Insights into the microbiology of selected drinking water treatment plants and sludge

by

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DECLARATION

I declare that the above thesis is my own work and that all the sources that I have used or quoted have been indicated and acknowledged by means of complete references. I further declare that I have not previously submitted this work, or part of it, for examination at the University of South Africa for another qualification or at any other higher education institution.

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DEDICATION

I dedicate this thesis to my beloved parents and siblings, whose steadfast love, support, and encouragement have been the foundation of my academic journey. Their efforts, perseverance, and faith in me have served as my paramount motivation. I am eternally thankful to my supervisors and mentors, whose guidance and wisdom have influenced this research, as well as to individuals who have inspired and supported me during this journey. This work is dedicated to all individuals pursuing knowledge and achievement, serving as a tribute to endurance and enthusiasm.

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TABLE OF CONTENTS

ABSTRACT

Drinking water treatment plants (DWTPs) play a crucial role in treating raw water sources to potable quality. Contaminants in drinking water can pose serious health concerns. The study evaluated the microbial diversity of raw water, the treatment stages, treated water, and the sludge of three DWTPs in the Northwest and Gauteng provinces over a 12-month monitoring period. Microbial and physicochemical parameters of the were analysed. Enumeration of *E. coli* and *Enterococcus* spp. was conducted using Colilert-18 and Enterolert Quanti-Tray/2000 IDEXX. Heterotrophic plate counts (HBC) were performed to quantify heterotrophic bacteria. Antibiotic resistance of the bacteriological indicators was assessed using the disc diffusion method. Microbial diversity and functional metagenomic profiles were analysed using the shotgun metagenomics sequencing approach. Correlation analysis was conducted to determine relationships between bacterial communities, treatment stages, raw water and the physicochemical parameters. All DWTPs achieved approximately 90% reduction in bacteriological indicator across the seasons. Most of the isolated bacteriological indicators showed resistance to beta-lactams class of antibiotics. Based on metagenomic analysis, Euryarchaeota and Nitrososphaerota (Archaea phyla), Pseudomonadota (Bacterial phyla), Ascomycota and Basidiomycota (Eukaryota phyla), were abundant in the water and sludge samples. Correlation analysis showed that pH, temperature, nitrates, sulphate, EC, TDS, and Fe were associated with the proliferation of the bacteria. Metabolism functional genes were abundant across the sampling points.

Keywords: Metagenomes, microbial diversity, treatment plants, shotgun sequencing, antimicrobial resistance, functional metagenomics

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AOA- Ammonia Oxidizing Archaea

ARB- Antibiotic Resistant Bacteria

ARG- Antibiotic Resistance Gene

BWSS-Building Water Supply System

CRB- Chlorine Resistant Bacteria

DAF- Dissolved Air Flotation

DBPs- Disinfection By Products

DWDS- Drinking Water Distribution System

DWTP- Drinking Water Treatment Plant

DWTS- Drinking Water Treatment Sludge

EC- Electrical Conductivity

EPS- Extracellular Polymeric Substrate

FIB- Faecal Indicator Bacteria

FTU- Formazin Turbidity Unit

GAC- Granular Activated Carbon

HBC- Heterotrophic Bacterial Count

MDR- Multi Drug Resistance

MPN- Most Probable Number

NOM- Natural Organic Matter

NTM- Non-Tuberculous Mycobacteria

NTU- Nephelometric Turbidity Unit

OTU- Operational Taxonomic Unit

SANS- South African National Standards

TDS- Total Dissolved Solids

TTC- Temperature Tolerant Coliform

WASH-Water, Sanitation and Hygiene

WHO- World Health Organization

Publications related to the study

The study relates to work done under the Water Research Commission (WRC) Project No. **2022/2023-00801**.

1. Mudau, K. L., **Ntobeng, L. R**., Kalu, C. M., Khumbudzo, M., Masindi, V., & Tekere, M. (2024). Water Treatment Stage Impacts on the Occurrence of Bacteriological Indicators and their Multiple Antibiotic Resistance Index. *Engineering, Technology & Applied Science Research*, *14*(5), 16911-16926.

2. Kalu, C. M., Mudau, K. L., **Ntobeng, L. R.,** Masindi, V., & Tekere, M. (2025). Antibiotic resistance genes profile of selected drinking water treatment plants in South Africa as impacted by different treatment stages. *Microbiology Resource Announcements*, *14*(2), e00934-24.

3. Ntobeng, L. R., Kalu, C. M., Masindi, V., & Tekere, M. (2026). Physicochemical Parameters and Heterotrophic Bacteria in Drinking Water Treatment Plant in Four Seasons Unveiled through Correlation Analysis. *Prepared Manuscript.*

CHAPTER 1: GENERAL INTRODUCTION

1.1 Study Background

South Africa has a severe water shortage and challenges in providing quality potable water to communities. Although a lot of progress has been made since democracy in providing communities with access to drinking water, it is still difficult to ensure that the water fulfils the standards set by South African National Standards (SANS 241:2015). It is estimated that approximately 83% of the population in SA has access to piped tap water in their homes, communal taps, water tankers, and merchants primarily sourced from surface water bodies such as rivers and dams (Statistics South Africa, 2019). Additionally, it is estimated that 2.2% of the population relies on untreated water from rivers, streams, and dams, with 3.6% of the population using boreholes as their primary source of drinking water (Statistics South Africa, 2019).

Raw water from rivers, lakes, and dams normally contains contaminants from human activities such as sewage effluent, landfills, mining, urban runoffs, and agriculture, hence treatment of this surface raw water is crucial. Conventional drinking water treatment processes such as pre-chlorination, coagulation-flocculation, sedimentation, filtration, and disinfection are used to remove these contaminants (Elfikrie et al., 2020; Fish et al., 2020). Together, these procedures ensure that surface raw water is purified by lessening the amounts of physical, chemical, and microbiological pollutants. Variations in the occurrence of antimicrobial resistant bacteria are associated with water treatment processes, particularly during disinfection stages where different types of oxidants (disinfectant) are applied by DWTPs globally (Gao et al., 2022; Tiwari et al., 2022). Furthermore, the antibacterial properties of these oxidants may facilitate the release of antibiotic resistance genes (ARGs) from lysed microbial cells, potentially contributing to the proliferation of antibiotic-resistant bacteria (ARB) variety.

Drinking water treatment sludge (DWTS) is also produced from the water treatment process and is discharged into the environment. Environmental issues can emerge from DWTS if not managed and treated appropriately before being released into aquatic or terrestrial environments (Ullmann et al., 2019; Kalu et al., 2024).

For more than a century, the microbiological quality of drinking water has been primarily measured by counting cultivable indicator bacteria, i.e. heterotrophic bacteria count (HBC), and faecal indicators. According to the World Health Organization (WHO), drinking water should be free of faecal indicators such as *E. coli* and intestinal *Enterococci*, although no strict upper limits are specified for heterotrophic bacterial counts (HBC) (WHO, 2011; Pinar-Mendez et al., 2022). Factors such as seasonal precipitation, land use in catchment areas (including agriculture and wastewater contributions), temperature variations, and hydrological disruptions are the reason why microbiological quality of raw water sources is becoming increasingly unpredictable (Shukla et al., 2023). These factors facilitate the increased influx and variability of microbial populations entering DWTPs. Conventional treatment techniques aim to systematically eliminate or inactivate microorganisms (Sumalatha et al., 2025). The effectiveness of these methods can be affected by factors such as the quality of the influent water, operational conditions, and the structural integrity of the system.

Culture-based approaches, although operationally effective, are constrained in their capacity to identify the entire microbial community, as a substantial proportion of environmental microorganisms are viable yet non-culturable (Brumfield et al., 2020; Han et al., 2023). Consequently, culture-independent techniques such as metagenomic sequencing are increasingly employed to provide a comprehensive characterisation of drinking water microbiomes and evaluate treatment efficacy (Moreno et al., 2024; Cholet et al., 2025). In this context, minimum alteration in microbiological water quality is characterised as biological stability, with the principal aim of delivering to consumers drinking water that maintains the quality established at DWTPs.

1.2 Problem Statement

DWTPs operations work to eliminate microorganisms from untreated water sources. However, the different treatment processes could select the microbes that grow in each process thus the need to understand drinking water treatment stages microbiology (Azamzam et al., 2021). Prior research has demonstrated the presence of residual microbial communities with potential to negatively impact the water quality that consumers receive (Fish et al., 2018; Potgieter et al., 2018; Favere et al., 2021). In reality, a 1ml of secure drinking water can contain anything from around one thousand to a few hundred thousand of microscopic organisms (Chan et al., 2018). This raises a concern as the final treated water contain a variety and abundance of organisms capable of deteriorating the quality of water with potential to negatively impacting the health of the end users (Potgieter et al., 2018; Douterelo et al., 2020). Different treatment stages remove microbial contaminants by various levels; however, some stages, such as disinfection stages while destroying microorganisms can promote the development of antibiotic resistance amongst resistant microorganism (Walczak et al., 2026). The occurrence of ARB in drinking water is thus a great concern. Seasonal changes, catchment activities, and physicochemical conditions, leading to swings in microbial load and diversity often affect the microbiological quality of raw water entering DWTPs (Prest et al., 2016; Azamzam et al., 2021; Ayu & Tony, 2022; Ma et al., 2022; Moreno et al., 2024, Cholet et al., 2025).

The production of sludge during the treatment processes is another environmental concern, as most sludge generated is released without any kind of treatment or is dumped in a landfill after being dewatered. The sludge likely, contains varieties of microorganisms in addition to chemicals that could potentially affect the microbial ecosystem of the environment where it is discharged (Hou et al., 2018). Therefore, it is essential to treat the sludge prior to discharge and to regularly evaluate the microbial diversity to ensure a decrease in the microbial community load that will be released into the environment during sludge discharge (Krishna et al., 2020; Zhao et al., 2020). To understand water quality changes in DWTPs, sludge and improve treatment efficiency and water quality to consumers, it is necessary to investigate the microbial diversity, potential of pathogenicity, antimicrobial resistance, regrowth, and the reasons for such occurrences. Previous

research has also demonstrated that bacteria contribute to the physical and chemical deterioration of water in DWTP and sludge (Chen et al., 2020; Pan et al., 2021). This study evaluated the microbiological aspects of specific drinking water treatment plants and their sludge in two provinces in South Africa. The findings in this study will provide insight into microbial communities in different raw water sources (Provinces), different treatment stages, influence of some physicochemical factors and treatment stages; and possibly guide future developments in sustainable drinking water treatment process.

1.3 Motivation of the Study

Occurrence of microorganisms, including ARBs, in water treatment plants and recurring microbial presence in the treated water, has been one of the topical issues in drinking water purification entities (Gerba & Pepper, 2019; Carolina et al., 2021). This is mainly attributed to water resources pollution and development of microbial communities that have established some resistance towards different disinfectants, i.e., chlorine, ozone, ultraviolet light, and chloramines (Carolina et al., 2021; Walczak et al., 2026; Xu et al., 2026). As such, microbial persistency within water treatment plants and resistance to different disinfectants, among other factors leads to recurring microbial contamination in the final treated water. The nature of these microorganisms needs to be understood. Furthermore, the development of biofilms in the DWTPs creates an environment that is conducive to the re-growth of microorganisms, including ARBs, by the provision of a shield to disinfectants and nourishing nutrients for microbial proliferation, as well as promoting the transfer of resistant genes (Li et al., 2026). As such, there is a need for continuous monitoring of the microbial communities in the unit processes of the treatment plants. This study therefore, aims to evaluate the microbial diversity and antibiotic resistance of bacteriological indicators, as well as functional metagenomes associated with the microbial communities in selected three DWTPs in two provinces in South Africa.

1.4 Aim and Objectives

1.4.1 Aim

This study aims to evaluate the microbiology of three drinking water treatment plants and sludge in the GP and NW provinces in South Africa, focusing on diversity, antibiotic resistance, and functional metagenomes.

1.4.2 Objectives

The specific study objectives are as follows:

- To analyse bacterial indicator counts and associated physicochemical properties across different treatment stages in DWTPs and DWTS.
- To determine the antimicrobial resistance of indicator bacteria, *E. coli* and *Enterococcus* spp. across the different treatment stages and DWTS.
- To assess the microbial composition and genes involved in the microbial functions influenced by treatment stages in three selected water treatment plants located in Gauteng and Northwest Province, along with the sludge
- To evaluate the correlation between physicochemical parameters and bacterial dynamics in DWTPs and DWTS.

1.5 Research Questions

- What is the prevailing bacterial indicator counts and physicochemical properties across the different stages of the DWTPs and DWTS?
- What is the level of occurrence of indicator bacteria and resistance to antibiotics across the different stages of the DWTPs and DWTS?
- How do the microbial composition and genes involved in the microbial functions change across the sampling points?
- What is the relationship between the physicochemical parameters and bacterial dynamics in the DWTPs and DWTS?

1.6 Dissertation Outline

The dissertation is made up of five chapters with details as follows:

Chapter 1: This chapter focuses on the introduction of the research study. The chapter includes a detailed background, motivation of the study, problem statement, as well as study aims and objectives.

Chapter 2: In this chapter, the literature review is presented. The literature review consists of a detailed review of literature on the understanding of DWTP, the microbial communities encountered in DWTPs, and DWTS, factors influencing the growth of microorganisms, as well as antimicrobial resistance.

Chapter 3: This chapter contains a detailed description of the study design, study area and methodology used to collect and analyse the samples and data for all the study's objectives.

Chapter 4: This chapter provides analysis and discussions of results obtained from the execution of the project in line with set objectives.

Chapter 5: Summary of the study, conclusions and recommendations are provided in this chapter.

References are provided at the end of the dissertation.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Production and supply of drinking water of acceptable quality is the primary objective of DWTP when providing drinking water to the populace. The rivers, dams, and groundwater sources in South Africa are experiencing growing strain because of the country's thriving economy and expanding population. Freshwater resources are faced with the challenge of deterioration due to increasing pollution caused by various activities, including industrialization, urbanization, afforestation, mining, agriculture, and power generation (Bain et al., 2008; Whelan et al., 2022). This significantly poses a lot of pressure on treatment plants, as they are the only facilities that ensure that the entire South Africa is supplied with water that is free from any pathogenic microorganisms and contaminants. Various research studies on water quality have been conducted, but more must be done constantly since water contamination and quality continue to worsen, in part as a result of advanced anthropogenic activities and other natural phenomena like climate change (Edokpayi et al., 2018; Mukherjee et al., 2024; Saxena,2025).

Although the quality of the treated water leaving the DWTPs may be at the most acceptable level, alteration of water quality can occur on distribution due to various variables beyond the control of the DWTP, such as pipe materials, temperature, stagnant portions, secondary reactions, and microorganism regrowth (Emerick et al., 2020; Fetyan & Salem Attia, 2020). Effort is therefore made to ensure good microbial stability including maintaining sustained levels of disinfectants in the final water until point of consumption (Alajlan et al., 2022; Nduli et al., 2025). Issues for further understanding in microbial studies for DWTPs and DWTS include the types of microorganisms present, their abundance, activities, the impact of environmental factors on the organisms, and impacts on the treatment process relating to how the treatment processes affect the microorganisms and vice versa. The qualities of raw water provide insight into the microorganisms and physicochemical properties that are introduced into treatment plants (Mararakanye et al., 2022; Wang et al., 2022; Wang and Wang, 2026).

2.2 Stages involved in the Water Treatment

DWTPs aim to eliminate undesirable impurities (physical, chemical, or biological in nature) (Akhtar et al., 2021). Treatment facilities get their water supply from surface water and groundwater systems. This water is then transported straight to the treatment plant, where it undergoes various treatment processes. The treatment procedures used in drinking water serve various functions and play diverse roles in reducing the levels of naturally occurring and human-activities related pollutants. The schematic illustration of the drinking water treatment process is summarized in Figure 2.1.

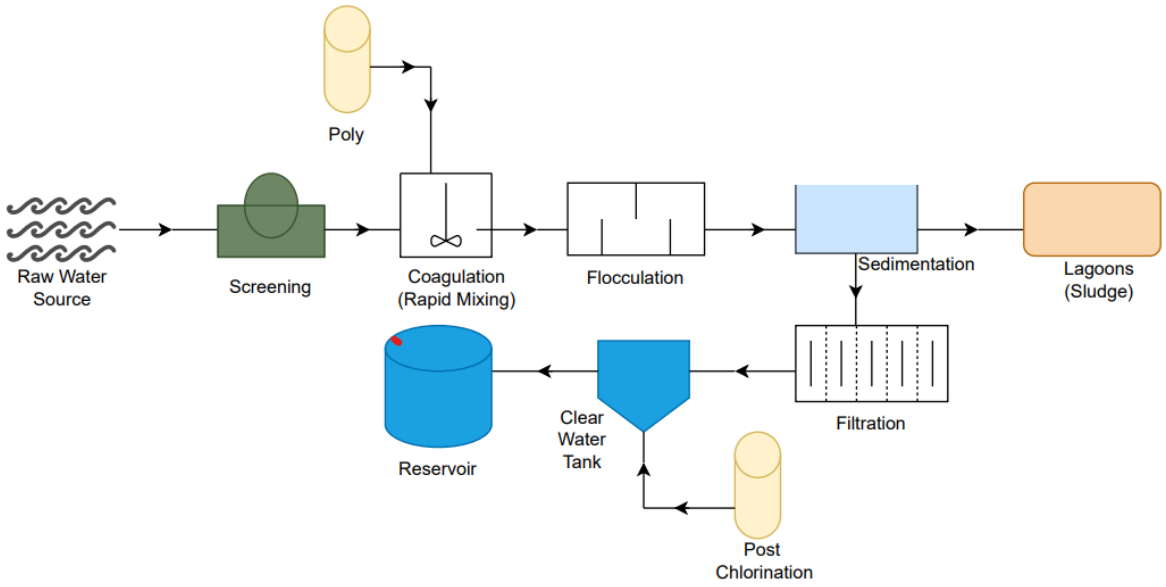


Figure 2.1: Schematic illustration of the typical drinking water treatment processes

The DWTP process stages are explained as follows:

Screening

Screening is conducted as the water enters the DWTP, where coarse or fine screens are used to capture plastics, plants, rags, diapers, huge debris, and other undesirable large and physical objects. These screens function as a sieve that can be operated either manually or mechanically. Microorganisms and dissolved chemicals progress to the next stage as they cannot be captured by screens.

Pre-chlorination/oxidation

The choice of disinfectant depends on the quality of raw water (Gilca et al., 2020). Chlorine gas, chlorine dioxide, or ozone, including their hybrid forms, are introduced as primary disinfectants and/or oxidants. The main purpose is to control the growth of algae and the formation of slime, which can cause taste and odour issues (Kalematsu et al., 2020; Mollo et al., 2022). Additionally, these disinfectants are used to eliminate biological contaminants (e.g. viruses) which can lead to human illnesses. Metals undergo oxidation and are transformed into insoluble forms at this stage (Kundu et al., 2024; Khan et al., 2025).

Coagulation and flocculation

Coagulation is defined as the destabilisation of colloidal particles by the addition of chemicals such as aluminium sulfate and ferric chloride and allow them to stick together through electrostatic forces/London forces, growing and forming floc. These chemicals or coagulants destabilize the colloidal particles by removing the forces which keep them apart and this is driven by affinity and charge difference (Sher et al., 2013; Katrivesis et al., 2019; Pivokonsky et al., 2024). This process takes place in less than 10 seconds. When colloidal particles are destabilized by colloids and stick together through electrostatic forces/London forces, they grow and form flocs. The floc particles then move together in a gentle motion of the water to grow even bigger. Therefore, this process of floc formation and growth is called flocculation, and it occurs for over a period of 20-40 minutes. While some particles passively settle down due to gravity and can be removed at the bottom of the tank, some are so small that they will not settle out and cannot be removed by filtration alone nor be seen by the naked eye. Only high-powered microscopes can be used to see them (bacteria, fine clays and silts). In advanced systems, the Dissolved Air Floatation (DAF) systems are used to enhance the removal of colloidal substances (Albarazanje, 2019; Gonzalez-Galvis, 2020; Ladouceur et al., 2023).

Sedimentation

In this process, particles are separated from the water through the force of gravity after the formation of flocs. A major part of the flocs is removed by settling. A sedimentation tank is provided where the flow and retention time is such that the particles can settle out. In some instances, the characteristics of the impurities is such that they float better than they settle. In such cases, the particles are separated from the water by flotation using the DAF as a polishing step (Edzwald, 2010; Lakghomi et al., 2015; Hell et al., 2024; Wang & Wang,2025). The separation can be sped up by attaching tiny air bubbles to the particles so that the density of the composite particles is appreciably less than that of the water (Edzwald, 2010).

Filtration

Filtration process is considered one of the central stages in water treatment (Cescon & Jiang, 2020). The pure water goes through filters constructed of various materials (such as sand, gravel, and charcoal) and various pore sizes during the filtration process. These filters eliminate germs and dissolved contaminants such as dust, chemicals, some bacteria and viruses. Bad odour is also eliminated by activated carbon filters. Although there is a tedious need for reactivation.

Disinfection

Disinfection is the most critical last step in the treatment plant. It is carried out to ensure that all microorganisms (pathogenic and opportunistic microorganisms included) which have passed all the processes mentioned above are eradicated to protect end users. Many chemicals can be used as disinfectants, namely chlorine, chlorine dioxide, ozone, and peracetic acid, but chlorine is the most commonly used one (Vosloo et al., 2018). Disinfection limits microbial growth within distribution systems. However, chlorine tends to react with organic compounds in water, leading to the formation of disinfection byproducts (DBPs) that could be mutagenic (Ebsa & Dibaba, 2022). Besides producing DBPs, chlorine generally inactivates bacteria by destroying the outer membrane and cytoplasmic components as a result (da Cruz Nizer et al., 2020). However, some bacterial species are resistant to chlorine and are called chlorine-resistant bacteria (CRB).

2.3 Treatment effectiveness of the DWTPs

DWTPs play a critical role in safeguarding public health by producing water that complies with established health and safety standards. These facilities operate most effectively when they can consistently eliminate physical, chemical, and microbiological contaminants from raw water sources while maintaining stable operations. Achieving optimal treatment results remains a challenging objective due to the constant variability in source water quality and the numerous factors that influence treatment processes. Studies have indicated that other than personal-related factors, factors such as chemical dosing, variation in source water, the condition of plant infrastructure and inadequate plant optimisation are major factors that contribute to the effectiveness of DWTP (Nawaz, 2018; Koley et al., 2024). Therefore, this section further examines key factors influencing treatment effectiveness.

2.3.1 Factors that affect the effectiveness of DWTP

2.3.1.1 Sources of raw water quality

The choice of raw water sources determines the quality of the treated water (Tyagi et al., 2020). Water used can be classified into two categories: groundwater and surface water (Khatri, 2015; Hamid et al., 2020). Both types of water can be susceptible to pollution by agricultural, industrial, and domestic activities. Treatment facilities mostly rely on pollution-prone water sources such as lakes, rivers, and wells, in which creates several complexities and difficulties (Ho, 2019; Mortazavi-Naeini et al., 2019; Pokhrel et al., 2022). Several researchers have identified that the release of nutrients into water sources is a primary factor responsible for the issues that result in the failure of DWTPs and DWDSs to providing clean water (Su et al., 2022; Abkar et al., 2024).

Numerous studies demonstrate that anthropogenic activities are the primary contributors to the degradation of raw water quality, along with the diversity of microorganisms in both influent water and DWTS (Akhtar et al., 2021). Runoff from fertilisers used in farming and manure from animals add nutrients including nitrates, phosphates, and organic matter, to surface waters. This causes eutrophication and the growth of bacteria, algae, and

cyanobacteria (Su et al., 2022). The nutrient-rich conditions make it hard for DWTPs to handle the water because nutrients and ultimately more microbes enter the facilities.

2.3.1.2 Climatic factors (temperature and rainfall)

Temperature and rainfall represent critical climatic factors that influence water quality and the efficacy of drinking water treatment processes. Climate variations influence the efficacy of physicochemical treatment and the viability of microbial populations, and this may facilitate the persistence of ARBs and ARGs throughout various phases of treatment (Mosaka et al., 2023).

Chemical reactions involved in disinfection, oxidation, and coagulation occur at a reduced rate at lower temperatures. This influences the elimination of microorganisms (Saleh et al., 2020). At low temperatures, coagulation and sedimentation effectiveness are impaired or rather reduced. This often led to increased amounts of suspended particles and organic material having a chance to be transported to other treatment processes, allowing for the proliferation and growth of microbial communities (Ye et al., 2019; Zhao et al., 2020).

Temperature variations influence the growth and composition of microbial communities within DWTPs. Reduced temperatures can impede the growth of most microorganisms while favouring psychrotolerant species, leading to changes in the community structure (Mathemani et al., 2019; Varma et al., 2021). Increased temperatures accelerate microbial metabolism and enhance biofilm formation. This promotes increased microbial diversity and biofilms formation (Wang et al., 2021).

The fluctuations in water viscosity and thermal stratification resulting from high temperature markedly affect the efficacy of flocculation and sedimentation processes. Minor variations in temperature (less than 1 °C) can lead to stratification in treatment basins, resulting in reduced effectiveness of floc interaction and settling (Hérard, 2023). Insufficient removal of solids in these environments increases the burden on subsequent filtration processes and alters the microbial communities present in sand filters, as well as the variety of microorganisms that accumulate in sludge.

The disinfection process is significantly influenced by temperature, which affects microbial survival and selection. The effectiveness of chlorine in eradicating bacteria is enhanced

at elevated temperatures, whereas it is reduced at lower temperatures (Zhao et al., 2025). This increases the likelihood of survival for bacteria capable of withstanding chlorine (Hu et al., 2022; Owesani, 2022). Sub-lethal disinfection conditions can impose selective pressure that promotes the proliferation of bacteria resistant to antibiotics, facilitating the persistence of ARGs in treated water and sludge. This demonstrates the importance of modifying activities based on weather conditions.

Changes in raw water quality due to rainfall patterns may influence the diversity of microbial populations. Intense precipitation leads to increased surface runoff, resulting in the transport of sediments, nutrients, faecal matter, and contaminants into source waterways (Ma et al., 2022). The inputs increase the microbial load and introduce a diverse array of environmental and faecal microorganisms, including ARBs, into the inflows of DWTPs. The rise in turbidity and organic matter during these events can diminish the effectiveness of treatment and promote the growth of different types of microbes in the treatment sludge.

Extreme weather events such as floods and droughts, driven by climate change, exacerbate microbial contaminants significantly. Flooding has the potential to compromise treatment equipment and destabilise processes. Drought conditions can lead to an increased concentration of pollutants and microorganisms in source waters (Babel et al., 2020; Duan, 2020). Climate-driven stressors alter the selection pressures on microbes in DWTPs, impacting community composition and increasing the likelihood of resistant bacteria surviving in both treated water and sludge. Sludge has the capacity to retain bacteria and ARGs. Consequently, variations in weather conditions can significantly impact the efficacy of treatment processes, which is essential for addressing the dissemination of antimicrobial resistance and the effective management of sludge.

2.3.1.3 Presence of contaminants

The elevated level of water pollution and diminished water quality can be related to a variety of sources. The reasons contributing to this issue encompass driving forces, such as the accelerated rate of population growth and industrialization, as well as the excessive discharge of pesticides, fertilizers, and chemicals into water supplies. These

contamination sources can result in the presence of many types of pollutants, with implications to changes in microbial dynamics and antimicrobial resistance.

Various pollutants based on their chemical composition, concentration levels, and persistence in the environment often influences aquatic microbiology. Industrial pollutants, fertilizers and herbicides in aquatic ecosystems, most of the time arise from runoff, leaching, and effluent discharge. They can either inhibit or promote the growth of microbial populations. Fertilizers rich in nutrients, particularly nitrogen and phosphorus, can induce microbial proliferation and eutrophication, resulting in a rise in bacterial populations and alterations in community composition. Many industrial chemicals and herbicides are extremely hazardous as they can damage cell membranes, impede enzymatic function, or modify cellular energy utilisation. This disrupts fragile microbial ecosystems and elevates the dominance of resistant species and creating a conducive environment for them (Speight, 2019; Li et al., 2020, Chabra et al., 2021).

2.4 Drinking Water Quality Assessment

Water is an essential and indispensable source of life (Hossain, 2015). It contributes to the equilibrium of nature and sustains the activities of human beings daily. However, ensuring the water is of high quality is as important. National and international guidelines are set to describe safe drinking-water as having no substantial health risks when consumed throughout one's lifespan, even accounting for variations in sensitivity during different periods of life (Havelaar & Melse, 2003; Tsaridou & Karabelas, 2021; SANS 241-1:2015). While water is available to people worldwide, it is often unsafe for human consumption and not available in adequate amounts to fulfil basic health requirements. According to the World Health Organization (WHO) 2022, over 1.1 billion individuals worldwide used water that is not suitable for consumption. Furthermore, a significant majority (88%) of diarrheal infections worldwide can be ascribed to the consumption of unsafe water, inadequate sanitation, and unsanitary habits. Furthermore, the water supply sector is encountering substantial obstacles because of climate change, global warming, and urbanization. The inadequate volume and substandard calibre of water have serious implications for the long-term progress, particularly in developing countries.

Treated water quality is assessed according to specific national criteria for drinking water established by the federal governments and other pertinent organizations. These guidelines prioritize specific physical, chemical and biological characteristics as crucial for the quality of drinking water, while considering others as less significant. The standard recommendations for drinking water quality state that no faecal indicator bacteria (FIB), namely *Escherichia coli* (*E. coli*) or temperature-tolerant coliform (TTC), should be present in any 100 ml sample of drinking water (Wen et al., 2020; Too, 2023).

Evaluating water quality before its distribution to customers is of utmost importance as it enables early identification of developing contaminants and reduces the potential risks posed to our consumers. The assessment also enables treatment plants to identify their shortcomings and take appropriate measures to address them. Assessing water quality is often carried out in different analyses, namely, microbial, physical and chemical analysis.

2.4.1 Microbial analysis in water

Microbiological analysis is the optimal method for identifying the presence of microbial contamination in water. Microbial contamination of water commonly occurs through faecal inputs from humans and animals, rendering it unsafe for consumption. Pathogenic microorganisms infiltrate water because of insufficient sanitation techniques. Interestingly, water has the capacity to support the development of various types of microorganisms, which can be advantageous for humans. Bacteria that are typically found in the digestive systems of humans and warm-blooded animals, including *E. coli*, *Salmonella*, *Vibrio*, and *Shigella*, have the potential to contaminate water via faecal matter, thus making it unsafe for consumption (Nwadike et al., 2024). In addition, the gastrointestinal tracts of endothermic animal's harbour viruses such as rotavirus and enterovirus, which pollute water and render it unsuitable for usage. Understanding the microbiological quality of water is crucial. Therefore, testing drinking water to ensure its safety should be a top concern in order to prevent any harmful consequences.

2.4.1.1 Methods used to test for microorganisms in water

E. coli

E. coli is a clearly classified member of the *Enterobacteriaceae* family characterised based on enzymes *β-galactosidase* and *β glucuronidase*, growth temperature range of 44-45ºC and lactose and mannitol fermentation. Full characterization of the organism is excessively intricate for regular application; however, some tests have been devised for swift and dependable identification with a satisfactory level of precision. Several of these approaches have been standardized at both international and national levels, such as ISO 9308-1 and ISO 9308-2, respectively. These methods have been widely approved for regular use. However, there are still other ways that are being developed or assessed.

Coliforms and other indicator organisms

Coliform is a collective term for a group of bacteria that are gram-negative and can break down lactose, producing gas within 48 hours at temperatures of either 35 ºC or 44/44.5ºC (Maina, 2020). These attributes provide convenient separation, identification, and quantification in the laboratory and are considered the benchmark for microbial water analysis. They consistently appear during water testing when enteric bacteria or viruses are identified (Devane et al., 2020, Opere et al., 2020). Nevertheless, an elevated count of 'total coliforms' does not automatically indicate the presence of faecal contamination. It necessitates an additional step to differentiate between the coliforms originating from faecal matter and those present in the surrounding environment. *Escherichia, Enterobacter,* and *Klebsiella* are types of bacteria commonly found in faeces, while *Citrobacter* and *Serratia* are typically found in plants and soil.

Enterococci and faecal streptococci

Previously, gram-positive cocci that formed chains were categorized under the genus *Streptococcus.* Faecal *Streptococci* referred to those *streptococ*ci commonly found in the faeces of both humans and animals. *Enterococcus* is a genus that includes a specific subgroup of faecal s*treptococci*. This subgroup is known for its ability to tolerate high levels of sodium chloride and alkaline pH (Wei et al., 2024). Majority of *Enterococcus*

species originate from faeces and can be considered reliable indicators of human l faecal contamination for most practical uses.

Detecting the existence of faecal *Streptococci/Enterococci* indicates the presence of faecal contamination. Faecal *streptococci* exhibit greater environmental persistence compared to thermotolerant or total coliforms and possess a strong resistance to drying (Martins et al., 2021). Consequently, it is feasible to separate faecal *streptococci* from water that has a low or non-existent presence of thermotolerant coliforms. This occurs when the origin of contamination is far away in either time or distance from the location where the sample is taken. Faecal *streptococci* can be cultivated in a medium that includes sodium azide, at a temperature ranging from 37 to 44 °C. Often, their presence is identified through the decrease in colour intensity of a dye (often a compound containing tetrazolium) or the breakdown of aesculin through hydrolysis (Sadek et al., 2021). Traditional approaches may produce "incorrect positive results," necessitating the need for further confirmatory tests.

Heterotrophic aerobic and aerobic spore-former bacterial counts

The enumeration of heterotrophic aerobic bacteria, commonly known as heterotrophic bacterial counts (HBC), and aerobic spore-forming bacteria, predominantly *Bacillus spp*., can be employed to evaluate the overall bacterial composition of water (Mabeo, 2020). The bacteria that are able to develop and generate visible colonies on the specific media employed, under the stipulated parameters of temperature and time of incubation, are the ones shown in the media. However, this does not include all the bacteria present in the water. Colony counts are often evaluated by incubating samples at both 22°C and 37°C in order to evaluate the presence of bacteria that are not associated with faecal contamination (Wight et al., 2020; Salamandane et al., 2021). While they may not have significant implications for sanitation, they can be valuable for evaluating the effectiveness of water treatment methods, such as coagulation, filtration, and disinfection. The goal in these processes is to minimize the presence of these microorganisms. Although the exact numbers have little importance, deviations from the usual counts at specific sites can indicate important trends. They can also be utilized to evaluate the sanitation and

coherence of the distribution system and the appropriateness of the water for use in the production of food and beverage items, where elevated counts may result in spoilage.

The cultural techniques employed to quantify heterotrophic aerobic bacteria can be modified to exclusively count spores by subjecting samples to temperatures ranging from 70 to 80 ºC for a duration of ten minutes prior to cultivation. The counting of aerobic spore-forming bacteria prior to and following a therapy is valuable for assessing the efficacy of the treatment (Wauters et al., 2021). They have been suggested as substitutes for the elimination of cysts of parasitic protozoa, although their effectiveness in this regard has not been confirmed.

2.4.1.2 Molecular methods of microbial identification

Molecular methods, such as shotgun metagenomics enable the analysis of genomic material from entire microbial communities within a sample (Kim et al., 2024). According to Kim et al. (2024), the untargeted DNA sequencing approach is the principle of this method. Sequencing reads are aligned to a database of microbial genomes to assign reads to closely related genes, pathways or genomes contained in a database or can be newly assembled larger genome fragments that are compared to the nearest genomes (Dai et al., 2020). A shotgun metagenomic approach provides a comprehensive composition of microbial communities in each sample, as well as the functional metagenomes, heavy metal resistance and antibiotic resistance genes. This is achieved by comparing sequence reads against specialized databases containing annotated functional genes, including antibiotic resistance genes (ARGs) and heavy metal resistance genes (HMRGs), researchers can identify and quantify these genetic determinants within the microbial community (Wang et al., 2024).

A culture-based technique, in conjunction with molecular analysis, can be utilised to identify antibiotic-resistant bacteria (ARB) in water samples. Kirby-Bauer disc diffusion method is an example of culture-based method used in the identification of ARB where the bacteria are exposed to different antimicrobial agents and zone of inhibition measured. Shot gun metagenomics analysis is a non-culture based method used in the identification of ARB through sequencing all DNA present in a sample and comparing the generated sequences against reference databases containing annotated antibiotic

resistance genes (ARGs). This method helps identify antibiotic resistance genes (ARGs) and their related microbial hosts. This gives a clearer picture of the presence and variety of antibiotic-resistant bacteria in the microbial community. Additionally, because it does not rely on growing microorganisms, shotgun metagenomics can find resistance genes in both culturable and non-culturable organisms. This provides a more complete assessment of antibiotic resistance in environmental samples.

2.4.2 Water chemical parameters and analysis in water quality

Water chemical parameters provide information on water quality, treatment performance, and the conditions that influence microbial survival and growth in DWTPs. Key chemical parameters routinely analysed in DWTPs include chloride, nitrate, nitrite, phosphate, ammonia, and water hardness. Monitoring of these parameters is mandatory as they can influence corrosion potential, aesthetic quality, treatment efficiency, and microbial processes within treatment systems (SANS 241:2015). Chemical analysis is therefore a core component of water quality assessment and is conducted in accordance with internationally recognised standard methods to ensure data reliability, comparability, and regulatory compliance (APHA, 2017; SANS 241:2015).

Ions of chloride are used to detect salinity, wastewater, and industrial pollutants. Chloride is a critical component of potable water treatment due to its association with chlorine-based disinfectants, which can inactivate microbes and reducing pathogens. Chloride is quantified through titrimetric silver nitrate procedures, such as ISO 9297 (Yildiz, 2024).

Nitrate (NO_3^-), nitrite (NO_2^-), and phosphate (PO_4^{3-}) are essential indicators of nutrient pollution associated with industrial effluents, wastewater discharges, and agricultural runoff. Nitrate and nitrite concentrations are closely linked to microbial proliferation in source water and treatment systems, altering microbial community composition and increasing the risk of nitrification within distribution networks (Alharbi et al., 2026; Pei et al., 2025; Zhao et al., 2025). Phosphate, as a key nutrient, strongly influences microbial and algal growth in surface water sources, and elevated concentrations can promote eutrophication, increase organic matter loads entering DWTPs, reduce treatment efficiency, and create favourable conditions for microbial survival in filters and sludge

(Nguyen et al., 2026). Because of their importance in water quality assessment and their links to algal blooms, taste and odour problems, and microbial regrowth, these parameters are routinely monitored using standard analytical methods, including spectrophotometric techniques, ion chromatography, and colorimetric analysis.

Water hardness, primarily determined by calcium and magnesium concentrations, influences both treatment processes and microbial ecology. While moderately hard water may be beneficial for corrosion control, excessive hardness can lead to scale formation, reducing hydraulic efficiency and providing surfaces for biofilm development. These biofilms can harbour diverse microbial communities and protect microorganisms from disinfectants, thereby influencing microbial persistence within DWTPs and distribution systems (Islam, 2020; Kaur et al., 2023).

Ammonia is frequently present in raw water due to the decomposition of organic matter and anthropogenic inputs. In DWTPs, ammonia is particularly significant because it reacts with chlorine to form chloramines, which are weaker disinfectants but more stable in distribution systems. The presence of ammonia can therefore promote nitrification, alter microbial community structure, and reduce disinfection effectiveness if not properly controlled. Ammonia is evaluated via established methodologies selected according to the concentration range and possible interferences, as outlined in the established Methods for the Examination of Water and Wastewater (APHA, 2017).

In general, chemical parameters are regulated not only to ensure that they comply with potable water standards, but also due to their direct and indirect impact on the stability of treated water quality, microbial dynamics, and treatment efficiency.

2.4.3 Water physical parameters and analysis

Physical water quality parameters provide essential information on treatment performance and the environmental conditions that affect microbial survival and transport in DWTPs (Singh et al., 2026).

pH is a fundamental parameter that controls chemical equilibria, disinfection efficiency, and microbial viability. In most natural waters, pH is regulated by the carbonate–bicarbonate buffering system and is influenced by temperature and dissolved carbon

dioxide. Drinking water standards typically specify a pH range of 6.5 to 8.5 to optimise treatment efficiency and minimise corrosion and microbial regrowth (WHO, 2017). Deviations from this range can reduce disinfectant effectiveness and favour the survival of specific microbial populations.

Turbidity and colour are key indicators of particulate and organic matter in water. Turbidity reflects the presence of suspended particles, including clay, silt, algae, and microorganisms, which can shield microbes from disinfection and reduce treatment efficiency. Colour is primarily associated with dissolved organic matter, iron, and manganese, and serves as an indirect indicator of organic loading. Elevated turbidity and colour are therefore closely linked to increased microbial loads and greater microbial diversity entering treatment processes. These parameters are routinely monitored to assess the effectiveness of coagulation, sedimentation, and filtration (WHO, 2017).

Electrical conductivity (EC) is an efficient method used to assess total dissolved ionic content in water and can be used to detect mineralisation and pollution. Changes in EC can indicate differences in source water quality and treatment efficacy. Elevated ionic concentrations can affect microbial community composition by modifying osmotic conditions and nutrition availability in treatment systems (Zhang et al., 2026).

Water temperature is a critical physical parameter influencing chemical reaction rates, microbial metabolism, and disinfection efficiency. Temperature greatly affects dissolved oxygen, microbial growth and opportunistic microbe survival. Water temperature in DWTPs is mostly dictated by raw water source parameters and cannot be economically managed. Thus, seasonal temperature changes affect microbial diversity, treatment performance, and sludge composition.

2.5 Microbial Diversity in Different Water Treatment Processes

The raw water source undergoes various treatment processes, which subject it to various physicochemical changes that alter the microbial ecology of the water source (Zupanc et al., 2019; Nguyen et al., 2021; Chakraborty, 2021).Typically, the initial two methods used to treat source water are flocculation and sedimentation, which have a limited impact on the structure of the microbial population (Lai et al., 2025; Zhao et al., 2025). This is

because both processes are fundamentally physicochemical and aim to eliminate suspended particles and colloidal substances rather than specifically targeting or inactivating microorganisms (Li et al., 2017; Zhang et al., 2026). In flocculation, microorganisms can be integrated into flocs by adhering to particle matter, thus allowing for their physical removal through sedimentation via gravity settling. However, these processes impose little selective pressures on microbial taxa, as they do not possess biological activity, oxidative stress, or sustained antimicrobial conditions (Lin et al., 2014; Singh et al., 2026). Consequently, numerous planktonic or loosely particle-associated microorganisms remain suspended in the water column, and the dominating microbial groups present in the source water often persist post-sedimentation with few changes in community composition (Gu et al., 2026). Previous studies have consistently shown that significant changes in microbial diversity and population structure usually occur at the later stages of treatment, such as filtration and disinfection, which employ more stringent selection and inactivation methods (Li et al., 2017; Singh et al., 2026). Consequently, while flocculation and sedimentation contribute to the reduction of turbidity and microbial load, their impact on the makeup of microbial populations is rather insignificant.

Proliferation of microorganisms not only result in a degradation of water quality and the presence of unpleasant tastes, odour, and visual cloudiness but can also lead to operational issues such as filter blockage, biological fouling, and biological corrosion (Fadini et al., 2021; Kumar, 2022). Comprehending the variety of microorganisms and their interactions in DWTPs is essential for developing inventive and efficient methods of control that guarantee the provision of safe drinking water (Collivignarelli et al., 2017; Pinar-Méndez et al., 2022; Ibarra et al., 2026). Microorganisms are naturally present in water and play a crucial role in the water environment and associated ecosystems (Some et al., 2021). Water acts as a reservoir for microorganisms that come from both the land and the atmosphere, mostly through processes like aerial deposition and the flow of water during rainfall and floods (Alla et al., 2021; Mishra et al., 2023). Notably, microorganisms are broadly present in the DWTPs, for example, in storage tanks, filter channels, as well as in pipelines (Lin et al., 2014; Li et al., 2021). Uncontrolled and unreasonable microbial development not just prompts deterioration in water quality and the related outcomes, smells, and visual turbidity, but can also cause process breakdown, for example,

obstructing of channels, biofouling and bioerosion (Farkas et al., 2012; Mulamattathil et al., 2014; Ibarra et al., 2026).

A study on the diversity and dynamics of microbial communities was conducted in the Chinese province of Hubei to reveal the dynamics of complex microbial communities in water samples going through various stages of treatment, as well as in biofilms connected to significant treatment steps like coagulation, flocculation, sedimentation, sand filtration, and disinfection by 454 pyrosequencing (Lin et al., 2014). Specifically, the 454-pyrosequencing results showed a variety of bacterial and eukaryotic populations in the collected samples. Albeit, following sand filtration and chlorination, these communities experienced a significant drop. At every stage of the DWTP, unique bacterial populations were found, and it is likely that specific processes used by DWTPs had a specific impact on bacterial composition and further afield. It was further stated that it is likely that specific DWTP procedures had a specific impact on the bacterial composition, i.e. overgrowth of bacteria. The study went on to clarify that the treatment's impact on the microbial community through coagulation-flocculation and sedimentation was minimal and that there was no discernible change in bacterial communities at these stages (Lin et al., 2014). The amount of *Acinetobacter* found in the sand filter biofilms was rather substantial. It is typical for all microorganisms to be killed off during chlorination, but in this study, Acinetobacter, a member of the *Gammaproteobacteria*, and *Chryseobacterium*, a member of the *Bacteroidetes*, were found, suggesting that they are either resistant to chlorine or that they can recover after chlorination (Lin et al., 2014). Similarly, another study conducted in Gaungzhou, South China and the findings denoted that the most prevalent phyla in DWTPs were *Pseudomonas*, *Cribrobacter*, and *Acinetobacter* (Hou et al., 2018). This study examined the spatiotemporal changes in bacterial community and microbial activity in a full-scale DWTP during the wet and dry seasons. The following were the key findings: 1) bacterial diversity and activity in drinking water were higher during the dry season than wet season; 2) the treatment process, specifically chlorination, had a significant impact on the microbial community composition; 3) the microbial communities present in finished water exhibited high levels of stability, and they were probably influenced by the bacterial communities present in the biofilter and 4) there were changes in the bacterial populations as a function of the ammonia and

CODMn concentrations. Furthermore, another study conducted in Milan, Italy, in the groundwater, identified *Parcubacteria (ODI), Superphylum, Saccharibacteria* to be the most abundant phyla in groundwater (Bruno et al., 2017). Bruno et al. (2017) study focused on investigating the "microbial dark matter" of drinking DWTP, which has received little attention. Even when considering the many sampling sites located throughout the entire sampling area, the groundwater samples had a consistent composition over the entire survey.

The different types of water used as source water and the different ways they are treated at each stage affect the overall microbial diversity in DWTPs. Flocculation and sedimentation constitute the initial stages in the removal of particles from water. The outcome is minimal selective pressure on microbial populations, allowing a broad diversity of bacteria from the source water to thrive downstream. Conversely, advanced treatment steps, particularly filtration and disinfection, significantly influence microbial populations by promoting biofilm development, facilitating selective retention, and eliminating susceptible taxa add ref. Extensive research on DWTPs indicates that the later phases significantly alter the types and activities of microorganisms, hence impacting the safety and stability of the final drinking water.

2.6 Drinking Water Treatment Plant Sludge (DWTS) and its Microbiology

When colour, turbidity and humic compounds are removed using inorganic alum-or ferric-based coagulants, DWTS is produced as a by-product. DWTS typically contains a lot of treatment chemicals in addition to a lot of impurities from the natural water sources, like suspended particles, colloidal debris, algae, and organics that cause colour (Xu et al., 2018). The sludge quality can be hazardous to the environment. It is frequently rich in various contaminants from raw water, including suspended particles, colloidal debris, algae, and organics that cause colour (Xu et al., 2018). Although the sludge's properties can vary, iron and aluminium hydroxide are frequently the dominant components.

Most sludge in undeveloped countries is released without any kind of treatment or is dumped in a landfill after being dewatered, which has the potential to severely harm aquatic and terrestrial ecosystems (Marenge et al., 2026). Through aggressive

pathogenic and ARBs that are likely to have adapted/resisted the treatment procedure and the physicochemical characteristics of the sludge, there is a chance that the sludge will have a negative influence on the environment. Thus, becoming an issue of concern in water treatment. While much research addresses the physicochemical characteristics, volatilization, and disposal of DWTS, there is little information available on microbial community investigations of DWTS (Hou et al., 2018). Understanding the sludge's quality and microbial diversity is crucial for understanding microbial ecology and its potential relationship to ecosystem function (Xu et al., 2018). Ullmann et al. (2019) conducted an examination of ARB in sludge extracts collected from Norwegian DWTPs and identified 20 bacterial genera showing resistance to aminoglycosides. *Mycobacterium* is recognized for its pathogenic characteristics, and the introduction of sludge into an environment requires heightened caution due to the potential transmission of this pathogen (Thom et al., 2022; Yu et al., 2024). Conversely, Xu et al. (2018) identified that the dominant phyla in the six DWTS samples under investigation were *Proteobacteria, Cyanobacteria, Bacteroidetes, Firmicutes, Verrucomicrobia, and Planctomycetes.*

2.7 Conclusion

Water is essential for our well-being. However, it is continually impacted by both human activities and natural processes that degrade its quality. Treatment plants are intended to purify contaminated water to prevent negative health consequences. However, there are instances where water becomes contaminated either at its origin or during the treatment process. Hence, it is vital to comprehend the microbiological fluctuations from the origin and through the treatment process.

CHAPTER 3: MATERIALS AND METHODS

3.1 Research Design

This was an empirical study that involved the collection of samples and analysis of physicochemical and microbiological parameters to investigate microbial diversity in DWTP and DWTS. Figure 3.1 shows the schematic flow of the experimental design.

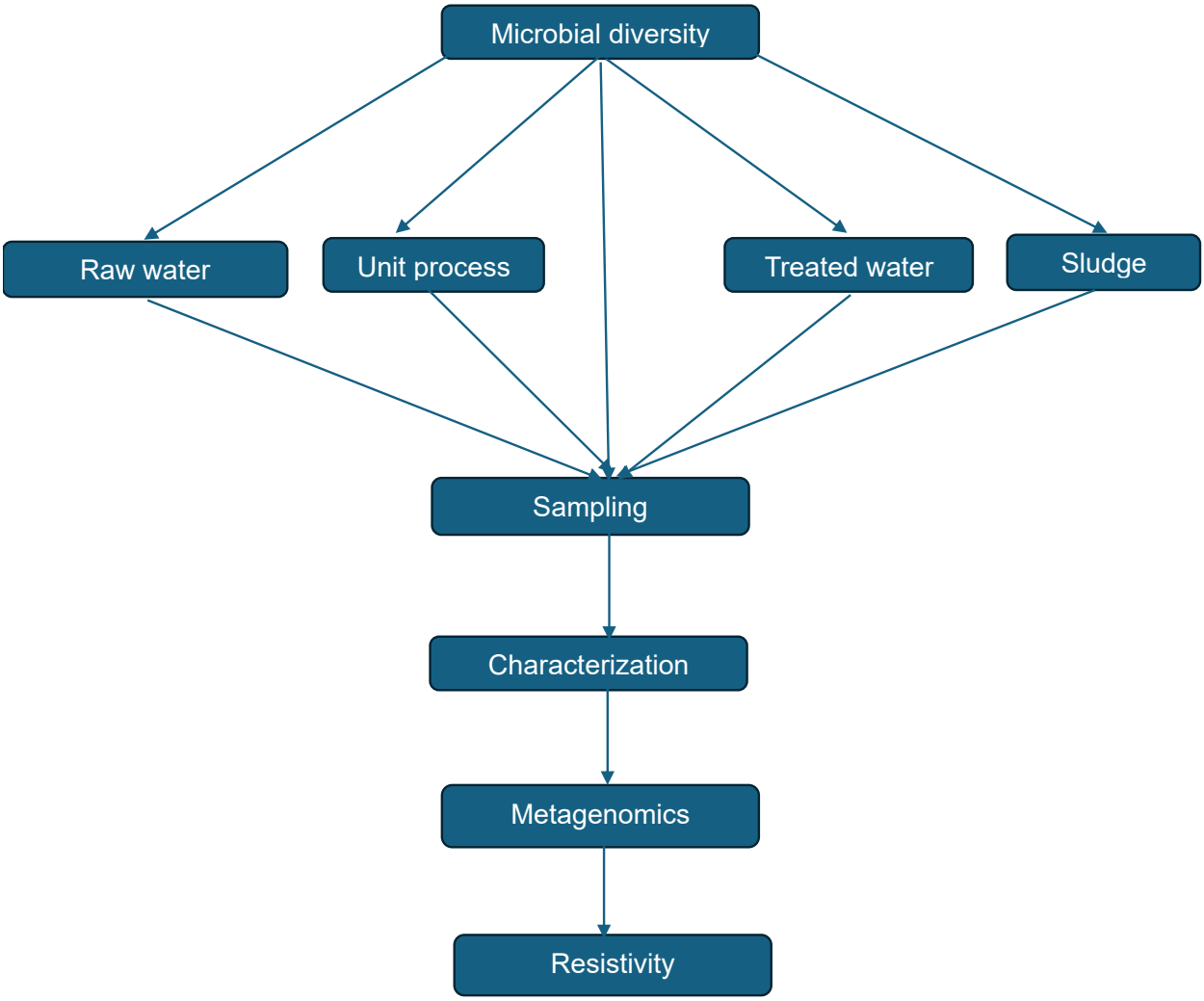


Figure 3.1: Schematic flow of the proposed experimental design.

3.2 Study Area

The study was conducted at three DWTPs located in GP and NW provinces. The plants, coded A, B and C, with different location and raw water sources, were selected to evaluate the impact of treatment stages on microbial dynamics. DWTPs A and B are located in Gauteng province with GPS coordinates of 25°34′ 34.22” S, 28°19′40.66” E and 25°40′ 30.81” S, 28°31′ 45.78” E respectively and DWTP C is located in Northwest province with GPS coordinate 25° 2' 21" S, 27° 16' 29" E.

3.3 Sampling Sites and Sample Collection

Composite water samples were collected for raw water sources before treatment, at each stage of the treatment process (pre-chlorination/disinfection, coagulation-flocculation, sedimentation, filtration and the final treated water) in triplicate. A total of 240 samples were collected across the raw water, treatment stages of the three plants, final treated water, and sludge for all four seasons. Sludge samples were collected across the profile of the sludge heaps (stockpiling facilities) and the lagoon. The treatment stages in DWTP A include raw water source, pre-chlorination, flocculation, sedimentation, filtration, final treated water, and sludge. DWTP B includes raw water source, disinfection, filtration, final treated water, and sludge. DWTP C includes raw water source, DAF, disinfection, filtration, GAC, final treated water, and sludge. Water and sludge samples were collected, stored in refrigerated cooler boxes and transported to the laboratory within 24 hours of sampling. Microbiological samples were stored in a refrigerator at the laboratory and analysed within 24 hours of sampling. Sampling was done quarterly over a period of 12 months.

3.4 Physicochemical Analysis

Samples were analysed at Magalies Waters ISO/IEC 17025 accredited chemistry laboratory for selected physicochemical parameters following standard methods for water and wastewater analysis (Baird et al., 2017). Electrical conductivity, pH and temperature were analysed using an HQ40d potable pH meter. Free chlorine was analysed using a potable DR890 colorimeter. Colour and turbidity were analysed using a benchtop 2100AN colorimeter. Total organic carbon (TOC) was analysed using a benchtop TOC L-series

analyser. A Gallery Plus automated-photometric analyser was used to analyse ammonia, nitrates and sulphates. Calcium, iron, magnesium and manganese were analysed using an inductively coupled plasma optical emission spectroscopy (ICP-OES) instrument.

3.5 Microbiological Analysis

3.5.1 Bacteriological indicators enumeration

The Colilert-18 and Enterolert Quanti-Tray/2000 (ISO 9308-2:2012) from IDEXX Laboratory (Pty) Ltd., Johannesburg, South Africa was used to quantify the total coliforms, *E. coli*, and *Enterococcus* spp. according to the manufacturer's guidelines. Decontamination of the equipment was done under ultraviolet (UV) radiation. A sachet of reagent powder was introduced into a 100 ml water sample, sealed, and subsequently incubated at specified temperatures: Colilert-18 at 35 ± 0.5 °C for 18 ± 1 hour, and Enterolert-DW at 41 ± 0.5 °C for 24 ± 1 hour. Trays were subsequently compared to comparators, and positive wells were enumerated and converted to ascertain Most Probable Numbers (MPNs) utilizing the supplied IDEXX MPN charts. A yellow coloration exceeding that of the comparator was noted as positive for total coliform, while blue fluorescence under a hand-held fluorescent light (4 W, 366 nm) was observed as positive for *E. coli* and *Enterococcus* spp. Heterotrophic bacteria counts were analysed by pipetting 1ml of the homogenized sample into a 90mm petri dish, followed by addition of approximately 20ml of molten nonselective R2A agar (Oxoid Limited, Basingstoke, Ha, and UK) and mixed by swirling. After the plates have solidified, they were incubated at 48 degrees for 48 hours. Upon completion of incubation, all visible colonies were counted and recorded as HBC (Baird, 2017).

3.5.2 Recovering Isolates and Confirmation test

After the incubation and enumeration of the positive wells, the outer surface of the tray was disinfected with 70% ethanol applied with a sterile swab. The backs of the three fluorescence-positive wells were punctured with a sterile razor blade on each tray. Three trays were utilised for testing each sample, and a loop containing the well content was streaked onto selective media specifically formulated for the detection of *E. coli*. Eosin methylene blue agar and *Enterococcus* spp. are utilised in ChromAgarTM *Enterococcus*

spp. The colonies were retrieved from the agar surface utilising a sterile loop for the purpose of conducting a confirmation test. The generation of gas and indole in lactose tryptose lauryl sulphate broth, along with growth observed in Brain Heart Infusion (BHI) broth, were utilised to confirm the presence of *E. coli* and *Enterococcus* spp., respectively. The confirmed *E. coli* and *Enterococcus* spp. were stored in a 20% (v/v) glycerol solution at −80°C for future analysis.

3.5.3 Antibiotic resistance analysis

Ten antimicrobial agents were employed for susceptibility testing: Ampicillin (AM) at a concentration of 10 µg, Erythromycin (E) at 15 µg, Ciprofloxacin (CIP) at 5 µg, Nitrofurantoin (F) at 300 µg, Amoxicillin/clavulanic acid (AMC) at 30 µg, Gentamicin (CN) at 10 µg, Imipenem (IMP) at 10 µg, Trimethoprim/sulphamethoxazole (SXT) at 25 µg, Levofloxacin (LEV) at 5 µg, and Vancomycin (VA) at 30 µg. The selection of antibiotics was conducted in accordance with the guidelines set forth by the Clinical Laboratory Standards Institute (CLSI, 2007, 2014, 2016) and their prevalent use in veterinary and human medicine. The Kirby–Bauer disc diffusion method was utilised, and the diameter of the inhibition zone was evaluated in accordance with the Clinical Laboratory Standards Institute (CLSI) breakpoint values (CLSI, 2007, 2014, 2016). Table 3.1 provides a summary of the antibiotic classes employed in this study. Isolates exhibiting multidrug resistance (defined as resistance to three or more classes of antimicrobials) were selected and stored in a 20% (v/v) glycerol solution at −80 °C for future analysis. The *E. coli* strain (Food and Drug Administration *E. coli* ATCC 25922) and *Enterococcus faecalis* (ATCC 29212) were utilised as control samples in compliance with CLSI control standards.

Table 3.1: Classes of antibiotics used in this study

Antibiotics class	Antibiotics used
Beta-lactams	Imipenem, Amoxicillin/clavulanic, Ampicillin,
Nitrofuran	Nitrofurantoin
Quinolone	Levofloxacin Ciprofloxacin
Aminoglycoside	Gentamicin
Macrolides	Erythromycin
Sulfonamides	Trimethoprim/sulphamethoxazol
Glycopeptide	Vancomycin

3.5.4 Multiple Antibiotic Resistance (MAR) Index

The MAR-index was calculated as (x/y), where "x" represents the quantity of antibiotics to which the isolate exhibited resistance, and "y" denotes the quantity of antibiotics to which the isolate was subjected. An isolate from a region of elevated antibiotic usage will have a MAR index greater than 0.2, whereas one with a value less than 0.2 originates from a region with reduced antibiotic usage.

3.5.5 Microbial community diversity and function analysis

Volumes ranging from 100 mL (raw water) to 1 L (treated water) were filtered through 0.22 µm polycarbonate membrane filters to accumulate the microbial biomass. The filters were sliced with a sterile scalpel and used for DNA extraction. DNA extraction kits obtained from Promolab Pty Ltd T/A Separations, RSA were used following the guidelines from the producers (Mhlongo et al*.,* 2020). The DNA concentration/purity and quality was

checked via a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA), and 1.5% agarose gel, respectively. The quality of the extracted DNA was assessed using absorbance ratios, which ranged between 1.8 and 2.0 (A260/A280), while DNA concentrations varied from 20 to 150 ng/μL. Samples meeting these quality criteria were subsequently subjected to shotgun metagenomic sequencing.

Shotgun metagenomic sequencing was performed using the MGI DNBSEQ-G400 platform at the Agricultural Research Council – Biotechnology Platform located in Pretoria, South Africa. The metagenomic shotgun data library preparation utilized the MGIEasy Universal DNA Library Prep Set V1.0 (MGI Tech Co., China). The library's quality control was conducted using a Qubit® fluorometer (Life Technologies, Carlsbad, CA, USA), with the sequencing depth determined to attain 5 million reads. The DNA nanoball (DNB) was generated by incorporating the pooled and circularized library, thereafter, fed into a PE150 flow cell for sequencing on the MGI DNBSEQ-G400 (MGI Tech Co., China) (2 × 150 bp). Raw FASTQ sequence files generated from the MGI DNBSEQ-G400 sequencing platform were analysed using the WGSA2.2 workflow implemented in Nephele (v2.2.8) with default settings (Weber et al., 2018). Sequence reads were clustered into operational taxonomic units (OTUs) using a 0.03 dissimilarity threshold, after which taxonomic assignment of representative sequences was performed. Classification was carried out using the Kraken2 classifier against the NCBI RefSeq reference genome database (Kraken2 Standard Reference Genomes DB; kr2_REFdb), together with the EuPathDB database containing genomes of known eukaryotic pathogens.

Microbial community diversity was evaluated by calculating alpha diversity indices, including Chao1, Shannon, Simpson, and ACE, using the plot_richness function in the phyloseq package, following the approach described by Ogola et al. (2021). Visualization of the dominant OTUs across different taxonomic levels was performed using heatmaps generated in ClustVis 2.0 (Tauno and Jaak, 2015), while stacked bar charts were created using the PAST version 4 (Smith et al., 2024).

Metagenomes were analysed for functional metagenomes, using the publicly available database in the WGSA2.2 pipeline implemented in Nephele (v2.2.8). Stacked bar charts

were created from functional metagenomes utilizing the Paleontological Statistics software package Version 4 (PAST 4) (Hammer and Harper, 2001). This study deposited the raw sequences in the National Center for Biotechnology Information Sequence Read Archive database: https://identifiers.org/ncbi/bioproject:PRJNA1090776.

3.6. Statistical

The assessment of water quality data was conducted in accordance with national guidelines and standards, specifically the South African water quality guideline for domestic use and the SANS: 241 standards for drinking water (SANS, 2015). Heterotrophic bacteria count data were analysed using one way analysis of variance (ANOVA) using Statistica version 12 (StatSoft Inc., Tulsa,OK, USA). Mean separation was done using Duncan' s multiple range test at 95% significant level. This action was performed to provide an assessment of the suitability for use. The analysis conducted using the IDEXX-defined substrate Colilert-18/QuantiTray 2000 yielded quantifiable data regarding the MPN of *E. coli*, *Enterococcus*, and the total coliforms, as referenced in the MPN table (IDEXX Quanti-tray/2000 MPN Table). A pairwise correlation was done to determine the relationship between the HBC and the physicochemical parameters. The value of the correlation coefficient (r) ranged from -1 to +1. For the strong positive correlations, the correlation diagrams were presented using the EzCorrGraph app (https://ezcorrgraph.firebaseapp.com/) (de Campos and Licht 2021), written in HTML5 and JavaScript.

CHAPTER 4: RESULTS AND DISCUSSIONS

4.1 Results

4.1.1 Physicochemical parameters of the samples

DWTP A (Gauteng Province)

Figure 4.1 illustrates seasonal variations in the physicochemical parameters across all treatment stages at DWTP A. During the spring season, the total hardness dropped from 37% at pre-chlorination to 19% at the sedimentation stage and rose to 34% at the final treated water. TDS, nitrate, Mn, Cu, and sulphate dropped from 79%, 85%, 90%%, 92 and 84% at pre-chlorination stage to 42%, 56%, 60%, 70%, and 61%, respectively, at sedimentation stage. In the summer season, there was no clear trend that shows decline in the measured parameter. During winter, ammonia, nitrate, sulphate, and Mn dropped from 78%, 81%, 90%, and 92% at the pre-chlorination stage to 60%, 60%, 70%, and 80%, respectively, at flocculation stage. In autumn, only TDS showed a clear trend of dropping from 74% at pre-chlorination to 58% at flocculation.

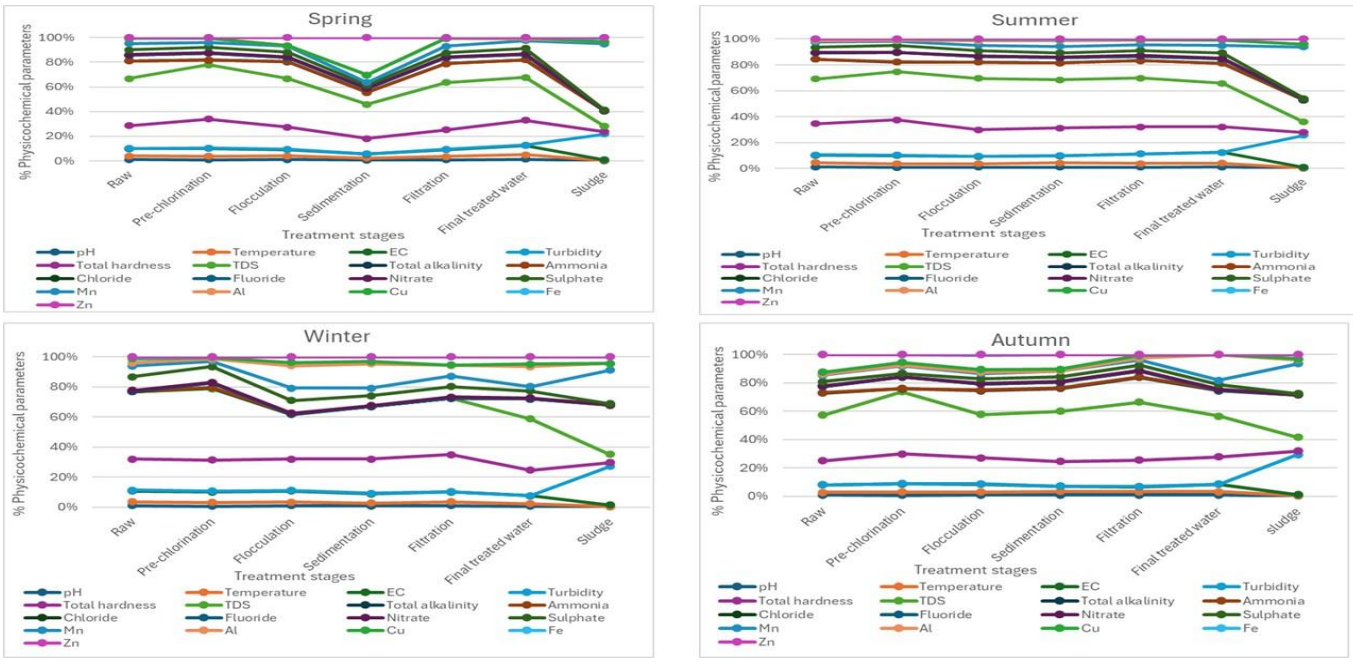


Figure 4.1: Variation in physicochemical parameters across treatment stages and seasons in DWTP A.

DWTP B (Gauteng province)

In DWTP B across all seasons, most physicochemical parameters showed a high percentage in raw water, followed by a noticeable reduction in the disinfection stage, particularly in summer and autumn (Figure 4.2). For example, in summer, total hardness, TDS, sulphate, and Mn dropped from 38%, 59%, 70%, and 80% at the raw water source to 21%, 38%, 51%, and 65%, respectively, at the disinfection stage. In autumn, TDS, Nitrate, and Mn dropped from 62%, 80%, and 83% at the raw water source to 40%, 58%, and 60%, respectively, at the disinfection stage.

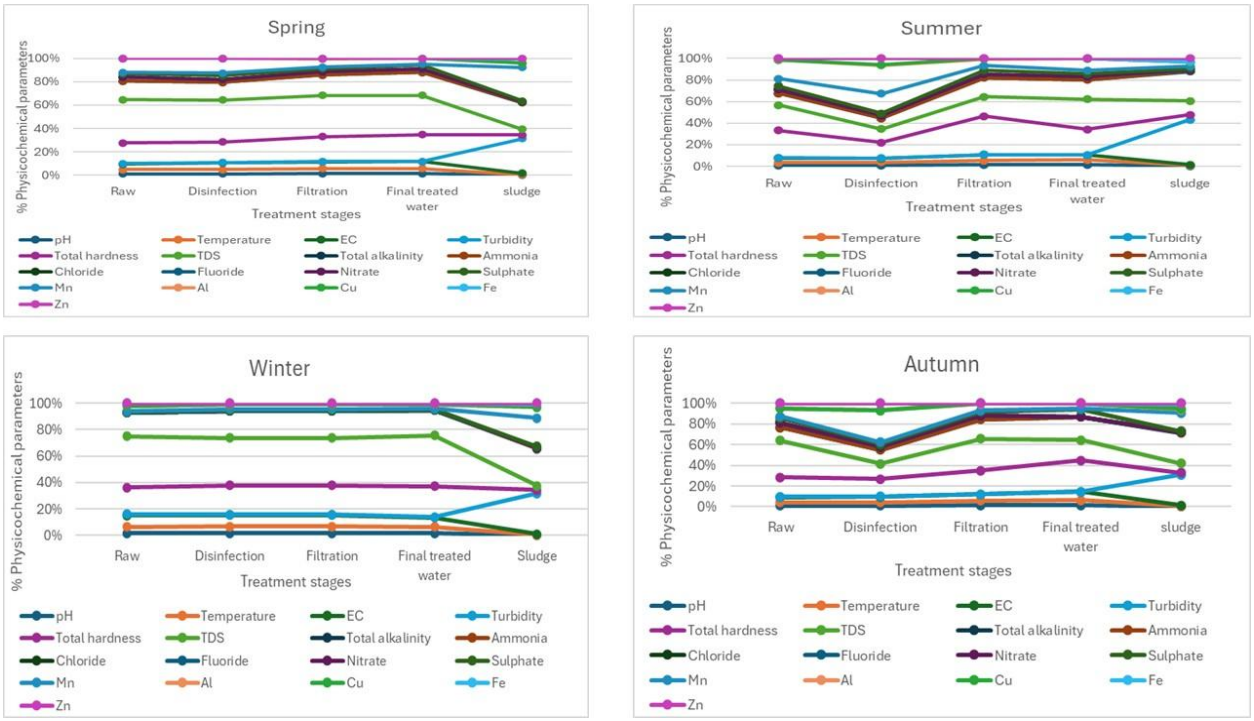


Figure 4.2: Variation in physicochemical parameters across treatment stages and seasons in DWTP B.

DWTP C (Northwest Province)

In Figure 4.3, a sharp reduction in various physicochemical parameters was observed in DAF, from the raw water source, especially, in spring, winter, and autumn. For example, in spring, total hardness and TDS dropped from 19% and 42% at the raw water source to 12% and 21%, respectively, at DAF. In winter, total hardness, TDS, and ammonia dropped from 30%, 65%, and 79% at the raw water source to 12%, 32%, and 62%, respectively, at DAF. In autumn, total hardness, TDS, and total alkalinity dropped from 22%, 58%, and 65% at the raw water source to 15%, 22%, and 57%, respectively at DAF.

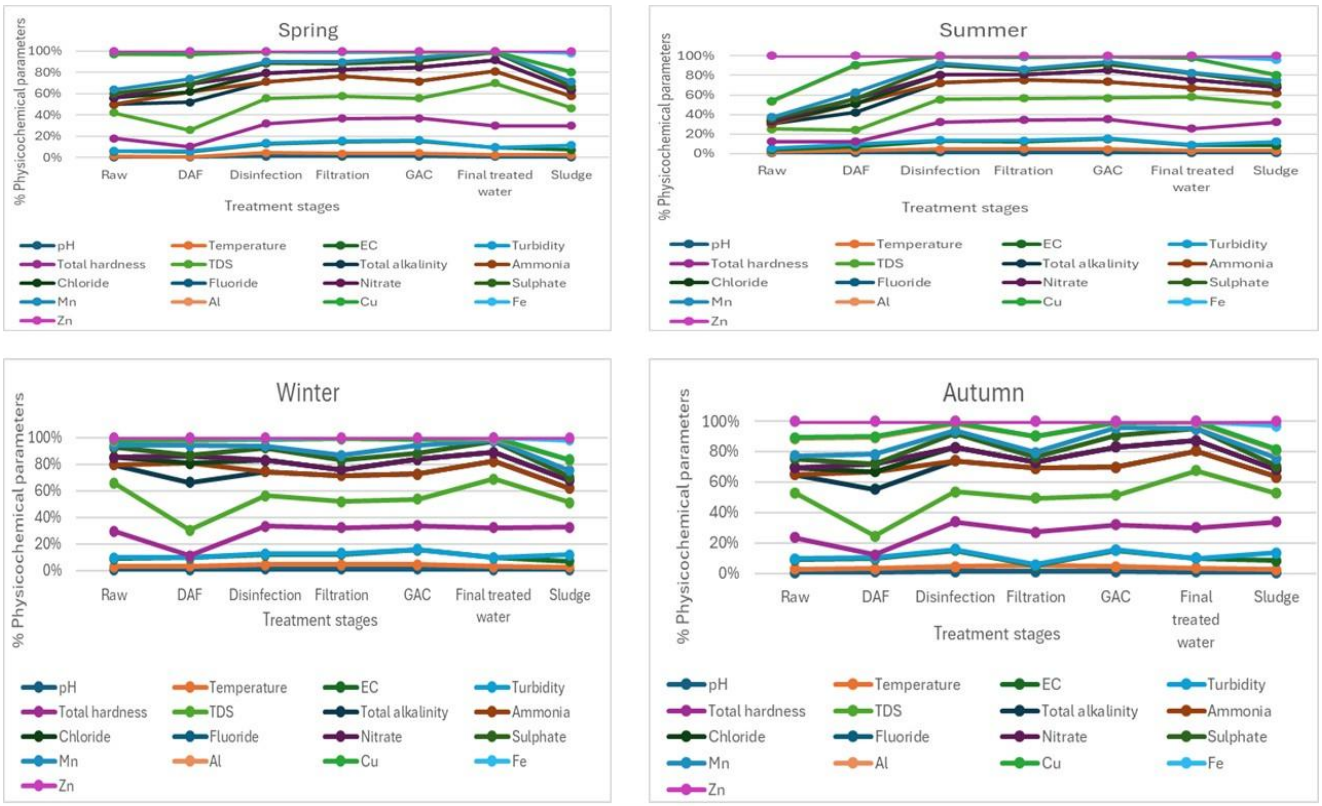


Figure 4.3: Variation in physicochemical parameters across treatment stages and seasons in DWTP C.

4.1.2 Bacteriological indicators

DWTP A (Gauteng Province)

The total coliforms in the raw water source for DWTP A were high ,as recorded at >2419.6 MPN/100ml throughout all seasons (Table 4.1). *E. coli* levels were 214.6 MPN/100ml during the summer season. The highest value for *Enterococcus* spp. was recorded in autumn at 2419.6 MPN/100 ml. In the flocculation stage, coliforms were high (2419.6 MPN/100ml) in all the seasons. *E. coli* levels varied, with a high of 48.1 MPN/100 ml in spring and a low of 4.3 MPN/100ml in autumn. Results for *Enterococcus* spp. during flocculation, indicated potential high microbial occurrence, with the highest concentration recorded in autumn at 53.8 MPN/100 ml. Throughout the sedimentation process, total coliforms were detected, and samples for the winter season recorded 248.9 MPN/100 ml. *E. coli,* in the sedimentation, had a value of 24.6 MPN/100ml observed during summer, while zero was recorded during winter. The highest recorded level of *Enterococcus* spp. was 7.1 MPN/100ml in winter, while the lowest was 2.5 MPN/100 ml in summer. The filtration stage showed the highest total coliform levels during spring and summer, while winter and autumn recorded values of 76.7 and 82.2 MPN/100ml, respectively. *E. coli* showed complete removal (0 MPN/ 100ml) in winter and autumn, while the peak concentration was observed in summer at 17.3 MPN/100ml. For *Enterococcus* spp. the highest recorded level was 3 MPN/100 ml in autumn, with zero recorded in summer and winter. In the final treated water samples, total coliforms, *E. coli,* and *Enterococcus* spp. were all recorded as zero across all seasons. The sludge recorded value of >2491.6 MPN/100ml for total coliforms during summer, winter, and autumn. The lowest value for *E. coli* was 727 MPN/100 ml in spring, while values were 2419.6 MPN/100ml in spring and winter, with a minimum of 1 MPN/100 ml in summer. The concentration of *Enterococcus* spp. was 2419.6 MPN/100ml during both summer and winter, and the lowest was 61.6MPN/100 mL in spring. Most of the indicated bacteria were above the stipulated standard in almost all the stages of treatment, except for *E. coli* in sedimentation (0 MPN/100ml) during winter and filtration in winter and autumn. *Enterococcus* spp., *E. coli* and total coliforms were compliant with SANS 241 standards (**0 MPN/100ml**) only at the final treated water stage during all seasons,

Table 4.1: MPN of the bacteriological indicators from the treatment stages in DWTP A using the IDEXX technique**.**

Sampling points	**Total coliform (SANS 241 limit: ≤ 10 MPN/100ml)**				***E. coli* (SANS 241 limit: ≤ 0 MPN/100ml)**				***Enterococcus* (SANS 241 limit: ≤ 0 MPN/100ml)**			
	Spring	Summer	Winter	Autumn	Spring	Summer	Winter	Autumn	Spring	Summer	Winter	Autumn
Raw	>2419.6	>2419.6	>2419.6	>2419.6	34.9	214.2	93.4	67.0	36.4	24.6	185	>2419.6
Pre-chlorination	>2419.6	>2419.6	>2419.6	>2419.6	22.4	235.9	30.5	4.1	21.7	11.4	2	870.4
Flocculation	>2419.6	>2419.6	>2419.6	>2419.6	20.3	48.1	10.2	4.3	16.1	5.3	8.6	53.8
Sedimentation	>2419.6	>2419.6	248.9	>2419.6	1.0	24.6	0	1.0	3.1	2.5	4	7.1
Filtration	>2419.6	>2419.6	76.7	82.2	1.0	17.3	0	0	1.0	0	0	3.0
Final treated water	0	0	0	0	0	0	0	0	0	0	0	0
Produced sludge*	727.0	>2419.6	>2419.6	>2419.6	>2419.6	1.0	>2419.6	6.2	61.6	>2419.6	>2419.6	454.1

*-indicates that there is no identified limit for sludge.

DWTP B (Gauteng Province)

For DWTP B, the total coliform counts in raw water were recorded at 2419.6 MPN/100ml during spring and summer, with a decrease observed in winter and autumn, resulting in values of 86.5 and 214.3 MPN/100ml, respectively (Table 4.2). *E. coli* levels were recorded at 235.9 MPN/100ml in spring, and a minimum concentration of 2 MPN/100ml recorded in autumn. The results demonstrated that the *Enterococcus* spp. levels peaked in summer at 30.9 MPN/100ml and declined to its lowest level in winter at 2 MPN/100ml. Total coliform levels in the disinfection stage were high during spring and summer and showed a decrease in winter and autumn. The filtration stage demonstrated improved outcomes in *E. coli* counts, with a recorded value of 0 MPN/100ml during winter and autumn. In contrast, spring and summer showed counts of 24.6 and 1 MPN/100ml, respectively. The total coliform levels during the filtration process were recorded at 2419.6 MPN/100ml in spring and summer, with a reduction observed in winter at 3 MPN/100ml and 45 MPN/100ml in autumn. The peak levels of *Enterococcus* spp. was recorded at 4.1 MPN/100ml during the summer, while the lowest levels, recorded in spring and winter, were zero.

The final treated water demonstrated no counts of *E. coli* throughout all seasons, consistent with the SANS 241 (2015) guideline of 0 MPN/100ml. Throughout the entire sampling period, every stage of the DWTP demonstrated consistently positive results for *Enterococcus* spp. The sludge produced showed *Enterococcus* spp. counts of 235.9 MPN/100ml in summer, decreasing to 159.7 MPN/100ml in autumn, while counts for the other two seasons were recorded as 0 MPN/100ml. Total coliforms were high in summer and spring at 2419.6 MPN/100ml, followed by a decline in winter and autumn (with values of 302.6 and 235.9 MPN/100ml, respectively). In addition, *E. coli* in filtration stage during winter and autumn recorded 0 MPN/100ml.

Table 4.2: MPN of the bacteriological indicators from the treatment stages in DWTP B using IDEXX technique

Sampling points	Total coliform (SANS 241 limit: ≤ 10 MPN/100ml)				*E. coli* (SANS 241 limit: ≤ 0 MPN/100ml)				*Enterococcus* (SANS 241 limit: ≤ 0 MPN/100ml)			
	Spring	Summer	Winter	Autumn	Spring	Summer	Winter	Autumn	Spring	Summer	Winter	Autumn
Raw water	>2419.6	>2419.6	86.5	214.3	235.9	88.6	11.7	2.0	3.0	30.9	2.0	10.7
Disinfection	>2419.6	>2419.6	41.1	179.3	21.8	3.0	4.1	0	1.0	18.7	1.0	2.0
Filtration	>2419.6	>2419.6	3	45.0	24.6	1	0	0	0	4.1	0	1.0
Final treated water	1	0	1	0	0	0	0	0	0	0	0	0
Produced sludge*	>2419.6	>2419.6	302.6	235.9	>2419.6	2	0	0	0	235.9	0	159.7

*-indicates that there is no identified limit for sludge.

DWTP C (Northwest province)

At DWTP C, the total coliform levels in raw water, following DAF treatment, disinfection, and sludge recorded the highest concentration in spring and summer at 2419.6 MPN/100ml across all processes (Table 4.3). Following GAC treatment, results decreased, achieving zero counts in all seasons except for the final treated water, which recorded 1 MPN/100ml in both spring and autumn. *E. coli* counts were recorded below the established limit, with a count of zero at the final treated water. An exception occurred in spring, where 1 MPN/100ml was noted in the filtration stage, alongside sludge, which recorded the highest across all seasons.

Enterococcus spp. counts in GAC stage and at the final treated water recorded 0 MPN/100ml. In contrast, raw water, DAF, disinfection, filtration stage, and sludge recorded the highest at 2419.6 MPN/100ml during summer and autumn in sludge, and

the lowest at 0.2 MPN/100ml in the filtration stage during winter. In addition, *E. coli* in GAC, filtration and final treated water recorded 0 MPN/100ml in spring, summer and autumn. *Enterococcus* spp. was compliant to the SANS 241limit in final treated water in all the seasons.

Table 4.3: MPN of the bacteriological indicators from the treatment stages in DWTP C using IDEXX technique

Sampling points	**Total coliform (SANS 241 limit: ≤ 10 MPN/100ml)**				***E. coli* (SANS 241 limit: ≤ 0 MPN/100ml)**				***Enterococcus* (SANS 241 limit: ≤ 0 MPN/100ml)**			
	Spring	Summer	Winter	Autumn	Spring	Summer	Winter	Autumn	Spring	Summer	Winter	Autumn
Raw water	>2419.6	>2419.6	189.7	187.0	90	78.0	92.0	100	46.7	26.7	37.3	43
DAF	>2419.6	>2419.6	106	101.7	10	6.0	4	4	8	2	1	0.3
Disinfection	>2419.6	>2419.6	104	99.7	22	13	19	23	0	4.1	0	1.0
Filtration	0.3	0.7	2.3	1	1	0	0	0	0.3	0.7	0.2	1
GAC	0	0	0	0	0	0	0	0	0	0	0	0
Final treated water	1	0	0	1	1	0	0	0	0	0	0	0
sludge	>2419.6	>2419.6	140.7	161.6	>2419.6	>2419.6	1	1	212.2	>2419.6	237.3	>2419.6

*-indicates that there is no identified limit for sludge.

4.1.3 Antibiotic responses by the indicator bacteria

To gain insights into the response of *E. coli* and *Enterococcus* spp. to frequently utilised antibiotics, an antibiotic resistance analysis was performed on isolates collected from the sampling points across the different seasons. Susceptibility testing was conducted on 121 confirmed isolates using a panel of ten empirical antibiotics. The results were interpreted

in accordance with the CLSI guidelines (Clinical and Laboratory Standards Institute, 2018).

A total of 6 out of 121 isolates were identified as non-MDR, comprising 6 *Enterococcus* spp. isolates. This indicates that most isolates exhibited multidrug resistance to three or more classes of antibiotics, raising significant concerns regarding environmental and public health implications. The occurrence of multidrug-resistant (MDR) strains indicates that resistant bacteria could disseminate via both treated and untreated water, contributing to the global challenge of antimicrobial resistance (AMR). Based on the MAR-index, there is potential exposure to environments with high antibiotic usage in the two provinces under this study.

DWTP A

A total of 32 isolates were tested, with 4 isolates (4- *Enterococcus* spp., all recorded in autumn) identified as non-MDR: 1 in pre-chlorination, 1 in sedimentation, 1 in filtration, and 1 in sludge (Table 4.4). *E. coli* isolates demonstrated resistance to the antibiotics tested (mostly, IMP, AMC, CN, AM, F, E, and SXT) from the raw water sources, treatment stages, and in the final treated water. Isolates of *Enterococcus* spp. from the treatment stages show resistance to IMP, AMC, LEV, CIP, CN, AM, F, E, and SXT. *E. coli* and *Enterococc*us spp. isolated from the raw water, treatment stages, final treated water, and sludge were resistant to many antibiotics throughout the seasons. *E. coli* from raw water, flocculation, sedimentation, final treated water, and sludge during spring were mostly resistant to IMP, AMC, AM, F, E, and SXT, and *Enterococcus* spp. from raw water, flocculation, sedimentation, filtration, and sludge was resistant to IMP, AMC, LEV, CIP, AM, VA, F, E, and SXT. During summer, *E. coli* from raw water, pre-chlorination, flocculation, sedimentation, filtration, and sludge resistance remained mainly to IMP, AMC, AM, F, E, and SXT. In winter, *E. coli* taken from raw water and sludge maintained resistance mainly toward IMP, AMC, AM, F, E, and SXT, while *Enterococcus* spp. from pre-chlorination, raw water, flocculation, sedimentation, and sludge were resistant against IMP, AMC, LEV, CIP, AM, VA, F, E, and SXT. In autumn, *E. coli* and *Enterococcus* spp. isolated along the treatment line (pre-chlorination, sedimentation, filtration, and sludge) showed high resistance to multiple antibiotics, especially IMP, AMC, AM, F, E, and SXT.

Most isolates (from all sampling points and seasons) were termed multidrug resistant with MAR indices from 0.1 to 0.9, which indicated that antibiotic resistance is not completely eradicated during treatment and there was very limited elimination of antibiotic-resistant bacteria in the plant.

Table 4.4: Antibiotic resistance of bacteriological indicators in DWTP A of Gauteng province

Sampling points	Isolates	Season	Inhibition zone (mm)										Status	MAR-Index
			Imp	AMC	Lev	CIP	CN	AM	VA	F	E	SXT		
Raw	*E. coli*	Spring	13	11	29	20	15	0	-	12	0	24	MDR	0.6
Pre-Chlorination	*E. coli*	Summer	15	0	25	23	15	0	-	10	0	0	MDR	0.6
Pre-Chlorination	*E. coli*	Autumn	18	15	23	27	20	0	0	20	0	0	MDR	0.4
Pre-Chlorination	*Enterococcus* sp.	Winter	13	17	25	25	17	0	0	13	0	0	MDR	0.6
Pre-Chlorination	*Enterococcus* sp.	Autumn	18	30	20	30	25	0	30	20	0	21	MDR*	0.2
Raw	*E. coli*	Summer	15	10	18	22	15	0	-	14	18	0	MDR	0.5
Raw	*E. coli*	Winter	8	14	22	22	15	0	-	8	8	19	MDR	0.6
Raw	*E. coli*	Autumn	20	13	25	30	22	20	-	18	0	0	MDR	0.4
Raw	*Enterococcus* sp.	Spring	10	12	27	24	17	0	0	10	0	0	MDR	0.7
Raw	*Enterococcus* sp.	Winter	10	10	18	30	15	0	0	0	0	0	MDR	0.7
Raw	*Enterococcus* sp.	Autumn	15	20	22	25	21	0	0	18	10	19	MDR	0.3

Flocculation	*E. coli*	Spring	12	16	16	26	17	0	-	10	10	14	MDR	0.6
Flocculation	*E. coli*	Summer	0	0	20	25	17	0	-	0	0	0	MDR	0.7
Flocculation	*Enterococcus* sp.	Spring	0	10	0	0	15	0	0.	0	0	0	MDR	0.9
Flocculation	*Enterococcus* sp.	Winter	10	8	8	7	12	0	9	0	19	19	MDR	0.8
Sedimentation	*E. coli*	Spring	9	8	29	30	15	0	-	0	7	0	MDR	0.6
Sedimentation	*E. coli*	Summer	10	16	20	30	20	8	-	15	0	0	MDR	0.5
Sedimentation	*E. coli*	Autumn	13	20	17	25	17	0	-	19	0	20	MDR	0.4
Sedimentation	*Enterococcus* spp.	Spring	10	0	0	0	22	0	0	0	0	0	MDR	0.9
Sedimentation	*Enterococcus* sp.	Winter	10	0	15	15	15	0	20	10	0	0	MDR	0.6
Sedimentation	*Enterococcus* spp.	Autumn	15	21	19	26	23	18	25	20	0	21	MDR*	0.1
Filtration	*E. coli*	Summer	10	15	20	23	15	0	-	13	10	18	MDR	0.5
Filtration	*Enterococcus* spp.	Spring	8	7	8	8	17	0	0	9	0	0	MDR	0.9
Filtration	*Enterococcus* spp.	Autumn	18	19	25	24	24	18	25	22	0	26	MDR*	0.1
Final treated water	*E. coli*	Spring	15	10	12	17	14	0	-	15	0	28	MDR	0.6
Sludge	*E. coli*	Spring	14	0	21	35	14	0	-	0	10	11	MDR	0.8
Sludge	*E. coli*	Summer	0	0	0	0	19	0	-	0	0	0	MDR	0.9
Sludge	*E. coli*	Winter	19	0	21	23	15	7	-	0	16	18	MDR	0.4

Sludge	*E. coli*	Autumn	17	22	17	19	20	0	-	17	0	0	MDR	0.4
Sludge	*Enterococcus* spp.	Spring	10	0	17	19	18	0	0	0	0	0	MDR	0.7
Sludge	*Enterococcus* spp.	Summer	0	0	0	25	20	0	0	0	0	0	MDR	0.8
Sludge	*Enterococcus* spp.	Winter	15	19	20	30	19	0	9	0	0	0	MDR	0.5
Sludge	*Enterococcus* spp.	Autumn	20	20	24	27	25	0	21	19	0	20	MDR*	0.2
ATCC25922	*E. coli*		27	18	29	34	20	18	-	22	0	23	MDR	0.3
ATCC29212	*Enterococcus faecalis*		15	25	21	23	12	20	20	20	20	25	MDR	0.2

MDR-Multi-drug resistance; MDR*-non-Multidrug resistance; MAR-Multiple antibiotic resistance

DWTP B

At DWTP B, among the 17 isolates tested, only 2 isolates of *Enterococcus* spp., both collected in autumn (1 from raw and 1 from filtration), were found to be non-MDR (Table 4.5). *E. coli* isolates demonstrating resistance to the antibiotics tested (mostly, IMP, AMC, CN, AM, F, E, and SXT) from the raw water sources, treatment stages, and in the final treated water. *Enterococcus* spp. was mostly resistant to IMP, AMC, LEV, CAN, AM, VA, F, and SXT across the raw water sources, filtration, and final treated water (Table 4.5). Both *E. coli* and *Enterococcus* spp. were resistant to various antibiotics in different seasons when the pathogens from raw water, disinfection, filtration, final treatment stages, and sludge were isolated. In the spring, *E. coli* isolated from raw water, disinfection, filtration, and sludge was essentially resistant to IMP, AMC, LEV, CIP, AM, F, E, and SXT, and no *Enterococcus* spp. was detected in this season except for the sludge, which showed resistance against IMP, AMC, AM, VA, F, and E. In summer, *E. coli* isolated from raw water, filtration, and sludge were mainly resistant to IMP, AMC, AM, F, E, and SXT. In winter, *E. coli* obtained from raw water and disinfection showed antimicrobial resistance mainly against IMP, AMC, LEV, AM, F, E, and SXT, while *Enterococcus* spp. from raw water, disinfection, and filtration showed resistance against IMP, AMC, LEV, AM, VA, F, E, and SXT. During autumn, *E. coli* isolated from raw water remained resistant to IMP, AMC, AM, F, E, and SXT. On the other hand, *Enterococcus* spp. isolated from raw water, disinfection, filtration, and sludge showed resistance against several antibiotics, including IMP, AMC, LEV, CIP, VA, and SXT. Most isolates across the treatment train were classified as multidrug resistant, and the MAR index ranged from 0.2 to 0.9, implying that antibiotic-resistant bacteria were ubiquitous and resistant across treatment stages.

Table 4.5: Antibiotic resistance of bacteriological indicators in DWTP B of Gauteng Province

Sampling points	Isolates	Season	Inhibition zone (mm)										Status	MAR-Index
			Imp	AMC	Lev	CIP	CN	AM	VA	F	E	SXT		
Raw	*E. coli*	Spring	0	0	0	0	20	0	-	0	0	0	MDR	0.9
Raw	*E. coli*	Summer	0	0	20	20	18	0	-	0	0	0	MDR	0.7
Raw	*E. coli*	Winter	0	10	10	22	19	0	-	0	0	0	MDR	0.8
Raw	*E. coli*	Autumn	7	0	21	30	15	0	-	0	0	0	MDR	0.7
Raw	*Enterococcus* spp.	Winter	0	7	15	21	10	0	0	6	0	0	MDR	0.8
Raw	*Enterococcus* spp.	Autumn	30	10	21	22	20	20	18	20	25	0	MDR*	0.2
Disinfection	*E. coli*	Spring	0	0	27	30	17	0	-	0	0	0	MDR	0.7
Disinfection	*E. coli*	Winter	0	0	20	0	19	0	-	0	0	0	MDR	0.8
Disinfection	*Enterococcus* spp.	Winter	13	13	20	21	13	0	0	16	0	22	MDR	0.6
Disinfection	*Enterococcus* spp.	Autumn	0	0	17	18	25	0	20	20	22	0	MDR	0.4
Filtration	*E. coli*	Spring	8	0	0	0	22	0	-	0	0	15	MDR	0.8
Filtration	*E. coli*	Summer	15	10	20	20	15	0	-	0	0	0.	MDR	0.6
Filtration	*Enterococcus* spp.	Winter	13	0	0	0	15	0	0	0	0	0	MDR	0.9
Filtration	*Enterococcus* spp.	Autumn	30	20	20	20	22	19	20	17	22	30	MDR*	0
Sludge	*E. coli*	Spring	15	11	20	23	18	13	-	0	0	0	MDR	0.6

Sludge	*E. coli*	Summer	0	9	35	26	26	0	-	0	0	0	MDR	0.7
Sludge	*Enterococcus* spp.	Autumn	25	12	21	21	20	10	20	19	30	0	MDR	0.3
ATCC25922	*E. coli*		27	18	29	34	20	18	-	22	0	23	MDR	
ATCC29212	*Enterococcus faecalis*		15	25	21	23	12	20	20	20	20	25	MDR	

MDR-Multi-drug resistance; MDR*-non-Multidrug resistance; MAR-Multiple antibiotic resistance

DWTP C

In DWTP C, out of the 38 isolates, none were recorded to be non-MDR (Table 4.6). *E. coli* strains were mostly resistant to IMP, AMC, CN, AM, F, E, and SXT while *Enterococcus* spp. were mostly resistant to IMP, AMC, CN, AM, F, E, and SXT. *E. coli* as well as *Enterococcus* spp. isolates recovered from raw water, DAF, disinfection, filtration, and GAC stages were multidrug resistant all seasons. *E. coli* isolates from raw water, DAF, disinfection, filtration, and GAC during spring were resistant mainly to IMP, AMC, AM, VA, F, E, and SXT, but *Enterococcus* spp. isolated from raw water, DAF, disinfection, and filtration indicated resistance to IMP, AMC, LEV, CIP, AM, VA, F, E, and SXT. *E. coli* and *Enterococcus* spp. were isolated in summer throughout the treatment train, resistance to IMP, AMC, AM, F, E, and SXT was still predominant. Isolates from raw water, DAF, disinfection, and filtration remained resistant to a variety of antibiotics in winter, including IMP, AMC, LEV, AM, VA, F, E, and SXT. In autumn, both *E. coli* and *Enterococcus* spp. resistance to multiple antibiotics including IMP, AMC, AM, VA, F, E, and SXT were observed in raw water, DAF, disinfection, and filtration stages. MAR indices varied between 0.5 and 1.0 across all treatment stages, indicating extremely high multidrug resistance and suggesting that the treatment steps, which included advanced treatment stages such as GAC and disinfection, were unable to effectively remove antibiotic-resistant bacteria.

Table 4.6 : Antibiotic resistance of bacteriological indicators in DWTP C of Northwest Province

Sampling points	Isolates	Season	Inhibition zone (mm)										Status	MAR-Index
			Imp	AMC	Lev	CIP	CN	AM	VA	F	E	SXT		
Raw	*E. coli*	Spring	0	15	32	42	18	0	0	0	0	0	MDR	0.8
Raw	*E. coli*	Summer	15	0	30	37	18	0	10	0	10	15	MDR	0.8
Raw	*E. coli*	Winter	0	10	11	23	21	0	0	0	0	0	MDR	0.8
Raw	*E. coli*	Autumn	18	15	23	27	20	0	0	20	0	0	MDR	0.6
Raw	*Enterococcus* spp.	Spring	15	10	22	25	20	0	15	20	35	0	MDR	0.5
Raw	*Enterococcus* spp.	Summer	9	0	23	25	15	0	10	0	0	0	MDR	0.8
Raw	*Enterococcus* spp.	Winter	11	10	21	22	18	0	12	0	0	0	MDR	0.8
Raw	*Enterococcus* spp.	Autumn	7	0	21	30	16	0	0	0	0	0	MDR	0.7
DAF	*E. coli*	Spring	16	10	9	26	14	0	0	17	0	0	MDR	0.9
DAF	*E. coli*	Summer	13	0	10	0	17	0	0	0	0	0	MDR	0.9
DAF	*E. coli*	Winter	0	0	15	0	18	0	10	0	10	0	MDR	0.9

DAF	*E. coli*	Autumn	0	0	10	0	15	10	12	0	0	0	MDR	1
DAF	*Enterococcus* spp.	Spring	0	9	22	24	9	0	0	15	0	0	MDR	0.8
DAF	*Enterococcus* spp.	Summer	12	0	17	22	15	0	0	0	0	0	MDR	0.8
DAF	*Enterococcus* spp.	Winter	10	10	15	0	10	0	15	0	0	0	MDR	1
DAF	*Enterococcus* spp.	Autumn	7	10	15	0	15	0	0	0	0	0	MDR	1
Disinfection	*E. coli*	Spring	10	10	20	20	20	0	0	0	0	0	MDR	0.7
Disinfection	*E. coli*	Summer	15	0	20	29	20	0	0	0	0	25	MDR	0.6
Disinfection	*E. coli*	Winter	10	10	15	0	15	0	0	0	0	0	MDR	1
Disinfection	*E. coli*	Autumn	7	10	15	0	10	0	10	0	0	0	MDR	1
Disinfection	*Enterococcus* spp.	Spring	12	10	21	20	25	0	0	0	0	0	MDR	0.7
Disinfection	*Enterococcus* spp.	Summer	12	0	27	25	21	0	0	0	0	0	MDR	0.7
Disinfection	*Enterococcus* spp.	Winter	10	10	19	22	20	0	0	0	0	0	MDR	0.7
Disinfection	*Enterococcus* spp.	Autumn	8	10	21	0	15	0	10	0	0	0	MDR	0.9
Filtration	*E. coli*	Spring	10	8	30	31	18	0	0	0	0	0	MDR	0.8

Filtration	*E. coli*	Summer	0	0	26	29	22	0	0	0	0	0	MDR	0.7
Filtration	*E. coli*	Winter	10	0	0	21	0	0	10	0	0	0	MDR	0.9
Filtration	*E. coli*	Autumn	10	0	0	0	15	0	0	0	0	0	MDR	1
Filtration	*Enterococcus* spp.	Spring	10	0	22	0	0	15	0	0	0	0	MDR	0.9
Filtration	*Enterococcus* spp.	Summer	0	0	25	26	15	0	10	0	10	0	MDR	0.8
Filtration	*Enterococcus* spp.	Winter	0	0	0	0	0	0	0	0	0	0	MDR	1
Filtration	*Enterococcus* spp.	Autumn	0	0	0	0	0	0	0	0	0	0	MDR	1
GAC	*E. coli*	Spring	0	0	10	21	19	0	0	10	0	0	MDR	0.9
GAC	*E. coli*	Summer	11	14	30	30	13	9	0	6	0	0	MDR	0.8
ATCC25922	*E. coli*		27	18	29	34	20	18	-	22	0	23	MDR	
ATCC29212	*Enterococcus faecalis*		15	25	21	23	12	20	20	20	20	25	MDR	

MDR-Multi-drug resistance; MDR*-non-Multidrug resistance; MAR-Multiple antibiotic resistance

4.1.4 Heterotrophic plate count for the DWTPs

DWTP A

Significant difference ($p<0.05$) across the different stages of treatment was observed on the HBC (Figure 4.4). In comparison to the raw water sources, treatment stages caused reduction in the heterotrophic bacteria especially the pre-chlorination stage and sedimentation. Sludge was observed to be the highest in comparison to other treatment stages. Seasonally, significant difference ($p<0.05$) was observed across the seasons with summer and winter recording the highest value of HBC.

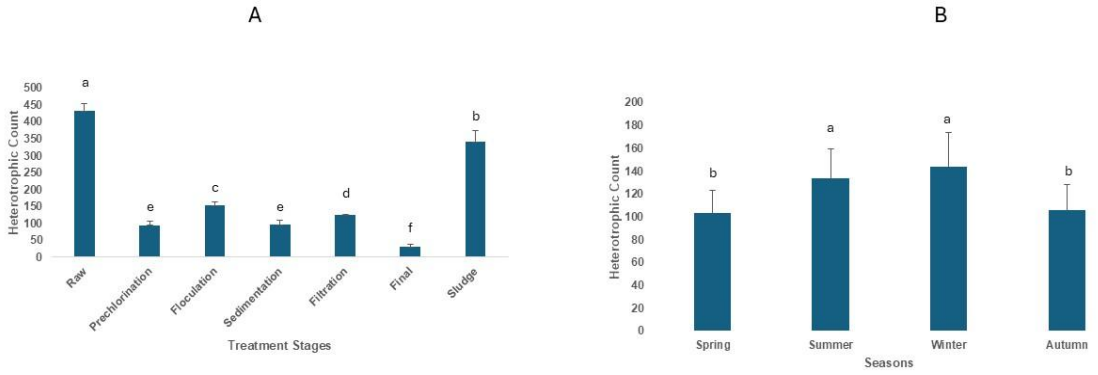


Figure 4.4: Mean value of heterotrophic plate count across the different treatment stages (A) and seasons (B) in DWTP A.

Plant B

The HBC for samples from DWTP B showed significant difference ($p<0.05$) across the various stages of treatment (Figure 4.5). There was significant reduction in the HBC in the treatment stages when compared to the raw water sources. Sludge showed a high abundance of HBC. In terms of seasons, a significant difference ($p<0.05$) was identified across the seasons, with summer and winter showing the highest levels of HBC.

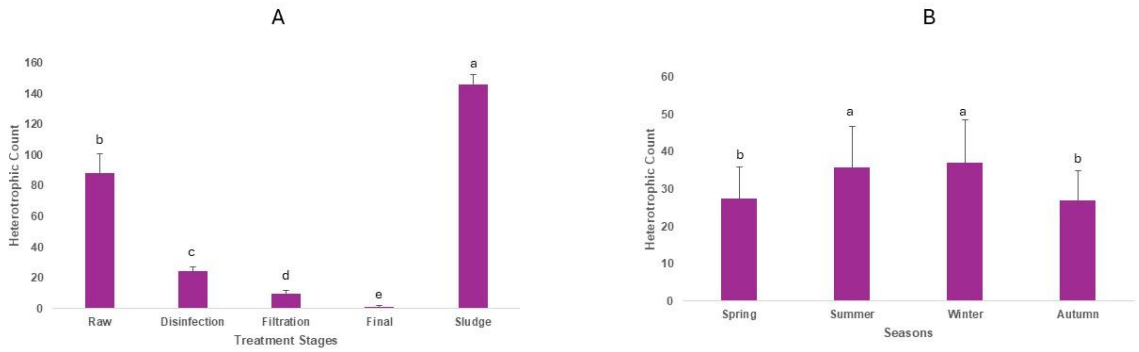


Figure 4.5: Mean value of heterotrophic plate count across the different treatment stages (A) and seasons (B) in DWTP B.

DWTP C

A significant difference ($p<0.05$) was observed across the various stages of treatment in relation to the heterotrophic plate count (Figure 4.6). The treatment stages demonstrated a significant reduction in HBC when compared to the raw water sources, with nearly undetectable levels at the final treated water. The analysis revealed the highest amount of HBC in the sludge. Seasonally, a slight difference ($p<0.05$) was identified across the seasons, with winter and autumn showing the highest levels of HBC.

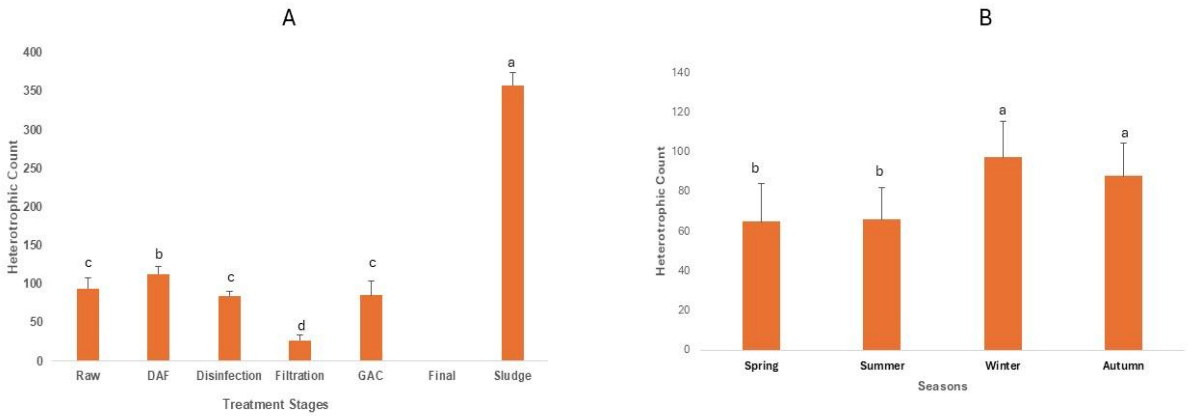


Figure 4.6 : Mean value of heterotrophic plate count across the different treatment stages (A) and seasons (B) in DWTP C.

4.1.5 Metagenomics microbial diversity assessment in DWTPs

Table 4.7 provides a summary of alpha diversity indices of DWTPs (A and B) in Gauteng and Northwest (C). The highest diversity indices according to the Shannon index were observed in the filtration stage of Plant A (2.98), disinfection stage of Plant B (1.94) and raw water source in Plant C (2.07). The indices indicated the impacts of treatment stages on the diversity and richness of the microorganisms in the DWTPs. Distribution of the three kingdoms (Archaea, Bacteria, Eukaryota) and the phyla, classes, and genera as detected in the DWTPs is discussed.

Based on shotgun metagenomic community analysis in all the plants, a total of 450453898 quality reads ranging from 2713003 to 78183780 were obtained from all the samples. Good's coverage across the samples was higher than 98%, suggesting that the sampling depth was adequate to estimate the microbial diversity containing the main microbial groups present in the water samples taken from the drinking water treatment plants. A total of 109576 OTUs (ranging from 5686 to 9814) were obtained from all the sampling plant sites (A, B and C). Both species richness (OTU, Chao1, and Evenness) and diversity estimates (Shannon and Simpson indices) varied among the treatment stages (raw water sources, filtration, disinfection, final treated water, sludge).

Table 4.7 : Microbial diversity and dynamics evaluation indices for DWTP A, B (Gauteng Province) and C (Northwest Province).

DWTP	Sampling points	Alpha diversity indices						
		Reads	OTUs	% Coverage	Evenness	Shannon	InvSimpson	chao1
(A)	Raw	50495462	9352	99.88	0.21	1.95	2.29	10194.0
	Filtration	44304102	6852	99.89	0.34	2.98	5.52	8106.06
	Disinfection	33312506	6285	99.94	0.27	2.36	2.73	7040.87
	Final treated water	48321716	8555	99.86	0.22	1.99	2.27	9475.47
	Sludge	78183780	9814	99.89	0.16	1.45	1.61	10450.7
(B)	Raw	5784777	6095	99.93	0.12	1.05	1.33	7870.1
	Filtration	23525604	8329	99.88	0.14	1.25	1.45	9757.49
	Disinfection	2713003	5686	99.93	0.22	1.94	2.02	6959.14
	Final treated water	21759126	9025	99.87	0.19	1.76	1.81	10083.8
	Sludge	21607162	7416	99.84	0.11	1	1.35	8534.84
(C)	Raw	25514037	6766	99.89	0.23	2.07	2.25	7916.11
	Filtration	23126064	7336	99.83	0.14	1.26	1.54	8438.17
	Disinfection	26988643	8844	99.9	0.08	0.69	1.23	10028.9

	Final treated water	44817916	9221	99.93	0.13	1.2	1.46	9942.54

DWTP A

Figure 4.7 provides a summary of the major phyla, classes and 25 genera for members belonging to archaea kingdom at DWTP A. Euryarchaeota and Nitrososphaerota were the two phyla which appeared the most in the treatment stages. Euryarchaeota were dominant in sludge, raw water source, disinfection stage, and filtration stage. Although Nitrososphaerota occurred in raw water sources, filtration and final treated, it was higher in the final treated water. Methanomicrobia, Halobacteria, Methanobacteria, and Nitrososphaeria were the most abundant classes. Halobacteria occurred the most in disinfection stage, final treated water, and raw water sources. Methanomicrobia were highest dominance in sludge and filtration stage. Methanobacteria showed highest abundance in the raw water sources and filtration stage. The abundance of Nitrososphaeria was observed in raw water source. At genera level, twenty-five most abundant genera were represented. Clustering based on the dendrograms showed similarity in the genera across the treatment stages. Final treated water and disinfection stages clustered together showing similar composition of the genera. Further, the raw water sources and sludge clustered together showing similarity in the abundance of *Halovivax*, *Methanobrevibacter*, *Halapricum*, *Halobaculum*, *Salinirubellus*, and *Natrinema*.

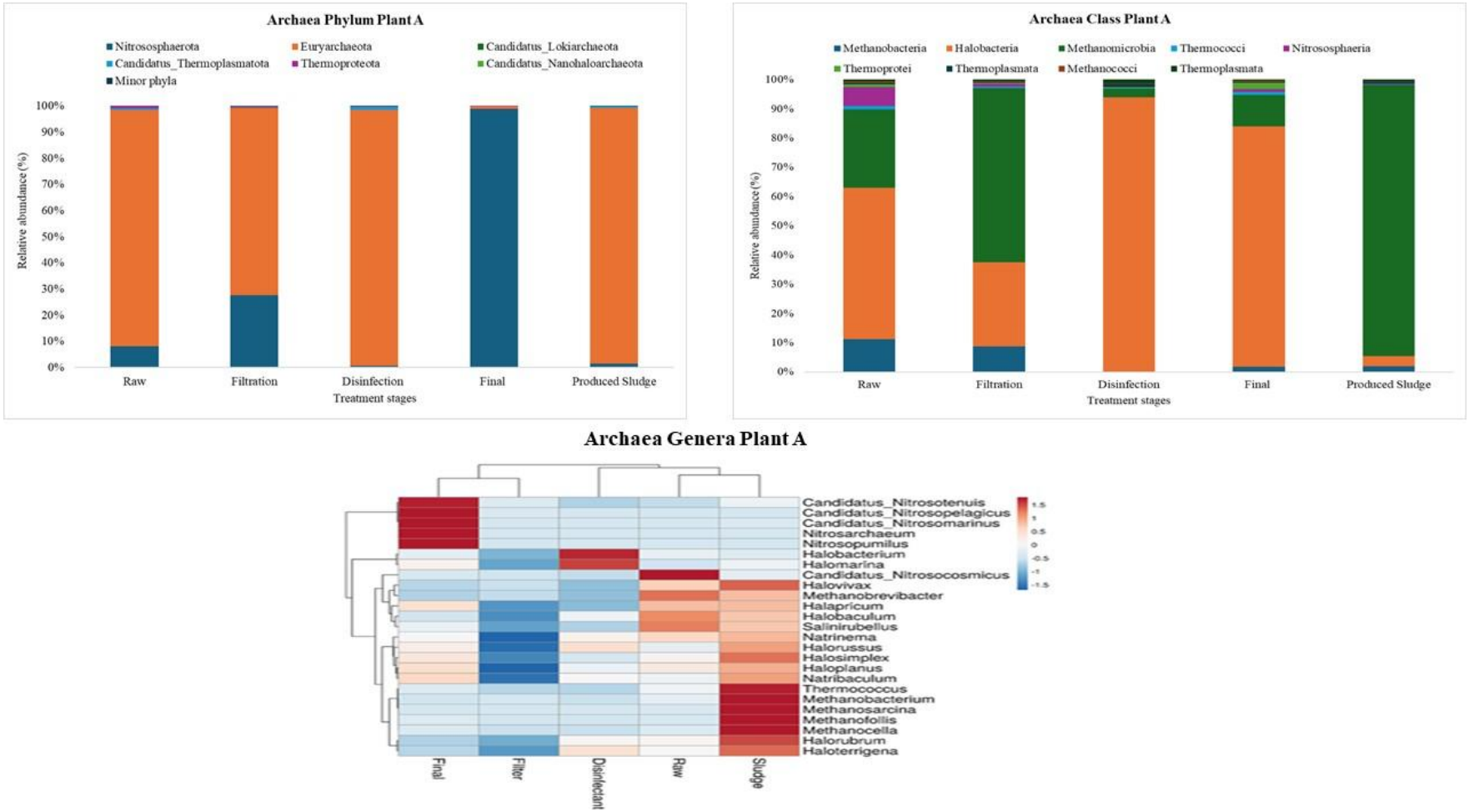


Figure 4.7 : Archaea phyla, classes and genera in Plant A (DWTP A) of the Gauteng Province.

In Figure 4.8 the occurrence of bacterial phyla, class, and genera of DWTP A were represented. At the phylum level, Pseudomonadota, Actinomycetota, and Bacteriodota were the most present in almost all the treatment stages. In comparison to the raw water source, Pseudomonadota were higher in disinfection stage, filtration stage, sludge, and final treated water. Actinomycetota occurred more in raw water sources than other treatment stages (disinfection and filtration stages), sludge, and final treated water. The abundance of Bacteriodota was observed in raw water source and filtration stage. Actinomycetes (occurred most in raw water source), Gammaproteobacteria (occurred most in final treated water), Alphaproteobacteria (occurred most in disinfection stage), and Betaproteobacteria (occurred most in sludge) were the abundant class members. At the genera level, final stage and sludge clustered together showing similarities in the abundance of *Rhodoferax*, *Acidovorax*, *Polynucleobacter* and *Caulobacter*. In addition, filtration stage and disinfection stages also similarities based on the clustering of the dendrogram.

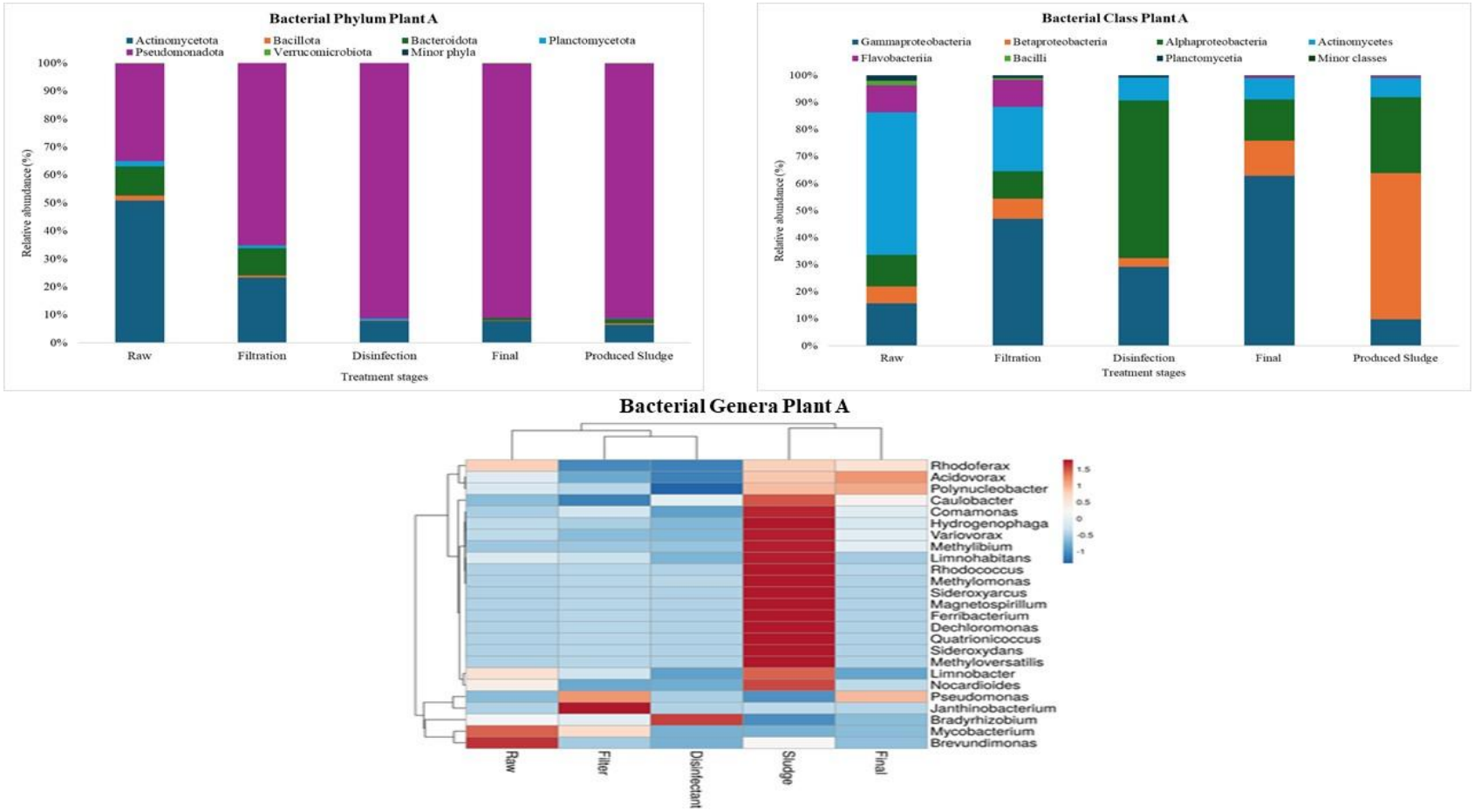


Figure 4.8 : Bacterial phyla, classes and genera in Plant A (DWTP A) of the Gauteng Province.

Figure 4.9 illustrates the occurrence of eukaryotic phyla, classes, and genera of DWTP A. The phyla that appeared in almost all treatment stages included Euglenozoa, Ascomycota, and Chordata. Euglenozoa predominantly showed high appearance during the disinfection stage, although it was also present in the sludge stage, filtration stage, final stage, and raw water source. Chordata showed a higher dominance during the filtration stage in comparison to the other stages. In the analysis at the class level, it was observed that Kinetoplastea, Mammalia, and Sordariomycetes were prevalent across all samples. Kinetoplastea predominantly appeared during the disinfection stage, while Mammalia were observed in the filtration stage, and Sordariomycetes were present in the raw water source. Saccharomycetes was prevalent in nearly all the samples, though in minimal abundance. At the genera level, raw water and sludge showed clustering, revealing significant similarities in the high abundance of *Dictyostelium, Trichomonas, Candida, Brettanomyces, Fusarium, Colletotrichum, Puccinia, Neurospora, Aspergillus,* and *Sporisorium*. Moreover, the disinfection stage showed a distinct microbial composition.

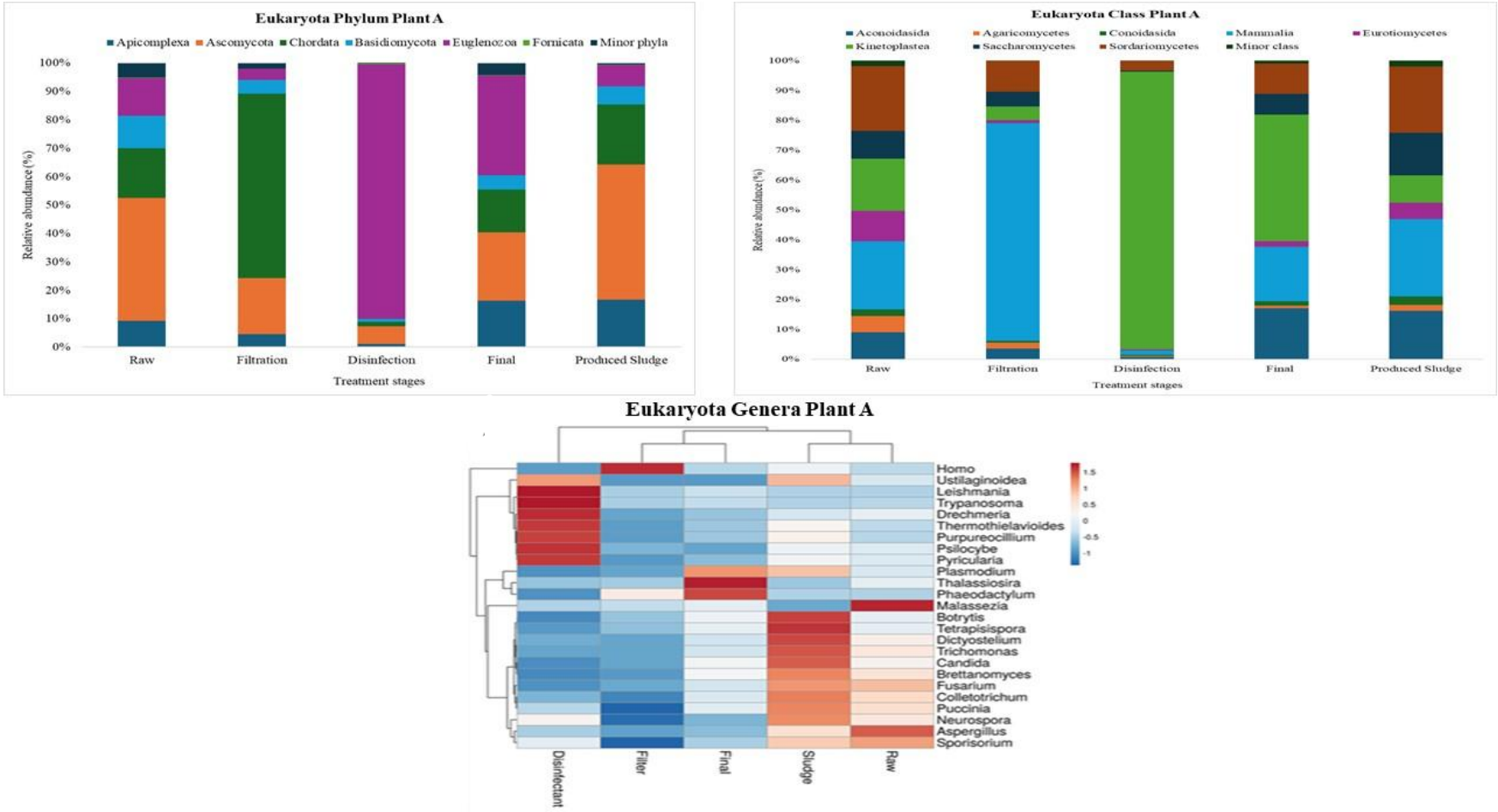


Figure 4.9 : Eukaryota phyla, classes and genera in Plant A (DWTP A) of the Gauteng Province.

DWTP B

Figure 4.10 illustrate the occurrence of the major phyla, classes and genera for members of archaea kingdom at DWTP B. Euryarchaeota and Nitrososphaerota were the most present phyla in all the samples. Euryarchaeota was mostly high in the filtration stage, disinfection stage, raw water source and sludge whereas Nitrososphaerota was mostly high in the final treated water. At class level, Halobacteria was noted in all the samples but highly dominant in the filtration stage. Similarly, Methanomicrobia was also noted in all the samples with high dominance in final treated water. Furthermore, Methanobacteria showed some dominance in all the samples though it was minimal. In the genera level, raw water stage and final stage clustered together showing similarities in the abundance of *Methanothrix.*

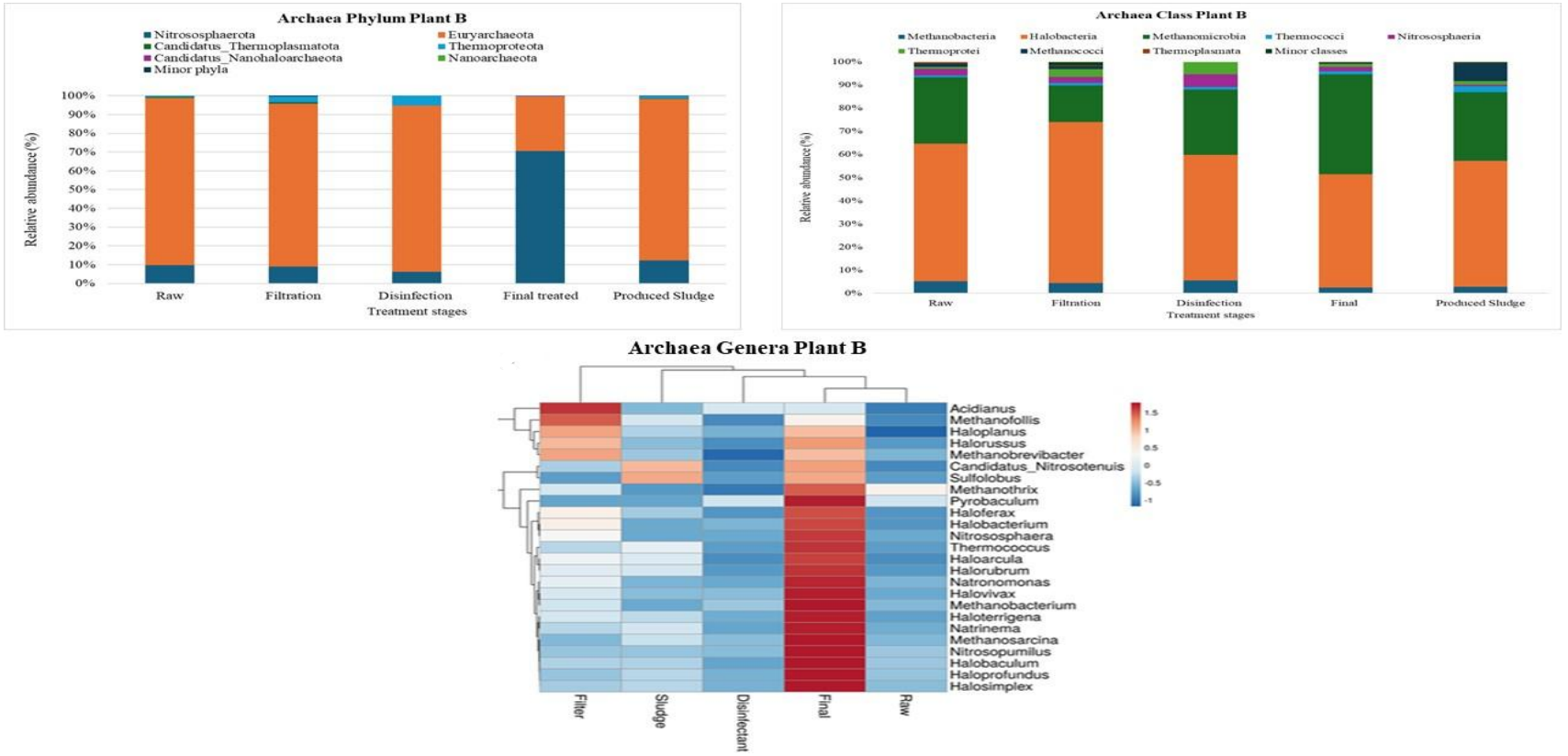
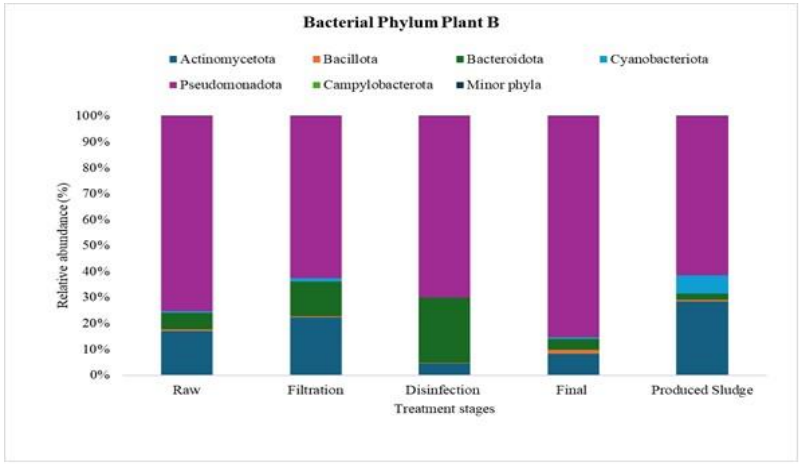
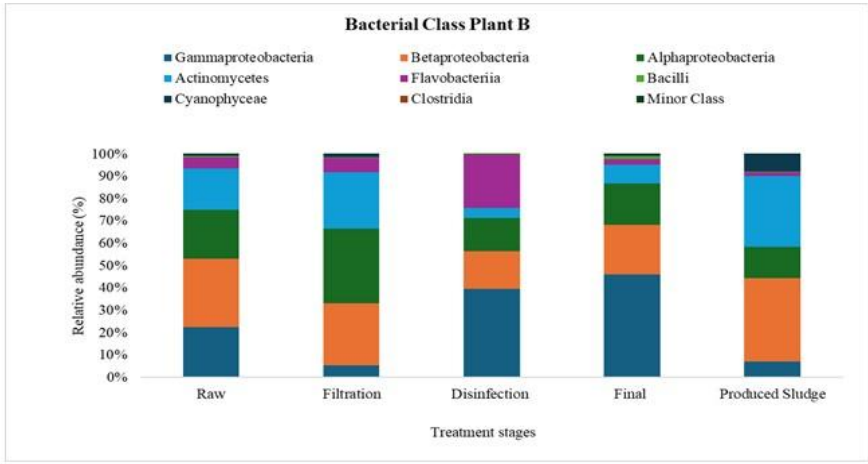


Figure 4.10 : Archaea phyla, classes and genera in Plant B (DWTP B) of the Gauteng Province.

Figure 4.11 illustrates the occurrence of various bacterial phyla, classes, and genera in DWTP B. The phyla which appeared in almost all the treatment stages were Pseudomonadota, Actinomycetota, and Bacteroidota. Pseudomonadota showed high abundance across all samples, peaking in the final treated water. Actinomycetota showed high abundance in the sludge. Lastly, Bacteroidota was mostly dominant in the disinfection stage. At the class level, both Betaproteobacteria and Actinomycetes showed high appearance in sludge stage, whereas Gammaproteobacteria was mostly dominant in the final treated water, although it was present across all samples. Flavobacteria was also dominant during the disinfection stage. At the genera level, raw water and sludge showed clustering, indicating significant similarities in the high abundance of *Halomonas*.





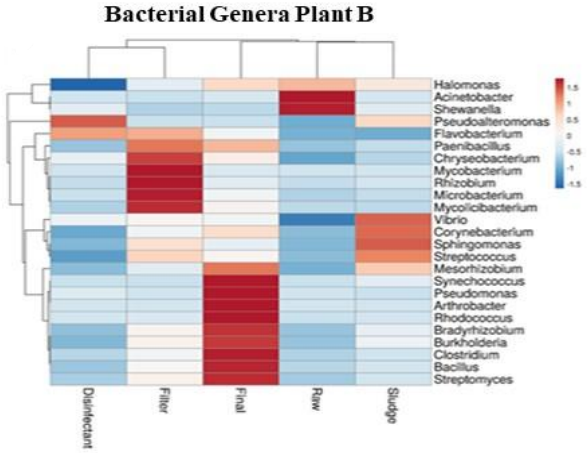
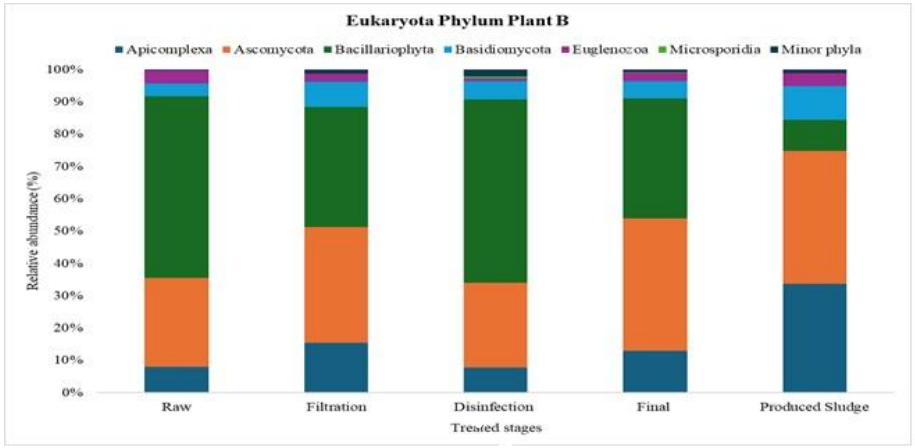
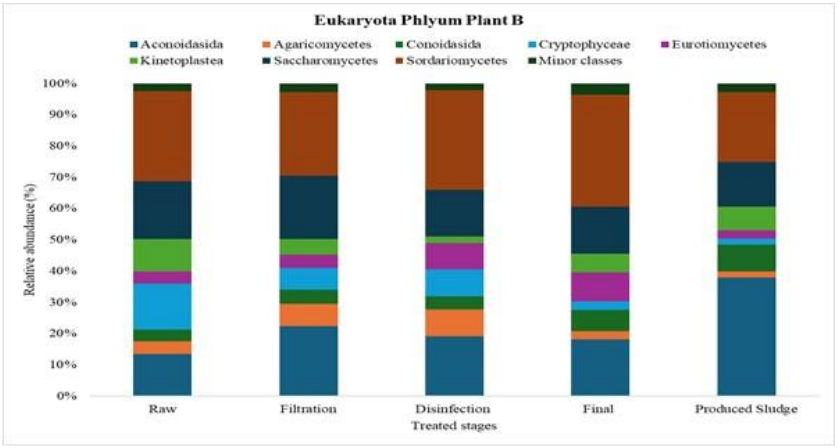


Figure 4.11 : Bacterial phyla, classes and genera in Plant B (DWTP B) of the Gauteng Province.

Figure 4.12 shows occurrence of eukaryota phyla, classes, and genera in DWTP B. Bascillariophyta, Ascomycota and Apicomplexa were the mostly noted phyla in treatment stages. Bascillariophyta dominated mostly in raw water source and disinfection stage. Ascomycota dominated mostly in the final treated water and sludge and lastly Apicomplexa was highly dominant in the sludge. At the class level, Sordariomycetes appeared in all the samples but highly dominant in the final treated water, Aconoidasida was highly dominant in the sludge, Saccharomycetes was found highly dominant in the filtration stage. Lastly, Cryptophyceae was highly dominant in the raw water source. In the genera level, raw and final stage clustered together showing similarities in the abundance of *Sugiyamaella*.





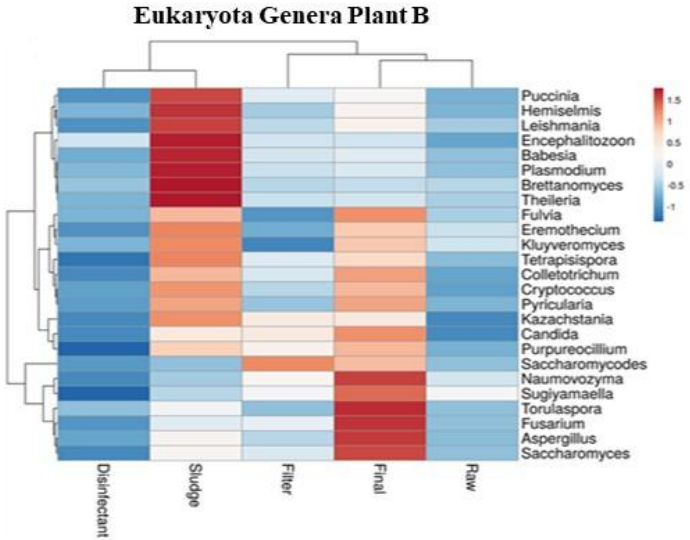


Figure 4.12 : Eukaryota phyla, classes and genera in Plant B (DWTP B) of the Gauteng Province.

DWTP C

Figure 4.13 provides a summary of the occurrence of the major phyla, classes, and genera for members belonging to archaea kingdom in DWTP C. Nitrososphaerota and Euryarchaeota were noted to be most present phyla in the treatment stages. Nitrososphaerota was dominant in all the samples but high in the final stage, whereas Euryarchaeota was high in raw water source though it was also present in all the samples. At the class level, Halobacteria and Methanomicrobia dominated in all the samples where Halobacteria occurred mostly in raw water source and Methanomicrobia occurred most in sludge. At the genera level, twenty-five most abundant genera are presented. Clustering based on the dendrograms showed how similar the genera were across the treatment stages. The raw water sources and final treated water clustered together showing similarity in the abundance of *Haloprofundus, Halosimplex, Halobaculum, Natronomonas, Halorubrum, Haloterriaena, haladaptatus, Natrinema* and *Haloarcula.* Furthermore, sludge and disinfection stage also clustered together showing abundance in *Methanobacterium* and *Methanobrevibacter.*

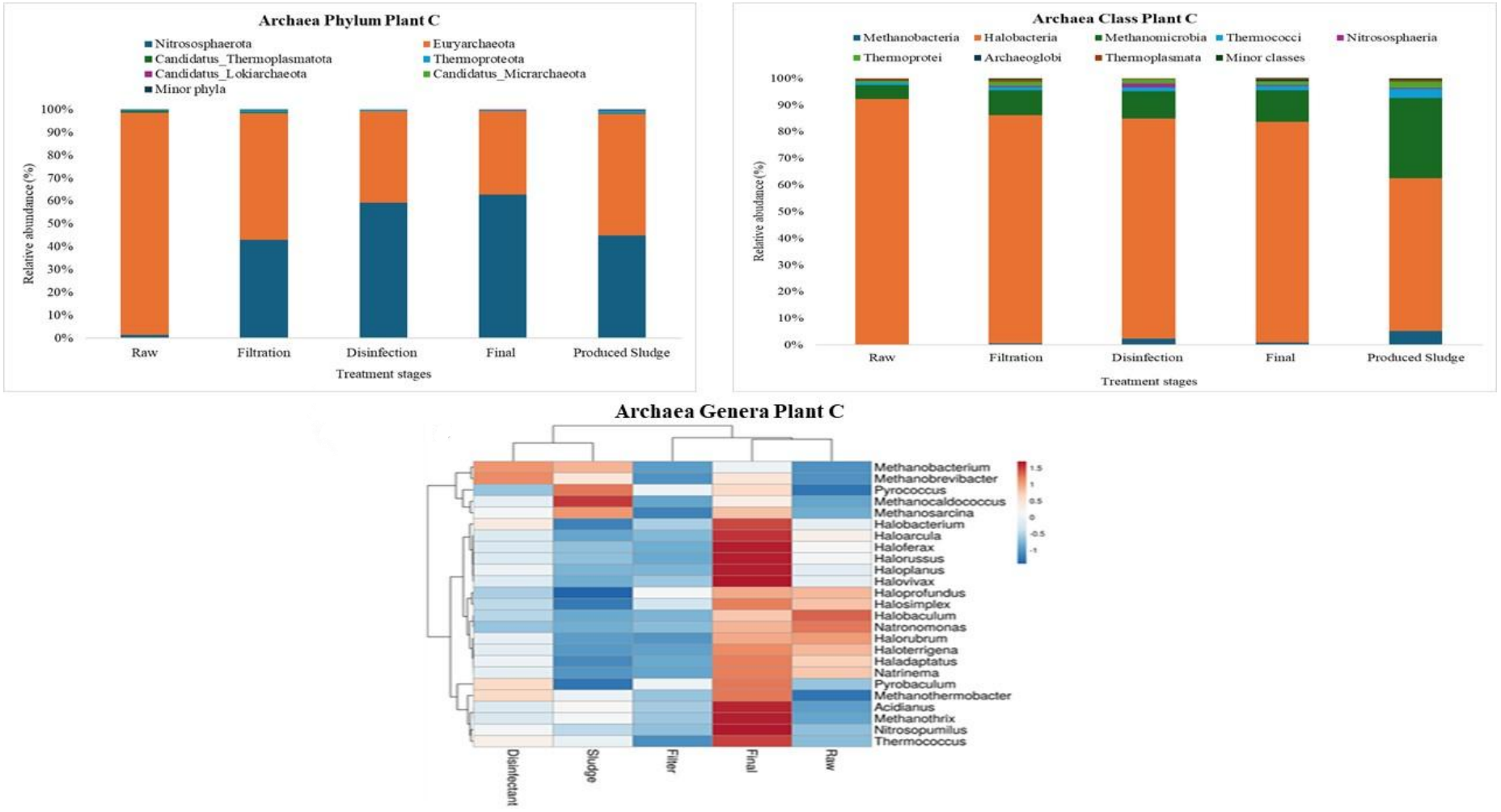


Figure 4.13 : Archaea phyla, classes and genera in Plant C (DWTP C) of the Northwest Province.

Figure 4.14 presents the comparative prevalence of different bacterial phyla, classes, and genera in DWTP C. The phyla identified to be present in most treatment stages were Pseudomonadota, which showed dominance across all samples, particularly in raw water source. Actinomycetota were predominantly found in the filtration stage, while Bacteroidota were mostly found in the sludge. In terms of class level, the most prevalent class identified were Alphaproteobacteria, Betaproteobacteria, Actinomycetes, and Gammaproteobacteria. The various classes of bacteria were prevalent throughout all samples, with variation across the samples. Alphaproteobacteria dominated in raw water source, Betaproteobacteria were predominant in final treated water, Actinomycetes showed high abundance in the filtration stage, and Gammaproteobacteria were mostly present in the disinfection stage. In terms of genera stage, final and disinfection stage clustered together showing similarities in the abundance of *Paenibacillus*.

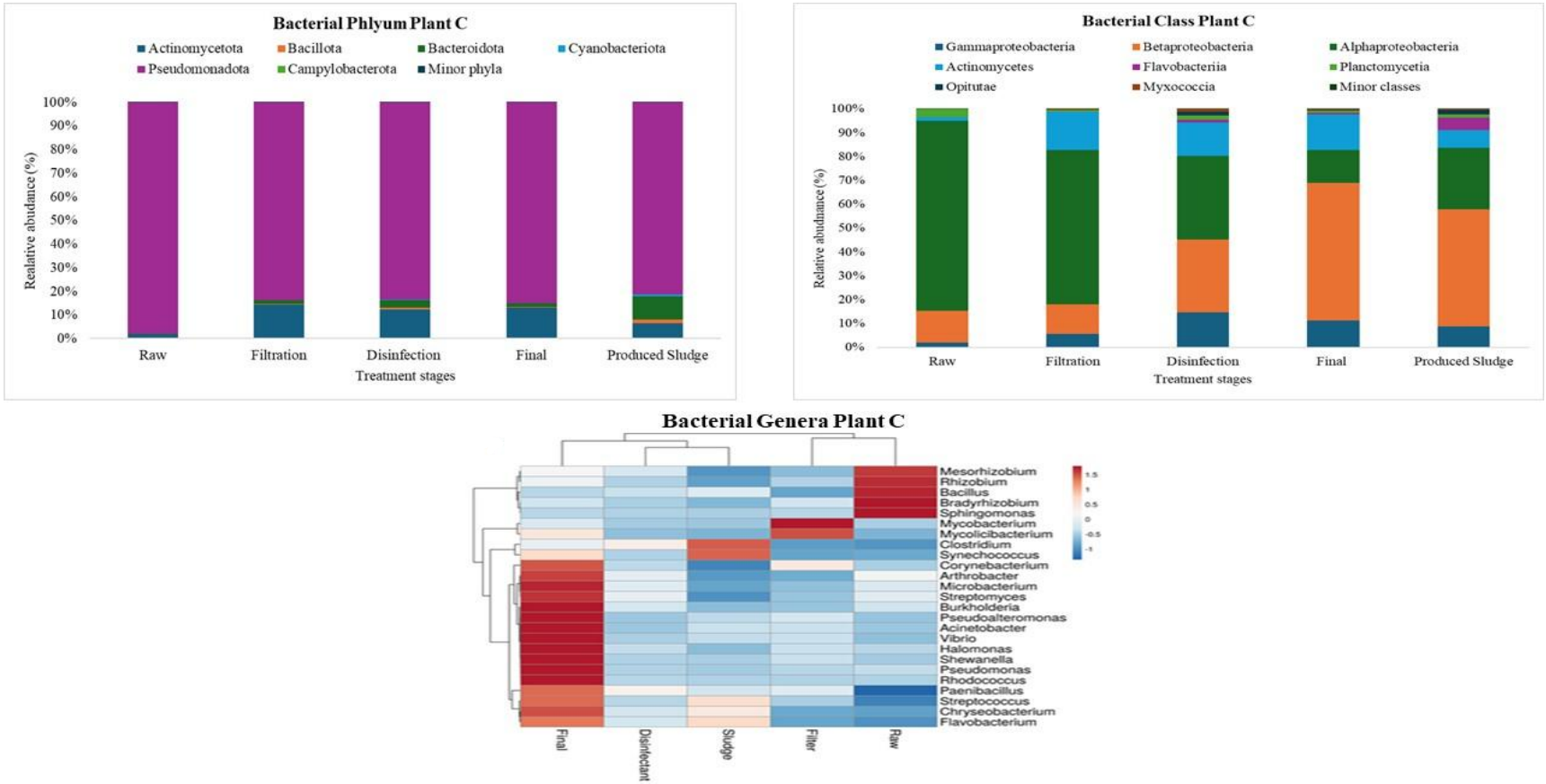


Figure 4.14 : Bacterial phyla, classes and genera in Plant C (DWTP C) of the Northwest Province.

Figure 4.15 illustrates the occurrence of eukaryotic phyla, classes, and genera in DWTP C. The phyla that were identified to be the most dominated in high abundance were Ascomycota, which was present throughout all the samples but was particularly prominent in the raw water source. Bacillariophyta and Apicomplexa were both highly dominant in the final treated water, while Chordata was mostly prevalent in the filtration stage. At the class level, Sordariomycetes appeared the most in raw water source, while Aconoidasida demonstrated high dominance in the final treated water. At the genera level, the raw water sources and final stage showed a clustered together, showing similarity in the abundance of *Pyricularia*.

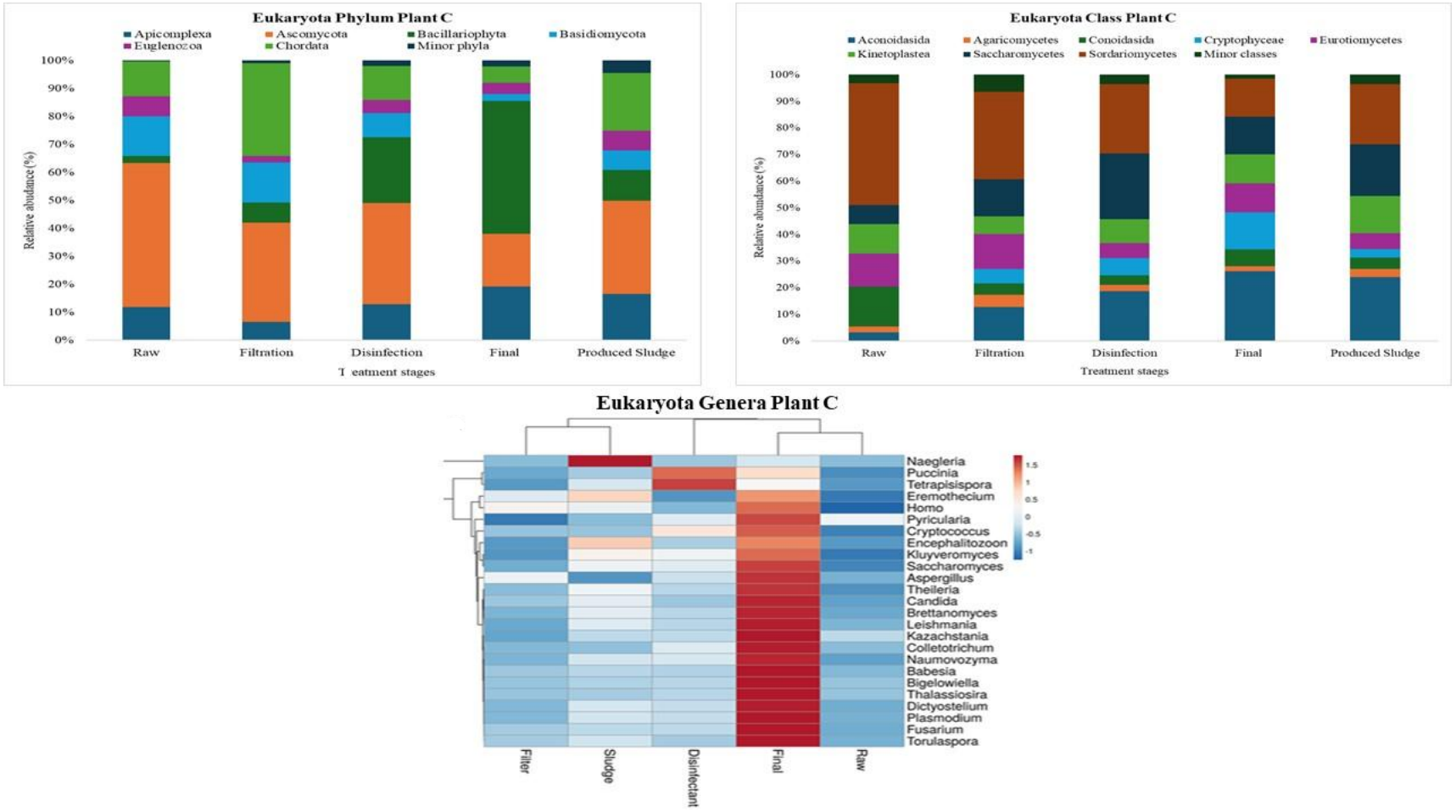


Figure 4.15 : Eukaryota phyla, classes and genera in Plant C (DWTP C) of the Northwest Province.

4.1.6 Functional Metagenomes across the three DWTPs

Metabolism, genetic information processing, organismal system, cellular processes, human diseases, and environmental information processing were the major functional metagenomes evaluated and identified across the raw water sources, filtration stages, disinfection stage, final treated water, and sludge of DWTP (A, B and C).

Variation in the presence of these functional metagenomes occurred in each of the treatment stages (Figure 4.16 A, B, C). Similarity in functional profiles between the stages were observed based on the clustering. Raw water and filtration clustered together having in common organismal system and cellular processes in abundance in DWTP A (Figure 4.16A). While in DWTP C, clustering patterns indicate that raw water source and filtration stage share similar metagenomic profiles, with a high prevalence of organismal systems and genetic information processing (Figure 4.16B). A further similarity was noted in DWTP B where sludge and the final stage formed a separate group, dominated by metagenomes associated with cellular processes and environmental information processing (Figure 4.16B). Moreover, in DWTP C, sludge stands out with the highest abundance of cellular processes and metabolism, linking it closely to the disinfection stage, which also contains notable levels of human disease-related metagenomes (Figure 4.16C).

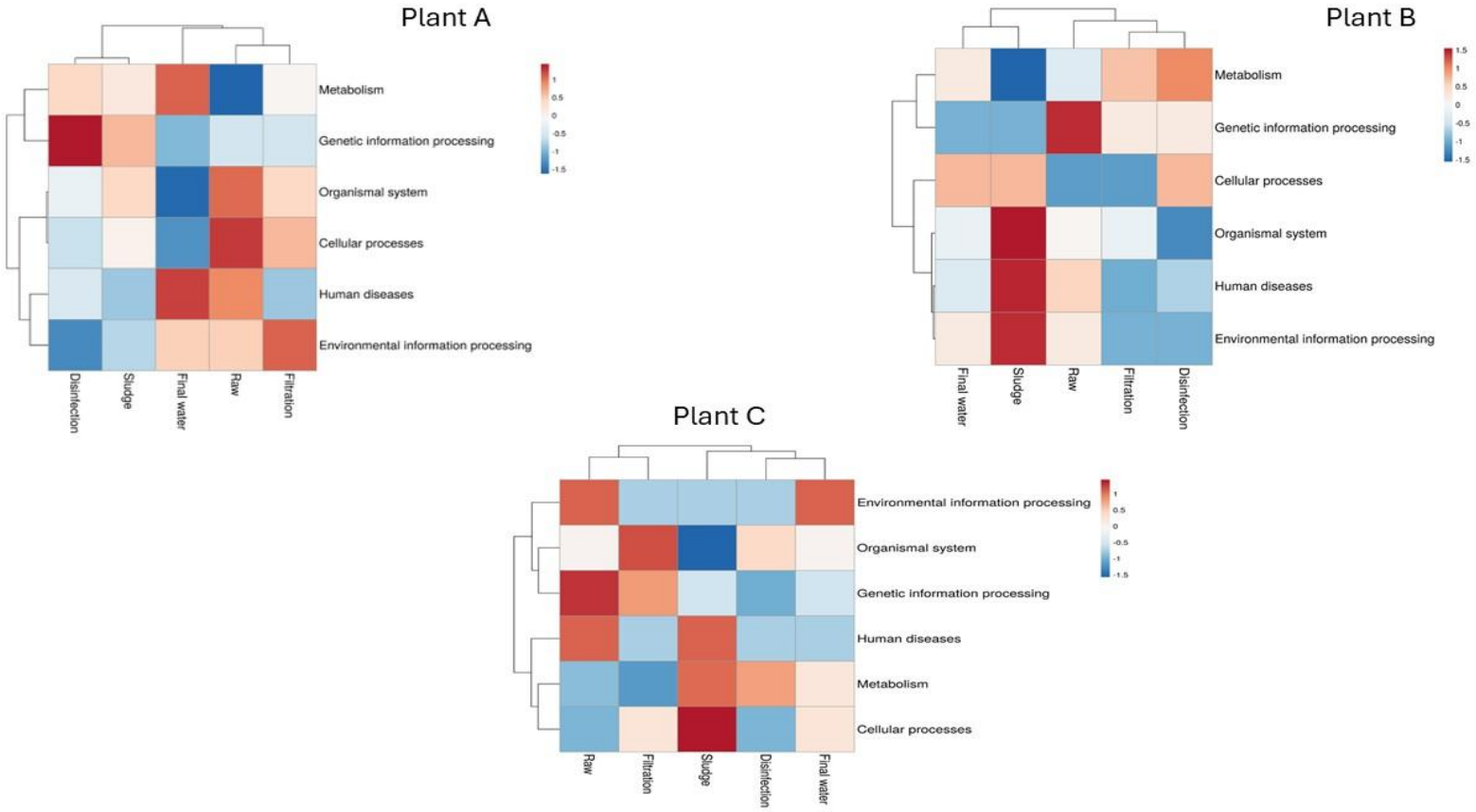


Figure 4.16: Heatmap of $\log_2$ normalized counts of the functional metagenomes across the treatment stages in the DWTPs A, B and C. The dendrogram shows complete-linkage agglomerative clustering based on a Euclidean distance.

4.1.7 Influence of physicochemical parameters on microbial communities

4.1.7.1 Bacteriological indicators

Table 4.8 provides a summary of Pearson's correlation coefficient (r) (at $P<0.05$) in DWTP A between *E. coli*, *Enterococcus* spp. and total coliforms and the physicochemical parameters (pH, temperature, EC,TDS, Fe, Mn, Cu, nitrates and chloride) of the raw water sources, samples from the disinfection stage, the filtration stage, final treated water, and sludge. In raw water source , the following strong positive correlations were observed : pH and *Enterococcus* spp. (0.77); temperature and *Enterococcus* spp. (0.64); EC and *E. coli* (0.98); EC and total coliform (0.83); TDS and *E. coli* (0.90); TDS and total coliform (0.65); Fe and *E. coli* (0.62); Fe and *Enterococcus* spp. (0.96); and Fe and total coliform (0.88). In the disinfection stage, pH correlated positively with *E. coli* (0.91) and *Enterococcus* spp. (0.89); EC correlated positively with *E. coli* (0.99) and *Enterococcus* spp. (0.99); TDS correlated positively with *E. coli* (0.84) and *Enterococcus* spp. (0.81); Fe with *E. coli* (0.94) and *Enterococcus* spp. (0.91); Mn with *E. coli* (0.83) and *Enterococcus* spp. (0.80); Cu and nitrate with total coliform (0.97 and 0.98, respectively). In the filtration stage, pH, EC, and Mn correlated positively with *Enterococcus* spp. (0.84, 0.92, and 0.87, respectively); Fe and Cu with E. coli (0.78 and 1.00, respectively); Fe with total coliform (0.79). In the final treated water, temperature and nitrate correlated positively with E. coli (0.90 and 0.92, respectively); pH and Cu with *Enterococcus* spp. (0.78 and 0.90, respectively); and EC, TDS, Fe, and Mn with total coliform (0.94, 0.68, 0.90, and 0.80, respectively). In the sludge, Fe correlated positively with E. coli (0.97); pH correlated with *Enterococcus* spp. (0.79); and Mn and chloride with total coliform (0.99 and 0.88, respectively).

Table 4.8: Pearson correlation coefficient showing the interaction between the bacteriological indicators and physicochemical parameters at DWTP A of Gauteng Province.

Sampling points	Physicochemical parameter	Correlation coefficient *E. coli*	Correlation coefficient *Enterococcus spp.*	Correlation coefficient Total coliform
Raw	pH	-0,30	**0,77**	0,09
	Temperature	-0,48	**0,64**	-0,11
	EC	**0,98**	0,18	**0,83**
	TDS	**0,90**	-0,08	**0,65**
	Fe	0,62	**0,96**	**0,88**
	Mn	-0,91	-0,71	-1,00
	Cu	-0,93	0,01	-0,71
	Nitrate	-0,38	-1,00	-0,71
	Chloride	-0,45	**0,66**	-0,07
Disinfection	pH	**0,91**	**0,89**	-0,29
	Temperature	-0,09	-0,04	-0,69
	EC	**0,99**	**0,99**	-0,58
	TDS	**0,84**	**0,81**	-0,16
	Fe	**0,93**	**0,91**	-0,35
	Mn	**0,83**	**0,80**	-0,14
	Cu	-0,82	-0,84	**0,97**
	Nitrate	-0,49	-0,54	**0,98**
	Chloride	-0,90	-0,92	**0,93**
Filtration	pH	-0,89	**0,84**	0,24
	Temperature	-0,03	-0,85	-0,98
	EC	-0,80	0,92	0,40
	TDS	0,28	-0,97	-0,87
	Fe	**0,78**	0,16	**0,79**
	Mn	-0,87	**0,87**	0,29
	Cu	**1,00**	-0,50	0,22
	Nitrate	-0,87	0,00	-0,68
	Chloride	0,00	-0,87	-0,97
Final	pH	-0,93	0,78	0,16
	Temperature	**0,90**	-0,83	-0,08
	EC	-0,19	-0,76	**0,94**
	TDS	0,30	-0,98	**0,68**
	Fe	-0,08	-0,82	**0,90**
	Mn	-0,92	0,11	**0,80**
	Cu	-0,83	**0,90**	-0,06
	Nitrate	**0,92**	-0,11	-0,80
	Chloride	-0,92	0,11	**0,80**
Sludge	pH	-0,19	0,79	-1,00
	Temperature	-0,94	-0,16	-0,55
	EC	-0,94	-0,73	0,11

	TDS	-0,78	0,21	-0,80
	Fe	**0,97**	0,22	0,47
	Mn	0,11	-0,83	**0,99**
	Cu	-0,98	-0,28	-0,42
	Nitrate	-0,82	0,14	-0,75
	Chloride	-0,24	-0,97	**0,88**

EC- electrical conductivity, TDS-total dissolved solids. +0.70 or higher (very strong positive); +0.40 to +0.69 (strong positive); +0.30 to +0.39 (moderate positive) +0.20 to +0.29 (weak positive); +0.01 to +0.19 (no correlation); 0 (zero correlation); − 0.10 to − 0.19 (no relationship); − 0.20 to − 0.29 (weak negative); − 0.30 to − 0.39 (moderate negative); − 0.40 to − 0.69 (strong negative); − 0.70 or higher (very strong negative).

DWTP B

As shown in Table 4.9, in the raw water source of DWTP B, EC, TDS, Mn and Cu correlated positively with *Enterococcus* spp. (0,98; 0,99; 0,97 and 0,77 respectively), nitrate with total coliform (0,72) and lastly chloride with *E. coli* (0,83). In disinfection, only pH correlated positively with *E. coli* (0,66). In the filtration stage pH correlated positively with *E. coli* (0,69), temperature, EC, TDS and Mn correlated with *Enterococcus* spp. (0,85; 0,92; 0,96 and 0,72 respectively), Cu correlated with *E. coli* (0,98), nitrate correlated with total coliform (1,00) and lastly chloride correlated with *Enterococcus* spp. (0,94). In the final treated water, pH correlated positively with total coliform (0,74), temperature, EC and TDS correlated with *Enterococcus* spp. (0,95;0,73 and 0,89 respectively), Mn and Cu correlated with *E. coli* (0,76 and 1,00 respectively). In sludge, pH, temperature and TDS correlated positively with Enterococcus spp. (0,97, 0,92 and 1,85 respectively), Mn correlated with *E. coli* (0,72) and total coliform (0,72), Cu correlated with *E. coli* (0,94), *Enterococcus* spp. (0,91) and total coliform (0,94). Nitrate and chloride correlated with *Enterococcus* spp. (0,91 and 0,98 respectively).

Table 4.9: The interrelationship between the bacteriological indicators and physicochemical parameters at DWTP B of the Gauteng Province

Sampling points	Physicochemical parameter	Correlation coefficient *E. coli*	Correlation coefficient *Enterococcus spp.*	Correlation coefficient Total coliform
Raw	pH	0,58	-0,90	0,02
	Temperature	0,51	-0,93	0,10
	EC	0,03	**0,98**	-0,62
	TDS	-0,31	**0,99**	-0,32
	Fe	-0,95	0,45	0,58
	Mn	-0,39	**0,97**	-0,24
	Cu	-0,75	**0,77**	0,21
	Nitrate	-0,99	0,28	**0,72**
	Chloride	**0,83**	0,42	-1,00
Disinfection	pH	**0,66**	-0,88	-0,85
	Temperature	-0,87	-0,29	-0,33
	EC	-1,00	0,19	0,15
	TDS	0,52	-0,94	-0,93
	Fe	0,21	-1,00	-1,00
	Mn	-0,48	-0,76	-0,78
	Cu	0,21	-1,00	-1,00
	Nitrate	0,21	-1,00	-1,00
	Chloride	-0,48	-0,76	-0,78
Filtration	pH	**0,69**	-0,97	-0,69
	Temperature	0,03	**0,85**	-0,03
	EC	-0,11	**0,92**	0,11
	TDS	-0,24	**0,96**	0,24
	Fe	-0,06	-0,83	0,07
	Mn	0,24	**0,72**	-0,24
	Cu	**0,98**	-0,66	-0,98
	Nitrate	-1,00	0,50	**1,00**
	Chloride	-0,19	**0,94**	0,19
Final	pH	-0,21	-0,74	**0,74**
	Temperature	-0,21	**0,95**	-0,95
	EC	0,23	**0,73**	-0,73
	TDS	-0,05	**0,89**	-0,89
	Fe	-1,00	0,50	-0,50
	Mn	**0,76**	0,19	-0,19
	Cu	**1,00**	-0,50	0,50
	Nitrate	-0,40	-0,59	0,59
	Chloride	0,63	0,36	-0,36
Sludge	pH	-0,45	**0,97**	-0,45
	Temperature	0,19	**0,92**	0,19
	EC	-0,74	-0,49	-0,74
	TDS	0,22	**0,85**	0,22
	Fe	-0,53	0,52	-0,53

	Mn	**0,72**	0,11	**0,72**
	Cu	**0,94**	**0,91**	**0,94**
	Nitrate	-0,60	**0,91**	-0,60
	Chloride	0,00	**0,98**	0,00

EC- electrical conductivity, TDS-total dissolved solids. +0.70 or higher (very strong positive); +0.40 to +0.69 (strong positive); +0.30 to +0.39 (moderate positive) +0.20 to +0.29 (weak positive); +0.01 to +0.19 (no correlation); 0 (zero correlation); − 0.10 to − 0.19 (no relationship); − 0.20 to − 0.29 (weak negative); − 0.30 to − 0.39 (moderate negative); − 0.40 to − 0.69 (strong negative); − 0.70 or higher (very strong negative).

DWTP C

In raw water sources (Table 4.10), the following positive correlations were observed: pH with *E. coli* (0,64), temperature with total coliform (0,78), EC with *E. coli* (0,97) and *Enterococcus* spp. (0,99), TDS, Fe and Cu with Total coliform (0,72, 0,98 and 0,68 respectively), chloride with *E. coli* (0,73) and *Enterococcus* spp. (0,66). In the disinfection stage, EC showed positive correlation with *E. coli* (0,89) and Total coliform (1,00), TDS (0,87), Fe (0,87), Mn (0,87), Cu (1,00), nitrate (0,98) and chloride (0,96) showed positive correlation with *Enterococcus* spp. In the filtration stage, pH showed positive correlation with *E. coli* (0,92), TDS with *Enterococcus* spp. (0,89), Mn with *E. coli* (0,92), Cu with *Enterococcus* spp. (0,96), nitrate and chloride with total coliform (0,82 and 0,99 respectively). In the final treated water, pH, temperature and EC showed positive correlation with *Enterococcus* spp. (0,92; 0,95 and 0,65 respectively), TDS with *E. coli* (0,77) and Total coliform (0,94), Mn with *E. coli* (0,98) and Total coliform (0,67), Cu with *E. coli* (0,99) and chloride with Enterococcus spp. (0,87). In sludge, the following positive correlations were observed: pH with *E. coli* (0.81) and *Enterococcus* spp. (0,79), TDS with *E. coli* (0,99) and *Enterococcus* spp. (0,98), Mn with *E. coli* (0,92) and *Enterococcus* spp. (0,90), Cu with total coliform (0,77), nitrate with *E. coli* (0,94) and *Enterococcus* spp. (0,92).

Table 4.10:The interrelationship between the bacteriological indicators and physicochemical parameters for DWTP C of the Northwest Province.

Sampling points	Physicochemical parameter	Correlation coefficient *E. coli*	Correlation coefficient *Enterococcus spp.*	Correlation coefficient Total coliform
Raw	pH	**0,64**	0,56	-0,68
	Temperature	-0,75	-0,68	**0,78**
	EC	**0,97**	**0,99**	-0,95
	TDS	-0,68	-0,61	**0,72**
	Fe	-0,99	-1,00	**0,98**
	Mn	0,17	0,26	-0,11
	Cu	-0,72	-0,78	**0,68**
	Nitrate	0,35	0,26	-0,40
	Chloride	**0,73**	**0,66**	-0,77
Disinfection	pH	-0,87	-0,24	-0,59
	Temperature	-0,99	0,12	-0,84
	EC	**0,89**	-0,69	**1,00**
	TDS	0,24	**0,87**	-0,18
	Fe	-0,72	**0,87**	-0,94
	Mn	0,24	**0,87**	-0,18
	Cu	-0,28	**1,00**	-0,65
	Nitrate	-0,47	**0,98**	-0,79
	Chloride	0,02	**0,96**	-0,39
Filtration	pH	**0,92**	-0,78	0,59
	Temperature	-0,76	0,54	-0,82
	EC	0,60	-0,80	-0,63
	TDS	-0,98	**0,89**	-0,42
	Fe	0,05	-0,33	-0,96
	Mn	**0,92**	-0,77	0,61
	Cu	-1,00	**0,96**	-0,24
	Nitrate	-0,36	0,60	**0,82**
	Chloride	0,10	0,18	**0,99**
Final	pH	-0,80	**0,92**	0,11
	Temperature	-0,74	**0,95**	0,21
	EC	-0,98	**0,65**	-0,34
	TDS	**0,77**	0,16	**0,94**
	Fe	-0,24	-0,72	-0,96
	Mn	**0,98**	-0,31	**0,67**
	Cu	**0,99**	-0,59	0,40
	Nitrate	-0,28	-0,69	-0,97
	Chloride	-0,87	**0,87**	0,00
Sludge	pH	**0,81**	**0,79**	-0,91
	Temperature	-0,02	-0,06	-0,85
	EC	0,09	0,06	-0,91
	TDS	**0,99**	**0,98**	-0,63
	Fe	-0,20	-0,23	-0,75

	Mn	**0,92**	**0,90**	-0,80
	Cu	0,16	0,20	**0,77**
	Nitrate	**0,94**	**0,92**	-0,77
	Chloride	-0,95	-0,96	0,22

EC- electrical conductivity, TDS-total dissolved solids. +0.70 or higher (very strong positive); +0.40 to +0.69 (strong positive); +0.30 to +0.39 (moderate positive) +0.20 to +0.29 (weak positive); +0.01 to +0.19 (no correlation); 0 (zero correlation); − 0.10 to − 0.19 (no relationship); − 0.20 to − 0.29 (weak negative); − 0.30 to − 0.39 (moderate negative); − 0.40 to − 0.69 (strong negative); − 0.70 or higher (very strong negative).

4.1.7.2 Heterotrophic bacteria count

DWTP A

A nodal representation indicating a strong positive correlation (P<0.05) between the physicochemical parameters and HBC in the five treatment stages (Figure 4.17). In raw water sources, HBC correlated positively with pH (0.73), temperature (0.72), turbidity (0.86), and ammonia (0.71). In the disinfection stage, HBC showed a strong positive correlation (p<0.05) with EC (0.91); total hardness (0.93); total alkalinity (1); ammonia (0.97); chloride (0.96); sulphate (0.72); Al (0.87); and Zn (0.97) (Figure 4.23). In the filtration stage, HBC showed strong positive correlations (P<0.05) with only Cu (0.77). In the final water, with respect to HBC, a positive correlation with [pH (1); EC (0.75); turbidity (0.84); Mn (0.95); nitrate (0.79); and Al (0.87)] was observed. During sludge stage, the correlation with HBC only showed positive correlations with pH (1).

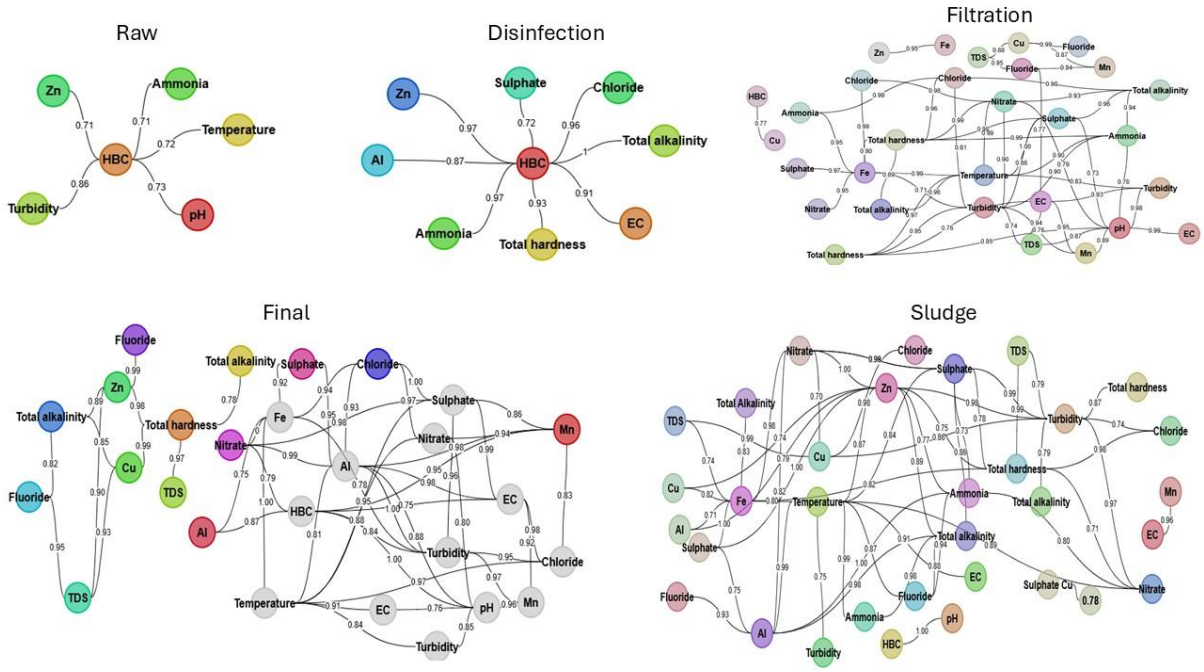


Figure 4.17: A nodal network showing positive correlation between the various measured physicochemical parameters and HBC for DWTP A. The number between

any two parameters is the correlation coefficient between those parameters. EC-Electrical conductivity; HBC-Heterotrophic bacteria count; TDS-Total dissolved solids.

DWTP B

In DWTP B, raw water sources showed positive correlations with [pH (0.94), Mn (1.00), Ammonia (0.74), Nitrate (1.00) and Al (0.99)] (Figure 4.18) During disinfection, HBC showed positive correlations with [EC (0.92), Turbidity (0.84), Fluoride (0.91), Fe (0.83), and Zn (0.96)]. In filtration stage HBC correlated positively with pH (0.73), Mn (0.75), Total alkalinity (0.79), Nitrate (0.99), Sulphate (0.71), and Zn (0.76). In the final treated water, the was no positive correlations between the HBC and the physicochemical parameters. Lastly, in the sludge stage, positive correlation with HBC was with pH (0.87), Temperature (0.84), Total hardness (0.91), Chloride (0.89), Fluoride (0.99), Sulphate (0.99) and Cu (0.97)].

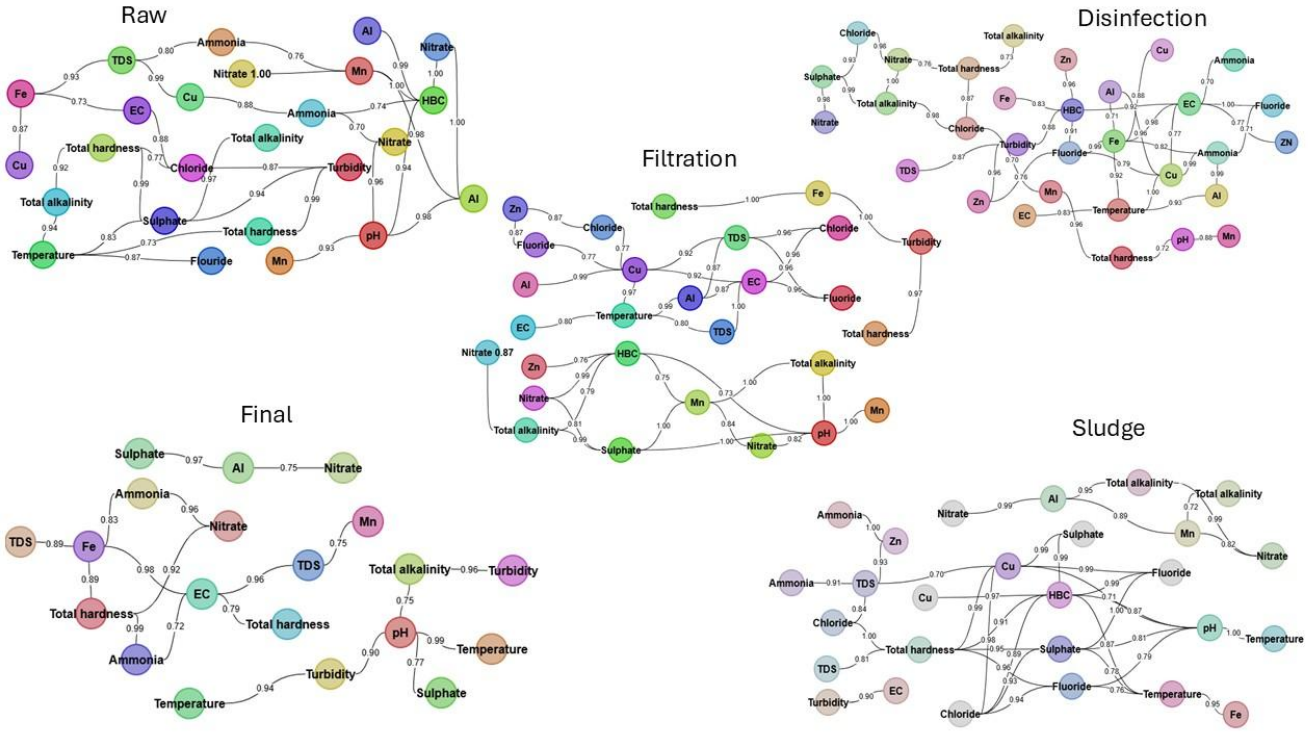


Figure 4.18: A nodal network showing positive correlation between the various measured physicochemical parameters and HBC for DWTP B. The number between any two parameters is the correlation coefficient between those parameters. EC-Electrical conductivity; HBC-Heterotrophic bacteria count; TDS-Total dissolved solids.

DWTP C

In DWTP C, raw water sources correlated with EC (0.99), Total alkalinity (0.96), Ammonia (0.91), Fluoride (0.80), Sulphate (0.97) and Zn (0.82)] (Figure 4.19). In disinfection stage, HBC showed positive correlations with Total hardness (0.82) and Zn (0.73). During filtration, a positive correlation with respect to HBC was with pH (0.97), temperature (0.96), total hardness (0.94), fluoride (0.92), sulphate (1.00) and Cu (0.92). In the final water, with respect to HBC there was no positive correlation observed. Lastly, during sludge stage, the correlation with HBC was with [Total alkalinity (0.99) and sulphate (1.00)].

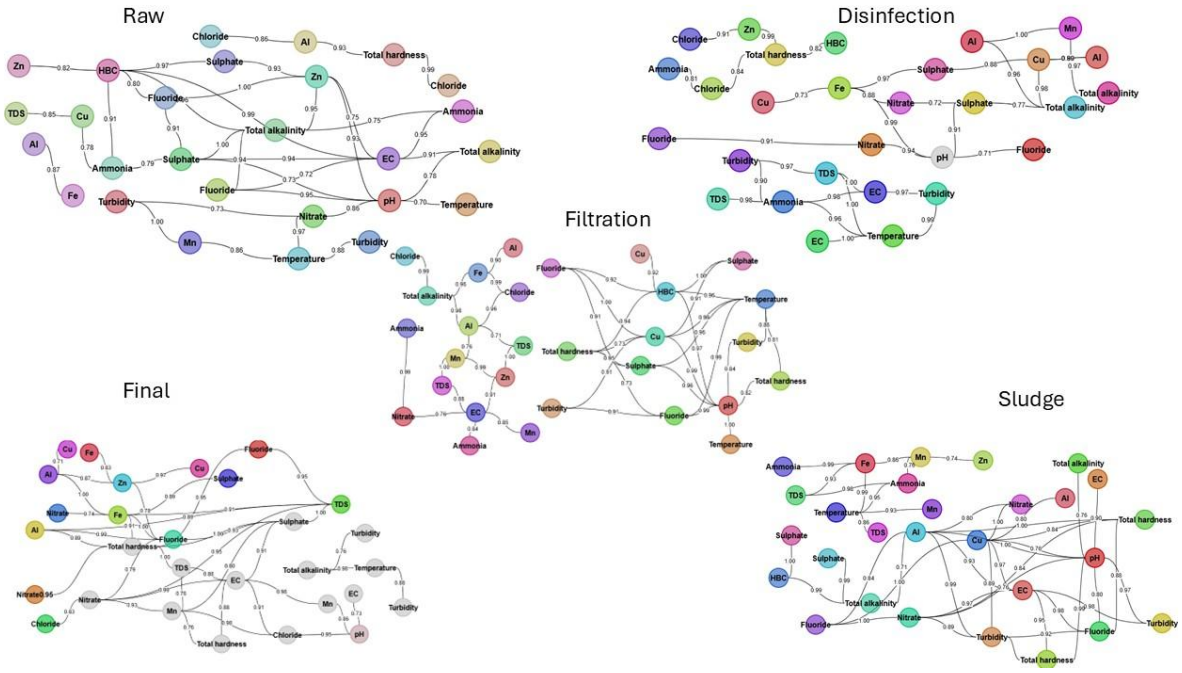


Figure 4.19: A nodal network showing positive correlation between the various measured physicochemical parameters and HBC for DWTP C. The number between any two parameters is the correlation coefficient between those parameters. EC-Electrical conductivity; HBC-Heterotrophic bacteria count; TDS-Total dissolved solids.

4.1.7.3 Shotgun metagenomics microbial diversity and correlation to physicochemical quality of samples

In DWTP A microbial Classes and the raw water source, the following significant ($P<0.05$) positive correlation were observed: pH with Clostridia and Flavobacteria, temperature with Alphaproteobacteria, Bacilli, Betaproteobacteria and Gammaproteobacteria, EC with Actinomycetes, TDS with Betaproteobacteria, Alphaproteobacteria, Bacilli and Cyanophyceae (Figure 4.20). In disinfection stage, pH showed positive correlation with Actinomycetes, Clostridia and Gammaproteobacteria, temperature with Alphaproteobacteria, Bacilli, Cyanophyceae and Gammaproteobacteria, EC and TDS with Flavobacteria. During filtration stage, pH correlated positively with Betaproteobacteria, temperature with Cyanophyceae and gammaproteobacteria, EC with Betaproteobbacteria and TDS with Bacilli and Betaproteobacteria. In final treated water, pH showed positive correlation with Betaproteobacteria,Clostridia, Flavobacteria and Gammaproteobacteria, temperature and EC with Actinomycetes, Bacilli and Cyanophyceae, TDS with Alphaproteobacteria. Lastly in sludge, pH showed positive correlation with Gammaproteobacteria, temperature, EC and TDS with Actinomycetes and Flavobacteria.

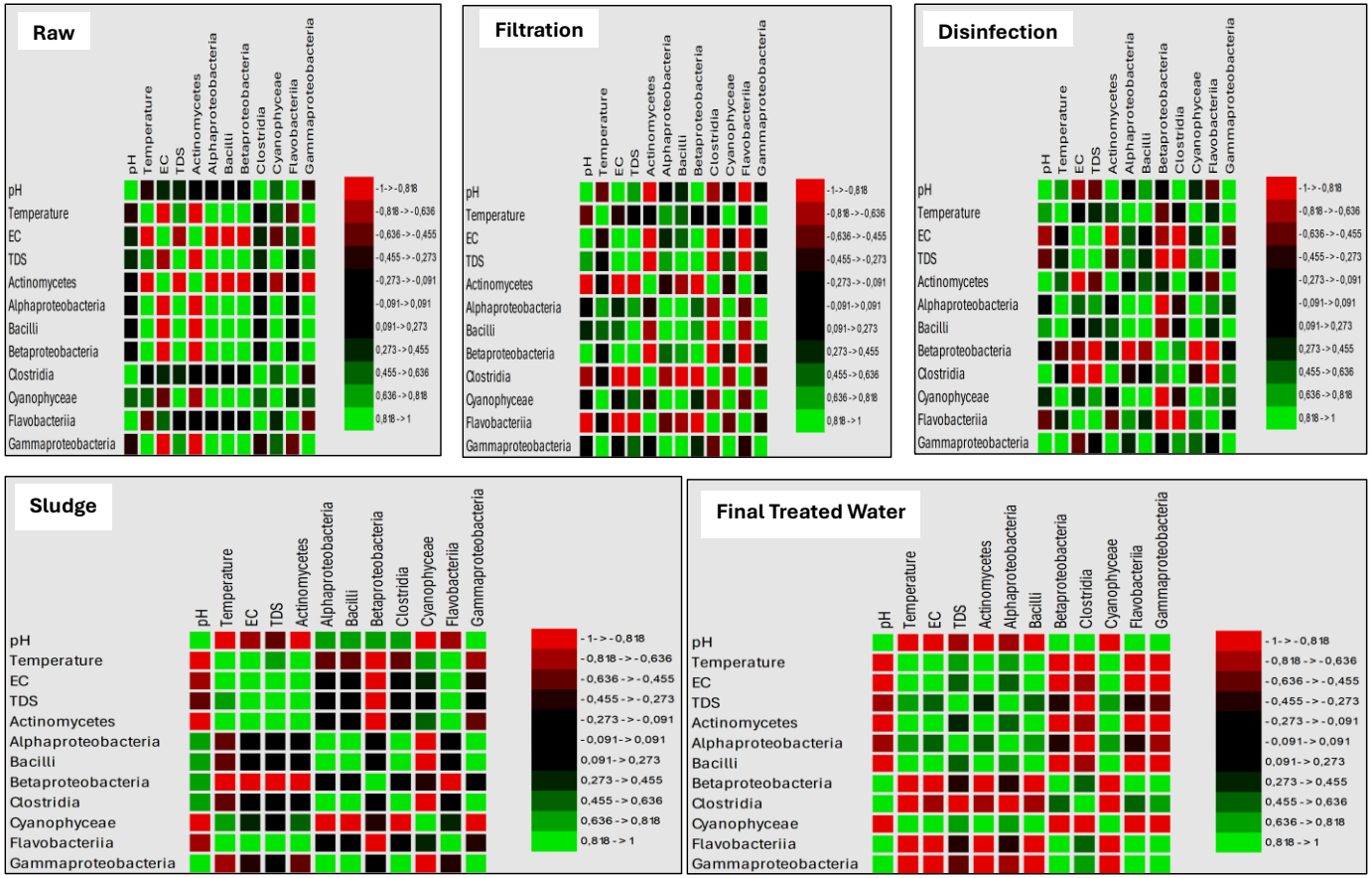


Figure 4.20: Correlation of selected physicochemical properties and bacterial communities in DWTP A of Gauteng Province.

A significant ($P<0.05$) positive correlation was discovered in the raw water source at DWTP B (Figure 4.21) between temperature and Alphaproteobacteria, EC and Actinomycetes, TDS and Alphaproteobacteria and Opitutae, however pH showed no positive correlations. In disinfection, pH showed positive correlation with Actinomycetes, temperature with Gammaproteobacteria, EC with Alphaproteobacteria, Betaproteobacteria and Myxococcia, TDS with Flavobacteria, Opitutae and Planctomycetia. In filtration,there was no positive correlarion observed with Ph while temperature showed positive correlation with Flavobacteria, and Gammaproteobacteria, EC and TDS with Actinomycetes and Betaproteobacteria. In final treated water, there were no positive correlation observed with pH and TDS, only temperature and EC showed positive correlation Alphaproteobacteria, Betaproteobacteria, Flavobacteria, Gammaproteobacteria and Planctomycetia. In sludge, pH showed positive correlation with Planctomycetia, temperature and TDS with Actinomycetes, Alphaproteobacteria, Flavobacteria and Myxococcia, EC with Betaproteobacteria and Planctomycetia.

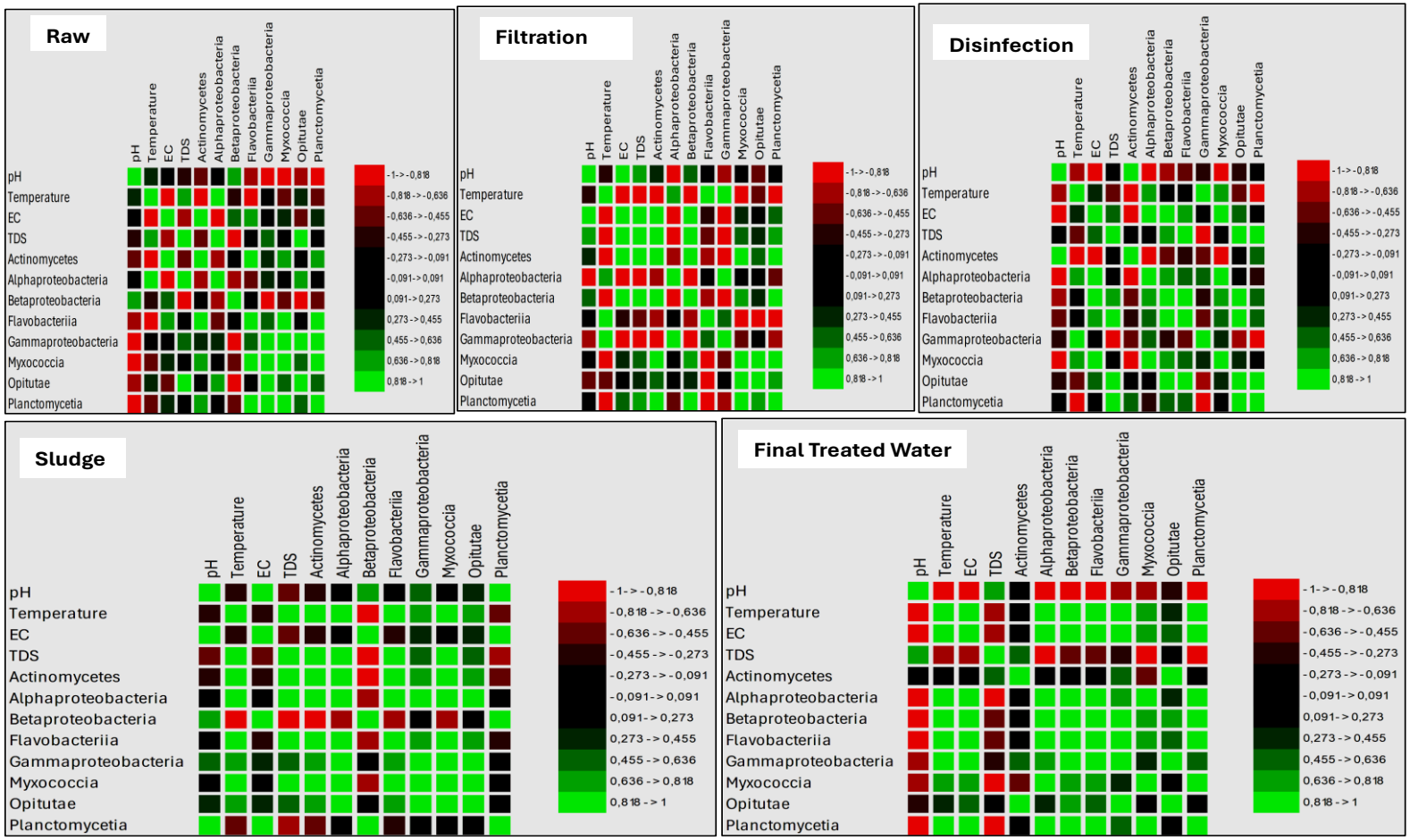


Figure 4.21: Correlation of selected physicochemical properties and bacterial communities in DWTP B of Gauteng Province.

In DWTP C (Figure 4.22) and the raw water source, the following significant ($P<0.05$) positive correlation were observed: pH with Bacilli and Gammaproteobacteria, temperature and TDS with Alphaproteobacteria and Cyanophyceae, EC with Betaproteobacteria. In disinfection, pH showed positive correlation with Flavobacteria, there was no correlation observed with temperature and TDS, EC with Actinomycetes, Alphaproteobacteria, Betaproteobacteria and Cyanophyceae. In filtration, pH showed positive correlation with Actinomycetes and Betaproteobacteria, temperature with Cyanophyceae, EC with Gammaproteobacteria, TDS with Alphaproteobacteria and Clostridia. In the final treated water, pH showed positive correlation with Alphaproteobacteria, Bacilli, Betaproteobacteria, Cyanophyceae and Gammaproteobacteria, EC with Alphaproteobacteria, Clostridia and Flavobacteria, lastly there was no positive correlation observed with TDS. In sludge, pH showed positive correlation with Betaproteobacteria, temperature and EC with Alphaproteobacteria, Flavobacteria and Gammaproteobacteria, TDS with Betaproteobacteria.

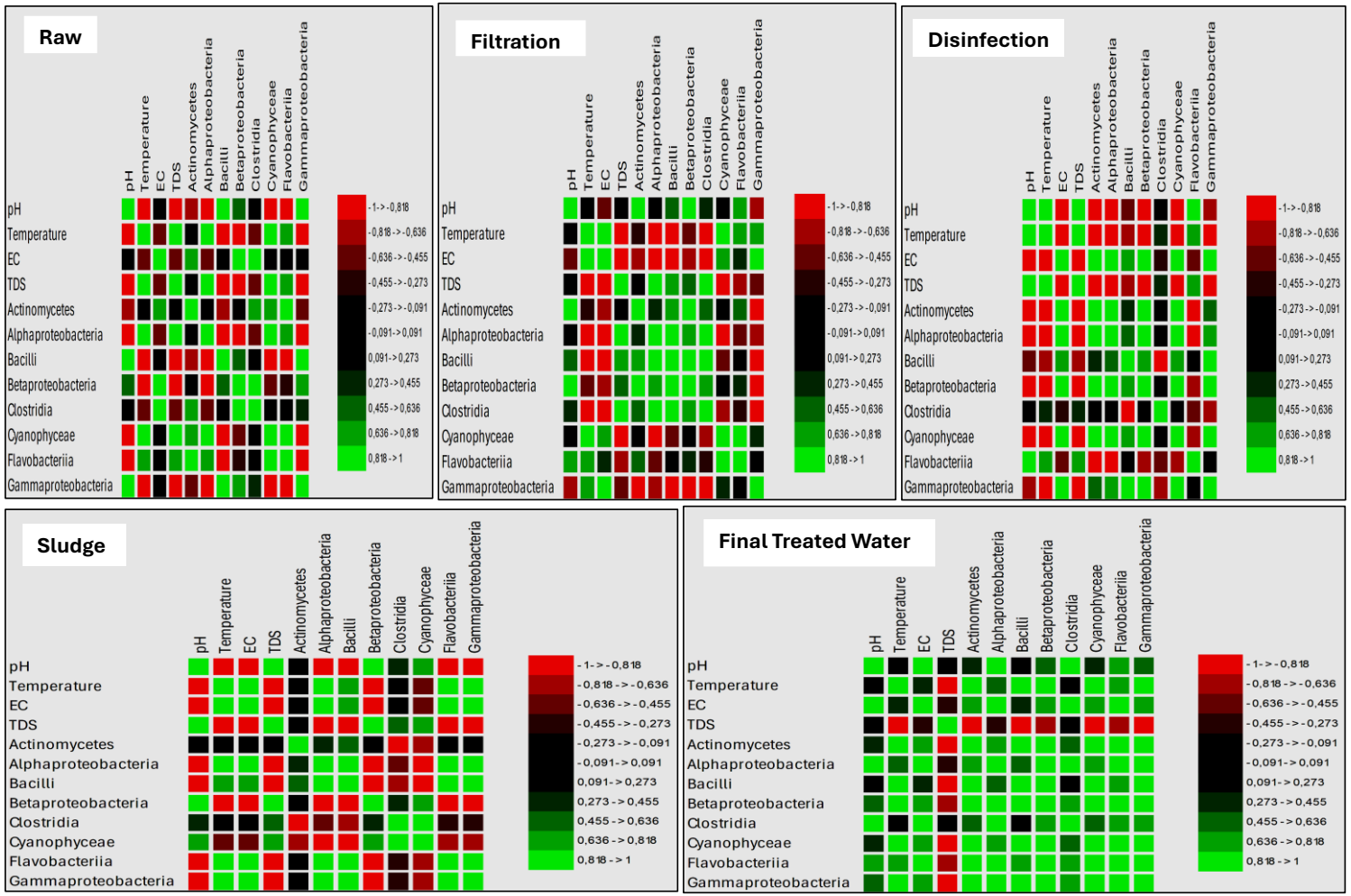


Figure 4.22: Correlation of selected physicochemical properties and bacterial communities in DWTP C of Northwest Province.

4.2 Discussion

4.2.1 Physicochemical parameters of the samples

This study revealed different seasonal and process-related trends in DWTPs A, B, and C, indicating that physicochemical characteristics vary from raw water to subsequent treatment stages. The study done by Fahes et al. (2025) on chemical, physical and microbiological analysis of drinking water in Lebanon, revealed that analogous trends, demonstrating that fluctuations in physicochemical parameters are significantly affected by the source of the drinking water and the treatment methods employed. This study noted consistent changes in physicochemical parameters during the initial treatment phases, especially in pre-chlorination and DAF, with significant variations observed in the spring and summer seasons. The notable decrease in physicochemical parameters observed seem to originate from the destabilisation of particles, oxidation reactions and the redistribution of both suspended and dissolved constituents (Viers et al., 2009). According to Cirovic et al. (2025), there are substantial links between physicochemical characteristics at different treatment stages due to the impact of the seasons and the nature of treatment reactions applied at each stage. Hence, the variations in the percentage occurrence of the physicochemical parameters in raw water could be associated with seasons or as well as treatment processes at different stages.

4.2.2 Bacteriological indicators in the drinking water samples

Enumeration of bacteriological indicators, as well as seasonal monitoring of drinking water, is crucial, as the presence of bacteria in the final treated water serves as an essential indicator of potential detrimental compromises to water quality (Tambi et al., 2023; Aydamo et al., 2024). Pinar-Méndez et al. (2022) indicated that observing bacterial communities in DWTPs could enhance understanding of their routine functions and efficiency of the treatment processes. In this study, high total coliform, *E. coli*, and *Enterococcus* spp., were recorded in raw water sources in all the DWTPs (A, B and C), particularly in spring, summer, and autumn. The abundance of the bacteriological indicators was also reflected in some of the treatment stages, i.e., flocculation and sedimentation stage, indicating the contribution of the bacterial load in the raw water sources on the subsequent treatment stages. Concurring with our findings, Pinar-Méndez et al. (2022) also discovered that high bacterial load and

diversity in the groundwater sources influenced their load on other treatment stages. In addition to the impact of raw water sources, the inherent conditions at different stages can influence the growth of these indicators (Ramírez-Castillo et al., 2015). For instance, Ramírez-Castillo et al. (2015) found that *E. coli* and *Enterococcus* spp. regrow in the sedimentation stage due to the changes in the physicochemical condition of the stage, leading to an increase in disease outbreaks. Like our study, *E. coli*, *Enterococcus* spp*.,* as well as total coliforms levels were very high during flocculation and sedimentation, particularly in spring, summer and autumn in DWTPA and B while in DWTP C, the indicators were high in the DAF unit, as well as in GAC in spring and summer. This may signify that the conditions like the different reagents, unit conditions, and physicochemical conditions in different stages could enhance the growth and regrowth of these bacteriological indicators. The decline in the quantity of the indicator was noted in later stages where in DWTP A, *E. coli* met the set limit in final treated water in winter and autumn, while *Enterococcus* spp*.* was fully compliant in the final treated water in all the seasons. In DWTP B, *E. coli* demonstrated better results during disinfection and filtration processes, as well as sludge in certain seasons. *Enterococcus* spp*.* demonstrated improved removal efficiency in the filtration stage and sludge during the spring and winter seasons, respectively. In accordance with the findings from DWTP A and B, additional studies indicate that *E. coli* concentrations generally comply with the established limits during winter, attributed to microbial regrowth and elevated chlorine residual levels (Gui et al., 2022; Domańska et al., 2023). Park et al. (2024) found that *E. coli* levels surpassed the established limits at all treatment stages, including post-chlorination, suggesting that some systems are not achieving complete bacterial removal. Lastly, at Plant C, levels of total coliforms, *Enterococcus* spp., and *E. coli* decreased after GAC filtration and in the final treated water, indicating better performance compared to what was observed in DWTP A and B. Although this study observed reductions following GAC treatment, other studies have reported opposing findings. For instance, Deck (2025) discovered that GAC filters can develop biofilms and release some bacteria into the water, resulting in high microbial count rather than removing the bacteria. To add on, Chabi et al. (2024) also reported that biological activity with GAC columns can occasionally increase heterotrophic bacteria in water.

4.2.4 Heterotrophic bacteria count for the DWTPs

Heterotrophic bacteria count (HBC) method are used to assess microbial quality of drinking water and help in determining the effectiveness or rather functionality of treatment plant (Carabin et al., 2024). The values obtained for the HBC does not directly indicate health risks but help pinpoint inefficiencies within treatment processes. The analysis in this study showed notable differences in HBC levels among all treatment plants. This could be because the processes were not as efficient or because heterotrophic bacteria could have regrown during treatment. In DWTP A and B, reductions were evident during pre-chlorination, sedimentation, and filtration; however, elevated HBC values in sludge indicate the accumulation or persistence of bacteria in solids-rich environments. Likewise, the seasonal peaks in summer and winter in these plants may be due to microbial activity and the availability of organic nutrients that depend on temperature. Other researchers discovered that high temperature and fluctuating disinfection concentration promote the regrowth of HBC in water (Waite et al., 2023). DWTP C, on the other hand, had more steady decreases in HBC, and the levels were nearly undetectable in the final treated water. This indicates improved process control, perhaps attributable to the implementation of advanced treatment barriers. These observations align with the results of Liu et al. (2020) and Ryu et al. (2021), who indicated that efficient multi-stage filtration and GAC units can markedly inhibit microbial regrowth in treated water. However, the continued presence of high HBC levels in sludge and occasional regrowth in specific treatment stages support previous research by Egli (2010) and Prest et al. (2013), which demonstrated that heterotrophic bacteria could proliferate on biofilms and within sedimentation basins, thereby diminishing overall disinfection efficiency. Additionally, the seasonal variations identified in this study align with prior research demonstrating that HBC dynamics are affected by temperature, organic matter concentration, and the decay of residual chlorine throughout the treatment process (Berry et al., 2015; Liu et al., 2020). These results collectively indicate that although HBC alone does not serve as a direct health risk indicator, its fluctuations across treatment and seasonal cycles offer essential insights into the microbiological stability and operational efficacy of drinking water treatment facilities.

4.2.3 Antibiotic responses by the indicator bacteria

Bacterial antibiotic resistance has emerged as a significant public health issue in recent years. The presence of antimicrobial compounds in drinking water treatment plants has facilitated the growth of ARBs and the spread of antibiotic resistance genes (ARGs) (Bessa et al., 2014; Duarte et al., 2022; Alegbeleye et al., 2022; Wang et al., 2026). Zhang et al. (2015) found that the largest scale of antibiotic production and consumption occurs in China, led to the spread of ARGs and ARBs across diverse environmental sources, including water, soil, and even the air. This necessitates the need to control antibiotics consumptions.

The current study illustrated the persistent presence of antibiotic-resistant *E. coli* and *Enterococcus* spp. across all treatment stages in the studied plants, indicating that even advanced treatment methods fail to entirely eradicate resistant bacteria. *E. coli* showed extensive resistance to many antibiotics, including Imipenem (IMP), Amoxicillin (AMC), Gentamicin (CN), Ampicillin (AM), Nitrofurantoin (F), Erythromycin (E), and Sulphamethoxazole (SXT) in DWTP A in most of the treatment stages. In DWTP C, both *E. coli* and *Enterococcus* spp. showed considerable resistance, even in the final treated water, indicating the continuity of antibiotic resistance throughout the entire treatment process. These results align with studies conducted by Hendriksen et al. (2019) where it was discovered that ARGs and MDR strains survive conventional chlorination and become active throughout the treatment stages, which then shows that they are resilient and are able to adapt. Similarly, studies conducted by Novo et al. (2013), Mutuku et al. (2022), and Wang et al. (2026) have demonstrated that chlorination and conventional disinfection methods often fail to completely inactivate resistant bacteria and may even promote resistance through oxidative stress and horizontal gene transfer under sub-lethal conditions. The increased resistance observed in the final treated water, especially among *E. coli* isolates resistant to IMP, AMC, CN, LEV, AM, VA, F, and SXT, may be associated with selection pressure during treatment process. In contrast, studies by Cai et al. (2021) and Luo et al. (2023) showed that treatment plants that used modern technologies like membrane filtration, UV disinfection, and granular activated carbon (GAC) systems had much lower levels of ARBs and ARGs than traditional treatment systems. This concur with the current study, as declined in the resistant bacteriological indicators were observed in GAC stage in DWTP C, emphasizing that indeed advanced treatment technologies can

effectively reduce resistance. Kalli et al. (2023) have demonstrated that even the most effective treatments may fail to eliminate all extracellular ARGs or viable but non-culturable resistant bacteria. This means that the effectiveness of these technologies depends on how they are set up, how they are used, and what kind of water they are used on. The ongoing presence of resistant *E. coli* and *Enterococcus* spp. identified in this study corroborates findings from both global and regional research (Thamer et al., 2026), underscoring the shortcomings of conventional disinfection methods and the imperative for comprehensive, multi-barrier strategies to effectively mitigate the proliferation of antibiotic resistance in drinking water treatment systems.

4.2.5 Metagenomics microbial diversity assessment in DWTPs

Organisms in full-scale water supply systems raises worries about their spread and potential dangers to human health (Zhou et al., 2021). The bacterial community in tap water is highly affected by the quality of the source water, the techniques used to treat the water, the DWDS, and the building water supply systems (BWSSs) (Wang et al., 2018; Gomez, 2019). To support this Soler et al. (2021) emphasized that changes in the form of the microbiome could throw the microbial population out of balance, which could lead to problems like an increase in pathogens, corrosive phenomena, or the appearance of smells and tastes that could make the water treatment less effective. This study employed the metagenomics technique to better understand the non-culturable microbial communities linked to the drinking water treatment processes in the current investigation.

In this study Euryarchaeota and Nitrososphaerot*a* were dominant in the raw water sources. This is consistent with global studies that demonstrate the dominance of these phyla in freshwater, wastewater, and engineered water treatment systems (Naberezhnykh et al., 2021; Vigneron et al., 2019). Euryarchaeota were most prevalent in the raw water source, filtration stage, and sludge in DWTP A and B, whereas DWTP C showed dominance solely in the raw water source. Other researchers support these findings by emphasizing that methanogenic Euryachaeota also thrive in nutrient-rich environments, which particularly would be in raw water sources and sludge (Zhang et al., 2020). Nitrososphaerota, which are ammonia-oxidizing archaea (AOA), are most common in the final treated water from all the plants. This suggests that they were able to adapt well to oligotrophic and disinfected

environments thus remain in the water. Advanced treatment systems have demonstrated comparable microbial patterns, as AOA persist due to their significant affinity for ammonia and their ability to withstand oxidative stress (Sauder et al., 2017).

DWTP A and C showed a high dominance of Halobacteria in the raw water source. This could be associated with the high TDS in the two plants that contribute to the salinity of the environment. The study carried out by Oren (2014) showed that halophilic archaea in survives in saline or mineral-rich environments. Methanomicrobia was found to be the most abundant bacterial group in the final treated water in DWTP C but also present in sludge and the filtration stage across all plants. Most researchers have emphasized that Halobacteria are mostly found in treatment plants characterized by low oxygen levels (Vigneron et al., 2019). Hence, the presence of Halobacteria in the treatment plant indicates that their process created a low oxygen level environment that promoted the growth of these archaea. Methanobacteria are considered to be anaerobes, so they are mostly dominant in environments where there is no oxygen or rather where it is very low (Conrad, 2020; Guerrero-Cruz et al., 2021). These align with observations here, where *Methanobacteria* are present in small amounts in DWTP B, indicating low oxygen levels rather than abundant availability in bulk water. *Halovivax*, *Halobaculum*, *Natrinema*, *Methanothrix*, *Methanobrevibacter* and *Halorubum* were common in both the disinfection stage and the final treated water. According to Shen and Zhou (2021), this could be attributed due to their ability to thrive and survive in advanced treatment processes.

The bacterial community showed patterns similar to those identified previously. Pseudomonadota was the most abundant phylum in all treatment stages of DWTP A and B. Their presence was high in the final treated water of Plant A and raw water source of Plant C. The presence of Pseudomonadota in final treated water was previously mainly due to poor performance of the treatment plant as well as the ability of the bacteria to modify their metabolism and resist disinfection (Hu, 2013; Bautista-de los Santos et al., 2016). Actinomycetota were identified in significant quantities in both raw water and sludge samples. This aligns with research that has connected Actinomycetes to inputs from soil and surface water, demonstrating their ability to survive in raw water sources and sludge (Huang et al., 2021). Bacteroidota, prevalent in environments rich in organic matter, were frequently observed in the raw water source and filtration stages. To support our findings, Prest et al. (2018) in their study

on monitoring changes in drinking water systems also found a decrease in Bacteroidota levels in coagulation-flocculation stages.

At the class level, Betaproteobacteria occurred both in sludge and final treated water. Liu et al. (2018) highlighted that Betaproteobacteria form biofilm, which aids in their survival. This could be attributed as the reason of their presence in the final treated water. Followed by Gammaproteobacteria, which were highly common in the final treated water and disinfection stage. According to Tong et al. (2021), this could be associated with disinfectant-resistant taxa such as Pseudomonas (Tong et al., 2021). The prevalence of Alphaproteobacteria during disinfection in DWTP A supports findings that suggest their relative resilience to chlorination (Hwang et al., 2012).

Ascomycota was very high in the final treated water of Plant B and in the raw water source of DWTP C. According to Belila et al. (2017), the notable presence of fungal communities in the final treated water demonstrates their ability to thrive well throughout different stages of the drinking water treatment process. Ascomycota are set to be phyla communities that multiply in harsh environments and can form biofilms and use various organic substrate (Behera et al., 2023). Assress et al. (2019) confirmed our findings in their study, where it was discovered that the presence of this organism in the final treated water indicates either inadequate removal during treatment or potential regrowth within the system, especially in areas with low disinfectant residuals. These findings align with the overall patterns identified by Zhang et al. (2022), who documented a variety of eukaryotic signatures, including metazoan sequences associated with Chordata, during the filtration stage of drinking water systems. Bascillariophyta was primarily identified in the raw water source and disinfection stages of DWTP B, along with the final treated water of DWTP C. They are considered to be the major components of phytoplankton (Diatoms) communities which most of the time dominate in raw water sources (Carvalho et al., 2025). Their re-occurrence in the disinfection stage is aligned to ineffectiveness of treatment processes in removing them. The study of Li et al. (2022) supports findings in further clarifying that diatoms and their tendencies to attach to particulate matter make it hard for treatment plants' processes to be able to remove them.

The most common phyla in both sludge and final treated water was *Apicomplexa*. According to Efstratiou et al. (2017), DNA from parasitic protists such as

Cryptosporidium and *Toxoplasma* persist even after treatments and can be detected through shotgun metagenomics. Furthermore, Apicomplexan DNA stays in treated water for a long time and can be transferred to other fungi through vertical and horizontal gene transfer (Li et al., 2020, Zahedi & Ryan, 2020). Ahmed et al. (2018) and Rodriguez-Manzano et al. (2022) have demonstrated that molecular techniques frequently detect residual DNA from non-viable parasites, particularly in environments with significant sludge accumulation. The results indicate that the presence of *Apicomplexa* in final treated water likely signifies DNA persistence instead of active contamination. This highlights the need for careful sludge management and regular molecular monitoring to tell the difference between pathogens that can and cannot survive.

Candida, *Aspergillus*, *Fusarium* and *Neurospora* were common in raw water source and sludge of DWTP A showing how eukaryotic patterns are influenced by treatment stages. Their presence represents the common fungal diversity that is found in DWTPs' treatment processes that are rich in nutrients or rather organic materials (Zhang et al., 2022). Furthemore, *Sugiyamaella* and *Pyricularia* were common in raw water source and final treated water of Plant B and C. According to Medeiros et al. (2020), fungal contamination in drinking water systems may be due to selective pressure that allows specific fungal genera to survive multiple treatment stages.

4.2.6 Functional metagenomes across the three DWTPs

The functional metagenomic profiles of DWTP A, B, and C comprised of metabolism, genetic information processing, cellular processes, organismal systems, environmental information processing, and pathways related to human diseases. The functional categories are established within drinking water systems. The presence of these functional metagenomes in the drinking water system is an indication of the processes that occur within the microbial communities which enable them to withstand treatment and possibly cause diseases upon consumption by humans (Wu et al., 2021; Zhang et al., 2023). The clustering of these metagenomes across different treatment stages, demonstrated variations specific to treatment conditions.

In DWTP A, the raw water source and filtration processes clustered together with cellular activity and metabolic processes in common. This aligns with the findings of Pinto et al. (2016), which indicated that raw water source and filtration stage alter the

functional metagenomes within the microbial communities to ensure their survival or pathogenicity. In contrast, sludge and the disinfection stage formed a distinct cluster, primarily defined by metabolic processes and the processing of genetic information. This study illustrates the mechanisms by which microbes respond to stress, repair DNA, and adapt their metabolism to endure exposure to disinfectants and coagulation (Shi et al., 2020). The similarity observed between sludge and final treated water suggests that both stages support the presence of resilient or highly adaptable organisms (Dottorini et al., 2023). This phenomenon is also noted in microbial groups that show resistance to disinfection, including *Mycobacterium* and *Sphingomonas* (Fitzgerald et al., 2021). The final treated water in DWTP A showed a unique functional profile, which corresponds to Jian et al. (2022) finding on the functional reshaping of microbial communities' study, which stated that treated water often has different functional metagenomes because of the strict selective pressure used during treatment.

Filtration and disinfection processes clustered together in DWTP B, sharing in common functional metagenomes such as metabolism and processing of generic information. This supports Graham et al. (2022) study of microbial functional triads across drinking water treatment processes, where they discovered that bacteria are capable of rapidly altering their DNA, which controls the functional metagenomes and efficiently utilizes nutrients to enhance their survival against both physical and chemical treatment processes. Sludge and final treated water also clustered together sharing cellular activities and improved environmental information processing in common. This indicates that microbial communities during these phases heavily rely on stress signalling, nutrient sensing, and adaptive pathways to survive in oligotrophic conditions (Wei et al., 2020). The unprocessed water exhibited a unique functional signature, indicating that it remained unaltered while containing a diverse array of microorganisms and chemicals.

DWTP C showed a different functional metagenomic distribution compared to the other two plants. The final treated water showed a notable functional profile, marked by increased processing of environmental information and decreased processing of genetic and metabolic information. This supports what other researchers have found, where they reported that microorganisms in final treated water often adopt low-energy survival strategies, prioritizing sensory and adaptive regulatory pathways (Oh et al.,

2023). Organismal systems and genetic information processing occurred in common in raw water source and filtration stage. This suggests that microbial communities in the raw water sources and filtration stages survived or adapted to the environment through altering their organismal system and genetic information processing system.

In all the studied DWTPs, it is evident that the functional metagenomics structure of each plant has its own unique signature and that it is shaped by the type of raw water source it receives as well as the treatment processes employed. These findings contribute to a growing body of evidence that microbial functional traits in treated water systems are shaped by a complicated relationship between environmental stress, treatment chemical composition, and microbial adaptation (Pinto et al., 2012; Shi et al., 2020).

4.2.7 Relationship between the physicochemical parameters and the bacterial community

4.2.7.1 *E. coli*, *Enterococcus* spp., and Total coliform

Analysing the relationships between indicator bacteria and physicochemical parameters in DWTPs is crucial for identifying conditions that promote microbial persistence or regrowth. Factors including pH, temperature, EC, TDS, and metal concentrations can affect bacterial survival, transport mechanisms, and resistance to treatment processes. Mudau et al. (2024) investigated the impact of water treatment stages on the occurrence of bacteriological indicators and their multiple antibiotic resistance index, observing that physicochemical variability often influences microbiological presence and removal efficacy across various treatment stages. Correlation analysis offered valuable insights into the potential factors influencing microbial occurrence throughout various treatment stages and within sludge. The current results from DWTP A, B, and C demonstrate multiple significant positive correlations between microbial indicators (*E. coli*, *Enterococcus* spp., and total coliforms) and essential water quality parameters.

Consistent robust positive correlations were observed between microbiological indicators and EC, TDS, iron, and manganese in raw water at all three plants. This trend indicates that higher concentrations of dissolved solids and metals could signify increased pollution in the watershed and potential faecal contamination. Ondieki et al.

(2025) in their study of assessing physicochemical and bacteriological quality of drinking water from source to household level provided evidence for this interpretation, indicating that higher EC and TDS frequently correspond with increased microbiological contamination in source waters, attributed to common pollution sources like runoff and wastewater intrusion. The strong correlation between iron and manganese and microbial indicators seems to be understandable, as metals can help bacteria survive by giving them places to adhere or by being present with particles that protect bacteria from stress in the environment. Similarly, Mesfun et al. (2024) in their study also reported positive correlations between increased Fe and Mn, emphasizing that the higher the Fe and Mn there more there will be high coliform counts in raw water sources. While on the other hand, other researchers found no correlation between microbial indicators (Hassard et al., 2017; Debassi et al., 2025). This fluctuation indicates that the strength of linkages varies significantly across each catchment.

During disinfection, a strong positive correlation was observed among DWTP A, B, and C between microbiological indicators and the parameters of EC, TDS, Fe, and Mn. The interaction in the disinfection stage clearly suggests that water chemistry plays a huge role in the effectiveness of disinfectants. Mudau et al. (2024) showed that elevated levels of dissolved metals and minerals can diminish the efficacy of disinfection operations by increasing chlorine demand or enhancing bacterial survival. This strengthens our findings by emphasising that physicochemical factors influence bacteria's survival throughout the disinfection phase. Barry (2023) and Doble et al. (2023) study support our findings of a correlation between pH levels and indicator organisms that is seen in all plants by indicating that suboptimal pH conditions may reduce chlorine efficacy and facilitate microbial persistence. On the other hand, Jiang et al. (2024) and De Marines (2025) studies have shown that physicochemical parameters and microbial indicators are completely dissociated after successful chlorination. The consistent correlations in the plants suggest fluctuating disinfection effectiveness.

In all the three treatment plants, filtration stage showed strong correlation between *Enterococcus* spp., TDS, EC and Mn, as well as *E. coli* and metals (Fe & Mn). These strong correlations clearly shows that the functionality of the filter and biofilm formation is determined by the ionic strength and metal concentration within the filter. These

interactions are likely because bacterial attachment, survival, and movement across granular media can be influenced by metals and dissolved ions. Li et al. (2022) in their study demonstrated that the chemical composition of water going into rapid sand and granular media filters significantly influences the resident microbial communities, particularly regarding nutrient and metal availability. Our findings corroborate Bai et al. (2022) study and suggest the adsorption of bacteria onto metal particles, biologically active filtration processes, or the possibility of filter breakthrough under certain operational conditions. In summary, iron and manganese, serve as substrates for microbial attachment or facilitate microbial mobility when destabilised. Conversely, Velmurugan et al. (2024) demonstrated in their study that properly maintained filters can substantially lessen microbial presence without impacting EC or TDS.

In final treated water, strong correlations were still observed between EC, TDS, Mn and Cu with Total coliform and *Enterococcus* spp. This clearly indicates the ineffectiveness of DWTPs, as ideally there should be minimal or rather no correlations observed in final treated water, as microbial indicators are expected to be removed during treatment. However, findings in this study corroborate that of Mudau et al. (2024) where it was discovered that there is occasional persistence of indicator organisms in treated water, particularly in plants experiencing operational fluctuations. These clearly explain the incomplete removal which promoted the survival of microorganisms. On the other hand, other researchers found no correlation in final treated water even with different physicochemical parameters (Thomas-Possee, 2023; Aghababaiyan et al., 2025).

In all the plants, strong positive correlations between microbial indicators and metals (Fe, Mn and Cu) are observed in sludge stage. This is totally acceptable as sludge serve as a home for microorganisms as it is rich in nutrients. Zhang et al. (2023) study corroborate our findings as they also emphasize that sludge is a home for indicator organisms and some metals. Whilst on the other hand, other researchers believe that when sludge is well maintained, the correlation between indicator organisms and metals can be poor (Maal-Bared, 2020; Feng et al., 2023).

4.2.7.2 Heterotrophic bacteria count

Understanding the factors that contribute to the proliferation of heterotrophic bacteria in the different stages of DWTPs is essential for the development of sustainable methods/approaches to ensure the production of potable water that has good aesthetic and microbiological status suitable for human consumption. Generally, in this study, the correlation analysis indicates that the physicochemical parameters played a crucial role in the proliferation of heterotrophic bacteria at different stages. Chigor et al. (2012) underscores the roles of natural physicochemical interactions in certain water bodies in the modulation of microbial proliferation.

Strong, significant positive correlations were observed between the physicochemical parameters and HBC. These factors indicate a complex relationship that contributes to the proliferation of HBC. In all the plants, pH and nitrates showed strongly significant positive correlation with HBC in raw, disinfection, filtration and sludge. Agedah et al. (2015) observed strong positive correlations between physicochemical parameters (pH and nutrients) of surface water that enhanced the proliferations of heterotrophic bacteria. Furthermore, Adesakin et al. (2020) identified notable positive correlations among pH, temperature, sulphate, phosphate, and heterotrophic bacteria in domestic water sources (well, borehole, and tap), as well as interactions between the physicochemical parameters that promote the growth of heterotrophic bacteria. This corroborates our findings as pH, temperature, sulphates and phosphates were observed to contribute to the proliferation of HBC in almost all the treatment stages. However final treated water in DWTP B and C showed no correlation to HBC, this clearly highlight the effectiveness of these treatment plants in removing microbial indicators. Prest et al. (2016) also confirms our findings were in their study they reported no consistent correlation with HBC. On the other hand, other researchers reported strong correlation between, nutrients, metals and HBC (Johnson et al., 2020; Ley et al., 2020).

4.2.7.3 Microbial classes identified by shotgun metagenomic for the DWTPs

In all the DWTPs temperature, EC, and TDS were the main factors that shaped the structure of microbial communities during the treatment operations. In the raw water sources, temperature favoured the Proteobacteria class (Alpha, Gamma, Beta). This corroborates Zhao et al. (2025) study, who reported temperature-driven enrichment of Proteobacteria in conventional DWTP influents due to enhanced enzymatic activity

and organic matter utilization at warmer conditions. Hirata et al. (2017) underscore the interaction between temperature and Gammaproteobacterial members as a potential factor that contributes to their increase metabolic rate in marine environment. This aligns with the current study where temperature correlated positively with Gammaproteobacteria in all the three plants in raw water source, disinfection and filtration stages, final treated water, and sludge. EC and TDS correlated positively with Actinomycetes and Alphaproteobacteria particularly in DWTP B. Similar findings were obtained in Momba and Sibewu (2022) study where EC act as indicator of minerals and nutrient loading that influences microbial communities in African DWTPs.

The chemistry of water plays a crucial role in influencing microbial life and the development of communities. Based on the results, it is necessary to enhance multi-barrier treatment approaches, improved sludge management practices, and advanced disinfection methods to effectively control microbial growth and reduce the spread of antibiotic resistance in drinking water treatment systems.

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

This study provided insight into the microbiology of three DWTPs in two provinces in South Africa. This helped ascertain how different raw water settings and water quality influence starting microbial communities in the plants. Also, the results showed that different treatment stages and seasons influenced the microbial dynamics of the treatment plants. Disinfection stages of all three treatment plants reduced the bacteriological indicators. In terms of seasons, the reduction was high in spring and summer in DWTP A. *E. coli* and *Enterococcus* spp. isolates from raw water sources, and the disinfection stage showed more resistance across all seasons. Metagenomics analysis identified intricate microbial communities predominantly composed of Archaea, bacteria, and eukaryotes, with their profiles differing based on treatment stage and raw water characteristics. Functional metagenomics profiling revealed unique clustering patterns among plants and developmental stages, indicative of microbial responses to environmental stressors, disinfection processes, and nutrient gradients. The presence of genetic information processing metagenomes and human diseases metagenomes could be associated with the antibiotic resistance observed in the bacterial isolates. The correlation study indicates that pH, temperature, nitrates, sulphate, EC, TDS, and Fe are associated with the proliferation high bacterial communities in the drinking water systems.

This study demonstrates that although DWTPs effectively manage physicochemical quality, microbiological stability is variable across the treatment processes. The presence of the bacterial communities in the final treated water in all the treatment plants creates a great concern. This is because consumption of such water could be detrimental to human health. Hence, the study recommends further studies to look into the development of real-time monitoring methods that can detect diverse microbial contaminants in the water as well as the factors that control their growth.

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