Impact of effluent discharges on water quality and invertebrates in the Apies River system

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ABSTRACT

Large populations in big and developing cities are exerting pressure on wastewater treatment plants. The inadequately treated sewage discharged into the aquatic environments deteriorates the quality of the receiving water resources. The Apies River system has received attention, and several studies have been conducted on the significant sources of pollution, particularly wastewater plants. However, nothing much has been done about the impact of sewage discharges into the Apies River. This study aimed to assess the impacts of effluent discharges and land use on water quality and macroinvertebrate diversity of the Apies River.

The study focused on how the effluent discharges affect water quality and macroinvertebrate diversity by comparison to the least affected site. This will therefore add to a significant assessment of the water and ecological quality of the Apies River system. According to literature, the Apies River is one of the most polluted rivers in South Africa; it is characterized by inadequate domestic sewage treatment, power generation, and agricultural and industrial activities. Such activities are known to reduce the diversity of macroinvertebrate assemblages as well as the community structures. According to aquatic health studies, aquatic invertebrates are sensitive to organic pollution, altered physicochemical factors, and habitat transformation; hence, it is appropriate to use them to assess the ecological health of rivers.

Four surveys were conducted during the dry and wet seasons at four sites for macroinvertebrate analysis; however, only two surveys were conducted for water and sediment analysis. Macroinvertebrates were collected at all four sites from various biotopes using standard sampling procedures, and ultimately they were counted and idntified. Water and sediments were collected at all sites following the standard method, while physical parameters (temperature, electrical conductivity, pH, TDS, and flow rate) were measured *in situ* at all selected sites. Sediments and water samples were digested and sent for inductively coupled plasma-mass spectrometry (ICP-MS) analyses for the determination of selected heavy metal concentrations. Chemical analyses for nutrients and selected major ion concentrations were conducted on water samples using ICMS as well.

All selected metals were detected in water and sediment samples; however, high concentrations were recorded in sediments. Seasonal variation was recorded during the study. Some metal concentrations were high during the low flow in water and sediments, while others were high during the high flow season.

A total of 28 families of macroinvertebrates were collected at the four selected sites during the four surveys. Macroinvertebrates were classified according to their sensitivity. Families classified as highly tolerant were abundant at 3 sites that are impacted by various anthropogenic activities (WWTWs), suggesting that there is a presence of organic enrichment from site 2 further downstream. Site 1 accommodated highly sensitive families and moderately sensitive and highly tolerant families, exhibiting the least levels of organic pollution. Habitat quality was good throughout the study, and all expected biotopes were available. Habitat scores ranged from 55% to 90% at all sites. The macroinvertebrate diversity was high at sites 1 and 3 compared to sites 2 and 4. Sensitive taxa were dominant at site 1, which is the headwater stream, and highly tolerant taxa were dominant at sites 2, 3, and 4. Based on the results obtained from this study, it is evident that the pollution in the Apies River has caused a shift in the macroinvertebrate structure across four sites. Constant monitoring on the river should be implemented going forward.

KEY WORDS: Apies River, Anthropogenic activities, Diversity, Metals, Water quality, Wastewater effluents

DECLARATION

I, Boikoketso Mary Ramokolo (student number: 35994193), do hereby declare that this research is my own and that all the contents presented here are original and that the same work has not been submitted for the award of a degree at this or any other university or institution of higher learning. Information sources and the work of other authors cited in this research have been duly acknowledged.

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DEDICATION

This study is dedicated to my family.

My two beloved kids, Ogorogile and Orenetswe, because of them I have a purpose to be a role model. It is my desire that they may not fall far from the tree. Ke tsholofela gore fa tlhako ya ntlha e gatileng teng, le ya morago e gata teng.

LIST OF ACRONYMS

ACRONYMS	DEFINITIONS
ASPT	Average Score per Taxon
DO	Dissolved Oxygen
DWWTW	Daspoort Wastewater treatment works
DWAF	Department of Water Affairs and Forestry
DWAF	Department of Water Affairs
DWS	Department of Water and Sanitation
EC	Electrical Conductivity
EPT	Ephemeroptera, Plecoptera and Trichoptera
mg/l	Milligrams per litre
MIRAI	Macroinvertebrate Response Assessment Index
NAEHM	National Aquatic Ecosystem Health Monitoring
NRHP	National River Health Program
PCA	Pearson’s Correlations Analysis
PES	Present Ecological Status
REMP	River Ecosystem Monitoring Program
RQO	Resource Quality Objectives
RHP	River Health Program
SASS5	South African Scoring System Version 5
SAWQG	South African Water Quality Guidelines
SPSS	Statistical Package for the Social Sciences
TWQR	Target Water Quality Range
TDS	Total Dissolved Solids
USEPA	United States Environmental Protection Agency
WWTW	Waste Water Treatment Works
WHO	World Health Organisation
WRC	Water Research Commission

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Chapter 1

1.1 Background

Water is the most critical natural resource, which covers approximately 71% of planet Earth, of which 1% covers freshwater ecosystems (Davis & Day, 1998). The majority of global users of water are agriculture, industrial, and domestic sectors, with agriculture being reported to use 70% of global water withdrawals, 20% in industry, and 10% in the domestic sector; however, these estimations differ from one country to another (UN-Water, 2014). Water scarcity is a global challenge, though it is mandatory to expedite various sectors (Akoth, 2018). In addition to the water scarcity problem, another threatening environmental problem observed is water quality deterioration due to pollution (Vorosmarty *et al.,* 2010; Lebepe, 2018). Factors such as rapid population growth, urbanization, unregulated effluent discharges, and increasing food production have been linked to water pollution as they exert pressure on the available water resources (Al-Musharafi *et al.,* 2013; Aahman *et al.,* 2018).

Pollution can either originate from point or non-point sources (Lebepe, 2018). Point sources can be controlled and regulated, such as industrial and wastewater treatment works, while non-point sources cannot be controlled, such as agricultural and urban runoff (Dallas & Day, 2004). According to Aahman *et al.* (2008), approximately 2 million tons of wastewater are discharged into freshwater and oceans each year, causing water-related diseases in humans as well as aquatic life disruptions and imbalances. Freshwater resources such as rivers and lakes are the most threatened aquatic habitats globally (Akoth, 2018; Lebepe *et al.,* 2020). Anthropogenic activities are the drivers of freshwater ecosystem deterioration and biodiversity decline, and these activities include agriculture, effluent discharge, power stations, mining, industrialization, and urbanization (Vorosmarty *et al.,* 2010). The impacts of the abovementioned activities in freshwater settings include physical and flow regime alterations, habitat loss, and pollution (Al-Musharafi *et al.,* 2013).

Freshwater can reduce pollution levels (Omole *et al.,* 2016); however, the continuous discharge of wastewater effluent results in water quality degradation (Omole *et al.,* 2016; Tau *et al.,* 2021). Wastewater contains pollutants such as heavy metals, organic chemicals, and microbial pathogens that can exhibit high levels of toxicity to human and aquatic life and ultimately result in environmental degradation (Aahman *et al.,* 2008; Al-Musharafi *et al.,* 2013). Even though pollution compromises aquatic and human health, biomonitoring programs have been developed to monitor past and present conditions of a river system to complement physicochemical monitoring (Dallas, 2000). Macroinvertebrates are considered good bioindicators to aid with the assessment of the biological integrity of stream ecosystems (Selvanayagam, 2015). These organisms complement physico-chemical parameters and exhibit a variation of responses to pollution (Selvanayagam, 2015).

In South Africa, the Apies River is one of the river systems that is threatened by pollution from effluent discharges (Abia *et al.,* 2015a; Omole *et al.,* 2016; Tau *et al*., 2020). Along the Apies River, effluent discharges from various anthropogenic activities are discharged into the river system (Ngobeni, 2020; Tau *et al.,* 2020). These include effluent from Daspoort Wastewater Treatmenet Works (WWTW) in Pretoria Central, Rooiwal WWTW, Laudium Industrial Estate, Pretoria West power station, and Rooiwal power station (Omole *et al.,* 2016; Enzwakala *et al.,* 2017a; Tau *et al.,* 2020; Ngobeni, 2020). Wastewater treatment works and other effluent discharges are the main key factors that negatively impact the quality of the South African rivers; hence, the health and quality of the Apies River are threatened (Ngobeni, 2020).

1.2 Problem statement

The Rooiwal WWTW receives domestic wastewater, stormwater runoff water, and commercial wastewater that undergo secondary treatment and are ultimately discharged into the Leeukraal Dam (DWS, 2019). Temba and Babelegi WWTW abstract water from the Leeukraal Dam and distribute it to the surrounding areas. Even though secondary treatment is accomplished by the Rooiwal WWTW, DWA (2019) has reported that the Rooiwal WWTW is poorly managed and lacks appropriate infrastructure. Tau *et al.* (2020) and SAHRC (2019) have reported high levels of coliforms in the Apies River, close to the Rooiwal WWTW, indicating fecal pollution resulting from multiple raw sewage discharges into the river. Besides disease-causing microorganisms that may be discharged from wastewater effluents, other contaminants such as heavy metals, organic matter, and hydrocarbons could be present in partially treated effluent, thus disrupting the eco-balance of the aquatic life (Al-Musharafi *et al.,* 2012).

Rivers undergo a natural auto-purification process; despite that, studies have reported that ceaseless discharge of poor-quality effluent, escalates the levels of undesirable pollutants in receiving water and therefore this would inhibit the river’s natural assimilative processes (Akoth, 2018). This would potentially lead to water quality deterioration and aquatic life imbalances (Lebepe *et al.,* 2020; Tau *et al.,* 2020). Considering the continuous discharge of partially treated effluent in the Apies River by the Rooiwal WWTW (DWA, 2019), metals could accumulate in the sediment and later be released into the water column (Al-Musharafi *et al.,* 2013); therefore, continuous monitoring of aquatic health and biota is crucial. Furthermore, there are fishing activities on this river system by local communities; it is thus important to keep target fish species under observation for metal concentrations and evaluate human health implications associated with the consumption of fish contaminated with heavy metals. Previous studies have been conducted on the Apies River system, with the focus of the study on microbiological pollution and drinking water quality from the Leeukraal Dam. However, none exist on the possible metal pollution from the effluent and their effect on macroinvertebrate communities. Wastewater treatment plants put extreme efforts into reducing metal loads in wastewater; however, they still release trace metals into the environment (Al-Musharafi *et al.,* 2013).

1.3 Hypotheses

The following three hypotheses will be tested during this study:

1. Water quality on the lower reaches of the Apies River is degraded due to effluents.
2. Anthropogenic activities upstream and downstream result in an increase in metal concentrations in the Apies River.
3. Macroinvertebrate assemblages are different in the lower reaches of the Apies River as compared to the upstream reaches.

1.4 Aims

The study aimed to assess the impact of effluent discharges on water quality and macroinvertebrate diversity of the Apies River.

1.5 Objectives

The following objectives were set out to achieve the aim of the project:

- To assess water quality by measuring selected constituents.
- To determine metal concentrations in water and sediment from selected sites.
- Determine the aquatic macroinvertebrate diversity within the study area.

1.5 Dissertation outline

Chapter 1

This chapter provides the background of the study, aims, and objectives. A motivation of the project is also included.

Chapter 2

This chapter involves literature from various research publications related to the research problem under investigation.

Chapter 3

This chapter gives a detailed description of the study area (Apies River) and sampling points.

Chapter 4

This chapter provides details on water quality analysis; it includes an introduction, methodology, results and discussion, and conclusions.

Chapter 5

This chapter provides details on metal assessment on the Apies River; it includes an introduction, results and discussion, and conclusions.

Chapter 6

This chapter provides details on aquatic macroinvertebrate diversity assessment; it includes the introduction, methodology, results and discussion, and conclusions.

Chapter 7

This chapter provides conclusions and recommendations for future research.

Chapter 2

2.1 Literature review

2.1.1 Overview of South Africa's water resources

Water resource management coupled with water availability are the main elements of environmental and socio-economic systems (Mukheibir & Sparks, 2003). South Africa has been announced to be a water-scarce country with highly seasonal and variable rainfall, with an estimation of water availability at 900 m in the year 2017 (Rodda et al., 2016; Stevens & Koppen, 2015). It was declared the 29th driest country of 193 driest countries in the year 2005 and the 30th driest country on a global scale (Muller *et al.,* 2009; Hornby *et al.,* 2016), with an average rainfall of approximately 450mm per annum (Stevens & Koppen, 2010). According to Mukheibir and Sparks (2003), barely a slight portion of the country receives a rainfall of more than 750mm per annum, usually on the southeastern coastlines; however, the western section is arid to semi-arid. Moreover, approximately 65% of the country acquires less than 500mm of rainfall per annum (Nkosi *et al.,* 2021).

Shulze (2007) reported that the conversion of rainfall to runoff is extremely slow in South Africa, whereby in wetter regions about 9% of rainfall reaches the rivers. Freshwater resources are grouped into three sources, which are surface water (77%), return flow (14%), and groundwater (9%) (Kahinda & Boroto, 2009). South Africa relies on surface water sources to maintain various sectors such as agriculture, domestic, and industrial sectors (Kahinda & Boroto, 2009).

2.1.2 The state of South African surface water

South Africa is having water quality challenges that are activated by various human and natural activities. The anthropogenic activities associated with various sectors introduce various pollutants to water resources, hence compromising water quality and quantity (DWA, 2011b). The commonly occurring water quality problems in South Africa include salinity, eutrophication, micropollutants, microbiological pollutants, and sediment (Ashton, 2009; DWA, 2011b). Water quality in South African water resources is continuing to deteriorate; for instance, the Vaal, Crocodile, and Olifants River systems are affected by salinity resulting from mining activities (DWAF, 2011b). Freshwater resources in coastal regions are highly affected by salinity due to seawater intrusion (DWAF, 2011b).

Fecal pollution is also a countrywide problem due to overloaded and malfunctioning WWTW municipal infrastructure, lack of appropriate sanitation, urbanization, and increasing informal settlements (Ashton, 2009; Enzwakala *et al.,* 2017). Fecal pollution poses health risks to humans as it can result in the emergence and transmission of waterborne diseases (Enzwakala *et al.,* 2017). Water resources with high nutrient concentrations are named eutrophic (Akpor, 2011; Agoro *et al.,* 2020). In South Africa, eutrophication occurs mainly as a result of inadequately and partially treated sewage effluents that are discarded into the river systems (Harding, 2011). Moreover, agriculture and domestic and industrial effluents are other contributing sources of high nutrient loads (Akpor, 2011; Agoro *et al.,* 2020).

Chemical pollution with micro-pollutants such as metals, carcinogens, synthetic chemicals, illicit drugs, and pharmaceuticals is another growing water quality challenge in South African water resources (Ashton, 2009; Olujimi *et al.,* 2010). Micro-pollutants coupled with endocrine-disturbing chemicals in aquatic environments affect and reduce aquatic biodiversity (DWA, 2011b). Pollution of this type results in human and animal health impacts following uncontrolled exposure to the micro-pollutants (Akpor, 2011; Olujimi *et al.,* 2010).

2.1.3 South African water legislation and management

In South Africa, environmental impacts originating from municipal wastewater disposals are regulated by different legislations and policies. These guidelines, policies, and standards have been established to protect the country's water resources and to ensure that water needs are met (DWS, 2014). This is accomplished by the Department of Water and Sanitation (DWS), which governs the country's water resources. The main aim of the legislation developed by DWS is to ensure that water that is disposed of into the national water resources meets the standards set to ensure the protection of human and aquatic health (Muller, 2013; Dube, 2020). The establishment of specific quality limits as part of the legislature has been implemented by the DWS due to water quality challenges to prevent pollution (DWS, 2014; Muller, 2013).

The South African legislation enacted to govern water management and wastewater effluents in water resources is broad and centered on various aspects of water use and protection (Muller, 2013). The Water Services Act (WSA) (No. 108 of 1997) and National Water Act (NWA) (No. 26 of 1998) give detailed information on various elements of wastewater treatment plants. According to Muller (2013), the challenge with water and wastewater laws and policies is that they have been documented separately, which is time-consuming and difficult to access; therefore, there is a need for one comprehensive document covering all the laws and policies.

The National Water Act regulates issues on resource management, and the Water Services Act sets authorizations for water services as well as setting criteria for discharge (Muller, 2013). These two laws focus only on disposals into streams and rivers; however, compliance levels of the treatment are not covered, which therefore means that it is crucial to set standards for wastewater discharges from WWTWs into water resources (Gaydon *et al.,* 2007; Muller, 2013)**.**

2.1.4 National Water Act (NWA)

The National Water Act (No. 38 of 1998) is based on elements of equity, efficiency, and sustainability (Muller, 2013). The purpose of the act is to promote the implementation of an Integrated Water Resource Management (IWRM) framework**.**

The act aims to promote sustainable use and protection of water resources; hence it is mandatory to manage and monitor wastewater treatment plants (Muller, 2013; Dube, 2020). A license is required by the NWA for discharging any treated water into the water resources, as well as consistent monitoring of water quality, because there are still people who use untreated water from the resources to meet their daily water needs. This implies that wastewater plants are expected to have the correct technology and adequate and fully functional infrastructure to prevent pollution from treated water effluents (WRC, 2002; Muller, 2013). The DWS issues approved licenses in the form of a general authorization or water use license for plants that discharge effluents into water resources.

Chapter 3 of the National Water Act focuses on preventing pollution with requirements and restrictions for users who may negatively impact water resources from the activities they carry out (Belcher & Grobler, 2014). The focus is not only on the chemical, physical, and biological alterations that may occur, but also on the potential impacts on the user. which makes it easy to assess and quantify pollution at various levels (Belcher & Grobler, 2014).

The NWA also covers the effluent quality standards for effluent disposal of treated water into water resources; these standards focus on the quality of the receiving water body, which then allows for disposal of effluent of a certain quality to be discharged. Different elements such as ammonia, nitrates, and *E. coli* have various discharge limits. The NWA requires the establishment and implementation of the Resource Quality Objectives (RQOs), which ensure the protection and sustainable use of the country's water resources for current and future generations. The purpose of the RQOs is to set goals about the quality of water resources. In addition, the act emphasizes the importance of having a balance between the protection and utilization of a water resource (DWA, 2014).

2.1.5 The South African Water Quality Guidelines (TWQR)

The Department of Water Affairs and Forestry (DWAF), currently known as the Department of Water and Sanitation (DWS), has established and implemented target ranges for water quality due to escalating degradation of rivers and streams in the country. The implemented water quality guidelines were developed based on the capacity and tolerance levels of water resources to any undesirable foreign materials (Naidoo, 2013b). The developed water quality guidelines consist of quality criteria target ranges to guarantee the protection and agility of water for any intended purpose, which is assessed with the provision of treatment options (Dube, 2020). Legislation is enforced to ensure compliance with the recommended water quality guidelines (DWAF 1996a; 1996a; 1996a; 1996a; 1996a;1996b; 1996c; Naidoo 2013b; Muller 2013). These guidelines and standards are used as a restriction of untreated municipal and industrial effluent discharges into the water to hinder damage to human and aquatic health (Naidoo, 2013b; DWAF, 1996a). Quality standards are used for the regulation of specific quality parameters in relation to specific uses and are based on observations and scientific approaches.

2.1.6 Water quality monitoring in South Africa

The River Eco-Status Monitoring Programme (REMP) originates from the River Health Programme (RHP), which was implemented in 2016 (DWS, 2016). According to DWS (2016), REMP was initiated for purposes of the establishment of a relative reference condition. The REMP is the constituent of the National Aquatic Ecosystem Health Monitoring Programme (NAEHMP) (DWS, 2015). The main focus and aim of REMP is the monitoring of ecological conditions as they are demonstrated by biological (instream

& riparian) as well as system drivers (DWS, 2015; DWS, 2016). Some of the monitoring implements used within REMP include the South African Scoring System Version 5 (SASS5) and the Macroinvertebrates Response Assessment Index (MIRAI); both indices rely on the utilization and application of macroinvertebrates for river monitoring (DWAF, 2016; Mnisi, 2018).

2.1.7 Biomonitoring indices

Biomonitoring relies on biological responses to assess any alterations in the environment (Dallas *et al.,* 2010). It is based on the biological integrity and bioindicators such as macroinvertebrates and diatoms, as well as other appropriate indices for the instream and riparian habitats (Dallas, 2010). This scientific procedure uses biota in relation to aquatic settings to assess and monitor the ecological state of an ecosystem (Lebepe, 2018). To run successful biomonitoring procedures, constant observations and evaluations of environmental data must be carried out (Dallas 2000; Taylor *et al.,* 2007). South Africa has a few indices that are used to monitor and assess aquatic ecosystems, such as the South African Scoring System (SASS5) (Dickers and Graham, 2002), the Biological Diatom Index (BDI) (De la Rey *et al.,* 2008), the Fish Assemblage Index (FAII) (Kleynhans, 1999), and the Riparian Vegetation Index (RVI) (Kemper, 2001). All the biological indices implemented in South Africa are legit and provide an inexpensive indication of any possible alterations occurring within a freshwater ecosystem (Lebepe, 2018).

2.1.8 The South African Scoring System (SASS)

The South African Scoring System (SASS) is a fast and user-friendly index implemented for monitoring water quality; the index was developed by Chutter in the 1990s in South Africa (Mnisi, 2018). SASS has been improved and amended multiple times by the SASS forum along with the South African Water Research Commission (Ngobeni, 2020). This method has been discovered to be rapid and cost-effective; hence, the majority of practitioners throughout the country prefer the method; therefore, it has become a biomonitoring standard over the years (Dickens & Graham, 2002). Currently, SASS is the integral component of the River Eco-Status Monitoring Programme (REMP), and at times it is composed within the determination of the Ecological Reserve (EC), which is among the requirements of the South African Water Act (1998) (Mnisi, 2018).

The SASS5 index yields good results mostly in places with wide biotope diversity, though valuable results can still be obtained from poor habitats (Dickens & Graham, 2002). According to Dickens and Graham (2002), SASS data should be interpreted considering factors such as habitat diversity and availability, the ecoregion, and seasonality, because natural variations occur throughout the year while others occur between the years. Although SASS is a reliable and cost-effective method for water quality monitoring, criticisms have been made towards it as it can assess pollution in rivers but fail to distinguish between types of pollution (chemical/physical). Therefore, it is critical for specialists to carry out chemical-based water quality analysis for the determination of the responsible pollutants causing alterations in water quality (Mnisi, 2018; Ngobeni, 2020).

2.1.9 Macroinvertebrate Response Assessment Index (MIRAI)

The Macroinvertebrates Response Assessment Index (MIRAI) was implemented as an integral part of the eco-status indices (fish response assessment index, Geomorphological Driver Assessment Index, Index of Habitat Integrity, and Riparian Vegetation Response Assessment Index) to be incorporated in the Ecological Classification Process (Thirion, 2007; Kleynhans, 2008)**.** The fundamental basis of MIRAI is the aquatic macroinvertebrate's responses incorporate the changes of the drivers (geomorphology, hydrology, and physico-chemical conditions) (Thirion, 2016). While having SASS data, MIRAI is incorporated with the provision of a strong 'habitat-based cause-and-effect' basis to determine modifications of macroinvertebrate assemblage from the reference state (Thirion, 2007; Thirion, 2016).

MIRAI provides an interpretation of SASS results with greater depth and complexity. In addition, SASS demonstrates water quality modifications of a particular site, whereas MIRAI incorporates habitat-based modifications as well (Mnisi, 2018). MIRAI calculates the degree of modifications on a six-point scale, of which 0 is recorded where there are no changes observed and 5 is recorded where there are maximum modifications (Thirion, 2007). MIRAI consists of four components that are measured; they include habitat modification, flow modification, water quality modification, system connectivity, and seasonality (Thirion, 2007; Kleynhans, 2008).

Table 1: Factors affecting macroinvertebrate productivity.

Seasonal variation and temperature	Manuel *et al. (*2004) reported that temperature influences macroinvertebrate abundance as well as disruptions in the life cycles of species that rely the most on food availability. During summer, there is a high abundance of macroinvertebrates that feed on algae due to high production of algae during that time (Manuel *et al.,* 2004).
Dissolved oxygen	During the immature stage of most aquatic macroinvertebrates, high levels of dissolved oxygen are mandatory for the survival and growth of species (Letort, 2010; Mnisi, 2018).
Excess nutrients	High nutrient concentrations from anthropogenic activities such as sewage spill and fertilizers promote algal blooms, which ultimately result in the depletion of dissolved oxygen, reducing macroinvertebrate survival due to the eventual death and decomposition of excessive algae (Cortelezi *et al.,* 2015)
Chemical pollution	Excessive concentrations of toxic pollutants such as heavy metals and pesticides can shift the relative abundance of macroinvertebrates toward pollution-tolerant species (Mnisi, 2018).
Turbidity and sedimentation	Increased turbidity and sedimentation in the waterbody resulting from the reduction of riparian vegetation or erosion can reduce food sources as well as suitable habitat for macroinvertebrates.
pH	pH alterations in waterbodies can occur due to disturbances by mining runoffs and industrial pollutants. Changes in pH (decreased pH) interfere with the survival of macroinvertebrates

	by weakening their shells and exoskeletons; however, when the pH is increased, the survival of alkaline-intolerant species occurs (Manuel *et al.,* 2004).
Riparian vegetation	Alterations in riparian vegetation can affect water quality and thus aquatic macroinvertebrate communities (Mnisi, 2018; Ngobeni, 2020).

2.1.10 Factors determining the productivity of stream organisms.

- **Water quality**

Water quality is of great significance characterized by physical, chemical, and aesthetic attributes (Ashton *et al.,*2008; Dallas, 2008). Various land use activities can remarkably modify chemical and physical water conditions and ultimately reduce biological integrity within aquatic ecosystems (Dallas, 2008). Substances that are either suspended or dissolved in water can subsequently impact the chemical, physical, and aesthetic elements, resulting in water quality deterioration (Lebepe, 2018). Impacts of anthropogenic activities place a significant strain on water quality and quantity as well as production of aquatic life. Aquatic macroinvertebrates vary in tolerance to withstand levels of pollution; therefore, high levels of pollution limit their productivity (Mnisi, 2018).

- **Physical habitat structure**

Aquatic community structures are somewhat determined by habitat in relation to quality and availability (Mnisi, 2018). It is therefore crucial to assess habitat quality and availability when conducting bio-assessments of stream ecosystems (Dickens and Graham, 2002). Habitat assessments are mandatory to carry out, as various aquatic macroinvertebrates vary in terms of biotope preferences (Mnisi, 2018). For instance, certain taxa can inhabit the GSM biotope due to nutritional preferences, such as bacteria/diatoms attached to detritus or sand grains (Mangold, 2001). Habitat quality and biotope availability can be the reason for the absence of certain taxa from a site (Mangold, 2001).

- **Flow regimes and riverine ecosystems**

Almost all features of flow regimes are fundamental for the proper functioning of a riverine ecosystem (Poff *et al.,* 2007). The structure and the functioning of riverine ecosystems, which include aquatic species and riparian vegetation, are determined by patterns of temporal variations in river flows (Poff *et al.,* 1997). Therefore, any interruptions in the natural flow can disturb the ecosystem function as well as the population size and spatial-temporal diversity of lotic organisms (Mnisi, 2018).

Natural flow inconstancy in rivers occurs as a result of natural flow features related to climate, geology, and topology (Naiman *et al.,* 2008). Extreme flow events (floods) and low flow events (droughts) are integral parts of riverine ecosystems; however, human disturbance of the natural flow can result in an unforeseen chain of events with negative impacts on the riverine ecosystem (Lytle& Poff, 2004; Poff *et al.,* 1997; Naiman *et al.,* 2008). For instance, during the high-flow events, the physical structure and stream ecosystems are most likely to be modified through riverbed scouring and flushing out certain organisms and ultimately disturbing the temporal variation of benthic communities (Jacobsen *et al.,* 2013).

- **Energy and nutrient inputs**

Energy flow is another important factor that determines the productivity of aquatic ecosystems (Poff *et al.,* 1997). Energy supply must remain at optimal levels, as excessive nutrient supply results in eutrophication (Kemp & Boynton, 2004; Sutton *et al.,* 2013; Shortle *et al.,* 2019). Aquatic macroinvertebrates depend on nutrient stocks, especially phosphorus; whereby at events with extremely low nutrients, a major reduction in macroinvertebrate abundance is observed (Struijs *et al.,* 2011).

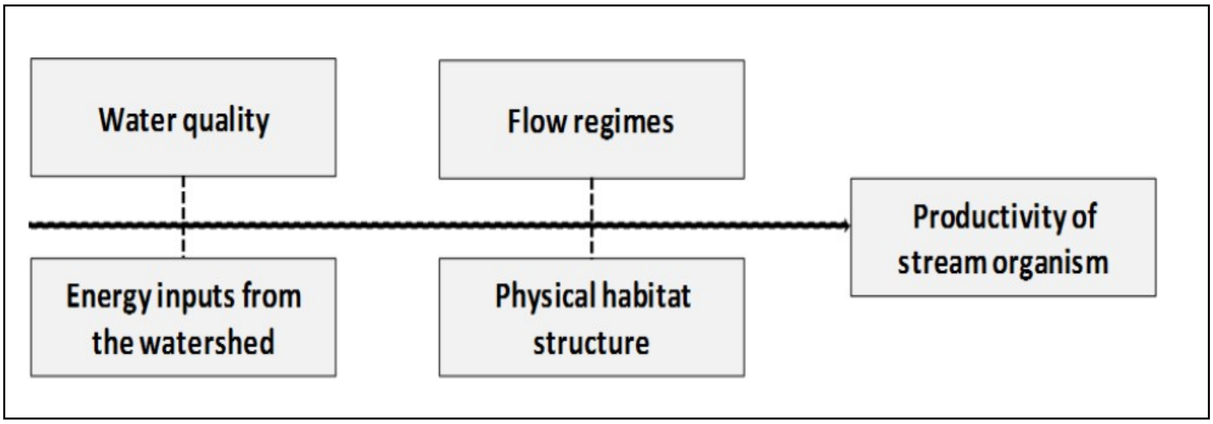


Figure 1: Factors affecting stream productivity (adapted from Mnisi, 2018).

2.1.11 Wastewater as a water source

Wastewater is a workable and feasible water resource, which, under operative management, is a reliable source for future water security (Akoth, 2018). Due to the rising demand for water, the reuse of wastewater for various water use purposes has been receiving attention globally (Garcia & Pargament, 2015). The recycling of treated wastewater is a crucial strategy that has been implemented in arid and semi-arid regions to mitigate water shortages (Al-Shamar & Shahalam, 2005). The majority of the driest countries have adopted wastewater recycling strategies to deal with water scarcity issues (Singh *et al.,* 2022).

2.11 Pollutants in wastewater effluents

Wastewater effluents are the end products of various treatment processes of the wastewater treatment plants (Singh *et al.,* 2022). The primary objective of WWTW is to provide intensive treatment of municipal and industrial water with the aim of protecting aquatic environments from harmful waste (Akpor *et al.,* 2014). Every country, in collaboration with international environmental agencies, has implemented laws and policies regulating pollutants in wastewater by establishing guidelines for accepted concentrations of specific pollutants in order to ensure effluent discharges that are environmentally friendly (Akoth, 2018). However, there have been reported cases of insufficiently and partly treated wastewater in South Africa due to factors such as malfunctioning treatment infrastructure, cost imbalances and shortages to carry out treatment processes, and the complexity of treatment processes (Akpor *et al.,* 2014).

Inadequately treated wastewater effluents contain contaminants such as heavy metals, microorganisms, nutrients and organic matter, which are toxic to humans and the environment (Akpor *et al.,* 2014; Giri & Singh, 2014; Singh *et al.,* 2022). Discharging inadequately treated effluents in receiving waterbodies leads to water quality deterioration, ecosystem degradation, and the spread of waterborne diseases (Akpor, 2011; Ali *et al.,* 2018). Environmental pollution can be observed following effluent discharge due to the fact that the quantitative removal of some pollutants cannot be fully guaranteed from some of the commonly utilized wastewater treatment processes (Cantinho *et al.,* 2016; Tytla *et al.,* 2019).

2.12 Metals

Even though heavy metals occur naturally throughout the Earth's crust, they are among environmental pollutants (Singh *et al.,* 2022; Ali *et al.,* 2018). Natural sources of heavy metals in the environment include wet and dry deposition of atmospheric salts, water interaction with rocks, and chemical weathering of rocks (Li & Zhang, 2010). Whereas anthropogenic activities include wastewater effluent, agricultural activities, urban storms, and runoffs (Singh *et al.,* 2022; El-Bouraie *et al.,* 2010). Heavy metals are released as both elements and inorganic or organic compounds, with the latter know of their high toxicity and the ability to affect aquatic environment quality (Cameron *et al.,* 2011; Camilo *et al.,* 2021) Metal pollution in surface water depends on the volume of flow as well as the proximity to point sources (Khan, 2011; Singh *et al.,* 2022) During precipitation season, metal concentrations in water decrease due to the diluting effects of non-pollutants run off water (Singh *et al.,* 2014). However, during the dry season high metal concentrations occur (Li and Zhang, 2010).

Various anthropogenic activities, such as domestic sewage, agricultural activities, mining, and industrial yields, wastewater compounds of high heavy metal concentrations and are ultimately discharged into surface water resources (Sankhla *et al.,* 2021). This practice is common in developing countries, though it's an environmental threat considering the nature of heavy metals, especially with discharges that are highly polluted with heavy metals (Camilo *et al.,* 2021; Sankhla *et al.,* 2021).

2.13 Nutrients

Nutrients are mandatory elements for plant growth and development and are not toxic (except for nitrite and ammonia), although excess concentrations can lead to eutrophication, which disturbs the structure and functioning of the entire aquatic ecosystem (Lebepe, 2018).

Nitrogen and phosphorus are elements that occur naturally in the soil and water; they are essential nutrients for plant growth (Sutton *et al.,* 2011). These nutrients occur in the environment as natural and anthropogenic activities such as the utilization of fertilizers and atmospheric deposition (Uchida, 2000; Sutton *et al.,* 2011). Nutrient pollution is a principal origin of global water quality challenges (Sutton *et al.,* 2013). Excess nutrients in surface water are the major cause of conspicuous water quality problems leading to impairments in water bodies (Sutton *et al.,* 2013).

Industrial and municipal wastewater effluents are point sources of nutrients established in surface water (Shortle *et al.,* 2020). The nutrient concentrations in surface water are determined by environmental components such as temperature, which varies based on seasons, oxygen, location, and light, among others (Quadra & Brovini, 2023). Although nutrients are crucial for maintaining and sustaining aquatic life, excess concentrations become harmful and unfavorable to both human and aquatic health (Quadra & Brovini, 2023). Eutrophication occurs when there is nutrient over-enrichment in water and has been a significant environmental concern for years (Shortle & Horan, 2017). This nutrient over-enrichment results in various problems such as toxic algal blooms, loss of aquatic life, food chain disruptions, and depletion of dissolved oxygen, which ultimately lead to the degradation of water quality, interrupting the overall water use, agricultural activities, and other purposes (Shortle *et al.,* 2019; Shortle *et al.,* 2020).

Global biodiversity and surface water have been reported to have been massively reduced due to this nutrient enrichment, with Southern Africa included (Sutton *et al.,* 2013). Changes in land use, climate, and population growth can have empowering consequences on biogeochemical processes that dictate fate and transport nutrients from point and non-point sources (Shortle *et al.,* 2019). Expeditious population growth may occur due to factors such as urbanization, which would ultimately generate intensive nutrient loadings that frequently surpass the assimilative capacity of local water bodies downstream (Shortle *et al.,* 2019). Excessive nutrient concentrations are often observed where much of urban development occurs (Shortle & Horan, 2017). Climate change has an impact on the rate of mobilization and bioavailability of these nutrients in water bodies, determining dead zones and eutrophication in freshwater resources (Shortle *et al.,* 2019). High air temperatures culminate in high temperatures in water, which favor and accommodate harmful algae and sunlight-absorbing compounds (Sutton *et al.,* 2013; Quadra & Brovini, 2023).

South Africa is one of the countries that are faced with nutrient enrichment in its water resources (Lebepe, 2018). This challenge of excess nutrients in water resources is one of the leading factors in water quality deterioration, hence affecting clean water supply (Quadra & Brovini, 2023). According to Matthews and Benard (2015), plenty of South African surface water bodies have been impacted by excessive nutrients as well as cyanobacterial blooms, whereby 62% are hypertrophic and 54% contain cyanobacteria surface scum, which poses health risks to aquatic life and humans.

Partly treated wastewater effluent discharges due to malfunctioning sewage treatment plant infrastructure have been reported as the leading source of eutrophication in South Africa (Akoth, 2018). Some studies suggest that even if wastewater can be adequately treated, it often contains high concentrations of phosphorus, which is detrimental to water quality due to the fact that phosphorus removal is not prioritized by wastewater treatment processes (Mangold, 2001). In South Africa, some wastewater treatment plants have inadequate and malfunctioning infrastructure problems and have been reported as not meeting the water quality standards and hence have a negative impact on surface water quality (Petterson, 2016).

Contaminant	Significance	Origin
Settleable solids (sand, grit)	Settleable solids may create sludge deposits and anaerobic conditions in sewers, treatment facilities or open water	Domestic, run-off
Organic matter (BOD); Kjeldahl-nitrogen	Biological degradation consumes oxygen and may disturb the oxygen balance of surface water; if the oxygen in the water is exhausted anaerobic conditions, odour formation, fish kills and ecological imbalance will occur	Domestic, industrial
Pathogenic microorganisms	Severe public health risks through transmission of communicable water borne diseases such as cholera	Domestic
Nutrients (N and P)	High levels of nitrogen and phosphorus in surface water will create excessive algal growth (eutrophication). Dying algae contribute to organic matter (see above)	Domestic, rural run-off, industrial
Micro-pollutants (heavy metals, organic compounds)	Non-biodegradable compounds may be toxic, carcinogenic or mutagenic at very low concentrations (to plants, animals, humans). Some may bioaccumulate in food chains, e.g. chromium (VI), cadmium, lead, most pesticides and herbicides, and PCBs	Industrial, rural run-off (pesticides)
Total dissolved solids (salts)	High levels may restrict wastewater use for agricultural irrigation or aquaculture	Industrial, (salt water intrusion)

Figure 1: Main classes of the contaminants of the municipal wastewater and their significance and origin (Source: Metcalf and Eddy Inc., 1991).

2.14 Wastewater treatment methods

Wastewater treatment plants are intended to treat wastewater originating from various sectors with the aim to minimize possible environmental and health impacts with regard to untreated or partially treated wastewater discharges into receiving surface water waterbodies (Edokpayi *et al.,* 2017; Akoth, 2018). Wastewater is treated at various levels of treatment with the ultimate purpose of eliminating harmful pollutants contained in the wastewater (Edokpayi *et al.,* 2017). For this to be achieved, municipal wastewater treatment plants have been placed in charge to collect and treat wastewater prior to discharge in receiving water waterbodies (Bundschun *et al.,* 2011; Edokpayi *et al.,* 2017). The major aim of WWTPs is to ensure the protection of human health as well as the prevention of environmental degradation (Bundschun *et al.,* 2011; Bansah & Suglo, 2016).

Municipal wastewater treatment (i) preliminary treatment, which involves the removal of debris; (ii) primary treatment, which entails partial removal of suspended solids and organic matter; (iii) secondary treatment, which involves the elimination of biodegradable and suspended solids; and (iv) tertiary treatment, which is the last stage involving the elimination of residual suspended solids, nutrients, and disinfection (Weber *et al.,* 2006; Bundschun *et al.,* 2011). In ancient times there were no wastewater treatment plants; therefore, wastewater was treated naturally through channelization from buildings to waterways and ultimately reached rivers and streams.

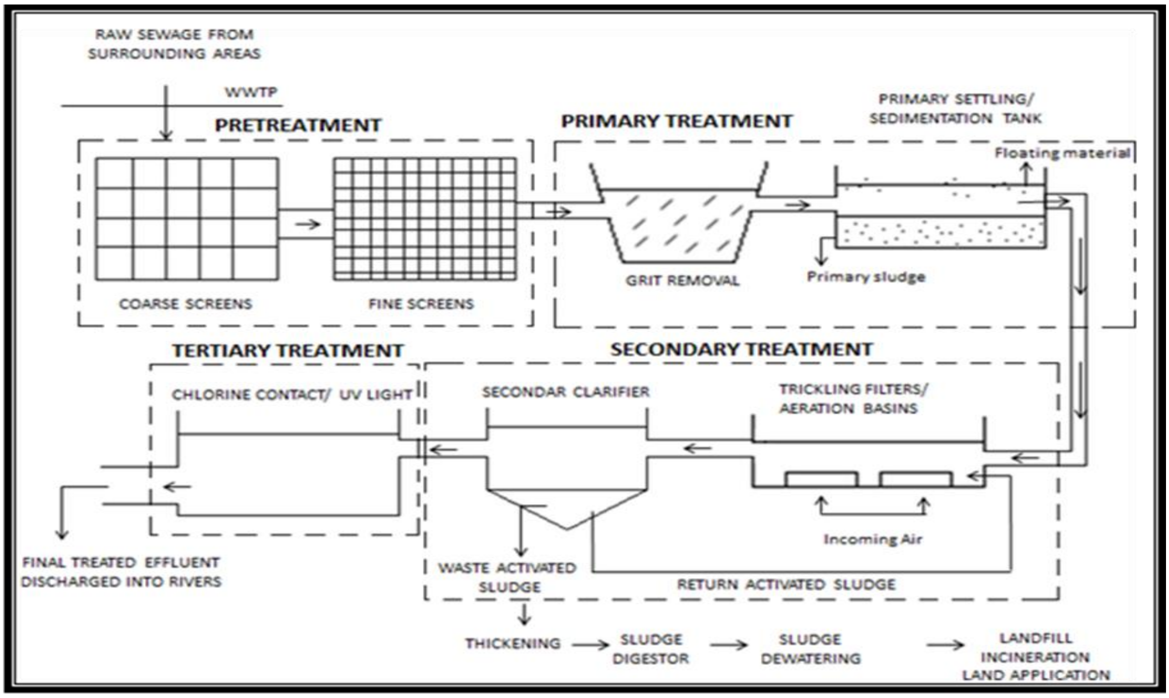


Figure 2: A general overview of treatment stages within a wastewater treatment plant (Naidoo, 2013b).

2.15 Challenges in wastewater treatment plants

Most South African rivers are contaminated with coliforms, nutrients, and metals due to the discharge of partially treated or raw wastewater (Akpor & Muchie, 2011). Different studies have reported that the major cause of pollution in South African rivers is the poor operational state and lack of maintenance of the municipal WWTWs (Al-Musharafi *et al.,* 2013; Tau *et al.,* 2020). Subsequently, this led to economic and health risks for people who rely on river water. Partially treated or raw sewage discharged directly into the rivers has a negative impact on the oxygen demand and nutrient concentrations, thus resulting in eutrophication and affecting aquatic species (Al-Musharafi *et al.,* 2013).

Key challenges faced by the wastewater treatment industry locally and internationally include (I) most of the wastewater treatment plants have aged, thus requiring new infrastructure, equipment repairs, and replacements in order to guarantee sustainability and longevity of use; (II) urbanization leads to population growth within a specific area, which affects the wastewater treatment plant infrastructure due to high demand of water and capacity issues; and (III) differences in volumes and compositions of effluents containing contaminants have been observed with threatening results compared to historical challenges (EPA, 2004).

Chapter 3: Study Area

3.1 Study area description

The Apies River flows through the City of Tshwane in the Gauteng Province of South Africa. It lies within the Apies River Makebasin that forms part of the Crocodile (West) Marico Water Management Area (Abia *et al.,* 2015a). The Apies River originates at the Fountains Valley, where there are springs that produce approximately 30 million liters of water daily (Yude, 2006; Vogel, 2016). There are 10 tributaries that contribute and flow into the Apies River. The river flows through three provinces of South Africa, which are Gauteng, North West, and Limpopo. When it reaches Hammanskraal, it connects with the Pienaars River, then flows until it reaches and connects with the Limpopo River (DWAF, 2008).

3.1.1 Crocodile West Marico Water Management Area

The Crocodile-West and Marico water management area is the second most populated in South Africa; the area generates almost a third of the country's GDP (Holtzhauzen, 2018). The catchment accommodates parts of the North West, Limpopo and Gauteng provinces (DWAF, 2004b)

3.1.2 Land use

The City of Tshwane Metropolitan Municipality comprises an area of approximately 2300 km². The area comprises various land uses ranging from dense urban development to rural agricultural and undeveloped (DWAF, 2008; Rude, 2006; Vogel, 2016; Dippenaar *et al.,* 2016; Ngobeni, 2020). The land use within the area consists primarily of coal-fired generation plants, wastewater treatment works, agricultural activities, and industries, as well as urban and rural settlements (figure 3) (DWAF, 2008; Ngobeni, 2020; Tau *et al.,* 2021).

The Apies River connects significant natural elements, including streams, nature areas, and ridges (Rude, 2006). Along the river, there are open spaces that include agricultural land and unused open areas from the Rooiwal until the Makapanstad area (Ngobeni, 2020). The river is under severe ecological pressures resulting from the invasion of exotic plant species, pollution, and physical disturbances (Ngobeni, 2020). Four WWTW effluents are in close proximity to the Apies River; the Rooiwal WWTW discharges approximately 100 million liters of treated sewage into the river per day (Tau *et al.,* 2021).

Figure 3 (I & II): Some of the land use activities along the Apies River include the coal power station and crop farming, which are both closely located to the Rooiwal Wastewater Plant.

3.1.3 Geology

The City of Tshwane along the Apies River is dominated by geological units that include Basement granite (tonalite and migmatite), Malmani dolomites, Meinhardskraal granite, and Sand River Gneiss (Dippenaar *et al.,* 2014). The Pretoria Group Quartzites and Pretoria Group Conglomerates dominate most parts of the City of Tshwane, as well as the Transvaal Supergroup (Dippenaar *et al.,* 2014).

3.1.4 Surface hydrology

The Apies River is the most prominent surface water feature in the City of Tshwane; it flows throughout the city, and stormwater is added to the natural river flow. There are 10 tributaries in total that feed into the Apies River as it flows through the city (Pretoria) until it reaches the Bon Accord Dam in Pretoria North (Vogel, 2016). It continues to flow out of the Bon Accord Dam until it reaches the Leeukraal Dam in Hammanskraal. It flows out of the Leeukraal Dam until it reaches the Makapanstad area in the North West Province and ultimately merges with the Pienaars River (Ngobeni, 2020).

The Apies River system is classified as perennial even though some of its tributaries experience low to seasonal flows (Vogel, 2016). The area receives summer rainfall, although the mean annual rainfall differs across regions of the catchment.

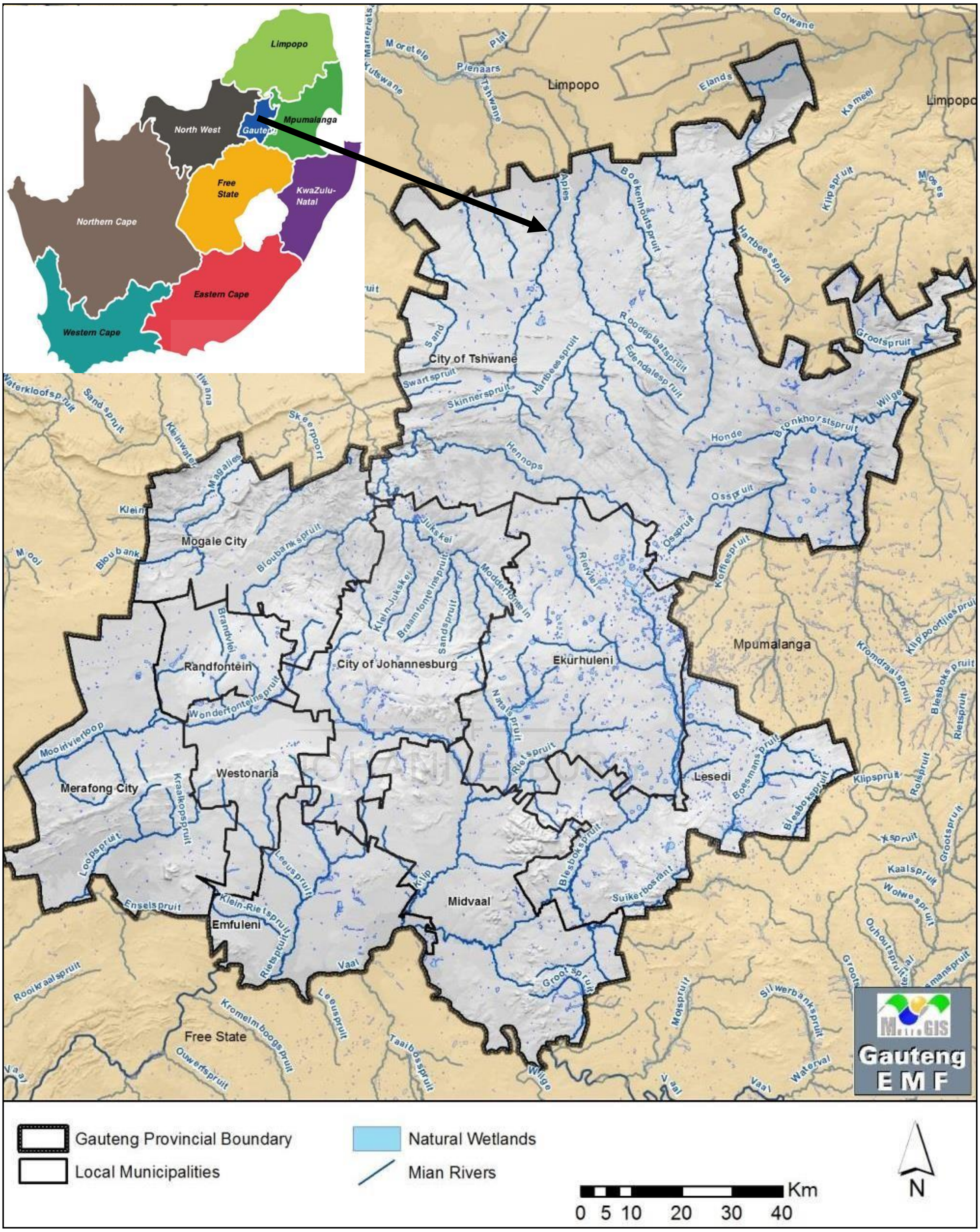


Figure 4: Study area illustrating where the Apies River originates in the City of Tshwane and as it flows through the city until it reaches the Pienaars River, which is a tributary of the Crocodile River. (Source: Armour, 2018)

3.2 Water quality problems on the Apies River system

Anthropogenic activities and human pressures on freshwater resources are a global environmental challenge, which threatens water quality and aquatic ecosystem health (Sutton *et al.,* 2011; Zaharia *et al.,* 2016). These human pressures are more common and prevalent in urban areas (Zaharia *et al.,* 2016). Human pressures are common in urban areas due to exploitation and land use activities in close proximity to the rivers with the ability to alter the natural state of rivers through channelizing, damming, and diverting (Sutton *et al.,* 2011; Ceola *et al.,* 2015). Furthermore, in urban areas the aquatic functions, structure, and health are affected due to the modifications that occur on the water chemistry, flow regime, and quality (Lamberth *et al.,* 2009).

The Apies River receives effluent discharges from various anthropogenic activities such as wastewater treatment plants and industries (Omole *et al.,* 2016). The concerning environmental issue with wastewater is that, at times, it is discharged into the river as it is, with no treatment performed prior (Singh *et al., 2022*; Sankhla *et al.,* 2021). In developing countries, approximately 90% of untreated wastewater is discharged into the environment, and this is one of the key causes of pollution of surface water (Singh *et al.,* 2022). Along the Apies River, effluent from various anthropogenic sources is the main origin of pollution, followed by natural sources such as weathering of geological formations and agricultural activities (Tau *et al.,* 2020).

Tau *et al.* (2020) have reported high concentrations of ammonia caused by the effluent from the Rooiwal WWTW and microbial pollution with high levels of *E. coli* from the Rooiwal WWTW, showing that there are other sources of microbiological pollution before the WWTW. Enzwakala *et al.* (2017a) and Abia *et al.* (2015b) have reported high concentrations of fecal coliforms and *Enterococcus spp.* in water and river sediment, which indicates high loads of external fecal pollution. Land users around the Apies River use various herbicides and insecticides, which end up in the river and negatively impact the water quality (Ngobeni, 2020). The informal settlements near the river also pollute the river water with illegal waste disposal (Abia *et al.,* 2015b). In certain areas along the river, vegetation has been removed due to anthropogenic and natural processes, resulting in the exposure of riverbanks and making them susceptible to erosion (Abia *et al.,* 2015b).

Sediment resuspension can occur due to extreme weather events as well as human activities releasing pathogenic agents into the water column and posing health risks to people using water directly from the river for domestic purposes (Abia *et al.,* 2015b). Sediments on the riverbed are a significant reservoir of pathogens in the Apies River (WRC, 2015; Enzwakala *et al.,* 2017). Omole *et al.* (2016) reported odor, overgrown aquatic plants, and slow stream flow along the Skinnerspruit in the Apies River, which indicates eutrophication. The slow stream flow can be resolved by clearing overgrown aquatic plants after wastewater discharges by the WWTW (Ngobeni, 2020). The pollution challenges along the Apies River can be resolved by determining and investigating the sources of pollution in the upstream region (Omole *et al.,* 2016).

Figure 5: Study area for the current study. This is the Apies River from site 1 to 4 (Image: Google Earth, 2023).

3.3 Site selection

The sampling sites were selected based on the aim and objectives of the study, as well as the accessibility and availability of habitat for macroinvertebrates. The more detailed site descriptions are provided below and also in the tables below.

Site 1 (Table 1): The site is situated at the Groenkloof Nature Reserve, also known as The Fountains Valley. It is highly protected and receives fewer impacts from anthropogenic activities. There are no visible signs of pollution; the stream is in good condition.

Site 2 (Table 2): The site is situated downstream from the Daspoort WWTW and in close proximity to the PPC cement industries. This site is heavily impacted by the combined effect of the effluent discharges from the Daspoort WWTW, storm water discharge from the cement industries, and illegal waste dumping. The area contains a bad odor and algal blooms and appears to be slightly eutrophic.

Site 3 (Table 3): This site is located upstream of the Rooiwal WWTW and has minimal human disturbances that may extremely impact and deteriorate the ecological integrity of the Apies River; however, there are few crop plantations around this area that might pose threats to the river's health.

Site 4 (Table 4): This site is dominated downstream of the Rooiwal WWTW. It was selected based on the idea that it will reveal the effects of pollution from the WWTW and coal power plant effluents.

Table 2: Description of Site 1: Fountain Valley.

Site 1 Fountains valley	
	
Coordinates	25.7823870 S 28.1938470 E
Site description	The site is situated at the Groenkloof Nature Reserve, also known as The Fountains Valley. It is highly protected and receives fewer impacts from anthropogenic activities. There are no visible signs of pollution; the stream is in good condition
Biotope description	Stones (SOC & SIC), Vegetation, Gravel Sand and Mud (GSM)

Table 3: Site 2 description: Daspoort WWTW

Site 2: Daspoort WWTW	
	
Coordinates	25.7823870 S 28.1938470 E
Site description	The site is situated downstream from the Darspoort CWWTW and in close proximity to the PPC cement industries. The area contains a bad odor and appears slightly eutrophic.
Site mainly impacted by:	● WWTW effluent discharges ● Dust pollutants from the nearby cement manufacture ● Urban and stormwater discharge ● The influence of all activities upstream ● Illegal waste dumping
Biotope description	Stones (SOC & SIC), Vegetation, Gravel Sand and Mud (GSM)

Table 4: Site 3 description: Rooiwal Upstream

Site 3: upstream Rooiwal Wastewater Treatment	
	
Coordinates	25.550889 S 28.243805 E
Site description	This site is dominated upstream of the Rooiwal WWTW and has minimal human disturbances that may extremely impact and deteriorate the ecological integrity of the Apies River; however, there are numerous crop plantations around this area that might pose threats to the river's health with nutrient pollution.
Site mainly affected by:	● Agricultural activities ● Industrial and stormwater runoff
Biotope description	Stones (SOC & SIC), Vegetation, Gravel Sand and Mud (GSM)

Table 5: Site 4 description: Rooiwal Downstream

Site 4: Downstream the Rooiwal WWTW			
			
Coordinates			25.53396 S 28.23135 E
Site description:			This site is downstream from the Rooiwal WWTW and the coal-fired power station. There are few industrial and agricultural activities around this area.
Site mainly affected by:			• Untreated sewage leaks • Partially treated effluent from the Rooiwal WWTW

			• Effluent from the coal power station • Agricultural activities
Biotopes			Stones (SOC & SIC), Vegetation (marginal), Gravel Sand and Mud (GSM)

Chapter 4 : Water Quality

4.1 Introduction

Surface water systems are important ecosystems with high ecological value; therefore, their quality and health are extremely important to those depending on them (Nguyen *et al.,* 2018). Humans and freshwater ecosystems rely on rivers for renewable water supply and important services such as domestic uses, food, and recreational activities (Berger *et al.,* 2016; Dickens *et al.,* 2018). Globally, anthropogenic activities are altering the availability and quality of freshwater (Nguyen *et al.,* 2018; Rodell *et al.,* 2018). For the past 3 decades, deterioration of water quality has been observed globally, which ultimately led to the water management and legislation for the available water (Abbasi & Abbasi, 2012). Streams and rivers are the most threatened freshwater ecosystem (Nguyen *et al.,* 2018).

Analysis of physicochemical parameters concurrently with the use of benthic macroinvertebrates has become a solid and dependable method for water quality assessment (Aklilu, 2011). Currently, the leading causes of water quality degradation include discharging toxic and hazardous chemicals into the water, over-pumping of aquifers, polluting water sources with substances that promote algal growth, land use and water management practices (WHO, 1996). Various methods have been developed to assess and analyze disfigurements in water quality. These methods analyze water quality through the measurement of chemical parameters (e.g., biochemical oxygen demand, alkalinity, pH, dissolved oxygen, organic compounds, nutrients, and metals) and physical parameters (e.g., temperature, turbidity, sedimentation, and patterns of water flow) (Aklilu, 2011; Aazami *et al.,* 2015; Tamiru *et al.,* 2017).

The aims of the chapter included the assessment of the state of water quality by measuring relevant constituents in order to estimate pollution levels and to determine if the water quality parameters comply with the South African water quality standards for aquatic ecosystems. The hypothesis is that the water quality of the Apies River is degraded downstream due to effluents.

4.2 Methods and materials

4.2.1 Water samples

In-situ analyses

The water samples were collected between March and July 2023, representing the wet and dry seasons. Physical parameters that included dissolved oxygen (DO), temperature, pH, electrical conductivity, and total dissolved solids were measured *in situ* using a HANNA multiparameter instrument (Model: HI 98195) (Figure 6). Distilled water was used to rinse the probe, and the probe was immersed in each sample for approximately one minute in order for the meter to reach equilibrium. After obtaining constant readings of each parameter, the obtained measurements were recorded on the data sheet. The meter probe was rinsed repeatedly with distilled water for each sample to avoid contamination. This procedure was followed for all samples collected at each sampling site.

Figure 6: HANNA multiparameter instrument (Model: HI 98195) used to measure physical variables at the Apies River in 2023.

4.2.2 Chemical variables

Chemical parameters were sampled following the method of Mwangi (2014). At all four sampling sites, water samples were collected using 1L bottles, which were labeled with all the necessary information, such as names, date, time, and sample location. Samples were then transported on ice and later refrigerated in the laboratory. Water samples were analyzed at the laboratory for the following parameters: nitrite (mg/L), nitrate (mg/L), ammonia (mg/L), calcium (mg/L), potassium (mg/L), and sodium (mg/L).

4.2.3 Laboratory analysis

4.2.3.1 Metal analysis in water

A clean 60mL syringe was used to extract 9.85 mL of water from polyethylene bottles that were used for sampling and were filtered through a 0.22 µm Whatman® filter into a 15mL plastic inductively coupled plasma mass spectrometry (ICP-MS) test tube. The 15mL of water transferred in the ICP-MS test tube was acidified by adding 0.15 mL of 65% nitric acid to an acid concentration of 1%. The test tubes were then covered with Parafilm and labelled correctly and sent for ICP-MS analyses to determine metal concentrations. Certified standard from De Bruyn spectroscopic solutions: 500MUL20-50 STD2 was used to determine analytical accuracy, with recoveries being within 10% of certified values. All of the metal concentrations in water samples during all sampling surveys were measured in µg/L (Wolmarans *et al.,* 2017).

4.2.3.2 Metal analysis in sediment

In the laboratory, sediment samples were dried at 60°C in an oven for 24 hours. From the dried sediment sample, only 0.5 g was used for digestion. Analyses of metals in sediment were done using ICP-MS. Samples were digested using nitric and hydrochloric acid ($HCl:HNO_3$ = 3:1) and a microwave digester. After digestion, 9.7 mL of water was added to a 15mL test tube, followed by the addition of 300 µL of supernatant. The test tubes were labelled correctly, sealed with Parafilm, and sent for ICP-MS analyses to determine metal concentrations in sediment and measured in mg/kg dry weight. Quality control and assurance were applied for each sample. Certified standard from De Bruyn spectroscopic solutions: 500MUL20-50 STD2 was used to determine analytical accuracy, with recoveries being within 10% of certified values.

4.2.3.3 Nutrients

The water samples were analyzed in the laboratory following methods adapted from Mwangi (2014). The samples were analyzed using the Merck Test kits and the Merck Spectroquant Pharo-100 spectrophotometer. This spectrophotometer has been designed to measure samples at a wavelength range of between 190-1100vnm using UV/VIS.

4.3 Data analyses

4.3.1. Pearson’s correlation analysis

Pearson's product moment correlation (r) is a measure of the direction and strength of the relationship that may exist between two measured variables on at least an interval scale (Esezi & Chikweru, 2018). The Pearson’s correlation was used to assess the impact of WWTW effluents on water quality, as it indicates the measure of relationship between factors (Bhandari & Nayal, 2008; Healey, 2014; Soko, 2014). For calculating correlation coefficients, the correlation matrix must be created through calculating the coefficients of different pairs of parameters, and correlation for significance must be tested by applying the p-value (Kebede & Kebedee, 2010; River *et al.,* 2013)

4.3.2 Principle Component Analysis

In a Principle Component Analysis (PCA) bi-plot, the variance of the variable is approximated by the length of the lines, meaning the variance will be high when the line is long. In a multivariate space, the distance between two points is called the Euclidean distance. The influence of each variable is shown by the length and direction of the vector to the two principal components in the biplot (Tripathi & Singal, 2019). Eigenvalues are normally used to determine the principal components (PCs) (Olsen *et al.,* 2012; Zeinalzadeh & Rezaei, 2017).

Statistical significance of the spatial and temporal variation of the selected physical and chemical variables was determined at $p < 0.05$ using R Studio version 4.4.1. Where data was parametric, ANOVA was applied, and where data was non-parametric, Kruskal-Wallis tests with Dunn’s Multiple Comparison Tests were performed to test for significant differences between sites and surveys (Olsen *et al.,* 2012).

4.2 Results and discussions

4.2.1 Physical variables

Temperature

Waterbodies experience daily and seasonal variations in temperature (Dallas & Day, 2004). Various organisms have various optimal temperatures suitable for their growth, reproduction, and survival (Palmer *et al.,* 2004). The temperatures in water have an effect on biological processes such as metabolic rate (chemical reaction rates). High temperatures in water result in elevation of organisms' respiration rates, which ultimately lead to increased oxygen consumption together with increased decomposition of matter (Bartman & Balance, 1996; DWAF, 1996a & c; Rajele, 2004). In South Africa, the temperature of inland water ranges between 5 and 30°C. Temperature changes from time to time since it's mostly dependent on climatic conditions; it also depends on vegetation cover as well as water depth. A temperature ranging between 28.6 and 31°C was recorded on the Apies River during the high flow season and 16.9 to 19.4°C during the low flow season, respectively (Figure 7).

According to Banacina *et al.* (2023), temperature fluctuations can affect the structure and the functioning of aquatic ecosystems; however, with regards to aquatic macroinvertebrates, their response to changes in temperature has received less attention. In addition, since temperature fluctuations occur due to natural causes, there is no specific target water quality range (TWQR), although suggestions have been made that daily changes at specific sites should have a difference of approximately 2°C to be considered as normal (Banacina *et al.,* 2023).

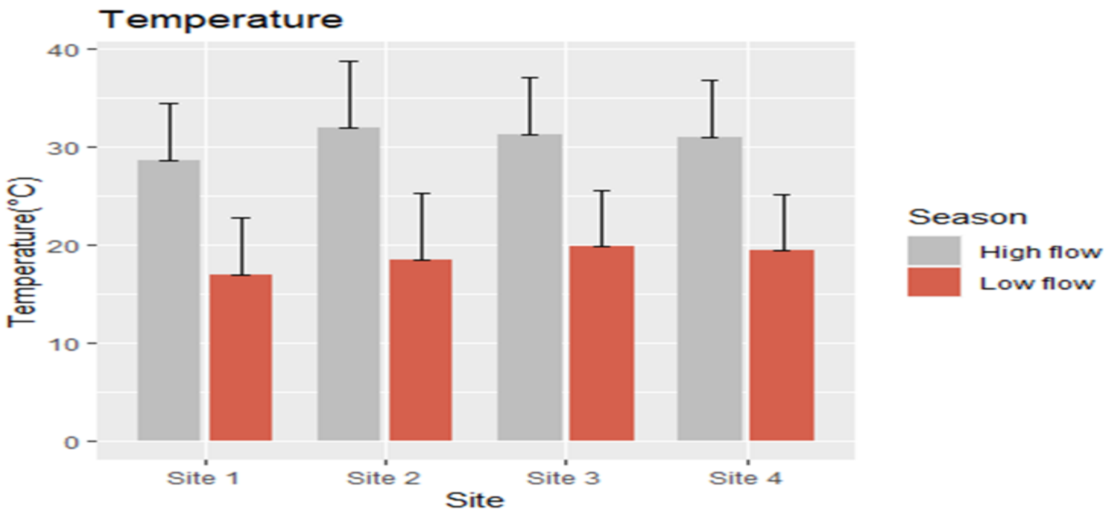


Figure 7: Temperature recorded at the Apies River during the high-flow and low-flow seasons (February/March and June/July 2023).

pH

pH is a measure of hydrogen-ion concentrations in water (DWAF, 1996a; Rajele, 2004). Most South African freshwater ecosystems have pH ranging between 6 and 8 (DWAF, 1996a). It is important to monitor pH in surface water since it's capable of affecting biological and chemical processes (DWAF, 1996a & c). pH of natural water is significantly determined by atmospheric and geological influences (DWAF, 1996a). Most of South Africa's freshwaters, with pH ranges between 6 and 8, are comparatively well buffered and nearly neutral, according to DWAF (1996a).The pH values recorded on the Apies River throughout the study ranged from 7 to 8 at site 1 and site 3. However, sites 3 and 4 recorded values slightly above 8 (Figure 8). Most species can survive and procreate in the 6.5–9 pH range, which is the TWQR for aquatic environments (DWAF, 1996a). According to Palmer *et al.* (2004), an increase in algae's biological activity and photosynthetic activity that are caused by anthropogenic activities and industrial effluents may have an impact on pH levels. pH values that are alkaline and higher than 10.8 have the potential to become lethal to certain aquatic organisms, including fish (Svobodóva *et al.*,1993; Palmer *et al.,* 2004). Therefore, in the present study, the pH was suitable for most aquatic organisms and within the TWQR for aquatic environments.

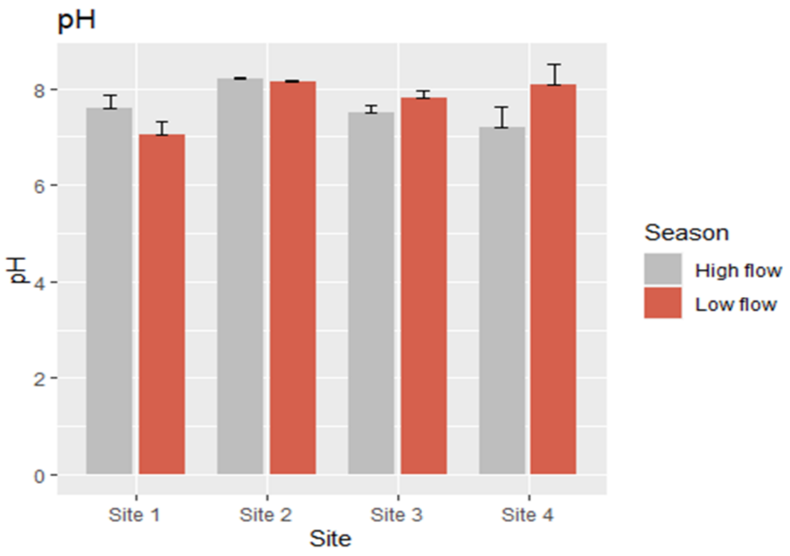


Figure 8: pH values recorded at the Apies River during the high-flow and low-flow seasons (February/March and June/July 2023).

Electrical conductivity

According to Dallas and Day (2004), electrical conductivity in water is defined as a measure of water samples to conduct electric current, and it is expressed in millisiemens per meter (mS/m). The ability to conduct electric current occurs as a result of the availability of ions such as calcium (Ca^{2+}), potassium (K^{+}), sodium (Na^{+}), magnesium (Mg^{2+}), chlorine (Cl^{-}), sulfate (SO_2^{-}), nitrate (NO^{-}), bicarbonate (HNO^{-}), and carbonate (CO_2^{-}), which carry electric charge (DWAF, 1996a). In most freshwater resources, electrical conductivity ranges between 0.1 and to 10mS/m; however, in cases of polluted water or those receiving large quantities of runoff it may exceed 10mS/m. In this study, EC mean ranged from 252 to 400 mS/m during the high flow season at all four sampling sites and ranged from 184.9 to 432 mS/m during the low flow season (Figure 9). Even though natural waters are considered healthy with EC ranges between 0.1 and 10 mS/m, there are no serious health implications with EC above 45 45mS/m (DWAF, 1996c).

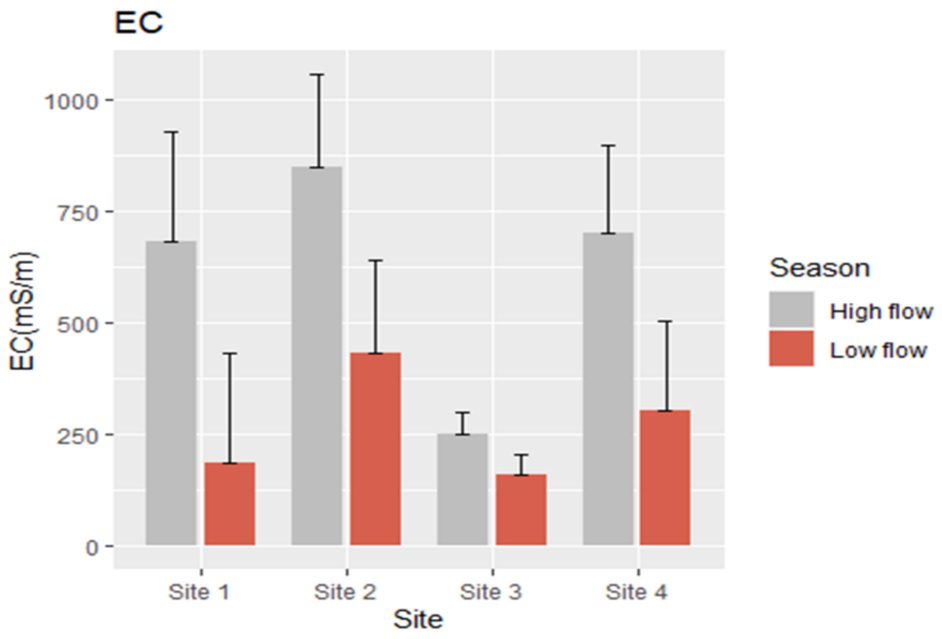


Figure 9: Electrical conductivity recorded at the Apies River during the flow and low-flow seasons (February/March and June/July 2023).

Electrical conductivity of all samples at all four sites ranged between 252 and 848 mS/m during the high flow season and 159.1 to 432 mS/m during the low flow season, respectively. According to Chapman (1998), in natural waters, EC ranges between 0.1 and 10 mS/m; in addition, DWAF (1996c) reported that there are no health threats with conductivity of <45 mS/m. Therefore, the results obtained in this study are above mS/m, indicating aquatic health threats. Omole *et al.* (2016) reported EC values ranging from 55.2 to 85.7mS/m on the Apies River, which differs greatly from the results obtained in the present study. According to Bhat *et al.* (2014), the higher conductivity recorded in this study could be attributed to anthropogenic activities along the sampling sites, such as agricultural runoff, waste disposal, WWTWs, and industrial effluents. In addition, Sasikala *et al.* (2015) reported that high values of electrical conductivity may result from high salinity and mineral content. Dilution and other naturally occurring processes along the river system may be the cause of the EC concentration drop at the downstream sampling site (site 3) during both seasons. Makuya (2022) , reported less EC concentrations downstream of the WWTW along the Juskei River compared to upstream concentrations. High readings at site 2 and site 4 could be attributed to the two WWTWs failing to remove the ions in the wastewater before discharging into the Apies River (Makuya, 2022).

Total Dissolved Solids (TDS)

Total dissolved solids are naturally occurring components in waterbodies, which are dependent on geochemical, hydrological, and physical processes (Davies & Day, 1998). The TDS refers to any salts, minerals, cations, anions, and metals in water. The concentration of TDS is defined as the total amount of soluble material in a water sample (Dallas & Day, 2004). In undisturbed aquatic environments, total dissolved solids are determined based on the degree of chemical composition and weathering of rocks as well as the influences of rainfall and evaporation within the catchment (Davies & Day, 1998). According to Dallas and Day (2004), increases in TDS are influenced by anthropogenic activities such as irrigation, water reuse, and industrial effluents.

The constituents that add up to the total dissolved solids can include calcium (Ca^{2+}), potassium (K^{+}), sodium (Na^{+}), magnesium (Mg^{2+}), chlorine (Cl^{-}), sulphate (SO_4^{2-}), nitrate (NO^{-}), bicarbonate (HNO^{-}), and carbonate (CO_3^{2-}) (DWAF, 1996a).

At all selected sites, TDS were high during the high flow season, which could potentially be attributed to evaporation since sampling was conducted prior to heavy rainfall. Site 2 had the highest recorded values of TDS during the high flow season, which was followed by sites 4, 3, and 1, respectively (Figure 10). During the low flow season, TDS values were increasing from site 1(124.8mg/L) to site 2 (618mg/L), and got reduced at site 3 (346mg/L) and increased again at site 4 with concentrations of 505mg/L. In the study of Omole *et al.* (2016), TDS concentrations ranged from 305 and 404mg/L, particularly at locations close to site 3 and site 4 of the present study; however, the results were slightly different from the one obtained in this study. Although the consequences of elevated TDS levels on freshwater species are poorly understood, juvenile stages are frequently more vulnerable than adult stages.

Therefore, where organisms are acclimated to clean waters, elevated TDS levels might have unfavorable effects (Davies & Day, 1998). According to Dallas and Day (2004), an excessively high or low TDS concentration might impede the growth of numerous aquatic creatures and ultimately cause their demise. Although there are no TDS TWQR standards for aquatic ecosystems, concentrations should never deviate more than 15% from the water body's typical cycles in unaffected conditions year-round (DWAF 1996a).

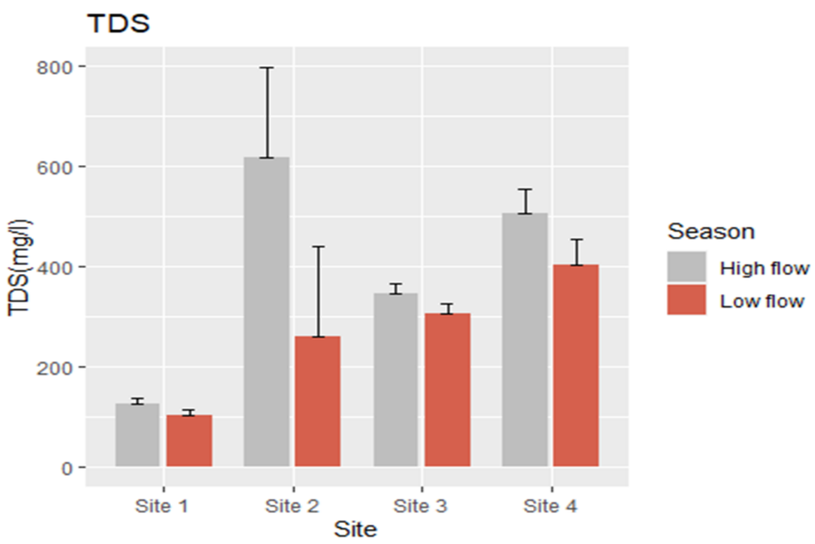


Figure 10: Total Dissolved Solids recorded at the Apies River during the high flow and low flow seasons (February/March and June/July 2023)

Dissolved oxygen

Dissolved oxygen (DO) is defined as the available oxygen dissolved in water at a given temperature, salinity, and atmospheric pressure. According to Dallas and Day (2004), it is one of the crucial abiotic factors for the survival of most aquatic organisms. Dissolved oxygen is expressed in milligrams per litre (mg/L), sometimes as a percentage of the saturation concentration during sampling time (DWAF, 1996a). Photosynthesis is carried out by plants, and phytoplankton is the major source of oxygen in surface water (DWAF, 1996a). Aquatic organisms use dissolved oxygen during respiration; therefore, the decline of DO results in migration or morbidity of sensitive species (Dallas & Day, 2004). Consequently, the DO concentration is a helpful indicator of the well-being of an aquatic ecosystem. The majority of the aquatic biota in southern Africa that is native to or adapted to aerobic warm water habitats can be protected at all life stages with a concentration of 80% to 120% saturation (DWAF 1996a).

Concentrations ranging between 5.52 and to 6.6mg/L were recorded between the four sampling sites during the high-flow season and 4.9mg/l to 6.4 mg/L during the low flow season (figure 11). The concentration of DO at site 2, 3 and 4 were almost the same during both seasons. Minimal differences were recorded at all sites with the exception of site 1. The mean values were 6.2 to 6.35 mg/L, 5.3 to 5.5 mg/L at site 3, and 6.1 to 6.2 mg/L at site 4 for both high and low seasons, which exceeded the TWQR for DO of >5.00 mg/L. The lowest mean of 4.8 mg/L was recorded at site 1 during winter and 6.6 mg/L during the high flow season, which may be due to the amount of suspended material in the water, which has an impact on the saturation concentration of dissolved oxygen in two ways: physically by reducing the volume of water available for solution or chemically by scavenging oxygen through the characteristics of the suspended particles (DWAF, 1996a; Palmer *et al.,* 2004). In the study of Omole *et al.* (2016) conducted on the Apies River, DO ranged between 6.81 and 8.0 mg/L; the results obtained were comparable to the results of the present study.

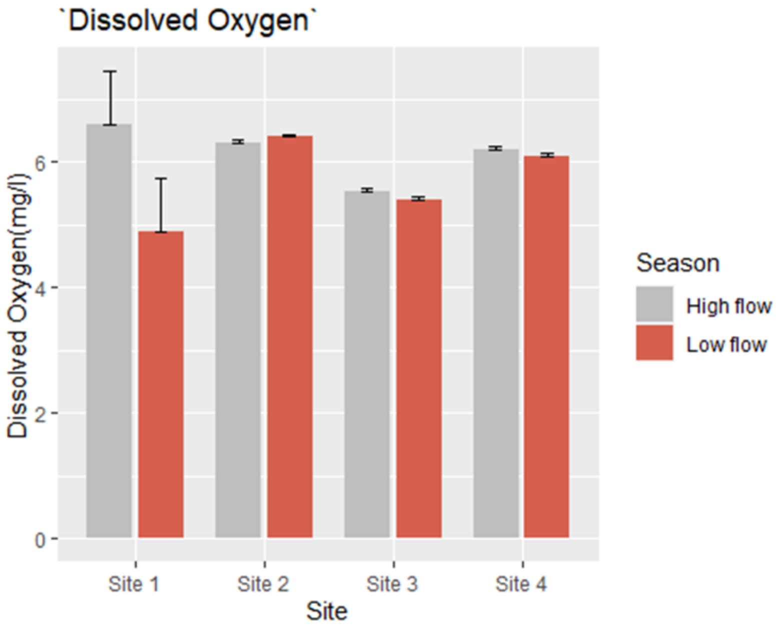


Figure 11: Dissolved oxygen recorded at the Apies River during the high-flow and low-flow seasons (February/March and June/July 2023).

Salinity

Salinity and total dissolved solids are similar in the sense that they both measure the mass of solutes in the water; they only differ in the components they measure (DWAF, 1996b). According to DWAF (1996b), salinity measures the dissolved inorganic content, while in contrast, TDS measures both organic and inorganic compounds in water. Salinity occurs naturally in aquatic ecosystems, and it is influenced by geological characteristics of the area (Chapman, 1996).

During this study, salinity varied for different sites and seasons; however, constant measurements of 0.2% were recorded for site 3 and site 4 during both seasons. High salinity concentrations of 0.3% and 0.35% were recorded at site 2 during the high-flow and low-flow seasons, respectively. Site 1 had the least recorded salinity concentrations during both seasons. Salinity concentrations reported in the water column in this study were comparable to those reported in other studies conducted at the Apies River (Ngobeni, 2018). There is limited literature on salinity tolerances of freshwater organisms; in addition, there are no TWQR sets for salinity on freshwater organisms. Furthermore, Dallas and Day (2004) have reported that certain amounts of salt in water can either protect or sensitize aquatic ecosystems from different types of pollutants, including biocides and heavy metals.

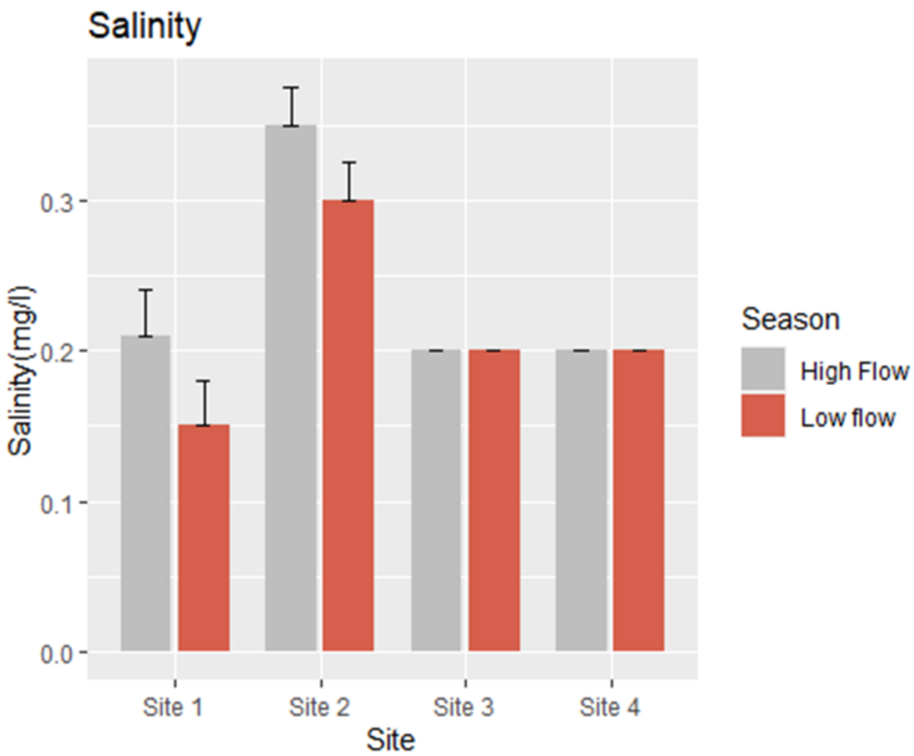


Figure 12: Salinity recorded at the Apies River during the high-flow and low flow seasons (February/March and June/July 2023).

4.2.2 Chemical parameters

Ammonia

The occurrence of ammonia usually results from activities such as atmospheric gas exchange, breaking down of organic and inorganic matter, microbes reducing nitrogen gas, and excretion by biota (Rajele, 2004). High levels of ammonia indicate organic pollution (Palmer *et al*., 2004; Rajele, 2004). Ammonia occurs in two forms, un-ionized form (NH_3) or ionized form (NH_4^+); in addition, the occurrence of these forms is dependent on the DO, pH, and temperature of the water. The concentrations of ammonia ranged between 0.03 and 0.28 mg/L during the high-flow season and 0.08 and 0.3 mg/L during the low-flow season at all four sites (Figure 13). Site 1 recorded between 0.21 and 0.28 mg/L during the low-flow and high-flow seasons, respectively, which were the highest concentrations of ammonia recorded in this study. Site 2 recorded 0.26 and 0.27 mg/L. Site 3 recorded the lowest concentrations of ammonia during the study, at 0.08 and 0.03mg/L, and site 4 recorded 0.2 to 0.3 mg/L. In the studies of Dube (2020) and Makuya (2022), the ammonia concentrations increased from upstream to downstream of WWTWs along the Mooi River and Juskei River, respectively; however, the concentrations escalated to up to 15 mg/L downstream after the sewage outfall. Moreover, in the present study, ammonia concentrations near the WWTWs were lower than at the reference site (Site 1), which is the site that is not affected by WWTW effluents. Dube (2020) reported higher ammonia and phosphates at Site 4 of the present study during winter, which could be linked to sewage contamination and agricultural activities around the Mooi River. The excessive ammonia content may have resulted from non-point sources of ammonia or from the WWTW's inability to process ammonia effectively.

Nitrite

Nitrite (NO_2) is the inorganic intermediate product of the oxidation of organic nitrogen and ammonia (DWAF, 1996a). Nitrite is toxic to aquatic biota even at low concentrations (Davies & Day, 1998). Nitrite occurs simultaneously with nitrate and ammonia; however, they usually occur at extremely low concentrations, owing to the fact that they are readily oxidized to nitrate or reduced to ammonia by bacteria (DWAF, 1996b). According to DWAF (1996b), the conversion of nitrogen to different forms both in water and soils involves two processes, which are nitrification and denitrification, which are regulated by two groups of highly aerobic, autotrophic bacteria (*Nitrobacter spp*. and *Nitrosonomas spp.*) that oxidize ammonia to nitrate and nitrite.

Nitrate

In aquatic ecosystems the most occurring nutrient is Nitrate (NO_3) contrasted to nitrite (NO_2) (Davies and Day, 1998). Sources of nitrate include animal and plant debris, weathering of igneous rocks, and land drainage (Rajele, 2004). Excess concentrations of these nutrients indicate pollution caused by animals and humans as well as runoff of fertilizers; furthermore, nitrate concentrations of 5 mg/L (5000 µg/L) indicate pollution resulting from the abovementioned sources (Rajele, 2004). In unpolluted waters, the concentration of nitrate rarely exceeds 0.1mg/L; however, it may reach approximately 0.2mg/L in lakes having algal growth. Nitrate is the end product of aerobic decomposition of organic nitrogen compounds (DWAF, 1996b). Nitrate is rarely plentiful in natural water, as it is incorporated into cells or microorganisms and converted to atmospheric nitrogen (Davies & Day, 1998).

Nitrate was the most abundant nutrient at all selected sites during both seasons (Figure 13). Nitrate concentrations started from 0.3 to 1.1 mg/L at site 1 and 8.5 to 10 mg/L at site 2; this could be attributed to urbanization, industrial activities, and poor-quality effluent discharged at this point. Concentrations ranging between 7.5 and 9mg/L were recorded at site 3, and those ranging from 8.2 to 10 mg/L at site 4, which could be resulting from agricultural runoff and animal and human wastes (Dube, 2020; Makuya, 2022). Tau *et al.* (2021) reported nitrate concentrations ranging between 0.75 and 0.98 mg/L on the Apies River between site 3 and site 4 of the present study, while Ngobeni (2020) recorded nitrate concentrations ranging between 0.2 and 11.8mg/L at different sites of the Apies River, including the Bon Accord Dam and Temba Dam. High concentrations of nitrate were recorded during the low-flow season as compared to the high-flow season. Site 2 and Site 4 recorded the highest nitrate concentrations during the low- flow season; this could be linked to poor quality of effluent from the two WWTWs that are located near these sites.

Phosphorus

Phosphorus is a fundamental part of DNA and is required for various life processes, as it is a crucial macronutrient for a variety of living organisms (Davies & Day, 1998). The sources of phosphorus include agricultural runoff, exposed soil erosion, human and animal wastes and industrial waste; however, naturally in surface water, phosphate gains access through weathering of rocks, leaching of phosphate rocks, and decomposition of organic matter (DWAF, 1996a&b; Rajele, 2004; Aklilu, 2011). Phosphorus concentrations above 0.03 mg/L stimulate algal and aquatic macrophyte growth, resulting in eutrophication and a major shift of aquatic biota to a decreased number of pollutant-tolerant species (Aklilu, 2011). Phosphorus occurs in organic and inorganic forms in the environment; it exists as orthophosphates, polyphosphates, metaphosphates, and pyrophosphates, as well as organically bound phosphates in natural waters (Rajele, 2004).

From this study, a high concentration of phosphorus was recorded at site 1 during the low-flow season, which could be attributed to weathering of rocks and urban runoff; however, it was recorded in lower concentrations at sites 2, 3, and 4 during both seasons (Figure 13). Site 3 recorded mean concentrations ranging from 0.1 to 0.026 mg/L during the low-flow and high-flow seasons, and site 4 recorded 0.05 during both seasons, representing mesotrophic conditions with typically high levels of species variety, productive systems, nuisance growth of aquatic plants, and blooms of blue-green algae; algal blooms are seldom toxic (DWAF, 1996a). A mean concentration of 0.31 mg/L was recorded at site 2 during the high flow season, explaining hypertrophic conditions, which are defined by usually very low levels of species diversity, very highly productive systems, nuisance growth of aquatic plants, and algal blooms often including species that are toxic to man, livestock, and wildlife (DWAF, 1996a). Site 3 improved from hypertrophic conditions during the high-flow season to mesotrophic conditions during the low-flow season. Ngobeni (2020) reported average concentrations of orthophosphates ranging from 0.04 to 4.67 mg/L along the Apies River during various seasons; furthermore, Tau *et al. (*2021) reported an average orthophosphate concentration of 0.055 mg/L at site 3 and 1.895 mg/L at site 4 on the Apies River, resulting in higher concentrations downstream of the Rooiwal WWTW compared to other sampling points upstream.

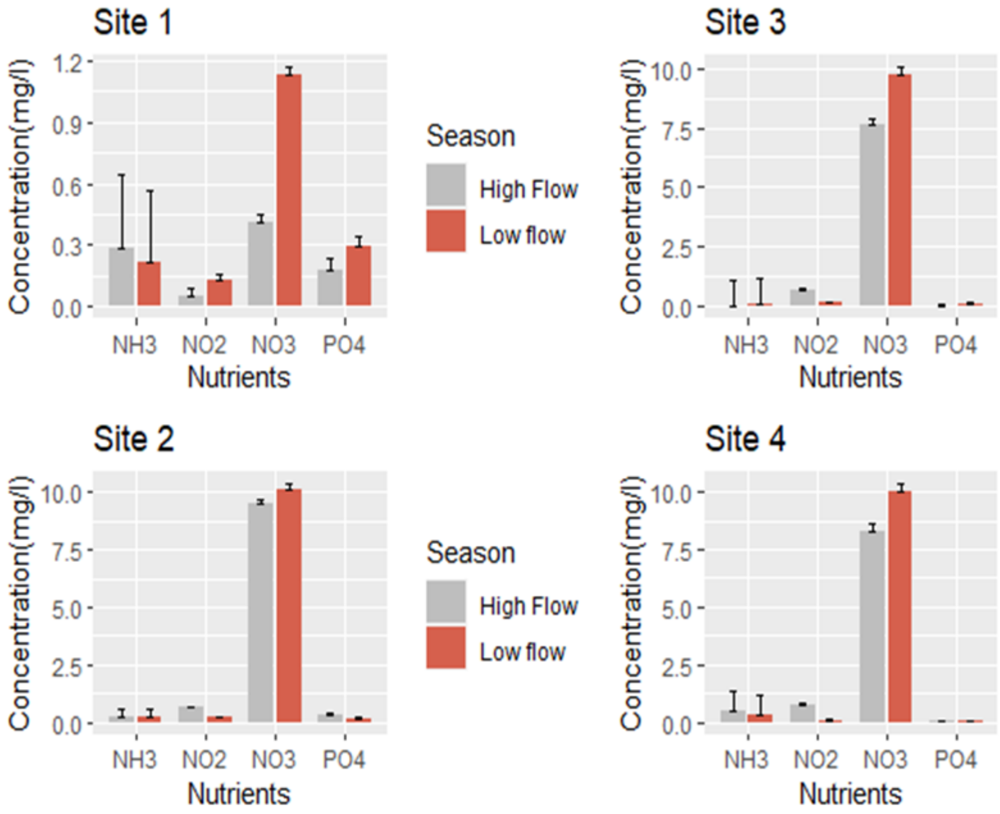


Figure 13: Nutrient concentrations recorded at all selected sites of the Apies River during the high flow and low flow seasons (February/March and June/July 2023)

Similar investigations on the influence of WWTW on water quality were conducted in other parts of South Africa, and the results showed that the guidelines and limitations for water and wastewater had been surpassed for the majority of the criteria, which is consistent with the findings of this study. A related study conducted by Naidoo (2013a) on the evaluation of the effect of wastewater discharges on river water quality in eThekwini (Durban) reported the following:

- The obtained results showed a difference in pH levels of river water at the upstream and downstream points, whereby the wastewater discharge located at the downstream point was linked to the increased levels. The same was reported for electrical conductivity, TDS, and pH, where higher concentrations were found downstream of the two WWTWs as compared to the upstream site.

The following results were reported in a study by Gitari *et al.* (2017) on the physico-chemical evaluation of an effluent-receiving stream (Mvudi River):

- The river's pH at the downstream location ranged from 7 to 7.95 after discharge, which were similar results obtained in this study at downstream points.

- The EC of the river water was reported to be between 39.4 and 316 mS/m, this was also reported on the Apies River, where the EC varied from 159 to 305.6mS/m, exceeding the established limits.
- The upstream point's nitrate profile ranged from 0.63 to 16.27 mg/L, whereas the downstream points ranged from 0.8 to 23.4 mg/L. A similar trend was observed in this study: low concentrations were obtained at the upstream point; however, increased levels were obtained downstream, which may be linked to the WWTWs and various anthropogenic activities occurring downstream.
- The orthophosphate levels at the downstream point were ranging from 1.37 to 13.63 mg/L, with the upstream point ranging from 0.37 to 3.2 mg/L; a similar trend was observed in this study during the high-flow season, although the opposite was recorded during the low- flow season, where concentrations upstream were higher than downstream.

Morrison *et al.* (2001) examined pH, EC, carbon-demanding substance (COD), and nutrients in a study that evaluated the effects of point source pollution from the Keiskammahoek Sewage Treatment Plant on the Keiskamma River. The following results were reported in the study:

- The pH readings were reported to be within the South African water quality recommendations; in addition, the similar trend of pH readings was discovered in this study.
- Although nitrate concentrations fluctuate throughout samples, 87% of the sampling period was compliant with the standards.
- Most of the time, orthophosphate levels were higher downstream of the dam (the discharge point), whereas in this study there were slight differences in the levels of orthophosphate downstream and upstream.

4.2.3 Major ions

Calcium (Ca)

Calcium is abundant and dissolves out of most rocks and it is present in aquatic environments.in waters affiliated with granite or siliceous sand, low calcium concentration may be observed (Lebepe, 2018). High calcium concentrations may be observed in water affiliated with limestone and gypsum. In addition, elevation of calcium concentrations in surface water can be observed resulting from industrial activities and wastewater treatment processes; however, the primary source of this element is the geological characteristics of the area.

There is limited knowledge about the effects of calcium in aquatic ecosystems; however, according to Dallas and Day (2004), decreased concentrations of calcium fail to support the lives of certain organisms, including crustaceans and mollusks. Calcium (Ca^{2+}) is an alkaline earth metal and exists as a double positive-charged ion, Ca (II) (DWAF, 1996). It is one of the crucial elements in living organisms and is found as a structural component in bones, teeth, mollusk shells, and crustacean exoskeletons. has The occurrence of calcium concurrently with magnesium contributes to the total hardness of water. For aquatic environments, neither DWAF (1996a) nor CCME (2012) has established any guidelines; nonetheless, Jooste *et al.* (2005) emphasized that concentrations up to 250 mg/L are suitable for all users.

Potassium (K)

Potassium is an alkali metal that forms positively charged potassium ions after a violent reaction with water (DWAF, 1996c). Potassium occurs in abundance in natural freshwater and is approximately 10mg/L or less (Chapman, 1996). However, Bartman and Balance (1996) reported that concentrations of potassium in unpolluted freshwater normally do not exceed 20mg/L. Potassium is often recorded in low concentrations in freshwater; this may be attributed to the fact that rocks containing this element are normally resistant to weathering (Chapman, 1996). The highest concentrations of potassium were recorded at site 3 during the high-flow season, followed by site 4,2 and 1 respectively.

The highest concentrations during the low-flow season were recorded at site 4, followed by site 3 and site 2. During both seasons, site 1 recorded the lowest concentrations; however, the high- flow season recorded the highest concentrations. For aquatic ecosystems, there are no set TWQR for potassium. Dissolved potassium and sodium in freshwater at all sites may have contributed to the TDS recorded at all sites.

Sodium (Na)

Sodium is another plentiful element on earth and exists in natural waters as sodium salts are highly water soluble. Moreover, concentrations of sodium in surface water depend on the geological properties of the area (Chapman, 1996). The anthropogenic sources of sodium include industrial and sewage effluents (Chapman, 1996). Sodium together with potassium are the most essential intracellular cations important to all living organisms (DWAF, 1996). Usually, potassium is measured in water for drinking and agricultural purposes; it is the least toxic metal cation with the least effects on aquatic ecosystems. The major effect of sodium in aquatic ecosystems is its contribution to TDS; however, there is no set TWQR for sodium for aquatic ecosystems and aquaculture (Dallas & Day, 2004). The highest concentrations of sodium were recorded at site 4 during both seasons, which could be linked to various effluents and agricultural activities around the area. Even though concentrations of sodium were high, they were within TWQR for domestic use. The current study could not corroborate the theory that surface waters always have a larger proportion of sodium than potassium. Sodium was higher than potassium at all sites during the low-flow season; however, different results were obtained during the high-flow season (Figure 14), potassium was slightly higher than sodium.

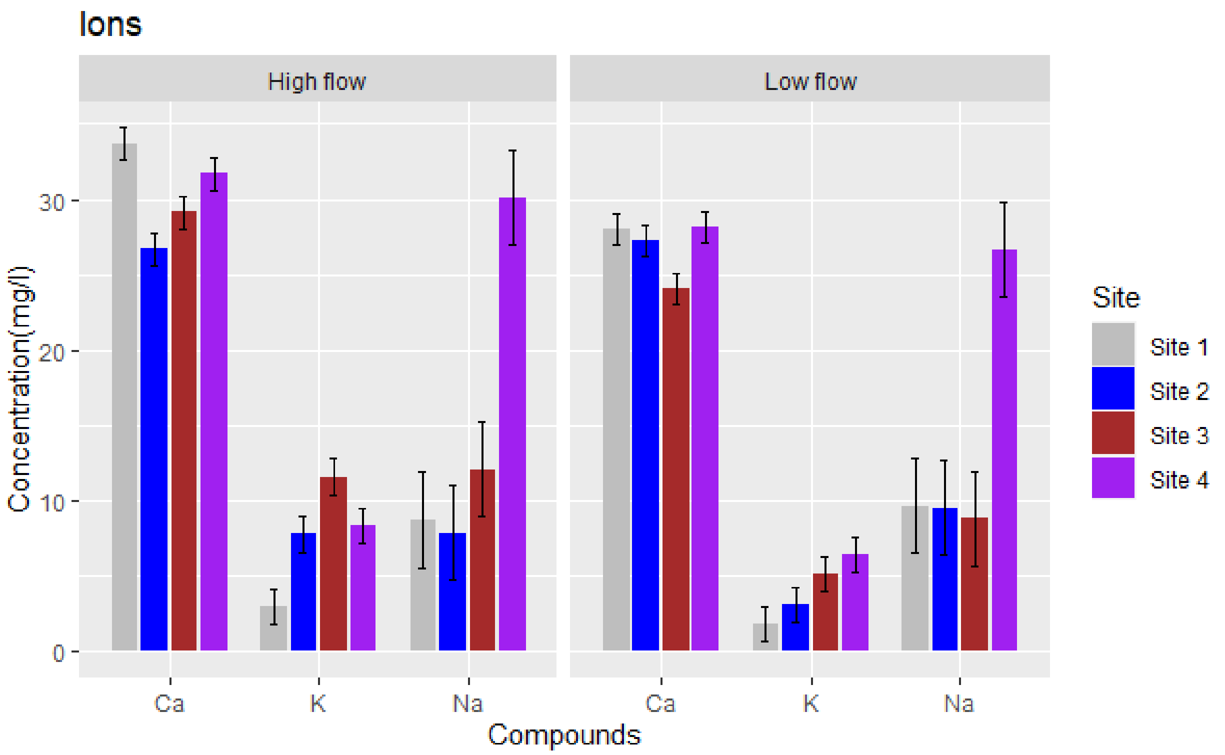


Figure 14: Mean values of the major ions recorded on the Apies River

The correlation analysis on water quality parameters showed that some parameters are more or less correlated with each other. It can therefore be concluded that some of the parameters do not have significant correlation between them, indicating the different origin source of pollution (Edokpayi *et al.,* 2016). The results indicate that there are significant correlations between some of the physico-chemical variables measured in the water samples from all sites during the two seasons. Electrical conductivity and pH have a strong negative correlation ($r = -0.8$, $p < 0.05$), meaning that as the electrical conductivity increases, the pH decreases. This could imply that the water is becoming more acidic due to the presence of ions that conduct electricity. Electrical conductivity and dissolved oxygen have a moderate positive correlation ($r = 0.65$, $p < 0.05$), meaning that as the electrical conductivity increases, the dissolved oxygen also increases.

Nitrite and temperature have a very strong negative correlation ($r = -0.9$, $p < 0.05$), meaning that as the temperature increases, the nitrite decreases Nitrite and total dissolved solids (TDS) have a moderate negative correlation ($r = -0.6$, $p < 0.05$), meaning that as the nitrite decreases, the total dissolved solids increase. This could imply that the water is becoming more saline due to the evaporation of water or the addition of salts from external sources. Salinity and pH have a weak positive correlation ($r = 0.3$, $P < 0.05$), meaning that as the salinity increases, the pH also increases slightly. Potassium and nitrite have no correlation ($r = 0$, $p < 0.05$), meaning that there is no relationship between these two variables in the water samples

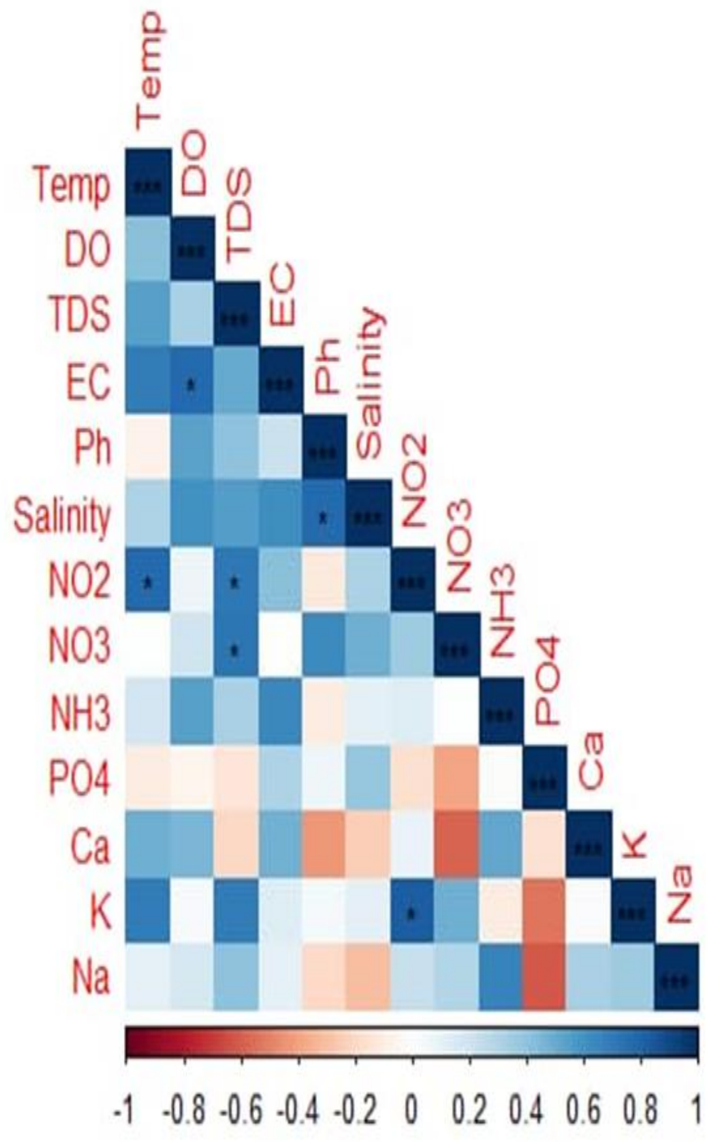


Figure 15: Pearson's correlation coefficients matrix among different physico-chemical parameters of the Apies River during both seasons (*p=0.05; ***p=0.001)

The PCA-Biplot (figure 16) shows clear seasonal separation of water quality parameters in the Apies River. TDS, EC, NO_2, and salinity have strong negative loadings; these are more associated with high-flow samples, suggesting dilution or runoff effects. Phosphate and pH are slightly positive on PC2. TDS, EC, and temperature are negative on PC2. TDS, EC, and salinity are strongly correlated, often reflecting mineral content or pollution (Singh *et al.,* 2004; Shrestha & Kazama, 2007).

NO_3, NO_2, and K are clustered, possibly tied to nutrient pollution from agriculture or sewage. During low-flow season, variables like PO_4, Ca, NH_3, and Na are more prominent, possibly due to concentration effects or anthropogenic sources (Camargo & Alonso, 2006). During high flow, TDS, EC, and salinity are more pronounced, possibly reflecting runoff, stormwater, or erosion. High-flow season is linked with dilution, higher TDS, EC, and salinity. Low-flow season is characterized by higher nutrient concentrations, especially phosphate, which may indicate pollution accumulation during dry periods (Jarvie *et al.,* 2006).

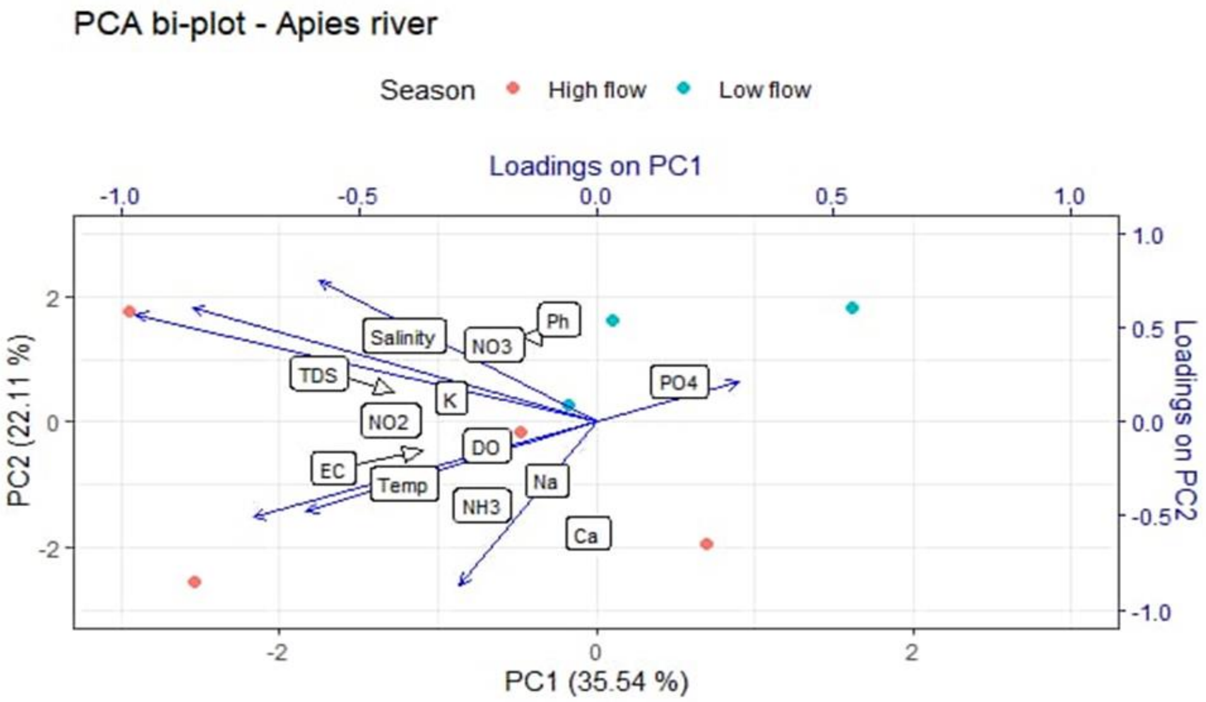


Figure 16: the PCA-biplot illustrating the possible associations between physical and chemical variables recorded on the Apies River during the two seasons

The South African Water Quality Guidelines

All sampled parameters met the requirements, except for conductivity, which was found to have exceeded the South African Water Quality Standards (SAWQS), Target Water Quality Range (TWQR) of 0-70 for domestic use (DWAF, 1996a); nitrate, which exceeded the SAWQS, TWQR of 0-6 for domestic use (DWAF, 1996a) and TDS, which exceeded the SAWQS (TWQR) of 0-450 for domestic use, particularly at site 2 and site 4 during the high flow season (DWAF, 1996a) (Table 6).

Table 6: Compliance of Apies River sampled parameters compared to the South African Water Quality Standards (DWAF, 1996a; 1996b; 1996c).

Parameters	**Range values of parameters**	**Compliance with the South African Water Quality Standards**
Nitrate	S1= 1.13 S2= 9.5	The concentration of Nitrate in all the sampled sites did not comply with the

	S3= 8.2 S4= 10.04	SAWQS (TWQR) of 0-6 for domestic use (DWAF, 1996a). Compliance was recorded only at site 1.
Nitrite	S1= 0.12 S2= 0.65 S3= 0.7 S4= 0.71	The concentration of Nitrite in all the sampled sites complied with the SAWQS (TWQR) of 0-6 for domestic use (DWAF, 1996a).
Ammonia	S1= 0.28 S2= 0.27 S3= 0.08 S4= 0.5	All of the measured sites' ammonia concentrations complied with the SAWQS (TWQR) of 0-1.0 for domestic use (DWAF, 1996a).
Phosphorus	S1= 0.25 S2= 0.28 S3=0.16 S4= 0.05	The values recorded for Phosphorus in all the sampled sites complied with the SAWQS (TWQR) of 0-70 for aquatic ecosystems (DWAF, 1996b).
Conductivity	S1= 184-681 S2= 432-849 S3= 159-252 S4= 305-700	The values for conductivity in all the sampled sites did not comply with the SAWQS (TWQR) of 0-70 for domestic use (DWAF, 1996a), during both seasons
pH	S1= 7.6 S2= 8.2 S3= 7.8 S4= 8.07	The pH levels at each location met the SAWQS (TWQR) range 6.0–9.0 for residential use and 6.5–8.5 for outdoor use (DWAF, 1996a; DWAF, 1996c).
TDS	S1= 124.8 S2= 600 S3= 346 S4= 490	TDS values in all the sampled sites were complying with the SAWQS (TWQR) of 0-450 for domestic use (DWAF, 1996a) except for site 2 and site 4, during the high flow season.

4.3 Conclusions

The Apies River system is known as one of the most polluted rivers in South Africa due to the discharge of inadequately treated and untreated effluents from the nearest WWTW (Tau *et al.,* 2021). In the present study, all nutrient concentrations were higher at sites 2 and 4, which are located downstream of the two WWTWs, and Tau *et al.* (2021) reported hypertrophic conditions on the Apies River downstream of the Rooiwal WWTW. However, the present study noted a mesotrophic state at site 2 and oligotrophic at sites 3 and 4. Sources of pollution along the Apies include WWTW and the discharge of treated, partially treated, and untreated domestic and industrial sewage from municipal WWTW. However, during the present study, these anthropogenic activities elevated the nutrient levels.

Water quality studies are complex as the solubility of certain constituents depends on one another. The present study demonstrated that the water quality at the selected locations was not always in acceptable condition, particularly at site 2, site 3 and site 4, as some constituents exceeded the TWQR for aquatic ecosystems, thus accepting the hypothesis that stated that the water quality of the Apies River is extremely poor to support aquatic ecosystems.

Chapter 5: Metal Assessment

5.1 Introduction

Heavy metals are known for their toxicity towards living organisms (Al-Musharafi *et al.,* 2013). Studies conducted by Jackson *et al*. (2007), Lebepe (2012), Al-Musharafi *et al.* (2013), Griffiths *et al.(*2015), Olujimi *et al.(*2015), Lebepe (2018), and Singh et al. (2022) have reported various types of heavy metals from terrestrial and aquatic environments. Municipal and industrial refuse are some of the known sources of metal pollution in aquatic environments (Al-Musharafi *et al.,* 2013; Tau *et al.,* 2020). Trace metals such as copper (Cu), cadmium (Cd), lead (Pb), and zinc (Zn) exhibit extreme toxicity levels and are the most environmentally concerning elements that have been reported as toxic contaminants of terrestrial and aquatic ecosystems as well as within the food chain (Al-Musharafi *et al.,* 2013).

The continuous discharge of heavy metals in freshwater is an environmental challenge, as they habitually accumulate throughout the ecosystem, surface water, sediments, soils and ultimately reach the food chains (Ali *et al.,* 2019). Madyiwa *et al.* (2002) reported that the continuous discharge of treated effluents in surface water can lead to heavy metal accumulation in sediment and nearby terrestrial grass ecosystems, which can be extremely threatening to the food chain. Organisms at a higher food chain level are most likely to consume toxic heavy metal compounds in tissue from their food (Al-Musharafi *et al.,* 2013).

It is crucial for water to contain various trace metals to support life within aquatic environments (Chapman, 1998). Metals such as copper (Cu), manganese (Mn), and zinc (Zn) are essential to carry out physiological functions as well as the regulation of distinctive biochemical processes in living organisms in aquatic resources (Chapman, 1998). The introduction of these metals from various anthropogenic sources, such as mining effluents or sewage discharges in excessive concentrations, may result in destructive repercussions on both humans and the aquatic environment (Hoffman *et al.,* 2002; Griffiths *et al.,* 2015; Singh *et al.,* 2022).

Metal pollution as a result of anthropogenic activities is a serious environmental health concern and has significant ecological effects on aquatic environments worldwide (Chapman, 1998; Griffiths *et al.,* 2015; Sankla *et al.,* 2021). For the purpose of this study, attention was given to the metals that are regarded as potentially toxic to aquatic life, such as aluminum (Al), arsenic (As), iron (Fe), lead (Pb), manganese (Mn), nickel (Ni), and zinc (Zn). The aim of the chapter was to (i) determine metal concentrations in the Apies River during two seasons. (ii) To determine metal concentrations in river water sediments from upstream and downstream and to compare the reported metal concentrations with freshwater management guidelines. The hypothesis was that metal concentrations are high both upstream and downstream due to anthropogenic activities.

5.2 Methods and materials

5.2.1 Water analysis

Metal concentrations were analyzed on all samples collected from four sites along the Apies River. After the water samples were transported to the laboratory, 9.85 ml of each sample was drawn up using a 60 ml syringe and filtered through a 0.22 µm Millipore® filter paper. The filtered samples were transferred to ICP-MS test tubes. The samples were labelled with the relevant information and acidified using 0.15mL of 65% nitric acid. After this, the samples were sent for ICP-MS analysis to determine the metal concentrations. All of the metal concentrations obtained from the water samples throughout the study were measured in µg/L.

5.2.2 Sediment analyses

Sediment samples were collected at a depth of 0–10 cm using a Friedlinger mudgrab at all four sampling sites (3 grab composites per site). At each site, three sub-samples were mixed together in order to form a composite sample (Bervoets and Blust 2003). The collected samples were then placed in 10% nitric acid pre-treated polyethylene Ziploc bags, transported to the laboratory, and frozen (−20°C) prior to chemical analysis (UNEP, 2005). The samples were put in acid-washed polypropylene, pre-weighed vials and dried at 60°C for 24 hours. The samples were then sieved through a 2-mm nylon sieve to remove any stones and coarse debris. Then, 0.1 g of each sediment sample was digested with 8 mL of 68% nitric acid (HNO_3) and 3 mL of 40% hydrochloric acid (HCl). It was then filtered through a membrane filter, and the concentrations of Al, As, Fe, Mn, Ni, Pb, and Zn were analyzed using inductively coupled plasma–optical emission spectrometry (ICP-OES) (Perkin Elmer, Optima 2100 DV). Concentrations of the metals in the sediments were calculated and expressed as mg/kg dry weight. Analytical accuracy was determined using certified standards (De Bruyn Spectroscopic Solutions 500 MUL20-50STD2) and recoveries were within 10% of certified values. Sediment samples were kept in acid-treated sampling bottles and frozen (-20°). Frozen samples were later sent to a SANAS accredited laboratory (ISO/IEC 17025:2005) in Germiston for metal analysis.

5.3 Data analyses

One-way analysis of variance (ANOVA) was used to test the significant variations of metals in water and sediment between sites and surveys. Normality and homogeneity of the data were tested using Levene's test (Hoffman, 2019).

Statistical significance of the spatial and temporal variation of the selected physical and chemical variables was determined at $p < 0.05$. Due to the fact that the obtained data was non-parametric data, Kruskal-Wallis tests with Dunn's Multiple Comparison Tests were performed to test for significant differences between sites and surveys (Kebede & Kebedee, 2010).

5.3.1 Games Howell Test

The Games-Howell method is an improved version of the Tukey-Kramer method and is applicable in cases where the equivalence of variance assumption is violated. It is a t-test using Welch's degree of freedom. The Games-Howell (GH) method gives the best performance for comparing data in pairs (Shingala, 2015; Sauder, 2017). The procedure recommends sample sizes that are greater than five (Shingala, 2015; Sauder, 2017).

5.4 Results and discussions

5.4.1 Aluminium (Al)

Aluminum (Al) is one of the most abundant elements in the Earth's crust; however, it exists in trace concentrations in natural waters (DWAF, 1996a). Aluminum is found in nearly all surface water due to its primary source, which is the geological characteristics of an area (Bartman & Balance, 1996). Aluminum ($p < 0.001$) this indicates that there is a significant difference in aluminium concentrations between high flow and low flow in water and sediments. In this study, sediments recorded higher concentrations of aluminium than water; Site 2 and site 4 recorded the highest concentrations of 930 mg/kg and 891 mg/kg, respectively. These are sites located downstream from the two WWTWs; therefore, these concentrations could be linked to direct pollution from the WWTWs. Site 1 and site 3 recorded less concentrations in sediment samples.

The recorded concentrations for aluminium in water were lower than the recommended concentrations for Al of 0.1 mg/L to 0.15 mg/L (DWAF, 1996b) and 0.005 mg/L to 0.1

mg/L (CCME, 2001) throughout the low flow season; however, site 2 and site 3 exceeded the limits set by DWAF (1996a) and CCME (2001) during the high flow season. Furthermore, there were no criteria for aluminum available for the world average. In comparison to this study, Jackson *et al*. (2009) reported aluminum concentrations that were above the recommended concentrations of 0.1 mg/L to 0.15 mg/L (DWAF, 1996b) and 0.005 mg/L to 0.1 mg/L (CCME, 2001). From this present study, aluminum concentrations recorded in sediment were the highest of all metals recorded from the four selected sites. Jackson *et al.* (2009) reported higher concentrations of aluminum in sediment as well as more than all other selected metals. Al, Fe, Mn, Pb, Ni, and Zn had no suggested sediment quality criteria available from DWAF (1996b) or the world average (Martin and Windom, 1991); the CCME (2001) only provided guidelines for Cu and Zn.

5.4.2 Arsenic (As)

Arsenic is a carcinogenic metalloid toxic to freshwater and marine life (DWAF, 1996). It gets introduced to aquatic ecosystems via anthropogenic activities and natural processes such as rock weathering and volcanic emissions (Lebepe, 2018). Extreme utilization of pesticides containing arsenic and improper disposal of domestic sewage, industrial and mining waste have led to ubiquitous and continuous contamination of soils and aquatic environments (Mahimairaja *et al.,* 2005; Singh *et al.,* 2021)

The toxicity of arsenic is influenced by constituents such as pH and redox potential (DWAF, 1996). Arsenic (III) and (V) are the most common forms of the element, which are capable of forming stable compounds with carbon, leading to various organo-arsenical compounds (DWAF, 1996). Arsenic can absorb and accumulate well in sediment and homogenize with dissolved organic carbon (Lebepe, 2018). In aquatic ecosystems, high concentrations of arsenic are derived from the sediment (Mahimairaja *et al.,* 2005).

Arsenic concentrations of 0.91 to 2.204 mg/kg were recorded in sediments during the high flow season and 1.78 to 3.4 mg/kg during the low flow season; high arsenic levels at these sites of this river may be due to storm water and wastewater effluents. Lower concentrations in water of 0.1mg/L were recorded at three sites during the high flow season at sites 1 and 4; however, concentrations of 0.02 and 0.01 mg/L were recorded at sites 3 and 4 during the low flow season, respectively. Arsenic ($p < 0.01$): This result shows that there was a significant difference in arsenic concentrations between high flow and low flow. The seasonal trend of arsenic in water shows that the high flow samples had the least concentrations, while the winter had the highest concentrations for all sites.

In addition, the same trend was reported by Olujimi *et al.* (2015). Concentrations recorded at all selected sites were above the human consumption, livestock watering, irrigation, and aquaculture uses (DWAF, 1996).

5.4.3 Iron (Fe)

Iron is a crucial micronutrient in all living organisms, occurring as part of the respiratory pigments such as hemoglobin and catalases (Dallas & Day, 2004). In aquatic environments, lead occurs in ferrous state II or ferric state III. The occurrence of the two forms of iron is reliant on oxygen concentrations in water, pH, and other chemical properties of the water (Svobodova *et al.,* 1993). Iron exhibits high levels of toxicity in less oxygenated water with low pH and occurs in soluble form (Svobodova *et al.,* 1993).

Iron concentrations in water had an average of 0.45mg/L and 0.8mg/L during the high-flow and low-flow seasons. From this study, iron recorded the highest concentrations in water of all other metals. High iron concentrations in river water can turn the natural colour of water to brown, affecting the biogeochemical cycling of nitrogen, phosphates, and organic matter, which may ultimately become toxic and life-threatening to aquatic biota (Jackson *et al.,* 2009). Iron ($p > 0.05$) means that there is no significant difference in iron concentrations between high-flow and low-flow seasons.

There were no suggested iron-related sediment quality criteria available from DWAF (1996b) or the "world average" (Martin and Windom, 1991). Iron concentrations in water exceeded the TWQR for domestic purposes, irrigation, and aquaculture during the low flow season; however, they were within the livestock watering limit. The results of this study were comparable to those of Jackson *et al.,* (2009), where the quantities of aluminum and iron in the water samples taken from the Plankenburg River were more than those of every other metal examined, and they also went above the limits set by the CCME and the DWAF (Al and Fe).

5.4.4 Lead (Pb)

Lead is another common and toxic trace metal that is capable of accumulating in living tissues and other invertebrates (Dallas & Day, 2004). According to the United States Environmental Protection Agency (USEPA), lead is hazardous and threatening to most forms of life (DWAF, 1996). In addition, toxicity exhibited by lead to aquatic biota is substantially influenced by pH and water hardness (DWAF, 1996). In most instances, lead original from industrial and municipal wastewater discharge, fossil fuel combustion, and mining. When lead enters the water system, it largely accumulates at the bottom sediment at concentrations approximately four times greater than in the water (Svobodova *et al.,* 1994).

Pb recorded the least mean concentrations at all sites with the highest concentrations of 7.26 mg/kg at site 4 and 7.02 mg/kg in sediments during the low flow season. Overall average Pb concentrations of 0.3075 mg/L and 0.0262 mg/L were recorded in both seasons respectively. The concentrations of Pb at all sites during the high flow season exceeded the SANS:241 and WHO drinking water acceptable limits, which could be related to wastewater effluents containing high Pb concentrations, especially at sites 2 and 3, which are located downstream of the two WWTWs (Tchounwou *et al.,* 2012). According to Solomon (2009), Pb concentrations above 0.5 mg/L inhibit enzymatic functions in algae photosynthesis in freshwater, which ultimately result in a decrease in algae growth and disturb the entire algae photosynthesis. The Pb concentrations in water at all sampling sites were beneath 0.5 mg/L; therefore, the obtained results show that the river water is safe from Pb poisoning. Lead ($p < 0.001$) This result indicates that there is a significant difference in lead concentrations between high flow and low flow in aquatic sediments. 0.01mg/L is the acceptable threshold level of lead for South African rivers (DWAF, 1996). The results obtained from this study reveal that the average value of lead for all the sampling sites of the river system was above the TWQR threshold level for human consumption, aquaculture and irrigation purposes. Nonetheless, the reported values for livestock watering fell within the TWQR. Moreover, TWQR standards of 0.0002 mg/L were exceeded, the water was inappropriate for the protection of aquatic ecosystems.

5.4.5 Nickel (Ni)

Nickel is omnipresent and is a nutritionally essential trace metal for several animal species, microbes, and plants (Lebepe, 2018). However, excessive concentrations either from anthropogenic releases or natural processes may be toxic to living organisms (Vandenbrouck et al., 2008; Lebepe, 2008). Anthropogenic activities that may contribute to nickel loadings in the environment include mining, refining, and waste incineration (Vandenbrouck *et al.,* 2008).

Nickel is often recorded at extremely low concentrations in the environment; however, it is toxic when the maximum tolerable level is exceeded (Addo-Bediako *et al.,* 2021). Nickel contamination in surface water is a great concern due to the fact that it can cause severe health complications such as cardiovascular diseases, kidney diseases, headaches, and dry cough (Addo-Bediako *et al.,* 2021). The highest means of 98.1 mg/kg and 120 mg/kg in sediment were recorded at sites 3 and 4, respectively, during the low-flow season. Sources of Ni include pesticides, chemical fertilizers, combustion of fossil fuels, and urban and industrial effluents, which might be sources of Ni at sites 3 and 4. Olujimi *et al.* (2015) and Addo-Bediako *et al.* (2021) reported that Ni enrichment in water and sediment could be attributed to WWTP effluents, which may be the case with Ni concentrations at Site 4, which is downstream from the Rooiwal WWTP and the coal-fired power station. Nickel ($p < 0.01$) this reveals that there is a significant difference in nickel concentrations between high flow and low flow in aquatic sediments.

There were no water quality guidelines set by the South African Department of Water Affairs and Forestry for human consumption, protection of aquatic ecosystems, or aquacultural uses; however, the reported concentrations in this study were within the TWQR of 0.2mg/L and 1.0mg/L for irrigation and livestock watering. According to this study, the WWTWs are some of the main sources of nickel in the freshwater system since the concentration of nickel was higher downstream than it was upstream of the treatment facilities. Sediment at site 2 and site 4 recorded the highest concentrations of nickel as compared to sites 1 and 3 during both seasons.

5.4.6 Manganese (Mn)

Manganese is found in various salts and minerals in aquatic ecosystems and does not occur as a metal. According to DWAF (1996a), Mn exists in two forms as a manganous (Mn^{2+}) form. Sources of manganese include soils and sediments as well as metamorphic and sedimentary rocks (Bartman & Balance, 1996). Mn is one of the most abundant trace metals in the soils and water; the major anthropogenic sources of Mn include industrial activities, fossil fuels, and pesticides (Addo-Bediako *et al.,* 2021). Mn concentrations of 544 mg/kg and 654 mg/kg in sediment were recorded at sites 3 and 4, respectively, during the low-flow season, and they could be resulting from the agricultural activities and industrial effluents in close proximity to both sites and from natural sources. Manganese ($p>0.05$) this result implies that there is no significant difference in manganese concentrations between high flow and low flow in aquatic sediments.

Manganese mean concentrations at site 1 were within the DWAF (1996) guideline in the water column, during both seasons; however, elevated concentrations were reported in sediment throughout the study for all selected sites. Mean concentrations recorded at site 2, site 3, and site 4 during the high-flow season exceeded the TWQR for aquatic ecosystem protection, irrigation, aquaculture, and domestic purposes; however, they were within the livestock watering limit. In addition, the results obtained during the low-flow season were within the TWQR limits set for manganese in surface water. From this study, manganese recorded high concentrations in sediment following aluminum concentrations, which were the highest. The recorded concentrations in water samples were, however, higher than the 'world average' of 0.0015 mg/L (Martin and Windom, 1991). The CCME (2001) did not have any environmental quality guidelines accessible for manganese.

5.4.7 Zinc (Zn)

Zinc is an important nutritional trace element for both plants and animals; however, other organisms such as fish are extremely susceptible to zinc poisoning, while humans have high tolerance levels to elevated concentrations of zinc (DWAF, 1996c). Zinc occurs naturally in various rocks and ores; it therefore gets into natural water through rock weathering and erosion as well as industrial activities (DWAF, 1996a; DWAF, 1996c). In aquatic ecosystems, zinc occurs in two oxidation states, that is, as a metal and as Zn (II) (DWAF, 1996a). Zn (II) ion is toxic to aquatic biota, including fish, even at lower concentrations (DWAF, 1996b). The concentration of zinc in freshwater is usually low, at approximately 0.015 mg/L.

The Apies River has been reported to be polluted by WWTP, particularly the Rooiwal (Omole *et al.,* 2016; Abia *et al.,* 2017; Enzwakala *et al.,* 2017; Tau *et al.,* 2021). The concentrations of Zn recorded in sediment were far higher than the concentrations in water. Zn was found to be higher at sites 2 and 4 during both seasons in sediment and in water samples; it could be from industrial and WWTW effluents, as both sites are located downstream from the Daspoort and Rooiwal WWTW. In addition, it could also be from urban runoff and coal burning from the coal power station upstream from site 4. Zinc ($p < 0.001$) This result shows that there is a ~~very~~ significant difference in zinc concentrations between high flow and low flow in aquatic sediment.

Figure 17 shows the seasonal averages of the zinc concentration in water samples from each of the 4 monitoring sites. The average seasonal sediment concentration ranged from 12.4 to 35.01 mg/kg during the high-flow season and from 17.53 to 53.04 mg/kg during the low-flow season. The low flow season recorded the highest concentrations of zinc in sediments, as well as in water, with concentrations ranging from 0.08 to 0.25 mg/L. Earlier research conducted in the Western Cape Province revealed varying levels of zinc in river water near WWTWs (Olujimi *et al.,* 2015). According to Jackson *et al.* (2007), Berg River's zinc concentration ranged from 100 µg/L to 2100 µg/L and Jackson *et al.* (2009) reported Zn concentrations ranging between 100 µg/L and 4400 µg/L for studies conducted on Plankenburg and Diep Rivers. For domestic purposes, the recommended TWQR for zinc in water is 0-3 mg/L (DWAF, 1996). Therefore, based on the stated values, using the water from the sampling sites for domestic purposes is not predicted to have any negative health effects. However, the TWQR for the protection of aquatic ecosystems, aquaculture purposes, livestock watering, and irrigation are 0.002 mg/L, 0.03 mg/L, 0 to 20 mg/L and 1mg/L (DWAF, 1996). According to this study, water from the river system's chosen locations is not adequate for aquaculture or for the protection of aquatic ecosystems.

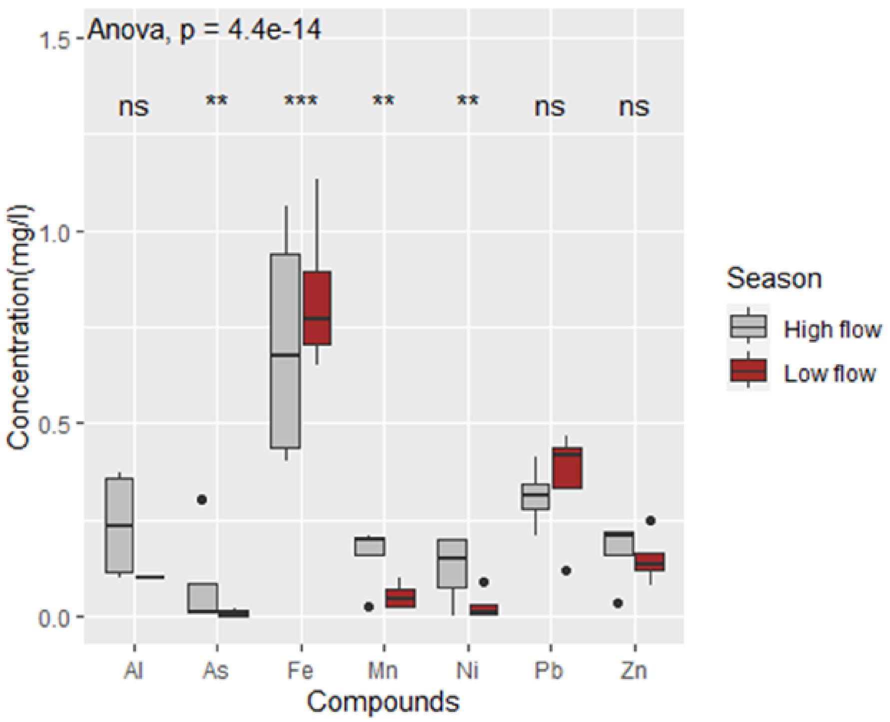


Figure 17: Average seasonal metal concentrations in water of the Apies River between (Feb/March 2023) and (Jun/Jul 2023).

There is no significant difference in aluminum concentrations between high-flow and low-flow seasons in surface water ($p>0.05$) (Figure 18). There is a significant difference in arsenic concentrations between high flow and low flow in surface water ($p<0.01$). There is a very significant difference in iron concentrations between high flow and low flow in surface water ($p < 0.001$). Manganese ($p < 0.01$) means that there is a significant difference in manganese concentrations between high flow and low flow in surface water. There is a significant difference in nickel concentrations between high flow and low flow in surface water ($p < 0.01$). Lead ($p > 0.05$): This result suggests that there is no significant difference in lead concentrations between high flow and low flow in surface water. Zinc ($p > 0.05$) means that there is no significant difference in zinc concentrations between high flow and low flow in surface water.

The results indicate that there is a significant difference in metal concentrations between high flow and low flow in surface water for some metals, but not for others. Arsenic, iron, manganese, and nickel have p-values less than 0.01, which means that the difference is very unlikely to be due to chance. These metals are more concentrated in high flow than in low flow, suggesting that they are influenced by factors such as erosion, runoff, or leaching from the soil. Aluminum, lead, and zinc have p-values greater than 0.05, which means that the difference is not statistically significant. These metals have similar concentrations in both high flow and low flow, suggesting that they are more stable and less affected by environmental conditions.

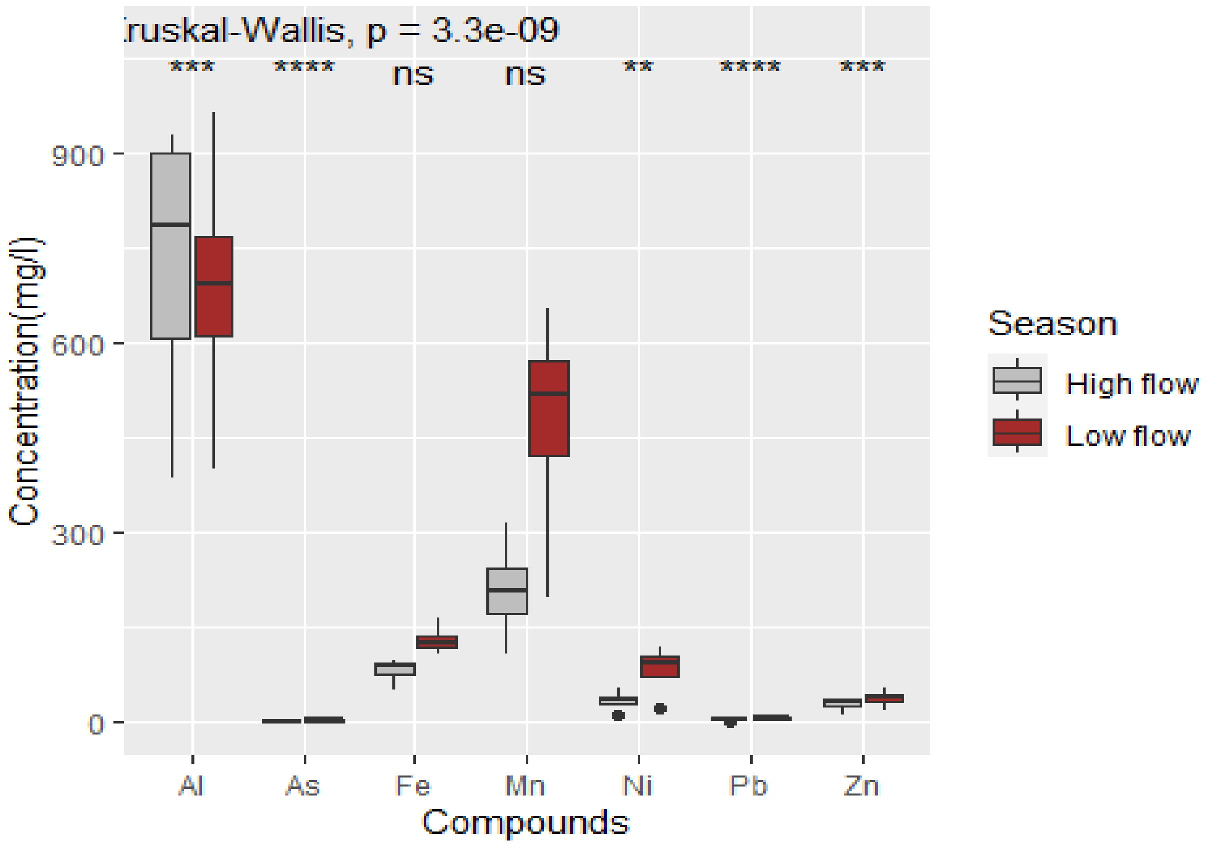


Figure 18: Average seasonal metal concentrations in sediment of the Apies River between (Feb/March 2023) and (Jun/Jul 2023).

One trend that seems to emerge from several studies is that metal concentrations in water tend to be higher in the dry season than in the wet season, while metal concentrations in sediment tend to be higher in the wet season than in the dry season. This could be explained by the fact that in the dry season, there is less water flow and dilution, which leads to higher metal accumulation and bioavailability in water. In contrast, in the wet season, there is more water flow and erosion, which leads to higher metal transport and deposition in sediment (Al-Musharafi *et al.,* 2013). One study that evaluated the seasonal variation in the Noyyal River in India found that the average Pb concentrations in water were higher in the dry season, while the average Pb concentration in the sediment was lower in the dry season than in the wet season (Edokpayi *et al.,* 2016; Karunanidki *et al.,* 2022). Another study that examined the seasonal variations of heavy metals in the Mvudi River in South Africa reported similar results, with higher concentrations of Hg, Cd, Zn, Pb, As, Cu, and Cr in the water in the dry season and higher concentrations of these metals in the sediment in the wet season (Edokpayi *et al.,* 2016).

However, there are also some exceptions and variations to this trend, as some studies have found no significant difference or even opposite results for some metals and some locations. For instance, one study that investigated the impacts of seawater physicochemical parameters and sediment metal concentrations on benthic communities in the Persian Gulf found that the concentrations of Cd, Cr, Cu, Ni, Pb, and Zn in the sediment did not show any significant seasonal variation (Zhu *et al.,* 2022). Another study that analyzed the seasonal and spatial variations of heavy metals in two typical reservoirs in China found that the concentrations of Cd, Cr, Cu, Ni, Pb, and Zn in the sediment were higher in the dry season than in the wet season in both reservoirs (Whang *et al.,* 2018).

The results of this study indicate that there are significant differences in metal concentrations between high flow and low flow in aquatic sediments for some metals, but not for others. Specifically, aluminum, arsenic, lead, and zinc have significantly higher concentrations in high-flow sediments than in low-flow sediments, as shown by the p-values below 0.05. On the other hand, iron and manganese do not show any significant difference in metal concentrations between high-flow and low-flow sediments, as shown by the p-values above 0.05. Nickel has a significant difference in metal concentrations between high-flow and low-flow sediments, as shown by the p-value of 0.01 (Figure 18).

Table 6: Mean concentrations of trace metal levels in water (mg/L) and in sediment (mg/kg) during the high flow and the low flow seasons on the Apies River

Metals	Water	Sediment

	High flow	Low flow	High flow	Low flow
Al	0.23	0.1	722.87	687.57
As	0.08	0.0075	1.645	2.7
Fe	0.7	0.82	80.45	129.25
Mn	0.16	0.05	209.25	472.5
Ni	0.125	0.027	33.17	81.41
Pb	0.31	0.35	2.61	5.84
Zn	0.148	0.123	27.51	37.09

5.5 Metals in water

During the course of the study, aluminum concentrations exceeded the recommended limit by DWAF (1996) and CCME (2001) at site 2 and site 3 during the high flow season (table 7). There is no world average limit available for aluminum in freshwater (Martin & Windom, 1991). Arsenic exceeded the limits stipulated by DWAF (1996), CCME (2001), and world average (Martin and Windom, 1991) at site 2 during the high flow season, and the other three sites were within the recommended guidelines for freshwater concentrations for arsenic. There are no recommended safe concentrations stipulated by DWAF for iron in freshwaters; however, it exceeded the environmental quality guidelines stipulated by CCME (2001) and the world average (Martin & Windom, 1991) at all sites during both seasons, showing extreme contamination across all sites.

Manganese was within the recommended limit stipulated by DWAF (1996) at all sites during both seasons as well as the world average. There are no recommended limits set by DWA (1996) for lead and nickel; however, nickel was within the CCME limits during the low flow season and exceeded the limit during the high flow season at site 3 and site 4. Lead exceeded the CCME (2001) limit at all sites during both seasons, showing high levels of lead contamination within the Apies River. Zinc exceeded all recommended limits set by DWAF (1996), CCME (2001), and the world average (Martin & Windom, 1991) at all sites during the course of the study, indicating high contamination at all sites.

Table 7: Metal concentrations recorded in water samples of the Apies River compared to recommended safe concentrations as stipulated by the Department of Water Affairs and Forestry (DWAF, 1996b), the Canadian Council of Ministers of the Environment Quality Guidelines (CCMe, 2001) and the 'World average' (Martin and Windom, 1991)

Metals	Recommended safe concentrations as stipulated by DWAF (1996b) (mg·l-1)	environmental quality guidelines as stipulated by CCMe (2001) (mg·l-1)	'World average' for metal concentrations in freshwater by Martin and Windom (1991) (mg·l-1)	Mean metal concentrations obtained in water (mg·l-1) of the (Apies River) during the low flow season	Mean metal concentrations obtained in water (mg·l-1) of the (Apies River) during the high flow season
Al	0.1 - 0.15	0.005 - 0.1	N/A	0.1 – 0.1	0.1 – 0.37
As	0.01	0.005	0.05	0 – 0.02	0.01 – 0.3
Fe	N/A	0.3	0.04	0.65 – 1.13	0.4 – 1.06
Mn	1.3	N/A	0.0015	0.025 – 0.098	0.02 – 0.21
Ni	N/A	0.025 - 0.15	0.0005	0.006 – 0.08	0 – 0.2
Pb	N/A	0.001 - 0.007	N/A	0.12 – 0.46	0.21 – 0.41
Zn	0.036	0.03	0.0006	0.035 – 0.22	0.08 – 0.134

N/A=Data not available

5.6 Conclusions

Metal concentrations were analyzed throughout this study on the Apies River. Metals in sediment and water were digested using the nitric acid digestion method and analyzed by spectrophotometer-IEC. The metals were analyzed during the high-flow and low-flow seasons of the year 2023 at various sites along the Apies River and were compared to relevant water quality guidelines. Results for metal concentrations varied over the course of the study period for every site along the Apies River, and no point sources of pollution could be located. Though the wastewater discharge from sites 2 and 4 may have had an impact on some metal concentrations, most metals were likely caused by soil erosion from nearby agricultural activities and natural weathering of the minerals and rocks in the catchment region.

The concentrations found in the study were mostly within the DWAF (1996c) irrigation water guidelines. Possible sources of contamination of the Apies River could be the leaching of household waste into the river from the residential settlements, agricultural runoff, and the leaching of industrial effluent from the industries close to the river. Furthermore, the overuse of insecticides and pesticides on farms that border the river system and the disposal of these pesticides into the rivers may have contributed to the contamination of the Apies River. To guarantee an accurate assessment of the current condition of the rivers, metal concentration analysis should be carried out on a regular basis. Even though metal concentrations varied during both seasons, higher concentrations were recorded downstream (sites 2, 3, and 4), thus accepting the hypothesis that stated that metal concentrations are influenced by anthropogenic activities downstream.

Chapter 6: Macroinvertebrate diversity and abundance in the Apies River

6.1 Introduction

Surface water settings are vital ecosystems with high ecological value; therefore, their quality and health are extremely important to those depending on them (Nguyen *et al.,* 2018). Humans and freshwater ecosystems rely on rivers for renewable water supply and important services such as domestic uses, food, and recreational activities (Berger *et al.,* 2016; Dickens *et al.,* 2018). Globally, anthropogenic activities are altering the availability and quality of freshwater (Nguyen *et al.,* 2018; Rodell *et al.,* 2018). For the past 3 decades, deterioration of water quality has been observed globally, which ultimately led to the water management and legislation for the available water (Abbasi & Abbasi, 2012). Streams and rivers are the most threatened freshwater ecosystems. Aquatic macroinvertebrates are essential tools to assess the overall water quality and aquatic ecosystem health (Tampo *et al.,* 2021).

Most macroinvertebrates complete their life cycles in water, from larvae of different insect species until they become adults (Thirion, 2007; Griffiths *et al.,* 2015; Mnisi, 2018).

Macroinvertebrates that complete their life cycles in aquatic ecosystems include snails, mussels, and aquatic worms (De Moor & Scott, 2003; Thirion, 2007; Griffiths *et al.,* 2015; Mnisi, 2028; Ngobeni, 2020). Even though macroinvertebrates can spend their entire lifetime in water, they occupy various biotopes in riverine habitats. Occupying various biotopes enables them to perform important ecological functions such as: 1) maintaining and regulating the decomposition of organic matter, which is mostly performed by Baetidae, Caenidae, Trichoptera, Tubificidae, Chironomidae, and some Mollusca species (De Moor & Scott, 2003; van Hoven & Day, 2002); 2) they act as the source of food for larger invertebrates such as families of Atyidae and Corixidae (Griffiths *et al.,* 2015) and 3) they play an integral role in maintaining aquatic ecosystems (Thirion, 2007; Farrell, 2014; Griffiths *et al.,* 2015; Thirion, 2016).

Aquatic macroinvertebrates exhibit a high sensitivity towards habitat transformation, organic pollution, and selected physico-chemical factors such as electrical conductivity, flow rate, pH, temperature, and turbidity (Dallas & Day, 2004; Wolmarans *et al.,* 2014). It is crucial for these physico-chemical parameters to be stable to suit the survival and reproduction of aquatic macroinvertebrates (Wolmarans *et al.,* 2014). According to Dallas & Day (2004), most aquatic macroinvertebrates prefer pH ranges between 6 and 8. Griffiths *et al.* (2015) and Mnisi (2018) reported that pH fluctuations may interfere with and disrupt normal metabolic processes and ultimately reduce the efficiency of the cells (interfering with ion exchange and osmotic balance of the organism) (Dallas & Day, 2004). A reduction of pH can exert an influence on macroinvertebrates to absorb metals, which have detrimental effects on their survival and reproduction (Griffiths *et al.,* 2015).

There is limited literature on conductivity tolerances of freshwater organisms; however, aquatic macroinvertebrates seldom have tolerances for larger alterations of electrical conductivity, as it affects salt and water movements on the cell membranes (Dallas & Day, 2004; Griffiths *et al.,* 2015).

In terms of temperature, there is adequate information on its impact on metabolic rate, respiration rate, growth and reproduction, and the toxicity of many substances, as well as the solubility of dissolved oxygen (Dallas & Day, 2004; Griffiths *et al.,* 2015). According to Dewson *et al.* (2007), flow rate exerts physical force on macroinvertebrates and has an impact on water chemistry, substrate composition, nutrient and organic particle availability, and suitable habitat availability. In addition, an increase in turbidity also affects macroinvertebrates in many ways. A reduction in photosynthesis occurs due to limited light penetration, which gives rise to limited food availability and oxygen in aquatic ecosystems (Dallas & Day, 2004; Griffiths *et al.,* 2015).

Aquatic macroinvertebrates have various biotope preferences; therefore, biotope availability determines the presence of the organisms in freshwater resources (Dallas & Day, 2004; Dallas, 2007; Thirion, 2007). Various biotopes in freshwater resources include vegetation, stones in and out of the current, riffles, pools, runs as well as gravel, sand, and mud (GSM). Vegetation biotope comprises aquatic and marginal vegetation; it is therefore another essential biotope that accommodates macroinvertebrates (Thirion, 2007). Macroinvertebrates that are found in the vegetation biotope include Belostomatidae, Coenagrionidae, Dysticidae, and Lymnaeidae. The stone biotope is characterized by hard surfaces, such as stones in and out of the current. The riffle biotope consists of shallow water with a rapid current, and it flows over substrates such as stones and cobbles (Dallas & Day, 2002). Macroinvertebrate families that can be associated with the abovementioned biotopes include Aeshnidae, Elmidae, Huridenea, Potamonautidae, and Simullidae. The pool biotope occurs at a reasonable depth, with no current, and usually accommodates families such as Caenidae, Vellidae, and Physidae (Thirion, 2007). The GSM biotope is characterized by gravel, sand, and mud (GSM) and normally accommodates the families of Chironomidae, Oligochaeta, and Tipulidae.

This chapter aimed to assess the effects anthropogenic activities and WWTW effluents on the structure of the benthic communities on the Apies River. The objectives were to assess the aquatic macroinvertebrate diversity within the study area; and investigate the relationship between macroinvertebrate assemblages and selected abiotic factors.

6.3 Methods and materials

During this study, four surveys for macroinvertebrate collection, March 2023 (two surveys) and July 2023 (two surveys), were conducted in the low-flow and high-flow seasons. Surveys were conducted in the Apies River in Pretoria at four selected sites based on methods and techniques described in the studies of Kemp et al. (2014) and Wolmarans et al. (2014). The aquatic macroinvertebrate sampling was completed using the South African Scoring System Version 5 (SASS5), which is a biomonitoring protocol implemented to carry out rapid water quality assessments (Dickens & Graham, 2002; Kemp *et al.,* 2014; Wolmarans *et al.,* 2014). This method relies on the use of macroinvertebrate communities to assess the impacts of stream and habitat modification (Dickens & Graham, 2002). Various macroinvertebrate families respond differently to pollution and environmental stressors; some families are highly tolerant to pollution, while others are highly sensitive (Manuel *et al.,* 2004; Kemp *et al.,* 2014; Mnisi, 2018). The coordinates were determined by means of a Garmin Nuvi 500 GPS for all the sampling sites. The collected species from all the biotopes were identified with the aid of a guide (Gerber *et al.,* 2004).

6.3.1 Sampling and identification

Biotopes

<u>Stones</u>

- Stones in current (SIC) were sampled for approximately 2 minutes; it included movable stones in the current, such as pebbles and cobbles (2-25 cm average size). In cases where rocks were highly embedded, an extension to 5 minutes was done on the sampling time. The SASS net was positioned to capture all macroinvertebrates that were transported by the water current.
- Stones out of current (SOOC): the sampling of SOOC was done immediately after the SIC were sampled. These stones were sampled for 1 minute of kicking and scraping of stone and sweeping the SASS net through the disturbed area to collect macroinvertebrates. The collected macroinvertebrates were placed in a SASS tray for identification.

<u>Vegetation</u>

Macroinvertebrates were sampled from any emergent and overhanging vegetation growing at the edge and inside the stream for approximately 2 minutes. Sampling was completed by following the protocol of Dickens and Graham (2002). The SASS5 net was pushed and pulled vigorously on vegetation to sample macroinvertebrates. The same procedure was followed to sample from aquatic vegetation that was submerged vegetation (roots, stems, and floating aquatics).

<u>Gravel Sand and Mud (GSM)</u>

Gravel, sand, and mud were sampled for 2 minutes. Gravel consisted of small stones of less than 2cm in size, and the sampling was completed by constant shuffling of feet and sweeping the net over the target area to catch dislodged biota. Sand consisted of particles that were less than 2mm diameter in size; sampling was completed in a similar way as the gravel by stirring and shuffling the feet in sand, which was followed by the sweeping of the net on the target area to catch dislodged biota. The exact procedure was used to sample mud, silt, and clay particles.

Visual observation

After sampling various biotopes, visual observation was done to identify samples using a macroinvertebrate guide (Gerber and Gabriel, 2002). This was followed by the calculation of the total SASS5 score, total number of taxa, and Average Score Per Taxon (ASPT) for each sample. The abundance was also estimated as per the SASS5 method (I=1, A=10, B=100, C=1000, D greater than 1000). All the samples collected were taken to the laboratory for further analysis. Upon arrival at the laboratory, all macroinvertebrate samples were transferred onto a rectangular Perspex® sorting tray (300 x 200 x 25 mm). All macroinvertebrates were sorted using a stereomicroscope, with lighting from above and beneath, and into macroinvertebrate groups. Further identification was done by making use of the microscope mentioned above, as well as the Guides to the Freshwater Invertebrates of Southern Africa (Davies & Day, 1998; Gerber & Gabriel, 2002a; Gerber & Gabriel, 2002b).

Sensitivity Values

Upon the completion of macroinvertebrate collection, identification, and data compilation, sensitivity scores were assigned to each family (Dickens & Graham, 2002). The SASS 5 values were adapted for this study to classify the collected macroinvertebrate families into three groups, namely highly tolerant (scores 1 to 5), moderately sensitive (scores 6 to 10) and highly sensitivity (scores 11 to 15) (Dickens & Graham, 2002).

Table 8: Sensitive category scores used to obtain SASS5 scores and ASPT.

Sensitivity category scores	
1-5	Highly tolerant
6-10	Moderately sensitive
11-15	Highly sensitive

6.3.2 Habitat integrity

The habitat integrity was determined by following McMillan (1998). The habitat was categorized into three parts: stones in and out of current, vegetation and gravel, sand and mud (GSM). The habitat score included physical characteristics and stream habitats for macroinvertebrates. The physical conditions of the stream consisted of depth, width, biotope, watercolor, and riparian vegetation. Table 9 below was followed to obtain the overall habitat conditions.

Table 9: Habitat integrity categories used to assess habitat within the study area in 2023.

IHAS score	Description	Ecological category
>75	Excellent/Natural - Unmodified or almost natural conditions; natural biotic template will not be modified. Minimal risk or reduction in habitat availability.	A
65 – 75	Good - Largely natural with few modifications; only a small risk of modifying the natural biotic template. Risk to the availability of habitat moderate, availability of unique habitats at risk	B
55 – 64	Adequate/Fair - Modified state; moderate risk of modifying the biotic template occurs. Habitat unavailable to certain aquatic invertebrates.	C
<55	Poor - Largely modified unnatural state; large risk of modifying the biotic template. Natural required habitat generally unavailable to most aquatic invertebrates.	D

6.4 Data analyses (Analysing macroinvertebrates)

Macroinvertebrates were analyzed according to Dickens & Graham (2002), Dallas (2007), and Bellingan *et al*. (2015), using two principal indices: Number of Taxa and Average Score per Taxa (ASPT). A score was allocated to the taxa based on the sensitivity of the taxa to pollution. Sensitive taxa have a high score (score of 15), while less sensitive taxa are given a lower score (score of 1) (Dallas & Day, 2004). The Average Score per Taxon (ASPT) was then calculated by dividing the SASS5 scores by the number of taxa for each sample at each site (Dallas & Day, 2004). The macroinvertebrate communities were then used to indicate the level of pollution and water quality of the river (Dickens & Graham, 2002).

6.4.1 Shannon-Wiener Diversity Index

Species Richness (SR) was used to determine the total number of species present in a community (Heip *et al.,* 1998), while the Shannon-Wiener index (H') was applied to characterize species diversity in a community (Heip *et al.,* 1998; Beals *et al.,* 2000). The higher the value of H', the greater the biodiversity is in each location. This value is calculated by the following equation:

$$\boxed{H = -\sum_{i=1}^{S} P_i \ln P_i}$$

P*i* = S/N

S = Number of individuals of one genus

N = Total number of all individuals in the sample

ln = Natural logarithm

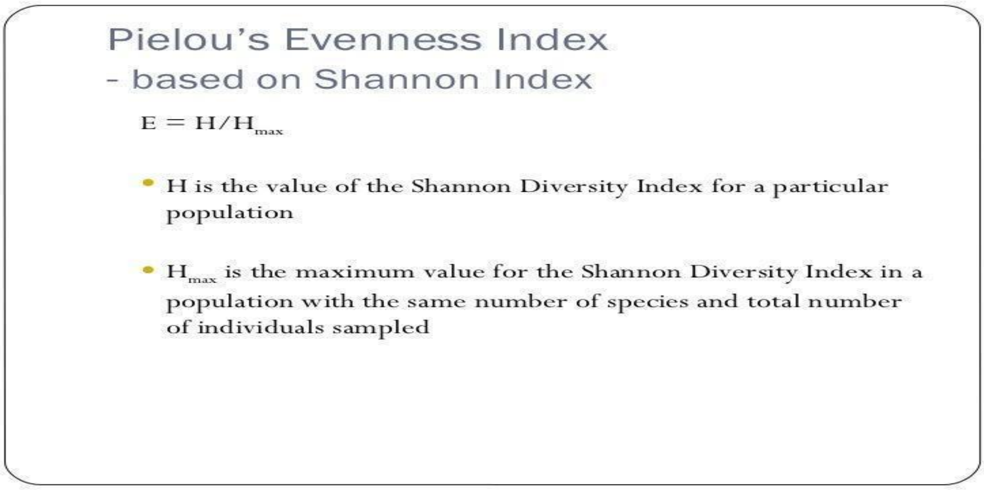
The Shannon-Wiener index score was interpreted according to Colour keys allocated to each level of pollution.

Table 10: The Shannon Wiener Diversity index score interpretation (adapted from Lad, 2015).

SPECIES DIVERSITY	POLLUTION LEVEL
3.0 - 4.5	Slight pollution
2.0 - 3.0	Light pollution
1.0 - 2.0	Moderate pollution
0.0 - 1.0	Heavy pollution

6.4.2 Pielou’s Evenness Index (J’)

Pielou’s evenness index (J’) describes the even distribution between species at a given sampling location and is defined as the Shannon-Wiener indices (H’) divided by the natural logarithm of the species richness (SR). In a healthy ecosystem it is expected that there will be an even distribution between species in a given community. Therefore, community stability can be related (Heip *et al.,* 1998). Values of J’ can vary between 0 and 1; thus, 1 indicates an even distribution and 0 an uneven distribution. To calculate J’, the following equation is used.



6.5 Results and discussion

Macroinvertebrate habitat

There were no seasonal variations observed in the habitat score across all four sampled sites due to the fact that most components remained unchanged, excluding water colour, water velocity, and stream depth. In terms of aquatic vegetation, there were tree logs and aquatic vegetation that provided habitat for macroinvertebrates. Habitat scores ranging from 70% to 78% were recorded throughout the four sampling surveys, indicating natural or unmodified conditions. Rocks covered with algae were dominant at site 2, together with stones with vegetation scattered along the riverbank; therefore, habitat scores ranged from 60% to 70%. Site 3 had good quality habitat scores ranging from 80% to 90%; this part of the river was wide with stones in and out of current covered with algae and gravel and sand, as well as marginal and aquatic vegetation. Site 4 had habitat scores ranging from 55% to 70% with pools, riffles, few stones, and limited marginal and aquatic vegetation.

Ecological categories

The four SASS5 surveys were conducted to collect macroinvertebrates at selected sites. The methods and guidelines of Dallas (2007) for SASS5 data interpretation were used to interpret the SASS5 information collected during the surveys. Table 9 summarizes the results of the aquatic macroinvertebrates and associated ecological categories.

Site 1 recorded the highest SASS score of 163 and ASPT of 5.62, categorizing the Apies River as an ecological category A/B (slightly modified) (Table 11).

A SASS score of 62 was recorded across 18 taxa with an ASPT of 2.12, categorizing the Apies River as an ecological category C (moderately modified) at site 2 (Table 9). Makuya (2022) reported the overall ASPT score of 3.4 on the Jukskei River, which placed the site into a PES of Category F (Severely Modified), which was a deterioration from Category C (Moderately Modified) and was potentially due to the impact of effluent from the WWTW.

At site 3, the ASPT score of 3.65 obtained placed the Apies River into a Present Ecological State (PES) of Category E/F (Severely Modified) (Table 9). This, therefore, indicates high modification from site 1 and site 2, with a dominant presence of pollution-tolerant macroinvertebrate species. The change in the ecological category could indicate the contamination from urban runoff and agricultural runoff from the farms around the area.

Site 4, which is downstream from the Rooiwal WWTW and the coal-fired power station, had a very low SASS score of 60 and ASPT of 1.72 (Table 11) with a dominant presence of pollution-tolerant macroinvertebrate species. The obtained ecological category could indicate that the WWTW wastewater effluent released into the Apies River has an impact on the water quality of the river as well as macroinvertebrate assemblages.

Table 11: Macroinvertebrate Assessment Results (Ecological Category) between February/March and June/ July 2023

Location	Site 1	Site 2	Site 3	Site 4
SASS Scores	163	62	106	60
ASPT	5.62	2.12	3.65	1.72
Ecological Category	**A/B**	**C**	**E/F**	**E/F**

Association between aquatic macroinvertebrates and selected abiotic factors is known to affect stream productivity. The GSM and vegetation biotopes were present at all sites during both seasons, although aquatic vegetation was minimal at site 1, 2 and 3 but dominant at site 4. Marginal vegetation was present at all four sites throughout the study. The stones in current (SIC) were present at all sites but it was minimal at site 4; however, it was available at all sites during both seasons. Algae varied between the four sites, but it was dominant at site 2 and 3 during the dry season which could be attributed to low water levels and high nutrient concentrations.

Table 12: Macroinvertebrates and selected physico-chemical factors

Abiotic factors	Low flow				High flow			
	Site 1	**Site 2**	**Site 3**	**Site 4**	**Site 1**	**Site 2**	**Site 3**	**Site 4**

pH	7.05	6.9	7.5	6.2	7.6	6.11	7.8	6.4
EC (mS/m)	184.9	432	159	305	681	849	252	700
Temperature (^{0}C)	16.9	18.5	18	19	24	26	26	27
Flow rate (m/s)	0.3	0.7	0.5	0.6	0.5	0.8	0.6	0.9
Marginal vegetation	X	X	X	X	X	X	X	X
Aquatic vegetation	X	X	X		X	X	X	
GSM	X	X	X		X	X	X	X
SIC	X	X	X	X	X	X	X	
SOC	X	X	X	X	X	X	X	X
Riffle	X	X	X	X	X	X	X	
Run	X	X		X	X	X	X	X
Pool	X	X	X	X	X	X	X	X

X represents the available biotopes at all sampling sites

6.6 Macroinvertebrate diversity

There was inconsistency of families in the assemblages collected throughout the four surveys of the study. Throughout the study, a total of 33 families were collected. The headwater stream (site 1) has unique features in a river system, which is characterized by good water quality as well as physical features; moreover, the headwater stream consists of higher taxa diversity in contrast with other sites of the river system (Clarke *et al.,* 2008). Throughout the four surveys of this study, the headwater stream (site 1) was dominated by taxa that are highly sensitive to pollution, such as Aeshnidae, Atydae, Lestidae, and Platycnemididae. The studies of Finn *et al.* (2011) and Matomela *et al.* (2021) have reported higher macroinvertebrate diversity at the headwater streams, owing to the fact that headwater streams are less disturbed or polluted and contain sufficient food. However, according to Richardson (2019), headwater streams are usually narrow with limited space to accommodate small and isolated communities.

For this study, site 1 exhibited good quality water as well as adequate physical habitat (all biotopes were present), which therefore explains higher diversity at this site. Site 2 is in the urban part of the city and is located approximately 1 km downstream of the Daspoort WWTW as well as other industrial activities that could potentially modify the physical structure and water quality (Zhang *et al.,* 2016). The diversity of macroinvertebrates at site 2 was poor due to limited biotopes. According to Erasmus (2017), biotope availability has a great influence on the macroinvertebrate community structure. Poor habitat quality usually has reduced biotic diversity as compared to good-quality habitats (Dickens & Graham, 2002; Soko, 2014; Bere *et al.,* 2016; Erasmus, 2017). According to Wilson *et al.* (2021), rivers that are polluted due to illegal dumping may result in higher macroinvertebrate diversity, as macroinvertebrates can colonize anthropogenic litter more than rocks and vegetation; this could be another reason why macroinvertebrates at site 2 were lower than at site 1, as site 2 was affected by litter.

Higher macroinvertebrate diversity at a site indicates good water quality (Norris & Thoms, 1999). Most families that occurred at all four sites had preferences for poor water quality, taking into account organic enrichment (Dickens & Graham, 2002; Thirion, 2007; Griffiths *et al.,* 2015). Macroinvertebrates are highly sensitive to anthropogenic impacts such as alterations in water quality and habitat; hence, they are good biological indicators to assess surface water. Therefore, poor macroinvertebrate diversity at site 2 and site 4 could also be linked to poor water quality.

Plecoptera and Trichoptera (EPT) were never sampled throughout the study; however, Ephemetoptera were sampled, and according to Mwangi (2014), these taxa are indicators of good water quality. The occurrence of Ephemeroptera, Plecoptera, and Trichoptera (EPT) is an indication of adequate oxygen as well as good water quality. These taxa exhibit high SASS and ASPT scores due to their sensitivity to water pollution (Norris & Thoms, 1999; Mwangi, 2014; Ngobeni, 2021). Park (2018) reported high diversity of Plecoptera species in streams that were not impacted by WWTWs and reduced diversity in streams impacted by WWTWs; however, Trichoptera and other taxa were more abundant in impacted streams, and the abundance of Ephemeroptera was similar in reference and impacted streams. Furthermore, comparisons of EPT assemblages between up and downstream of the WWTWs outfalls revealed decreased abundance in downstream reaches, while the abundance of other taxa increased (Park, 2018). The results of Ngobeni (2018) were comparable to the results of this study in terms of collecting EPT families, as they never collected any species of the EPT along the Apies River, including the Bon Accord Dam and Leeukraal Dam.

The families that occurred in all four sites were Dystidae, Belostomatidae, Chironomidae, Aeshinidae, Coenagrionidae, and Aytidae. Pretorious (2017) reported having collected Chironomidae at almost all the selected sites of the study and Dystidae at 8 of the 11 selected sites. According to the findings of Lundkvist *et al.* (2001), the degree of shadowing was the most significant factor in identifying Dytiscidae presence. A study conducted by Makuya (2022) collected Chironomidae, Oligochaeta and Hirudinea (tolerant taxa) from all selected sampling sites

Macroinvertebrate families that were dominant at site 3 included Aeshnidae, Atyidae, Coenagrionidae, Oligochaeta, and Hirudinea. This community structure is comparable to those observed in other related studies (Vermonden *et al.,* 2009; Wiederkehr *et al.,* 2020). Atyidae were mostly collected at site 3 during the first and third surveys and were never collected at site 4. The least collected family was Thiaridae, which were only sampled at site 1 during the first survey and were never collected from any of the other three sites. Although in low numbers, the occurrence of Coenagrionidae at all four sites during all three surveys provides evidence that the family can populate a variety of waterbodies with various physical and chemical properties; moreover, this could be explained by their biotope preferences rather than water quality preferences (Griffiths *et al.,* 2015; Thirion, 2007). In this study, Coenagrionidae was collected from marginal vegetation biotopes at all four sites during all four conducted surveys. Pretorious (2017) reported Coenagrionidae at seven, nine, and ten out of the possible 11 sites during the three surveys, respectively, in the Mooi River.

Table 13: Macroinvertebrates collected during the four surveys during the high flow (Feb/Mar) and low flow (Jun/Jul) seasons of 2023 on the Apies River

Families	**Functional feeding Group**	**Survey**			
		1	**2**	**3**	**4**

Baetidae	Predator	8	0	0	0
Oligoneuridae	Collector/ Gatherer	4	12	6	0
Tricorythidae	Collector/ Gatherer	6	12	8	6
Caenidae	Collector/ Gatherer	7	8	9	6
Heptaneiidae	Scrapper	6	0	0	0
Tricorythidae	Collector/ Filter	3	10	2	2
Hydropsychidae	Predator	2	7	13	12
Dystidae	Collector/ Gatherer	6	8	15	4
Elmidae	Scavenger	0	0	13	4
Hydrophilidae	Predator	4	12	8	3
Belostomatidae	Predator	4	13	9	3
Pleidae	Predator	0	6	2	0
Hydometridae	Predator	4	3	12	7
Anisoptera	Predator	3	0	0	0
Libellulidae	Predator	5	18	18	12
Gomphidae	Predator	7	3	13	0
Aeshnidae	Predator	5	6	17	0
Calopterygidae	Predator	5	0	6	1
Coenagrionidae	Collector	12	15	14	8
Lestidae	Collector	8	0	4	0
Platycnemidae	Collector/Gatherer	6	6	10	0
Protoneuridae	Shredder	10	0	8	0

Amphipoda	Collector/ Gatherer	8	0	0	0
Potamoutidae	Predator	2	13	17	15
Atyidae	Scrapper	15	13	12	9
Oligochaeta	Scrapper	13	10	22	21
Huridinae	Collector/ Gatherer	7	8	18	8
Lymnaeidae	Scrapper	4	7	12	3
Physidae	Scrapper	4	13	16	0
Chironomidae	Collector/ Gatherer	12	12	13	10
Thiaridae	Scrapper	11	0	0	0
Total		193	226	297	134

The second most occurring family was Planorbidae, which were collected in all four sites during all four surveys; this family can survive in various aquatic habitats in different biotopes, such as vegetation and GSM, as well as standing and flowing freshwater. The ability to survive in various habitats may be the reason why it occurred at all four sites (Brown, 1994; Thirion, 2007; Griffiths *et al.,* 2015). According to Dickens & Graham (2002) and Thirion (2007), Planorbidae is highly tolerant and contains hemoglobin as a blood pigment, which enables them to thrive and survive at different levels of organic enrichment in different types of habitats.

Baetidae were present only at site 1 (Table 7) during the first survey; however, this family can survive in different water bodies and occupy a variety of biotopes, including vegetation, rocks, riffles and GSM (Thirion, 2007; Griffiths *et al.,* 2015). The occurrence of this family at site 1 could be linked to nutrient availability as well as suitable biotopes; however, according to Dickens and Graham (2002) and Thirion (2007), the occurrence of more than two species of Baetidae is an indication of higher water quality, which was not the case in this study. Erasmus (2017) collected a maximum of 551 specimens of Baetidae whereby two species of Baetidae were collected at 2 of 11 selected sites.

Chironomidae were collected in all four sites of the study during all four surveys. Harrison (2003) reported that Chironomidae are among the most abundant aquatic macroinvertebrates and make up to 50% of the biomass. Chironomidae are found in most aquatic habitats as well as different biotopes ranging from riffles, pools, GSM and aquatic vegetation (Harrison, 2003; Griffiths *et al.,* 2015). The same as Planorbidae, Chironomidae contain hemoglobin as a blood pigment to support oxygen uptake, which is a biological factor enabling the survival of larvae in organically enriched environments (Griffiths *et al.,* 2015); this therefore allows this family to inhabit various environmental conditions.

Dytiscidae is among the largest beetle families in freshwater food webs (Griffiths *et al.,* 2015). Because they may function as both predators and prey, Dysticidae are regarded as keystone species in the freshwater food web (Biström, 2007; Griffiths *et al.,* 2015). Dysticidae were collected at all four sites during the first survey but were never collected at sites 3 and 4, during the third and fourth surveys. Aquatic and marginal biotopes were available at all four sampling sites, which are suitable biotopes for Dytiscidae; however, this family was never collected at site 3 and site 4 during the winter season. Erasmus (2017) reported a collection of Dytiscidae from 4 of 10 sites during 3 surveys at the Mooi River; all of the sites were characterized by slow flow rate (0.01 – 0.5 m/s), marginal vegetation in common, and algae, except for one site.

The Heptageniidae (Mayflies) were only collected at site 1 during all four surveys and were never collected from the other three sites; furthermore, this is one of the most sensitive families, and this is an indication that other sites were not suitable for this family in terms of pollution levels. According to Voshell and Reese (2002), the presence of this family in an aquatic ecosystem is a strong indicator of good water quality (clean to moderately polluted water). In addition, this family is moderately tolerant to nutrient enrichment. Erasmus (2017) reported a collection of Dytiscidae from 4 of 10 sites during 3 surveys; all of the sites were characterized by slow flow rate (0.01 – 0.5 m/s), marginal vegetation in common, and algae, except for one site.

Table 14: Macroinvertebrates collected at different sites during both seasons at the Apies River

	Sites							
Families	Site 1		Site 2		Site 3		Site 4	
	High flow	Low flow	High flow	Low flow	High flow	Low flow	High flow	Low flow
Baetidae	8	0	0	0	0	0	0	0
Oligoneuridae	4	0	0	4	6	0	0	0
Tricorythidae	4	2	0	2	3	3	0	0
Caenidae	3	6	1	0	3	0	0	0
Heptageniidae	0	4	8	4	8	3	0	0
Hydropsychidae	4	0	3	3	5	0	3	0
Dystidae	10	2	14	6	4	0	4	0
Elmidae	0	0	0	0	11	7	4	0
Hydrophilidae	0	4	8	4	8	3	0	3
Belostomatidae	6	2	0	13	0	0	0	0
Pleidae	0	0	6	0	2	0	0	0
Hydrometridae	4	0	6	6	4	4	0	4
Anisoptera	3	0	0	0	0	0	0	0
Libellulidae	3	2	8	12	10	8	7	5
Gomphidae	2	5	3	0	13	0	0	0
Aeshnidae	3	2	0	6	12	8	0	0
Coenagrionidae	10	11	20	15	9	10	8	0

Lestidae	2	8	0	0	4	0	0	0
Platycnemidae	7	5	0	0	0	4	0	0
Protoneuridae	9	7	0	0	5	3	0	0
Amphipoda	7	5	0	0	0	0	0	0
Potamonautidae	6	4	13	10	12	15	8	7
Atyidae	15	10	10	13	14	8	9	3
Hirudininae	6	5	10	6	18	10	2	6
Lymnaeidae	4	0	3	3	10	2	0	3
Physidae	5	0	13	10	10	9	0	0
Chironomidae	10	8	11	5	12	8	3	7
Thiaridae	11	0	0	0	0	0	0	0
No. of Organisms	**143**	**101**	**126**	**123**	**178**	**102**	**48**	**38**
Species Richness	**25**	**20**	**14**	**16**	**22**	**15**	**10**	**8**
Shannon-Wiener (H')	**3.056**	**2.858**	**2.476**	**2.644**	**2.939**	**2.576**	**2.125**	**2.02**
Pielou's Evenness (J')	**0.95**	**0.954**	**0.938**	**0.954**	**0.951**	**0.951**	**0.923**	**0.971**

Site 1 seems to be the least polluted with organic pollution, as it accommodates the most sensitive families to organic such as Baetidae, Heptageniidae, and Hydropsychidae. Table (8) displays the results obtained from biodiversity indices, the Shannon-Wiener Diversity Index (H'), Pielou's Evenness Index (J'), and the total number of organisms collected. Site 1 had the highest species richness with a mean of 22 species while site 4 had the lowest species richness with a mean of 9 species.

Site 1 is located in an undisturbed area that is relatively natural, and site 4 is located where there are various anthropogenic activities, such as WWTW, agriculture, urban and industrial activities, that had an influence on the macroinvertebrate's diversity. The highest H' and J' values were recorded at site 1, followed by site 3, which accounts for the highest diversity and an even distribution of species. All four sites had different numbers of organisms, with the lowest mean of 43 at site 4 and the highest mean of 140 at site 3.

Macroinvertebrate family abundance

Site 1

Figure 19 shows percentages of abundance values obtained from the sampling sites. Of the collected macroinvertebrates, 46% were highly tolerant families, 44% were moderately tolerant, and 10% were highly sensitive families. Site 1 is the only site that had a high percentage of sensitive families as compared to other sites. Macroinvertebrates that were found to be highly tolerant included Chironomidae (8%), Protoneuridae (7%), and Thiaridae (5%); these families are tolerant to organically enriched waters (Thirion, 2007; Griffiths *et al.,* 2015). Amphipoda were collected only at this site during both seasons and were never collected at other sites; the presence of this family could be attributed to low organic enrichment as well as the suitable biotopes (aquatic and marginal vegetation/rifles). The presence of pollution-intolerant families is an indication that this site was minimally or moderately polluted. Based on the collected families and SASS5 scores, site 1 was found to be the least polluted sampling site within the study area.

Site 2

A total of 20 families were collected at site 2 during both seasons. During all four surveys at site 2, the families of Baetidae, Caenidae, Elmidae, Lestidae, Amphipoda, and Platycnemididae were never sampled, adding up to 0%. This site was dominated by highly tolerant families (62%) and moderately tolerant families (28%) (Figure 19), including Potamonautidae and Coenagrionidae. Chironomidae is a highly tolerant family, and they were the most dominant highly tolerant family (19%) at this site, followed by Potamonautidae (10%) and Coenagrionidae (9%).

Site 3

Figure 19 shows that the majority of the families collected at site 3 were highly tolerant to organic pollution, including Hirudinea (10%), Potamonautidae (9%), Chironomidae (9%), Coenagrionidae (8%), Physidae (7%), and Libellulidae (6%), adding up to 49% of the collected families at this site during both seasons. Moderately tolerant families at this site included Aeshnidae, Atyidae, and Elmidae. Water quality deterioration at this site of the river could be attributed to agricultural practices, although that is solely dependent on the types of fertilizers used by nearby farmers. Interestingly, a total of 11 Heptageniidae, which constitute 11%, were collected at this site during both seasons; in addition, this family prefers flowing water and is highly intolerant to organic pollution.

Site 4

A total of 12 families were collected at site 4, 67% constituted highly tolerant families, and 33% were moderately tolerant families; moreover, none of the sensitive families were recorded at this site, indicating organic pollution during both seasons. This could be attributed to the impacts of effluent from the Rooiwal WWTW and the coal-fired power station as well as the intensive agricultural activities around this site. The above-mentioned activities are the cause of eutrophication, which results in the restriction of the diversity of pollution-intolerant species.

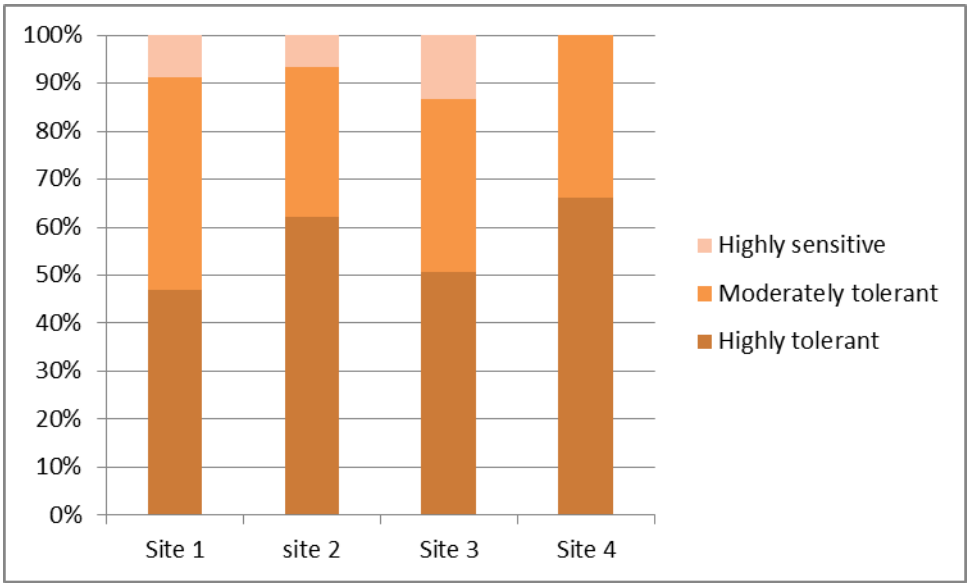


Figure 19: Percentage composition of highly sensitive, moderately sensitive, and highly tolerant taxa collected during all four surveys at all four sampling sites.

Functional feeding groups

The community structure of macroinvertebrates in a river system is known to be influenced by the physical habitat. The river begins to receive enough sunshine as it widens and the canopies open, which causes the aquatic biota to produce autochthonous food (Park, 2018). Because the food production in the headwater streams is predominantly allochthonous and is characterized by leaves dropping into the river from completely closed canopies, it is reported that these streams are home to collectors, shredders, and gatherers (Erdozain *et al.,* 2022; Labed-Veydert *et al.,* 2023). Throughout this study, predators (42%) and collector-gatherers (32%) during the high flow season dominated site 1, the headwater stream. In comparison to the headwaters, Damanik-Ambarita *et al.* (2016) found that there were more scrapers and predators in the lower Guayas River basin in Ecuador, while there were more scrappers in the lowland Amazonian streams; however, in this study, predators were dominant at the headwater stream while scrappers were less dominant with only 7% (figure 20). Functional feeding groups are particularly important to include in biomonitoring because they provide a clear insight of the dynamics of a stream (Park, 2018). Impacted stream sites, that are located downstream of the WWTWs, were all dominated by predators, collectors and collector-gathers. Shredders and scavenges were recorded in extremely low abundance at all sampling sites, even though many sites were in well-forested areas. All sampling sites were dominated primarily by predators (60%), collector-gatherers were typically co-dominant with (24%), followed by Scrapers, shredders and scavengers (figure 20 & 21). Scavengers comprised a small amount of the sampled macroinvertebrates as well as collector-filters, which were only sampled at site 2 during the low flow season. All sites were in primarily well forested watersheds, and although riparian land cover varied, most received considerable CPOM inputs, therefore the reduced shredder abundance was unexpected. A higher quantity of collector-gatherers has been reported downstream from WWTWs in previous investigations; this is probably due to the increased effluent's fine particulate organic matter concentrations; however, in this study collector-gathers were dominant at site 1 and site 3 during the high flow season (figure 20). According to Birge *et al.* (1989), it's also possible that nutrient enrichment from WWTP effluent modifies stream nutrient budgets and causes changes in the assemblages of primary producers and macroinvertebrates. In all sampled sites in the Apies River, shredders and scavengers were uncommon, and predators constituted the predominant FFG; this pattern persisted in sites upstream and downstream from WWTP effluent sources. Predators are thought to be a sign of lower water quality because of human activity, whereas detritivores and scrapers are thought to be indicators of locations with higher water quality (Jiménez *et al.,* 2021). According to Carrasco-Badajoz *et al.* (2021), in areas affected by urban activity, the number of collectors increases while that of predators, filter-feeders, and scrapers (functional groupings) decreases.

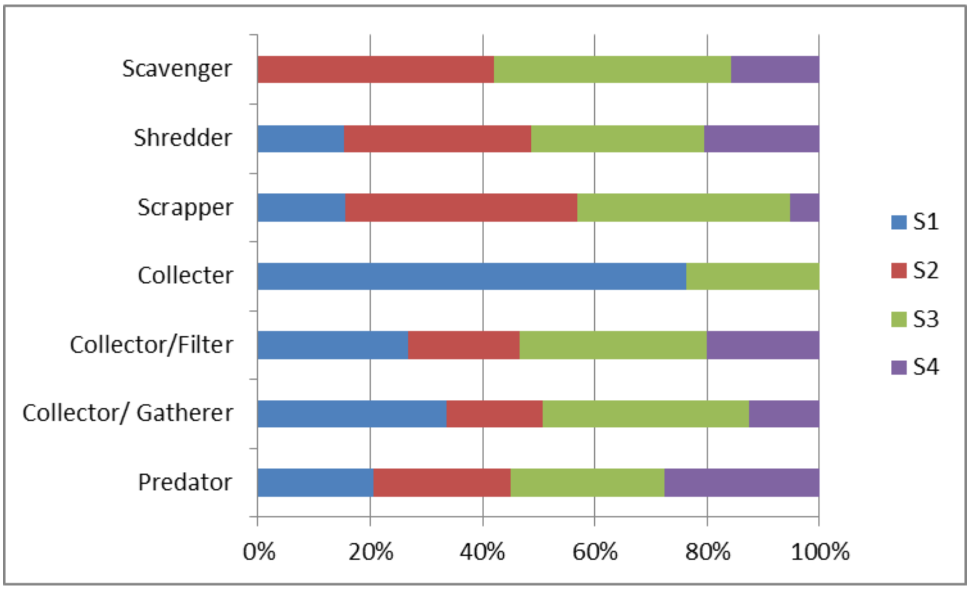


Figure 20: 100% bar plots showing the percentage and progression of functional feeding groups in all four sites during the high flow season of 2023 on the Apies River

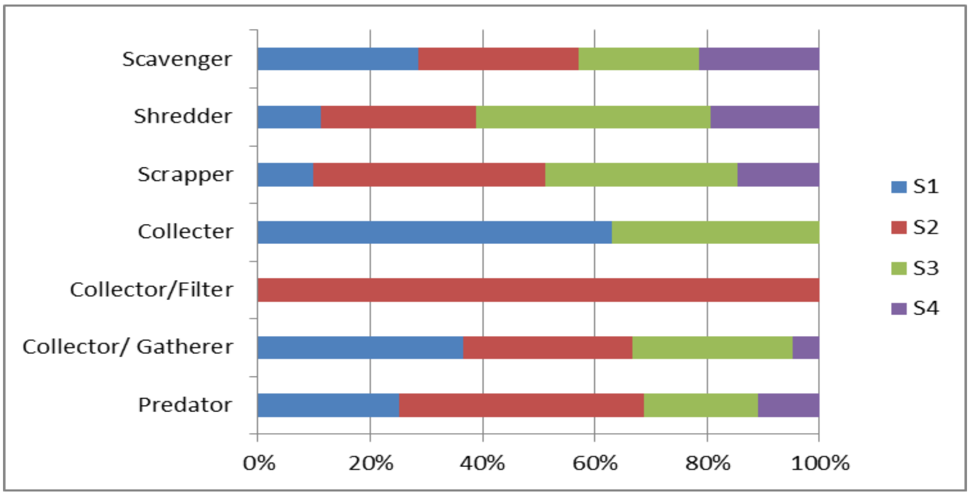


Figure 21: 100% bar plots showing the percentage and progression of functional feeding groups in all four sites during the low flow season of 2023 on the Apies River

6.9 Conclusion

In this study, various families of macroinvertebrates were collected, consisting of several taxa. According to the results obtained, the macroinvertebrate families varied between sites during the four surveys of two seasons. Even though the majority of the families that occurred were tolerant and moderately sensitive to organic pollution;

highly sensitive taxa occurred at two sites, although they were dominant at only one site. The results obtained from the biological indices indicated that the Apies River is organically enriched from various sources, which subsequently allow tolerant taxa to thrive; however, they also accommodate the survival of moderately sensitive taxa. The sites downstream of the Daspoort WWTW (Site 2) and the site downstream of the Rooiwal WWTW (Site 4) are the sites where the most tolerant taxa were collected. The origin of the Apies River (Site 1) is the only site where highly sensitive taxa were collected; this is an indication that the headwater stream is in a relatively natural state with few anthropogenic impacts. Even though sensitivity towards organic enrichment should be considered, environmental and abiotic factors can also influence the macroinvertebrate community structure.

In terms of habitat availability, all sites had good habitat quality including all necessary biotopes throughout the course of the study. Low species richness, diversity and even distribution between species were recorded at site 2 and site 4, where the WWTW effluents enter the river. High EC was recorded at Site 2 and 4 which indicate organic enrichment, which therefore serves as the source of food for tolerant species found at these sites. From the taxa collected and ASPT scores recorded, it may be concluded that the river is highly modified downstream. For this chapter, it was hypothesised that the macroinvertebrate diversity will be altered by anthropogenic activities in the lower reaches of the Apies River and is thus accepted.

Chapter 7: Conclusions and Recommendations

7.1 Conclusions and Recommendations

Extensive research has been conducted with regard to the monitoring of rivers due to an increase in anthropogenic activities such as agriculture, industry, mining, and urbanization. The management of water resources is important even though water monitoring plans have been designed and implemented.

The aim of this study was to determine water quality and macroinvertebrate diversity at the impacted sites on the Apies River during the two seasons.

Data collected from this study revealed that water of the Apies River had high concentrations of some physical and chemical indicators. The overall obtained results revealed that on several occasions the two WWTWs and other effluents were discharging poor-quality wastewater, resulting in the elevation of these concentrations as well as values of water quality indicators. It is also possible that the WWTWs have discharged effluents that were non-compliant with the wastewater guidelines and water use license for authorized discharge. The hypothesis in chapter 4 stated that, due to an increase of anthropogenic activities on the urban stretch, the water quality on the Apies River is too poor to support aquatic life; thus, the hypothesis was rejected as the present study demonstrated that the quality of water at all sampled sites is in an acceptable condition for most tested variables.

The hypothesis for chapter 6 stated that the macroinvertebrate community structure will be altered by the effluent discharges in the lower reaches of the Apies River. The aim of this chapter was to establish the diversity of aquatic macroinvertebrates during the low and high flow seasons at the selected sites of the river. Varieties of families were collected during the study, and the majority of tolerant taxa were collected at Sites 2, 3, and 4, which were followed by the moderately sensitive families. Site 1, which is the headwater stream, was the least impacted and accommodated families with different sensitivities and had high species richness, diversity, and even distribution between taxa. This therefore indicated that the effluents had a negative effect on the macroinvertebrate community structure at the sites impacted by the effluents. Therefore, the hypothesis for this chapter was accepted. Macroinvertebrate monitoring was used to assess the water quality and confirmed the river's poor ecological condition. Based on the documented taxa and ASPT scores, it can be inferred that the river is significantly altered at every sampling location. Poor water quality was the main cause for poor biodiversity in the river since suitable habitat and biotopes were present throughout the study.

7.2 Recommendations

The following are the recommendations for improving the downstream water quality of the Apies River:

- To continue sampling further downstream to determine the self-cleaning capacity and the reclamation of sensitive taxa.
- Continual water quality monitoring along the Apies River should be implemented. The monitoring plan should incorporate all the physical variables and be extended to other variables such as *E. coli*, various chemical variables such as metals and major ions that could not be included in the study due to financial constraints. This will therefore provide a comprehensive assessment of the overall water quality status of the Apies River.
- In-depth studies focusing on determining point source and non-point source pollution should be conducted to address water quality problems on the Apies River and reinforce water quality legislation to prevent further pollution.
- Responsible authorities should ensure compliance monitoring and enforcement of laws and regulations governing effluent discharges to minimize pollution in rivers.
- On several occasions the Rooiwal WWTW has been reported for discharging raw sewage into the Apies River; therefore, mitigation measures such as compliance with water legislation and the upgrading as well as increasing capacity of the plant are recommended in order to minimize the impacts of raw and partially treated effluents on the river's health.
- A planned continuous operation and maintenance program for wastewater treatment facilities and associated infrastructure must be established.

- There is a need for constant monitoring of effluent quality from the Rooiwal and Daspoort WWTWs as well as other industrial/residential effluents to determine if they comply with the DWA wastewater effluent quality standards.
- Public awareness and education campaigns are necessary to inform the people about the value of water resources, the pollution levels in the Apies River, and health risks associated with using polluted water.

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