Water Quality Monitoring of Biological and Chemical Pollutants into the Wakkerstroom Wetland

By

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Abstract

Wetlands are among the most important ecosystems in the world due to the array of ecosystem services that they provide, such as flood attenuation, ground water restoration, water filtration and pollutant removal, climate change regulation and reducing erosion.

The Wakkerstroom wetland in the Mpumalanga Province South Africa, plays a major role in improving downstream water quality. The wetland is threatened due to excess nutrient loading and microbiological contaminants entering the water column from a sewage ground runoff and a malfunctioning wastewater treatment plant. This study aims to determine the water quality and nutrient removal from the wetland by assessing chemical parameters and coliform and *E.coli* contamination as faecal indicators. Eight sampling sites were chosen along the length of the wetland, and water sample collection occurred once a month between August 2021 and July 2022. The results showed a neutral pH between 6 and 8 with no spatial or temporal significant differences. The mean Total Dissolved Solids (TDS) ranged between 50 – 145 mg/L. The mean nitrate concentrations (NO_3^-) ranged between 1.50 and 7.00 mg/L across the sampling sites. During the sampling period, a net total of 17.53 mg/L of nitrate was removed from the water column by the wetland. The mean phosphate concentration (PO_4^{3-}) ranged between 0.40 – 1.40 mg/L, showing a total net removal of 2.14 mg/L during the sampling period. Coliform abundance was high closest to the wastewater treatment plant and sewage runoff and decreased along the length of the wetland, with an estimate removal of 1.17, calculated from a qualitative colour changing scoring system. *E.coli* bacteria was present in the wetland at all of the sampling sites between March 2022 to July 2022. This study resulted in the confirmation of a healthy, functioning wetland system where the wetland is able to reduce excess nutrients and coliform contamination. The wetland water quality shows similarities to studies conducted on wetlands in South Africa. The wetland is still under threat through possible reduced functioning as a result of excessive exposure to untreated domestic and sewage waste. A need exists for improved management and more stringent standards of water quality control from the wastewater treatment plant to reduce the risk of wetland degradation through excessive sewage exposure.

Keywords: Water quality monitoring, wetland retention, wastewater treatment plant, faecal contamination, chemical and biological pollutants

Declaration

I (Celine Klinkert) hereby declare that all the research done for my master's dissertation titled Water Quality Monitoring of Biological and Chemical Pollutants into the Wakkerstroom Wetland. All the relevant sources have been cited in a complete reference list.

19th March 2024

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Chapter 1. Introduction

1.1 General Overview

Wetlands are among the most important ecosystems in the world due to the ecosystem services that they provide. Wetlands provide an array of ecosystem services such as flood prevention, ground water restoration, water filtration and pollution removal, climate change regulation and erosion reduction (Rebelo *et al*., 2019). Wetland ecosystems play a crucial role in providing important, cost effective services for human well-being and longevity (Turpie *et al*., 2010a). Wetlands help to improve water quality by removing and reducing excess nutrients as well and removing and/or reducing chemical, physical and biological pollutants (Turpie *et al*., 2010b). Despite this, wetlands are threatened ecosystems due to overuse, excess nutrients (Nyenje *et al*., 2010; Atkins *et al*., 2022) and changing climates threating to deplete water sources (Dallas & Rivers-Moore, 2013). The freshwater ecosystems in South Africa, such as river and wetland systems are a valuable resource as South Africa relies on fresh water ecosystems for the majority of our water sources (Dallas & Day, 2004). In addition to this, wetlands are among the most threatened ecosystems in the southern African region due to the geographical and climatic nature of this arid region, than compared to wetlands found in the northern hemisphere. Despite their vulnerable state, many of the freshwater systems in South Africa, including wetlands are subjected to high pollution loading (Edokpayi *et al*., 2017; Reynolds & Ryan, 2017; Rimayi *et al*., 2018) through point source contamination as water is discharged from wastewater treatment plants directly into water bodies (Dungeni *et al*., 2010; Edokpayi *et al*., 2017).

Wetlands are able to improve water quality by reducing nutrients, microbial pathogens and heavy metals from the water column, and are recognised as “sinks” (Kotze *et al*., 2010). Wetlands are able to improve water quality by the nature and structure of wetlands, as they are highly productive systems, with dense vegetation that reduce the flow of water moving through the wetland (Collins, 2005). Wetland systems in South Africa are valued for their ecosystem service of nutrient and microbial retention as a means to improve downstream water quality (Edokpayi *et al*., 2017). As such, wetlands are more susceptible to increased pollutant loads and reduced water quality as a result of this (Dallas & Day, 2004). This places a pressure on wetlands to reduce biological and chemical pollutants from the water to improve water quality for downstream water bodies. The reliance on wetlands to improve water quality could lead to further wetland degradation as wetlands act as sinks, making wetlands more susceptible to accumulated nutrients from anthropogenic activities (Kock *et al*., 2019).

1.2 Rationale

The Wakkerstroom wetland, located in Mpumalanga Province South Africa, is an unchanneled valley bottom wetland, where the main stretch of the wetland is approximately 5km long. The upper 1km and lower 2km of the wetland are categorized as floodplain wetland (Joubert & Ellery, 2013). The wetland has a high vegetation biomass, but low vegetational species diversity. The two main plant communities that occur throughout the wetland *are Typha capensis* (Bulrush), distributed across 48% of the wetland, and is the most abundantly found species in the Wakkerstroom wetland. The second plant species is *Phragmites australis* (common reed) that is distributed across 30% of the wetland, and lastly wetland grasses distributed throughout the wetland (Stockdale *et al*., 2021).

1.1.1 Water Quality Monitoring

Many of South Africa's freshwater ecosystems such as rivers, lakes and wetlands are subjected to biological and chemical waste. However, a percentage of the population still relies on freshwater ecosystems for domestic use water (Dallas & Day, 2004) Therefore, it is imperative that the water quality and health of these freshwater ecosystems are monitored in order to understand the pollutants entering freshwater ecosystems, and to ensure correct management and sustainable use of these ecosystems (DWAF, 1996).

Comprehensive water quality monitoring assesses physical and chemical parameters (collectively known as physio-chemical) as well as biological indicators of water quality and wetland ecosystem health. Physical parameters include temperature and turbidity, and chemical parameters include, but are not limited to, pH, total dissolved solids, suspended solids, nutrient ion concentrations and dissolved oxygen (Malan & Day, 2005). Biological indicators include assessing the species diversity and abundance of diatoms, bacterial communities and macroinvertebrates. In addition to this, the use of microbial indicators allows us to determine the presence of harmful or pathogenic bacteria, often as a result of wastewater input (Luyt *et al*., 2012). Many of these parameters are affected by one another, in conjunction with having an effect on the biological organisms. Monitoring the physio-chemical parameters is an important factoring when determining pollutants and the surface water quality, however the condition of water is limited to the period of time that the measurements were taken. Therefore, the monitoring of biological indictors in addition to chemical parameters gives one an improved understanding of the overall water quality (Dallas & Day, 2004). Testing for the presence of coliforms, a group of bacteria of both faecal and non-faecal origins allows for the suggestive occurrence of faecal contamination (Odonkor & Ampofo, 2013). When determining the possible presence of faecal contamination, an indicator that is present in animal and human faecal matter is needed, to represent faecal contamination from livestock as well as waste water deposits from sewage treatment plants (Luyt *et al*., 2012). *Escherichia coli* (*E.coli*) bacteria is found in the intestinal tract of warm blooded animals, including humans, and is therefore an ideal determinant of faecal contamination. Secondly, detecting the presence of coliforms, especially faecal coliforms as an efficient and effective determinant of probably (Luyt *et al*., 2012; Odonkor & Ampofo, 2013).

1.1.2 The Wakkerstroom Wetland

The Wakkerstroom wetland is categorized as an unchannelled valley bottom wetland, as the wetland lacks a channel running through. Some general characteristics which are prevalent in unchannelled valley bottoms include the absence of channel banks, diffuse flows and the main water input is usually an upstream channel and diffuse runoff water moving down the hillslopes. The four characteristics mentioned above fit the Wakkerstroom wetland, where the main water input is the Wakkerstroom River just upstream of the wetland.

The Wakkerstroom wetland was chosen as a study site as several years of speculation from the Wakkerstroom community (Wakkerstroom Community Members, 2021) have suggested that the sewage plant, located a few hundred metres upstream of the wetland, has been leaking untreated or partially treated wastewater into the wetland, exposing the wetland to chemical and biological pollutants. As such, two things were important to confirm from these community statements:

1. Whether or not the sewage plant was leaking untreated/partially treated wastewater into the Wakkerstroom wetland
2. If the above statement is true – revealed by data collection – whether the Wakkerstroom wetland was assisting in the removal of excess nutrients and microbiological pathogens to improve downstream water quality, or if the quantity of the pollutants entering the wetland were at too high a volume to be removed/reduced by the wetland, therefore rendering the water waste leak unsustainable and causing increasing degradation to the Wakkerstroom wetland.

As such, a Master of Science study was conducted on the Wakkerstroom wetland, where a relevant set of data and tests were completed in order to answer the above mentioned concerns of the Wakkerstroom wetland.

1.2 Aim

To assess the physical and biological properties along the Wakkerstroom wetland between August 2021 and July 2022 and to calculate the amount of nutrient removal by the wetland.

1.3 Objectives

1. To determine and compare the spatial and temporal differences of nutrient loading and faecal contamination from wastewater inputs into the Wakkerstroom wetland.
2. To determine the chemical parameters of the Wakkerstroom wetland by collecting water samplings at eight sampling sites along the Wakkerstroom wetland between the sampling period August 2021 to July 2022.
3. To determine the presence and abundance of Coliforms and *E. coli* using qualitative methods.

4. To calculate the net removal or addition of total dissolved solids, nitrate, phosphate and coliform abundance as a result of wetland retention and release.

Chapter 2. Literature Review

2.1 Wetland Dynamics

Wetlands are categorized as aquatic ecosystems which are transitional between terrestrial ecosystems such as grasslands and forests and fully aquatic ecosystems such as rivers and oceans (Ollis *et al*., 2013). Wetlands are made up of surface or ground water, saturated soils and support faunal and floral species adapted to and tolerant of aquatic conditions (Mitsch & Gosselink, 2016). The hydrological cycle of wetlands is crucial for understanding wetland functioning and the biotic life that is influenced by the hydrology of wetlands (Ollis *et al*., 2013). This refers to the balance between water inputs, outputs and storage capacity of ground water affecting soil type, water chemistry, pH, water depth and vegetation (Baker *et al*., 2009).

2.1.1 Wetland Hydrology

Wetlands within South Africa are classified by their geomorphic characteristics (Ollis *et al*., 2013), however, the hydrological characteristics of a wetland are a major factor to the functioning and hydrochemical properties of the wetland (Ellery *et al*., 2016). Wetland ecosystems are complex systems shaped by changing seasonal rainfall, soil saturation, the topography of the landscape and the water movement through the wetland from upstream water inputs, all controlled by the hydrological characteristics of the wetland (Mitsch & Gosselink, 2016). In general wetlands have five avenues of hydrological input, namely, precipitation, runoff, inundation, subsurface flow and ground water discharge. These are all factors that influence an increase in the water balance of the wetland system (Baker et al., 2009). The three outputs that reduce the water balance of the wetland include evapo-transpiration, overland flow and ground water recharge (Baker et al., 2009).

In South Africa specifically due to the semi-arid climate - the three main water inputs include surface flow, precipitation and ground water recharge (Batzer & Sharitz, 2007), In the case of the Wakkerstroom wetland, the main hydrological input is the Wakkerstroom river, followed by seasonal precipitation and small streams entering the wetland (Joubert & Ellery, 2013). The surface water level and the duration of soil saturation is controlled by the balance of water inputs and water outputs. This can explain the duration and patterns of wetland flooding, referring to permanent or seasonal wetland flooding (Mitsch & Gosselink, 2016).

2.1.2 Wetland Soils

The three main factors that control the formation of wetland soils are:

- Geomorphic relating to the landscape and topography of the area;
- Hydrological relating to the movement of water through the soils and the depth and length of time the soils remain saturated; and
- Biogeochemical which relates to the physical, mineral and organic properties of the wetland soil (Jackson e*t al.*, 2014)

The topography of an area influences wetland soils through the movement of water, as water flows to the lowest point, usually at the bottom of a hillslope or valley. These areas of the landscape usually possess increased wet areas and increased soil saturation due to the flow of water. Therefore, the increased volume and period of wetness in these areas drives wetland soil characteristics. Secondly, the two main characteristics of wetland soils are that the soils are saturated with water either perennially or non-perennially and are anaerobic or lack oxygen as oxygen cannot diffuse through the lower depths of water at the bottom of wetlands (Mitsch & Gosselink, 2016). Physical properties of wetland soil include soil texture and structure as well as porosity and particle size (Jackson e*t al.*, 2014).

Soils play an important role in the regulation of excess nutrients and microbial contamination entering the wetland. This occurs through sedimentation or sorption, where the nutrients interact with the suspended solids in the wetland soils (Villa *et al*., 2023), or through the physical settling of microbial contaminants and nutrients in the non-reactive form (Smith *et al*., 2008). The type of nitrate removal that occurs is controlled by the availability of oxygen in the soil, as anaerobic soils promote dentification (Hefting *et al*., 2004).

2.1.3 Wetland Vegetation

Wetland vegetation is a key component to the healthy functioning of a wetland system. Wetland impacts reduced through dense vegetation cover include the lateral movement of water through the wetland, reducing the rate of the flow and limiting the risk of erosion and downstream sedimentation. Vegetation in the wetland also supports the improvement of water quality by reducing excess nutrients as plants use these minerals for growth (Ollis *et al.*, 2013). Wetlands contain gradients of water saturation, where the perimeter of the wetland contains unsaturated soils and the lower regions of the wetland contain increased periods of inundated soil. In response to this, the vegetation shows variation and adaptability depending on the soil saturation (Mitsch & Gosselink, 2016). These reed communities are commonly found at the edge of the wetland with their roots submerged. These plant communities are successful due to readily available nutrients in the water, often in an inorganic form, allowing for fast paced plant growth, and therefore these reeds grow to between 2-3 meters tall. It is also characteristic for wetland plant communities to be a part of the nutrient cycle of the wetland as the plants rely on nutrient uptake for growth (Romero *et al*., 1999; Batty, 2003).

2.2 Wetlands in South Africa

The naming and classification system used to classify wetlands in South Africa developed in conjunction with the South African Biodiversity Institute (SANBI) to further understand and categorize the types of wetlands found in South Africa (Ollis *et al*., 2013). This classification manual only relates to inland wetlands. Wetlands in South Africa can be broadly divided into three groups which can be differentiated by their distance from the sea: marine, estuarine and inland wetlands (Ollis *et al*., 2013). For the sake of the topic of this project only inland wetlands shall be discussed further. Inland wetlands are defined as wetlands with no direct connection to the sea, such as tidal inputs, as defined by the 6th edition Ramsar Convention manual (Ramsar Convention Secretariat, 2013). The classification system has five different levels of wetland classification, level 1 being an inland wetland system. From level 1 onwards are level 2 to 5, illustrated in Figure 1 below:

- Level 2 - Regional setting;

- Level 3 - Landscape unit;
- Level 4 – Hydrogeomorphic unit; and
- Level 5 – Hydrogeological regime.

Level 4, the hydrogeomorphic unit is the level where most of the focus revolves around as this level differentiates the main types of inland wetlands in South Africa (Ollis *et al*., 2013).

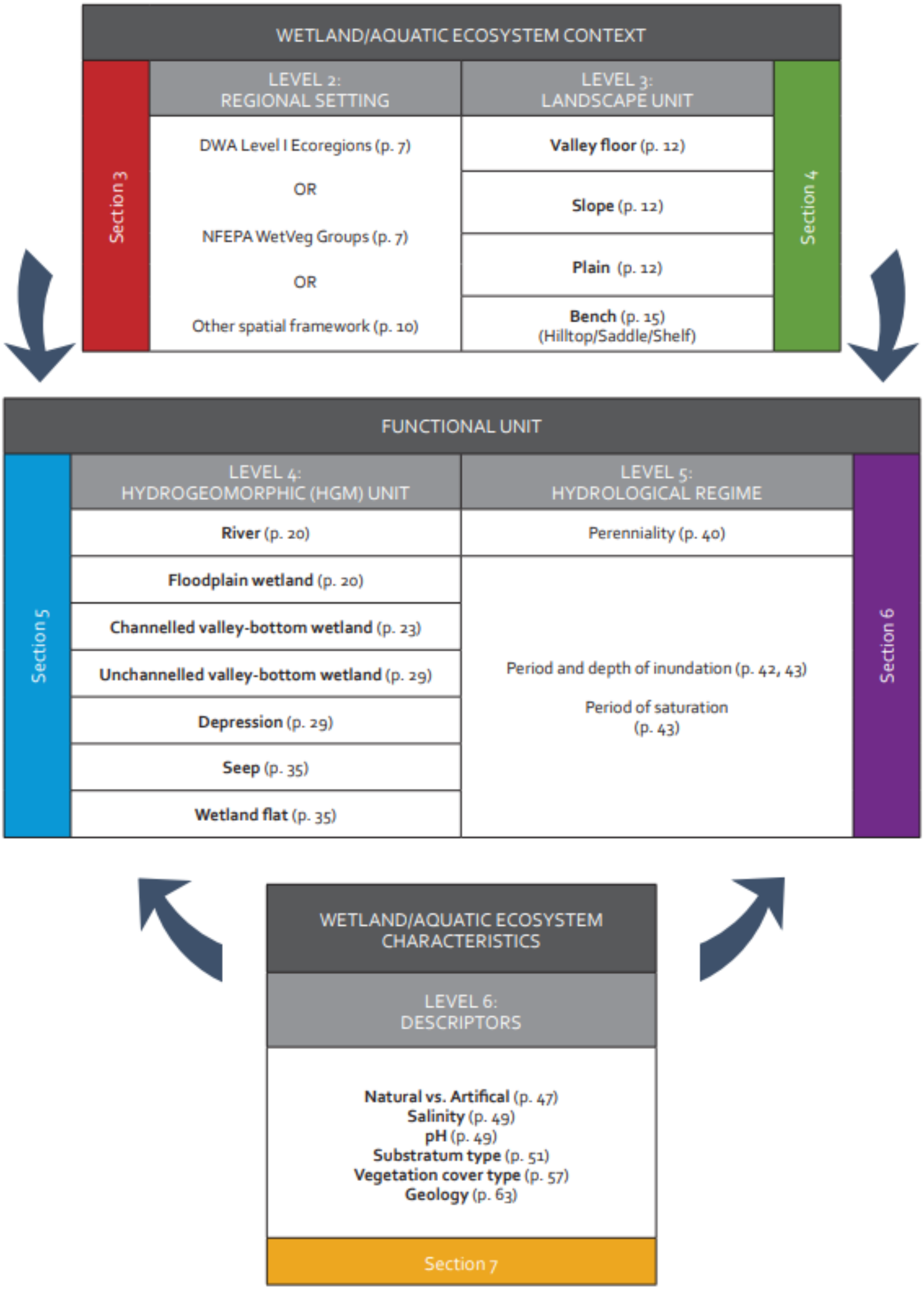


Figure 1. A diagram of the different levels of wetland classification from Ollis *et al*. (2013)

Five main groupings of inland wetlands are recognised in South Africa – floodplain, valley-bottom, depression, seep and wetland flats. Valley bottom wetlands can be further divided into channelled and unchanneled valley bottoms. As the name highlights, these types of wetlands are located between two hillslopes, within 500 m of the wetland system and typically occur on the valley floor (Ollis *et al*., 2013). Unchannelled valley bottom wetlands are categorized by diffuse surface or sub-surface flows into the wetland, and these wetlands usually have a main hydrological input source such as river flowing into the wetland (Ollis *et al*., 2013). The Wakkerstroom wetland is an unchanneled valley bottom wetland with the main hydrological source being the upstream Wakkerstroom river flowing into the wetland.

South Africa is a semi-arid country with hot summers with periods of high rainfall and cold low-rainfall winters. Despite this, South Africa's landscape is rich with diverse wetlands, with approximately 300 000 wetlands and 791 types of wetlands found in South Africa (DWA, 2021). Wetlands found in South Africa differ from northern hemisphere wetlands due to differing climates, levels of radiant energy and rainfall (Ellery *et al*., 2011). Northern Hemisphere wetlands are mainly sustained by rainfall and reduced evaporation rates. In contrast to this, Southern Hemisphere wetlands, especially wetlands in South Africa are not sustained solely on rainfall due to South Africa's semi-arid climatic conditions and differing seasonal rainfall inputs (Brinson, 1993). The main hydrological inputs for wetlands in South Africa are surrounding water bodies such as dams, rivers and streams which flow downstream (Turpie *et al*., 2010). Fluctuations in hydrological input cause large changes in the wetland conditions, as wetland areas are largely controlled by their hydrological regime.

The general threats wetlands in the South African region are decreased precipitation and increased surface temperatures, resulting in reduced water quality (Dallas & Rivers-Moore, 2013) as well as human caused threats such as increased population which results in the demand for more good quality water and increased untreated wastewater flowing into wetland (Mitchell, 2013; Edokpayi *et al*., 2017). Therefore, it is imperative to understand the sustainable use of wetlands as well as preserving healthy wetland functioning by ensuring wetlands are not furthered threatened.

2.3 Ecosystem Services Provided by Wetlands

Ecosystem services are defined as any direct or indirect services provided by a natural ecosystem that benefits human well-being. Ecosystem services are classified by regulating, provisioning, supporting and cultural services (Fisher *et al*., 2008). Wetland ecosystems play a crucial role in providing important, cost effective services for human well-being and longevity. Wetlands help to improve water quality by removing and reducing excess nutrients as well as chemical and biological pollutants (Turpie *et al*., 2010). Wetlands also ensure flood protection for low lying towns and cities near water sources, decrease loss of water through evaporation and host a variety of biodiversity dependant on wetlands, summarised in the Millennium Ecosystem Assessment (2005). Over the past decade, wetlands have been valued far more highly than in the 20th century. Turpie *et al.* (2010) found that the value of improved water quality through water purification by wetlands ranged between $2 100- 2 325/ha per year. However, entire South African wetlands, providing a variety of ecosystem services can be valued to at least R275 billion per annum if these ecosystem services were to be replaced due to total degradation. This value includes provisioning services such as grazing for livestock, cultural services through ecotourism and regulating services such as erosion control and carbon sequestration through vegetation cover and high biomass (Turpie *et al*., 2017). Globally it is estimated that wetland ecosystem services are valued at $47.4 trillion per annum. Of this, inland wetlands are estimated to provide $27.1 trillion of ecosystem services (Davidson *et al*., 2019).

2.4 Pollutant Removal

Excess nutrients reduced by wetlands, and removed from the water column through nutrient retention allows wetlands to improve water quality (Kotze et al., 2010; Mandishona & Knight, 2022). This is where the external loading of nutrients are retained within the wetland and removed from the water column due to a number of chemical and biological processes (Collins, 2005). This is done through several wetland processes:

- The reduced flow rate of water through a wetland system allows for a physical and/or chemical exchange between nutrients and the sediment, leading to nutrients and microbial contaminants being incorporated into the sediment;

- Wetlands are highly productive systems with dense vegetation and increased biomass, which encourages nutrient uptake from the water via plants; and
- Saturated soil conditions allow for the anaerobic process, denitrification, where nitrate is released into the atmosphere as gaseous nitrogen (N_2) (Collins, 2005; Mitsch & Gosselink, 2016). Under specific conditions, when wetlands become saturated from excess nutrients and as wetlands begin to degrade, wetlands can also act as nutrient sources, where nutrients are released from the wetland into the water column, known as internal loading of the wetland (Fisher & Acreman, 2004; Sánchez-Carrillo *et al.*, 2010; Mitsch & Gosselink, 2016).

2.5 Nutrient Cycling in Wetland

Nutrient cycling and removal of excess nutrients is one of the most crucial ecosystem services that wetlands provide. Nutrient cycling through a wetland are controlled by chemical, biological and physical processes involving several inorganic nutrients and ions such as phosphate (PO_4^{3-}), nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), calcium (Ca^+), sulphate (SO_4^{2-}) and sodium (Na^+), among many others. Wetlands allow for many of these process to occur as wetlands support anaerobic and aerobic conditions in concurrence with one another (Mitsch & Gosselink, 2016). Nutrient cycling occurs through processes such as the storage of nutrients and physical pollutants in the sediment, nitrification, denitrification, and the transformation of inorganic nutrients to organic nutrients and plant uptake (Mitsch & Gosselink, 2016).

2.5.1 Nitrogen Cycle

Nitrogen can enter the wetland in an organic state (not soluble in solution), through plant matter that decomposes, or from external sources, either as organic or inorganic nitrogen. The first process is nitrogen mineralization, where organic nitrogen (usually NH_3) is mineralized to inorganic ammonium (NH_4^+). This process is known as ammonification and requires oxygen to be available and is completed via microbial activity (Lamers *et al.*, 2012). Following ammonification, further oxidization occurs, where nitrate (NO_3^-) is produced as the end product. Ammonium and nitrate can be taken up via plant roots. Denitrification occurs when nitrate is converted to nitrogen gas (N_2), which is released into the atmosphere. The denitrification occurs by bacteria and is an anaerobic process, as it occurs in the lower anoxic layers of the wetland soil (Hefting *et al*., 2004). Nitrification is the process of nitrate being converted back into ammonium, and this process occurs in the upper aerobic wetland soils, as well as along the rhizomes on plant roots, all enabled by bacteria found in the soil and on plant roots *(*Collins*, 2005 and* Mitsch & Gosselink*, 2016)*.

2.5.2 Phosphorus Cycle

The major difference between the nitrogen and phosphorus cycle, is phosphorus can be removed from the water column, but remains within the wetland either in sediment or within plant biomass, whereas nitrogen can be released in a gaseous state into the atmosphere. The phosphorus cycle is therefore referred to as a sedimentary cycle. The main inorganic compounds of phosphorus are phosphate ions (PO_4^{3-}), hydrogen phosphate (HPO_4^{2-}) and dihydrogen phosphate ($H_2PO_4^-$). These three ions are collectively known as orthophosphates. Inorganic phosphorus is removed from the water column by chemically bonding with suspended solids in the water, or in ions in the sediment. This mainly occurs with iron, calcium and aluminium ions. Next, inorganic phosphorus, as it is negatively charged, bonds to positively charged sediment molecules, such as clay (Cui *et al*., 2018). When re-suspension of phosphate occurs from the sediment layer and phosphates are re-introduced into the water column, this is due to the sorption and release process of inorganic phosphorus with the sediment (Collins, 2005 and Mitsch & Gosselink, 2016).

2.6 Microbiological Indicators

The National Microbial Monitoring Programme for Surface Water (NNMP, 2002), set up by the Department of Water and Sanitation (DWS) in South Africa, was established to assess faecal contamination in water bodies in South Africa, such as rivers and dams, to monitor the bacterial load and presence of faecal indicator organisms, such as total coliforms, faecal coliforms and *E.coli* (Department of Water and Sanitation, 2015)

When determining whether faecal contamination is occurring by using culture based methods, there are several challenges involved. Firstly, the procedure requires specific complex techniques in order to correctly culture the bacterial species found in the human colon. Secondly, the process is costly and can take several days to culture bacteria, therefore it is not time sensitive (Lapworth *et al*., 2017). As such, indicator bacteria are used to indicate whether faecal matter is likely present. These tests are efficient (between 18 – 24 hour procedure) and do not require the use of complicated techniques or equipment. Therefore, the water quality monitoring of microbiological contamination is more accessible (Lapworth *et al*., 2017).

Faecal coliforms – a branch of coliform bacteria found in the intestinal tracts of warm blooded animals including sheep, goats, cattle and humans. When faecal coliforms are present in water, this could indicate the presence of pathogen causing bacteria (Edokpayi *et al*., 2017). Faecal coliforms enter water ways when untreated wastewater and sewage or livestock faecal matter enter water ways through malfunctioning wastewater plants (Momba & Mfenyana, 2004; Dungeni et al., 2010), a lack of proper sanitation facilities such as those found in informal settlements, and the incorrect treatment from livestock agricultural farms are exposed to natural water bodies. Therefore, pathogenic bacteria can be transported through river and wetland land systems, degrading the water quality and health of the river and wetland systems (Namugize *et al*., 2018)

2.6.1 Escherichia coli Bacteria (E.coli)

Using the presence of faecal coliforms such as *E.coli* as a method for detecting faecal contamination in water has been widely used since the early 1900's (Odonkor & Ampofo, 2013). *Escherichia coli* bacterium is a well-regarded faecal indicator organism as it naturally occurs in the large intestine of warm blooded animals. However, not all strains of *E.coli* are pathological, therefore, *E.coli* present in water does not immediately suggest ingesting the water will result in illness – but rather that there is an increased risk if *E.coli* is present in water. Likewise, the coliform group of bacteria do not all originate from faecal matter and many can be naturally found in the environment such as in soil, plant matter and river beds. Therefore, a positive result of coliform presence does not confirm faecal contamination, but rather suggests possible faecal contamination which can then be followed up by more specific faecal identification test such as bacterial cultures and Polymerase Chain reaction (PRC) for *E.coli* strain identification (Odonkor & Ampofo, 2013).

Freshwater systems in South Africa have regularly been identified with *E.coli* present in the watercourse (Namugize *et al*., 2018), sometimes so much so that the amount of coliforms and faecal bacteria indictors such as *E.coli* exceeds the maximum limit according to the South Africa water guidelines (DWAF 1996). In Kwa-Zulu Natal, it has been shown that the Umhlangane River (Marie & Lin, 2018) and the Umgeni River (Namugize *et al*., 2018) and in Gauteng the Klip River and its tributaries (Makumbe, 2018) all showed E.coli present in the water column, which suggests the presence of faecal contamination.

2.7 Water Quality Degradation

Wetland degradation can occur in a number of ways, many of which result in reduced water quality. The most destructive form of wetland degradation is the physical transformation of the wetland, through land transformation or altering the hydrological input such as the draining or diverting of wetland water (Kindsford *et al*., 2016). Following this, is the excessive exposure to nutrients through fertilizer/pesticide runoff from agricultural lands (Buah-Kwofie *et al*., 2018) and untreated waste, from human domestic waste (Adeeyo *et al*., 2022) and livestock waste from pasture lands.

Untreated wastewater, agricultural runoff containing fertilizers, excess nutrients and introduction of domestic discharge due to the lack of running water, large quantities of pollutants and excess nutrients flow downstream into wetlands. This places a pressure on wetlands to reduce biological and chemical pollutants from the water to improve water quality for downstream water bodies. The reliance on wetlands to improve water quality could lead to further wetland degradation as wetlands act as sinks, making wetlands more susceptible to accumulated nutrients from anthropogenic activities (Kock *et al*., 2019).

2.7.1 Wastewater Treatment Plants

In South Africa 89% of the population has access to clean safe drinking water (Statistics South Africa, 2018), with the majority of those without access living in rural areas of South Africa. According to the Sustainable Development Goals for South Africa, only 67% of those in rural areas have access to clean safe drinking water from boreholes, piped into their houses or local community water storage facilities in 2017 (Department of Statistics South Africa, 2017). However, many of South Africa's freshwater ecosystems do not contain water that is safe for consumption as the indicators of faecal contamination are frequently observed in wetlands, rivers and dams in South Africa (Momba & Mfenyana, 2004; Obi *et al*., 2004; Dungeni *et al*., 2010; Teklehaimanot *et al*., 2014; Edokpayi et al., 2017 ; Madilonga *et al*., 2021). Several studies in South Africa have shown that water bodies in South Africa receive effluents from wastewater treatment plants, however the water quality does not comply with South Africa's guidelines. The water quality parameters of receiving water bodies shows several pathogenic bacterial species and excessive nutrient concentrations (Momba & Mfenyana, 2004; Dungeni *et al*., 2010; Teklehaimanot *et al*., 2014). This implies that wastewater treatment plants are inadequately reducing excess nutrients and microbiological pathogens before being released into surface water bodies such as dams and rivers (Adams *et al*., 2016 and Gani *et al*., 2019).

The purpose of wastewater treatment plants is to reduce the chemical and biological contaminants to prevent the risk of disease through reduced water quality and degradation of receiving ecosystems, such as wetlands and rivers (Edokpayi *et al*., 2017).. Edokpayi *et al*. (2015) found that many of the wastewater treatment plants are discharging polluted and inadequately treated water into water bodies is a result of a lack of investment for maintenance, reduced capacity of the treatment plant to effectively improve the water quality for the incoming volume of wastewater and a lack of regular testing of the water quality before being discharged into receiving water bodies (Edokpayi *et al*., 2017).

Chapter 3. Study Area and Sampling Sites

3.1 Study Area

The Wakkerstroom Wetland is located in the south east of the Mpumalanga province, neighbouring the border of KwaZulu-Natal, South Africa (Fig. 1). The Wakkerstroom Wetland is west of the small town Wakkerstroom and runs parallel to the town, which is well known for its eco-tourism and birding activities. East of the main town Wakkerstroom and the Wakkerstroom Wetland, there is an informal settlement, eSizamenleni, which has varying access to clean drinking water and established sanitation facilities. The Wakkerstroom Wetland (27°20’S; 30°07’E) is a natural wetland reserve which is approximately 1000 hectares, five kilometres in length, 200 – 500 m in width with an average depth of 2m. The wetland is located within the Mpumalanga highveld at an altitude of approximately 1780 m and is categorized as an unchanelled valley bottom wetland. The wetland has one main hydrological input, the Wakkerstroom River which flows directly into the wetland. Immediately upstream of the Wakkerstroom River is the Wakkerstroom Dam, known as Martin’s Dam which feeds the Wakkerstroom River (Joubert & Ellery, 2013). The wetland receives secondary hydrological inputs from precipitation and surface runoff water flowing downwards into the wetland from the surrounding hills. The lower portion of the wetland leads into the Thaka River flowing out of the wetland, it then flows into the Zaaihoek Dam (Joubert & Ellery, 2013).

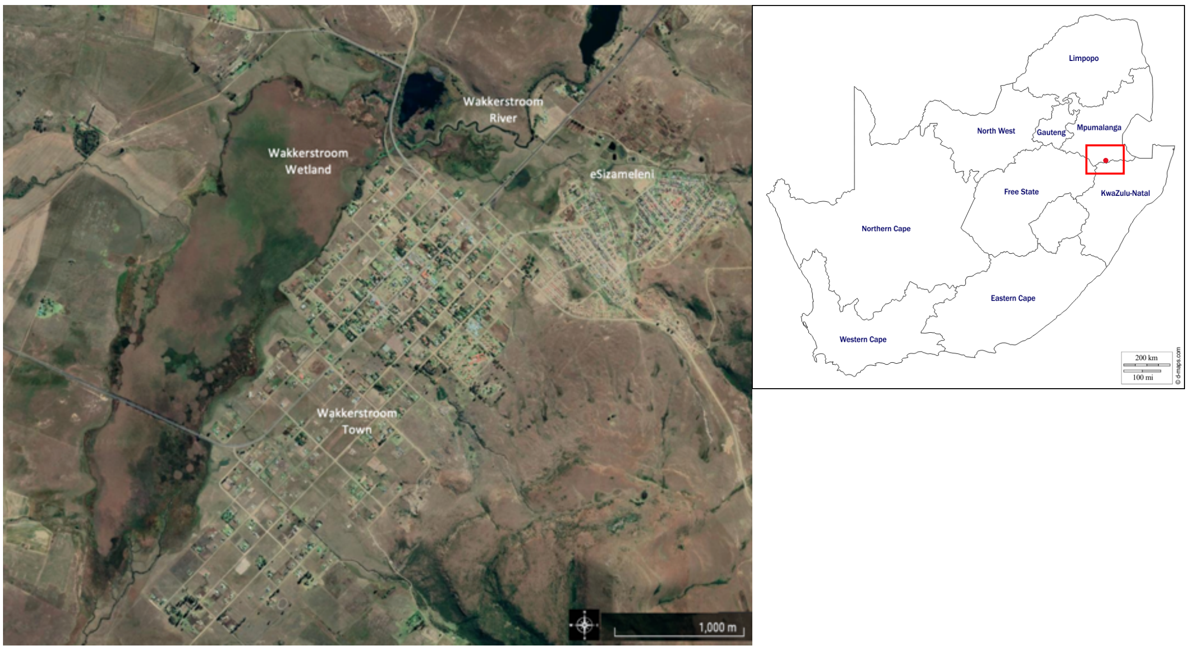


Figure 2. Google Earth. Wakkerstroom. [27° 21’ 04’’ S 30° 07’ 27’’ E]. Wakkerstroom Wetland showing the relation to the Wakkerstroom Town, the informal settlement eSizameleni and the Wakkerstroom River.

3.*1.1Wakkerstroom annual rainfall*

The average long term annual rainfall for Wakkerstroom is 1073 mm – with December usually having the highest average rainfall in a year, and June having the lowest average rainfall (Weather and Climate, 2023). Illustrated below is the long term monthly average rainfall in Wakkerstroom and the observed monthly rainfall patterns for the sampling period in 2021 and 2022 (Fig. 2). The monthly rainfall for 2021 followed the expected rainfall pattern and experienced 777.16 mm of annual rainfall. The monthly rainfall patterns in 2022 showed an extended high rainfall period as high rainfall occurred late into April 2022 with increasing rainfall between January 2022 to April 2022. The low rainfall period began in May 2022 with reduced rainfall as expected.

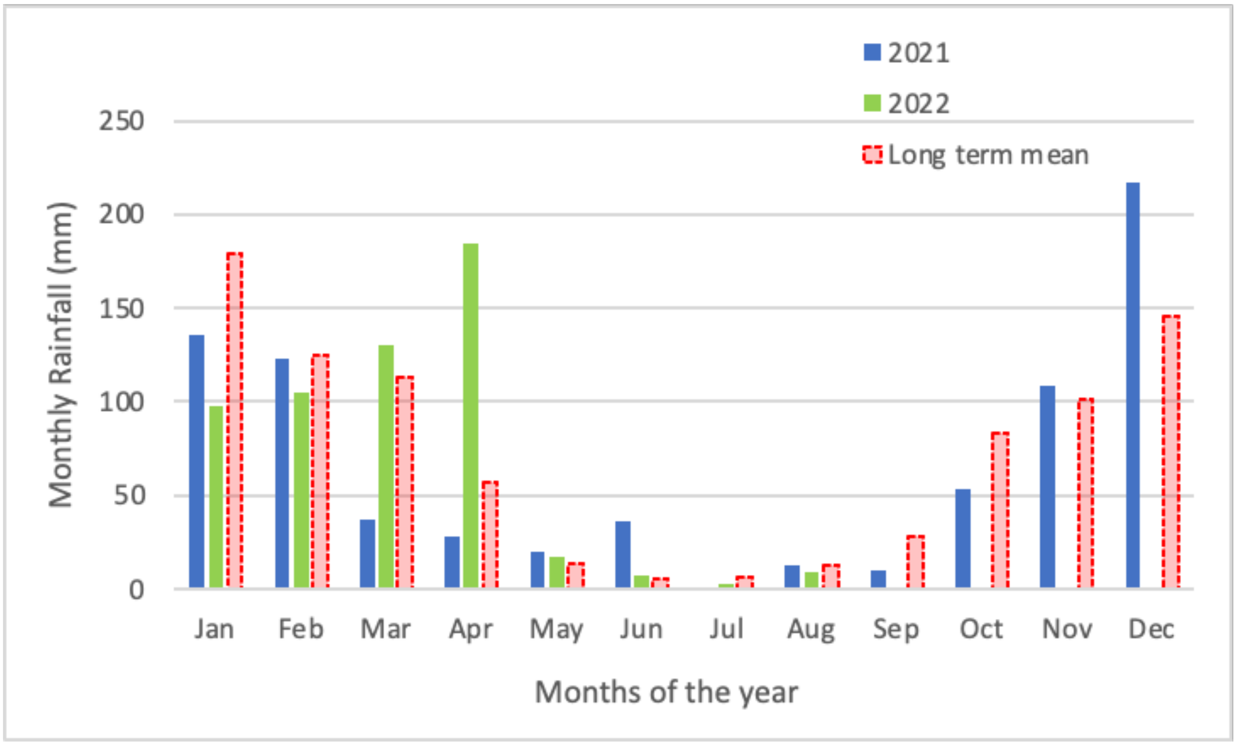


Figure 3. The monthly rainfall during the sampling period for 2021 and 2022 compared to the long-term average annual rainfall in Wakkerstroom, South Africa (Climate Data, 2022).

3.2 Site selection

Eight sampling sites were chosen for data collection of this study. All of the sampling sites run along the length of the Wakkerstroom wetland (Fig. 3). The sampling sites were selected for ease of accessibility and as an estimate measure of the water quality of the wetland. The sampling sites were spaced approximately 500 – 1000 m from one another.

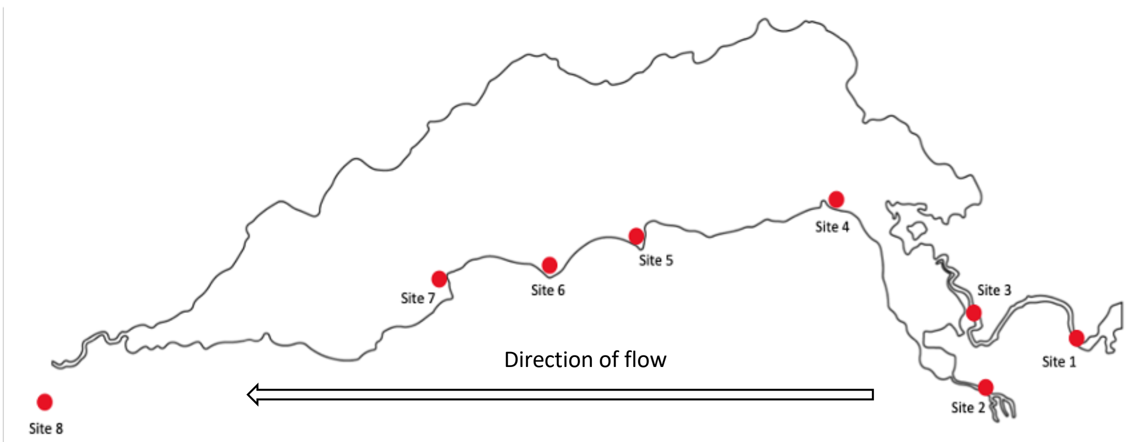


Figure 4. Outline of the Wakkerstroom wetland showing the eight sampling sites along the length of the wetland ***Image not to scale***

Illustrated below is land cover use with a ten kilometer radius surrounding the Wakkerstroom wetland (Fig. 5). The Land Cover map was generated using the South African National Land Cover 2020 dataset (SANLC, 2020).

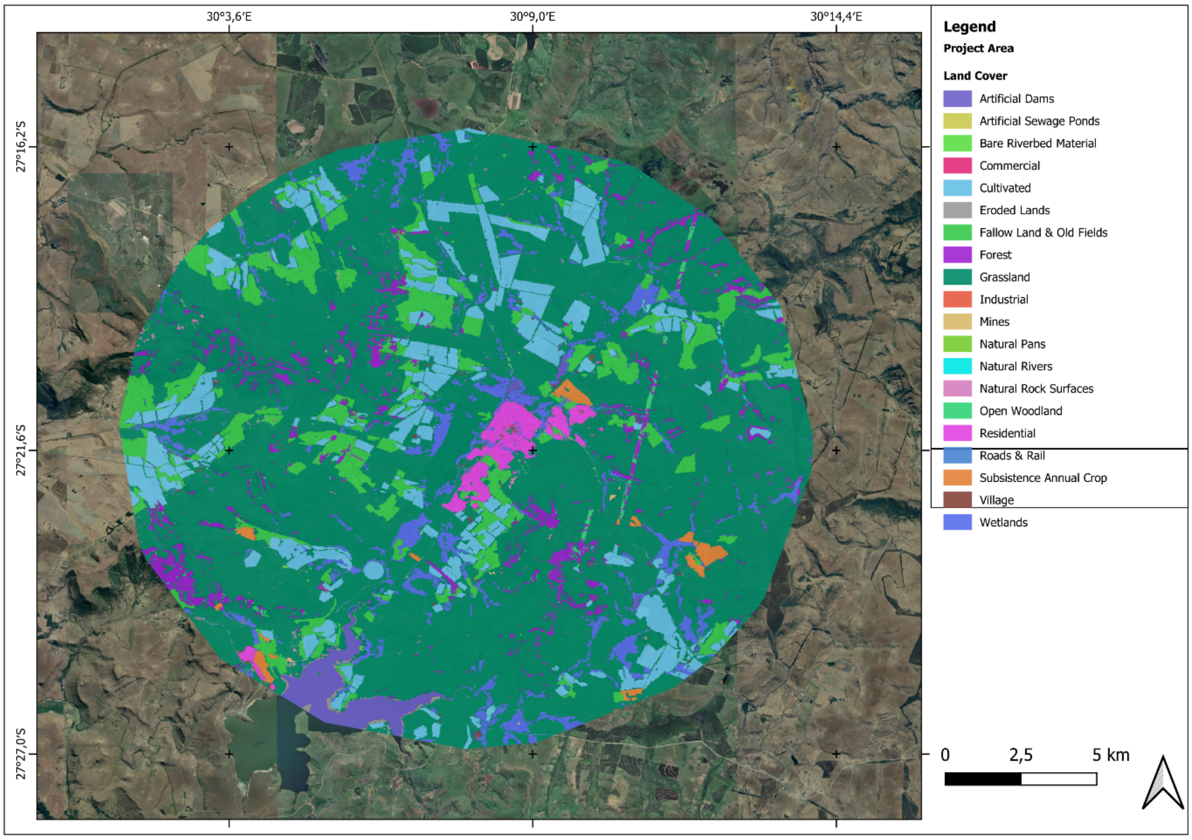


Figure 5. A land cover map showing the land cover types situated around the wetland within a 10 km radius (SANLC, 2020)

Site 1

Site 1 [27° 20′ 25′′ S 30° 09′ 21′′ E] is the first sampling site and is upstream of the Wakkerstroom Wetland, approximately 700 m from the upper region of the wetland (Fig. 6). Site 1 is located along the Wakkerstroom River, immediately downstream of Martin’s Dam. It is a perennial river and throughout the 2021 – 2022 sampling period for this study, the river maintained water flow. The river had a slow and gentle flow which increased to a moderate flow during the months of high rainfall. The riverbank was sparsely lined with hydrophytes such as wetland grasses. Throughout the sampling period, no buildup of algae or floating water plants such as Water hyacinth (*Eichhornia crassipes)*, Water Lettuce (in the family *Araceae*), or Duckweed (*Lemnoideae*) were observed. These invasive aquatic plant species are common indicators of excess nutrients. Therefore, at Site 1 low levels of nutrients such as nitrates and phosphates were anticipated. The land immediately upstream of the Wakkerstroom river is not known to be used for agricultural purposes, and as such it is expected that the Wakkerstroom River water does not have increased nutrients.

Figure 6. Photographs of Site 1. The Wakkerstroom River during the high (A) and low (B) rainfall season

Site 2

Site 2 [27° 20’ 48’’S 30° 09’ 06’’E] is along the R543 travelling Southwest towards Wakkerstroom town. Site 2 is a high pollutant loading site, containing untreated wastewater, grey water and runoff water from the informal settlement eSizameleni. Site 2 is located east of the Wakkerstroom wetland and there is no exchange of water between Site 1 and Site 2. Site 2 was a large marshy area, with several small streams flowing into storm water channels entering the wetland as a point source pollutant, as the storm water channels lead directly into the wetland. The polluted water streams were shallow and often contained murky or cloudy water (Fig. 7). Livestock such as cattle and goats were frequently present at Site 2, grazing the surrounding grasses. As the water samples collected at Site 2 were from polluted water streams, it was expected that the water would contain high levels of nutrients from greywater (containing dissolved domestic detergents) and possible microbiological contaminants from untreated wastewater and livestock faecal matter.

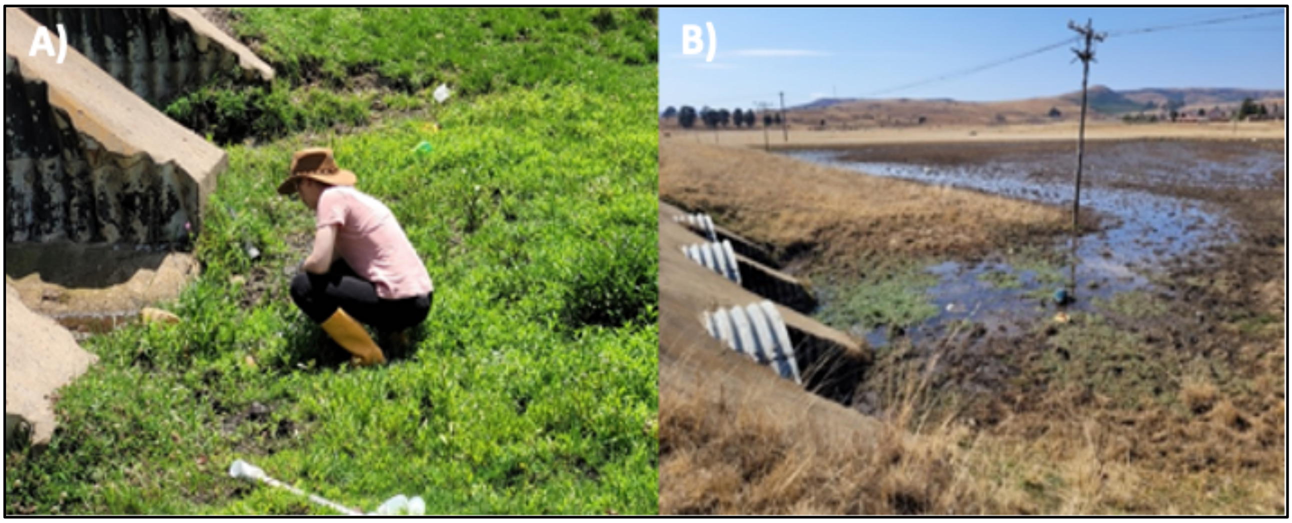


Figure 7. Photographs of Site 2. The domestic and sewage runoff from eSizameleni informal settlement, during the high (A) and low (B) rainfall season

Site 3

Site 3 [27° 20' 39''S 30° 08' 34 E] is located in the upper region of the wetland. It is a perennial narrow stream leading into a water channel within the wetland. Site 3 receives polluted water from Site 2 to the east, as well as polluted runoff water from eSizameleni via several smaller streams entering the upper region of the wetland. Therefore, Site 3 receives several additional sources of polluted water. The water conditions for the site were expected to show a similar concentration of pollutant loading to that in Site 2, as well as microbiological contamination in the form of coliforms and possibly *E.coli* as a result of faecal matter either from livestock or untreated wastewater. Over the course of the sampling period, it was noted that a mat of floating plants, identified as duck weed (*Lenma spp)* was occasionally present (Fig 8).

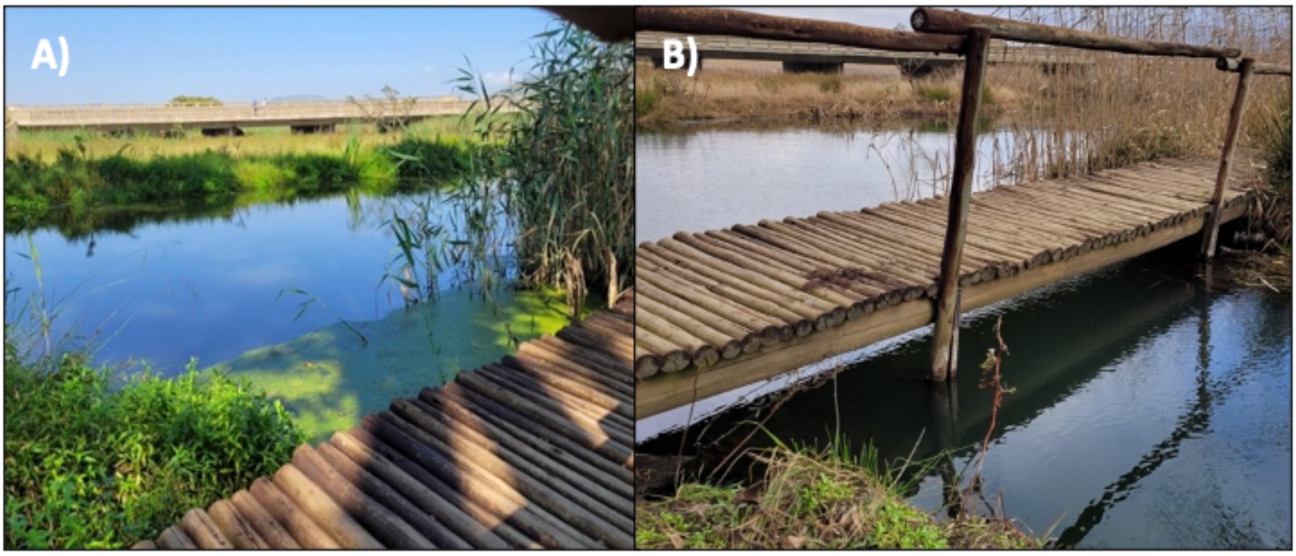


Figure 8. Photographs of Site 3. A dam that forms part of the Wakkerstroom wetland due during the high (A) and low (B) rainfall season

Site 4

Site 4 [27° 20' 49''S 30° 08'23'' E] is the next site along the Wakkerstroom Wetland and is approximately 450 m downstream of Site 3. A thick stand of *Phragmites sp.* reeds grows all along the border of the wetland at Site 4. The mature *Phragmites* were up to 2 m tall and there was limited access to sample water through the dense reeds. Site 4 was chosen for sampling as there was a pool of water, approximately 3 - 5 m in length forming a small and mostly still water body where the water was accessible for samples to be collected (Fig 9). The water at Site 4 was often slightly murky. Indicator floating plants and excessive algae were not noted throughout the sampling period. The water was expected to have a slightly lower concentration of nutrients than Site 2 and bacterial contamination of coliforms due to the close proximity to Site 3.

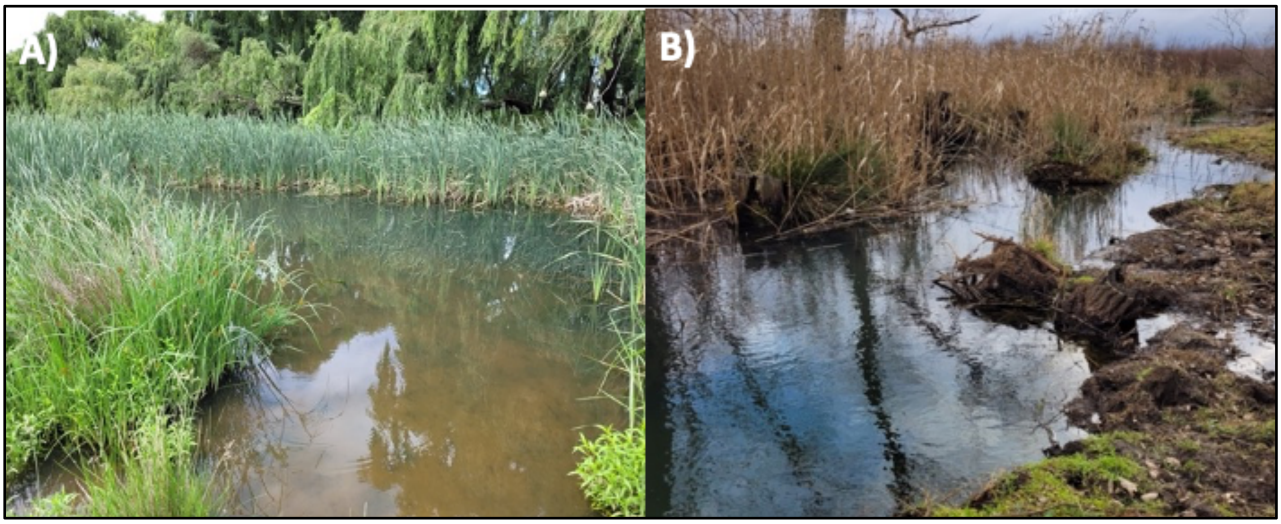


Figure 9. Photographs of Site 4. A small waterbody as a relic channel during the high (A) and low (B) rainfall season

Site 5

Site 5 [27° 21′ 19′′ S 30° 08′ 01′′ E] was located 1100 m from Site 4 and approximately 2000 m from the upper region of the wetland. It is permanently flooded with saturated soils all year round and is densely populated mainly with wetland grasses (Fig. 10). In addition, this location does not contain thick reeds such as *Phragmites* or *Thypha*, as observed in some of the upstream sites. There was no open flowing water located around Site 5 as the water filters through the wetland very slowly. The water remains fairly shallow and during the high rainfall months of sampling the water reached a maximum of 40 – 50 cm in depth. During the sampling period it was observed that cattle were frequently grazing and walking along the edges of the wetland. For most of the sampling months, a thin oil-like substance was observed on the surface of the water. The water at this site was often shallow and murky or muddy, especially during the low rainfall period. It was speculated that the water would contain reduced nutrients due to the slow flow of water through the wetland, which allows for plant uptake of excess nutrients.

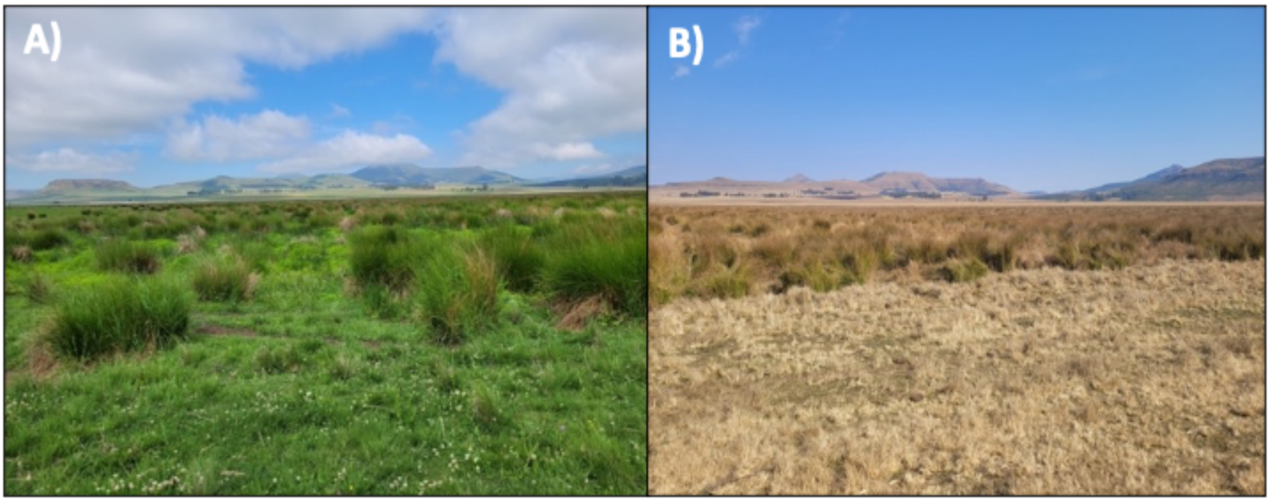


Figure 10. Photographs of Site 5. A temporarily saturated site along the wetland border dominated by wetland grass species during the high (A) and low (B) rainfall season

Site 6

Site 6 [27° 21’ 38’’ S 30° 07’ 52’’ E] was situated approximately in the middle of the wetland in terms of length and was around 600 m downstream of Site 5. Site 6 was very similar to Site 5 with a high density of wetland grasses, an absence of *Phragmites* or *Thypha* and reduced flow of water through the site (Fig. 11). A concrete sewage drum was located on a small hill just above the water sampling site and possibly groundwater sewage leaks could have occurred that seeped into the wetland. There were no signs of surface leaks from the sewage concrete drum.

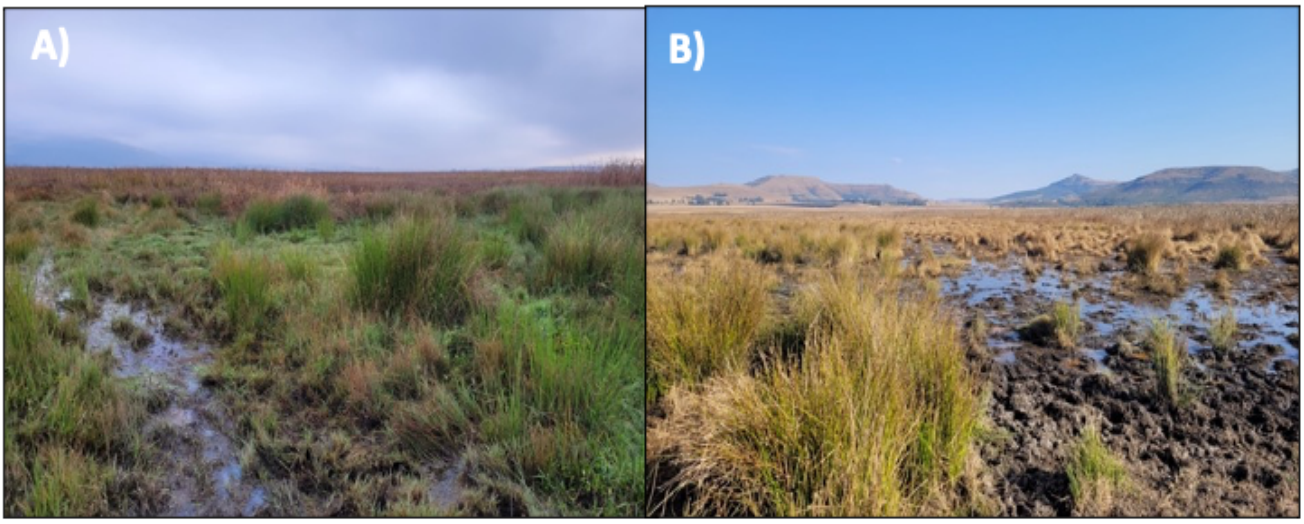


Figure 11. Photographs of Site 6 A seasonally saturated site along the wetland border dominated by wetland grass species during the high (A) and low (B) rainfall season

Site 7

Site 7 [27° 21′ 53′′ S 30° 07′36′′ E] is situated on the lower end of the wetland underneath the R543 road from Volksrust heading towards Wakkerstroom and was the last sampling site along the wetland. Site 7 has a narrow but deep stream of water flowing through the wetland. The stream had *Phragmites*, *Thypha* and wetland grasses lining the edges. Sampling took place from a slow flowing perennial section of the river. The water was often a murky brown colour, possibly as a result of disturbed sediment, with no algal blooms or *Lemna* present (Fig. 12). The water quality at Site 7 was expected to be improved as compared to the upper region of the wetland due to the wetland filtration processes reducing excess nutrients and microbiological contaminants.

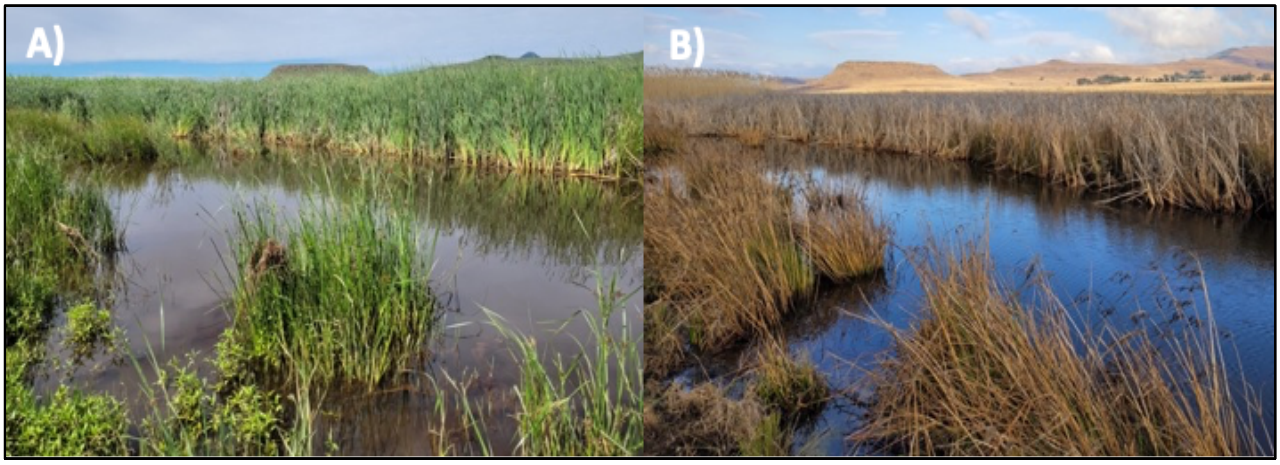


Figure 12. Photographs of Site 7. A permanent body of water within the wetland flowing underneath the R543 during the high (A) and low (B) rainfall season

Site 8

Site 8 [27° 23′ 56′′ S 30° 06′ 30′′ E] is located along the Thaka river, downstream from the main wetland and approximately two kilometers from the lower region of the wetland. A meandering stream develops from the wetland which becomes the Thaka River flowing south west. Site 8 is located in the Kwa-Zulu Natal province. The river is wide with varying waterflow depending on the season (Fig. 13). Thaka River flows into the Zaai-Hoek Dam (Joubert & Ellery, 2013). The river is surrounded by grasslands grazing cattle. The water samples were taken from a small bridge, where several previous studies have sampled the water, to allow for comparison. *Phragmites* and *Thypha* were absent, however some wetland grasses were present along the edges of the river. The water was always clear with no algal blooms or

Lemna. It was expected that the water quality would be similar to that of Site 1, with reduced nutrients and a reduction of microbial contamination as a result of wetland filtration processes. No pollutant inputs were suspected between the wetland and the downstream Thaka River.

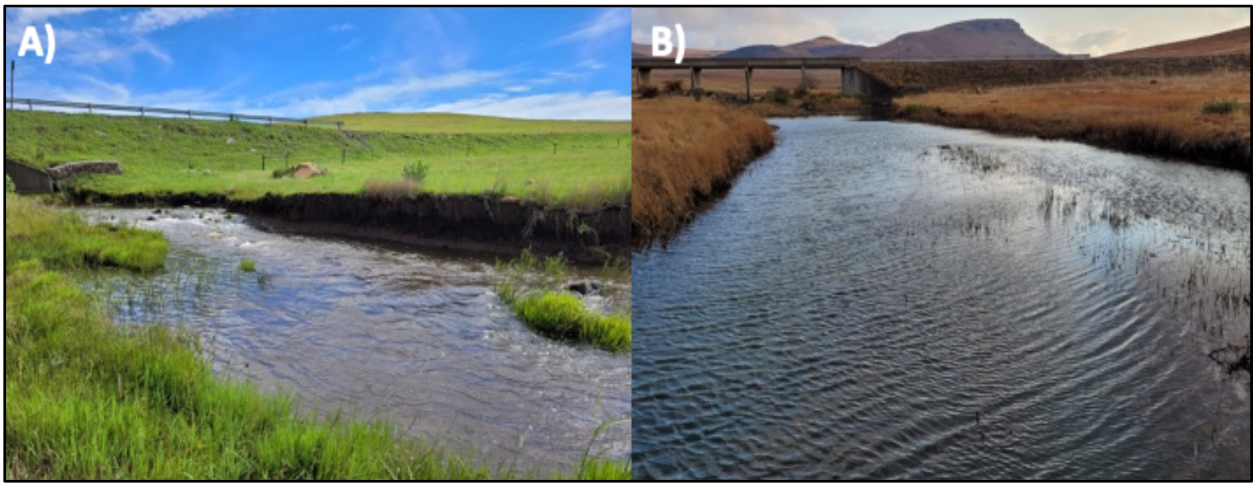


Figure 13. Photographs of Site 8. The Thaka River downstream of the Wakkerstroom wetland during the high (A) and low (B) rainfall season

Chapter 4. Methodology

4.1 Collection of water samples

Data were collected once a month between the period of August 2021 to July 2022 except in September 2021 due to transport difficulties, resulting in 11 months of data. Water samples were collected for two types of data analysis – chemical and microbiological analysis. Microbiological data are presented from October 2021 to July 2022, where as data for the chemical properties of the water samples are presented from August 2021 to July 2022. Microbiological data from August 2021 were not included as there was contamination from dirty glassware. Bottles used for sampling were cleaned thoroughly using a rigorous cleaning procedure of multiple washes.

4.1.1 Water sample collection in the field

At each sampling site, three water samples were collected to ensure a representative sample. Each repeat was collected 3 – 5 m away from the previous one. A 1 m sampling stick with a 125 mL Nalgene bottle secured to the end within an open plastic cylinder was gently lowered into the water to around 20 cm below the surface. At sites 1 and 8, where the water was flowing, the sample bottle was placed into the water facing the direction of water flow, to ensure that any disturbances caused by lowering the bottle and sampling stick into the water flowed downstream, rather than directly into the sampling bottle. At the sampling sites where the water was shallow, the Nalgene bottle was placed into the water just above the sediment layer, with care not to disrupt any sediment such that sediment did not enter the sampling bottle. The bottles were filled and removed from the water and capped immediately. The water samples were then placed into a cooler bag to prevent temperature changes. Analyses of the water samples were conducted in the Wakkerstroom research house within 3 hours of sample collection.

4.2 Water Sample Analysis

4.2.1 Measurement of pH and Total Dissolved Solids (TDS)

pH measurement: The samples were measured for pH using the HACH HQ40D Portable Multimeter probe and meter. The probe was calibrated using standard pH buffer solutions of pH 7 and 10.

Total dissolved solids measurement (TDS): The samples were measured for TDS using the HACH HQ40D Portable Multimeter probe and meter. The probe was calibrated using a clean sample of 10 mL of distilled water.

4.2.2 Analysis of Nitrate (N- NO_3^-) and Phosphate (P-PO_4^{3-})

Nitrate analysis: Water samples were analysed using the procedure described in the HACH DR 900 User Manual. This is a colourimetric reaction using reagents provided by the company, where the intensity of the yellow colour developed is measured using a colorimeter. The Hach meter which is a colourimeter is calibrated using prepared standard solutions (KNO_3) of known concentrations and nitrate is measured as N-NO_3^- mg/L. The programme "335" was selected for a nitrate measurement range between 0.1 to 30 mg/L. The accuracy of the instrument ranged between 0.3 to 0.8 mg/L depending on the concentration of N-NO_3^- within the sample (see user guide file:///C:/Users/Daniel/Downloads/DOC316.53.01066.pdf).

Phosphate analysis: Water samples were analysed using the procedure described in the HACH DR 900 User Manual. This is a colourimetric reaction using reagents provided by the company, where the intensity of the blue colour developed is measured using a colorimeter. The Hach meter which is a colourimeter is calibrated using prepared standard solutions (H_2PO_4) of known concentrations and nitrate is measured as P-PO_4^{3-}- mg/L. The programme "490" was selected for a phosphate measurement ranged between 0.02 to 2.50 mg/L. The accuracy of the instrument is 0.02 mg/L of P-PO_4^{3-} within the sample (see user guide file:///C:/Users/Daniel/Downloads/DOC316.53.01119.pdf).

4.2.3 Trophic status of nutrient concentrations

Using the South African Water Quality Guidelines Volume 7: Aquatic Ecosystems, developed by the Department of Water Affairs and Forestry, the trophic status of the wetland with reference to the nitrate (Table 2) and phosphate (Table 3) concentrations were determined. Using above-mentioned guidelines, we were able to determine the general trophic status of the wetland and the possible effects that higher concentrations of nutrients could have on the water quality, vegetation and supported biodiversity.

Table 1. A table showing the trophic status of average field water conditions for NO_3^- and their general effects on the surrounding aquatic environmental as categorized by the South Africa Water Quality Guidelines – Aquatic Ecosystems (DWAF, 1996).

NO_3^- concentration (mg/L)	Classification	Effects
< 0.50	Oligotrophic	Moderate levels of species diversity; rapid nutrient uptake and cycling; low algae growth and problematic aquatic plant growth
0.50 – 2.50	Mesotrophic	Moderate levels of species diversity; low algae and problematic aquatic plant growth
2.50 – 10.00	Eutrophic	Low levels of spices diversity; high algae and problematic aquatic plant growth; can be toxic to humans and livestock
> 10.00	Hypertrophic	Very low levels of spices diversity; high algae and problematic aquatic plant growth; toxic to humans and livestock

Table 2. A table showing the trophic status of average field water conditions for PO_4^{3-} and their general effects on the surrounding aquatic environmental as categorized by the South Africa Water Quality Guidelines – Aquatic Ecosystems (DWAF, 1996).

PO_4^{3-} concentration (mg/L)	Classification	Effects
< 0.005	Oligotrophic	Moderate levels of species diversity; rapid nutrient uptake and cycling; low algae growth and problematic aquatic plant growth
0.005 – 0.025	Mesotrophic	Moderate levels of species diversity; low algae and problematic aquatic plant growth
0.025 – 0.250	Eutrophic	Low levels of spices diversity; high algae and problematic aquatic plant growth; can be toxic to humans and livestock
> 0.250	Hypertrophic	Very low levels of spices diversity; high algae and problematic aquatic plant growth; toxic to humans and livestock

4.3 Coliform and *E.coli* analyses

4.3.1 Development of the coliform colouration scale

A qualitative coliform bacterial analysis was conducted to test for the presence of the coliform group of bacteria and estimate the abundance of these bacteria. A change in the colour was described on a scale from one to five. This scale shows the absence or relative abundance of the coliform bacterial group in the collected water samples. "1" on the scale shows no presence of coliforms. "5" on the scale shows the presence of coliforms, described in Table 3 and using Figure 14.

4.3.2 Detection of Coliforms and E.coli

Water samples were tested for coliforms and *E.coli* using the EC Blue technique and reagent. After the colour had developed, the intensity of the colour was measured against the colour scale (Fig. 14) to estimate the coliform abundance for each water sample (Table 1). Following this, the same water samples were used to test for the presence of E.coli bacteria, as it was a two-part qualitative test. The *E.coli* analysis was conducted on the coliform water samples after the 24 hour period when the colour had developed and the samples had been taken out of the water bath. After which the samples were taken to a darkened room within the research house and each sample was checked for *E.coli*. This was done using an ultra violet (UV) light where the light was held up against the glass sample bottles. If the coliform samples fluoresced, the water samples contained the bacteria *E.coli,* if the water sample did not fluoresce, *E.coli* bacteria was not present in the water sample (Fig. 15).

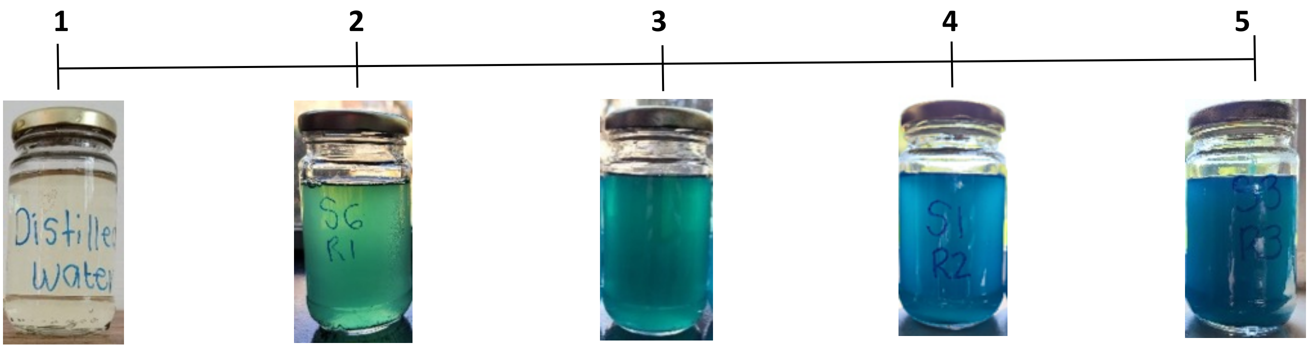


Figure 14. Photographs of coliform water samples showing the presence and abundance of coliforms in ascending order of coliform abundance from 1 to 5.

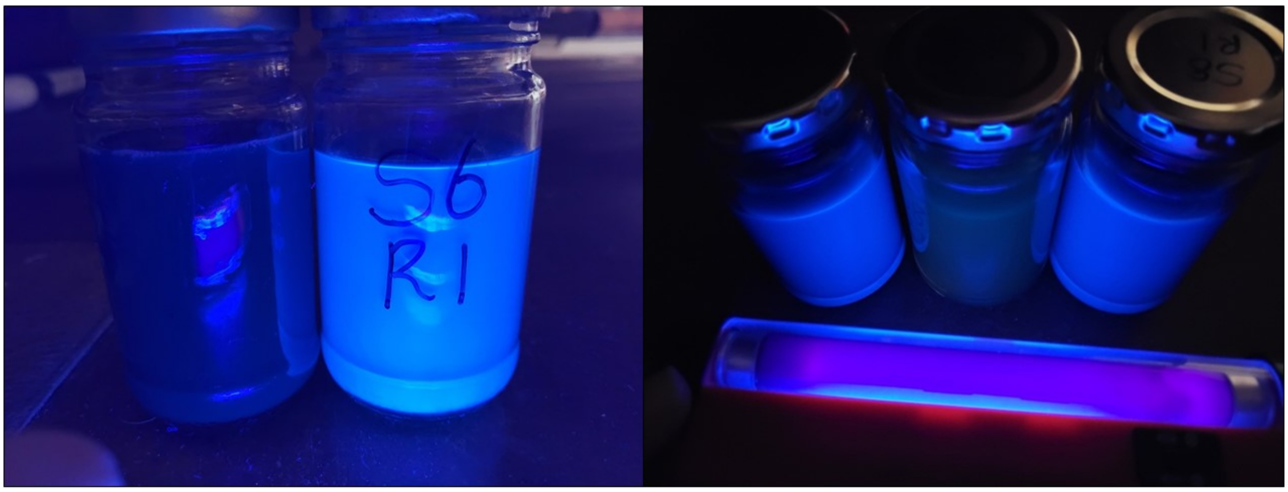


Figure 15. Photographs of the *E.coli* analysis procedure showing the difference between a positive, which fluoresced, and a negative *E.coli* water sample that did not fluoresce, with the UV light that was used at the bottom of the right image.

Table 3. Colour descriptions of the coliform samples indicating the presence and abundance of coliforms in the water samples.

Score	Colour Description	Coliforms present	Coliform Abundance
1	Clear pale yellow	No	Absent
2	Light turquoise	Yes	Low
3	Darker turquoise /blue-green	Yes	Low - Moderate
4	Blue	Yes	Moderate to High
5	Dark blue	Yes	Very high

4.4 Net Removal or Addition

The removal or addition of the nutrient concentrations and coliform abundance were calculated from the monthly means of each sampling site along the wetland for each month. The removal or addition of the concentration of nutrients or coliform abundance were calculated using the difference in concentration or coliform abundance between each site, from Site 1 to Site 8. The difference for nutrient concentrations and coliforms were calculated between Site 1 and Site 3. This is done as Site 1, the Wakkerstroom River does not flow into Site 2, and therefore does not influence Site 2. After this, the difference between Site 2 and Site 3 was calculated, followed by the difference between Site 3 and Site 4, and so on. This was repeated until the difference between Site 7 and Site 8, the downstream sampling sites was calculated. The net removal for the duration of the sampling period was calculated through the summation of the removal or addition of nutrient concentrations and coliform abundance of all the sampling months.

4.5 Statistical Analyses

All data were tested for normality using the Shapiro-Wilk test in RStudio and R Statistical Software (version 1.1.4. R Core Team, 2018), which revealed all data to not be normally distributed. The data could not be transformed into a normal distribution, therefore all statistical analyses were done using non-parametric statistical tests. A non-parametric two-way analysis of variance (Kruskal Wallis) test was run in RStudio for the chemical parameters between months and sites to test if there were significant differences for chemical parameters and coliform abundance. The Kruskal Wallis test was run with a 95% confidence interval. Thereafter, the Dunn Test (non-parametric post hoc test) using the Bonferroni correction was performed to determine where the significant differences lay between the sites and sampling months. Spearman's rank correlation tests were run in Microsoft Excel between the four chemical parameters to test for a linear relationship between the variables, and to test the relationship between the presence of *E.coli* and the abundance of coliforms. Lastly, to test if there were significant differences between the net addition and net removal of nitrate and phosphate, a Mann – Whitney U test (non-parametric independent t-test) was run in Phyton (The Phyton Tutorial Version 2, 2009).

Chapter 5. Results

Water quality can be monitored through biological and chemical parameters – such as assessing for the presence of bacterial contamination, and nutrient loading concentrations. This chapter will outline the results collected between the period August 2021 to July 2022 from the Wakkerstroom Wetland, Mpumalanga, South Africa. The data were collected on a monthly basis across eight sampling sites along the Wakkerstroom wetland. First, the chemical parameters: pH, total dissolved solids (TDS)(mg/L), nitrogen - nitrate (mg/L) and phosphorous - phosphate (mg/L) will be explored. Secondly, the qualitatively scored microbiological data including coliform abundance and *E. coli* presence will be assessed, and finally, the Wetland's removal efficiency for total dissolved solids, nitrate, phosphate and coliforms will be presented.

5.1 Chemical Analysis

5.1.1 pH

pH displayed little temporal and spatial change across the sampling period and sampling sites, remaining consistent with little variability ($p > 0.05$) throughout the sampling period. The pH measurements all fell within a range of 6 – 8, summarized in Table 4. The measurements taken for the eight sampling sites can be categorized as neutral acidic (pH = 6 -7) to neutral basic (pH = 7- 8). The highest mean pH (7.40 ± 0.20) was most frequently recorded in October 2021 at six of the eight sampling sites and the lowest mean pH (6.40 ± 0.18) across the sampling sites was most frequently recorded in April 2022 at seven of the eight sampling sites. The mean pH measured across the sampling period ranged between 6.80 ± 0.37 and 7.03 ± 0.22, displaying low variation of 0.09 between the mean pH measurements across sampling sites. Site 2 showed the highest pH but lowest variance in pH measurements with a range between 6.85 and 7.44 while Site 1 showed the highest pH variance with a range between 6.09 and 7.87. Site 4 displayed the lowest mean pH between the sampling sites (6.40 ± 0.37). The overall trend of pH showed a decrease in pH between August 2021 to July 2022, while the month to month pH distribution shows an irregular pattern of increasing and decreasing pH seen at all eight sampling sites (Table 4).

Table 4. Monthly mean pH of water samples for the sampling sites along the Wakkerstroom wetland between August 2021 and July 2022 with the overall mean pH and standard deviation (SD) for each sampling site.

Month / Site	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8
Aug 2021	6.73	7.29	7.21	6.98	7.12	6.55	6.58	6.77
Oct 2021	7.12	7.08	7.55	7.45	7.57	7.54	7.38	7.40
Nov 2021	7.18	6.82	7.14	6.98	6.78	7.02	6.58	6.95
Dec 2021	7.46	7.25	7.29	7.30	7.02	7.16	7.40	7.30
Jan 2022	6.40	7.03	6.45	6.33	6.51	6.46	6.57	6.98
Feb 2022	7.11	6.90	7.07	6.67	6.33	6.99	6.82	7.27
Mar 2022	6.49	6.93	6.96	6.86	6.72	6.81	6.88	6.89
Apr 2022	6.26	6.69	6.43	6.25	6.46	6.41	6.27	6.24
May 2022	7.29	7.10	6.86	6.78	6.80	7.04	6.99	6.92
Jun 2022	7.77	7.31	7.30	7.06	6.72	6.76	7.06	6.99
Jul 2022	6.34	6.89	6.51	6.53	6.83	6.49	6.31	6.30
Mean ± SD	6.92 ± 0.51	7.03 ± 0.22	6.98 ± 0.38	6.40 ± 0.37	6.81 ± 0.34	6.84 ± 0.35	6.80 ± 0.38	6.91 ± 0.36

The mean pH measurements were significantly higher during the low rainfall period (August 2021 - October 2021 and May 2022 – July 2022), than the high rainfall period (November 2021 – April 2022) ($p = 0.03$), where the mean pH of the high rainfall period was 6.81 ± 0.11, and the low rainfall period was 6.98 ± 0.10 (Table 4). This pattern is observed across the first seven sampling sites (Site 1 – Site 7), however at Site 8 a reduced pH was observed during the low rainfall season (Table 4). At each sampling site, the pH during the low rainfall season is closer to a neutral pH of 7, whereas the mean pH recorded for the same sampling site during the high rainfall period is lower on the pH scale and therefore more acidic. The highest mean pH during the high rainfall period was recorded at sites 2 and 8, 6.94 ± 0.21 and ± 6.94 0.37, respectively. The highest pH during the low rainfall period was also observed at Site 2, 7.13 ± 0.20. The lowest mean pH during the low rainfall period was recorded at Site 7, 6.86 ± 0.39, while the lowest mean pH for the high rainfall period was recorded at Site 5, 6.64 ± 0.24 (Table 4).

5.1.2 Total Dissolved Solids

Total dissolved solids showed high variability between the sampling sites, and showed high temporal variability at sites 3, 4 and 5, with differences between all sites summarized in Table 5. Site 1 had significantly lower TDS measurements which all clustered around 50 mg/L, when compared to sites 2, 3, 4 ,5 and 6 ($p < 0.01$). Site 1 (53.54 ± 7.13), Site 7 (60.47 ± 13.38 mg/L) and Site 8 (68.81 ± 15.30 mg/L) had similar mean TDS measurements across the sampling period ($p > 0.05$). Site 1 remained consistently low throughout the sampling period, suggesting low pollutant loads coming from upstream sources that enter the Wakkerstroom River. Site 1, showed low variability through the seasons, suggesting rainfall input does not majorly affect the TDS of the Wakkerstroom River (Table 5). Site 2, the input pollution site had an increased TDS, with a higher concentration of dissolved solids due to the water streams categorized as grey and wastewater containing detergents, soaps, other household cleaning products and untreated sewage. The mean TDS measurements at Site 2 were above 110 mg/L for all the sampling periods except in May 2022 (88.40 ± 1.95 mg/L). Similar elevated TDS measurements were recorded at Sites 5 and 6, where more than half of the sampling months had TDS measurements above 100 mg/L, however Site 5 showed the highest variability in TDS across the sampling period with a range between 50 – 350 mg/L. In addition, Site 5 (143.29 ± 104.78), Site 2 (142.22 ± 34.13) and Site 6 (142.16 ± 47.21) had near identical mean TDS values across the sampling period ($p = 0.95$). Site 3 – the first sampling site of the Wakkerstroom Wetland, followed by Site 4 both showed reduced TDS measurements from Site 2 ($p < 0.01$). Site 3 and 4 showed almost identical overall mean TDS measurements across the sampling period, 79.26 ± 42.91 and 79.20 ± 28.83 mg/L, respectively ($p = 0.98$). Site 7 regularly showed reduced TDS following sites 5 and 6 ($p < 0.001$). Site 7 had the second lowest overall mean TDS (58.02 ± 12.08 mg/L), following Site 1 ($p = 0.95$), suggesting TDS removal is occurring through the wetland (Table 3a and 3b) after chemical pollutants in the form of dissolved solids enter the wetland. Site 8 – the downstream river, also had reduced TDS (65.34 ± 14.22 mg/L) similar to sites 1 and 7 ($p = 0.62$). This indicates no pollutant loading is occurring between the wetland and the downstream river where sampling at Site 8 occurs.

Five of the eight sampling sites had the lowest TDS measurements occur in March 2022, followed by Sites 6 and 7 where the lowest TDS measurements were recorded in August 2021. No consistent temporal patterns were observed for the high TDS measurements between months, other than sites 3, 4 and 8 had high monthly TDS in October 2021. The increase observed during October's data collection period corresponded with increased pH (Table 4), high nitrate (Table 6) and phosphate (Table 8) concentrations as well. A weak positive relationship was present for all three chemical parameters against TDS, however, no significant relationship was found between TDS and pH (rho = 0.54), TDS and nitrate (rho = 0.46) or TDS and phosphate (rho = 0.42) when a correlation analysis was done between the chemical variables.

Table 5. Monthly mean total dissolved solids (TDS)(mg/L) of water samples for the sampling sites along the Wakkerstroom wetland between August 2021 and July 2022 with the overall mean TDS and standard deviation (SD) for each sampling site.

Month / Site	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8
Aug 2021	59.63	143.90	113.13	107.57	75.37	50.43	43.83	56.33
Oct 2021	57.83	185.57	177.73	140.17	219.57	142.00	80.73	93.63
Nov 2021	57.40	141.00	83.87	84.50	78.90	166.17	59.63	90.00
Dec 2021	51.87	129.30	55.67	72.70	147.37	137.87	56.07	61.37
Jan 2022	46.10	191.20	67.37	62.13	207.13	167.93	59.43	61.03
Feb 2022	45.27	121.13	54.50	56.90	99.00	185.27	82.17	77.50
Mar 2022	41.03	174.73	43.57	48.67	58.83	94.53	49.77	52.37
Apr 2022	55.93	163.17	59.37	73.53	79.27	127.40	52.10	58.27
May 2022	54.40	88.40	63.07	63.17	79.87	125.00	51.03	54.03
Jun 2022	59.30	112.90	72.57	79.23	298.17	206.80	53.00	56.83
July 2022	60.13	113.10	81.07	82.67	232.70	160.33	50.40	57.33
Mean ± SD	53.54 ± 7.13	142.22 ± 28.53	79.26 ± 42.91	79.20 ± 28.83	143.29 ± 64.37	142.16 ± 46.06	58.02 ± 60.47	65.34 ± 15.30

There is an inconsistent change between mean TDS at each site over the high and low rainfall period (Fig. 15). Sites 1, 3, 4 and 5 show increased TDS during the low rainfall period while sites 2, 6, 7 and 8 show decreased TDS during the low rainfall period. Sites 3, 4 and 5 all show noticeable differences in mean TDS between the high and low rainfall periods - greater than 25 mg/L. Sites 1, 7 and 8 which all have reduced TDS, show no significant change ($p = 0.06$) in TDS across the high and low rainfall periods – suggesting rainfall had an inconsistent effect on the total dissolved solids of the sites.

5.1.3 Nitrate (NO_3^-)

The nitrate concentrations recorded across the eight samplings sites showed high variability, with several extreme upper values between Site 2 and sites 4 to 7, summarized in Table 6. Site 1 had the lowest nitrate concentrations across the sampling period with significant differences between sites 2, 4, 5 and 6 ($p < 0.01$). Site 2, the input site, had high variability in nitrate concentrations where the mean monthly concentrations were categorized from oligotrophic in December 2021 (0.47 ± 0.15 mg/L) to hypertrophic in November 2021 (13.07 ± 6.64 mg/L) and February 2022 (14.23 ± 1.79 mg/L). Site 3 displays a similarly low mean nitrate concentration (1.90 mg/L) to Site 1 ($p > 0.05$) – categorized as mesotrophic (Table 7). Despite the low mean nitrate concentration at Site 3, an elevated nitrate concentration was observed at Site 3 during March 2022 that was uncharacteristically high when compared to the other sampling months showing a monthly mean of 7.80 ± 0.46 mg/L – which results in eutrophic water conditions. Site 4 had an increased mean nitrate concentration following Site 3, however the mean nitrate concentration at Site 4 remained lower than the input site (Site 2). The nitrate concentration at Site 4 also had an uncharacteristically high NO_3^- concentration during February 2022 (17.00 ± 4.32 mg/L), along with Site 2. Sites 5 and 6 showed elevated nitrate concentrations throughout the sampling period ($p > 0.05$). During December 2021 and January 2022 hypertrophic conditions were observed at Sites 5 and 6. There was a reduction in mean nitrate concentrations after March 2022. There was a decrease in nitrate concentrations from Sites 5 and 6 to Site 7, however Site 7 was not significantly different to Site 6 ($p = 0.08$) Site 7 showed similar increased mean raised NO_3^- concentrations at Site 4 (p

> 0.05). Site 8 had lower nitrate concentrations than Site 7 ($p > 0.05$) with a similar mean nitrate concentration to Site 1 and 3. The water conditions at Site 8 showed high temporal variation with monthly means between 0.53 – 4.00 mg/L, ranging between mesotrophic and eutrophic conditions, however the mean nitrate concentration across the sampling period shows predominately mesotrophic conditions for 70% of the sampling period. Site 8 is not significantly different to Site 1 ($p > 0.05$), suggesting reduced nitrate concentrations after nutrient loading occurs between Site 2 and Site 6. Between sites 1 and 4 and Site 8, February or March 2022 showed elevated nitrate concentrations. February 2022 also had the highest monthly mean across the sampling sites (8.36 ± 5.69 mg/L). August 2021 had the lowest mean nitrate across the sampling period (0.98 ± 0.88 mg/L).

Table 6. Monthly mean nitrate (NO_3^-)(mg/L) of water samples for the sampling sites along the Wakkerstroom wetland between August 2021 and July 2022 with the overall mean NO_3^- and standard deviation (SD) for each sampling site.

Month / Site	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8
Aug 2021	0.00	2.33	1.23	0.90	1.90	0.07	0.83	0.53
Oct 2021	0.67	6.33	3.07	5.77	3.50	2.30	11.53	4.00
Nov 2021	1.27	13.07	1.83	8.53	3.40	2.70	9.33	- -
Dec 2021	0.47	0.47	0.00	0.20	12.90	15.17	0.00	1.17
Jan 2022	1.73	1.20	1.53	0.27	20.00	13.77	1.03	1.83
Feb 2022	1.77	14.23	1.83	17.00	11.13	6.83	10.00	4.07
Mar 2022	7.50	1.83	7.80	3.07	3.33	3.87	1.20	2.97
Apr 2022	1.03	5.57	0.90	1.63	5.83	4.93	1.27	2.27
May 2022	1.30	0.80	1.10	0.90	3.03	3.00	0.73	0.97
Jun 2022	0.43	3.77	0.20	1.17	4.27	6.27	0.70	0.93
Jul 2022	0.73	1.67	1.43	1.70	2.87	2.37	0.80	1.63
Mean ± SD	1.54 ± 2.01	4.66 ± 5.13	1.90 ± 2.07	3.74 ± 5.14	6.98 ± 7.58	5.35 ± 5.43	3.40 ± 4.39	2.04 ± 1.32

Table 7. A table showing the overall mean NO_3^- (mg/L) concentration at each sampling site and the water quality classification according to the nitrate concentration (DWAF, 1996).

Sampling Site	Mean NO_3^- concentration (mg/L)	Water Quality Classification
Site 1	1.54 ± 2.08	Mesotrophic
Site 2	4.66 ± 5.13	Eutrophic
Site 3	1.90 ± 2.07	Mesotrophic
Site 4	3.74 ± 5.14	Eutrophic
Site 5	6.98 ± 7.58	Eutrophic
Site 6	5.35 ± 5.43	Eutrophic
Site 7	3.40 ± 4.39	Eutrophic
Site 8	2.04 ± 1.32	Mesotrophic

The nitrate levels across the wetland at the different sampling sites showed no oligotrophic conditions (NO_3^- concentrations < 0.05 mg/L). Sites 1, 3, and 8 showed mesotrophic conditions where the NO_3^- concentrations ranged between 0.50 – 2.50 mg/L. Mesotrophic conditions usually do not promote high levels of algal bloom or unfavorable vegetation growth. Mesotrophic conditions can however quickly lead to eutrophic conditions if nitrate concentrations are not managed. Site 2, 4, 5, 6 and 7 all showed eutrophic conditions (Table 7) where the NO_3^- concentrations are between 2.50 – 10.00 mg/L, however sites 4 and 7 are on the lower end of the eutrophic NO_3^- concentration classification and with correct management, NO_3^- concentrations could be reduced such that the nitrate concentrations fall within a safer range for water conditions. Sites 2, 5 and 6 are highly eutrophic which can readily lead to algal blooms, high density of unfavorable vegetation and are toxic conditions for drinking and irrigation use.

The high and low rainfall periods reveal a clear difference in nitrate concentrations (Fig. 16) ($p < 0.01$). Nitrate concentrations are consistently higher during the high rainfall period across all sampling sites with a mean between 2.30– 10.00 mg/L at sites 1 and 5, respectively. The highest difference in mean nitrate concentration between the high and low rainfall periods is seen at Site 5 and 6, while sites 3 and 8 show the lowest difference in the mean nitrate. During the low rainfall period, Site 2 is the leading contributor of nitrates into the wetland system with an mean of 2.98 ± 2.37 mg/L, while Site 5 had the highest mean nitrate during the high rainfall period 10.51 ± 9.17 mg/L. Reduced concentrations of nitrate (1.70 mg/L) are entering the wetland at Site 1 between the high and low rainfall periods, however there is a less evident difference between the high and low rainfall concentrations at Site 7 and 8.

5.1.4 Phosphate (PO_4^{3-})

The phosphate concentration occurred in high concentrations throughout the sampling period at every sampling site (Table 8). Phosphate concentrations (mg/L) can also be measured in μg/L as they naturally occur in low concentrations. The Wakkerstroom Wetland had phosphate concentrations above 1000 μg/L and therefore were measured in mg/L. These high phosphate concentrations measured throughout the wetland system results in the water conditions being categorized as hypertrophic (Table 3). The phosphate concentrations showed high temporal and spatial variation, with the majority of phosphate concentrations ranging between 0.20 and 2.50 mg/L – excluding extreme upper and lower measurements. Site 1 had the lowest mean phosphate concentration suggesting low pollutant loading is occurring from upstream water sources, significantly lower than sites 2, 3,4 and 5 ($p < 0.02$). Site 2 had the highest mean phosphate concentration across the sampling period and is therefore the source of the pollutant load into the wetland system for phosphate, and had significantly elevated phosphate concentrations than sites 1, 7 and 8 ($p < 0.01$). Sites 3 and 4 have a decreased phosphate concentration following Site 2, where sites 3 and 4 had identical mean phosphate concentrations, (1.08 mg/L)($p > 0.05$). Site 5 had the next highest mean phosphate concentration after Site 2. This was also observed for nitrate concentrations (Table 6) and total dissolved solids (Table 5) – where elevated concentrations occurred at Site 5 throughout the sampling period. Site 6 shows little reduction in phosphate concentrations following sites 3, 4 and 5 ($p > 0.05$). At sites 7 and 8, the majority (82%) of the mean monthly phosphate concentrations are below 1.00 mg/L and the mean phosphate concentration across the sampling period for site 7 and 8 is 0.60 ± 0.56 mg/L and 0.63 ± 0.63 mg/L, respectively. Site 7 and Site 8 are not significantly different to each other or to Site 1 ($p > 0.05$). This implies phosphate concentrations are reduced during the wetland filtration process and thus lower concentrations of phosphate are present downstream of the wetland. Peaks in phosphate concentrations were measured in either January 2022 (1.90 ± 1.52 mg/L) or February 2022 (1.23 ± 0.64 mg/L) for all sites, except Site 8 which showed an elevated concentration in November 2021, also seen at Sites 2, 3 as 4. June (0.33 ± 0.15 mg/L) and July 2022 (0.46 ± 0.25 mg/L) showed reduced phosphate concentrations across all of the sampling sites.

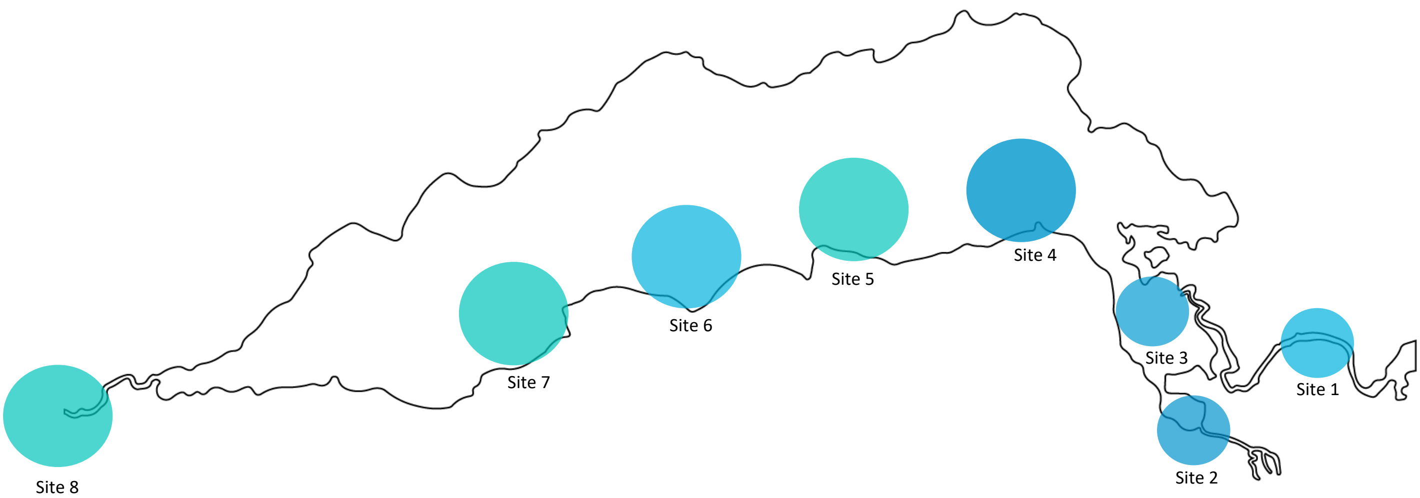
Table 8. Monthly mean phosphate (PO_4^{3-})(mg/L) of water samples for the sampling sites along the Wakkerstroom wetland between August 2021 and July 2022 with the overall mean PO_4^{3-} and standard deviation (SD) for each sampling site.

Month / Site	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8
Aug 2021	0.41	0.80	2.12	0.76	0.83	0.53	0.63	0.09
Oct 2021	0.00	0.45	2.37	1.18	0.34	0.21	0.59	0.38
Nov 2021	0.30	2.03	1.25	1.47	1.09	0.43	0.30	1.70
Dec 2021	0.21	1.00	0.21	0.36	1.43	2.50	0.27	0.95
Jan 2022	0.59	2.61	2.34	2.52	2.57	3.72	0.44	0.39
Feb 2022	0.84	1.93	0.74	1.71	1.95	0.92	1.15	0.55
Mar 2022	0.70	1.74	0.73	0.36	0.93	0.74	0.33	0.68
Apr 2022	0.59	2.06	0.41	1.47	1.61	0.70	1.10	0.33
May 2022	0.45	1.56	0.36	1.42	1.34	0.51	1.21	1.31
Jun 2022	0.26	0.42	0.41	0.27	0.28	0.46	0.21	0.33
July 2022	0.32	0.40	0.95	0.37	0.69	0.34	0.38	0.25
Mean ± SD	0.42 ± 0.32	1.36 ± 0.97	1.08 ± 0.88	1.08 ± 0.82	1.19 ± 1.06	1.01 ± 1.07	0.60 ± 0.56	0.63 ± 0.63

During the high rainfall period six of the eight sampling sites show a higher mean phosphate concentration than the low rainfall period ($p < 0.01$). An exception to this is at Site 3, where the mean phosphate concentration during the low rainfall period is 0.30 mg/L higher than the mean during the high rainfall period. At Site 7 there is no difference in mean phosphate concentrations between the high and low rainfall periods as the mean phosphate concentration for both rainfall periods are identical (0.95 mg/L). Sites 2 and 6 show the greatest difference between high and low rainfall periods where the high rainfall period had a mean phosphate concentration greater than 1.00mg/L than the low rainfall period for both sites. This distinct pattern between high and low rainfall periods was also measured at Site 6 for nitrate concentrations (Table 7). During the high rainfall period, Site 2, the pollution input site, had the highest addition of phosphate into the wetland system, whereas during the low rainfall period, Site 3 contributed on mean an additional 0.52 mg/L more than Site 2

5.2 Coliform and *E.coli* Bacterial Analyses

The mean scores for each sampling site are presented in Figure 20 across the sampling period, which represents the mean score from the colour scale. Site 1 and Site 2 had a moderate to high abundance of coliforms present, with a mean coliform abundance rating of 3.67 ± 0.70 and 3.80 ± 0.61 but showed no significant difference ($p > 0.05$) (Fig. 20). Site 3 had a slightly increased mean abundance of coliforms, 4.03 ± 0.62, when compared to the other seven sampling sites, and coliform abundance was significantly increased from sites 5, 7 and 8 ($p = 0.02$). For 7 months of the sampling period (October 2021, December 2021, January 2022, February 2022, March 2022, June 2022 and July 2022), Site 3 had a higher abundance of coliforms than Site 2, therefore, for 70% of the sampling period, Site 3 was the main contributor of coliform contamination into the wetland (Fig. 20 and Table 9). Site 4 had a lower coliform abundance from Site 3 during November 2021, December 2021 and March 2022 through to July 2022. Over the sampling period the coliform abundance at Site 4 was predominately moderate to high with a mean coliform abundance of 3.73 ± 0.83. Site 5 showed the lowest means in coliform presence throughout the wetland – where eight of the ten sampling months showed low or low to moderate levels of coliforms with a reduced mean of coliform abundance of 3.27 ± 1.01, however Site 5 and 6 showed the highest variability in coliform abundance when compared to the other sampling sites. Site 6 showed an increase in the abundance of coliforms from Site 5 for six of the ten sampling months with a mean coliform abundance of 3.63 ± 1.00. Coliform abundance predominantly increased or remained the same from Site 5 to Site 6 ($p > 0.05$). Similarly, Site 7 showed a lower coliform abundance as Site 5 ($p > 0.05$) with an equal mean coliform abundance as site 5 of 3.27 ± 0.74. Eight of the ten sampling showed the coliforms at Site 7 were between "2" and "3" on the colour scale – hence the coliform abundance was between low – low to moderate. Site 8 had a small increase in mean coliform abundance following Site 7 (3.35 ± 0.59). Site 8 also showed the least variability in coliform abundance, followed by sites 2 and 3. There was a non-significant increase from Site 7 to Site 8 ($p > 0.05$), suggesting no further coliform contaminations were entering the water column downstream of Site 7.



Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8
3.67 ± 0.70	3.80 ± 0.61	4.03 ± 0.61	3.73 ± 0.83	3.27 ± 1.01	3.63 ± 1.00	3.27 ± 0.74	3.35 ± 0.59

Figure 20. Outline of the Wakkerstroom wetland showing the mean coliform abundance between October 2021 and July 2022 at the eight sampling sites along the Wakkerstroom Wetland – using the scoring system **Image not to scale*.

Table 9. A table showing the coliform abundance of each sample sampling collected at the eight sampling sites along the Wakkerstroom Wetland between October 2021 and July 2022 using the scoring system between 1 to 5.

Sites	Oct 2021	Nov 2021	Dec 2021	Jan 2022	Feb 2022	Mar 2022	Apr 2022	May 2022	Jun 2022	Jul 2022
	4	4	3	4	4	4	3	4	4	2
1	4	4	4	4	3	5	2	4	4	4
	4	4	4	4	3	3	3	5	4	3
	4	4	5	4	3	4	3	4	4	3
2	3	4	4	3	3	4	4	4	3	4
	4	4	4	4	3	5	4	4	3	5
	4	4	5	4	4	5	3	3	4	4
3	4	3	4	4	4	5	4	4	4	4
	4	4	5	4	4	5	3	3	4	5
	5	2	4	5	4	4	3	2	3	4
4	5	4	4	4	4	5	3	3	3	4
	5	3	4	4	4	4	3	3	3	4
	3	4	4	4	3	5	4	2	2	2
5	2	4	3	4	3	5	4	3	2	2
	3	3	3	3	4	5	4	3	4	2
	2	3	3	3	5	3	3	2	3	4
6	2	4	4	4	4	5	5	3	4	2
	2	5	4	4	5	4	4	4	4	5
	2	3	4	3	3	3	4	2	3	4
7	2	3	4	4	3	3	4	4	3	3
	2	4	4	4	4	4	4	2	3	3
	3	4	3	4	3	4	4	3	3	4
8	3	4	4	4	3	3	3	3	3	2
	3	4	3	3	3	3	4	3	3	4

Throughout the sampling period, all of the water samples collected at each sampling site contained coliforms – from Site 1 through the wetland to Site 8. Spatial and temporal variability was observed for the abundance of coliforms. Site 1 had a moderate abundance (Table 9) of coliforms present in the water throughout the sampling period. Moderate to high coliform abundance was the most frequently observed at Site 1, 63% of the time, followed by "3", occurring 23% of the data collection period, and a "5" in March 2022 and May 2022 and "2" occurring 12% of the time in April 2022 and July 2022.Site 2 had a very similar presence of coliforms as Site 1 where the majority of the water samples showed an abundance of coliforms, showing "4" on the colour scale 60% of the time, followed by "3" 30% (Table 4). The remaining samples collected in December 2021, March 2022 and July 2022 showed a rating of "5" on the colour scale. No water samples collected at Site 2 showed a low abundance of coliforms. Site 3 had an elevated abundance of coliforms present across the sampling period – where 20% of water samples collected from Site 3 were "5" on the colour scale. This could be contributed to additional streams of grey and black water entering the wetland from the eSizameleni, resulting in an increased accumulation of bacterial contamination at Site 3. Most frequently the water samples collected had a high to moderate coliform abundance (63%) and a reduction of samples showing “3” following sites 1 and 2. Site 4 showed a reduction in the “4” rating when compared to Site 2 and 3. During October 2021, all samples collected had a rating of “5” – showing a high abundance of coliforms. The samples collected in April 2022, May 2022 and June 2022 were all low to moderate coliform abundance (rating of 3), where one sampling in May 2022 was rated “2”. Moderate to High coliform abundance was still the most frequent coliform water sample with a rating of “4” recorded 47% of the time. Site 5 had the highest frequency of water sampled rated “2” with a low abundance compared to the other seven sampling sites, 23% of the sampled collected at Site 5. In July 2022, all samples collected were rated “2” with a low abundance of coliforms. An equal number of water samples collected were rated “3” and “4” (30%). In March 2022, all samples collected at Site 5 were rated “5” with a high abundance of coliforms. This was the only month during the sampling period where a high abundance of coliforms were recorded. Site 6 had elevated levels of coliforms present across the sampling period following Site 5. November 2021, February, March April, May and July 2022 all had samples with a high abundance of coliforms, rated “5”. The water sampled collected at Site 6 were most frequently rated “4” (40%) followed by the abundance ratings “3”, “5” and “2”. All samples collected in October 2021 were rated “2” with a low abundance of coliforms. The water samples collected at Site 7 were predominately rated “3” and “4” on the colour scale. Site 7 also had a reduced abundance of coliforms when compared to Site 6, as there were no samples with a high abundance of coliforms. Samples that were rated “2” with a low abundance occurred 17% of the time. Similar to Site 6, all samples collected in October 2021 were rated “2” with a low abundance of coliforms. Reductions in coliforms frequently occurred between Site 6 and 7. This is evident in November 2021, February, March, May June and July of 2022. Site 8 showed further reductions in coliforms following Site 7, as the water samples collected most frequently had low to moderate coliforms, rated “3”. Site 8 was the only sampling site where a greater number of samples were rated “3”, than “4”. Site 8 only had one sample with a low coliform abundance, rated “2”. The remaining water samples collected at Site 8 were rated “4”, 37% of the time. During October 2021, the coliform abundance between Site 7 and 8 increased from low to moderate, however this did not occur again across the sampling period.

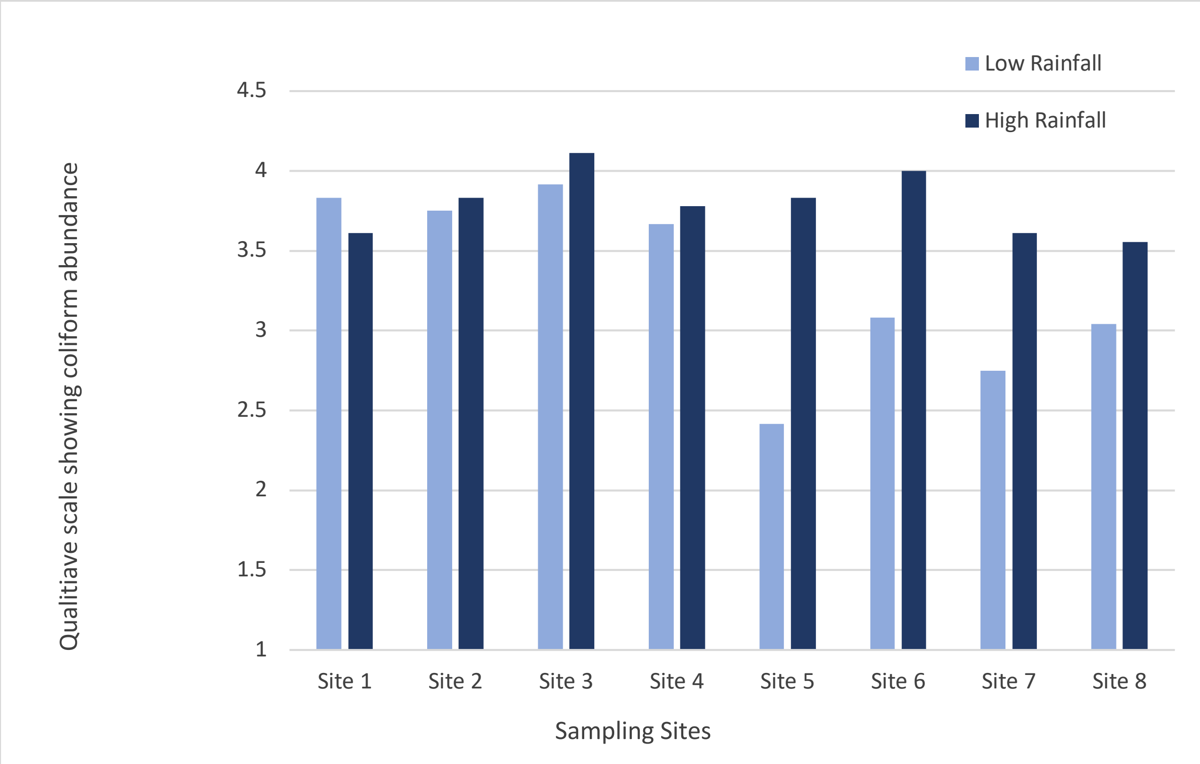


Figure 21. The mean coliform abundance using the scoring system between 1 and 5 at the sampling sites along the Wakkerstroom Wetland over the high and low rainfall periods during the sampling period in 2021 and 2022.

During the high rainfall period seven of the eight sampling sites show an increased mean coliform abundance than the low rainfall period ($p < 0.01$). The most notable differences between the high and low rainfall periods were seen at Sites 5, 6, 7 and 8. The greatest difference was observed at Site 5 where the mean coliform abundance during the low rainfall period was 2.42 ± 0.74 and 3.83 ± 0.71 during the high rainfall period. The difference between the high and low rainfall for sites 1 to 4 ranged between 0.08 and 0.19. Between sites 1 and 4, the effects of increased rainfall did not increase the coliform contamination, however this did occur between sites 5, 6, 7 and 8 during the high rainfall period. This may be a possible influence of coliform contamination entering the wetland through runoff and it is observed from Site 5 onwards as this is where observable reductions in coliform abundance begins to occur in the wetland (Table 9).

5.3.2 Presence of *E.coli* Bacteria

Table 10. A table showing the presence (√) or absence (x) of *E.coli* in the water samples from eight sampling sites along the Wakkerstroom Wetland between October 2021 and July 2022.

Month/Site	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8
Oct 2021	x	x	x	x	x	x	x	x
Nov 2021	x	x	x	x	x	x	x	x
Dec 2021	x	x	x	x	x	x	x	x
Jan 2022	x	x	x	x	x	x	x	x
Feb 2022	x	x	x	x	x	x	x	x
Mar 2022	√	√	√	√	√	√	√	√
Apr 2022	√	√	√	√	√	√	√	√
May 2022	√	√	√	√	√	√	√	√
Jun 2022	√	√	√	√	x	√	√	√
Jul 2022	√	√	√	√	x	√	√	√

No *E.coli* was detected at any of the sites from October 2021 to February 2022. All sites showed the presence of *E.coli*, based on the fluorescence of the samples under UV light, from March 2022 to July 2022 with 2 exceptions, namely June and July 2022 at Site 5 (Table 10). The degree of confidence in these *E.coli* data is low in that it seems extremely unlikely that there was no *E. coli* for 5 months, especially from Site 2, from the township where fecal contamination is highly likely and then from March to July, *E.coli* presence is measured at all sites except for 2 exceptions. The low confidence is probably due to operator inexperience. For a more accurate reading, I suggest that another method is used, for example to culture *E.coli* colonies which would allow for the quantitative collection of *E.coli* present within the water samples. This would be a more accurate measure of the presence and abundance of *E.coli* present in the samples.

5.4. Net Addition and Removal

In order to calculate the net removal or addition of nitrate, phosphate and coliforms across the entire wetland, removal and addition calculations were made from site to site, starting at Site 1 and moving downstream through the wetland. In the tables below, the positive values in orange indicate an increase in the concentration of nutrients or abundance of bacteria present from one site to the other. The negative values in green indicate a decrease. The overall net concentration or abundance is a summation of all the reductions and additions between each site.

Table 11a. A table showing the net removal (-) or addition (+) of total dissolved solids (TDS)(mg/L) from site to site, moving downstream from Site 1 to Site 8 along the Wakkerstroom Wetland between August 2021 to February 2022.

Site / Month	Aug 2021	Oct 2021	Nov 2021	Dec 2021	Jan 2022	Feb 2022
Sites 1 – 3	+ 53.50	+ 119.90	+ 26.47	+ 3.80	+ 21.27	+ 9.23
Sites 2 – 3	- 30.77	- 7.83	- 57.13	- 73.63	- 123.83	- 66.63
Sites 3 – 4	- 5.57	- 37.57	+ 0.63	+ 17.03	- 5.23	+ 2.40
Sites 4 – 5	- 32.20	+ 79.40	- 5.60	+ 74.67	+ 145.00	+ 42.10
Sites 5 – 6	- 24.93	- 77.57	+ 87.27	- 9.50	- 39.20	+ 86.27
Sites 6 - 7	- 6.60	- 61.27	- 106.53	- 81.80	- 108.50	- 103.10
Sites 7 – 8	+ 12.50	+ 12.90	+ 30.37	+ 5.30	+ 1.60	- 4.67
Net TDS	**- 34.07 ± 30.37**	**+ 27.97 ± 73.00**	**- 24.53 ± 62.78**	**- 64.13 ± 54.04**	**- 108.90 ± 89.87**	**- 34.40 ± 63.54**

Table 11b. A table showing the net removal (-) or addition (+) of total dissolved solids (TDS)(mg/L) from site to site, moving downstream from Site 1 to Site 8 along the Wakkerstroom Wetland between March 2022 to July 2022.

Site / Month	Mar 2022	Apr 2022	May 2022	Jun 2022	Jul 2022
Sites 1 – 3	+ 2.53	+ 3.43	+ 8.67	+ 13.27	+ 20.93
Sites 2 – 3	- 131.17	- 103.80	- 25.33	- 40.33	- 32.03
Sites 3 – 4	+ 5.10	+ 14.17	+ 0.10	+ 6.67	+ 1.60
Sites 4 – 5	+ 10.17	+ 5.73	+ 16.70	+ 218.93	+ 150.03
Sites 5 – 6	+ 35.70	+ 48.13	+ 45.13	- 91.37	- 72.37
Sites 6 -7	- 44.77	- 75.30	- 73.97	- 153.80	- 109.93
Sites 7 -8	+ 2.60	+ 6.17	+ 3.00	+ 3.83	+ 6.93
Net TDS	**- 119.83 ± 55.64**	**- 101.47 ± 54.12**	**- 25.70 ± 37.46**	**- 42.80 ± 116.57**	**- 34.83 ± 82.84**

Overall TDS removal =	**- 564.70 ± 43.91**

The net overall total dissolved solids removal totaled 564.70 mg/L as a result of the monthly net additions and net removal of TDS. This finding implies the wetland is functioning healthily and is able to reduce chemical pollutants through its various purification processes. The wetland consequently improves water quality and reduces dissolved solids for downstream water sources. Of the eleven sampling months, all except one month – October 2021 showed a net removal of TDS across the wetland. The net TDS removal ranged between 24 – 120 mg/L. In January 2022, March 2022 and April 2022 the wetland showed net TDS removals above 100 mg/L. In October 2022, an addition of TDS occurred in the wetland, as the net addition of TDS was 24.97 mg/L. This could be due to the increased addition of TDS between sites 1 and 3 where an addition of TDS more double the other sampling months was observed in October 2021. Throughout the sampling period, Sites 1 to 3, 3 to 4 and 4 to 5 showed increases in TDS ranging between 7.00 and 65.00 mg/L. This is likely a result of the high pollutant wastewater site situated between Site 1 and Site 3. Consistently throughout the sampling period, there was a reduction in TDS from Site 2 to Site 3 and from Site 6 to Site 7. Sites 2 to 3, 3 to 4, 5 to 6 and 6 to 7 showed a negative mean, resulting in a mean monthly removal of TDS. Therefore, between sites 1 to 3, 4 to 5 and 7 to 8 there was a mean addition of TDS between 0.05 and 85.00 mg/L.

Table 12a. A table showing the net removal (-) or addition (+) of nitrate (NO_3^-)(mg/L) from site to site, moving downstream from Site 1 to Site 8 along the Wakkerstroom Wetland between August 2021 to February 2022.

Site / Month	Aug 2021	Oct 2021	Nov 2021	Dec 2021	Jan 2022	Feb 2022
Sites 1 – 3	+ 1.23	+ 2.40	+ 0.56	- 0.47	- 0.20	+ 0.67
Sites 2 – 3	- 1.10	- 3.27	- 11.23	- 0.47	+ 0.33	- 12.40
Sites 3 – 4	- 0.33	+ 2.70	+ 6.70	+ 0.20	- 1.27	+ 15.17
Sites 4 – 5	+ 1.00	- 2.27	- 5.13	+ 12.70	+ 19.73	- 5.87
Sites 5 – 6	- 1.83	- 1.20	- 0.70	+ 2.27	- 6.23	- 4.30
Sites 6 -7	+ 0.77	+ 9.23	+ 6.63	- 15.17	- 12.73	+ 3.17
Sites 7 -8	- 0.30	- 7.53	--	+ 1.17	+ 0.80	- 5.93
Net NO_3^-	**- 0.57 ± 1.06**	**+ 0.07 ± 4.96**	**- 3.17 ± 6.33**	**+ 0.23 ± 7.54**	**+ 0.43 ± 9.20**	**- 10.10 ± 8.17**

Table 12b. A table showing the net removal (-) or addition (+) of nitrate (NO_3^-)(mg/L) from site to site, moving downstream from Site 1 to Site 8 along the Wakkerstroom Wetland between March 2022 to July 2022.

Site / Month	Mar 2022	Apr 2022	May 2022	Jun 2022	Jul 2022
Sites 1 – 3	+ 0.30	- 0.13	- 0.20	- 0.23	+ 0.70
Sites 2 – 3	+ 5.97	- 4.67	+ 0.30	- 3.57	- 0.23
Sites 3 – 4	- 4.73	+ 0.73	- 0.20	+ 0.97	+ 0.27
Sites 4 – 5	+ 0.27	+ 4.20	+ 2.13	+ 3.10	+ 1.17
Sites 5 – 6	+ 0.53	- 0.90	- 0.03	+ 2.00	- 0.50
Sites 6 -7	- 2.67	- 3.67	- 2.27	- 5.57	- 1.57
Sites 7 -8	+ 1.77	+ 1.00	+ 0.23	+ 0.23	+ 0.83
Net NO_3^-	**+ 1.43 ± 3.13**	**- 3.43 ± 2.77**	**- 0.03 ± 1.19**	**- 3.07 ± 2.85**	**+ 0.67 ± 0.87**

Overall NO_3^- removal =	**- 17.53 ± 3.14**

The overall net nitrate removal totaled 17.53 mg/L over the course of the sampling period. There was a significant difference between the total addition of nitrate (2.83 ± 0.53 mg/L) and the total removal of nitrate (20.37 ± 3.59 mg/L)($p = 0.02$). During February 2022 a significant concentration of nitrate (10.10 mg/L) was removed from the water column via the wetland, more than double the other sampling months which showed removal concentrations between 0.03 and 3.43 mg/L of nitrate. In contrast, in March 2022 the highest addition of nitrate into the water column was observed at 1.43 mg/L. No patterns of consistent removal or additions were observed from one site to another. From Site 4 to 5, there are constant additions of nitrate between March 2022 and July (Table 3b). However, between the sampling months August 2021 and February 2022 fluctuating addition and removal of nitrate was observed. A similar pattern is observed from Site 6 to 7, where March 2022 to July 2022 showed consistent removal of nitrate, however between August 2021 and February 2022 an addition and removal was observed. High concentrations of nitrate above 15.00 mg/L were added to the wetland between sites 3 to 4 and sites 4 to 5 during January 2022 and February 2022, respectively. The highest concentrations of nitrate removal occurred from site 6 to 7 during December 2021 (15.17 mg/L) and January 2022 (12.73 mg/L) (Table 3a). Corresponding to the nitrate analysis between sites, Site 8 is not significantly different to Site 1 ($p = 0.93$), suggesting reduced nitrate concentrations after nutrient loading occurs between Site 2 and Site 6. Therefore, the wetland is successfully reducing excess nutrients through the wetland purification process of nitrogen uptake, supported by the net nitrate removal.

Table 13a. A table showing the net removal (-) or addition (+) of phosphate (PO_4^{3-}) (mg/L) from site to site, moving downstream from Site 1 to Site 8 along the Wakkerstroom Wetland between August 2021 and February 2022.

Site / Month	Aug 2021	Oct 2021	Nov 2021	Dec 2021	Jan 2022	Feb 2022
Sites 1 – 3	+ 1.71	+ 2.37	+ 0.95	+ 0.00	+ 1.75	- 0.09
Sites 2 – 3	+ 1.32	+ 1.92	- 0.79	- 0.79	- 0.28	- 1.19
Sites 3 – 4	- 1.36	- 1.19	+ 0.22	+ 0.15	+ 0.18	+ 0.97
Sites 4 – 5	+ 0.07	- 0.84	- 0.37	+ 1.07	+ 0.05	+ 0.24
Sites 5 – 6	- 0.30	- 0.12	- 0.66	+ 1.07	+ 1.15	- 1.03
Sites 6 -7	+ 0.10	+ 0.38	- 0.13	- 2.23	- 3.28	+ 0.24
Sites 7 -8	- 0.54	- 0.21	+ 1.40	+ 0.68	- 0.05	- 0.60
Net PO_4^{3-}	**+ 1.00 ± 1.06**	**+ 2.30 ±1.35**	**+ 0.61 ± 0.82**	**- 0.05 ± 1.18**	**- 1.81 ±1.41**	**- 1.47 ± 0.77**

Table 13b. A table showing the net removal (-) or addition (+) of phosphate (PO_4^{3-}) (mg/L) from site to site, moving downstream from Site 1 to Site 8 along the Wakkerstroom Wetland between March 2022 and July 2022

Site / Month	Mar 2022	Apr 2022	May 2022	Jun 2022	Jul 2022
Sites 1 – 3	+ 0.02	- 0.18	- 0.09	+ 0.15	+ 0.63
Sites 2 – 3	- 1.01	- 1.65	- 1.20	- 0.01	+ 0.55
Sites 3 – 4	- 0.36	+ 1.06	+ 1.06	- 0.14	- 0.58
Sites 4 – 5	+ 0.56	+ 0.14	- 0.08	+ 0.01	+ 0.32
Sites 5 – 6	- 0.18	- 0.19	- 0.83	+ 0.19	- 0.34
Sites 6 -7	- 0.42	+ 0.40	+ 0.71	- 0.25	+ 0.04
Sites 7 -8	+ 0.36	- 0.77	+ 0.10	+ 0.12	- 0.13
Net PO_4^{3-}	**- 1.03 ± 0.53**	**- 1.90 ± 0.91**	**- 0.33 ± 0.79**	**+ 0.06 ± 0.16**	**+ 0.48 ± 0.46**

Overall removal PO_4^{3-} =	**- 2.14 ± 1.29**

The overall net removal of phosphate totaled 2.14 ± 1.29 mg/L over the course of the sampling period. The net addition of phosphate into the water column versus the net removal of phosphate via the wetland was not significantly different ($p = 0.01$). The summation of phosphate added to the water was 4.45 ± 1.64 mg/L while the total removal of phosphate was 5.26 ± 0.72 mg/L. Between December 2021 and May 2022 consistent removal of phosphate was observed between 0.05 and 1.90 mg/L. August 2021 to November 2021, June and July 2022 showed additions of phosphate into the water column between 0.60 and 2.30 mg/L. During August 2021 to March 2022 there were additions of phosphate between Site 1 to 3, likely due to the increased phosphate input from Site 2. April and May 2022 showed removal of phosphate, and June and July 2022 showed additions. The remaining sampling sites showed temporal fluctuations between additions and removals of phosphate. The highest net removal concentration occurred in April 2022, and the highest net addition of phosphate occurred in October 2021, likely due to the increased phosphate input between Site 1 to 3 and Site 2 to 3. During August 2021, November 2021 and July 2022 the net phosphate concentration was positive– suggesting an additional 1.00 mg/L, 0.62 mg/L and 0.49 mg/L respectively, of PO_4^{3-} entered the wetland than the phosphate that was reduced through the wetland via plant uptake and other wetland filtration processes. In January 2022, March 2022 and May 2022 a net reduction in phosphate was measured, suggesting phosphate retention in the wetland and a removal of phosphate from the water passing through the wetland. The majority of phosphate reduction occurred between Site 5 and 6 as five of the six sampling months showed reduced phosphate concentrations between sites 5 and 6. The majority of phosphate additions into the wetland occurred between sites 1 and 3 as a consequence of the sewage and grey water runoff from the township coming into the wetland from Site 2.

Table 14a. A table showing the net removal (-) or addition (+) for the abundance of coliforms from site to site, moving downstream from Site 1 to Site 8 along the Wakkerstroom Wetland between October 2021 to February 2022.

Site / Month	Oct 2021	Nov 2021	Dec 2021	Jan 2022	Feb 2022
Sites 1 - 3	0	- 0.33	+ 1.00	0	+ 0.67
Sites 2 - 3	+ 0.33	- 0.33	+ 0.33	+ 0.33	+ 1.00
Sites 3 - 4	+ 1.00	- 0.67	- 0.67	+ 0.33	0
Sites 4 - 5	- 2.33	+ 0.67	- 0.67	- 0.67	- 0.67
Sites 5 - 6	- 0.67	+ 0.33	+ 0.33	0	+ 1.33
Sites 6 - 7	0	- 0.67	+ 0.33	0	- 1.33
Sites 7 - 8	+ 1.00	+ 0.67	- 0.67	0	- 0.33
Net Coliforms	**- 0.67 ± 1.15**	**- 0.33 ± 0.59**	**0 ± 0.67**	**0 ± 0.33**	**+ 0.67 ± 0.96**

Table 14b. A table showing the net removal (-) or addition (+) for the abundance of coliforms from site to site, moving downstream from Site 1 to Site 8 along the Wakkerstroom Wetland between March 2022 to July 2022.

Site / Month	Mar 2022	Apr 2022	May 2022	Jun 2022	Jul 2022
Sites 1 - 3	+ 1.00	+ 0.67	- 1.00	0	+ 1.33
Sites 2 - 3	+ 0.67	- 0.33	- 0.67	+ 0.67	+ 0.33
Sites 3 - 4	- 0.67	- 0.33	- 0.67	- 1.00	- 0.33
Sites 4 - 5	+ 0.67	+ 1.00	0	- 0.33	- 2.33
Sites 5 - 6	- 1.00	0	+ 0.33	+ 1.00	+ 2.00
Sites 6 - 7	- 0.67	0	- 0.33	- 0.67	- 0.33
Sites 7 - 8	0	0	+ 0.33	0	- 0.17
Net Coliforms	**0 ± 0.79**	**+ 1.00 ± 0.50**	**- 2.00 ± 0.52**	**- 0.33 ± 0.71**	**+ 0.50 ± 1.39**

Overall Net Coliforms =	**- 1.17 ± 0.83**

The totaled removal of coliform was reduced by 1.17 on the colour change scale. As a qualitative test was performed on coliforms, and analyzed using a semi quantitative scoring system, the net addition or removal serves an as estimate of the coliform abundance.

Reductions in coliforms occurred four of the ten sampling months, namely October and November 2021 and May and June 2022. Net additions of coliforms occurred in February, April and July 2022, suggesting an addition of polluting factors into the wetland. Three months showed no net change in coliform abundance in December 2021, January and March 2022. Between sites 3 and 4, there was a reduction in the coliform abundance for seven of the ten sampling months. May 2022 had the highest net coliform reduction, where-as the previous sampling month, April 2022, had the highest net addition of coliforms. In January and April 2022, no change in the coliform abundance was observed from Site 5 to Site 8.

Chapter 6. Discussion

This study sought to investigate the status and functioning of the Wakkerstroom wetland by quantifying the pollutant removal capabilities of the wetland using chemical parameters and microbiological indicators. This discussion will explore comparisons from existing literature of the chemical parameters and microbiological contaminants, followed by the effects of rainfall patterns and factors that result in nutrient and microbiological retention in the wetland. This will then allow for discussion around wetland health, functionality and conservation and lastly limitations of the study and research that could arise from it. Each section will briefly summarize the main findings from the study.

6.1 Chemical parameters

6.1.1 pH

Chemical parameters are a well-used, common practice when determining water quality, which can be measured against existing water quality guidelines (Kora *et al*., 2017; Fentaw *et al*., 2022; Zhu *et al*., 2022). The measurement of pH allows us to determine the acidity and alkalinity of water – which is a good indicator of the presence of heavy metals present in the water as lower pH parameters lead to the release of heavy metals from sediment, for example iron (Fe) and zinc (Zn) (Kamzati *et al*., 2019) . The pH along the Wakkerstroom wetland showed minimal to no spatial and temporal changes with all the measurements taken resulting in a neutral acidic to neutral alkaline status between 6 and 8. According to the target water quality range (TWQR), the ideal range for freshwater systems is between 6 – 8, therefore, the Wakkerstroom wetland pH fell within the ideal range. South African freshwater systems are usually well buffered and therefore do not experience rapid fluctuations due to external changes in the hydrological system and external environmental factors. Increased pH can occur as a result of eutrophic conditions in standing waters such as wetlands, however the pH observed at the Wakkerstroom wetland did not reflect this (DWAF, 1996). Most studies conducted on wetland systems have a neutral pH (Atnafu *et al*., 2011; Dalu *et al*., 2022), with a small range of variability towards a lower pH (Rebelo *et al*., 2018) and higher pH Gordon *et al*., 2011; Burger *et al*., 2018). Two tropical wetlands studied in Ethiopia showed lower pH at both wetlands during the rainy seasons and a higher pH during the dry seasons (Atnafu *et al*., 2011). The differences in pH between the rainy and dry seasons were similar to the differences seen in this study – which were more acidic in the high rainfall season.

6.1.2 Total Dissolved Solids (TDS)

Total dissolved solids (TDS) are the ions dissolved in the water column, usually carrying an electrical charge (Malan & Day, 2002). The TDS of a water column are naturally controlled by the geology and chemistry of the water body through underlying rocks releasing minerals, cycling of dissolved solids in the sediment layer and decomposition of decaying vegetation. Secondly, TDS is influenced by evaporation and rainfall input into the water column and lastly anthropogenic influences such as runoff from artificial fertilizers, untreated sewage inputs and waste from industrial processes, among many others (DWAF, 1996; Flaková *et al*., 2014). The most common anions and cations that make up TDS measurements from natural water bodies include Na^{+}, Ca^{2+}, Mg^{2+} and K^{+} and Cl^{-}, HCO_3^{-}, SO_4^{2-} and CO_3^{2-} . The target water quality range for TDS is such that, the TDS of the water, under normal conditions should not fluctuate more than 15% (DWAF, 1996), where the mean TDS measurements observed for the Wakkerstroom wetland ranged between 40 – 300 mg/L. However, it has long been speculated that there has been untreated sewage leaking into the Wakkerstroom river for several years now, hence the Wakkerstroom wetland system can no longer be considered an unaffected or pristine wetland system as it is subjected to continual elevated TDS conditions (DWAF, 1996).

Three sampling sites along the Blesbokspruit Wetland which are downstream of sewage treatment plants had mean TDS ranges between 550 – 950 mg/L, where the TDS measurements were consistently higher than for the Wakkerstroom wetland (Phaleng, 2009). This suggests several pollutants entering the Blesbokspruit such as acid mine drainage, which the Wakkersteroom wetland is not subjected to. Several depression wetlands in Gauteng, South Africa showed TDS measurements between 200 – 3000 mg/L, elevated well past the TDS for the Wakkerstroom wetland, with selected pans as high as 35 000 mg/L, however, these pans were subjected to industrial water inputs (Burger *et al*., 2018). Nylsvley Wetland in the Limpopo Province, had a mean TDS between 75.00 mg/L and 205.00 mg/L, varying across three different seasons. The higher TDS measures occurred during the hot and wet season (Dalu *et al*., 2022). The Wakkerstroom wetland had slightly reduced TDS for Site 1, and the downstream sites, Site 7 and 8, which had a mean TDS below 65.00 mg/L. Sampling sites 2 to 6 had similar mean TDS values values to Nylsvley Wetland

6.1.3 Nutrients

Inorganic nitrogen, such as nitrate (NO_3^-), nitrite (NO_2^-) and ammonia (NH_4^+), are commonly found in polluted surface water, in high concentrations (above 0.50 mg/L). These concentrations that are higher than naturally occurring NO_3^- are a consequence of a number of different factors such as excessive use of fertilizers, ground runoff, domestic waste and untreated sewage. (Li *et al*., 2023).

The majority of the mean nitrate concentrations observed along the Wakkerstroom wetland are eutrophic (2.50 – 10.00 mg/L), however Site 8, downstream of the wetland is mesotrophic (0.50 – 2.50 mg/L). Site 2, the sewage runoff site showed maximum nitrate concentrations of 1.50 mg/L for Sanderson (2018), while the sampling period in 2021 - 2022 at Site 2 had increased nitrate concentrations between 0.50 and 15.00 mg/L. The 2021 – 2022 sampling period also showed greater variability of the nitrate concentrations at Site 2 than Sanderson (2018). This suggests increasing nutrient loads are entering the Wakkerstroom wetland through sewage and domestic wastewater runoff. Four reed pans in the Mpumalanga province subjected to input pollutants had nitrate concentrations between 3.00 – 9.0 mg/L (Klerk & Wepener, 2013). Goedverwatching se Pan, the reed pan with the highest nutrient input (8.83 mg/L) was comparable to Site 5 at the Wakkerstroom wetland with a mean nitrate concentration of 6.98 mg/L. In the case of Wakkerstroom, there are no large-scale agricultural lands directly upstream the wetland, therefore, the main source of excess nitrate seen in the wetland is likely not a result of fertilizers, further supported by the lack of high nitrate concentrations frequently observed in waters with chemical fertilizer input (Rhymes *et al*., 2015). A wetland in Uganda, had low nitrate concentrations, ranging between 0.00 and 1.20 (Okot-Okumu, 2011), in contrast the Site 1 with the lowest mean nitrate concentration at the Wakkerstroom wetland was 1.54 mg/L – suggesting a higher pollutant load entering the

Wakkerstroom Wetland. The Rietvlei Lake, in Gauteng, South Africa, displayed high nitrate concentrations, between 42.00 and 12.00 mg/L, which were rated severely degraded to mildly impacted due to the degree of eutrophication within the river system (Oberholster *et al*., 2008). The lowest nitrate concentrations measured at the Rietvlei wetland area (2.50 – 2.60 mg/L) were comparable with the nitrate concentrations measured at the Wakkerstroom wetland for Sites 1 (1.54 mg/L), Site 3 (1.90 mg/L) and Site 8 (2.04 mg/L).

Phosphorus needs to be in an inorganic form in order for plants to utilize it, which is usually in the form of phosphate ions (PO_4^{3-}). Excessive inorganic phosphorus is usually the main contributor to eutrophication as phosphorus is the more likely limiting nutrient to plant growth than inorganic nitrogen (Malan & Day, 2002). Phosphate naturally occurs in low concentrations (< 5 μg/L) in unaffected water columns. The trophic status of the Wakkerstroom wetland with respect to phosphate is eutrophic, using the South Africa Water Quality Guidelines, as observed mean concentrations were greater than 400 μg/L (0.40 mg/L). Malan and Day (2005) stated that the phosphate target concentration is too cautious for natural wetland systems, and should be increased to 0.02 mg/L. Therefore, the phosphate concentrations recorded for the Wakkerstroom wetland are still above the target range, stated by the DWAF, however, the phosphate concentrations could be deemed less disproportionate or in excess.

Sanderson (2018) showed comparable phosphate concentrations at Site 2, ranging between 1.50 – 2.50 mg/L, between 2017 - 2018. The sampling period of 2021 – 2022 showed slightly reduced phosphate concentrations between 0.40 – 2.60 mg/L. However, the mean phosphate concentrations at Site 1 and 8 were reduced in 2016 – 2018 (< 0.25 mg/L), compared to the 2021 – 2022 sampling period, with a mean phosphate concentration of 0.42 and 0.63 mg/L for sites 1 and 8 respectively. Kock *et al*. (2019) observed reduced total nitrogen (0.50 – 0.80 mg/L) and comparable phosphate concentrations (1.00 – 1.70 mg/L) in Lake Sibaya, Kwa – Zulu Natal, South Africa, than compared to the Wakkerstroom wetland. Although the phosphate concentrations were within the eutrophic range, corresponding with the Wakkerstroom phosphate concentration range, Lake Sibaya had an overall mesotrophic trophic status. Lake Sibaya also presented diatom communities which are associated with nutrient rich waters and are known to be less sensitive to increased pollution (Kock *et al*., 2019). A study was also conducted on the Rietvlei Lake in Gauteng, South Africa, with water inputs from the Hennops river and sewage contamination upstream of the lake. The phosphate concentrations measured at Rietvlei Lake ranged from 0.57 to 2.60 mg/L. The lower concentrations are comparable with Site 1, 7 and 8 with mean phosphate concentrations between 0.40 – 0.65 mg/L. In addition these sites were classified as severely degraded when compared to the high pollutant sites along the Wakkerstroom wetland. These sites would likely not be classified as “severely degraded”, but rather “moderately” to “mildly impacted”, using the Australian river assessment system (Oberholster *et al.*, 2008). The downstream sites of the Rietvlei Lake had comparable nutrient concentrations to the downstream Wakkerstroom wetland sites. However, wetland systems in South Africa have reduced concentrations of phosphates such as in the Western Cape with the highest concentrations between 0.20 – 0.30 mg/L (Gordon *et al*., 2011). The water quality of four receiving water bodies after being discharged from waste treatment plants, showed excess nutrients between 4.00 - 7.00 mg/L for total nitrogen and 3.10 – 6.80 mg/L for phosphate (Momba & Mfenyana, 2004). The phosphate measured at the Wakkerstroom wetland had reduced concentrations of phosphate, even at the Site 2 (1.36 mg/l), which had the highest mean phosphate concentration.

6.2 Microbiological Contaminants

Coliforms are common biological indicators used to assess possible faecal contamination in water sources, in combination with *E.coli* bacteria, which is exclusively found in the colon of warm blooded mammals. As such, *E.coli* is a good indicator of faecal contamination. Faecal contamination can enter the wetland through a point source, such as sewage leakage from malfunctioning treatment plants (Sidhu *et al*., 2013), or diffuse sources such as ground water runoff from land used for livestock grazing (Wilkinson *et al*., 2011; Rhymes *et al*., 2015). It was stated in the Literature Review section that the *E. coli* measurements taken during this study were only an initial indication of contamination and more precise techniques should be used.

In order to determine the main source of contamination into a water column (point source or diffuse), the coliform abundance should be measured, either as faecal coliforms or total coliforms, from runoff sites and from input water sources where faecal contamination is speculated (Rhymes *et al*., 2015). As is the case with the Wakkerstroom wetland, point sources of faecal contamination were considered to be entering the wetland upstream of Site 1, and Site 2, eSizameleni waste-water runoff. This is consistent with these findings, as Site 2 and 3, followed by Site 4 had the highest coliform abundance measures across the sampling period. This finding however only confirms that increased coliform contamination is occurring at those sites, and the coliform analysis alone cannot show evidence for faecal contamination – however studies have shown a correlation between the presence of *E.coli* and coliforms, therefore suggesting faecal contamination as a probable cause (Asfi *et al*., 2023).

Escherichia coli and the bacterial genus *Enterococcus* are commonly used as indicators of faecal contamination (Sibanda *et al*., 2013). Sidhu *et al*. (2013) showed that high levels of *E.coli* were present in storm water runoff, present in six storm water sites in Brisbane, Melbourne and Sydney, Australia, all of which were within a sewage pipe network. Therefore, this suggests malfunctioning sewage plants. These conclusions were made as markers were identified in stormwater that were specific to contaminations only found in human faecal matter because of sewage leaks into stormwater from urban infrastructures. As *E.coli* was present at Site 1 from March 2022 onwards – coming straight from Martin's Dam located immediately next to a waste treatment plant, it is likely that the faecal contamination is a result of a point source contamination, such as sewage leaking into the water column upstream of the wetland. These findings are consistent with Sanderson (2018) where *E.coli* samples were quantified from Site 1, 2 and 8 where *E.coli* was present throughout the five month sampling period. These findings are also consistent with studies where known faecal contamination is occurring as a result of sewage contamination into the water column (Kora *et al*., 2017).

The external conditions of the environment either favour or discourage *E.coli* bacteria to survive outside of its host. Optimal temperatures, around 37°C, and increased suspended solids allow *E.coli* bacteria to survive and reproduce in water (Asfi *et al*., 2023). Dry seasons with shallow water depths, increased sun exposure and reduced water movement all favour an increased rate of *E.coli* die-off (Wilkinson *et al*., 2011). This is a possible explanation for why *E.coli* was not present at Site 5 in June and July 2022. The consistent presence of *E.coli* in the wetland during the dry period suggests a constant reintroduction of faecal contamination, further supporting evidence of a sewage leak.

Another concern of *E.coli* presence is ground water contamination. Faecal contamination can enter ground water sources (Lapworth *et al*., 2017) and pose a serious health risk for locals using ground water supply for irrigation, drinking and cooking water or recreational uses (Lehloesa & Muyima, 2000; Teklehaimanot *et al*., 2014). Many Wakkerstroom residents rely on borehole water, for domestic use, from ground water sources, which have shown and continue to show high coliform contamination and *E.coli* presence (Phili, 2020; NHWA unpublished data, 2022), posing health risks and illnesses as a result of ingesting the water.

Several water bodies were tested for the presence of *E.coli*, within the rural Venda communities in Northen Cape, of which only one had *E.coli* present, illustrating effective removal of the indicator bacteria *E.coli* for the majority of the wastewater treatment plants. However, several proposed pathogenic species were present in the water bodies from all four of the treatment plants, suggesting an inadequate removal of harmful microbes and ineffective water treatment facilities (Momba & Mfenyana, 2004). Obi *et al*. (2004) conducted a microbial water quality study on several river systems in Northern Cape, including the Vuwani, Mutshindudi, Tshinane, Ngwedi, Mutale, Mudaswali and Lebuvu river systems. Data collected from these rivers revealed a poor and unsafe water quality as a result of several bacterial species present in seven rivers in the Northern Cape. All seven of these rivers had *E.coli* present, suggesting faecal contamination occurring within the Venda region. The presence of *E.coli* in water bodies downstream of wastewater treatment plants is not an uncommon finding in natural water bodies in South Africa, however the quantity of *E.coli* present in the wetland has not yet been determined.

6.3 Rainfall

Seasonal changes from rainfall patterns greatly influence the concentrations of nutrients and coliform contamination present in the water column (Sibanda *et al*., 2012; Asfi *et al*., 2022). The greatest influence is that increased rainfall generally results in increased coliform contamination and increased nutrient loading in the water column. This may be a consequence of surface runoff where nutrients are accumulating during the dry season and rainfall causes the relocation of these nutrients. Increased nutrient concentrations during the high rainfall period could also be a result of litter decomposition and sediment disturbance, where minerals and nutrients are released back into the water column (Okot-Okumu, 2011). The second factor is through increased input from upstream water sources, which cause disturbance to the sediment and reintroduce coliforms and organic minerals into the water column (Uwimana *et al*., 2018; He *et al*., 2020; Silva *et al*., 2020). This raises concern for eutrophication and decreased water quality as a result of excess nutrients entering water bodies during high rainfall periods (Rhymes *et al*., 2015).

Differing concentrations of nutrients between rainfall seasons is a common phenomenon in studies done on wetlands and rivers – where inorganic nitrogen (Klerk & Wepener, 2013) and phosphorus concentrations (Dalu & Chauke, 2020) increase during rainfall periods (Atnafu *et al*., 2011). Nitrate concentrations have a specific interaction with hydrological cycles, as periods of high rainfall increase the nitrate concentration, however, specific flooding events or singular instances of heavy rainfall can also reduce nitrate concentrations (Deemy & Rasmussen, 2017; Primost *et al*., 2022). This is a result of the dilution effect, as well as increased interaction with upstream water sources (Malan & Day, 2002). However the opposite has been documented as well, where increased nutrient concentrations are recorded during periods of reduced rainfall. For example, Dalu *et al*. (2022) observed increased concentrations and variability in phosphate during the dry period as a result of reduced macroinvertebrate abundance altering the availability of nutrients.

Consistent with the Wakkerstroom wetland Dalu & Chauke, (2020) had phosphate concentrations between 0.30 – 0.70 mg/L during the low rainfall period and increased to 1.0 – 2.0 mg/L during the high rainfall period. Wakkerstroom wetland had phosphate concentrations between 0.25 – 1.25 mg/L during the low rainfall period, and 0.50 – 1.90 mg/L during the high rainfall period. It has been found that the dilution affect is more applicable for total phosphorus, and the pattern is less discernable for phosphates in the water column (Malan & Day, 2002). Increased nitrate concentrations were recorded at four reed pans in the Mpumalanga Province, where the summer nitrate concentrations (7.5 mg/L) were more than double the spring nitrate concentrations (3.0 mg/L), and elevated from the winter and autumn nitrate concentrations (4.7 – 4.8 mg/L), showing seasonal differences consistent with the findings from the Wakkerstroom wetland (Klerk & Wepener, 2013). The mean nitrate concentrations recorded at the Wakkerstroom wetland were slightly decreased during the low rainfall (2.13 mg/L) than the high rainfall period (5.14 mg/L), due to the lack of ground runoff entering the Wakkerstroom wetland. Nlyslvey Wetland, located in Limpopo province, South Africa had a neutral to alkaline pH, and a mean pH of 7.60 during the dry period and 6.80 during the rainy season (Dalu *et al*., 2022). These values are parallel to the measurements recorded during this study, with a decreased mean pH during the high rainfall period.

The Mpumalanga Highveld is known for having increased anthropogenic sources of nitrogen dioxide (NO_2) and sulfur dioxide (SO_2) (Kai *et al*., 2022), and are deposited into water sources in the form of organic acids through wet deposition (Conradie *et al*., 2016). Wet deposition is the process of gases in the air interacting with water molecules and brought down to the surface through rainfall. As a result, the acidification of rain increases, especially in highly air polluted regions such as the Mpumalanga Highveld, from coal power plants, and decreases the pH of water when rainfall increases (Conradie *et al*., 2016).

In addition, seasonality also affects faecal contamination (Chávez-Díaz *et al*., 2020). During the low rainfall period, point source contamination is increased due to the lack of dilution from rainfall and interaction with the main input water source (Sibanda *et al*., 2013). This is not clear at the Wakkerstroom wetland, as coliforms during the low rainfall season did not noticeably increase at Site 1, 2, 3 and 4. Conversely, increased rainfall and flooding events increase faecal contamination (Asfi *et al*., 2023). This occurs in two ways, firstly ground runoff transfer through diffuse faecal contamination, and secondly as increased water flow allow bacteria to remain attached to particles as there is increased water movement and turbidity. As such less faecal contamination is removed from the water column into the sediment (Chávez-Díaz *et al*., 2020). This is a possible explanation for the increased coliform abundance seen during the high rainfall periods at Site 5, 6, 7 and 8.

Asfi *et al*. (2023) showed higher values of *E.coli* during the dry periods than the high rainfall periods, similar to the presence of *E.coli* found in the Wakkerstroom wetland. The sudden occurrence of *E.coli* in the wetland from March 2022 can be explained from consistent sewage contamination from the waste treatment plant upstream (Sarkar *et al*., 2009). However, it is not known why Site 2, the runoff site from eSizameleni followed the same trend, as there is no clear connection from Site 1 to Site 2 for faecal contamination to occur. It has been noted though, that it is common for the concentration of *E.coli* to show high variation (Asfi *et al*., 2023).

6.4 Retention of nutrients

The reduction of excess nutrients and biological contamination to improve water quality has long been recognised as a valuable ecosystem service that wetlands provide to humanity (Collins, 2005). Several factors contributing to wetland degradation such as anthropogenic activities, wetland draining for land conversion, water input diversion and increased chemical and pollutant loadings can disturb a healthy functioning wetland system resulting in decreased potential for wetlands to improve water quality. In South Africa, several studies have been conducted on the water quality of wetlands, showing the physio-chemical parameters and microbial counts of water (Oberholster *et al*., 2008; Gordon *et al*., 2011; Klerk & Wepener, 2013; Dalu & Chauke, 2020).However, very few studies have been conducted on the removal capabilities of these wetlands, which could show a quantitative record of chemical and microbial retention within the wetland (Kotze *et al*., 2010; Cowden *et al*., 2014).

6.4.1 Removal of Nutrients

Inorganic nitrogen cycling occurs through two main processes which are denitrification and nitrification and several main factors are responsible for the efficiency of nitrate removal. The degree of soil moisture controls inorganic nitrogen removal in a wetland due to oxygen availability in the soil and dictates nitrogen removal through denitrification (anaerobic) or nitrification (aerobic). Therefore, denitrification would be the predominate nitrate removal process during high rainfall periods with elevated soil moisture and lowered soil oxygen conditions. Conversely, low rainfall periods would allow for greater rates of aerobic nitrification for inorganic nitrogen removal to occur (Fisher & Acreman, 2004; Hefting *et al*. 2004; Burgin & Groffman, 2010).

Sanderson (2018) showed annual nitrate removal rates of 73%, 74% and 81% in 2016, 2017 and 2018, respectively, for the Wakkerstroom wetland. The nitrate removal between the 2021 – 2022 sampling period showed a net nitrate concentration removal of 17.50 mg/L. This is consistent with the nutrient retention data between 2016 – 2018 from the Wakkerstroom wetland, as nitrate removal has been and is still occurring in the wetland. Two rehabilitated wetlands in Kwa-Zulu Natal, South Africa, had a nitrate removal between 25 – 45% while the phosphate removal through sedimentation was between 25-28% (Cowden *et al*., 2014). Overall, a higher concentration of nitrate (mg/L) was removed from the water column than phosphate, within the Wakkerstroom wetland. The Mngazi Wetland in the Eastern Cape, South Africa, was studied to assess the status of ecosystem services provided. The wetland was found to have a moderate removal in its nitrate removal and phosphate sedimentation status (Ndlazi *et al*., 2017).

Unlike nitrate removal, phosphate removal occurs mainly through sedimentation as an abiotic chemical process (Das Gupta *et al*., 2016; Bastami *et al*., 2021). Phosphate is found in greater concentrations at deeper levels of wetland soil, as a result of phosphate accumulation into the sediment (Villa *et al*., 2023). Iron oxides and aluminum oxides (Fe and Al) are a major contributor towards phosphate sorption into the sediment through ion exchange (Zhang *et al*., 2012). Increased phosphate concentrations in shallow soil layers are found in areas with high vegetation. This is because of the vegetation growth and decomposition cycle resulting in a continuous uptake and release of phosphate (Cui *et al*., 2018).

The Wakkerstroom wetland had a net phosphate removal of 2.14 mg/L over the 2021 – 2022 sampling period. Sanderson (2018) had phosphate removal rates between 95 – 100% between the 2016 – 2018 sampling period. The results from this study had comparable phosphate loading at Site 2, however Sanderson (2018) showed reduced phosphate concentrations at Site 8 across all three years of sampling. Therefore, it is likely that phosphate retention in the wetland has reduced since 2018, however phosphate retention is still occurring through the wetland.

It is plausible that there was a greater removal of phosphorus through sedimentation, and this would be revealed through a reduction in total or organic phosphorus (Rücker &

Schrautzer, 2010). Moreover, reactive phosphorus removal, such as phosphate, has a much slower and lower removal rate from the water column than particulate phosphorus (Das Gupta *et al*., 2016).

In this study, the correlation analysis between pH and phosphate did not reveal any significant relationship between the two variables. However, Cui *et al*. (2018) found that pH and the absorption of inorganic phosphorus (such as PO_4^{3-}) were negatively correlated, therefore as pH increases above 6, the removal of inorganic phosphorus decreases as there are greater amounts of OH^- ions competing with PO_4^{3-} ions to bond with available inorganic molecules. Phosphate interactions with ions in the water, contribute to suspended solids, and are therefore removed from the water column (Primost *et al*., 2022).

It has been documented that phosphate release from sediment back into the water column is a consequence of high phosphate pollutant loads (Cui *et al*. 2018). Secondly, increased soil moisture from the initial part of the rainfall period decreases soil capabilities for phosphate absorption and will therefore reduce phosphate removal rates (Song *et al*., 2007). The results from the Wakkerstroom wetland contradict these findings, as phosphate reduction occurred during the rainfall period. Conversely, June and July 2022 had the lowest mean phosphate loading and both months showed a net addition of phosphate. Therefore, the predominate phosphate removal mechanisms resulting in phosphate reduction were likely not from phosphate absorption into the sediment. In contrast to several studies, Rücker & Schrautzer (2010) showed increased retention rates during the early summer and summer rains, and reduced phosphate retention during low hydrological input. It is also evident that external factors other than rainfall and nutrient loading influence phosphate retention.

The phosphate concentration is usually higher than the nitrate concentration in raw sewage, due to organic matter (Edokpayi *et al*., 2015). Therefore, less diluted raw sewage inputs should show greater phosphate concentrations than nitrate concentrations (Sarkar *et al*., 2009) . This was observed at Site 3, where the mean PO_4^{3-} concentration was greater than the mean NO_3^- concentration. This suggests a high amount of untreated waste entering the wetland at Site 3. These results are similar for Site 3 showing the highest mean coliform abundance.

The Wakkerstroom wetland nutrient reduction could be an underestimation of the genuine removal abilities, as several studies with several sampling had reduced removal efficiencies when compared to studies with fewer sampling sites which showed higher removal efficiencies (Fisher & Acreman, 2004; Phili, 2020).

6.4.2 Removal of Microbiological Contamination

Numerous studies exist on quantifying coliform and *E.coli* contamination in wetlands (Kayima *et al*., 2018; Seo *et al*., 2019) and tracing the source of these faecal contaminations (Whitehead *et al*., 2016). However, few studies focus on how the natural wetland system improves water quality through the reduction of faecal contamination and the mechanisms by which it is removed. The net removal of coliforms did not show temporal consistency, while *E.coli* was only removed on two sampling occasions at Site 5, over the five month sampling period when *E.coli* was detected in the wetland. This suggests two possible outcomes, 1) there is a constant source of *E.coli* entering the wetland, reducing the ability of reduction for microbiological contaminants, or 2) the microbiological contaminant load is too large for the wetland to make reductions, thereby constituting towards wetland degradation.

Algae and water plants (e.g. *Lemna*) can play an important role in the removal of *E.coli* as the bacteria attach to the algae an die off, removing E.coli from the water column. This is especially relevant in eutrophic water bodies where algae build up is high (Ansa *et al*., 2011). While it was not a measured trait, it was noted that no surface algae was present at any of the eight sampling sites, which could have contributed to the low coliform and *E.coli* removal . It has also been found that elevated pH leads to the death of *E.coli* due to the change in chemical parameters (Ansa *et al*., 2011). The pH at the Wakkerstroom wetland remained stable and below 8.00, possibly creating conditions for the survival of the bacteria.

Chávez-Díaz *et al*. (2020) found higher volumes of coliforms present in the sediment than the surface water indicating sediment can act as a storage basin for coliforms. Therefore, the loading of coliforms increase in the sediment and as a result coliforms present in the surface water are reduced. Though, coliforms can be reintroduced into the water column from high rainfall, flooding events or large physical disturbances thereby increasing the abundance of coliforms in the water column (Smith *et al*., 2008; Jeon *et al*., 2019; Chávez-Díaz *et al*., 2020).

6.5 Wakkerstroom wetland status and management

The findings from this study reveal a functioning wetland, able to reduce excess nutrients, and coliforms depending on certain factors such as the pollutant load, rainfall, increased water input and flow rate. The entire wetland system is also not categorized as a eutrophic system, which would result in major wetland degradation and reduced filtration capability (Ansari *et al*., 2010; Kanyiginya *et al*., 2010).

6.5.1 Threats to the Wakkerstroom wetland

It is more effective with less resources needed to maintain and conserve a healthily functioning wetland than to restore damaged and degraded wetlands (Land *et al*., 2016). Several studies have shown the difficulties of restoring ecosystem services of degraded wetlands back to the capabilities of the original services provided (Cowden *et al*., 2014; Ambani & Annegarn, 2015), which, even successfully restored wetlands fall short of providing ecosystem services to the wetland's full capacity (Cui *et al*., 2018). Therefore, it is of paramount importance for the Wakkerstroom wetland to be effectively managed to ensure all possible effects that could degrade the wetland are managed sustainability. The main threats facing the degradation of the Wakkerstroom wetland include overgrazing from cattle (Fentaw *et al*., 2022), excess biological and chemical pollution (Mckay *et al*., 2018; Soro *et al*., 2023), ensuring the main river input is not diverted or dammed (Cowden *et al*., 2014), and controlling wetland draining measures that may occur as a result of droughts, agricultural land use, or other land transformation efforts. A common global and regional trend for degraded wetlands is the loss of land, as wetlands have shown major size decreases (Mckay *et al*., 2018; Orimoloye *et al.*, 2020). Fortunately, the Wakkerstroom has not experienced any size reductions in the last three decades, and rather has shown a slight increase in wet area (Stockdale *et al*., 2021).

The two biggest concerns that may be actively leading towards the possible downscaling of the functionality of the Wakkerstroom wetland are high pollutant loading, with an emphasis on inorganic phosphorus and untreated waste water entering the wetland resulting in faecal contamination and *E.coli* presence in the wetland (Sanderson, 2018).

It is a country wide concern that wastewater treatment plants are insufficiently removing excess nutrients and pathogenic microbes (Momba & Mfenyana, 2004; Obi *et al*., 2004; Dungeni *et al*., 2010). Several cases of wastewater treatment plants are releasing below standard water quality, into receiving water bodies which are used recreationally, and in some cases for domestic and drinking water sources in the rural areas of South Africa where clean safe running water is not accessible (Momba & Mfenyana, 2004; Obi *et al*., 2004; Dungeni *et al*., 2010). The ineffective removal of bacterial contamination poses health risks for people ingesting or using the water from receiving water bodies. The ineffective reduction of nutrient concentrations can cause excess nutrients in the water bodies, resulting in adverse effects to the wetland ecosystem as well as potentially toxic water conditions to humans if ingested (DWAF, 1996).

Secondly, further threats include the unsustainable use of wetland resources, namely overgrazing from cattle (Fentaw *et al*., 2022). Cattle do continuously graze at various sites of the wetland, throughout the year, as well as downstream of the wetland. Moreover, it was reported by local residents in Wakkerstroom that the local farmers have agreements with the council for the Wakkerstroom wetland to be used as a grazing area for livestock despite the wetland being a conserved natural area (Stockdale *et al*., 2021).

Other factors that could affect wetland functionality are the transformation of the wetland for alternative land uses and the draining or removal of wetland water (Fentaw *et al*., 2022). The Wakkerstroom wetland has previously been under this threat, where a pipeline project running through the Wakkerstroom Town was proposed by the municipality. This pipeline project would have diverted the Martin's Dam water source from the Wakkerstroom river, causing a reduction of hydrological input into the wetland. This would have had major adverse effects on the town and the natural surrounding environment that is dependent on the wetland (Fentaw *et al*., 2022). The issue was taken to court and it was ruled that the pipeline project should be discontinued (Wakkerstroom National Heritage Associate v Dr Pixley ka Isaka Local Muncipality, 2019).

6.5.2 Improving sustainable management

Over the past three decades of research, information and guidelines on wetland management and sustainable measures have been emphasized (Mccartney & Houghton-Carr, 2009). However, more recent research has shown that the limiting factor to sustainable wetland management is not the lack of management guidelines but rather limitations that prevent the implementation of these practices for various reasons (Ostrovskaya *et al*., 2013). These limitations include the lack of financial and training resources available to implement proposed management strategies. This is especially true for wetlands located in more remote areas of South Africa, where the local communities have an influence on the land (Ostrovskaya *et al*., 2013). There are also high developmental pressures from government as the need for food, housing and development increases. In order to overcome this, there needs to be a specialized focus on local implementation and community participation (Ncube, 2022). One important factor to consider for wetland conservation is the harmonious balance between the locals using the wetland for their livelihood, such as livestock grazing, especially during the winter months and the control of ensuring overgrazing of the wetland grasses do not occur (Libala *et al*., 2022). A successful method of livestock grazing management is to implement rotational grazing areas (Libala *et al*., 2022).

High and low altitude wetlands differ in the ecosystem services that they provide. Wakkerstroom is located in the highveld grasslands, and the wetland is therefore considered a high altitude wetland. High altitude wetlands are effective at nitrate removal and phosphate trapping and improving overall water quality (Chatanga *et al.*, 2020). This is particularly relevant as the Wakkerstroom wetland leads into Tugela Catchment in Kwa-Zulu Natal. In addition, high altitude wetlands are effective at supporting biodiversity. The Wakkerstroom town is highly regarded for its ecotourism, as the wetland provides recreational activities to the community and tourists such as hiking, biking, walking, birding and photography, among others. The Wakkerstroom wetland is regarded as a birding hotspot and a nationally and internationally important bird and biodiversity area, as the wetland is home to many threatened and rare bird species (Bird Life South Africa, 2018). The promotion of these ecotourism activities supports the conservation of the wetland and the surrounding natural areas as there are economic and social benefits to the conservation of the Wakkerstroom wetland (Hausmann *et al*., 2017).

6.5.3 Limitations and recommendations for future studies

The current study has brought clarity to speculated sewage leaks into the wetland, as well as provided an estimate of the wetland water quality and reduced nutrient loading into downstream water bodies.

Future research following the outcomes of this study that could be done at the Wakkerstroom wetland is the monitoring of biological indicators such as diatoms, macroinvertebrates and microbial species abundance and diversity which will significantly improve our understanding of the general health and functioning of the Wakkerstroom wetland (Wang *et al*., 2023). Secondly, water quality monitoring could incorporate the use of more precise indicators used to track human and cattle faecal matter. This could be done using markers for chemicals found in common pharmaceutical products such as paracetamol and anti-biotics, as well as commonly ingested household foods such as caffeine, as well as cattle feed and supplements (Sidhu *et al*., 2013). A much more precise method is needed for a quantitative estimation of the trends across sites and months in *E coli.*

Chapter 7. Conclusion

The aim of this research was to determine the water quality of the Wakkerstroom wetland along the length of the wetland at eight sampling sites from August 2021 to July 2022. This was achieved by assessing chemical parameters pH and total dissolved solids, as well as the nutrient concentrations nitrate (NO_3^-) and phosphate (PO_4^{3-}) to determine spatial and temporal differences. Secondly, the coliform contamination and presence of *E.coli* was tested for, using a qualitative assessment as an indicator of faecal contamination into the wetland. Lastly, the net removal or addition of the nutrient concentrations and coliform abundance were determined to estimate the filtration capabilities of the Wakkerstroom wetland.

The objectives of this study were to determine the water quality of the Wakkerstroom wetland, and to determine spatial and temporal differences and trends. The pH remained neutral throughout the study. The total dissolved solids and nitrate concentrations were highest at Site 2, 5 and 6. Reduced concentrations for TDS nitrate were recorded at Site 7 and 8, similar to Site 1, where no significant differences were obtained between sites 1,7 and 8 for TDS and nitrate. The phosphate concentration was the highest at Site 2, suggesting sewage runoff from eSizameleni informal settlement was the largest contributor of phosphate into the wetland system. Reduced phosphate concentrations were recorded at Site 7 and 8, similar to the TDS and nitrate concentrations. Coliform abundance was the highest at Site 2 and 3, suggesting greater contamination occurring in those areas, however, from these qualitative tests alone, it can not be determined the type of contamination, i.e. faecal. *E.coli* bacteria was present at all of the sampling sites from March 2022 to July 2022.

The nutrient concentrations for nitrate and phosphate as well as the abundance of coliforms seen throughout the wetland for the duration of the sampling period showed reductions. The Wakkerstroom wetland had a high net nitrate retention within the wetland, followed by phosphate, which also had a net reduction of phosphates in the wetland. There were no consistent patterns observed for the reduction of nutrient concentrations occurring at particular sites, but rather nutrient retention occurred throughout the wetland, thereby reducing excess nutrients from the water column. Coliform abundance consistently showed the reduction of coliforms at Site 5, which confirms that microbiological contamination is being reduced by the wetland. Site 6 had an increased coliform abundance throughout the study period, suggesting a reintroduction of contamination into the wetland around Site 6.

Overall, the Wakkerstroom wetland showed improved water quality for downstream water sources through the removal of excess nutrients and microbiological contamination. Improved management and more a stringent standard of water quality monitoring of the wastewater treatment plant upstream of the Wakkerstroom wetland is advised, in order to prevent wetland degradation through excessive sewage exposure, resulting in reduced functionality of the Wakkerstroom wetland.

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