several factors discussed below.

2.2.4.1 Factors affecting biofilm formation

All factors that affect bacterial growth, including but not limited to factors discussed in section 2.2.2, affect bacterial growth and regrowth in biofilms. Apart from factors that fuel growth, survival of microorganisms in biofilm community is further dependant on system hydraulics, pipe materials, and sediment accumulation as discussed below (U.S.A EPA, 1992; van der Kooij, 1999; Lehtola *et al*., 2006; Fang *et al*., 2009; Lautenschlager *et al*., 2010; Simoes, 2013; Henne *et al*., 2013).

Hydraulic conditions

Drinking water distribution systems are designed for velocities between 0.2-0.5m/s (Manuel *et al*., 2007). Flow velocity can be altered by using pipes with varying diameters (U.S.A EPA, 1992). Laminar, turbulent and stagnant flow can be observed in DWDSs. Stagnant conditions can be due to low water consumption and reservoirs in buildings (Manuel *et al*., 2007; Simoes, 2013). A study carried out by Lehtola *et al*. (2006) showed an increase in biofilm formation with increasing flow velocity. This is a result of better mass-transfer of growth-limiting nutrients at the higher flow velocity of water. When it comes to the effect of flow velocity on biofilm there have been contradicting results (Lehtola *et al*., 2006; Manuel *et al*., 2007). An experiment carried out by Lautenschlager *et al*., (2010) showed that bacterial growth increased after stagnation which means that longer detention

times are conducive to bacterial growth and should be avoided (Simoes and Simoes, 2013; Shamsaei *et al*., 2013).

Materials

Pipe material has been shown to affect water quality and bacterial growth and regrowth (Zhou *et al*., 2009; Wang *et al*., 2014). In the past, pipes in distribution networks were made of iron or cement-based material. Over time polyvinylchloride (PVC) and polyethylene (PE) have become more common (Simoes, 2013). There are many studies that document the effect of pipe materials on water quality and biofilm formation (Percival *et al*., 1998; Zacheus *et al.,* 2000; Lehtola *et al*., 2004; Lehto *et al*., 2006; Wang *et al*., 2014). Lehtola *et al*., (2004) showed that biofilms formed faster in plastic pipes compared to copper because plastic pipes leach phosphorus which is used up by microorganisms and increases the rate of biofilm growth. Zacheus *et al*., (2000) showed that PVC, PE and stainless steel had similar result with regards to the formation of biofilm. There are also studies that give opposing results for the same material (Simoes, 2013). The main factors to consider with regards to pipe material that affect biofilm growth are roughness and surface physicochemical properties (surface tension, chemical composition and surface charge) (Percival *et al*., 1998; Simoes, 2013).

Sediment accumulation

Sediments can consist of organic matter or insoluble materials. Sediments and debris in distribution systems can form habitats for microbial growth and provide protection from disinfectants in distribution systems with low flows and dead zones (USEPA, 1992; Simoes, 2013). Different sediments have different effects on biofilm. Inorganic particles like sand will promote erosion of biofilm and clay particles would result in thicker and stronger biofilms (Simoes, 2013).

2.2.4.2 Other biofilm microorganism

It is important to note that while bacteria make up the largest proportion of distribution system biofilms other organisms such as fungi, protozoa and some invertebrates can also thrive in these biofilm communities (USEPA, 1992; Manuel *et al*., 2010; Flemming and Wingender, 2010). For this reason, any efforts aimed at controlling biofilm should be made after considering the effect of the strategy on the survival of all types of microorganisms that can thrive in biofilms. The use of water quality models could be useful in determining the effect of biofilm control mechanisms on different microorganisms.

2.3 Water quality modelling

Water quality models as defined by Kirmeyer, 2000 are “extensions of distribution system hydraulic models and are capable of evaluating various water quality parameters throughout the distribution system.” The accuracy of the water quality model is dependent on the results of the hydraulic model, since flow in pipes, the volume of the water in tanks and reservoirs and source inflow rates will impact water quality parameters (Kirmeyer, 2000).

2.3.1 Types of models

Water quality models used by drinking water distribution systems can be categorized as steady state or dynamic models. Steady-state modelling represents external forces as constant in time and determines solutions that would occur if the system is allowed to reach equilibrium. This type of modelling uses the law of mass conservation to determine the critical spatial distribution of dissolved substances under static hydraulic conditions (Kirmeyer, 2000). In dynamic modelling, demands and supplies are allowed to vary over time to simulate distribution system conditions, thus providing more accurate results when compared to steady state

models (kirmeyer, 2000). Figure 4, shows some of the different processes, with respects to particulate and dissolved substances that take place within a pipe in a drinking water distribution system. These processes have to be accounted for when creating water quality models.

In steady-state and dynamic modelling, a distribution system is represented as links and nodes, where the links are the pipe and the nodes are tanks or pipe intersections (Clark and Coyle, 1990).

Difference between hydraulic and water quality models

Hydraulic models are used to determine flows and velocities in pipes. They are used to help size storage facilities, improve energy management and to evaluate alternative operating conditions. Water quality models use numerical algorithms to determine the accumulation and loss of reactive substances as well as the growth and death of bacteria as the water travels through the distribution system (Clark and Coyle, 1990; Kirmeyer, 2000). The generation and depletion of constituents within the distribution system are as a result of reactions taking place in the bulk water and at the pipe wall, see Figure 4. Water quality models can also be used to determine the most favourable location for disinfection stations and storage facilities by modelling water age or chlorine residual. They can also be used to help water treatment facilities comply with regulatory requirements within drinking water distribution systems (Kirmeyer, 2000). Some programmes combine the water quality and hydraulic models while others input data from hydraulic models into the water quality models (Clark and Coyle, 1990).

To determine the accuracy level and relevance of the water quality model you have, the following characteristics need to be known. These traits were derived from Tsakiris and Alexakis, (2012).

- The type of approach used (physical, conceptual, empirical)
- Pollutant item (nutrients, sediments, microorganisms, etc.)
- Area of application (drinking water distribution systems, river system, coastal water, groundwater, etc.)

- Nature (deterministic or stochastic)
- State analysed (steady state or dynamic)
- Spatial analysis (lumped or distributed)
- Data requirements (extensive database, minimum requirements model)

It should be noted that the more complex and detailed a model is the more computational effort is required to solve equations, making it more difficult to get accurate estimates for the different parameters tested (Rauch *et al*., 1999). The difficulty in modelling arises when choosing which parameters are important and which are not in order to make the model less complex and reduce the time in which model predictions are obtained (Rauch *et al*., 1999). When modelling water quality in DWDSs the main properties to consider are transport, mixing, constituent generation and depletion (Blokker *et al*., 2008).

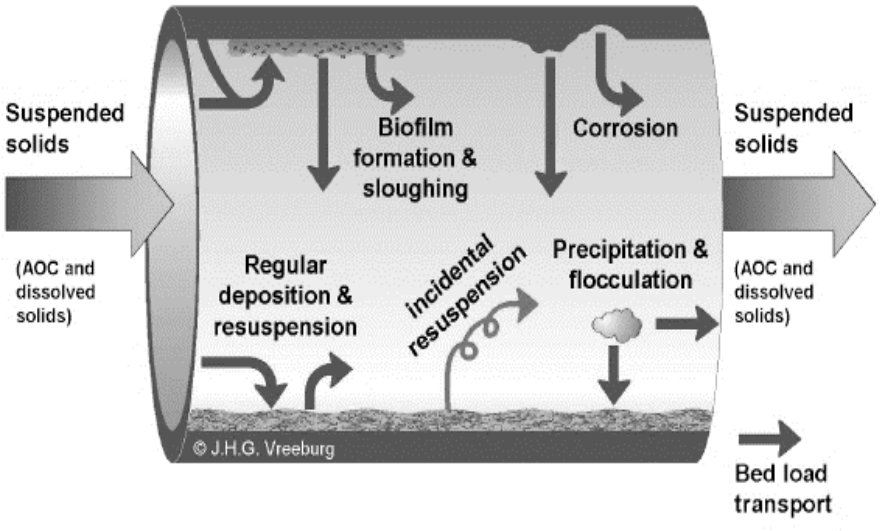

Figure 4: Processes taking place within a pipe in a drinking water distribution system. (Source: Blokker *et al*., 2008)

The comprehensive water quality and disinfection model

The comprehensive water quality and disinfection (CDWQ) model was developed by Woolschlager (2000). CDWQ is a full scale model capable of predicting the loss of residual disinfectants (chloramine and free chlorine) and bacterial growth (heterotrophs and nitrifiers) under aerobic conditions.

The expanded comprehensive disinfection and water quality model

The expanded comprehensive disinfection and water quality (CDWQ-E) model was developed by Biyela (2010). This model is an adaption of the CDWQ model with the following unique characteristics:

- The CDWQ-E incorporates updated information in modelling microbial systems (Biyela, 2010).

- The CDWQ-E model allows for change in heterotrophic respiration, from aerobic respiration to anoxic denitrification under conditions typical of drinking water distribution systems (Biyela, 2010).
- The CDWQ-E is able to monitor the growth of a protozoan pathogen, *N. fowleri,* within drinking water distribution systems (Biyela, 2010).

- The CDWQ-E model looks at both nitrification and denitrification compared to the CDWQ model which only considers nitrification

A schematic for the flow of electrons for bacterial metabolism in the CDWQ-E model is depicted in Figure 5. The electron donors are used to aid in the following:

- Respiration of electro acceptors (oxygen)
- Creation of new cells
- Creation of EPS
- Creation of utilization associated products (UAP) by all active biomass

The new cells (active biomass) that are formed through the oxidation of electron donors can be lost through endogenous respiration (cell decay) and through disinfection. The total biomass is made up of the new cells (active biomass), inert

biomass (dead biomass) and EPS. Within the model EPS gets hydrolysed to biomass associated products (BAP). UAP and BAP, make up soluble microbial products (SMP). SMP can be recycled and used as an electron donor substrate by heterotrophic bacteria.

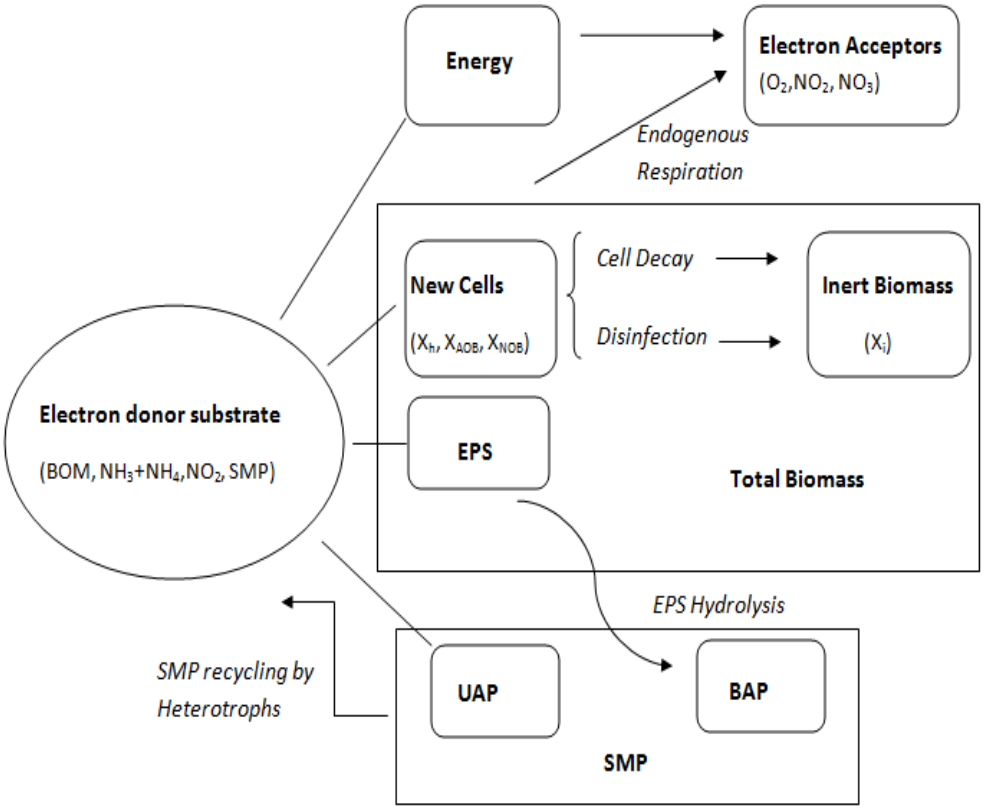

Figure 5: Electron flow schematic for bacterial metabolism in the CDWQ-E model (Biyela, 2010)

The expanded comprehensive disinfection and water quality model, version 2

The expanded comprehensive disinfection and water quality model, version 2 (CDWQ-E_2), is the model that will be calibrated at a later stage, as part of a separate research study, from the data generated in this study. The CDWQ-E_2 is an upgrade of the CDWQ model created by Woolschlager (2000) and the CDWQ-E model created by Biyela (2010). CDWQ-E_2 model was developed by Biyela and Culligan (2014). This model is able to simulate processes within a distribution system and is able to account for biofilm.

The CDWQ-E_2 is able to describe post-treatment changes in the quality of chlorine and monochloramine treated drinking water. The CDWQ-E_2 model has the same electron flow pathway for bacterial metabolism as the CDWQ-E (see Figure 5). Central to this model is the oxidation of different biological substrates (BOM, nitrite and ammonia, and SMP) by heterotrophs, ammonia-oxidizing bacteria, and nitrite-oxidizing bacteria. These oxidation reactions fuel the synthesis of new bacterial biomass, EPSs, and soluble microbial products; depletion of dissolved oxygen; and nitrification. This model also accounts for biomass disinfection and the loss of residual chlorine.

The CDQW-E model, which is a batch model, was used to create the CDWQ-E_2 model. As a logical place to start, the mass balance equations used in the batch version had to be updated to incorporate flow and transportation of nutrients and microorganisms.

This model is different from other models because it is said to be capable of not only predicting water quality changes, but also the reasons for such changes (Biyela, 2014). The incorporation of an advanced model such as the CDQW-E_2 in water utilities could be a profitable addition to their drinking water monitoring routine.

2.4 Literature review summary and conclusions

The literature reviewed in this chapter shows the importance of water quality standards, as these standards are what determine the potability of water sources. The monitoring and testing of drinking water is used to determine areas with poor water quality, as poor water quality could lead to loss of biostability. Biological stability in drinking water is affected greatly by the method of disinfection, environmental factors (pH and temperature), the presence of nutrients and water age. There have been several methods used to determine biostability in potable water, some of these methods include monitoring HPC, AOC and BDOC. From previous research studies, it has been shown that some of the best ways to control biostability was to ensure there was sufficient disinfection residual and to limit the nutrients in the water. Previous research has also shown that in cases where biostability was not possible, it led to, and increased the potential for biofilm formation. This increase in bacterial growth and/or regrowth can negatively impact human health as the microorganisms within biofilms are protected from disinfectants. Water utilities have struggled, and continue to struggle to deal with the problems that arise due to biological instability and biofilm formation in their systems. In an effort to address the problems that arise from the lack of biostability, water quality models have been created over the years. One such model was the CDWQ-E_2 model which could be used to improve the water quality in drinking water distribution systems in the near future.

In conclusion, this review has outlined water quality parameters that are important in determining biostability and how to control it. In this study, the water quality parameters that were analysed are, pH, temperature, conductivity, Alkalinity, DO, free and total chlorine, nitrogen species (total nitrogen, total ammonia, nitrate, and ammonium), HPC, faecal and total coliforms, DOC and BDOC. The study design laid out in chapter 3 of this report is based on this review and as a result, it assisted in meeting the objective that was set out in section 1.5 of chapter 1 of this report. More details on the methodology used to carry out this research can be seen in Chapter 3 of this report.

3. Study Design

In this chapter the experimental design is explained based on information obtained from the literature review in chapter 2. The sampling plan and sites are discussed as well as appropriate laboratory experiments and instruments that were used to analyse the field data.

3.1 Sampling procedures

When choosing sampling sites (points) there are several factors that need to be taken into consideration. The sampling points should vary in their proximity to the treatment plant, average hydraulic retention times, and flow conditions. All these factors affect water quality including but not limited to biostability and biofilm formation potential (Biyela, 2010). Having sampling points that vary in these parameters will produce a wide range of results that would, in future, be used to test the accuracy of the CDWQ-E_2 model for varying quality and hydraulic conditions.

For the purposes of this research, 13 sampling points were used. These points were all located within a small segment of JW's distribution system. This sample size created a balance in the number of sampling points and data that was required for modelling robustness as well as the financial implications of in-depth water quality testing. The data generated from this study was enough to provide a clear picture of the causes and effects of the loss of biostability within the tested distribution network. From section 1.1 in chapter 1, it was mentioned that this programme spanned one of the seven regions supplied and monitored by JW, which is approximately 25% of the entire distribution network (Johannesburg Water, 2011). Within this region are area 5 (Fairland and Northcliff) and area 10 (Blairgowrie) situated within the greater Randburg area, which can be seen in Figure 2. These areas were chosen because of their history of poor water quality. These 13 sampling sites within the trouble zone were chosen based on the several factors mentioned above. In addition to the fact that these sites were chosen based

on their history of poor water quality in the area, the selection of 2 sites from area 10 and 11 sites from area 5 was based on the size of the reservoirs supplying those areas respectively. This gave an indication of the population distribution between the two areas. Area 5, situated in and around Northcliff has a reservoir with a capacity of 45.5 million litres, while area 10 has a reservoir with a capacity of 6.8 million litres, as seen in Table 4. In Figure 6, a google image of the sampling points in relation to each other and the Rand Water treatment plant can be seen. It should be noted that the image shown in Figure 6 does not give an accurate depiction of the distances between the sampling points and the treatment plant, as the distribution system layout does not follow the same layout as the roads seen on the map. The image (Figure 6) is to be used as a guide, to see the relationship between sampling points and the treatment plant. For a more detailed view of the sampling area, Appendix B shows a map provided by JW, of the city of Johannesburg's existing water districts and bulk supply system. Table 5 shows the exact locations and description for all 13 sites. Table 4 shows the reservoirs and tower capacities. The sites labelled as distribution points, (Distr) in Table 5, of the 13 sites, were taps in public spaces or caged taps along the road side that only JW employees have access to.

Table 4: Tower and reservoir capacities. (Source: JW, 2011)

Site	Description	Capacity (million litres)
RW80 and RW81	Northcliff Reservoir	45.5
RW83 and RW84	Northcliff Tower	1.1
RW104 and RW105	Corriemoor Reservoir	18.6
RW107 and RW108	Fairland Reservoir	6.3
RW251	Blairgowrie Reservoir	6.8

The lack of access to valuable information, such as water demand data made it difficult to provide a more accurate representation of the processes within the distribution system and how these processes affect biostability and biofilm formation potential. However, a monitoring plan was still formulated based on the

parameters that affect biostability the most. The monitoring schedule can be seen later on in this chapter.

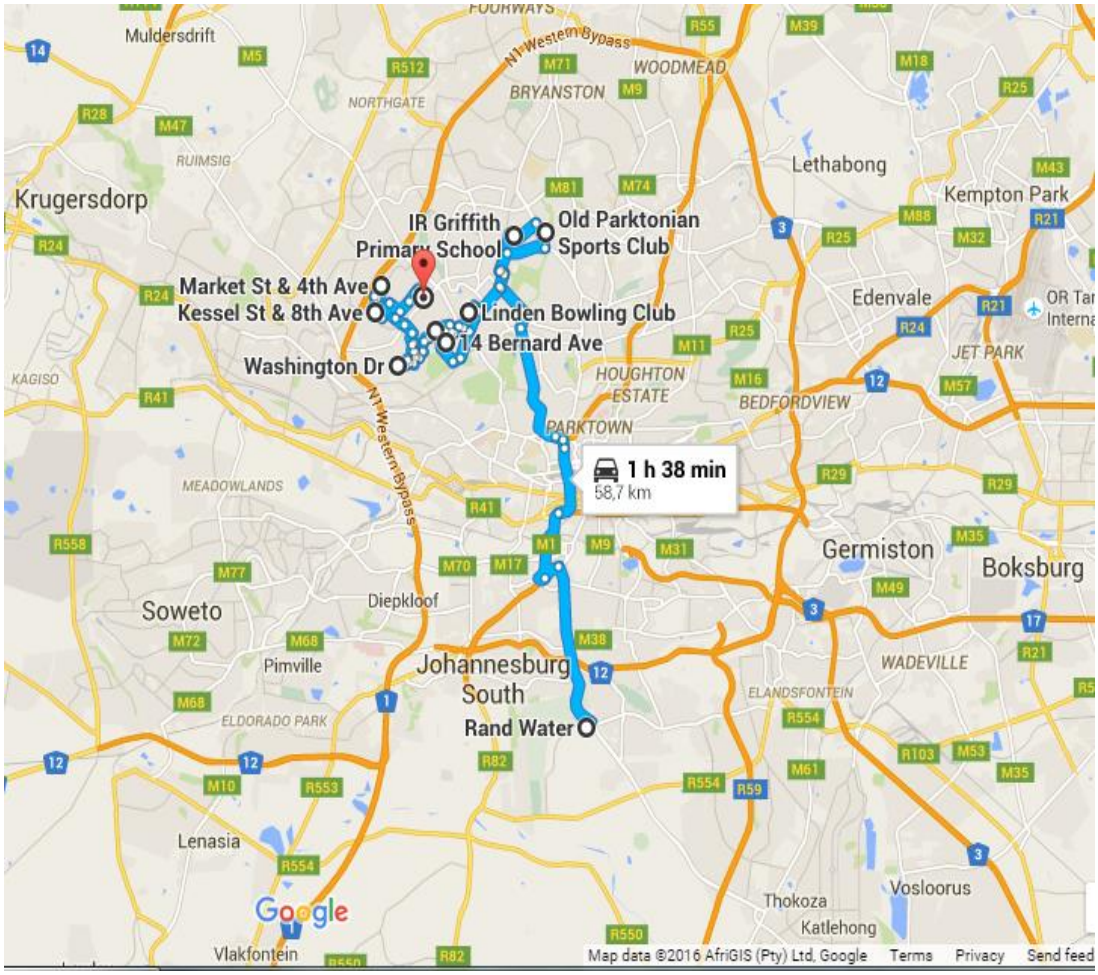

Figure 6: Sampling points in relation to each other and the Rand Water treatment plant. (Source: Google Maps, 2016)

Table 5: Sample location and description (Source: JW, 2015)

Sites	Latitude				Longitude		Code	Description	Address
1	26	8	53	27	58	24	RW80	Northcliff Reservoir Inlet	14 Bernard Lane Northcliff
2	26	8	53	27	58	24	RW81	Northcliff Reservoir Outlet	14 Bernard Lane Northcliff
3	26	8	9	27	58	58	RW82	Northcliff Reservoir Distr - Linden Bowling Club	Emma Park 1st St Linden
4	26	8	40	27	58	10	RW83	Northcliff Tower Outlet	Lucky St Northcliff
5	26	8	40	27	58	10	RW84	Northcliff Tower Inlet	Lucky St Northcliff
6	26	9	22	27	56	49	RW104	Corriemoor Reservoir Inlet	Washington Dr, Northcliff
7	26	9	22	27	56	49	RW105	Corriemoor Reservoir Outlet	Washington Dr, Northcliff
8	26	7	39	27	56	41	RW106	Corriemoor Reservoir Distr - Fairland	c/r 4th & Market Fairland
9	26	8	20	27	56	27	RW107	Fairland Reservoir Inlet	8th Ave., off Kessel St Fairland
10	26	8	15	27	56	31	RW108	Fairland Reservoir Outlet	6th Ave., off Kessel St
11	26	7	56.76	27	57	49.31	RW109	Fairland Reservoir Distr – Fairland	273 Castle Hill St Northcliff
12	26	6	36	28	0	20	RW251	Blairgowrie Reservoir Inlet	Equity & Susman
13	26	6	59	28	1	35	RW253	Blairgowrie Reservoir Distr Old Parks	Old Parktonians Sports Club

3.1.1 Sampling protocol

The water samples were collected in glass Schott bottles that were sterilized. When the samples were collected gloves were worn by the samplers to protect the integrity of the samples. The samples were collected in a way that prevented sample contamination (e.g. when collecting the water sample, the sampler held the body of the bottle and not the neck of the bottle). After sample collection the bottles were labelled with the name of the sample site, the day the sample was collected and the batch number. The samples were placed in a cooler box with dry ice packs to prevent them from deteriorating during transportation to the laboratory for analysis. All samples were analysed within 24 hours from the date of collection.

3.1.2 Sampling plan

The sampling plan included general water quality schedules. The water quality parameters that were considered were pH, temperature, conductivity, Alkalinity, DO, free and total chlorine, nitrogen species (total nitrogen, total ammonia, nitrate, and ammonium), HPC, faecal and total coliforms, DOC and BDOC. These tests were chosen based on the results from previous research conducted on biostability for drinking water systems (Woolschlager 2000; Biyela 2010; Hammes *et al*., 2010; Lu *et al*., 2014). Samples were collected biweekly (i.e. every 2 weeks) for parameters that are more sensitive to changes in environmental conditions. Monthly samples were collected for parameters that are less sensitive to changes in environmental conditions. Water samples were collected over a period of twelve months to account for seasonal variations. Table 6 below shows the different parameters measured, the sampling frequency and the regulatory standards.

Johannesburg water's sampling frequency is also biweekly for every site, but they do not test the parameters of interest in this research as frequently or at all. This observation is based on the historical water quality data provided by JW. With regards to regulation, JW has difficulty adhering to the regulatory standards for free

chlorine and heterotrophic plate count at some sampling sites. That is one of the reasons why this research was conducted. JW's water quality data, in addition to the data collected in this research could be used in a separate research, at a later stage.

Table 6: Sampling frequency and regulation of the measured parameters (Source: SANS241, 2011)

Parameter	**Sampling Frequency**	**Regulated by Law**	**Limits**
Temperature (°C)	biweekly	NO	
pH	biweekly	YES	≥5-≤9.7 operational
Conductivity (mS/m)	biweekly	YES	≤170 aesthetic
Alkalinity (CaCO3)	monthly	NO	
DO (mg/l)	biweekly	NO	
Free chlorine (mg/l)	Biweekly	YES	0.2* - ≤5
Total chlorine (mg/l)	Biweekly	NO	
Total nitrogen (mg/l)	monthly	NO	
Nitrate (mg/l)	monthly	YES	≤11
Ammonium (mg/l)	monthly	NO	
Total ammonia (mg/l)	monthly	YES	≤1.5
HPC (count/ml)	biweekly	YES	≤1000
Faecal coliforms (count/100ml)	monthly	YES	0
Total coliform (count/100ml)	monthly	YES	<10
DOC (mg/l) C	biweekly	NO	
BDOC (mg/l) C	monthly	NO	

* *lower limit derived from Monteiro et al., 2015*

As important as DOC and BDOC are in determining biostability it is not regulated by the South African drinking water standards, as this is an expensive procedure to perform.

3.1.3 Sample preparation

Schott glass bottles ranging from 100ml to 1 liter were used to collect water samples. The bottles used to collect samples for physical and chemical tests (excluding BDOC and DOC) were washed with soap and rinsed with tap water and the final rinse was with distilled water. The bottles were then autoclaved at 121 degrees Celsius for 15 minutes (Autoclave model number: OT 40L; Nuve; Ankara, Turkey). When the bottles had cooled down they were put in cooler boxes with ice packs.

The BDOC and DOC bottles were washed with soap and rinsed with tap water. The bottles were rinsed further with 10% hydrochloric acid (HCL). The final rinse was with distilled water, once the bottles had dripped dry they were put in cooler boxes with ice packs.

The bottles used to collect samples for microbiology tests were washed with soap and rinsed with water and distilled water then autoclaved at 121 degrees Celsius for 15 minutes. After the bottles had cooled down, 0.1N (0.1M) sodium thiosulfate preservative standard solution was made in a 100ml conical flask. The solution added to the sampling bottles corresponded to 0.5% of the sample bottle volume to neutralize the chlorine (e.g. in a 250ml sample bottle, 1.25ml standard solution of sodium thiosulfate was added). The bottles were put in cooler boxes with ice packs.

In the field, before samples were collected, the taps were left to run for 3 minutes at full speed. The taps were then turned off and flamed. Gloves were worn before opening the sampling bottles to collect water samples. Caution was taken to make

sure that the rim and neck of the bottle and inside of cap were not touched. Once the samples were collected the bottles were closed and labelled with the date, time, sampler's name, sample location and preservative. After all the data was recorded, the bottles were put back into cooler boxes.

Once the samples were transported back to the laboratory, they were taken out of the cooler boxes and allowed to reach room temperature before any analyses was carried out, using the instruments and methods discussed in section 3.2 below.

3.2 Methods and instruments for data gathering

In this section, the methodology used to analyse the samples for the parameters listed in the sampling plan is explained in detail. These parameters are broken down into three groups, namely, physical, chemical and microbiological.

3.2.1 Measurement of physical water quality parameters

Temperature

Temperature was measured in the field with a calibrated alcohol thermometer (Thermometer product number: 5120P110-qp; PromoLab; Chemille, France) twice a month. A water sample was collected from each sampling point in a clean 100ml beaker and the thermometer was placed in the beaker for 30 seconds to a minute to allow the thermometer to stabilize. Once stability was reached, the temperature reading was recorded in degrees Celsius.

3.2.2 Measurement of chemical water quality parameters

pH

pH was measured in the laboratory twice a month using an electric pH meter, InoLab: pH level 1, as per method 4500 H, in *"Standard Methods for the Examination of Water and Wastewater, 2005"* (pH serial number: 01320044; Wissenschaftlich Technische Werkstatten; Weilheim, Germany). The pH meter was calibrated using solutions with known pH levels of four, seven and ten respectively. After calibration a water sample was poured into a 100ml beaker and stirred using a mechanical stirrer. The meter was switched on and the probe was inserted into the beaker containing water. The pH was recorded when the meter stabilized and has no units.

Conductivity

Conductivity was measured in the laboratory using a Jenway conductivity meter 4071(Conductivity product number: CU600-10; Jenway; UK) twice a month. A water sample was poured into a 100ml beaker and stirred using a mechanical stirrer (product number: 771432; Janke and Kunkel, IKA; Staufen, Germany). The probe of the conductivity meter was inserted in the beaker containing the water sample. The meter was turned on and the conductivity was recorded after a stable reading was obtained. The unit for conductivity is milli-Siemens (mS).

Chlorine

Chlorine was measured twice a month in the field as free and total chlorine using a HACH: Pocket colorimeter II, (Chlorine product number: 5870000; HACH; Loveland Colorado, USA) The USEPA accepted method for drinking water analyses with regards to free and total chlorine was used. USEPA DPD method from standard methods for the examination of water and wastewater, method 4500 Cl-G for drinking water by the American Public Health Association (APHA).

Dissolved oxygen

DO was measured in the field biweekly with a DO meter, as per method 4500 O, in "*Standard Methods for the Examination of Water and Wastewater, 2005*" (model: 550A; YSI a Xylem brand; Yellow Springs, OH, USA). A water sample was collected in a 100ml beaker, the DO meter was switched on and its probe was inserted into the beaker. Once a stable reading was established it was recorded in units of mg/l.

Alkalinity

Alkalinity was measured once a month in the laboratory in accordance with SI: 3025 (Part 23) - Reaffirmed 2003 method. A 100ml of a water sample was poured into a 250ml conical flask. A burette was filled with sulphuric acid with a normality of 0.02N and the initial reading was recorded. A few drops of phenolphthalein were added to the water sample using a pipette. This made the water sample turn pink. The sample was then titrated with sulphuric acid from the burette until the sample turned colourless. At this point the final reading on the burette is recorded (f_1). A few drops of a mixed indicator were then added to the sample, making it turn blue-green in colour. The sample was titrated again with sulphuric acid until the colour of the sample changed to red. At this point the reading on the burette was taken (f_2). Total alkalinity was then calculated as:

$[(f_2-f_1)$ *0.02N*50*1000]/100. The unit for alkalinity is mg/L as $CaCO_3$ equivalent.

Nitrogen species

All nitrogen species monitored in this study were measured once a month.

For total nitrogen, the procedure for the Merck cell test (Total Nitrogen product number: 1.14537.0001; Merck, Spectroquant; Darmststadt, Germany) with a measuring range of 0.5-15 mg/l N was followed. This method was derived from (APHA 4500 -N_{org} D). A 10ml volume of the water sample was poured in an empty vial and one level blue microspoon of reagent N-1K was added and mixed. Six

drops of N-2K was added to the vials and the contents were mixed. The vials were put in the Lovibond RD 125 thermoreactor, (thermoreactor product number: 2418940; Lovibond, Tintometer Group; Dortmund, Germany) for 60 minutes at a temperature of 120 degrees Celcius to digest. After the 60 minutes were over and the vials cooled down one level microspoon of reagent N-3K was added to a reaction vial and the contents were mixed. 1.5ml of the digested sample was added to the reaction sample and mixed. After 10 minutes the vials were read in the Spectroquant, Pharo 300 (Spectroquant product number: 1.00707.0001; Merck, Spectroquant; Darmstadt, Germany) and their concentrations were recorded.

For nitrate, the procedure for the Merck cell test (Nitrate product number: 1.14542.0001; Merck, Spectroquant; Darmstadt, Germany) with a measuring range of 0.5-18 mg/l NO_3-N was followed. This method was based on the reaction of nitrate ions in sulphuric acid with a benzoic acid to form a red nitro compound that is determined photo-metrically. This method is based (APHA 4500- NO_3 C) on ISO 8466-1 and DIN 38402 A51. One level yellow microspoon of reagent NO_3-1K was added to reaction cell. 1.5ml of the water sample was added to the reaction vial and mixed. After 10 minutes the vial was measured in a Spectroquant, Pharo 300 and its concentration was recorded.

For ammonium, the procedure for the Merck cell test (Ammonium product number: 1.14544.0001; Merck, Spectroquant; Darmstadt, Germany), in accordance to APHA 4500-NH_3 F, with a measuring range of 0.5-16 mg/l NH_4-N was followed. A 0.5ml volume from the water sample was poured into a reaction vial, the one dose of reagent NH_4-1K was added to the reaction vial and mixed. The cell was left to sit for 15 minutes for the reaction to take place before it was measured in a Spectroquant/ photometer and its concentration was recorded.

For total ammonia, the procedure for Fluka/Aquanal cell test (Total ammonia product number: 70057 and 70059; Aquanal/Merck; Darmstadt, Germany) in accordance with, APHA 4500-NH_3 F, with a measuring range of 0.02-2 mg/l NH_4^+, was followed. A 10ml water sample was filled into an empty vial and an ammonia no.1 tablet was crushed and added to the vial. Ammonia no.2 tablet was added

and the contents mixed until the tables were completely dissolved. The vial was left to sit for 10 minutes for the reaction to take place. The vial was measured in the Spectroquant, Pharo 300 and its concentration was recorded.

Biodegradable dissolved organic carbon

BDOC was measured once a month. A 500ml water sample was poured in a 1-liter amber Schott bottle that contained biologically active sand (BAS). After the water sample was poured into the amber Schott bottles they were covered with cotton wool that was secured with twine. The use of cotton wool and not bottle caps was to allow for aeration. The bottles were then placed on a shaker, Orbit Shaker, (model: 3520; Lab-Line; Illinois, USA) that rotates continuously at 100rpm. The shaker can take six 1liter amber Schott bottles at a time. A sample of the water poured into the amber Schott bottles with BAS was filtered using a 0.45µm pore filter into 40ml total organic carbon (TOC) amber vials and analysed in the Torch combustion TOC analyser (Teledyne Tekmar; Mason, OH, USA) using the combustion-infrared technique per Standard Methods 5310 B The BDOC test is based on the batch reactors initially created by Joret- Levi (1991) which was then modified by Allgeier et al., 1996.

After five days another sample was taken from the 1 liter amber Schott bottles and filtered into the 40ml amber TOC vial and analysed in the TOC analyser, using the method previously mentioned. To calculate BDOC the day zero reading was subtracted from the day five reading. The units used for BDOC are parts per million (ppm) or mg/l of carbon.

Dissolved organic carbon

DOC was measured twice a month, using the combustion-infrared technique per Standard Methods 5310 B .A water sample was filled into an amber TOC 40ml vial and analysed in the TOC analyser. The units used were parts per million (ppm).

3.2.3 Measurement of microbiological water quality parameters

Heterotrophic plate count

Simplate test kits for HPC (product number: 98-05760-01; IDEXX; Maine, USA) were used to detect HPC bacteria in water. This method correlates with the pour plate method (9215B) using total plate count agar incubated at 35°C for 48 hours as described in *"Standard Methods for the Examination of Water and Wastewater, 2005"*. A 10ml volume of the water sample was poured into the media tube and mixed until the contents dissolved. The content of the media tube was poured into the center of the Simplate plate and covered. The plate was then swirled to distribute the sample in all the wells. The plate was tipped at an angle to drain the excess sample into the absorbent pad. The same process was followed to make a control sample using distilled water. The plate was inverted and put in the incubator (Scientific: Series 2000) at 37°C for 48 hours. After the incubation period was over the plate was held under a fluorescence analysis cabinet with a long wave ultraviolet (UV) light of 365nm and the number of florescent wells were counted and converted to number of heterotrophic bacteria present in a 10ml sample. HPC was measured twice a month.

Total and faecal coliform

Colilert 18 test kits (Colilert product code: 98-08877-00; IDEXX; Maine, USA) were used to test for the presence of total and faecal coliforms in water. The method used was the, Colilert-18/Quanti-Tray/200 method (9221B, E), which is an alternative method to the standard ISO 9308-3. Media was added to a 100ml water sample; the sample was mixed until the content dissolved. The sample was poured into a Quanti-Tray labelled with the date, sampler's name and sample location and sealed with an IDEXX Quanti- Tray sealer, Model X2 (tray sealer product number: 89-10894-05; IDEXX; Maine, USA). A control sample was made using distilled water following the same process. The Quanti-Trays were put in the oven, Scientific: Series 2000, (oven model: 220; Scientific; Industria, South Africa) for 18 hours at 37°C. After incubation in the oven was over, to determine faecal coliforms, the number of yellow wells were counted and converted to most probable number (MPN) of bacterial cells in a 100ml water sample. This gave an indication of the

number of faecal coliforms present in 100ml of water sample. To determine the total coliforms present in that sample, the same tray way placed inside the fluorescence analysis cabinet (fluorescence analysis cabinet model: CM-10A; Spectroline; New York, USA) with a long wave ultraviolet (UV) light (UV light model: EA-160/FE; Spectroline; New York, USA) of 365nm to see how many wells were florescent. The florescent wells were counted and converted to MPN in a 100ml sample. Water was sampled once a month to conduct this test.

Table 7 shows the number of replicates done for each parameter. Internal replicates are replicates taken from the same sample, while external replicates are replicates of the sample itself collected in different sample bottles.

Table 7: Internal and external replicates for each parameter tested

Parameter	**Replicates**	**Internal/ external**
Temperature	1	
pH	2	Internal
Conductivity	2	Internal
Alkalinity	1	
DO	2	Internal
Free chlorine	1	
Total chlorine	1	
Total nitrogen	3	Internal
Nitrate	3	Internal
Ammonium	3	Internal
Total ammonia	3	Internal
HPC	2	External
Faecal and total coliforms	2	External
DOC	3	Internal
BDOC	3	Internal

3.3 Research ethics

In any research or experiment conducted an ethical code of conduct should be adhered to. This improves the quality of the research performed. This research study adhered to the University of the Witwatersrand research integrity standards, which is known as the Singapore statement on research integrity (Steneck and Mayer, 2010).

Results obtained might assist with the improvement of all water related infrastructure in South Africa not just for JW. No incentives were offered by JW that affected the decisions made for this project. Given the confidential nature of some of the data provided by JW for this study, legal documentation cannot be shared in this report. The findings of this study shall be communicated to JW to assist with water improvements.

3.4 Data analysis

The data gathered was analysed using Microsoft Excel, 2016. To describe the relationship between parameter sets, the data was analysed using the Pearson correlation on Microsoft Excel, 2016.

4. Results and Discussion

In this chapter the field and laboratory results obtained from the one year monitoring programme are presented in section 4.1 as graphs and tables and analysed and discussed in section 4.2.

4.1 Results

For each parameter analysed, a line graph (Figures 7 - 23) was created to depict trends over the one-year duration of the monitoring program. Each plot included sampling points in both area 5 and area 10. The measured parameters were plotted on the y-axis and the time line, in months, was shown on the x-axis.

The parameters that were analysed on a biweekly basis were averaged out to give one reading every month. This was done to create a uniform depiction of all parameters that were tested. In so doing, the plots will not be 100% accurate but will still be sufficient in depicting the trends observed within a 12 month span. However, if the raw data is required, this can be found in appendix A of this report.

The biweekly temperature readings for each site were averaged out to give one reading every month and then plotted in Figure 7. The graph shows that temperature increases in the warmer months and decreases in the cooler months as was expected. The minimum temperature recorded was 11°C and the maximum was 26°C, with an average temperature of 19°C.

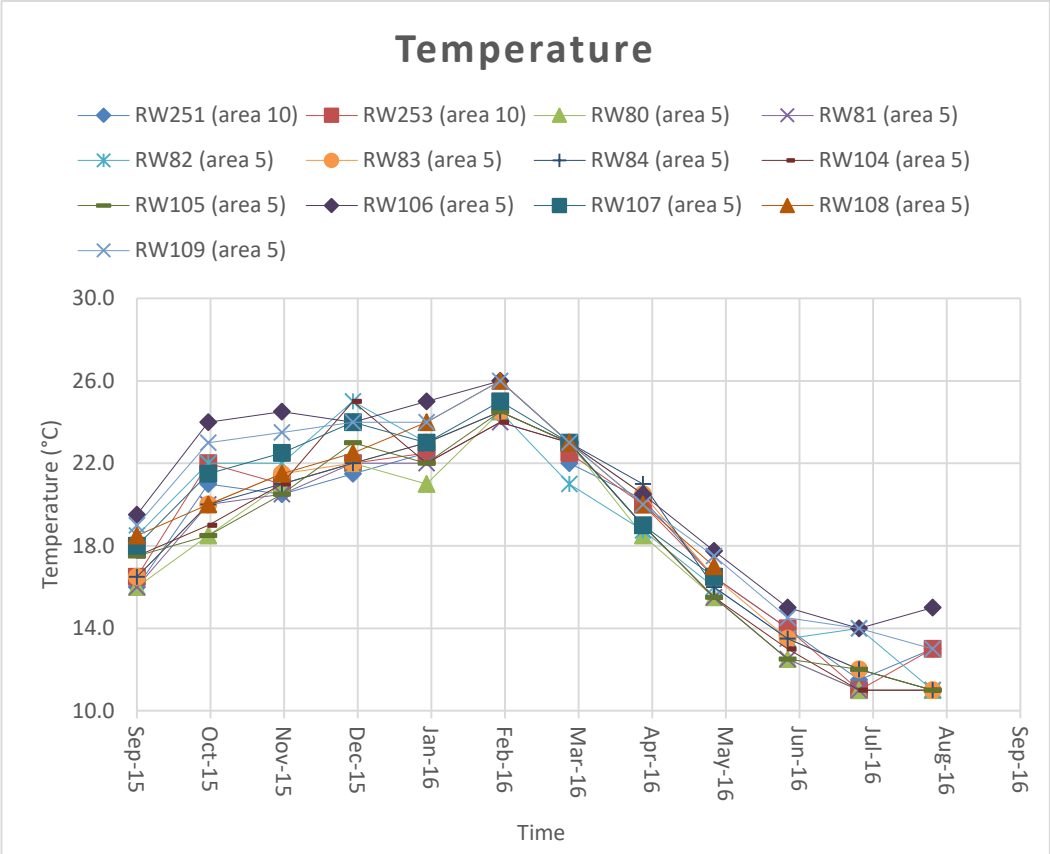

Figure 7: Temperature over time for all 13 sites

The biweekly pH readings for each site were averaged out to give one reading every month and then plotted in Figure 8. As can be seen from the graph, the pH stayed within the range of 7-9. The minimum pH reading was 7.1, the maximum reading was 8.7, with an average of 8.1.

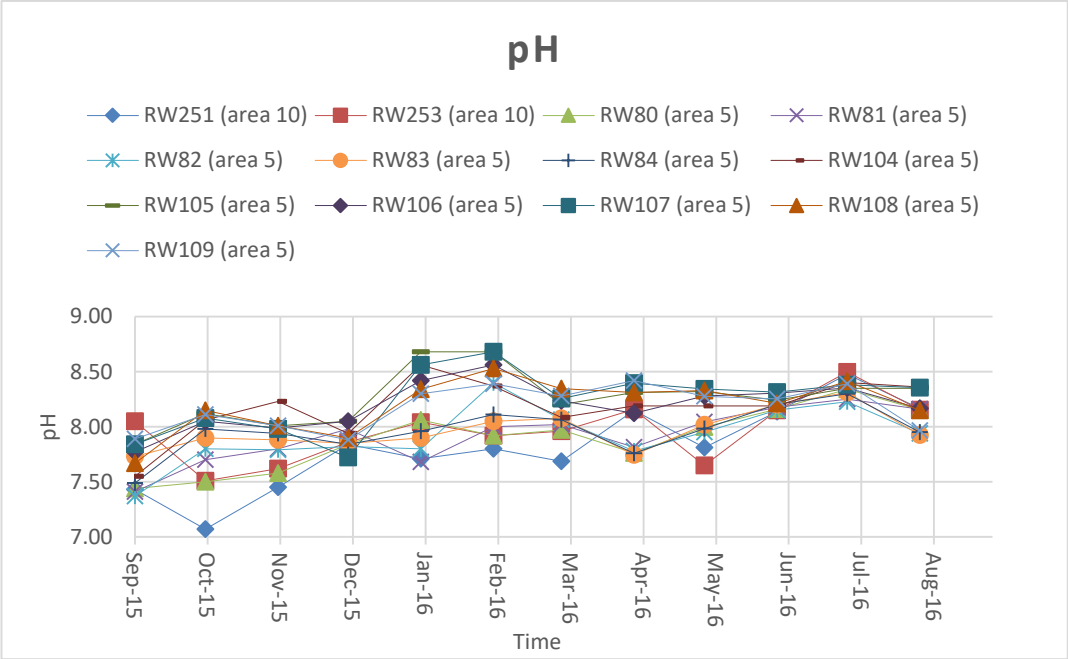

Figure 8: pH over time for all 13 sites

The biweekly conductivity readings for each site were averaged out to give one reading every month and then plotted in Figure 9. As can be seen from the graph, conductivity stayed between 15-26.5mS/m over the monitoring period. The minimum recorded conductivity was 15.5mS/m, the maximum was 26.25, with an average conductivity of 21.1mS/m.

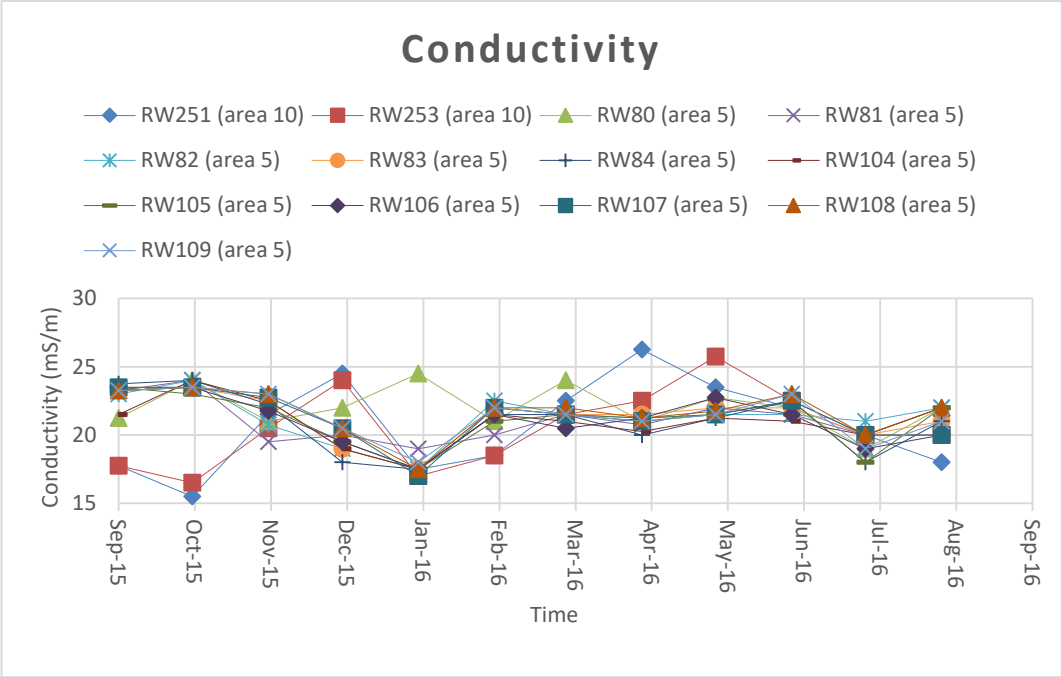

Figure 9: Conductivity over time for all 13 sites

The biweekly DO readings for each site were averaged out to give one reading every month and then plotted in Figure 10. As can be seen from the graph, overall DO was between 3.5-9 mg/l over the period monitored. The minimum recorded DO was 3.2mg/l, the maximum was 8.5 mg/l and the average was 5.9 mg/l.

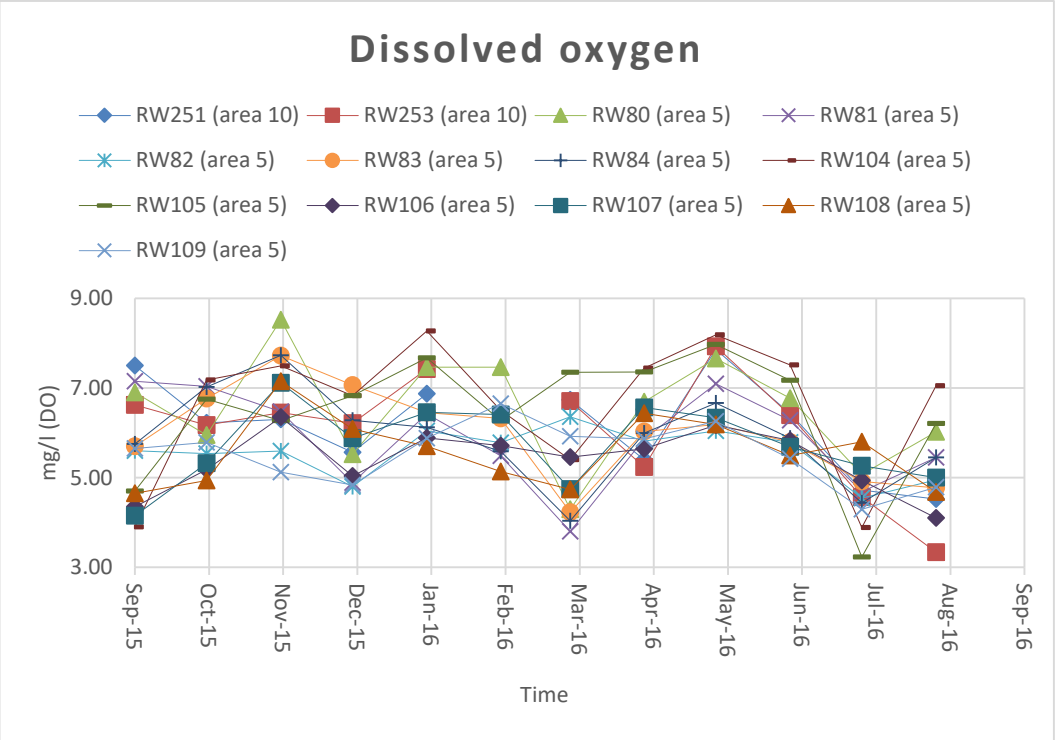

Figure 10: Dissolved oxygen over time for all 13 sites

The monthly alkalinity readings for each site are plotted in Figure 11. As can be seen from the graph, alkalinity decreased in the warmer months and increased in the cooler months. The minimum alkalinity recorded was 39mg/l $CaCO_3$, the maximum was 85mg/l $CaCO_3$ and the average was 56mg/l $CaCO_3$.

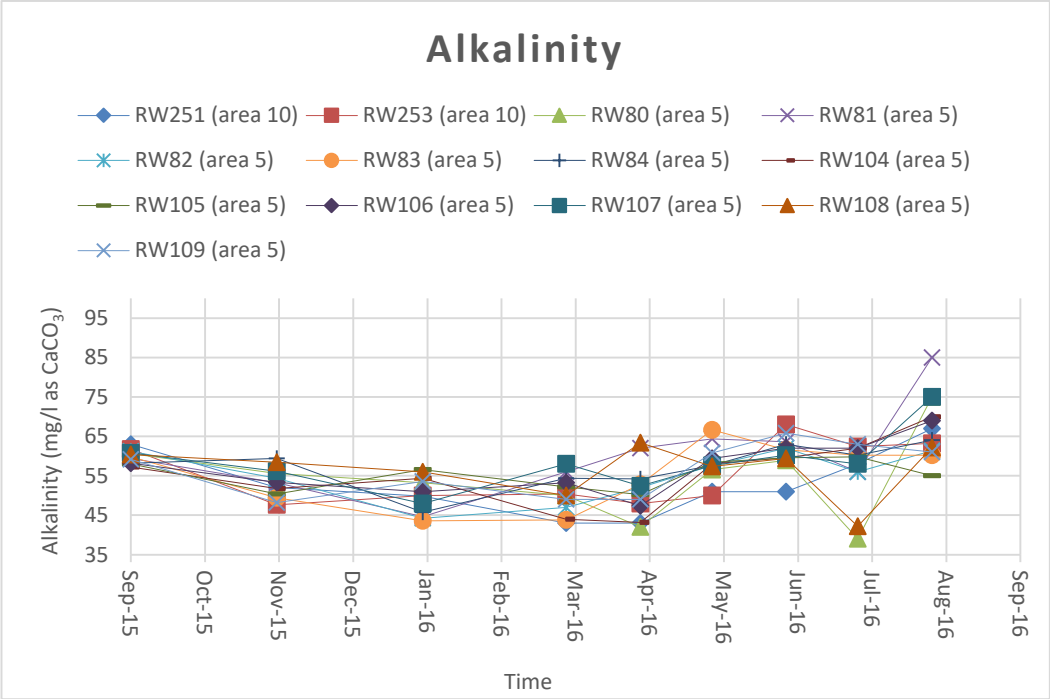

Figure 11: Alkalinity over time for all 13 sites

The monthly total nitrogen (TN) concentrations for each site are plotted in Figure 12. As can be seen from the graph, TN increased over the warmer months and decreased in the cooler months. The minimum TN concentration recorded was <0.5mg/l N, the maximum was 5.1mg/l N and the average was 1.0mg/l N.

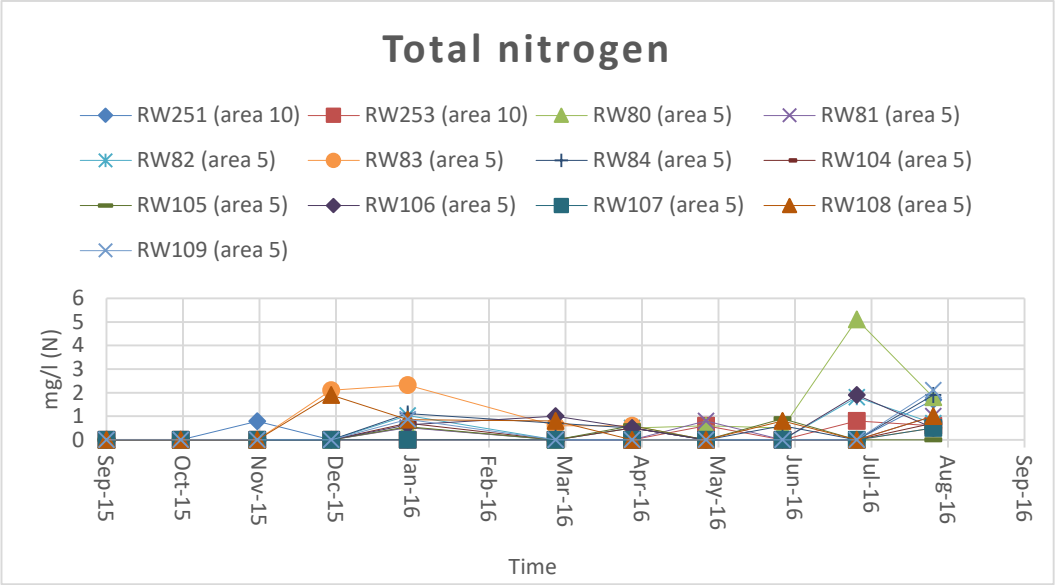

Figure 12: Total nitrogen over time for all 13 sites

The monthly nitrate (NO_3) concentration for each site are plotted in Figure 13. As can be seen from the graph, NO_3 concentrations stayed around 0.8mg/l N over the entire period monitored. The minimum NO_3 concentration was <0.5mg/l N the maximum was 1.1mg/l N and the average was 0.7mg/l N.

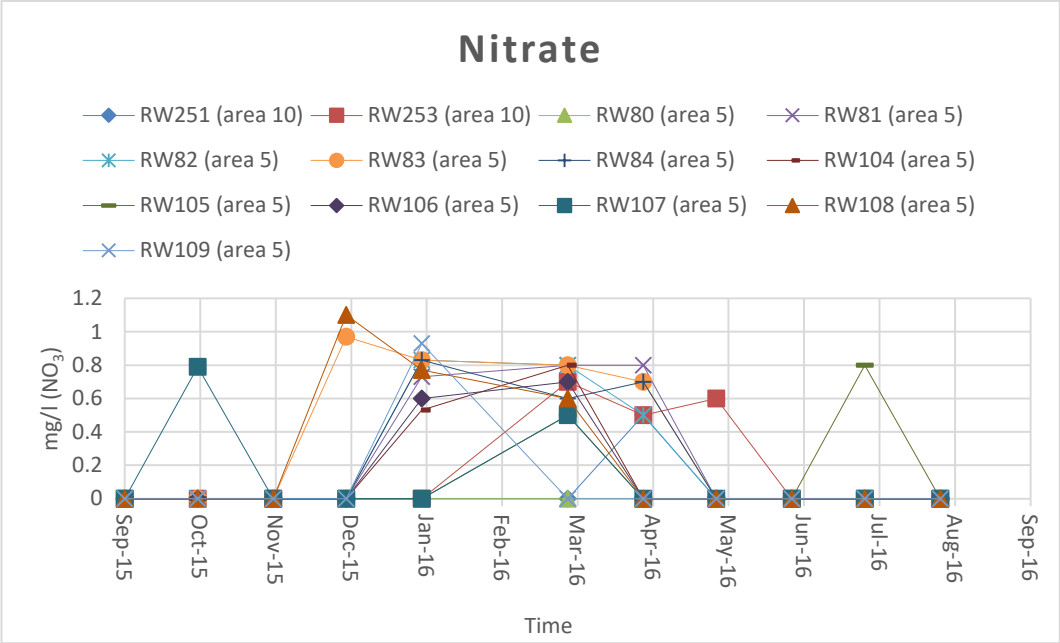

Figure 13: Nitrate over time for all 13 sites

The monthly total ammonia (NH_3) concentrations for each site are plotted in Figure 14. As can be seen from the graph, NH_3 increased over the warmer months and decreased over the cooler months. The minimum NH_3 concentration was <0.02mg/l N, the maximum was 0.17mg/l N and the average was 0.07mg/l N.

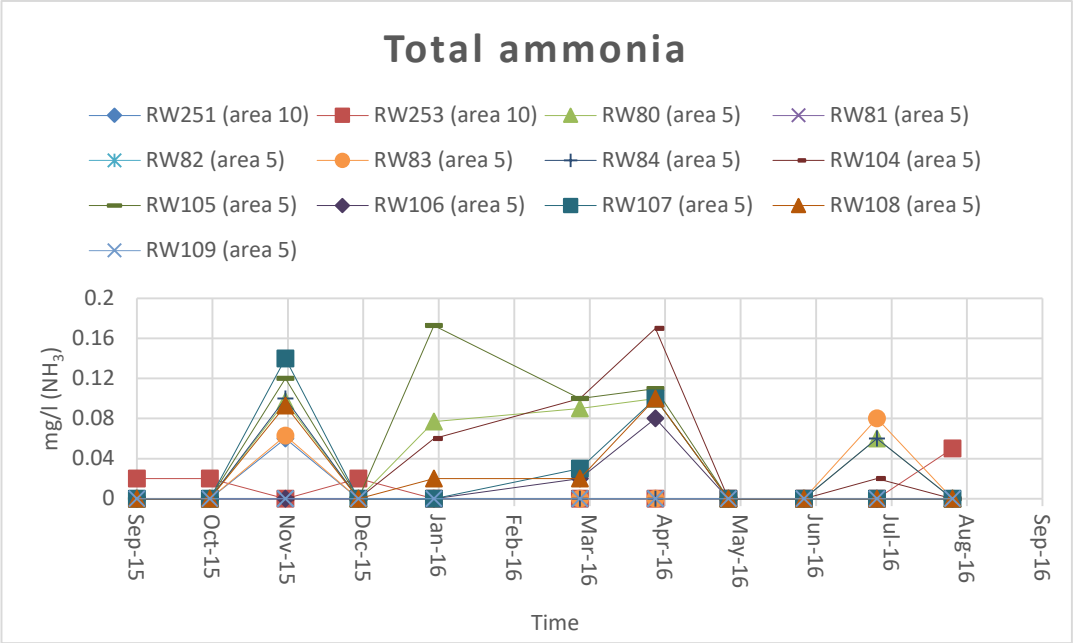

Figure 14: Total ammonia over time for all 13 sites

The monthly ammonium (NH_4) concentration for each site was plotted in Figure 15. As can be seen from the graph, ammonium concentrations increased over the warmer months and decreased over the cooler months with most of the readings being below the readable limit (0.5mg/l N) of the test kit. The minimum NH_4 concentration was <0.5mg/l N, the maximum concentration recorded was 0.6mg/l N, and the average was <0.5mg/l N.

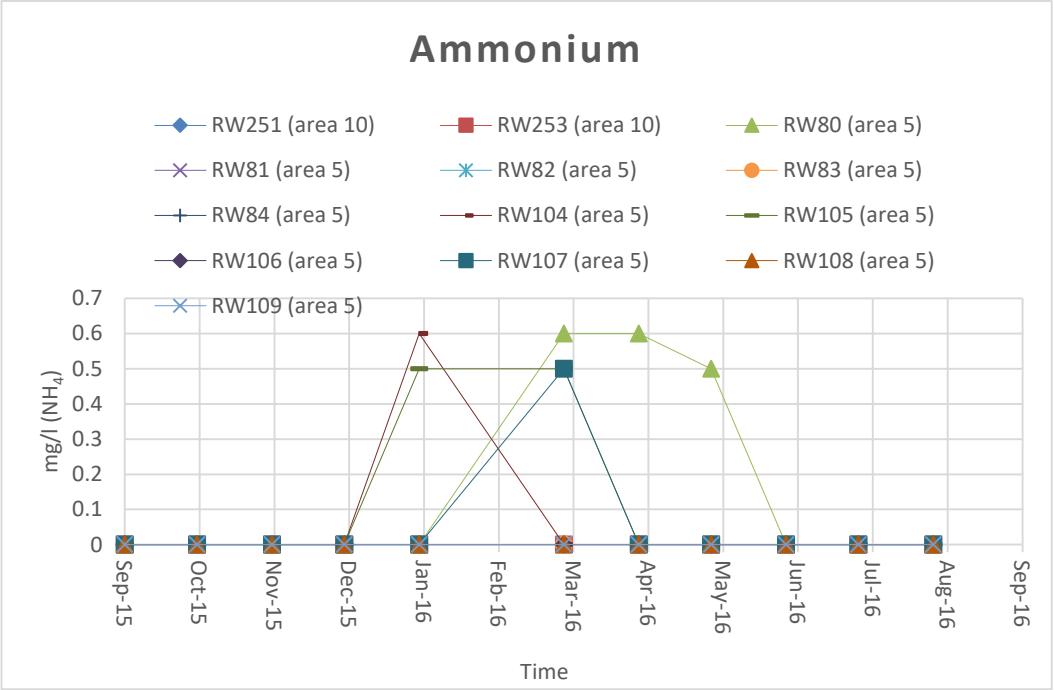

Figure 15: Ammonium over time for all 13 sites

The biweekly free chlorine readings for each site were averaged out to give one reading every month, then plotted in Figure 16. As can be seen from the graph, free chlorine decreased in warmer months then increased in cooler months. The minimum free chlorine concentration was 0mg/l Cl, the maximum free chlorine concentration was 0.95mg/l Cl and the average reading was 0.2mg/l Cl.

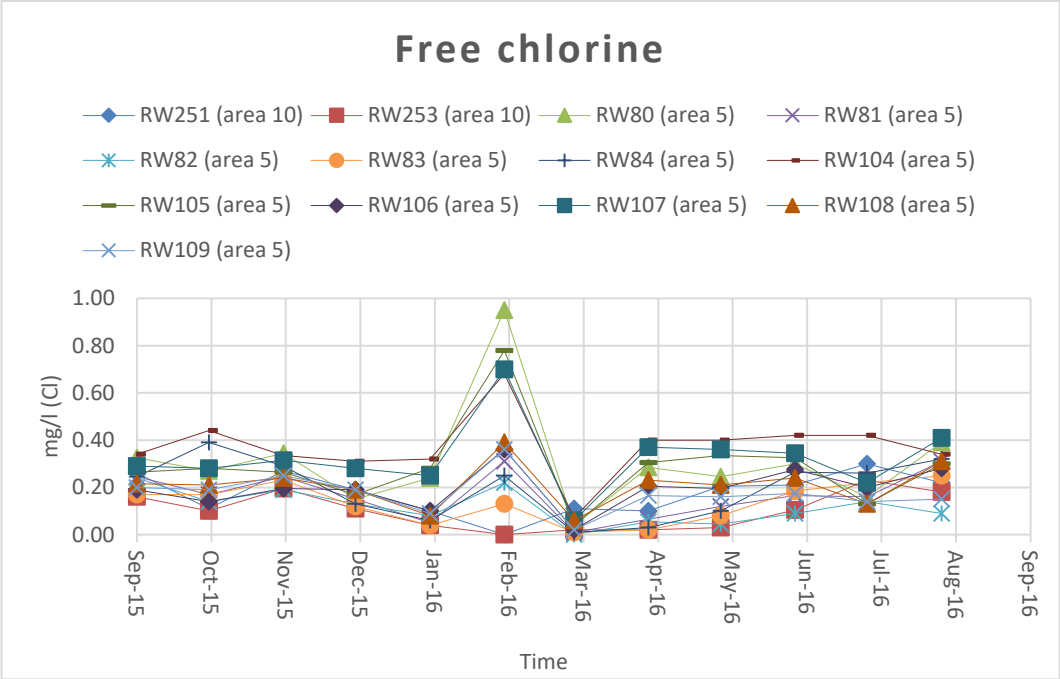

Figure 16: Free chlorine over time for all 13 sites

The biweekly monochloramine readings for each site were averaged out to give one reading every month, and then plotted in Figure 17. As can be seen from the graph, monochloramine decreased in warmer months and increased in cooler months. The minimum monochloramine concentration was 0.02mg/l Cl, the maximum was 1.75mg/l Cl, and the average was 1.09mg/l Cl.

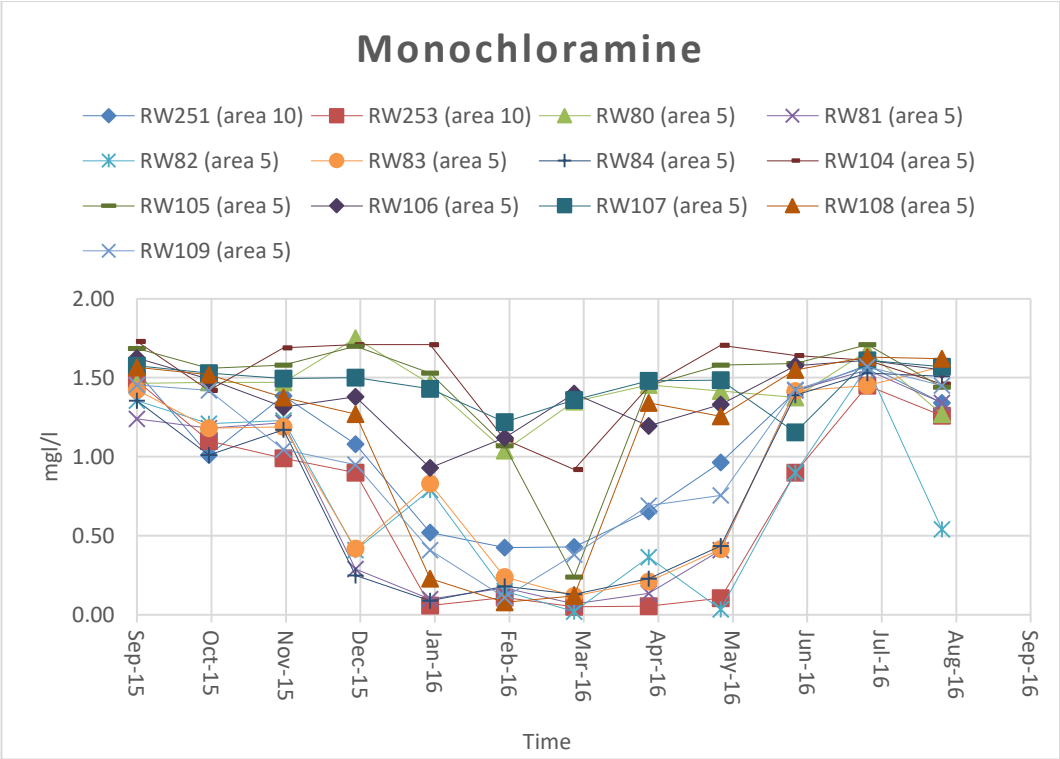

Figure 17: Monochloramine concentration over time for all 13 sites

The biweekly total chlorine readings for each site were averaged out to give one reading every month, then plotted in Figure 18. As can be seen from the graph, total chlorine decreased in warmer months and increased in cooler months. The minimum total chlorine concentration was 0.02mg/l Cl, the maximum was 2.1mg/l Cl and the average was 1.3mg/l Cl.

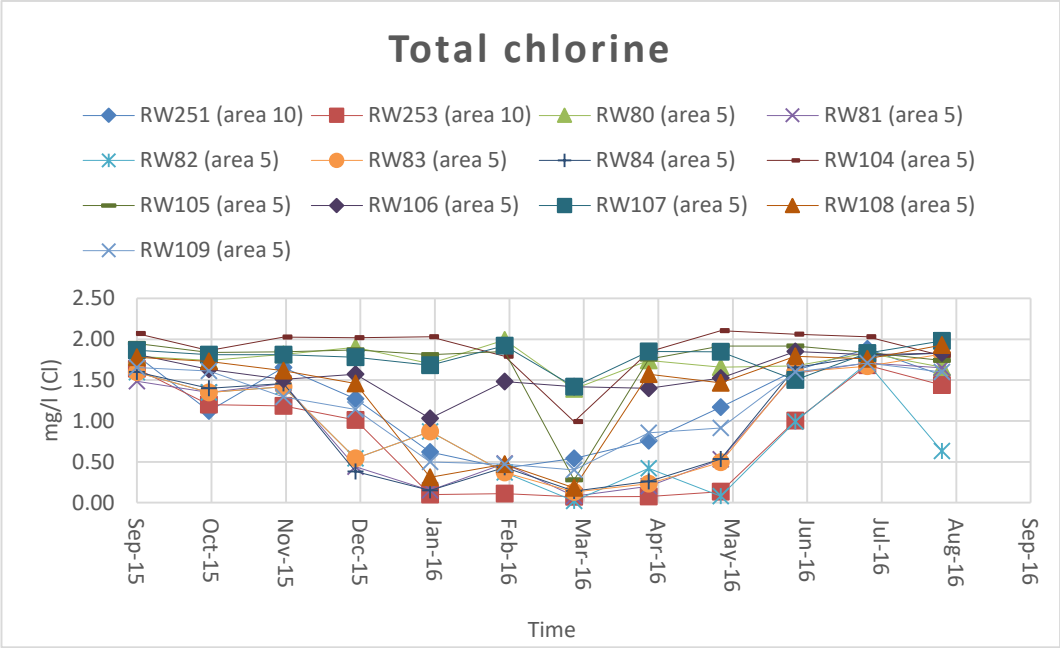

Figure 18: Total chlorine over time for all 13 sites

The biweekly heterotrophic plate count (HPC) readings for each site were averaged out to give one reading every month, and then plotted in Figure 19. As can be seen from the graph, HPC increased in warmer months and decreased in cooler month. The minimum HPC was <0.2 MPN/ml, the maximum was >73.8 MPN/ml, and the average was 26.9 MPN/ml.

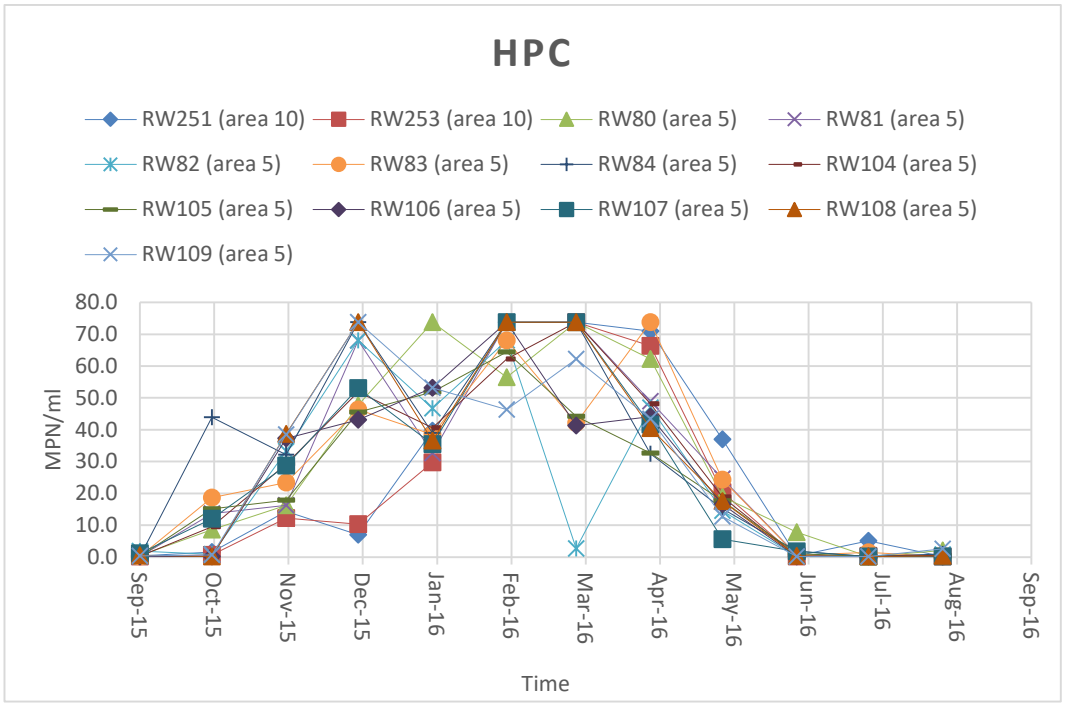

Figure 19: HPC over time for all 13 sites

The monthly faecal coliform readings for each site were plotted in Figure 20. As can be seen from the graph, there was no detectable faecal coliform in the drinking water.

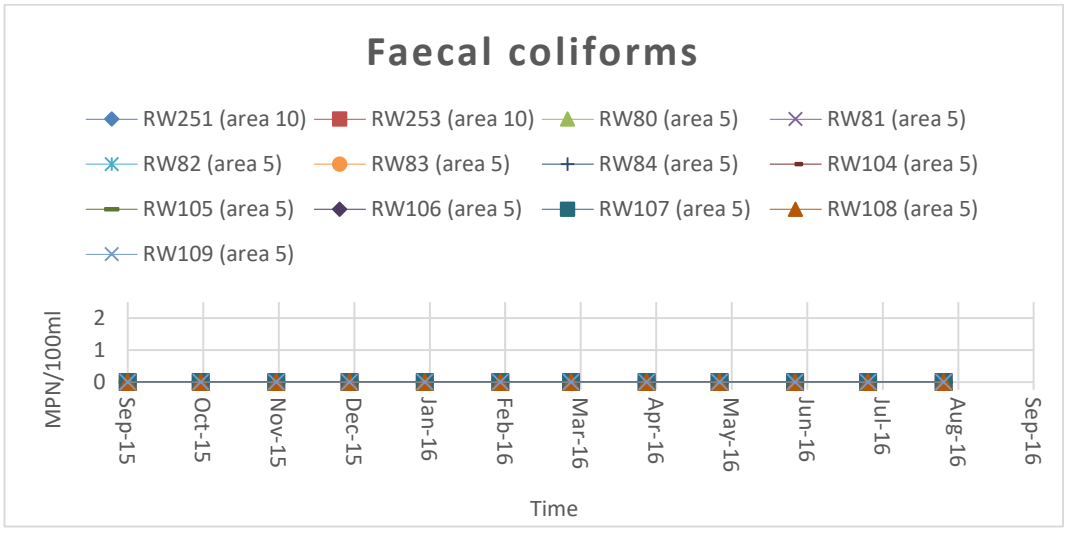

Figure 20: Faecal coliform over time for all 13 sites

The monthly total coliform readings for each site were plotted in Figure 21. As can be seen from the graph, there was hardly any total coliform detected. The minimum total coliform reading was <1 MPN/100ml, the maximum was 2 MPN/100ml and the average was <1MPN/100ml.

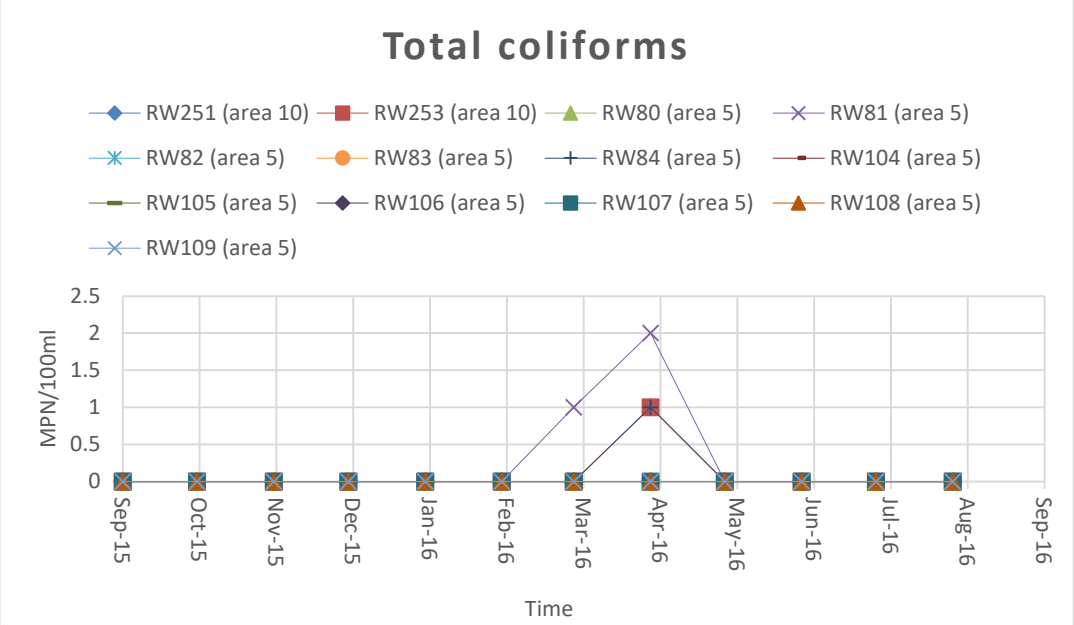

Figure 21: Total coliform over time for all 13 sites

The biweekly DOC concentrations for each site were averaged out to give one reading every month, and then plotted in Figure 22. As can be seen from the graph, DOC increased in the warmer months and decreased in the cooler months. The minimum DOC concentration was 1.87mg/l C, the maximum was 6.49mg/l C and the average was 4.04mg/l C.

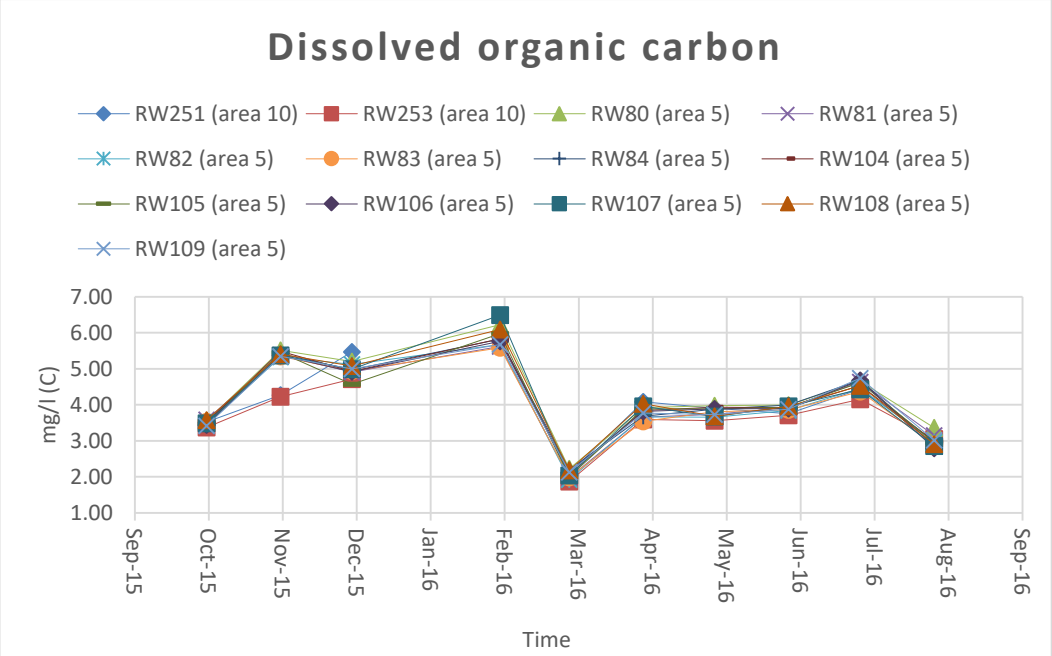

Figure 22: Dissolved organic carbon over time for all 13 sites

The monthly BDOC concentrations for each site were averaged and plotted in Figure 23. As can be seen from the graph, BDOC shows an increase in concentration over the cooler months and a decrease in the warmer months. The minimum BDOC concentration was 0mg/l C, the maximum was 3.05mg/l C and the average was 0.7mg/l C.

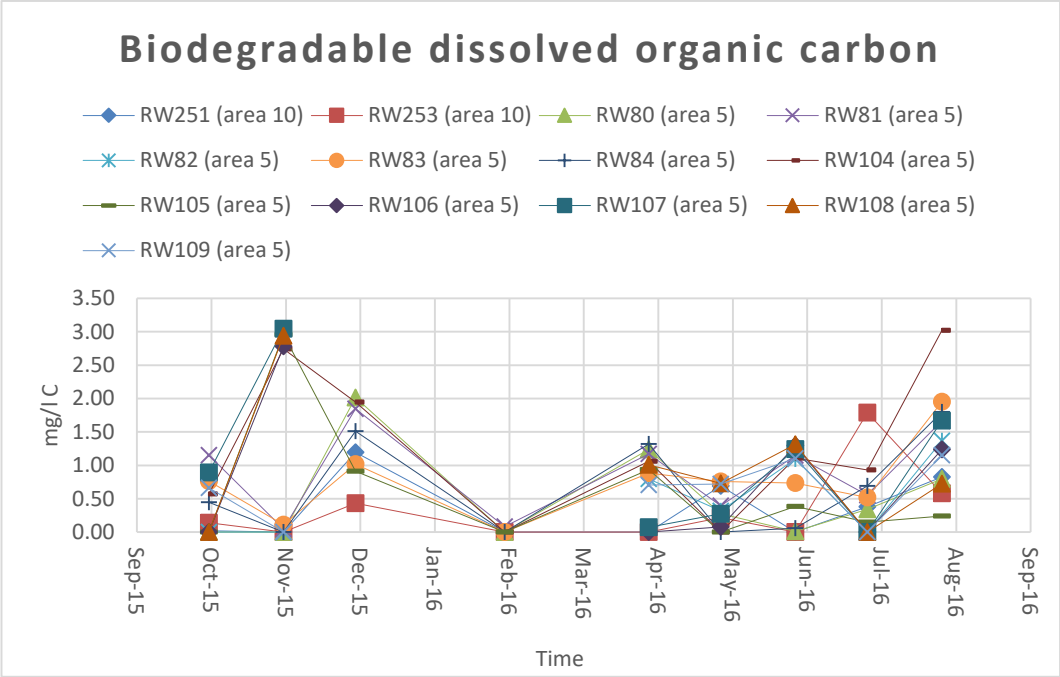

Figure 23: BDOC concentration over time for all 13 sites.

Relationship between parameter sets

Table 8 shows the relationships between various sets of parameters. All of these are based on Pearson correlation. Parameters were paired based on their known ability to influence each other as recorded in the literature. For example, bacterial growth is driven by the oxidation of such electron donor substrates as BDOC and ammonia. As a result, increases in bacterial densities are expected to be accompanied by drops in levels of electron donor substrates. These relationships are analysed, in depth, in the discussion section (4.2). The coefficients marked as N/A were for parameters that showed no change over time and could not be correlated using Microsoft Excel, 2016. The table shows the overall relationships between the chosen parameter sets over the one-year sampling duration for all 13 sites. The negative coefficients showed that there was an inverse relationship between the parameter set over the sampling period and a positive coefficient shows a direct relationship between the parameter set. Values close to one in Table 8 (negative or positive) signal a strong relationship for a parameter set, while values close to zero (negative or positive) signal a weak relationship for a parameter set. The use of these correlation coefficients provides a clearer picture

of how different water quality parameters worked together and against each other to affect water quality in general and biostability in particular.

Table 8: Correlation coefficients between different sets of parameters for all 13 sites over one year

Parameter	RW251 (area 10)	RW253 (area 10)	RW80 (area 5)	RW81 (area 5)	RW82 (area 5)	RW83 (area 5)	RW84 (area 5)	RW104 (area 5)	RW105 (area 5)	RW106 (area 5)	RW107 (area 5)	RW108 (area 5)	RW109 (area 5)
Chlorine & Temperature	-0.8	-0.5	0.1	-0.7	-0.1	-0.7	-0.8	-0.3	-0.3	-0.7	-0.2	-0.8	-0.6
HPC & DOC	-0.4	-0.6	0.0	0.0	0.7	0.2	0.2	0.1	0.3	0.4	0.2	0.3	0.2
pH & Alkalinity	0.1	0.6	-0.1	0.5	0.0	0.2	0.2	0.4	0.0	0.2	-0.3	-0.6	-0.2
BDOC & DOC	0.4	0.4	0.4	0.2	-0.3	0.0	0.0	0.4	0.6	0.5	0.4	0.5	-0.7
Chloramine & Total Ammonia	0.3	0.5	0.7	N/A	N/A	0.5	0.4	-0.5	-0.3	0.0	0.3	0.0	N/A
Total Ammonia & Nitrate	-0.1	-0.3	N/A	N/A	N/A	-0.4	-0.4	0.4	0.1	0.0	0.0	-0.1	N/A
Temperature & HPC	0.5	0.5	0.8	0.8	0.7	0.8	0.9	0.8	0.9	0.8	0.8	0.8	0.7
Chlorine & HPC	-0.8	-0.8	-0.2	-0.9	-0.3	-0.9	-0.7	-0.6	-0.3	-0.8	-0.3	-0.7	-0.8
BDOC & HPC	-0.2	-0.4	0.3	0.0	-0.3	-0.1	0.1	-0.3	-0.1	0.4	-0.1	0.3	-0.6
BDOC & Chlorine	0.2	0.6	0.5	0.1	-0.2	0.3	0.0	0.3	0.2	0.2	0.2	0.2	0.1

4.2 Discussion

Temperature

The temperature of drinking water is not regulated by the SANS241 (2011) code, but based on previous research (U.S.A EPA, 1992; Lautenschlager *et al*., 2010; Henne *et al*., 2013), distribution system water temperatures above 15°C promote bacterial growth. The temperature recorded in Figure 7 ranged between 11 and 26°C. First, it should be noted that the sites that consistently had high temperatures, for each day, were the sites that were sampled last, between 10:00 and 11:00 am, while the other sites were sampled between 7:30 and 10:00 am. Apart from the variations that could be directly tied to time of day when the samples were collected Figure 7 shows a stronger temporal (seasonal) variation than a spatial (site to site) variation. Research conducted by Henne *et al*. (2013) agreed with this observation.

From the correlation table, it is evident that a strong positive correlation exists between temperature and HPC (0.5 to 0.9), with HPC densities increasing as temperature increases. With water temperature as high as 26°C, attention should be paid to all sites during the summer months, as research has shown that, members of the *Legionella* genus, which are not only opportunistic pathogens but have also been shown to thrive within distribution system biofilms, are able to withstand temperatures between 15 and 60 °C and pH values between 5 - 8.5 (Moritz *et al*., 2010; Rakic *et al*., 2012). The higher water temperatures in the study, were closely associated with the loss of residual disinfectants and by extension, reduced disinfection power. These results show that seasonal changes had a large impact on temperature variations and that personnel managing the distribution network should take extra precautions to monitor temperature during the warmer months. In this work, this can be seen in Figure 7. From the results, it was seen that high temperatures led to loss of biostability, as it aids in the loss of chlorine residual which in turn, fuels bacterial growth.

pH

SANS241 (2011) regulates pH for drinking water. The pH values should stay in the range of 5 - 9.7. Figure 8 shows pH values ranging from 7 - 9. This result was expected and is acceptable for drinking water. Figure 8 also shows minimal seasonal and spatial variation in pH distribution. A possible cause for the stability of pH across space and time is the buffering effect of alkalinity, low levels of metals, mineral concentrations (sodium, calcium, and magnesium) and oxygen levels. However, site RW251 consistently had the lowest pH reading out of all the sites. The low pH in comparison to the other sites could be due to the absorption of carbon dioxide over time. As the water sample was collected early in the morning, which suggests low water demand, hence longer residence time with low oxygen supply.

To make sure pH in the distribution network is not an issue, points with pH greater than 7.5 should be boosted with chlorine (van der Walt *et al*., 2009; EPA, 2011). These pH measurements (7-9) are within a range that would not cause corrosion or the negative water quality impacts associated with corrosion (bitter taste, colour, scales, biofilm growth), as it falls within the range specified by SANS241, (2011) (LeChevallier *et al*., 2015). As previously mentioned, members of the *Legionella* genus are able to withstand temperatures between 15 and 60 °C and pH values between 5 - 8.5 (Moritz *et al*., 2010; Rakic *et al*., 2012). The pH range given falls within the measured pH range of the results. The above in addition to the results for temperature, means that both temperature and pH need to be monitored in the warmer months.

Overall, pH was important in tracking biostability as it is indicative of chemical, physical and biological processes, such as corrosion and nitrification (LeChevallier *et al*., 2015).

Conductivity

Conductivity is regulated by SANS241 (2011) and should not exceed 170mS/m. The results, seen in Figure 9 ranged between 15 and 26mS/m. Conductivity, like pH and temperature, shows minimal spatial and seasonal variation. This result indicates that the water had minimal particles and or salts, which minimises the

risk of pipe corrosion and its harmful side effects (Zakowski *et al*., 2014). Overall, conductivity did not play a large role in loss of biostability and biofilm formation potential.

Dissolved oxygen

Dissolved oxygen is not regulated by SANS241 (2011). In this study, DO ranged between 3 and 8.5mg/l (Figure 10), a range that is considered normal for drinking water in distribution systems in other jurisdictions (Alaska Department of Environment Conservation, 1979; Lu et al., 2014). The higher DO concentrations observed in the warmer months were due to the fact that some sites (RW80, RW104 and RW105) were not exposed to direct sunlight and the samples from the 3 sites mentioned were collected early in the day, when the temperature was lower. RW80, RW104 and RW105 all had high water pressures, which also accounts for the higher DO. This shows that DO has seasonal and spatial variation.

These DO measurements are good for water quality because, oxygen improves the taste of water (Masters and Ela, 2008). Provided that organic matter and nutrients are limited and that DO stays within an acceptable limit, biostability can be obtained and biofilm growth potential minimized. However, from the 13 sites sampled, DO did not significantly impact biostability or bacterial growth.

Alkalinity

Alkalinity is not regulated according to SANS241 (2011). Figure 11 shows that alkalinity stayed between 40 and 90 mg/l $CaCO_3$. In India (IS: 10500: 1991) alkalinity is limited to no more than 200 mg/l $CaCO_3$. In the United States of America, the USEPA limits alkalinity in terms of total dissolved solids to no more than 500mg/l $CaCO_3$. Low alkalinity water usually results in pH levels being close to or above 8 (EPA, 2011). This can be seen in the results obtained, as the average pH was 8.1. The measurements also show that there was minimal spatial variation in alkalinity concentrations. The correlation coefficient table, (Table 8), shows a weak to moderate relationship (0 to 0.6) between alkalinity and pH. Alkalinity

concentrations at RW80 and RW108 dropped significantly in comparison to the other sites in July 2016. The reason for this is not clear, as it did not appear to affect any other parameter. With these measurements, alkalinity has minimal effect on biostability and was not considered one of the most important water quality parameters.

Nitrogen species

Total nitrogen

The SANS241 (2011) does not regulate total nitrogen (TN). The TN measurements can be considered to be acceptable seeing that it did not surpass the regulatory limit for nitrate (≤11mg/l). This is because organic nitrogen is a source for total ammonia and nitrate and if the total nitrogen concentrations are high, then there is a chance that ammonia and nitrate concentrations will be high as well (van der Walt *et al*., 2009). Figure 12 shows that TN concentrations ranged between <0.5 and 6mg/l for all samples assayed in this study.

Figure 12 shows spatial and seasonal variation in TN. TN concentrations were within the readable limit during the warmer months, see Figure 12. Sites RW83 and RW108 peaked in the warmer months and RW80 peaked in the cooler months. The peaks could have been as a result of nutrient entering the bulk water supply. The low nitrogen concentrations suggest low ammonia and nitrate loads (van der Walt *et al*., 2009). Overall these results show that total nitrogen did not have a huge impact on biostability.

Nitrate

The regulatory limit for nitrate by SANS241 (2011) is ≤11mg/l N. In Figure 13, the nitrate concentrations ranged between 0 and 1.1mg/l N, which is acceptable. Similar to total nitrogen, Figure 13 shows nitrate concentrations varied seasonally and spatially. Sites RW83 and RW108 were again noted to have the highest nitrate concentrations in the warmer months. The nitrate levels observed in this study did not pose a health risk to humans and are expected, as a study carried out by

Lautenschlager *et al*. (2013) had nitrate results between 0.3 and 1.7mg/l N. Table 8 shows a weak relationship between nitrate and total ammonia (0 to -0.4). The nitrate concentrations also indicate low nutrient loads. Nitrate is a growth factor for biofilms in anoxic conditions (Fang *et al*., 2009; Hammes *et al*., 2010; Krishna *et al*., 2013; Lu *et al*., 2014).

Total ammonia

The SANS241 (2011) only accounts for free ammonia and not the ammonia already combined with chlorine to form monochloramine. The limit given is ≤1.5mg/l N. Figure 14 shows that total ammonia ranged between 0 and 0.2mg/l N. This range is below the limit given for free ammonia which implies that the results obtained are acceptable. These results are expected, as drinking water is not expected to contain high levels of ammonia (WHO, 2003). Similar to total nitrogen and nitrate, Figure 14 shows seasonal and spatial variation in total ammonia concentrations. Total ammonia concentration was low but increased over the warmer months, especially RW105 and RW80. This increase is not enough to be alarming, as the concentration did not reach 0.2 mg/l N, see Figure 14. A few reasons for the low concentrations could be that it was converted to either nitrate or nitrite, by nitrifying bacteria. Some ammonia combined with chlorine to form chloramines (van der Walt *et al*., 2009; EPA, 2011; Metcalf and Eddy, 2014).

These measurements are low enough to not create an excessive demand on residual chlorine and did not significantly impact biostability or influence bacterial growth (van der Walt *et al*., 2009; EPA, 2011).

Ammonium

Ammonium is not regulated by SANS241 (2011) but according to the European food safety authority (EFSA, 2012), ammonium concentrations between 0.5 and 5mg/l N is considered safe and does not pose any health risk, even to vulnerable groups (e.g. old people and infants) if consumed over a long period of time. Figure 15 shows ammonium ranged between 0 and 0.6mg/l N. These measurements are

within the range suggested by EFSA, 2012 and can be considered to be non-problematic in terms of maintaining good water quality and limiting biofilm growth potential. Seeing that most of the ammonium concentrations were below the readable limit of the test kit, a clear picture cannot be painted and it is unclear whether or not ammonium showed significant seasonal and spatial variation. Sites RW80, RW104, RW105 and RW107 were the only sites that had readable measurements however low they may have been. The peak noticed in free chlorine coincides with these 4 sites mentioned. This shows a link between free chlorine and ammonium. These results show that higher ammonium results can assist in prolonging the effect of free chlorine residual, thus limiting bacterial growth. Ammonium's impact on biostability and bacterial growth was negligible.

Chlorine residuals

Free chlorine

Free chlorine is regulated by the SANS241 (2011) and should not exceed 5mg/l. Figure 16, shows that free chlorine is well below this limit (0 - <1mg/l). Free chlorine concentrations above 1mg/l have been associated with the formation of toxic disinfection by-products, taste and odour complaints, therefore, the upper limit of the results is acceptable, as it does not exceed 1 (Bowden *et al*., 2006; Monteiro *et al*., 2015). The fact that sites RW253, RW81, RW82 and RW83 had little to no measurable chlorine residual is a cause for concern, especially during the warmer months. These 4 sites (RW253, RW81, RW82 and RW83) were flagged for poor water quality because of the low free chlorine residuals and corresponding high heterotrophic plate counts. This indicates that the water could be biologically unstable and thus encourage heterotrophic bacterial growth. The loss of residuals could be due to, long travel distances for the water, high residence time of the water in the distribution network, a high chlorine demand (due to presence of organics), or a combination of these factors (Shamsaei *et al*., 2013).

A chlorine residual of at least 0.2mg/l is necessary to combat bacterial regrowth (Hallam *et al*., 2001; American Water Works Association, 2006, Sarbatly and Krishnaiah, 2007; Monteiro *et al*., 2015). The lack of a detectable level of

disinfectant will inevitably lead to the proliferation of microorganisms within the drinking water distribution system. This can lead to a health risk (Sarbatly and Krishnaiah, 2007; Van der Walt *et al*., 2009; EPA, 2011; Rakic *et al*., 2012; Wang *et al*., 2014).

There is a very strong seasonal variation in the availability of chlorine, with the correlation between chlorine and temperature being as strong as -0.8 for some sites. This result is in agreement with literature (Sarbatly and Krishnaiah, 2007; Simoes, 2013; Monteiro *et al*., 2015).

Overall, chlorine residual had a large impact on biostability, thus to ensure biostability and reduce biofilm growth potential, the release of substances that exert a chlorine demand into the network should be limited (Ndiongue *et al*., 2005; Monteiro *et al*., 2015).

Chloramines

Monochloramine is regulated by SANS241 (2011) and should not exceed 3mg/l. Figure 17 shows that the chloramine concentrations in the sections of the network that were monitored did not exceed 2mg/l. Monochloramine concentration profiles mimicked free chlorine and total chlorine (Figure 16 and 18) concentration profiles as was expected. Monochloramine is lost to chemical and microbial reactions (Krishna *et al*., 2013). The decay of monochloramine increases the ammonia concentration which in turn will increase bacterial growth, particularly autotrophic nitrifiers which will lead to nitrification in the distribution system (Krishna *et al*., 2013). Table 8 shows a weak to moderate relationship between total ammonia and monochloramine concentration of ranging from 0 to 0.7.

Monochloramine had a significant impact on biostability in the same way that free chlorine did, as it affects bacterial growth.

Total chlorine

Total chlorine is not regulated by SANS241 (2011). Figure 18 shows that the total chlorine concentrations in the sections of the network that were monitored did not exceed 2.1 mg/l Cl. Total chlorine concentrations for the following sites, RW253, RW 82, RW 83 and RW 84 were low during warmer months, due to an increase in the rate of chlorine decay. These low chlorine concentrations leave these sites susceptible to heterotrophic bacterial growth. The increase in bacterial growth at these sites can be seen in Figure 19. RW80, RW104 and RW107 have higher concentrations due to the peak experienced in free chlorine at these same sites. The correlation coefficient table also shows a moderate to strong relationship between chlorine and temperature and chlorine and HPC. Total chlorine had a significant impact on biostability in the same way the free chorine did, as it affects bacterial growth.

HPC

The colony forming unit (CFU) and most probable number (MPN) are two of the most common methods used when monitoring water quality (Flemming, 1999; Beściak and Surmacz-Górska, 2011). Colony forming unit (CFU) is a measure of viable bacterial cells that grow into visible clusters on a solid medium (e.g. petri dish). Most probable number (MPN) refers to a method that uses dilution cultures and a statistical table to determine the approximate number of viable bacterial cells in a given volume of water sample.

HPC is regulated by SANS241 (2011) and should not exceed 1000 count/ml (CFU/ml). Figure 19 shows that the measured HPC density ranged between <0.2 and >73.8 MPN/ml. Although the regulated method for counting HPC differs from the method used, both methods give an indication of the viable number of bacterial cells in a given volume.

Figure 19 shows significant seasonal and spatial variation in HPC concentrations. The elapsed time between the water treatment plant and sampling locations has been known to have an effect on HPC counts due to the removal of disinfectant

residual over time (LeChevallier *et al*., 1996; Allen *et al*., 2004; Ndiongue *et al*., 2005; Al-Jasser, 2007; van der Walt *et al*., 2009; Shamsaei *et al*., 2013; LeChevallier *et al*., 2015). However, since the exact distance between the treatment plant and each of the 13 sites is unknown, this cannot be verified, but from the results, the sites (RW253, RW81, RW82 and RW83) with lower chlorine residuals had high HPC counts, except for RW82 which experienced a dip in HPC concentration, which was unexpected (Allen *et al*., 2004; Bowden *et al*., 2006; Al-Jasser, 2007). RW80 consistently had high HPC concentration regardless of the higher chlorine residuals recorded at that site. This could be due to longer residence times (Shamsaei *et al*., 2013). Sites RW253, RW80, RW81, RW82 and RW83 all had relatively high HPC concentrations and were flagged for poor water quality. The relationship between chlorine residual and HPC concentration has been mentioned previously. This is also shown in Table 8, where chlorine residual and HPC have a medium to strong relationship (-0.2 to -0.9), temperature and HPC also showed a medium to strong relationship (0.5 to 0.9). This result was expected because a study by Henne *et al*., (2013) showed that HPC densities were directly affected by temperature and a study by Shamsaei *et al*., (2013) showed an inverse relationship between chlorine residuals and HPC.

The results show that bacterial growth is more prevalent in the warmer months regardless of chlorine residuals (Kerneis *et al*., 1995). Some studies have shown that limiting substrates, in addition to increasing disinfectant residuals, could decrease bacterial growth (Ndiongue *et al*., 2005; Lu *et al*., 2014). Overall, HPC had a huge impact on heterotrophic biostability.

Total and faecal coliforms

The SANS241 (2011) regulates total and faecal coliform. Total coliform may not exceed 10 count/100ml and faecal coliform may not exceed 0 count/100ml. Figure 20 shows that there was no faecal coliform detected, and Figure 21 shows that there was a 0 to 2 count/100ml of total coliform detected. These results were expected, as drinking water should not have high concentrations of these parameters (LeChevallier *et al*., 1996; SANS241, 2011). These results are acceptable because these parameters are regulated by SANS241 (2011).

There was no impact of seasonal and spatial variation on the occurrence of coliform bacteria. These results show that the water quality was good and that biological instability and biofilm growth potential is limited with regards to coliforms (van der Kooij, 1999). Total and faecal coliform did not have a huge impact on biostability, the use of HPC as a biostability indicator would have sufficed.

DOC

The SANS241 (2011) regulates TOC, and it should not exceed 10 mg/l. DOC is the portion of TOC that can pass through a filter. Figure 22 shows the DOC results ranged between 1.5 and 6.5 mg/l. This result was considered acceptable as several distribution systems studies have reported similar DOC readings between 0.3-15mg/l. (Volk *et al*., 2000; van der Kooij, 2003; Allpike *et al*., 2005; van Leeuwen *et al*., 2005). There was seasonal variation, with the DOC concentrations higher in the warmer months, and, minute spatial variation over all 13 sites.

Since a fraction of DOC fuels heterotrophic growth there is supposed to be a clear relationship between the concentration profiles of DOC and HPC within the distribution network (Allen *et al*., 2004; Hammes *et al*., 2010). The results did not show this relationship clearly, the correlation coefficient table had two sites (RW251 and RW253) supporting this claim, both having moderate relationships respectively (-0.4 and -0.6). This claim was further supported by research carried out by Kernies *et al*., (1995) and Lu et al., (2014) which showed that organic carbon had a weak relationship with HPC count. The peak in DOC, while equally maintaining high HPC counts can be explained by a possible introduction of dissolved organics into the system from the bulk water. Table 8 shows a weak relationship between BDOC and DOC. With only two sites (RW82 and RW109) having an inverse relationship between the two parameters, -0.3 and -0.7, respectively. It is not clear how much of the DOC was biodegradable from the results obtained. As the results stand, DOC was not a good parameter to use for measuring biostability as it had little to no effect on the HPC concentrations (Kernies *et al*., 1995).

BDOC

BDOC is not regulated by SANS241 (2011). Other research showed that BDOC in drinking water had to be less than 0.3mg/l in order to maintain biostability (LeChevallier, 1998, p.160; Escobar and Randall, 2001; Van der Kooij *et al*., 2013, p.263). Figure 23 shows the BDOC concentration for this study. The results obtained were between 0 and 3mg/l. Figure 23 also shows temporal and spatial variation in BDOC concentrations. RW104 had one of the highest BDOC concentrations throughout, making it one of the sites with poor water quality regardless of the high chlorine residuals at that site. Table 8 shows a weak to moderate relationship (0 to 0.6) between chlorine residual and BDOC concentration. The relationship between BDOC and HPC was also weak to moderate (-0.1 to -0.6). With BDOC concentration at its highest at sites RW104, RW107 and RW108, these were the same sites where HPC concentration was at its maximum. This occurred in the warmer months as BDOC degrades more at higher temperatures and fuels bacterial growth. This was also observed in a study conducted by Hammes *et al*. (2010).

Overall BDOC was an important parameter in determining biostability and biofilm formation potential in a distribution system as microorganisms use BDOC as a food source.

5. Conclusions and Recommendations

5.1 Conclusions

The decline of water quality in distribution systems in general and the loss of biological stability in particular can potentially lead to the proliferation of pathogens and opportunistic pathogens and can also result in the breaching of regulatory standards. The major causes of the loss of biological stability and an increased biofilm formation potential in distribution systems are bacterial growth and regrowth. Bacterial growth and regrowth are both fuelled by the loss of disinfectant residuals and the presence of growth fuelling substrates.

In this study, a one-year water quality monitoring programme was carried out from September 2015 to August 2016 on a portion of JW's drinking water distribution system. This report aimed to meet the objective set out in the beginning of this study. The objective of this study was to monitor the parameters that are known to significantly affect biostability and biofilm formation potential in drinking water distribution systems. This objective was achieved as shown by the results presented in chapter 4 of this research report. The data obtained from the monitoring programme show that the portion of JW's drinking water distribution system that was monitored adhered to the standards set out by SANS241 (2011) for the most part. However, the field data also highlighted several problems in JW's drinking water system. These problems include the inability to maintain sufficient disinfectant residual and keep nutrients and organic matter low enough to maintain stability and the potential for biofilm growth in sites RW80, RW81, RW82, RW83, RW104 and RW253. The results also show that the location of the sampling points and the time of sample collection can also affect water quality. Based on these results, to answer the research question, the major triggers of the loss of biostability in the water are elevated temperatures, low residual chlorine concentrations, and high concentrations of oxidizable substrates. If these results are left unattended, the water quality would continue to deteriorate. At this stage, only aesthetic problems such as, taste and odour are likely. However, the potential for biofilm formation will increase, thus increasing the chances of severe illnesses, such as gastroenteritis, if the water is consumed.

The information gathered from this study can be used to improve management strategies that will limit bacterial growth in water distribution systems. To address the issues that have been raised in this study, some recommendations have been made.

5.2 Recommendations for future research

- The water quality data generated from this research should be used in combination with JW's hydraulic data, to calibrate and test the CDWQ-E_2 model for a full scale distribution system. If this model can be tested successfully, it will be possible to determine the best way to ensure biostability and minimise biofilm growth potential.

- A monitoring programme aimed at directly looking at the effect of the loss of biostability on the rate of biofilm formation should be undertaken and data from this programme will have to be incorporated in the CDWQ-E_2 model. If biofilm samples cannot be obtained from full-scale distribution systems, they will have to be grown in the laboratory either on a model distribution system or on annular reactors.

- JW should incorporate DOC, BDOC, and AOC testing in its routine water quality monitoring programme in order to continuously have access to data that can be used to monitor the biostability of distributed water.

- JW should pay attention to all the sites with poor water quality during warmer months to monitor the water quality, as high water temperatures have been linked to heightened rates of residual chlorine depletion and heightened bacterial growth rates.

- JW should at all times strive to maintain residual chlorine at concentrations no less than 0.2mg/l as free chlorine residual and limit BDOC to no more than 0.3mg/l to ensure biostability of the distributed water. BDOC

concentrations can be decreased by, flushing the pipes, regular cleaning of reservoirs, further treating the feed water before distributing. Chlorine residuals can be increased by increasing the dosing interval and concentration.

- As much as is possible JW should also attempt to keep the water's residence time low, as high residence times have been shown to worsen water quality in distribution systems. The distribution system architecture could be modified to reduce the distance from the distribution system to consumer taps. The disinfectant residuals could also be increased.

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Appendix A – Raw data

Averaged monthly raw data																		
Site	**Time**	**Temperature, °C**	**Conductivity, mS/m**	**ph**	**Alkalinity, mg/l CaCO3**	**DO, mg/l**	**TN, mg/l**	**Nitrate, mg/l**	**Total Amonia, mg/l**	**Ammonium, mg/l**	**free Chlorine, mg/l**	**total chlorine, mg/l**	**Chloramines, mg/l**	**Avg. HPC, MPN/ml**	**Avg. Total coliform,**	**Avg. Faecal coliform,**	**DOC, mg/l**	**BDOC, mg/l**
RW 251	**Sep-15**	16	17.75	7.43	63	7.5	<0.5	<0.5	<0.02	<0.5	0.26	1.8	1.54	0.35	<1	<1		
	Oct-15	21	15.5	7.07		6.23					0.11	1.12	1.01	1.6	<1	<1	3.52	0.02
	Nov-15	20.5	21.5	7.45	52	6.305	0.79	<0.5	0.06	<0.5	0.27	1.655	1.385	14.35	<1	<1	4.27	0.00
	Dec-15	21.5	24.5	7.84		5.565					0.19	1.27	1.08	6.95	<1	<1	5.47	1.20
	Jan-16	22.5	17.5	7.71	49.8	6.875	<0.5	<0.5	<0.02	<0.5	0.1	0.62	0.52	39.7	<1	<1		
	Feb-16		18.5	7.8							0	0.43	0.425		<1	<1		
	Mar-16	22	22.5	7.685	43	6.74	<0.5	<0.5	<0.02	<0.5	0.11	0.54	0.43	73.8	<1	<1	2.10	
	Apr-16	20.25	26.25	8.148	43	5.4	<0.5	0.5	<0.02	<0.5	0.1	0.755	0.655	70.925	<1	<1	4.09	0.00
	May-16	16.5	23.5	7.81	51	7.86	<0.5	<0.5	<0.02	<0.5	0.21	1.17	0.97	37	<1	<1	3.90	0.69
	Jun-16	14.0	22	8.14	51	6.43	<0.5	<0.5	<0.02	<0.5	0.21	1.62	1.41	0.35	<1	<1	3.76	0.00
	Jul-16	11.5	20	8.477	58	4.72	<0.5	<0.5	<0.02	<0.5	0.3	1.88	1.58	5.05	<1	<1	4.44	0.38
	Aug-16	13	18	8.165	67	4.525	1.7	<0.5	<0.02	<0.5	0.22	1.56	1.34	0.2	<1	<1	3.00	0.8246

Site	Time	Temperature, °C	Conductivity, mS/m	ph	Alkalinity, mg/l CaCO3	DO, mg/l	TN, mg/l	Nitrate, mg/l	Total Amonia, mg/l	Ammonium, mg/l	free Chlorine, mg/l	total chlorine, mg/l	Chloramines, mg/l	Avg. HPC, MPN/ml	Avg. Total coliform,	Avg. Faecal coliform,	DOC, mg/l	BDOC, mg/l
RW 253	**Sep-15**	16.5	17.75	8.05	61.8	6.62	<0.5	<0.5	0.02	<0.5	0.16	1.64	1.48	0.2	<1	<1		
	Oct-15	22	16.5	7.51		6.17					0.1	1.2	1.1	0.7	<1	<1	3.37	0.14
	Nov-15	21	20.5	7.62	47.6	6.455	<0.5	<0.5	<0.02	<0.5	0.195	1.185	0.99	12.225	<1	<1	4.23	0.00
	Dec-15	22	24	7.86		6.215					0.11	1.01	0.9	10.3	<1	<1	4.71	0.43
	Jan-16	22.5	17	8.04	50	7.425	<0.5	<0.5	<0.02	<0.5	0.04	0.1	0.06	29.7	<1	<1		
	Feb-16		18.5	7.92							0	0.11	0.11		<1	<1		
	Mar-16	22.5	21.5	7.96	50.4	6.71	<0.5	0.7	<0.02	<0.5	0.02	0.07	0.05	73.8	<1	<1	1.87	0
	Apr-16	20	22.5	8.158	48	5.235	<0.5	0.5	<0.02	<0.5	0.02	0.075	0.055	66.35	1	<1	3.59	0.00
	May-16	16.5	25.75	7.65	50	7.93	0.6	0.6	<0.02	<0.5	0.03	0.14	0.11	20.4	<1	<1	3.56	0.22
	Jun-16	14.0	22.5	8.15	68	6.40	<0.5	<0.5	<0.02	<0.5	0.11	1.01	0.90	0.2	<1	<1	3.71	0.00
	Jul-16	11	20	8.4965	62.4	4.565	0.8	<0.5	<0.02	<0.5	0.23	1.68	1.45	0.4	<1	<1	4.16	1.79
	Aug-16	13	20	8.155	63.2	3.34	0.6	<0.5	0.05	<0.5	0.18	1.44	1.26	0.2	<1	<1	3.05	0.58
RW 80	**Sep-15**	16	21.25	7.44	60.6	6.9	<0.5	<0.5	<0.02	<0.5	0.325	1.79	1.465	0.4375	<1	<1		
	Oct-15	18.5	24	7.5		5.96					0.27	1.74	1.47	8.65	<1	<1	3.55	0.00
	Nov-15	21	21	7.58	55.6	8.52	<0.5	<0.5	0.097	<0.5	0.345	1.815	1.47	16.3	<1	<1	5.51	0.00
	Dec-15	22	22	7.84		5.52					0.15	1.9	1.75	47.7	<1	<1	5.21	2.01
	Jan-16	21	24.5	8.06	53.2	7.465	0.533	<0.5	0.077	<0.5	0.24	1.7	1.46	73.8	<1	<1		
	Feb-16	24.5	21	7.92		7.465					0.95	1.99	1.04	56.5	<1	<1	6.22	0

Site	Time	Temperature, °C	Conductivity, mS/m	ph	Alkalinity, mg/l CaCO3	DO, mg/l	TN, mg/l	Nitrate, mg/l	Total Amonia, mg/l	Ammonium, mg/l	free Chlorine, mg/l	total chlorine, mg/l	Chloramines, mg/l	Avg. HPC, MPN/ml	Avg. Total coliform,	Avg. Faecal coliform,	DOC, mg/l	BDOC, mg/l
	Mar-16	23	24	7.97	50	4.295	<0.5	<0.5	0.09	0.6	0.04	1.39	1.35	73.8	<1	<1	2.23	
	Apr-16	18.5	21	7.763	42	6.693	0.5	<0.5	0.1	0.6	0.285	1.74	1.455	62.25	<1	<1	3.93	1.24
	May-16	15.5	22.75	8.00	56.6	7.66	0.6	<0.5	<0.02	0.5	0.25	1.66	1.42	19	<1	<1	3.97	0.27
	Jun-16	12.5	22	8.16	58.9	6.78	0.5	<0.5	<0.02	<0.5	0.30	1.68	1.38	7.8	<1	<1	4.00	0.01
	Jul-16	11	19	8.3405	39	5.07	5.1	<0.5	0.06	<0.5	0.16	1.83	1.67	0.2	<1	<1	4.65	0.34
	Aug-16	11	22	8.155	75	6.025	1.8	<0.5	<0.02	<0.5	0.39	1.66	1.27	2.1	<1	<1	3.36	0.795
RW 81	**Sep-15**	16	23.25	7.41	59.4	7.15	<0.5	<0.5	<0.02	<0.5	0.245	1.485	1.24	0.2	<1	<1		
	Oct-15	20	24	7.7		7.04					0.17	1.35	1.18	13.6	<1	<1	3.59	1.15
	Nov-15	20.5	19.5	7.8	53.2	6.475	<0.5	<0.5	<0.02	<0.5	0.245	1.46	1.215	16.25	<1	<1	5.38	0.05
	Dec-15	22	20	7.98		4.89					0.15	0.44	0.29	68.05	<1	<1	4.91	1.85
	Jan-16	22	19	7.68	44.6	6.425	0.51	0.73	<0.02	<0.5	0.05	0.15	0.1	32.65	<1	<1		
	Feb-16	24	20	8		5.49					0.31	0.48	0.17	73.8	<1	<1	5.62	0.08
	Mar-16	23	21.5	8.02	56	3.8	<0.5	0.8	<0.02	<0.05	0.01	0.08	0.07	73.8	1	<1	1.98	
	Apr-16	19	20.75	7.813	62	5.863	<0.5	0.8	<0.02	<0.05	0.065	0.2	0.135	48.975	2	<1	3.66	1.17
	May-16	15.5	22	8.05	64.4	7.10	0.8	<0.5	<0.02	<0.5	0.12	0.53	0.41	24.625	<1	<1	3.73	0.39
	Jun-16	12.5	21.5	8.18	63.6	6.28	<0.5	<0.5	<0.02	<0.5	0.17	1.59	1.42	0.675	<1	<1	3.92	1.15
	Jul-16	11	20	8.2505	56	4.69	<0.5	<0.5	<0.02	<0.5	0.16	1.71	1.55	0.2	<1	<1	4.63	0.55

Site	Time	Temperature, °C	Conductivity, mS/m	ph	Alkalinity, mg/l CaCO3	DO, mg/l	TN, mg/l	Nitrate, mg/l	Total Amonia, mg/l	Ammonium, mg/l	free Chlorine, mg/l	total chlorine, mg/l	Chloramines, mg/l	Avg. HPC, MPN/ml	Avg. Total coliform,	Avg. Faecal coliform,	DOC, mg/l	BDOC, mg/l
	Aug-16	11	22	8.16	85	5.45	1	<0.5	<0.02	<0.5	0.31	1.65	1.34	0.2	<1	<1	3.15	1.6625
RW 82	**Sep-15**	18.5	23	7.37	61.2	5.6	<0.5	<0.5	<0.02	<0.5	0.24	1.595	1.355	1.85	<1	<1		
	Oct-15	22	24	7.8		5.54					0.14	1.35	1.21	0.85	<1	<1	3.45	0.00
	Nov-15	22	20.75	7.79	54.4	5.595	<0.5	<0.5	<0.02	<0.5	0.19	1.42	1.23	33.25	<1	<1	5.33	0.00
	Dec-15	25	19	7.82		4.8					0.13	0.54	0.41	68.05	<1	<1	5.13	
	Jan-16	23	17.5	7.8	44.2	6.045	1.03	0.83	<0.02	<0.5	0.08	0.87	0.79	46.7	<1	<1		
	Feb-16	24.5	22.5	8.4		5.78					0.22	0.37	0.15	68.05	<1	<1	5.67	
	Mar-16	21	21.5	8.065	47	6.36	<0.5	0.8	<0.02	<0.5	0	0.02	0.02	2.65	<1	<1	1.93	
	Apr-16	18.75	21.25	7.778	52	5.813	0.5	0.5	<0.02	<0.5	0.055	0.42	0.365	46.975	<1	<1	3.67	0.79
	May-16	16.0	21.5	7.95	57.6	6.05	<0.5	<0.5	<0.02	<0.5	0.05	0.08	0.04	14.55	<1	<1	3.66	0.32
	Jun-16	13.5	21.5	8.15	62	5.79	<0.5	<0.5	<0.02	<0.5	0.09	0.99	0.90	0.25	<1	<1	3.85	1.10
	Jul-16	14	21	8.2275	56	4.545	1.8	<0.5	<0.02	<0.5	0.14	1.71	1.57	0.2	<1	<1	4.45	0
	Aug-16	11	22	7.94	61.4	4.95	0.7	<0.5	<0.02	<0.5	0.09	0.63	0.54	0.8	<1	<1	2.96	1.3686
RW 83	**Sep-15**	16.5	23.5	7.73	59.8	5.7	<0.5	<0.5	<0.02	<0.5	0.17	1.595	1.425	0.2	<1	<1		
	Oct-15	20	23.5	7.9		6.77					0.17	1.35	1.18	18.7	<1	<1	3.54	0.76
	Nov-15	21.5	22.5	7.88	49.4	7.72	<0.5	<0.5	0.063	<0.5	0.23	1.42	1.19	23.35	<1	<1	5.35	0.11

Site	Time	Temperature, °C	Conductivity, mS/m	ph	Alkalinity, mg/l CaCO3	DO, mg/l	TN, mg/l	Nitrate, mg/l	Total Amonia, mg/l	Ammonium, mg/l	free Chlorine, mg/l	total chlorine, mg/l	Chloramines, mg/l	Avg. HPC, MPN/ml	Avg. Total coliform,	Avg. Faecal coliform,	DOC, mg/l	BDOC, mg/l
	Dec-15	22	19	7.85		7.065					0.12	0.54	0.42	46.35	<1	<1	4.93	1.02
	Jan-16	23	17.5	7.9	43.6	6.45	2.32	0.83	<0.02	<0.5	0.04	0.87	0.83	38.45	<1	<1		
	Feb-16	24.5	22	8.05		6.325					0.13	0.37	0.24	68.05	<1	<1	5.58	0
	Mar-16	23	21.5	8.075	43.8	4.23	0.6	0.8	<0.02	<0.5	0.01	0.13	0.12	42.1	<1	<1	1.99	
	Apr-16	20.5	21.5	7.745	52.8	6.03	0.6	0.7	<0.02	<0.5	0.02	0.23	0.21	73.8	<1	<1	3.54	0.88
	May-16	16.5	22	8.02	66.6	6.19	<0.5	<0.5	<0.02	<0.5	0.08	0.50	0.42	24.275	<1	<1	3.81	0.76
	Jun-16	13.5	22	8.20	61.6	5.80	<0.5	<0.5	<0.02	<0.5	0.19	1.60	1.42	0.55	<1	<1	3.86	0.73
	Jul-16	12	20	8.3105	60.2	4.915	<0.5	<0.5	0.08	<0.5	0.22	1.67	1.45	1.5	<1	<1	4.37	0.52
	Aug-16	11	21	7.925	60.2	4.77	0.5	<0.5	<0.02	<0.5	0.25	1.82	1.57	0.2	<1	<1	2.89	1.9513
RW 84	**Sep-15**	16.5	23.75	7.49	58.2	5.75	<0.5	<0.5	<0.02	<0.5	0.245	1.6	1.355	0.2	<1	<1		
	Oct-15	20	24	7.98		7.03					0.39	1.4	1.01	43.95	<1	<1	3.53	0.45
	Nov-15	21	22.25	7.94	59.4	7.73	<0.5	<0.5	0.1	<0.5	0.29	1.46	1.17	32.175	<1	<1	5.42	0.00
	Dec-15	22	18	7.84		6.28					0.13	0.38	0.25	73.8	<1	<1	4.97	1.51
	Jan-16	23	17.5	7.96	45.8	6.125	1.11	0.83	<0.02	<0.5	0.06	0.15	0.09	38.95	<1	<1		
	Feb-16	24.5	21.5	8.11		5.585					0.25	0.43	0.18	73.8	<1	<1	5.82	0
	Mar-16	23	21.5	8.065	54.4	4.04	0.7	0.6	<0.02	<0.5	0.01	0.14	0.13	73.8	<1	<1	2.20	
	Apr-16	21	20	7.76	54.2	5.995	0.5	0.7	<0.02	<0.5	0.03	0.26	0.23	32.4	1	<1	3.68	1.32
	May-16	16.0	21.25	7.98	57.6	6.67	<0.5	<0.5	<0.02	<0.5	0.10	0.54	0.44	15.1	<1	<1	3.88	0.00
	Jun-16	13.5	22.5	8.20	63	5.88	0.6	<0.5	<0.02	<0.5	0.27	1.66	1.39	1.875	<1	<1	4.00	0.06
	Jul-16	12	18	8.301	60.2	4.435	<0.5	<0.5	0.06	<0.5	0.26	1.79	1.53	0.2	<1	<1	4.69	0.69

Site	Time	Temperature, °C	Conductivity, mS/m	ph	Alkalinity, mg/l CaCO3	DO, mg/l	TN, mg/l	Nitrate, mg/l	Total Amonia, mg/l	Ammonium, mg/l	free Chlorine, mg/l	total chlorine, mg/l	Chloramines, mg/l	Avg. HPC, MPN/ml	Avg. Total coliform,	Avg. Faecal coliform,	DOC, mg/l	BDOC, mg/l
	Aug-16	11	21	7.95	64	5.455	1.9	<0.5	<0.02	<0.5	0.32	1.83	1.51	1	<1	<1	2.87	1.7977
RW 104	**Sep-15**	17.5	21.5	7.55	57.2	3.9	<0.5	<0.5	<0.02	<0.5	0.34	2.07	1.73	0.3	<1	<1		
	Oct-15	19	24	8.06		7.18					0.44	1.86	1.42	9.55	<1	<1	3.50	0.57
	Nov-15	21	22.5	8.23	51.8	7.495	<0.5	<0.5	<0.02	<0.5	0.335	2.025	1.69	29.2	<1	<1	5.48	2.75
	Dec-15	25	19	7.94		6.83					0.31	2.02	1.71	51.85	<1	<1	4.92	1.95
	Jan-16	22	17.5	8.56	54.4	8.275	0.7	0.53	0.06	0.6	0.32	2.03	1.71	40.6	<1	<1		
	Feb-16	24	21.5	8.37		6.5					0.68	1.79	1.11	62.25	<1	<1	5.83	0
	Mar-16	23	21	8.085	44	5.4	<0.5	0.8	0.1	<0.5	0.07	0.99	0.92	73.8	<1	<1	2.06	
	Apr-16	19	20.25	8.19	43.2	7.445	0.5	<0.5	0.17	<0.5	0.4	1.845	1.445	48.1	<1	<1	3.84	1.06
	May-16	15.5	21.25	8.19	58	8.19	<0.5	<0.5	<0.02	<0.5	0.40	2.11	1.71	18.825	<1	<1	3.93	0.00
	Jun-16	13.0	21	8.19	59.6	7.52	<0.5	<0.5	<0.02	<0.5	0.42	2.06	1.64	0.3	<1	<1	3.92	1.11
	Jul-16	11	20	8.4025	62	3.89	<0.5	<0.5	0.02	<0.5	0.42	2.03	1.61	0.2	<1	<1	4.54	0.93
	Aug-16	11	22	8.36	70	7.05	0.7	<0.5	<0.02	<0.5	0.34	1.8	1.46	0.6	<1	<1	2.99	3.0198
RW 105	**Sep-15**	17.5	23.5	7.84	58	4.7	<0.5	<0.5	<0.02	<0.5	0.265	1.95	1.685	0.2	<1	<1		
	Oct-15	18.5	23	8.12		6.75					0.28	1.84	1.56	15.15	<1	<1	3.46	0.00
	Nov-15	20.5	22	8.01	50.4	6.27	<0.5	<0.5	0.12	<0.5	0.265	1.845	1.58	17.875	<1	<1	5.45	2.96
	Dec-15	23	19.5	8.05		6.83					0.17	1.87	1.7	45.5	<1	<1	4.57	0.91
	Jan-16	22	17.5	8.68	56.6	7.675	0.55	<0.5	0.173	0.5	0.28	1.81	1.53	51.85	<1	<1		
	Feb-16	24.5	21	8.68		6.27					0.78	1.85	1.07	64.4	<1	<1	5.97	0

Site	Time	Temperature, °C	Conductivity, mS/m	ph	Alkalinity, mg/l CaCO3	DO, mg/l	TN, mg/l	Nitrate, mg/l	Total Amonia, mg/l	Ammonium, mg/l	free Chlorine, mg/l	total chlorine, mg/l	Chloramines, mg/l	Avg. HPC, MPN/ml	Avg. Total coliform,	Avg. Faecal coliform,	DOC, mg/l	BDOC, mg/l
	Mar-16	23	21.5	8.2	52.2	7.35	<0.5	0.5	0.1	0.5	0.04	0.28	0.24	44.2	<1	<1	2.00	
	Apr-16	19	21.25	8.31	50.2	7.36	0.6	<0.8	0.11	<0.5	0.305	1.755	1.45	32.675	<1	<1	3.89	0.94
	May-16	15.5	21.5	8.32	58.4	7.97	<0.5	<0.5	<0.02	<0.5	0.34	1.92	1.58	17.65	<1	<1	3.88	0.00
	Jun-16	12.5	22.5	8.24	59.6	7.17	0.9	<0.5	<0.02	<0.5	0.33	1.92	1.59	1.1	<1	<1	3.93	0.38
	Jul-16	12	18	8.348	59.8	3.23	<0.5	0.8	<0.02	<0.5	0.13	1.84	1.71	0.2	<1	<1	4.61	0.15
	Aug-16	11	22	8.35	55	6.21	<0.5	<0.5	<0.02	<0.5	0.3	1.74	1.44	0.2	<1	<1	2.95	0.2401
RW 106	**Sep-15**	19.5	23.25	7.77	58.2	4.35	<0.5	<0.5	<0.02	<0.5	0.19	1.815	1.625	0.2	<1	<1		
	Oct-15	24	23.5	8.05		5.18					0.14	1.63	1.49	0.2	<1	<1	3.44	0.00
	Nov-15	24.5	21.75	7.99	53.2	6.35	<0.5	<0.5	<0.02	<0.5	0.195	1.51	1.315	37.25	<1	<1	5.38	2.78
	Dec-15	24	19.5	8.05		5.04					0.19	1.57	1.38	43.1	<1	<1	4.92	
	Jan-16	25	17.5	8.42	51	5.885	0.63	0.6	<0.02	<0.5	0.1	1.03	0.93	53.15	<1	<1		
	Feb-16	26	21.5	8.56		5.715					0.36	1.48	1.12	73.8	<1	<1	5.76	
	Mar-16	23	20.5	8.235	53.2	5.46	1	0.7	0.02	<0.5	0.02	1.42	1.4	41.25	<1	<1	2.13	
	Apr-16	20.5	21.25	8.123	47.4	5.643	0.5	<0.5	0.08	<0.5	0.205	1.4	1.195	44.075	<1	<1	3.82	0.00
	May-16	17.8	22.75	8.28	59.4	6.17	<0.5	<0.5	<0.02	<0.5	0.20	1.53	1.33	16.575	<1	<1	3.89	0.08
	Jun-16	15.0	21.5	8.31	62	5.83	<0.5	<0.5	<0.02	<0.5	0.28	1.85	1.58	0.2	<1	<1	3.89	1.30
	Jul-16	14	19	8.3525	62	4.935	1.9	<0.5	<0.02	<0.5	0.2	1.81	1.61	0.2	<1	<1	4.68	0
	Aug-16	15	20	8.165	69	4.105	0.5	<0.5	<0.02	<0.5	0.28	1.83	1.55	0.2	<1	<1	2.79	1.2387

Site	Time	Temperature, °C	Conductivity, mS/m	ph	Alkalinity, mg/l CaCO3	DO, mg/l	TN, mg/l	Nitrate, mg/l	Total Amonia, mg/l	Ammonium, mg/l	free Chlorine, mg/l	total chlorine, mg/l	Chloramines, mg/l	Avg. HPC, MPN/ml	Avg. Total coliform,	Avg. Faecal coliform,	DOC, mg/l	BDOC, mg/l
RW 107	**Sep-15**	18	23.5	7.84	60.8	4.15	<0.5	<0.5	<0.02	<0.5	0.29	1.865	1.575	1.15	<1	<1		
	Oct-15	21.5	23.5	8.08		5.32					0.28	1.81	1.53	12.05	<1	<1	3.49	0.89
	Nov-15	22.5	22.75	7.98	56.2	7.115	<0.5	<0.5	0.14	<0.5	0.315	1.81	1.495	28.675	<1	<1	5.37	3.05
	Dec-15	24	20.5	7.72		5.88					0.28	1.78	1.5	53.1	<1	<1	4.99	
	Jan-16	23	17	8.56	48	6.46	<0.5	<0.5	<0.02	<0.5	0.25	1.68	1.43	35.5	<1	<1		
	Feb-16	25	22	8.68		6.41					0.7	1.92	1.22	73.8	<1	<1	6.49	
	Mar-16	23	21.5	8.255	58	4.745	<0.5	0.5	0.03	0.5	0.06	1.42	1.36	73.8	<1	<1	2.04	
	Apr-16	19	21	8.395	52.4	6.565	<0.5	<0.5	0.1	<0.5	0.37	1.85	1.48	41.575	<1	<1	3.96	0.07
	May-16	16.5	21.5	8.34	57.6	6.34	<0.5	<0.5	<0.02	<0.5	0.36	1.85	1.49	5.575	<1	<1	3.67	0.28
	Jun-16	14.0	22.5	8.31	60.2	5.69	<0.5	<0.5	<0.02	<0.5	0.35	1.50	1.16	1.775	<1	<1	3.96	1.24
	Jul-16	13	20	8.3825	58	5.265	<0.5	<0.5	<0.02	<0.5	0.22	1.83	1.61	0.2	<1	<1	4.43	0
	Aug-16	12	20	8.355	75	5	0.5	<0.5	<0.02	<0.5	0.41	1.98	1.57	0.2	<1	<1	2.86	1.6705
RW 108	**Sep-15**	18.5	23.25	7.67	60.4	4.65	<0.5	<0.5	<0.02	<0.5	0.215	1.78	1.565	0.525	<1	<1		
	Oct-15	20	23.5	8.15		4.94					0.21	1.73	1.52	0.2	<1	<1	3.57	0.00
	Nov-15	21.5	23	8.01	58.4	7.155	<0.5	<0.5	0.093	<0.5	0.24	1.615	1.375	38.725	<1	<1	5.41	2.94
	Dec-15	22.5	20.5	7.9		6.086					0.19	1.46	1.27	73.8	<1	<1	5.08	
	Jan-16	24	17.5	8.34	56	5.7	0.85	0.77	0.02	<0.5	0.08	0.31	0.23	36.55	<1	<1		
	Feb-16	26	22	8.53		5.14					0.39	0.47	0.08	73.8	<1	<1	6.09	
	Mar-16	23	22	8.345	50	4.735	0.8	0.6	0.02	<0.5	0.06	0.18	0.12	73.8	<1	<1	2.18	
	Apr-16	20	21.25	8.31	63.4	6.438	<0.5	<0.5	0.1	<0.5	0.23	1.57	1.34	40.45	<1	<1	4.03	1.01
	May-16	17.0	21.75	8.33	57.4	6.19	<0.5	<0.5	<0.02	<0.5	0.21	1.47	1.26	17.55	<1	<1	3.69	0.73

Site	Time	Temperature, °C	Conductivity, mS/m	ph	Alkalinity, mg/l CaCO3	DO, mg/l	TN, mg/l	Nitrate, mg/l	Total Amonia, mg/l	Ammonium, mg/l	free Chlorine, mg/l	total chlorine, mg/l	Chloramines, mg/l	Avg. HPC, MPN/ml	Avg. Total coliform,	Avg. Faecal coliform,	DOC, mg/l	BDOC, mg/l
	Jun-16	14.5	23	8.21	59.4	5.50	0.8	<0.5	<0.02	<0.5	0.24	1.79	1.55	0.35	<1	<1	3.96	1.32
	Jul-16	14	20	8.414	42.2	5.8	<0.5	<0.5	<0.02	<0.5	0.13	1.76	1.63	0.2	<1	<1	4.52	0
	Aug-16	13	22	8.15	62.2	4.68	1	<0.5	<0.02	<0.5	0.31	1.93	1.62	0.4	<1	<1	2.91	0.7253
RW 109	**Sep-15**	19	23.25	7.89	59.2	5.65	<0.5	<0.5	<0.02	<0.5	0.2	1.655	1.455	0.4	<1	<1		
	Oct-15	23	23.5	8.11		5.79					0.19	1.61	1.42	0.7	<1	<1	3.42	0.66
	Nov-15	23.5	23	8.01	48.2	5.12	<0.5	<0.5	<0.02	<0.5	0.25	1.295	1.045	38.475	<1	<1	5.34	0.00
	Dec-15	24	20.5	7.88		4.84					0.19	1.14	0.95	73.8	<1	<1	5.00	
	Jan-16	24	18	8.3	53.6	5.88	0.87	0.93	<0.02	<0.5	0.09	0.5	0.41	53.15	<1	<1		
	Feb-16	26	22	8.39		6.655					0.36	0.47	0.11	46.35	<1	<1	5.67	
	Mar-16	23	21.5	8.28	49	5.925	<0.5	<0.5	<0.02	<0.5	0.02	0.4	0.38	62.25	<1	<1	2.13	
	Apr-16	20	21	8.42	49	5.868	<0.5	<0.5	<0.02	<0.5	0.165	0.855	0.69	43.375	<1	<1	3.77	0.71
	May-16	17.5	21.50	8.28	60.8	6.26	<0.5	<0.5	<0.02	<0.5	0.16	0.92	0.76	12.75	<1	<1	3.73	0.72
	Jun-16	14.5	23.00	8.25	65.8	5.42	<0.5	<0.5	<0.02	<0.5	0.18	1.60	1.43	0.2	<1	<1	3.88	1.11
	Jul-16	14	19	8.3895	63	4.295	<0.5	<0.5	<0.02	<0.5	0.14	1.71	1.57	0.2	<1	<1	4.74	0
	Aug-16	13	21	7.965	61	4.79	2.10	<0.5	<0.02	<0.5	0.15	1.6	1.45	2.6	<1	<1	3.02	1.1519

Appendix B – Map of City of Johannesburg existing water districts and bulk supply system