Investigating the effects of nutrients and chlorophyll concentrations on the water quality of the Nuwejaars River, Cape Agulhas, Western Cape.

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Master of Science

In

Environmental and Water Science

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DECLARATION

I, Agnes Nolitha Khungwayo declare that ***Investigating the relationship between nutrients and chlorophyll in the Nuwejaars Catchment, Cape Agulhas, Western Cape*** is my work, that it has not been submitted for any degree or examination in any other university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

Full name: Agnes Nolitha Khungwayo

Date…...............................

Signed….....................................

ABSTRACT

The Nuwejaars River flows into the largest natural freshwater lake in South Africa, Soetendalsvlei, which relies on and interacts with the Nuwejaars River. Therefore, the water quality of the Nuwejaars River plays a role in the health of the surrounding ecosystem.

Over a period of three years, concentrations of nutrient constituents, physicochemical parameters, chlorophyll and phycocyanin, were measured in an attempt to investigate the water quality of the river. This study aimed to identify the patterns of nutrients, chlorophyll and phycocyanin down the length of the Nuwejaars River by assessing the physico-chemical parameters, nutrients and chlorophyll concentrations in the surface water to determine the temporal and spatial variations of these parameters, and to examine the relationships between the concentrations of selected nutrients, chlorophyll a and phycocyanin in the river water.

Seven sampling campaigns in all were conducted during the dry and wet seasons. Physicochemical parameters, phycocyanin and chlorophyll a were measured *in situ* while water samples were collected from selected sampling sites for nutrient analysis in the laboratory. Descriptive statistics and correlation analyses were used to describe the nature of the data as well as to describe relationships between variables, while multivariate techniques were applied to determine the degree of similarity in water chemistry between the the sampling sites.

The results indicated that electrical conductivity was higher downstream than upstream, and some values downstream exceeded 1000 mS/m. The pH generally ranged between 6 and 8, although lower values were recorded, the lowest being 4.34 during the dry season. The dissolved oxygen readings downstream were above 12 mg/l, upstream values were fairly low with values ranging between 2 mg/l and 4 mg/l.

Nitrate concentrations in the Nuwejaars River ranged between undetectable and 4.2 mg/l and total nitrogen between 0.1 and 41.4 mg/l. Total nitrogen concentrations were fairly low except downstream of a dairy farm in four consecutive separate sampling trips, suggesting that the farm might be an active source of nitrogen.

Phosphate concentrations ranged between 0.1 and 18.6 mg/l. It was observed that the highest phosphate concentrations were measured in the dry season, whereas during the wet season concentrations were mostly low. Total phosphorus concentrations ranged between 0.21 and 20 mg/l during the sampling period. Total phosphorus concentrations were high during the dry season and low during the wet season, as was the case with phosphate concentrations.

The highest chlorophyll a concentration was 139.43 mg/l, measured during the dry season at a site downstream of the dairy farm. Phycocyanin concentrations ranged from 0.1 to 8.6 mg/l, with the highest concentrations also being measured downstream of the dairy farm. The high concentration of phycocyanin and chlorophyll a at this particular site can be a reflection of the high total phosphorus and total nitrogen concentrations also measured at this site

The results of the Pearson Correlation showed that phycocyanin concentrations correlated significantly with total phosphorus (p = 0.89); chlorophyll a correlated significantly with total phosphorus (p = 0.98). With total phosphorus being the only nutrient that has significant correlations with both phycocyanin and chlorophyll a, it can be inferred that total phosphorus plays a significant role in the concentrations of phycocyanin and chlorophyll a in the Nuwejaars River. Also, phycocyanin concentrations significantly correlated with chlorophyll a (p= 0.83).

The results obtained from this study provide an assessment of patterns of nutrients, chlorophyll and phycocyanin concentrations along the length of the Nuwejaars River and on the factors that influence nutrient concentrations in water resources in the Nuwejaars Catchment. From this current study, it can be concluded that the in-situ fluorometric method of measuring chlorophyll is a suitable surrogate for the traditional spectrometric method as it was able to yield statistically significant and informative results on the relationship between nutrients, phycocyanin and chlorophyll a.

DEDICATION

This thesis is dedicated to my late father Steven Maphosa, my late grandmother Winterose Khungwayo and late Aunt Noncedo Khungwayo, thank you for being my guardian angels and always watching over me.

To my mother, Ntombomzi Khungwayo, thank you for always supporting me and making sure I go after my dreams and for the endless sacrifices you had to make for me to get to where I am.

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Table of Contents

List of Tables

List of Figures

List of Appendices

CHAPTER ONE: INTRODUCTION

1.1 Background

South Africa is a semi-arid country with the western parts drier than the eastern parts, has recently suffered from rainfall becoming less reliable (Dabrowski *et al.*, 2017). During the time of unreliable rainfall, many rivers cease to flow from time to time; these are known as non-perennial rivers (NPRs) (Day et al., 2019).

NPRs differ from perennial rivers in their flow regimes, periods of cessation of flow and sometimes in their overall water chemistry (Seaman *et al.*, 2016). The cessation of flow is caused by processes such as evapotranspiration, seepage and lowered groundwater levels (Larned *et al.*, 2010). Hence, groundwater plays an important role in NPRs, most importantly during the dry season by contributing through base flow. During the dry season, NPRs cease to flow and pools form along the river channel. The persistence of these pools is a result of groundwater discharging into the surface, groundwater is, therefore, likely to influence the water quality of pools (Seaman *et al.*, 2013).

Water quality has been defined as the physical, chemical, biological and aesthetic properties of water that determine its suitability for a variety of uses as well as the protection of the health of aquatic ecosystems (DWAF, 1996). Water quality in a river can be affected by a reduction in the water level, decreasing dilution that results from flowing water and increasing the concentration of chemicals, including nutrients such as nitrates, nitrites, ammonium and phosphorus (e.g. Utete and Tshamba, 2017).

Increased nutrient concentrations in surface water are mainly caused by land-use activities such as agricultural activities, inflow of sewage effluent, and industrial effluents. The increase in nutrient concentrations may lead to algal blooms, a phenomenon known as eutrophication (De Villiers, 2007a; Dodds and Smith, 2016a; Filstrup and Downing, 2017). Therefore it is important to measure and quantify nutrient concentrations in rivers because of their potential to cause eutrophication. Furthermore, it is important to evaluate the extent of eutrophication and the best way to do this is by measuring chlorophyll concentrations in surface water as a surrogate for algal production because the direct measurement of algal production is expensive and time-consuming.

The traditional measurement of chlorophyll, which is by spectrophotometry, is labour and time-intensive, and therefore relatively costly. A significant improvement has been made to the traditional way of measuring chlorophyll concentrations. The new improvement is *in situ* measurement using a hand-held fluorometer. Chlorophyll is a molecule that can be made to fluoresce when excited at a particular wavelength (Falkowski and Kiefer, 1985). It then emits light at another specific wavelength which is then measured to determine the chlorophyll concentration (Kalaji *et al.*, 2017). This property of chlorophyll allows for fluorometric methods that measure real-time data in the field (Falkowski and Kiefer, 1985).

In this current study, the fluorometric method was used to measure chlorophyll concentrations in river water and also to determine if it is a suitable substitute for the traditional methods of measuring chlorophyll and a suitable surrogate for nutrient concentrations. This is important not only because measuring nutrient and chlorophyll concentrations requires fairly sophisticated laboratory-based equipment which is often not available (e.g. in municipal laboratories), while the fluorometric measurements are easy, cheap (once the instruments have been purchased) and easy to interpret – therefore being useful additions to the suite of field-based instruments measuring aspects of water quality.

1.2 Problem Statement

The Nuwejaars River is an intermittent river, it would normally be considered to be a perennial river but the drought of 2015 to 2018 years has proven otherwise. Intermittent rivers experience the cessation of flow, this results in the degradation of water quality in the river causing a decline in the quality of the water and a loss of biodiversity. The Nuwejaars River flows into the largest natural freshwater lake in South Africa, Soetendalsvlei, which relies on and interacts with the Nuwejaars River. Therefore, the water quality of the Nuwejaars River has an impact on the health of the surrounding ecosystem.

According to a study conducted by De Villiers and Thiart (2007), nutrients are increasing in catchments throughout South Africa. This is a cause for concern because eutrophication caused by increased nutrient loads is regarded as one of the most serious threats to water quality in natural aquatic ecosystems (Dabrowski and de Klerk, 2013). Thus it is important to identify the sources of nutrients in catchments in order to manage and prevent eutrophication.

The Nuwejaars Catchment falls within the Cape Floristic Region which has protected biodiversity hotspots. Also the river carries outflow into Soetendalsvlei and runs through the Agulhas National Park (Blokker et al., 2015; Mkunyana, 2018). The floodplains and pools of the Nuwejaars River support livestock and a variety of crops including grapes. There is therefore a major concern that the land-use activities on the floodplain contribute to water contamination and have the potential to affect biodiversity along and within the river channel (Mazvimavi, 2018). However, there is limited understanding of the influence of anthropogenic activities on the water quality within the catchment of the Nuwejaars River.

Phosphorus and nitrogen released from dairy farms can persist in water bodies (Cho et al, 1999), hence it is important to evaluate the effect that farms have on the water quality of a particular water resource. Excess concentrations of nutrients can cause eutrophication i.e. the excessive growth of algae; eutrophication is usually measured either as chlorophyll a concentrations or as nutrient concentrations.

Nutrients were targeted for analysis because agricultural activities located close to the river often act as point sources of nutrients (Smith, Tilman and Nekola, 1999). An important component of the project was to examine the relationship between nutrients and chlorophyll a because they are both indicative of land-use activities effects on the environment. Over a period of three years, concentrations of nutrient constituents and the magnitudes of the physico-chemical properties, as well as concentrations of chlorophyll and phycocyanin were measured *in situ* in an attempt to investigate the water quality of the river as well as to investigate the relationship between chlorophyll a and nutrients in the Nuwejaars River.

1.3 Research questions

The main research question of the current study is, what are the patterns of nutrients, chlorophyll a and phycocyanin concentrations along the length of the Nuwejaars River?

To answer the above-mentioned question, two sub-questions were selected:

1.3.1 To what extent do patterns in water quality, specifically those of nutrients and chlorophyll a, reflect riparian land use down the river?

1.3.2 Can any of these variables be useful surrogates for each other, given the cost and time involved in chemical analysis?

1.4 Aims and objectives

1.4.1 Aim

The study aims to identify the patterns of nutrients, chlorophyll and phycocyanin down the length of the Nuwejaars River by using secondary data collected by the Institute for Water Studies at the University of the Western Cape.

1.4.2 Objectives of the study are to:

1.4.2.1 Assess the physico-chemical parameters, nutrients and chlorophyll concentrations in the Nuwejaars River to determine the temporal and spatial variations in the water quality.

1.4.2.2 Assess the correlation between fluorometric methods for measuring chlorophyll and nutrient concentrations with the eventual aim of using fluorometric data as a surrogate for measurement of nutrient concentrations.

1.5 Structure of this thesis

- Chapter 1 provides background and introduction to the topic. It outlines the main concepts and scope of the study, the research problem, the research question, and the aims and objectives of the study.
- Chapter 2 provides a review of the literature concerning the water quality of rivers in semi-arid areas and anthropogenic sources of pollution in NPRs in semi-arid areas. The chapter also focuses on physicochemical parameters, nutrients and chlorophyll and reviews the different methods for measuring chlorophyll.
- Chapter 3 gives an insight into the location of the study area and a description of the specific study sites. This chapter also outlines the methods and procedures used to collect, prepare and analyse the water samples. It provides information on the software packages used in the numerical analyses. Furthermore, this chapter acknowledges the limitations of the study.
- Chapter 4 provides the results of the chemical analysis, highlighting spatial and temporal variations in water chemistry. This chapter attempts to identify the relationship between nutrient and chlorophyll concentrations.
- Chapter 5 provides a summary of the main findings and also provides recommendations for water resource managers and future research.

CHAPTER TWO- LITERATURE REVIEW

2.1 Introduction

This chapter provides a critical review of the literature concerning non-perennial rivers and anthropogenic sources of pollution in NPRs in semi-arid areas. The chapter also focuses on physicochemical parameters that affect the production of phytoplankton with emphasis on nutrients, chlorophyll and the effects of nutrient enrichment. Lastly, it reviews the different methods of measuring chlorophyll a in aquatic systems, mostly focusing on fluorometric methods.

2.2 Non-perennial rivers

Rivers are longitudinal systems that are divided into zones with distinct physical, chemical, and biological properties (Malan and Day, 2005). Some rivers stop flowing from time to time. Such rivers are known as non-perennial rivers (NPRs) because they do not behave like 'normal' rivers that flow all of the time. NPRs are typically found in arid to semi-arid regions with high ambient air temperatures and little rainfall (Day et al., 2019).

According to Mosley (2015), the number of NPRs is expected to increase globally, as more permanent rivers become seasonal as a result of increased river abstractions, droughts, unpredictable rainfall patterns and high evaporation rates. The pools that form along the river channel of NPRs are of ecological importance in that they act as a source of water for terrestrial animals during dry periods (Hughes, 2008; Seaman et al., 2013, Hargey, 2019).

Uys and O'Keeffe (1997) defined NPRs as *temporary* rivers that experience the cessation of flow yearly or within 2 or more years in a period of 5 years, *intermittent* rivers as those that cease to flow in certain sections of the channel for a variable period and may flow seasonally; *ephemeral* rivers as those that flow briefly, usually for a period of less than the one which they are dry, and lastly *episodic* rivers those that flow only after a flood or a high-rainfall event. Rossouw and Watson (2005) emphasised the need to clearly define NPRs and to distinguish them from perennial rivers since differences in their functionality had been established. Thus for this study, the term *intermittent* river will be used.

2.3 Hydrochemistry of intermittent rivers

In this section, a summary is provided of the important water quality constituents discussed in the thesis. Surface water quality has been reported to be declining due to increasing industrialization, overpopulation and poor management of water resources that ultimately has led to severe environmental degradation which is more severe in some places than others (e.g. Atique *et al.*, 2019). Water quality is defined as the combined effect of a sample of water's physical and chemical properties on its suitability for a specific purpose, such as drinking or irrigation (DWAF, 1996). Water quality parameters can be physical (e.g., turbidity, suspensiods, and temperature) or chemical (e.g. TDS, salinity, EC, nutrients, organic enrichment and DO, and toxic chemicals such as biocides and many trace elements) (Dallas and Day, 2004).

Water quality is determined by analysing water samples collected on a regular basis from monitoring stations. The results of water quality monitoring are crucial in determining spatial and temporal trends in surface water and groundwater (e.g. Khatri and Tyagi, 2015).

2.3.1 Electrical conductivity (EC) and Total dissolved solids (TDS)

Total dissolved solids (TDS) refers to the total amount of dissolved material in a water sample, both organic and inorganic, ionized and unionized. TDS is proportional to the electrical conductivity (EC) of water (DWAF, 1996; Dallas and Day, 2004), both EC and TDS reflect salinity levels in the water.

The ability of water to conduct an electrical charge is measured by its electrical conductivity. Water contains ions such as carbonate, bicarbonate, chloride, sulphate, nitrate, sodium, potassium, calcium, and magnesium, which allow an electrical charge to be carried (DWAF, 1996). Organic compounds dissolved in the water that did not dissociate into ions do not affect the EC.

In South Africa, considerable variations in EC exist as a result of variations in geology (Seaman *et al.*, 2013). For example, rivers that flow over the weathered Table Mountain Group sandstones are characterised by low EC values, while those underlain by Malmesbury Shales that leach more ions exhibit higher EC values (Malijani, 2020). There is variation in EC values in the Nuwejaars River, the variations being attributable to rainfall, groundwater discharge and underlying geology (Mazvimavi, 2018).

2.3.2 Dissolved oxygen (DO) and Biochemical oxygen demand (BOD

Water contains a moderate amount of oxygen that enters by direct absorption from the atmosphere, it also absorbs the oxygen released by aquatic plants and phytoplankton during photosynthesis (DWAF, 1996). Dissolved oxygen (DO) consumed by microorganisms during oxidation is measured as the biochemical oxygen demand (BOD); BOD directly affects the amount of DO available in surface water as the greater the BOD the more rapidly DO is consumed (Bhateria and Jain, 2016). It is critical to keep dissolved oxygen levels high for aquatic organisms to function and survive, as it is required for the respiration of all aerobic organisms.

DO in natural waters varies diurnally, exhibiting low values at dawn and increasing concentrations during the day, particularly mid-afternoon (Rossouw and Watson, 2005). During summer DO levels are generally lower than in other seasons because DO decreases with an increase in temperature (Dallas and Day, 2004; Rossouw et al., 2005). The rate of oxygen diffusion between air and water has a distinct effect on the oxygen content of water, which is strongly influenced by turbulence (Campbell, 1982) and wind. The extent to which pools formed during the dry period of intermittent rivers may become anoxic is determined by site-specific factors such as temperature and solar radiation (Day et al, 2019).

Clean stream water is usually saturated with DO but the concentration decreases as sewage and other organic matter are added because the bacteria responsible for the breakdown of organic matter consume DO in the process and the water may become anoxic. The cycle continues as oxygen from the atmosphere dissolves into the water as it is no longer saturated (Malan and Day, 2002). Since intermittent rivers are periodically characterised by slow-flowing or stagnant water they are prone to anoxic conditions. In the pools DO decreases due to respiration and reduced dissolved oxygen solubility due to higher temperatures (Day et al, 2019).

2.3.3 Temperature

Temperature is defined as a body's state that governs heat transfer to or from other bodies (DWAF, 1996). Temperature in water is significant because it influences the rates of chemical reactions and, as a result, the metabolic rates of organisms. As a result, temperature is one of the most important factors influencing both the distribution of aquatic organisms and the rates of non-biological interactions. Natural changes in water temperature occur as a result of seasonal and diel cycles, and some organisms use these changes as signals for activities such as migration, emergence, and spawning. Aquatic species have a temperature tolerance range in which they can survive and thrive, so artificially induced temperature changes in water can affect both individual organisms and entire aquatic communities (DWAF, 1996; Dallas and Day, 2004)

Under drought conditions, thermal stratification in rivers and streams could become more evident; thus, thermal stratification in deep pools of intermittent rivers is highly probable (Malan and Day, 2002; Mosley, 2015) and may lead to reduced DO levels as well as chemical changes in the environment. In a study conducted in the Vaal River, it was found that high temperatures coincide with high chlorophyll concentrations (Pieterse *et al.*, 1997).

2.3.4 pH

The pH value is a measure of the hydrogen ion activity in a water sample (DWAF, 1996). Natural water's pH is influenced by lithological, biological, and atmospheric factors. Most freshwaters are relatively well buffered and neutral, with pH values ranging between 6 and 8 (Dallas and Day, 2004).

The pH of water can have a significant impact on the chemical species of metal ions and nutrients, and thus on their bioavailability (Filella et al., 1995). As a result of desorption from the surfaces of suspended particles, decreasing pH frequently leads to increased levels of metal cations dissolved in the water column. As pools dry up, the pH of some intermittent rivers drops to as low as 4.5 due to the leaching of organic acids from leaf litter (Boulton et al., 2000).

Severe changes in pH affect the organisms that live within river systems, altering the rate of ionic exchange within the body, as well as affecting the toxicity of metals in water (Dallas and Day, 2004). Biological activities such as photosynthesis and anthropogenic activities that generate industrial effluents have the potential to increase or decrease pH values outside of the normal tolerance limits of many taxa. During algal blooms, photosynthetic activity causes an increase in pH (>9) due to carbonic acid Anthropogenic activities affecting water quality in intermittent rivers

Freshwater resources have been subjected to consequences of an increasing number of anthropogenic activities over the last few decades, including the construction of reservoirs to generate electricity, municipal water supply for drinking and household use, transportation, flood control, recreation, agriculture, fishing, and so on (Atique *et al.*, 2019). Figure 1 below shows some of the major sources of water pollution in both rural and urban areas.

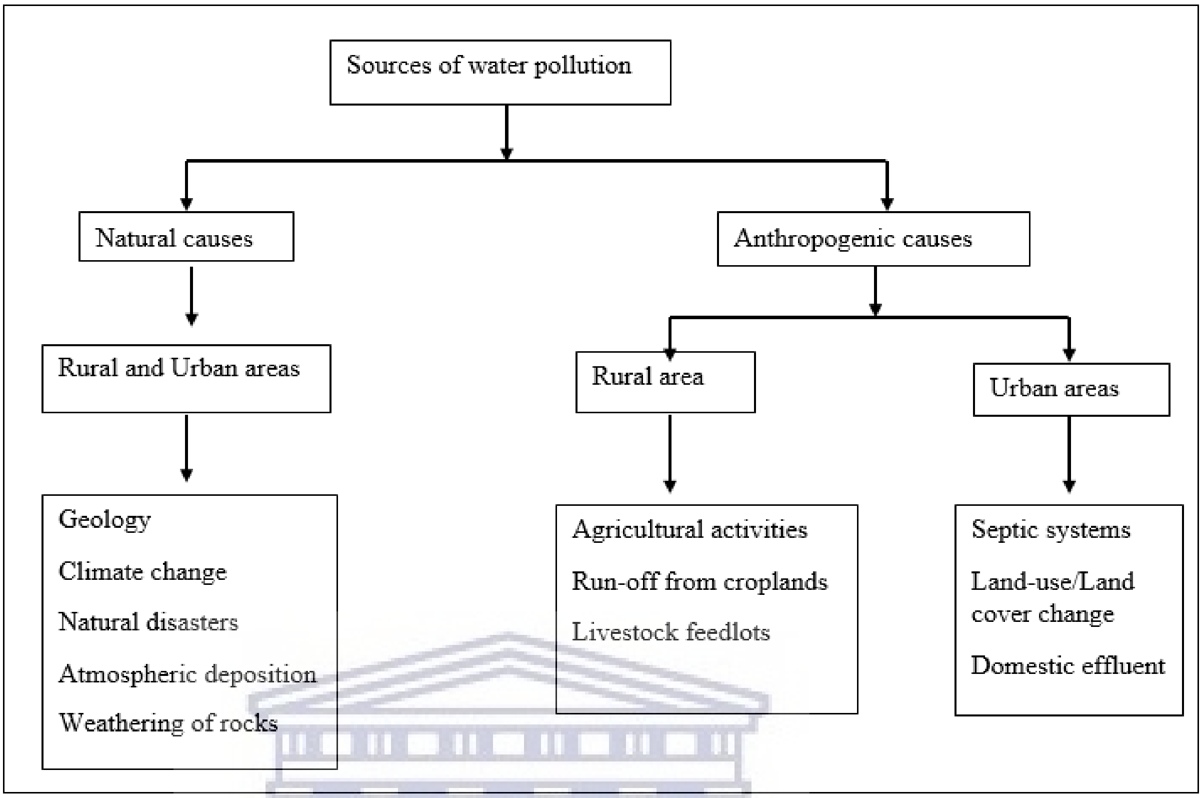
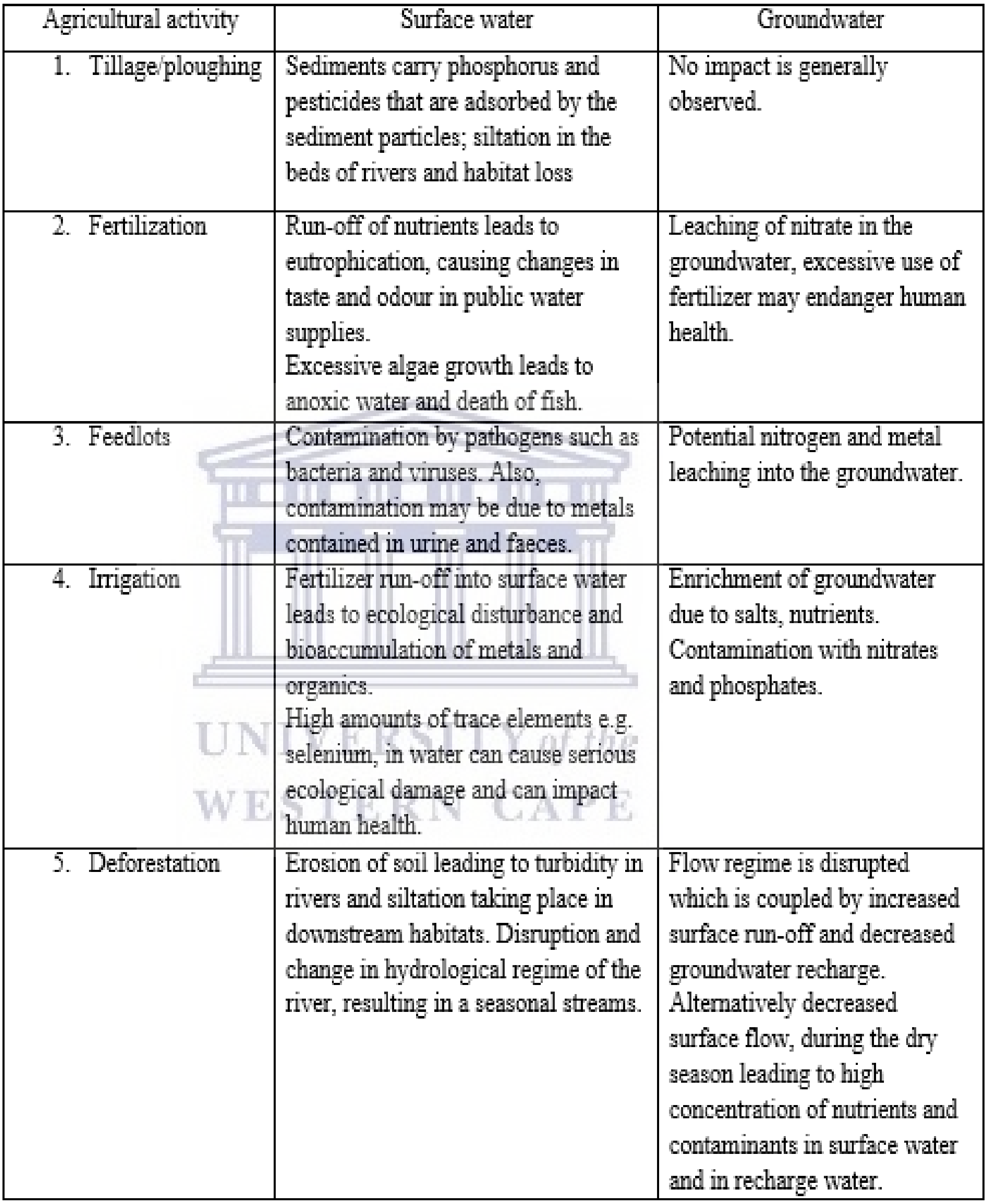


Figure 1: Sources of water pollution in rural and urban areas (adapted from Khatri and Tyagi, 2015).

Natural and anthropogenic processes both affect ground and surface water quality in rural and urban areas (Khatri and Tyagi, 2015) – refer to Figure 1. As a result of these anthropogenic influences, high-quality water is becoming increasingly scarce as the world's population grows. Surface water contaminants may have toxic effects on aquatic plants and animals, as well as human health if they are consumed (Khatri and Tyagi, 2015; Malijani, 2020).

Anthropogenic factors that often result in the nutrient enrichment of surface water are livestock farming, inefficient irrigation practices, deforestation of woodlands, aquaculture, pollution caused by industrial effluents and domestic sewage (Khatri and Tyagi, 2015). Agricultural activities are the main contributors to water pollution in this current study area. Table 1 details the different activities that lead to water pollution from agriculture (Khatri and Tyagi, 2015).

Table 1: Impacts on water quality due to agricultural activities (adapted from Khatri and Tyagi, 2015).



Agricultural activity	Surface water	Groundwater
1. Tillage/ploughing	Sediments carry phosphorus and pesticides that are adsorbed by the sediment particles; siltation in the beds of rivers and habitat loss	No impact is generally observed.
2. Fertilization	Run-off of nutrients leads to eutrophication, causing changes in taste and odour in public water supplies. Excessive algae growth leads to anoxic water and death of fish.	Leaching of nitrate in the groundwater, excessive use of fertilizer may endanger human health.
3. Feedlots	Contamination by pathogens such as bacteria and viruses. Also, contamination may be due to metals contained in urine and faeces.	Potential nitrogen and metal leaching into the groundwater.
4. Irrigation	Fertilizer run-off into surface water leads to ecological disturbance and bioaccumulation of metals and organics. High amounts of trace elements e.g. selenium, in water can cause serious ecological damage and can impact human health.	Enrichment of groundwater due to salts, nutrients. Contamination with nitrates and phosphates.
5. Deforestation	Erosion of soil leading to turbidity in rivers and siltation taking place in downstream habitats. Disruption and change in hydrological regime of the river, resulting in a seasonal streams.	Flow regime is disrupted which is coupled by increased surface run-off and decreased groundwater recharge. Alternatively decreased surface flow, during the dry season leading to high concentration of nutrients and contaminants in surface water and in recharge water.

It has been understood that anthropogenic inputs associated with land cover and land use have a significant influence on increased nitrogen and phosphorus concentrations in surface waters (e.g. Dabrowski and de Klerk, 2013; Petersen *et al.*, 2017; Kimambo, Gumbo and Chikoore, 2019). Nutrients, sediments, pathogens, pesticides, metals, and salts are among the pollutants produced by farming and cattle breeding. Agricultural activities are thought to be significant contributors to the eutrophication of aquatic ecosystems (Dallas and Day, 2004).

Overgrazing by livestock leads to the exposure of soil and increased erosion (Rossouw and Watson, 2005). This can result in the degradation of both land and aquatic ecosystems encouraging the invasion of alien species, destruction of stream banks, flood plain vegetation and fish habitats (Khatri and Tyagi, 2015).

The removal of trees and vegetation along the stream bank results in exposed soil and increased erosion and may increase the number of pollutants entering the stream, such as nitrogen, phosphorus, Escherichia coli, pesticides, and sediments (Khatri and Tyagi, 2015). Water temperature may rise, and as a result, DO levels in the water may decline (Khatri and Tyagi, 2015). As the temperature is critical to many aquatic species, the death of fish may result.

Atique and An (2019) in their study assessing reservoir water quality in the Chungju Reservoir, South Korea, discovered that monsoon rainfall plays a significant role in influencing water quality change. It is also the primary source of nutrients entering the reservoir from a variety of sources, including domestic sewage, wastewater treatment plants, industrial effluents, and livestock and crop production activities. In the current study area, the wet season is likely to have the same effect as the monsoon i.e. it will result in the runoff of nutrients from livestock and crop production, albeit at a smaller scale.

2.4 Nutrients in intermittent rivers

Nutrients are elements that are required for plant growth. Carbon, nitrogen, phosphorus, potassium, calcium, magnesium, sulphate, and silicate are some examples of nutrients, as some elements are known as micro-nutrients, which are required in much smaller quantities. Nutrients of greatest importance in aquatic ecosystems are nitrogen and phosphorus as they are the nutrients most implicated in excessive plant growth resulting in eutrophication of aquatic systems (De Villiers, 2007b; Dabrowski and de Klerk, 2013; Dodds and Smith, 2016a; Prayitno and Afdal, 2019). Nitrogen and phosphorus are known as limiting nutrients because a lack of them limits plant growth (Larned, 1998). Silicon is a limiting nutrient concerning the growth of diatoms. Most nutrients are not toxic, but in high concentrations, they can have a significant impact on the structure and functioning of aquatic systems (Dallas and Day, 2004), ammonium becomes toxic ammonia and nitrate at high concentrations is toxic to infants.

Anthropogenic nutrient sources can be single-point (e.g., wastewater treatment plants, industry, intensive animal enterprises) or non-point (agricultural runoff, urban runoff, atmospheric deposition). Point sources are relatively easy to measure and monitor because water managers at the source can re-route excess nutrients away from lakes and streams or use herbicides or algaecides to prevent algal blooms (Aczel, 2019), whereas non-point sources are diffuse and harder to quantify and monitor. After all, it is often difficult to pinpoint the exact source of the nutrients being released into the aquatic environment. Agricultural and industrial activities are the main sources of nitrogen and phosphorus to aquatic ecosystems, with crop fertilization and human waste being the primary sources, respectively. Phosphorus as phosphate ions (PO_4^{3-}) and nitrogen as nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+) ions are major nutrients that contribute to eutrophication (Rossouw and Watson, 2005).

2.4.1 Phosphorus

Phosphorus plays an important role in the structure of nucleic acids, such as DNA and RNA, as well as molecules involved in the storage and utilization of energy in cells (Addiscott et al., 1991). ATP (adenosine triphosphate), the molecule used for energy transfer, contains phosphorus occurring mostly in dissolved form as the inorganic PO_4^{3-} ion. Soluble reactive phosphorus (SRP) is often measured as orthophosphate, it is a dissolved form of phosphorus (Malan and Day, 2005) that is immediately available for plant uptake; various forms of phosphorus can be transformed into available PO_4^{3-} by naturally occurring processes and is often found in quantities <0.01mg/l in nonpolluted water (Dallas and Day, 2004). Understanding the processes and mechanisms that control the supply of bioavailable phosphate is critical for preventing eutrophication in catchments, rivers, and lakes (Webster et al., 2001).

Phosphorus, which cycles through the environment between the land and aquatic systems, originates from terrestrial rocks. Plants absorb dissolved inorganic phosphorus and convert it to organic phosphorus in their tissues. Animals obtain the organic phosphorus they require by consuming plants, other animals, or decomposing plant and animal matter (USEPA, 2012).

When plants and animals expel waste or die, the organic phosphorus they contain sinks to the bottom of a river or the sea, where bacterial decomposition converts it back to inorganic phosphorus, both dissolved and attached to particles. When the bottom of the water column is stirred up by animals, human activity, chemical interactions, or water currents, this inorganic phosphorus returns to the water column. Then it is taken up by plants, and the cycle starts all over again (USEPA, 2012). In an aquatic system, the phosphorus cycle tends to move phosphorus downstream as the current carries decaying plant and animal tissue as well as dissolved phosphorus. It only becomes stationary when it is taken up by plants or stuck to particles that settle to the bottom of pools.

Total phosphorus is a measure of all the forms of phosphorus, dissolved and particulate phosphorus - that is, orthophosphate, phosphate minerals and organic phosphate. A large fraction of the total phosphorus that enters a river is usually adsorbed onto particulate matter and a smaller fraction of the total load is incorporated into dissolved organic matter (DOM) in the water (Holton, Kamp-Nielson and Stuanes, 1988). Most of the total phosphates are delivered during storms when discharge and velocity are high and scouring of bottom sediments and soil wash-off occur. During low flows, sediments act as sinks for phosphorus entering the stream from point sources.

The concentrations of inorganic ions dissolved in the water column can be affected by changes in the number of suspended particles (Dallas et al., 1998). Phosphates adsorb onto particles, resulting in phosphorus deficiency in rivers carrying high loads of suspended solids. Sediments can therefore be of major importance in determining nutrient availability in the water column (Sweeting, 1994).

Due to increased sediment loads, particulate phosphorus is likely to increase in the water column during a flood. Phosphates are likely to decrease or remain constant in nutrient-poor areas in response to increased discharge in the absence of point sources of pollution. Because of the wash-off effects of pollutants from phosphate fertilizers, the trend may be reversed in areas with intensive land use. Dissolved phosphate levels may rise during low-flow periods as the amount of effluent in river water rises. (Malan and Day, 2002).

Dissolved phosphate often shows a dilution effect (Larned ST, 1998). Cahili, Imperato and Verhoff (1974) found a distinction in the behaviour of total phosphate (TP) and soluble orthophosphate. During steady state condition, both TP and orthophosphate show a dilution effect as a result of the low flows and low sediment supply. During storm events, only TP was increased by increased flow and orthophosphate concentrations remained constant. Changes in pH and EC resulting from altered discharge may also affect the absorption/ desorption equilibrium and thus the proportion of dissolved to bound phosphorus.

Hoobie and Likens (1973) found that there was little seasonal change in TP concentration in an undisturbed forested catchment and concluded that the ecosystem was strongly conserving phosphorus. It is likely that in nutrient-poor ecosystems, phosphate will be strongly bound either to the soil or within the living organisms and stream water concentrations will not vary greatly.

2.4.2 Nitrogen

Nitrogen is the fourth most abundant element in cellular biomass and makes up the vast majority of the Earth's atmosphere. It is available as both organic and inorganic nitrogen. Organic nitrogen refers to nitrogen compounds derived from living matter, whereas inorganic nitrogen includes nitrate, nitrite, and ammonium. It can enter the soil as decomposed plant or animal tissue; it is not available to plants until microorganisms convert it to ammonium (NH_4^+); total nitrogen (TN) is a measurement of the sum of ammonia, organic nitrogen, nitrates, and nitrites present in either a soil or water sample.

The nitrogen cycle is a continuous cycle in which nitrogen moves through both living and non-living things, including the atmosphere, soil, water, plants, animals, and bacteria. Nitrogen undergoes a transformation as it moves through the various stages of the cycle (Aczel, 2019). In the atmosphere, nitrogen exists as any of several gases (nitrogen oxide (NO), nitrogen dioxide (NO_2) and molecular nitrogen) but in soils, it exists as nitrate (NO_2), nitrite (NO_3) and ammonium (NH_4). It can also be found in other forms, such as ammonia (NH_3), which can be further processed into a fertilizer, ammonium nitrate (NH_4NO_3).

There are five stages in the nitrogen cycle: fixation, mineralisation, nitrification, immobilization and denitrification (Stein and Klotz, 2016; Aczel, 2019).

1- Nitrogen fixation: fixation is the process by which gaseous N_2 in the atmosphere is converted into forms that plants can absorb through their root systems. When lightning strikes, it provides the energy needed for N_2 to react with oxygen, producing nitrogen oxide (NO) and nitrogen dioxide (NO_2), which when dissolved form nitrates and nitrites. Nitrogen fixation is mostly carried out naturally in the soil by bacteria. Photosynthesis provides energy to the bacteria, which allows them to fix nitrogen into a form that plants can use.

2- Mineralisation: takes place in the soil. Bacteria convert nitrogen from organic materials such as manure or plant materials to an inorganic form that plants can use. It occurs when microbes interact with organic material, such as animal manure, and begin to convert it to a form of nitrogen that plants can use. Ammonia is the first form of nitrogen produced by the mineralisation process (NH_3). After that, the NH_3 in the soil reacts with water to form ammonium, NH_4^+. This ammonium is stored in the soil and is available for plant uptake.

3- Nitrification: during nitrification, ammonia in soils produced during mineralisation is converted into nitrites (NO_2^-) and nitrates (NO_3^-). Nitrates can be used by plants as well as animals that consume plants. Ammonia can be converted into nitrites by soil bacteria.

4- Immobilization: reverse mineralisation is a term that is often used. The two processes work together to regulate the amount of nitrogen in soils. It is significant because it helps to control and balance the amount of nitrogen in soils by tying it up or immobilizing nitrogen in microorganisms.
5- Denitrification: At this stage, nitrogen is returned to the atmosphere as bacteria convert nitrates to atmospheric nitrogen (N_2). This results in a net loss of nitrogen from soils as a gaseous form of nitrogen enters the atmosphere, and the cycle begins again.

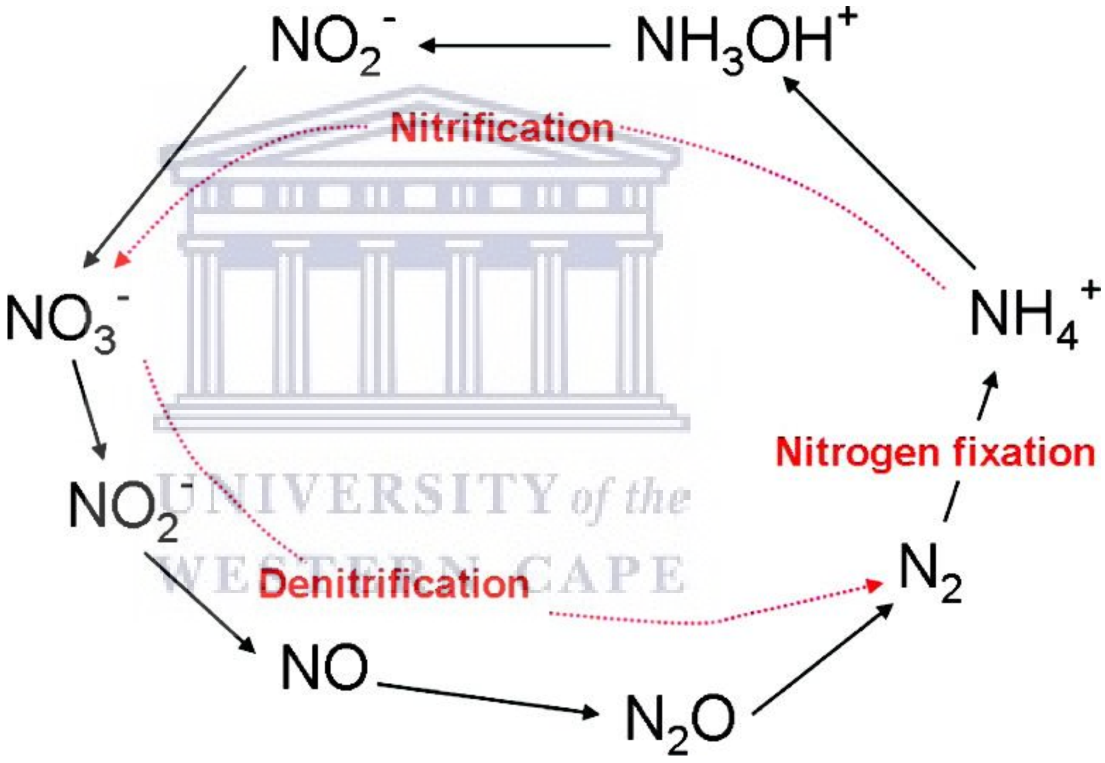


Figure 2: Nitrogen cycle. Source (Rosca *et al.*, 2009).

According to Burt et al. (1998), during dry periods especially during the summer, there is a build-up of mineralised inorganic nitrate from organic nitrogen in the soil. Such nitrates can be leached or transported in run-off (Blanchard and Lerch, 2000). Nitrates are strongly linked to agricultural land and grasslands (Ferrier et al. 2001), often resulting from fertilizer runoff (Hughes, 2014; Mamun, Lee and An, 2018). Due to the high degree of mobility in the soil, nitrate is likely to increase during storm events and also depending on the nutrient status of the soils a flushing effect may be experienced in regions of intense agricultural activity meaning nitrate is likely to be washed out of the soils (Rossouw and Watson, 2005).

Several studies have found that during the re-wetting phase of the hydrological cycle nitrate is flushed from soils in the immediate post-drought period (Mosley, 2015; Dabrowski *et al.*, 2017). This is most likely due to the concentration of both inorganic and organic nitrogen in the catchment during the drought phase. When dried soils are rewetted following cessation of flow, the stimulation of mineralisation and nitrification can result in the release of significant amounts of organic nitrogen back into the river system, i.e. in the form of decomposed organic matter (McLaughlin, 2008).

Wang and Evans (1970), in examining changes in ammonium concentrations in the Illinois River, found that ammonium increased with discharge due to runoff effects and then decreased at the highest flow events due to dilution. The ammonium ion is moderately mobile in soil. In the absence of point sources, a limited wash-off effect would be expected for ammonium as discharge increases followed by a levelling off as the nutrient becomes limiting. At very high discharges, a dilution effect may be observed. The potential impact of ammonia on the aquatic environment is influenced by the chemical species present, their relative proportions, and other factors such as pH, temperature, and DO concentrations(Dallas and Day, 2004).

2.4.3 Effects of nutrient enrichment

Codd (2000) described eutrophication as "the nutrient enrichment of waters which result in the growth of algae and aquatic macrophytes, deterioration of water quality and other symptomatic changes that are found to be undesirable and interfere with water use''. Eutrophication is one of the global hot issues for aquatic ecosystems and therefore has become a serious challenge. It has, however, become less of an issue in highly developed countries because effluent control measures have been put in place.

Human activities are considered to be the main cause of eutrophication, as increased concentrations of phosphorus and nitrogen are discharged into water bodies from agricultural run-off, organic waste from humans and stock animals, and industrial effluents. The high concentrations of nutrients and potential variability in climate can lead to an increased frequency of phytoplankton blooms (see section 2.6 below) and an alteration in the trophic state. “Trophic state” characterizes aquatic systems according to nutrient concentrations, it is commonly used to characterize lakes and wetlands. It ranges from oligotrophic to eutrophic, the oligotrophic state being characterised by low nutrient concentrations, limited plant productivity and moderate DO levels (Carlson, 1977). Eutrophic conditions are characterised by high nutrient concentrations and high plant productivity, resulting in DO concentrations fluctuating wildly and algal productivity reaching problematic levels (Komatsu et al., 2007).

Nutrient loading of the watershed directly or indirectly affects trophic state, phytoplankton communities and algal productivity (Kennedy and Walker, 1990; Rezo et al, 2016). When algal bloom densities increase in the water body, they may produce toxins and reduce light availability, affecting phytoplankton and plant communities directly as well as zooplankton and fish communities indirectly (Kotak et al, 1994). It is important to note that not all algal blooms produce toxins, it is mostly produced by a few cyanobacteria (blue-green algae) organisms.

A reduced flow often leads to enhanced autotrophic production resulting in increased biomass of benthic algae, floating algae and submerged angiosperms (Petts, 1987). In a system enriched with nutrients, high turbidity levels can be considered to be beneficial in limiting excessive algal growth and ironically these high turbidity levels are often due to excessive blooms of phytoplankton. The correlation between TSS in the water and the extent of light penetration is important. Reduced light penetration limits the potential for primary production by phytoplankton and rooted macrophytes because the depth of the photic zone is limited (Utete and Tsamba, 2017). Increasing salinity has also been shown to cause the flocculation of suspended sediment and can lead to an increase in the depth of the photic zone.

During algal blooms, sunlight is blocked from reaching the organisms responsible for producing oxygen in the water and cause a decrease in DO levels. Because of the reduced oxygen and elevated pH, this may inhibit the growth of macroinvertebrates and fish, resulting in fatalities (Dabrowski *et al.*, 2013). Cyanobacteria blooms (blue-green "algae") are visible signs of eutrophication, and these blooms contribute to a variety of water-related issues, including contaminating the water with toxins and killing the fish and animals that drink the water (Pieterse *et al.*, 1997).

In a study conducted in the Vaal River, South Africa (Pieterse *et al.*, 1997), indications of pollution and eutrophication were found to be reflected in the high concentrations of chlorophyll-a, inorganic nitrogen and phosphorus. It was also found that the concentration of chlorophyll was highly dependent on the nutrient concentrations as the nutrients are essential for primary plant production. Therefore, in the present study, the relationship between nutrients and chlorophyll a concentrations will be investigated.

2.5 Chlorophyll and methods of measuring chlorophyll in surface water

2.5.1 Chlorophyll

Chlorophyll is present in many organisms including algae and some species of bacteria i.e. Cyanobacteria. Cyanobacteria has the ability to conduct photosynthesis as it is the only bacteria that has chlorophyll a, which is required for photosynthesis. Cyanobacteria also contain phycobiliproteins which are protein pigments and amino-acid storage complexes, that act as photosynthetic accessory pigments (Horváth *et al.*, 2013). These phycobiliproteins are restricted to cyanobacteria and a few other eukaryotic algal classes, and they include phycocyanin.

Chlorophyll plays a central role in the process of turning light energy into usable chemical energy. It absorbs light in both the red-orange and blue-violet spectrums (Walsh, 2019). Chlorophyll a, b, c, and d are only found in specific types of organisms. They influence the colour of the organism, and certain types of chlorophyll can only be found in algae (Walsh, 2019). Chlorophyll a is a green pigment found in organisms that photosynthesize, including both land plants and algae. Chlorophyll a concentration in the water column is directly dependent on the light intensity, penetration, turbidity and nutrient concentration (Zohary and Ostrovsky, 2011).

Chlorophyll b is another green pigment. It is found in plants and green algae and absorbs light in the blue-violet spectrum. It is not found in the same concentrations as chlorophyll a and is not found in all photosynthetic organisms, so it is referred to as an "accessory pigment." Chlorophyll c is found only in certain types of algae. It is primarily found in marine algae such as diatoms, dinoflagellates, and brown algae. The pigment is an accessory pigment that appears as a blue-green colour. Chlorophyll d is a rare type of photosynthetic pigment found only in a few marine species of red algae and cyanobacteria (Walsh, 2019).

Chlorophyll a is required for the survival of phytoplankton. The term phytoplankton refers to a community of chlorophyll-containing suspended aquatic microorganisms. Phytoplankton can be used to assess the overall health of a body of water. Monitoring chlorophyll a levels is a direct way of tracking algal abundance because the ratio of chl: phytoplankton biomass is fairly consistent; thus, measuring chlorophyll can be used as a surrogate for measuring photosynthetic activity (Wang and Liu, 2005; Prasad *et al.*, 2010). Surface waters that have high chlorophyll concentrations are typically high in nutrients, generally phosphorus and nitrogen i.e. phytoplankton responds to nutrient availability (Higgins, 2014; Qiao *et al.*, 2017; Chen *et al.*, 2018).

Chlorophyll measurements can be used to estimate the entire phytoplankton concentration, while phycocyanin can be used to specifically measure cyanobacteria. Since cyanobacteria are the only phytoplankton that contains phycocyanin, this makes the pigment to be a good indicator of cyanobacteria concentrations in surface water.

Surface water algal content can be monitored, and databases and quality control protocols can be developed over time to characterize the trophic status of lakes and streams. These characterizations have the potential to be used for indirect monitoring and detection of pollutant indicators like phosphorus and nitrogen. Monitoring chlorophyll a concentrations in the field is a rather affordable alternative to the frequent collection of grab samples regularly for costly and time-consuming laboratory analysis of chlorophyll a (see section 2.6.2 below) (Higgins, 2014).

Fluorometry, spectrophotometry, and high-performance liquid chromatography (HPLC), which requires chlorophyll extraction for analysis, are some techniques for measuring chlorophyll (Higgins, 2014). Chlorophyll fluoresces, which means that when exposed to light of a specific wavelength, it emits light of a higher wavelength (lower energy). All commercial fluorometers capable of measuring chlorophyll *in situ* are based on its ability to fluoresce. The determination of cyanobacterial presence in surface water can also be done by detecting phycocyanin through in-situ fluorometric field applications, by remote sensing, and by in-vitro extraction (Horváth *et al.*, 2013).

2.5.2 Laboratory methods

The traditional method of determining the amount of chlorophyll in surface waters is spectrophotometry. It entails collecting relatively large amounts of water, filtering the samples to extract the chlorophyll-containing organisms, mechanically rupturing the collected cells, and extracting the chlorophyll from the disrupted cells into an organic solvent such as acetone. The extract is then analysed using either a spectrophotometric method (absorbance or fluorescence) based on chlorophyll's known optical properties or by HPLC (Higgins, 2014).

Morgan et al. (2006) detailed the process of analysing for chlorophyll content in water samples,"Sestonic chlorophyll a samples were collected for laboratory analysis. All processing and analysis were done in low-light conditions to avoid the degradation of photosynthetic pigments. High concentrations of chlorophyll a in samples were diluted to bring them within the instrument's acceptable range. Each sample's chlorophyll a concentration was calculated and expressed in milligrams per cubic meter''.

2.5.3 In-situ methods

Chlorophyll is a fluorescent molecule that absorbs and emits light of a specific region in the spectrum. The fluorometric instruments used *in situ* induce chlorophyll to fluoresce by shining a beam of light of an appropriate wavelength into the sample and then measure the intensity of the emitted light as a result of the fluorescence process. This property of chlorophyll a and phycocyanin is what allows for the fluorometric method to be able to measure real-time data. The data produced shows chl a concentrations in µg/l and phycocyanin in ppb (Turner Designs, 2014), however, the two units can be used interchangeably as 1 µg/l = 1 ppb.

To quantify fluorescence, a high-sensitivity photodiode is typically used, which is screened by an optical filter that limits the detected light. When the 470 nm exciting light is backscattered off particles in the water, the filter prevents it from being detected (Higgins, 2014; Turner designs, 2016). Without the filter, turbid (cloudy) water would appear to contain fluorescent phytoplankton even though none were present.

YSI has developed optical sensors (fluorometers) for chlorophyll determination. The sensor is designed for in-situ use and can collect large amounts of chlorophyll data in either point sampling or continuous monitoring applications (Hayashi et al., 2005).

Turner Designs have developed fluorescence measurement equipment claimed to offer high sensitivity, specificity, simplicity and speed (Turner designs, 2016). When compared to spectrophotometers, fluorometers achieve ‘1000 to 500 000 times' better detection limits. Because fewer materials absorb and emit light, fluorometers are also highly specific and less susceptible to interferences. The fluorometers’ sensitivity and specificity reduce the sample preparation procedures that are often required before analysis in spectrophotometry (Fleming, 2016). For this study, the Turner designs Aqua Fluor Handheld Fluorometer/Turbidimeter was used for the measurement of chlorophyll a for the reasons mentioned in section 2.6.3.

2.5.4 Advantages and disadvantages of the methods

Most commercial fluorometers are divided into two types: benchtop instruments, which have superior optical versatility and capability but are comparatively costly and hard to use in the field, and field-type fluorometers, which have a fixed optical configuration, are more easily used in the field and are usually compatible with data-collection platforms (Higgins, 2014).

The spectrophotometric methods have downsides in that they are time-consuming and typically require experienced and efficient analysts to produce consistently accurate and reproducible results. Furthermore, they do not lend themselves easily to continuous chlorophyll monitoring because collecting and processing samples at reasonable time intervals would be time-consuming (Morgan et al., 2006; Higgins, 2014). The biggest disadvantage of spectrophotometers is that they cannot easily be used in the field.

Fluorometers that are hand-held are fairly easy to use even *in situ*. It does not require the collection of large volumes of water and there are virtually no running costs aside from the costly standards used to calibrate the machine (Marion *et al.*, 2012). Fluorescence detection using Turner designs cyclops-7 Submersible Fluorometer is more sensitive, faster and less expensive than other quantitative measures and requires no sample handling and it is the one used in this study.

CHAPTER THREE - SITE DESCRIPTION AND METHODS

3.1 Introduction

This chapter describes the study area in terms of climate, geology, hydrology and land-use activities. The description includes spatial maps that show differences in land use and the locations of the sampling points selected for this study.

3.2 Study area - Nuwejaars catchment

The study area is located in the Nuwejaars catchment (Figure 3) which in turn is located within the greater Heuningnes catchment. It has an area of 760km^2 and falls within the Cape Agulhas Municipality. The catchment is located on the southernmost point of Africa and falls within the Breede-Gouritz Water Management Area. The catchment of the Nuwejaars River covers quaternary catchments G50B (A) and G50C (B), as defined by the national Department of Water and Sanitation.

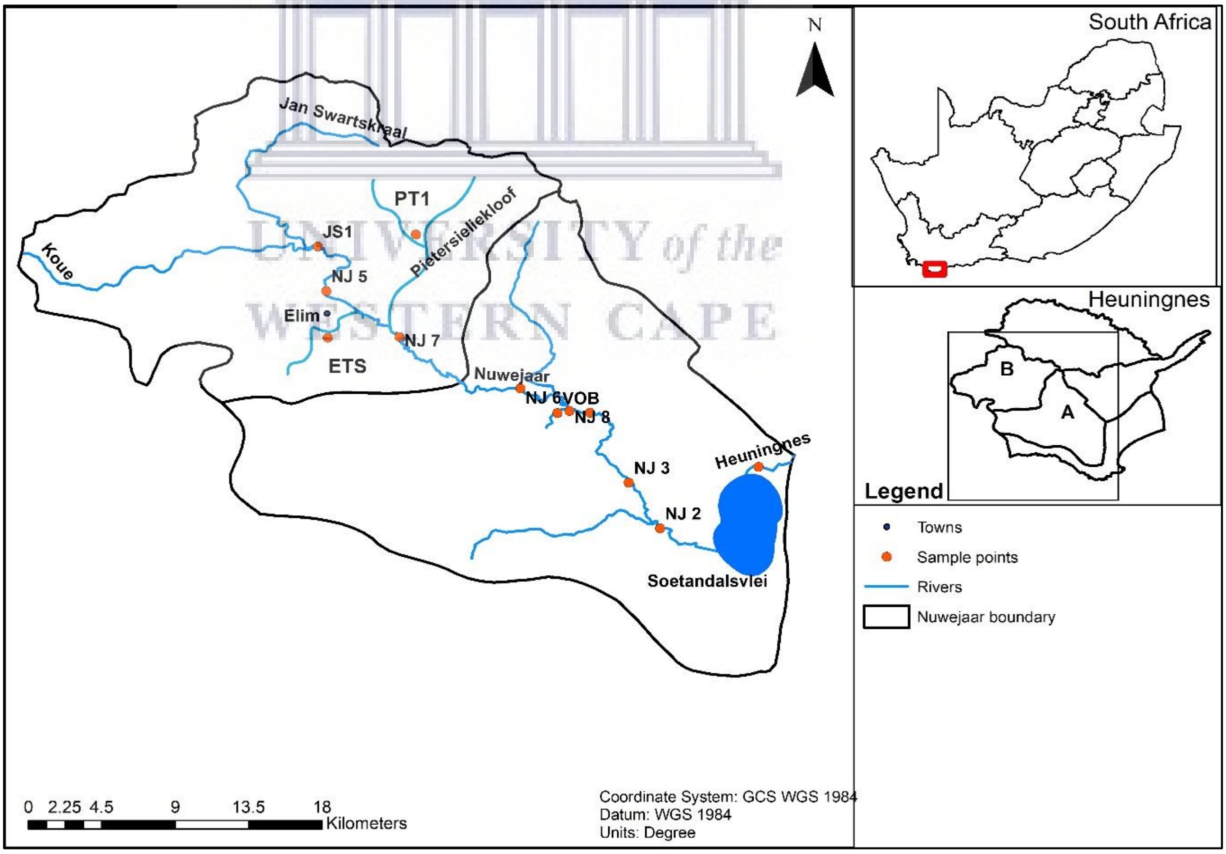


Figure 3: The Nuwejaars Quaternary Catchment (G50B and G50C) with surface water sampling points

3.2.1 *Climate*

During summer, from December to March, the average maximum temperatures are about 25°C and in winter, from June to August, 18°C (Mazvimavi, 2018). The average minimum temperatures during the winter months are about 9°C. Sub-zero temperatures are rarely experienced due to the warming influence of winds from the Indian Ocean (Kraaij et al, 2009). The study area lies in a region that receives its highest rainfall during winter, although rainfall is received throughout the year. The winter months, June to August, receive the highest rainfall of about 50-56mm/month. From December to February, the average monthly rainfall is 18-23mm/month. The average annual rainfall varies from 455mm//year in the lower part to about 600mm/year in the northern mountainous areas (Herdian et al., 2005; Kraaij et al., 2009). It must be noted that drought conditions were experienced during the study period in the study area as well as the greater Western Cape Province. During this period the river ceased to flow for some months, which is extremely unusual for the normally perennial river.

3.2.2 *Geology*

The dominant geologies in the Nuwejaars Catchment are the Table Mountain Group (TMG), Bokkeveld Group and the Bredasdorp Group. The upper catchment of the Nuwejaars River is dominated by sandstone, quartzite and shales of the TMG. Within the catchment outcrops of intrusive Cape Granite Suite and Malmesbury Group occur (Bickerton, 1984; Kraaij et al. 2009).

The Table Mountain Group overlies and is intruded by the crystalline basement units of the Malmesbury and Cape Granite Suite groups. The northern and western part of the catchment is dominated by quartz, sandstone and shale which are TMG rock types.

The Bokkeveld Group is sandwiched between the Bredasdorp Group and the TMG. The Bokkeveld Group is intruded by basement lithologies and is prone to weathering. Shale and sandy shale of the Bokkeveld Group dominate the catchment area between Elim and Soetendalsvlei.

The Bredasdorp Group is overlain by the Bokkeveld Group of the Cape Super Group units. It is made of quaternary deposits, calcified dune sand and coastal limestone dominating the areas around Soetendalsvlei and the Heuningnes Estuary (Bickerton and Pierce, 1984).

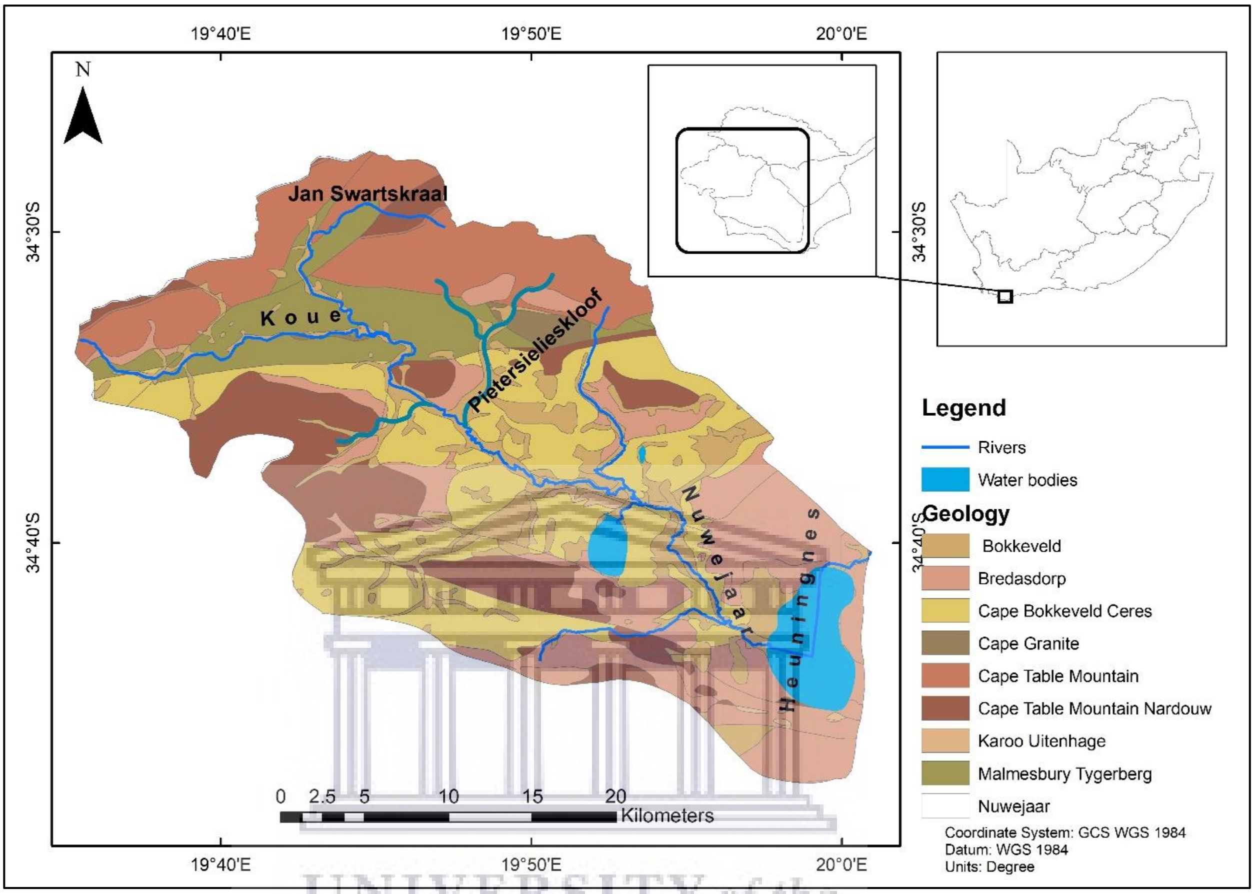


Figure 4: Geology of the Nuwejaars Catchment.

3.2.3 *Vegetation*

The Nuwejaars Catchment falls within the Cape Floristic Region, which is a plant-rich area located in the Western and Eastern Cape Provinces of South Africa. The Cape Floral Region has protected biodiversity hotspots that consist of a variety of endemic vegetation types (Blokker et al., 2015).

The Agulhas area has a wide range of contrasting soil types and systems (Thwaites and Cowling, 1988 as referenced by Cowling et al, 1988). These include the Bredasdorpberg land system, which has acid colluvial sands; the Hagelkraal land system, which consists of limestone hills and neutral colluvial sands; the Elim land system, with rolling shale lowlands with duplex soils; the Moddervlei land system, alluvial valleys and vleis with seasonally or permanently waterlogged duplex soils;

and the Die Dam land system which has calcareous coastal dunes (Cowling *et al.*, 1988).

Four natural vegetation types are recognised in the region, namely, Strandveld, Limestone Fynbos, Elim Fynbos and Mesic Mountain Fynbos (Moll et al, 1984 as referenced by Cowling et al 1988). The fynbos vegetation all leach weak organic acids into natural waters, providing the dark colour and low pH in the river.

Forest and thicket were most common on deep colluvial sands derived from the Bredasdorp Formation limestone and on calcareous coastal dunes; these have however been transformed by agriculture. Renoster shrubland is confined to the moderately fertile duplex soils derived from the Bokkeveld Group shale in the Elim land system. It also occurred in the valleys of the Moddervlei land system where acid infertile sands overlie saline clay.

Colluvial sands of mixed origin are dominant and are always seasonally waterlogged and usually neutral to alkaline in nature. The underlying bedrock is either TMG sandstone or Bredasdorp Formation limestone. The shallow sandy soils overlying the outcrops are nutritionally similar to those derived from TMG sandstones and are very oligotrophic.

Alien vegetation, including such species of *Acacia*, *Eucalyptus* and *Pinus* trees, have severely invaded the upper reaches of the catchment, especially along the riparian zone. They change the water balance and take up a lot of nutrients (Mkunyana, 2018).

3.2.4 Land cover and land use

Fynbos shrublands cover about 40% of the Heuningnes catchment. These are followed by cultivated lands, which cover about 39%. Surface water bodies such as pans and lakes cover a significant part, especially of the Nuwejaars catchment (Mazvimavi, 2018). Unvegetated land occurs mostly on mountaintops, as rocky outcrops. The Agulhas National Park, which is located in the catchment, lies at the southern extremity of Africa and it supports diverse fauna and flora, including numerous indigenous species of plant and aquatic animals (Cowling *et al.*, 1988).

Except for the national park, nearly all of the land in the Nuwejaars catchment is privately owned by farmers who grow crops and raise livestock. Barley, canola, and wheat are the main crops. Grape cultivation, particularly for winemaking, is becoming an increasingly important land use. On the majority of farms, cattle and/or sheep are raised, and dairying is practised on a small number of them (Mazvimavi, 2018).

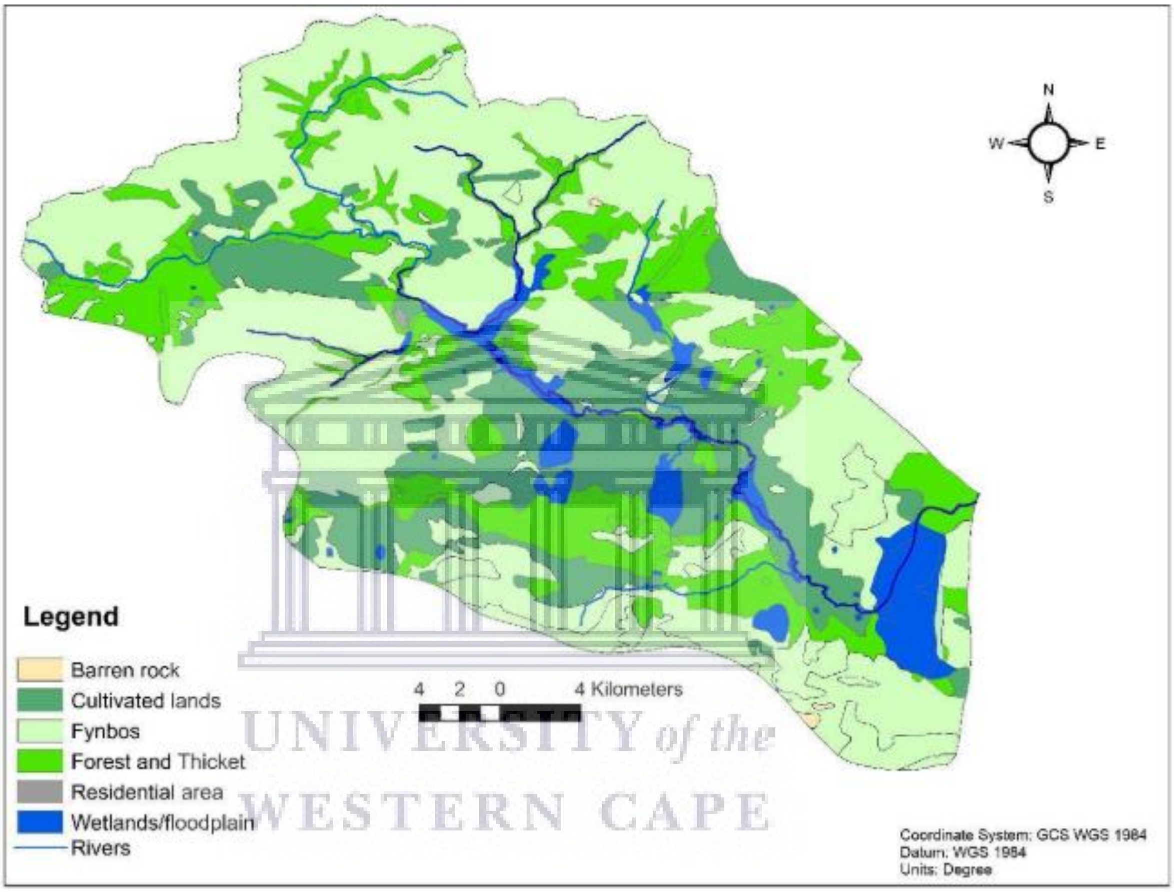


Figure 5: Land-use map of the Nuwejaars Catchment (from Malijani, 2020).

3.2.5 Groundwater

The study area is underlain by several primary (intergranular) and secondary aquifers. The primary aquifer exists along the river and in areas of Bredasdorp Group rocks. The secondary aquifers are mostly associated with fractures in the hard consolidated sediments of the Bokkeveld and the Table Mountain Group formations (Banda, 2019). The Table Mountain Group holds regional sandstone aquifers that primarily control groundwater flow through fractures. Due to the fractured nature of the Table Mountain Group Formation, groundwater recharge rates are expected to be relatively higher than in the Bredasdorp Group rocks, especially where it outcrops in the higher mountains (Mazvimavi, 2018; Banda, 2019).

According to Mazvimavi (2018), connections exist between groundwater and surface water in the upper reaches of the catchment. Springs upstream provide water from deep aquifer systems and are a source of constant base flow for the tributaries. On the other hand, due to low yielding geological formations and low gradients downstream, groundwater and surface water in the lower sections of the catchment are poorly connected (Banda, 2019).

Springs upstream within the TMG region of the catchment are closely linked to fault systems and are valuable sources of freshwater. They supply the town of Elim with water for domestic use all year round and feed rivers during the dry season. Springs in the upper catchment also provide water for irrigation purposes (Mazvimavi, 2018).

3.2.6 Hydrology

The Nuwejaars River has the following tributaries: the Koue, Wolwegatskloof, Jan Swartskraal, Boskloof and Uintjieskuil (Bickerton, 1984). The Nuwejaars River flows through the north-eastern reaches of the Agulhas National Park into one of South Africa's largest freshwater lakes, Soetendalsvlei (Russel and Impson, 2006). The Kars River drains the eastern part of the Heuningnes catchment and the Nuwejaars River, the western, before entering Soetendalsvlei (Hoekstra and Waller, 2014).

Due to the relatively un-impacted nature of the Nuwejaars and Kars rivers, the upper reaches have been identified as priority rivers for conservation initiatives (Herdien et al., 2005), while the lowermost part of the catchment on the Agulhas Plain is of the greatest conservation interest from a plant point of view. The Nuwejaars River has a system of riparian wetlands, the development of the wetlands being largely influenced by the low gradient of the area. The wetland system drains into the Agulhas Plain forming seasonal and permanent water bodies, including Soetendalsvlei and Voevlei (River Health Programme, 2011). Downstream of the Nuwejaars River is a system of smaller pans not linked to any fluvial system; these pans are intermittent in nature in that they flood during rainy winters and dry up during summer as high temperatures result in higher evaporation rates (Russel and Impson, 2006).

Due to the nature of the Mediterranean climate experienced in the study area, the rivers are subject to highly seasonal flows. The area experiences winter seasons that are extremely wet and summer seasons that are dry with little to no rain for consecutive months. Therefore, during the dry season, the river may have sections with permanent flows, a series of isolated pools, and even completely dry areas. This occurred during the study period, although extreme drought conditions seldom occur in the area.

3.2.7 Description and location of the study sites

The number of sampling points varied during the duration of the sampling period (July 2017 to October 2019) because in 2017 the area suffered from a severe drought, which led to substantial drying of rivers and soils.

The sampling stations were selected to cover the upper, middle and lower reaches up to the point of entry to Soetendalsvlei to examine variations in the river concerning land-use, geology and surface water-groundwater interactions.

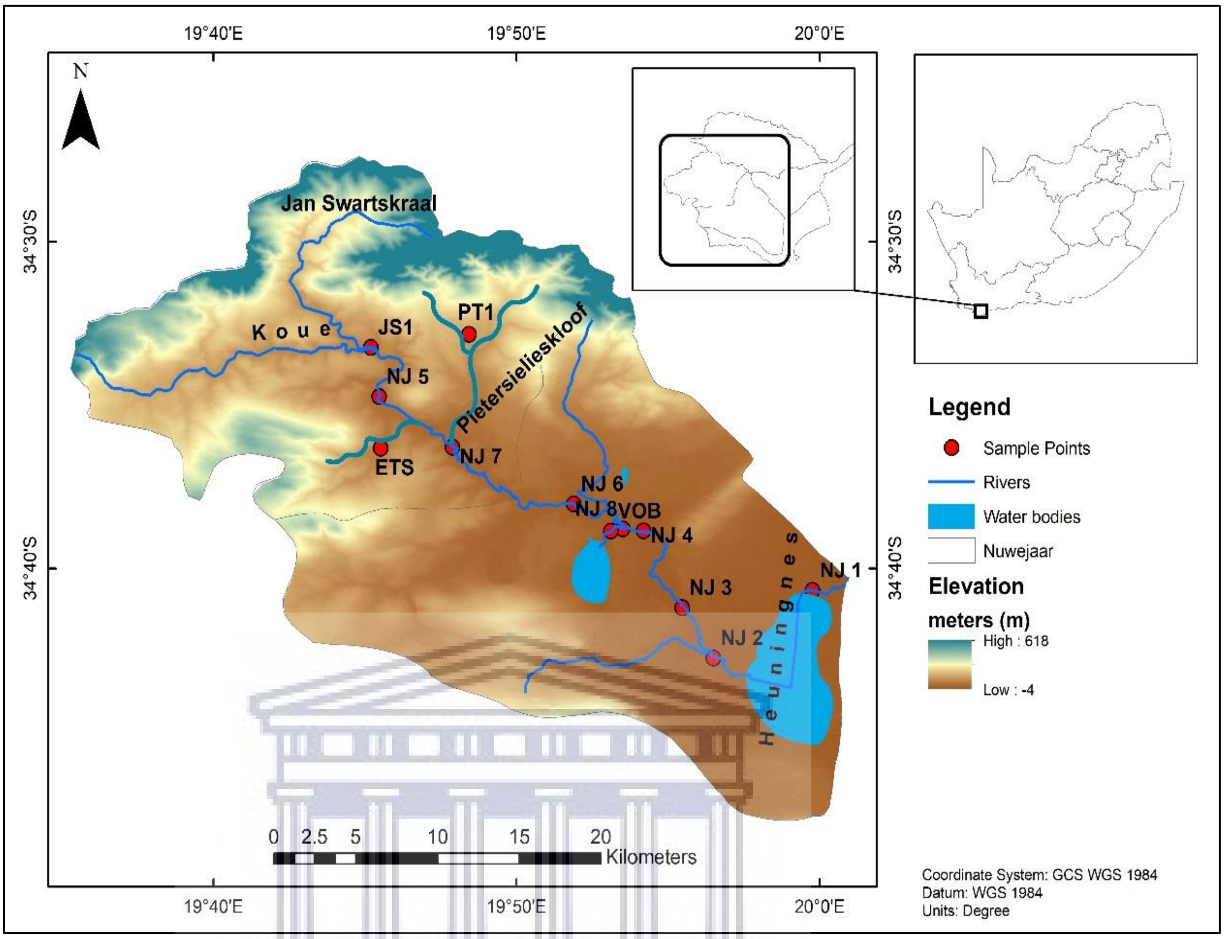


Figure 6: Location and distribution of sampling sites in the catchment.

*NJ= Nuwejaars river, PT= Pietersieslieskloof river, VOB= Voevlei outlet bridge, JS= Jan-Swartskraal river, ETS= Elim flow diversion.

Table 2: Location and description of surface water sampling sites. See Figure 6 for the site map.

Site Name	Description of location	Latitude	Longitude
NJ 1*	At Soetandalsvlei outlet bridge	-34.6779	19.9961
NJ 2	Close to SANParks offices	-34.7125	19.9417
NJ 3	At a dairy farm	-34.6867	19.9245
NJ 4	At a hay field close to Elandsdrift farm	-34.6475	19.9032
NJ 5	Near Voelvlei outlet bridge	-34.5791	19.7579
NJ 6	At Nuwejaars river bridge	-34.6337	19.8648
NJ 7	At Moddervlei site	-34.6048	19.7981
NJ 8	At the bridge in Elim	-34.6464	19.8918
VOB	At Voelvlei outlet	-34.6476	19.8852
PT1	Pietersieslieskloof River	-34.5472	19.8073
JS1	Jan-Swartskraal River	-34.5540	19.7532
ETS	Elim flow diversion	-34.6053	19.7587

* Downstream of Soetendalsvlei.

3.3 Data collection

For this study secondary data was used. The samples were collected by the Institute for Water Studies at the University of the Western Cape and most were analysed by Malijani (2020).

Seven sampling campaigns were conducted Studies to characterize the chemistry of the surface water during dry and wet seasons. Samples collected in July 2017 and July 2018 represent the onset of the wet season, while samples collected in October 2017, November 2018 and October 2019 represented the beginning of the dry season and those collected in March 2018 and February 2019 represent the driest months of summer. All samples collected in 2017 and 2018 were collected during the drought period.

3.3.1 Field sampling

Field parameters, i.e. pH, EC, temperature, DO, phycocyanin and chlorophyll a, were measured *in situ* during daylight hours while water samples were collected for nutrient analysis in the laboratory. Surface water samples were collected at the selected sampling sites along the river while it flowed, but during the dry season in the absence of flow, water samples were sometimes collected in pools that formed along the river channel.

Samples for laboratory hydro-chemical analysis were collected in 250 ml polypropylene plastic bottles that had been pre-cleaned with 10% HCl and rinsed with deionized water. Samples for analysis in the laboratory were filtered through a 0.45 µm Munkell filter and kept cool in a fridge at a temperature of 6°C until they could be analysed.

3.3.2 Field measurements of physicochemical parameters and chlorophyll

A HACH HQ40d multi-meter was used to measure temperature, EC, pH and DO. The calibration of the multi-meter is done using the Hach® buffer and standard solutions for pH, DO and conductivity. To determine the accuracy of the multi-meter and to determine if calibration is satisfactory, the measured parameter (pH, EC or DO) value should agree with the known parameter value of the buffer solution.

Chlorophyll-a and phycocyanin were both measured *in situ* without filtration or extraction using the Turner Design Model 10-000R AquaFluor® Handheld fluorometer/turbidimeter. Prior to the measurement of phycocyanin and chl a, the instrument was calibrated using the liquid dye standards used to calibrate the

AquaFluor, converting relative fluorescence which is the emitted light in nm to concentration estimates in µg/l.

The fluorometry procedure involved pipetting 4ml of water into a cuvette, which was then placed in the fluorometer. Two separate channels were used, one for phycocyanin and without changing the cuvette, the channel was changed to B and relative chlorophyll-a concentrations were measured. The detection limit for chlorophyll a is 0.3µg/l and for phycocyanin is 1 µg/l.

3.3.3 Laboratory analysis of water samples

Nutrients were analysed using the HACH DR 6000 UV/VIS spectrophotometer and a HACH DR 20 reactor for digesting samples for analysis for total nitrogen and total phosphorus. Low-range methods were selected to ensure that detection limits (Table 3) would be suitable for samples with low concentrations (Malijani, 2020). When measured concentrations were below the measuring range of the method, samples were recorded as below the detection limit of 0.3 mg/l. Standards recommended by the HACH Company claim a precision of 95% confidence. A 95% confidence interval is based on the notion that if a study were repeated as many times as possible and the results will provide a 95% confidence. Table 3 summarises the methods, detection limits, accuracy check methods and standards used during the analysis of nutrients.

Table 3: Analysis methods and standards for accuracy checks used during nutrient analyses performed with the HACH DR 6000 UV/VIS spectrophotometer and a HACH DR 20.

Parameter	**Method**	**Measuring range mg/l**	**Standard for accuracy checks**
Nitrate (NO_3^{-}-N)	Cadmium Reduction	0.3-30	Nitrate Nitrogen
Total Nitrogen	Persulfate Digestion	0.5-25	Ammonia Nitrogen
Total Phosphorus	PhosVer3 with Acid Persulfate Digestion	0.003	Phosphate standard solution
Phosphorus (PO_4^{3-}-P)	Molybdovanadate	0.3-45	Phosphate standard solution

Details are available from https://www.hach.com/

3.4 Data analysis

3.4.1 Statistical analysis

Descriptive statistics (minimum, maximum, median, standard deviation) and correlation analyses were used to describe the nature of the data as well as to describe relationships between variables. The Pearson Correlation was applied to measure the strength of the relationships between two variables. A strong relationship between two variables is represented by an $R^{2 \text{ value}}$ close to +1 or -1 while a weak relationship is depicted by an R^2 value close to 0. An R^2 value close to +1 means that there is a strong positive correlation while one that is close to -1 means there is a strong negative correlation between variables; p values > 95% (or 0.95) are taken to be statistically significant.

Descriptive data analysis of the water quality dataset was performed through Microsoft EXCEL software and multivariate analysis such as the cluster analysis and the principal component analysis were performed through IBM® SPSS® Statistics 21 software. Principal component plots were produced using the NCSS 2021 Statistical Software.

Multivariate statistical analysis is a quantitative approach that allows for the classification of samples, studies the correlations between their chemical constituents and evaluate the similarity between the sampling sites (Solomon, 2013). Multivariate analytical methods are frequently used for a better understanding of data that includes several variables, such as water chemistry, or environmental and biological data (Solomon, 2013; Barakat *et al.*, 2016; Atique and An, 2019).

To achieve the objectives of this study multivariate techniques were applied to:

1. determine the degree of similarity in water chemistry between the sampling stations.
2. look for correlations between concentrations of the two plant pigments and each of the nutrients.

Principal component analysis (PCA), Cluster analysis(CA), Discriminate analysis (DA) and Factor analysis (FA) were used in the scientific literature because of their ability to treat large datasets of temporal and spatial parameters obtained from various study sites. PCA and CA have proven to be important statistical tools for identifying relationships among various physicochemical parameters and sources of pollutants, as well as for grouping sites or parameters into similar clusters (e.g. Barakat *et al.*, 2016; Atique and An, 2019).

Multiple regression analyses were performed using the IBM® SPSS® Statistics 21 software to group variables that best predict the magnitude of a dependent variable. The procedure examines the degree of significance of each independent variable in predicting the dependent variable (Mertler and Reinhart, 2016). Prior to conducting the regression analyses, data were screened for missing values and outliers, as well as evaluated for test assumptions. Test assumptions were that there is a linear relationship between the variables, in the test the residuals are independent, the residuals have constant variance at every level and the residuals of the model are normally distributed. The regression output included three parts i.e. model summary, ANOVA summary table, and coefficients table.

To investigate similarity and dissimilarity in temporal and spatial variability between sampling stations, PCA and CA were used. Prior to conducting the PCA and CA data was screened for missing data and outliers as well as evaluated for test assumptions of linearity and normality. PCA is a data analysis tool that is used to reduce the number of variables of a large number of interrelated variables while retaining as much information as possible (SPSS, 2020). PCA based on the correlation matrix was performed to understand the underlying relationships between the water quality variables of all the monitoring stations and to identify their characteristics (Barakat *et al.*, 2016).

Cluster analysis (CA) is a method that provides a means of classifying a given set of variables into clusters, based on similarity or closeness measures. It differentiates members of one group from another by representing them in a graphical form called a dendrogram, which makes data interpretation easy and understandable (Solomon, 2013). The similarity between variables is measured according to Euclidean distance, which is the square root of the sum of the squared differences between values of the parameters (Fredline, 2012).

3.5 Limitations of the study

- Due to the drought at the beginning of the study, the river was not flowing at all sites on each sampling occasion. Instead, where possible surface water samples were collected from pools within the river channel. Where water had entirely dried up, there are therefore gaps in the data set.
- Due to lockdown regulations enforced by the Covid-19 pandemic, no samples could be collected for the year 2020. This resulted in the study being a desktop study using solely secondary data collected by the Institute for Water Studies at the University of the Western Cape.
- Also, if it had not been for Covid-19 the traditional method for measuring chlorophyll concentrations i.e. spectrophotometry would have been conducted to allow for

comparison with concentrations obtained with the fluorometer. As it was, I did not have access to the UWC laboratories and so was unable to perform these analyses.

CHAPTER FOUR – RESULTS AND DISCUSSION

4.1 Introduction

In the following sections, the water chemistry of the Nuwejaars Catchment is characterised by assessing temporal and spatial differences in the chemical constituents of the surface water. The key results obtained are discussed and compared to previous studies findings.

4.2 Physico-chemical parameters

Table 4 provides a descriptive summary of the data, while detailed results are presented in Appendix 1.

Table 4: Statistical summary of physicochemical properties of the surface waters (n=12) in the Nuwejaars catchment, July 2017- October 2019 (Units: Temperature in °C, EC in mS/m, DO in mg/l).

Parameter	Sampling month	Mean	SD	Min	Max
EC (mS/m)	July (2017)	841.05	1111.98	54.80	3740.00
	October (2017)	166.16	277.03	50.10	998.00
	March (2018)	700.50	880.28	35.50	2590.00
	July (2018)	681.94	967.50	26.50	2744.40
	November (2018)	319.40	574.92	43.13	2091.20
	February (2019)	1937.24	3785.16	51.67	12724.37
	October (2019)	286.60	538.21	32.90	1961.73
Temperature (°C)	July (2017)	15.92	2.41	12.20	19.70
	October (2017)	19.44	2.94	13.60	24.50
	March (2018)	23.41	1.71	19.40	26.00
	July (2018)	15.07	2.48	11.40	19.30
	November (2018)	22.18	2.26	18.57	26.70
	February (2019)	23.32	2.85	19.27	30.47
	October (2019)	16.63	1.52	14.00	18.64
pH	July (2017)	7.39	0.67	6.44	8.41

	October (2017)	7.15	0.64	6.23	7.90
	March (2018)	7.54	0.85	6.11	8.84
	July (2018)	6.88	0.95	5.56	8.08
	November (2018)	7.40	0.92	6.10	8.30
	February (2019)	7.73	0.83	6.01	8.48
	October (2019)	6.69	0.83	4.34	8.38
DO (mg/l)	July (2017)	8.12	2.68	4.67	12.75
	October (2017)	7.65	1.72	3.80	9.51
	March (2018)	7.25	2.42	3.63	11.93
	July (2018)	6.30	2.09	3.31	10.23
	November (2018)	5.13	1.60	2.36	6.92
	February (2019)	5.36	2.77	0.42	8.98
	October (2019)	6.35	3.18	2.74	12.44

4.2.1 Electrical Conductivity (EC)

Electrical conductivity (EC) values recorded for surface water during the sampling period (Table 4) ranged between 26.5 mS/m and 12 724.4 mS/m.

The highest EC values were recorded at downstream river sites, where some values exceeded 1000 mS/m. EC values were particularly low upstream in the catchment, as this section is underlain by the Table Mountain Group sandstone and the upstream area is still somewhat pristine, i.e. the water quality is not greatly impacted by human activities.

During the dry season the river had become disconnected and formed pools along the river channel, hence some water samples were collected from the pools. Pools are characterised by standing water are susceptible to chemical changes (Larned *et al.*, 2010). The chemical changes are primarily driven by evapotranspiration influences the amount of water available within the system, thus resulting in the variation in the water chemistry of the pools. The boxplot for EC data was log-transformed to minimise the effect of outliers as well as to allow for a better representation of the distribution of results.

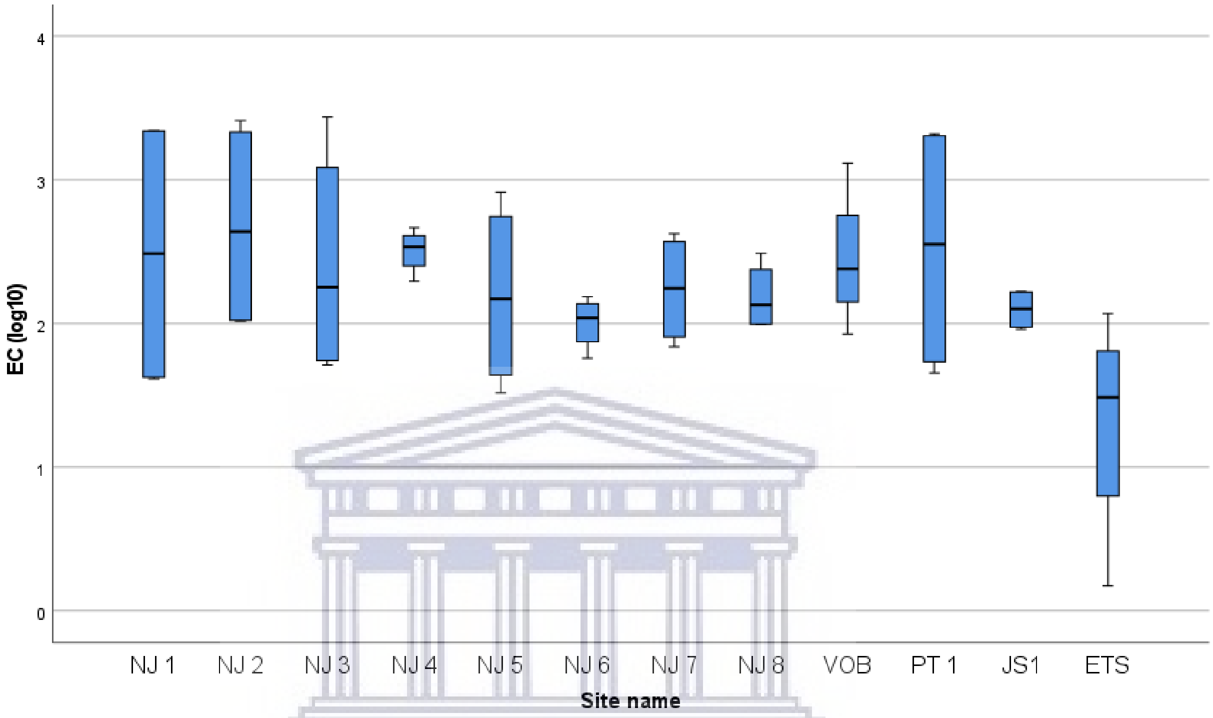


Figure 7: Boxplots ($\log_{10}$) for average EC values at the sampling sites (n=12) during the study period July 2017- October 2019.

4.2.2 *Temperature*

The measured temperature values for surface water are presented in Table 4. The surface water temperature ranged between 11.4°C and 30.4°C during the period of the study. The lowest temperatures were recorded during the winter wet seasons of July 2017 and July 2018. The highest surface water temperature was recorded during the dry summer season in March 2018, November 2018 and February 2019, with February 2019 having the highest temperature of 30.4°C. Although high temperatures were expected for the dry season, samples in October 2019 had surprisingly low temperatures with a mean of 16.6°C. A mean difference of 7.7°C between the lowest and highest mean values recorded was observed in the surface water temperatures.

There were strong variations in surface water temperature in the Nuwejaars River. The variations can be attributed to the time of sampling, that is the season and actual time of day the measurement was done. Water temperatures were slightly lower upstream. This difference in water temperature can be attributed to differences in elevation i.e. water temperatures at higher elevations are lower because temperature generally decreases with an increase in elevation.

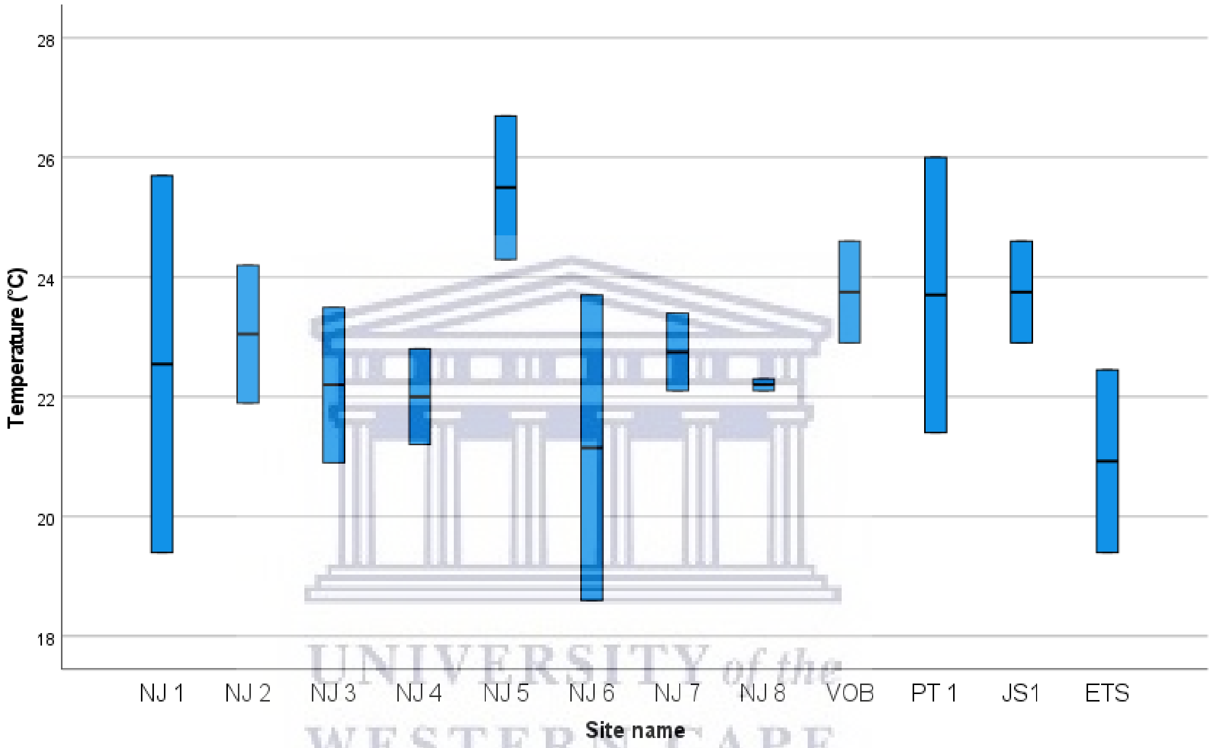


Figure 8: Boxplots for average temperature at the sampling sites (n=12) during the study period July 2017- October 2019.

4.2.3 pH

The pH values recorded for surface waters during the sampling period are presented in Table 4. The pH values ranged between 4.34 and 8.84.

The pH of most natural waters is commonly close to neutral, ranging between 6 and 8 (Dallas and Day, 2004), as was the case with most of the obtained results during the study period. Lower pH values were recorded, however, the lowest being 4.34 during the dry season in October 2019. The low pH value can be attributed to the fact that during this month samples were collected from a pool rather than a flowing river. In the pool, the water is not flowing and this results in the concentration of ions due to the lack of dilution. Furthermore, streams in the south Western Cape are more acidic in the winter than in the summer (Britton, Day and Henshall-Howard, 1993) because of the release of acidic humic substances from the soil and groundwater during winter rains (Day et al., 2019).

Lower pH values were detected in the higher-altitude streams, waters becoming more alkaline at lower altitudes (Bickerton, 1984), which can be attributed to the presence of leachates from fynbos vegetation (Raubenheimer and Day, 1991) in the upper catchment. Surface water pH values towards the coast were close to 8.2. The alkaline nature of the water can be attributed to the calcareous nature of the southern Agulhas plain (Mazvimavi, 2018). The presence of alkaline water downstream can be partly attributed to photosynthetic processes that occur as the river flows through the floodplain of the river but more so to the alkaline sands over which they flow.

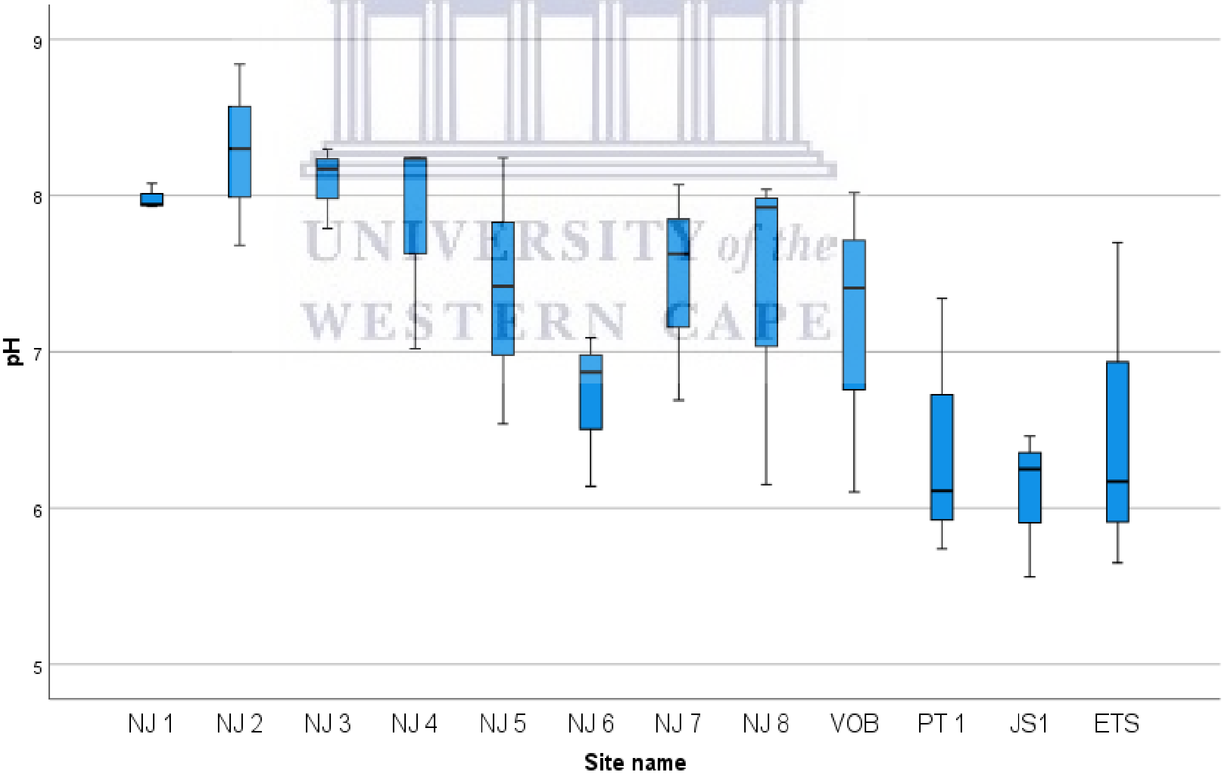


Figure 9: Boxplots for average pH at the sampling sites (n=12) during the study period July 2017- October 2019.

4.2.4 Dissolved Oxygen (DO)

Dissolved oxygen (DO) concentrations recorded during the sampling period are presented in Table 4. DO concentrations ranged between 0.42 mg/l and 12.75 mg/l.

DO concentrations are expected to be higher during winter months when temperatures are lower because [DO] increases with a decrease in temperature (Dallas and Day, 2004). The detection of lower DO concentrations during the winter period of July was thus not expected, but the low DO concentration may be attributable to other factors such as the decomposition of organic material or changes in photosynthetic rates by plants, both of which influence the amount of DO present in the water.

In October 2019 DO concentrations were distinctively high because on the day of the sampling trip the temperatures were low hence the high concentration of DO on this sampling occasion. Furthermore, the time of day at which DO is measured has an impact on the values recorded, because the concentration of DO varies diurnally due to temperature fluctuations. The level to which pools formed during the drying out period of NPRs might be anoxic is likely to depend on site-specific features. If algae and macrophytes are abundant, diurnal DO and pH may increase during the day due to photosynthesis (Williams, 2006), whereas in pools with little to no primary production, DO concentrations may decrease due to respiration and decreased solubility.

Low DO concentrations in the lower regions of the catchment are caused by the decrease in flow velocity from the high lying to the low lying sections, as a result of changes in surface topography (Mazvimavi, 2018). DO readings downstream that were above 12 mg/l can be attributed to being a result of photosynthesis of riparian vegetation, photosynthesis causes an increase in DO concentrations (Dallas and Day, 2004). High DO concentrations recorded upstream can be attributed to the lower temperatures at high elevations and also to turbulence caused by flowing water resulting in more O_2 dissolving in the water. Sites JS1, NJ6 and NJ7 had fairly low DO concentrations, dissolved oxygen is often used up by aerobic decomposer bacteria when the algae die they take up oxygen in the decomposition process. However, this was not evident in the phycocyanin and chlorophyll a concentrations obtained at this site.

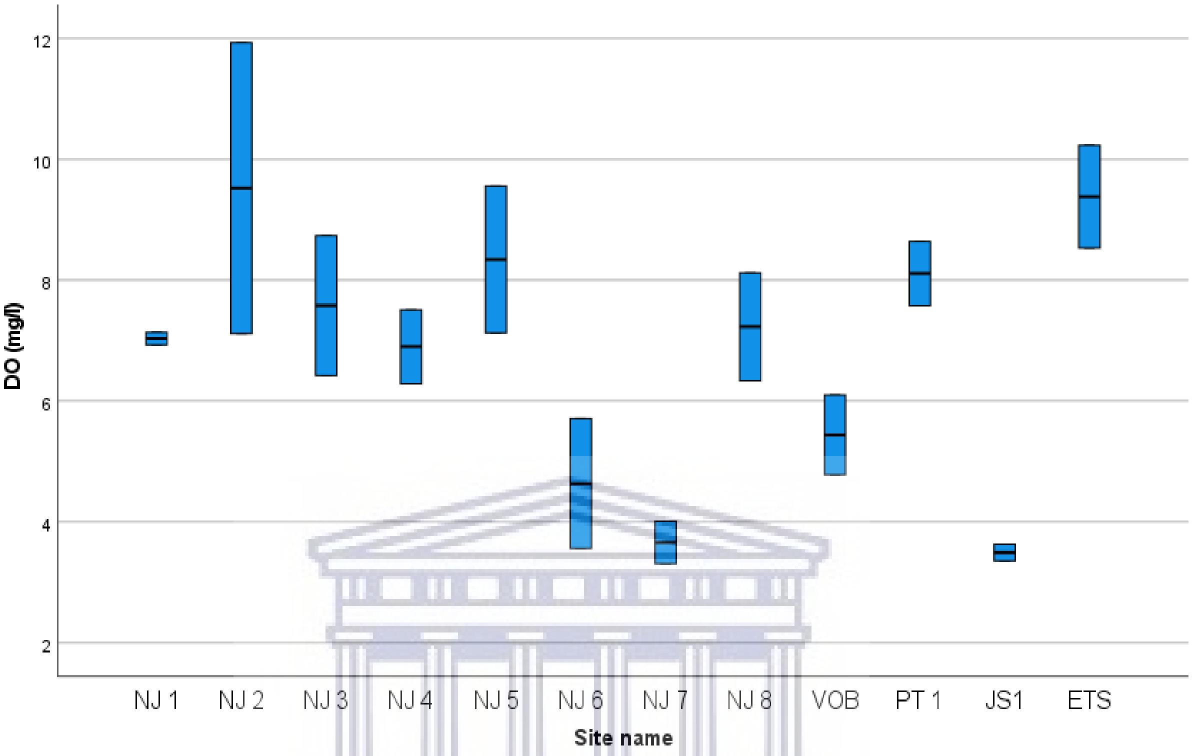


Figure 10: Boxplots for average DO concentrations at the sampling sites (n=12) during the study period July 2017- October 2019.

4.3 Nutrients, Chlorophyll a and Phycocyanin.

Concentrations of nutrients, chlorophyll a and phycocyanin recorded for surface waters are listed in Table 5. The table provides a summary of the descriptive data obtained and detailed results are presented in Appendix 2.

Table 5: Statistical summary of nutrient, phycocyanin and chlorophyll a concentrations (mg/l) in the surface waters (n=12) of the Nuwejaars Catchment.

Parameter	Sampling month	Mean	SD	Min	Max
Nitrate (NO_3^- -N)	July (2017)	0.23	0.18	0.10	0.70
	October (2017)	0.11	0.12	<0.1	0.30

	March (2018)	0.30	0.15	0.10	0.60
	July (2018)	0.51	1.18	<0.1	4.20
	November (2018)	0.59	1.00	0.10	3.50
	February (2019)	1.25	1.08	0.10	4.20
	October (2019)	0.79	0.62	0.10	2.30
Phosphate (PO_4^--P)	July (2017)	1.73	0.61	0.30	2.30
	October (2017)	2.63	0.63	1.40	3.60
	March (2018)	3.23	5.77	0.20	18.60
	July (2018)	1.70	0.72	1.00	3.70
	November (2018)	3.10	1.88	0.10	7.40
	February (2019)	3.54	2.42	1.00	9.70
	October (2019)	5.15	4.33	1.00	15.60
Total Nitrogen	July (2017)	0.72	0.55	0.20	2.00
	October (2017)	0.45	0.07	0.30	0.50
	March (2018)	0.93	1.14	0.40	4.40
	July (2018)	2.26	5.30	0.10	18.90
	November (2018)	0.93	1.15	0.20	4.30
	February (2019)	5.28	11.49	0.10	41.40
	October (2019)	6.13	1.45	4.70	7.60
Total Phosphorus	July (2017)	2.11	0.57	0.70	2.70
	October (2017)	2.89	0.58	1.90	3.80
	March (2018)	3.74	5.99	0.50	20.00
	July (2018)	1.95	0.73	1.30	3.90
	November (2018)	3.83	1.94	0.40	7.80
	February (2019)	3.36	1.57	1.32	6.95
	October (2019)	1.20	2.47	0.21	9.23
Chlorophyll a	March (2018)	0.34	0.42	0.03	1.35
	July (2018)	4.07	2.69	0.01	8.69
	November (2018)	4.65	1.73	1.85	8.28
	February (2019)	19.56	39.62	0.35	139.43

	October (2019)	4.18	1.44	2.51	8.04
Phycocyanin	March (2018)	0.94	1.25	0.11	3.87
	July (2018)	1.54	1.12	0.29	4.07
	November (2018)	1.13	0.62	0.20	2.14
	February (2019)	4.25	2.47	1.68	8.60
	October (2019)	2.87	2.19	1.34	8.01

4.3.1 Nitrate and Total Nitrogen (TN)

Nitrate concentrations in the Nuwejaars Catchment ranged from undetectable to 4.2 mg/l. The highest concentrations were observed in July 2018 (wet season), November 2018 and February 2019 (dry season). The standard deviations of the nitrate concentrations in the years of measurement show that in the year 2018, both dry and wet seasons had larger deviations than the year 2017 and 2019.

Total Nitrogen (TN) concentrations in the Nuwejaars Catchment ranged between 0.1 and 41.4 mg/l, with some samples exceeding the minimum and maximum detection limits of the HACH DR 6000 UV/VIS spectrophotometer used to measure the concentrations. High concentrations were observed in July 2018 (wet season) and February 2019 (dry season), with February 2019 having the highest concentration. The samples collected in 2019 had the highest standard deviation, indicating that TN concentrations in February and October 2019 were more variable than those recorded in the year 2017 and 2018.

High concentrations of 4.2 mg/l nitrate were observed at river site JS1, obtained on two different sampling trips, one in July 2018 and one in February 2019. It is important to note that at this particular site water was collected from a pool rather than a flowing river, thus the increased concentrations of nitrate can be attributed to the lack of flow of water in the pool which results in a lack of dilution. Since a high concentration of nitrate was measured on two separate events it is important to determine the source of the nitrate. Since the pool is located upstream of the dairy farm and there are no other apparent sources of nitrate addition, it can be assumed that the high concentrations are solely a result of the stagnation and lack of dilution of water in the pool.

TN concentrations were fairly low, except for increases at site NJ2 in four consecutive sampling trips i.e. July 2018, November 2018, February 2019 and October 2019, suggesting that there might be an active source of nitrogen in the catchment, for example, the dairy farm. Nitrate in the Nuwejaars River thus has the potential of promoting the growth of phytoplankton that may, in turn, be detrimental to the water quality of the river. The boxplot for TN data was log-transformed to minimise the effect of outliers as well as to allow for a better representation of the distribution of results.

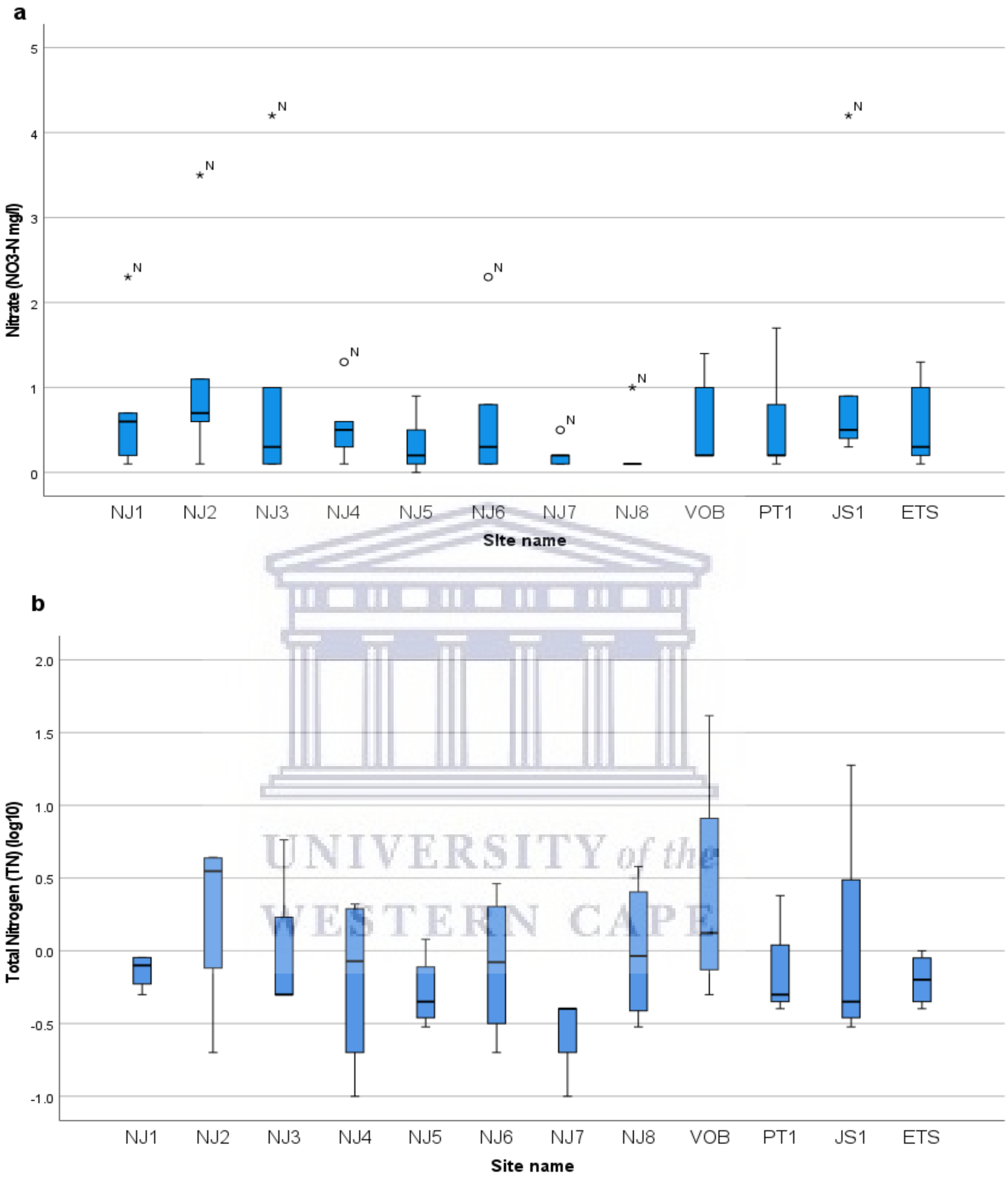


Figure 11: Boxplots for average NO_3^--N (a) and total nitrogen (b) concentrations at the sampling sites (n=12) during the study period July 2017- October 2019.

It is evident in the spatial distribution map (Figure 12) and the boxplot (Figure 11a) that sites NJ2, NJ3 and JS1 had the highest concentrations of NO_3^--N, with sites NJ2 and JS1 containing outliers that were measured in March 2018 and July 2018 respectively. It is important to note that the dairy farm is located right next to site NJ3 and the dairy farm has the potential of acting as a non-point source of nutrients. All the sites downstream of the dairy farm had high nitrate concentrations, as did JS1.

Sites VOB and JS1 had the highest measured concentrations of TN since these sites are located at confluences between the Nuwejaars River and tributary rivers i.e. Voevlei and Jan-Swartskraal. It can be inferred that the source of TN is from the said tributaries as there are no apparent anthropogenic sources of TN along the Nuwejaars River close to these sites.

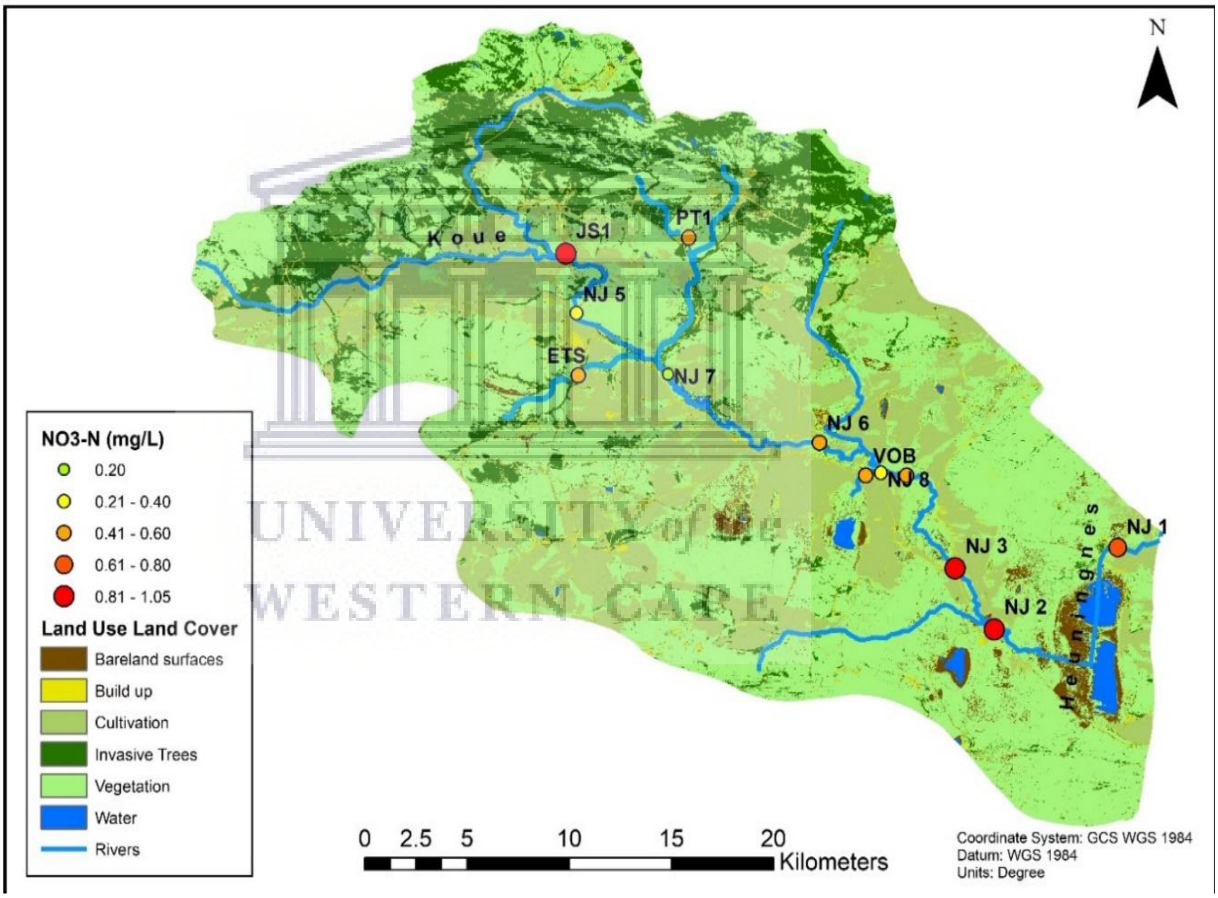


Figure 12: Spatial distribution map of average* NO_3^--N concentrations in relation to land use and land cover in the Nuwejaars Catchment. *average of all results for a site during the study period.

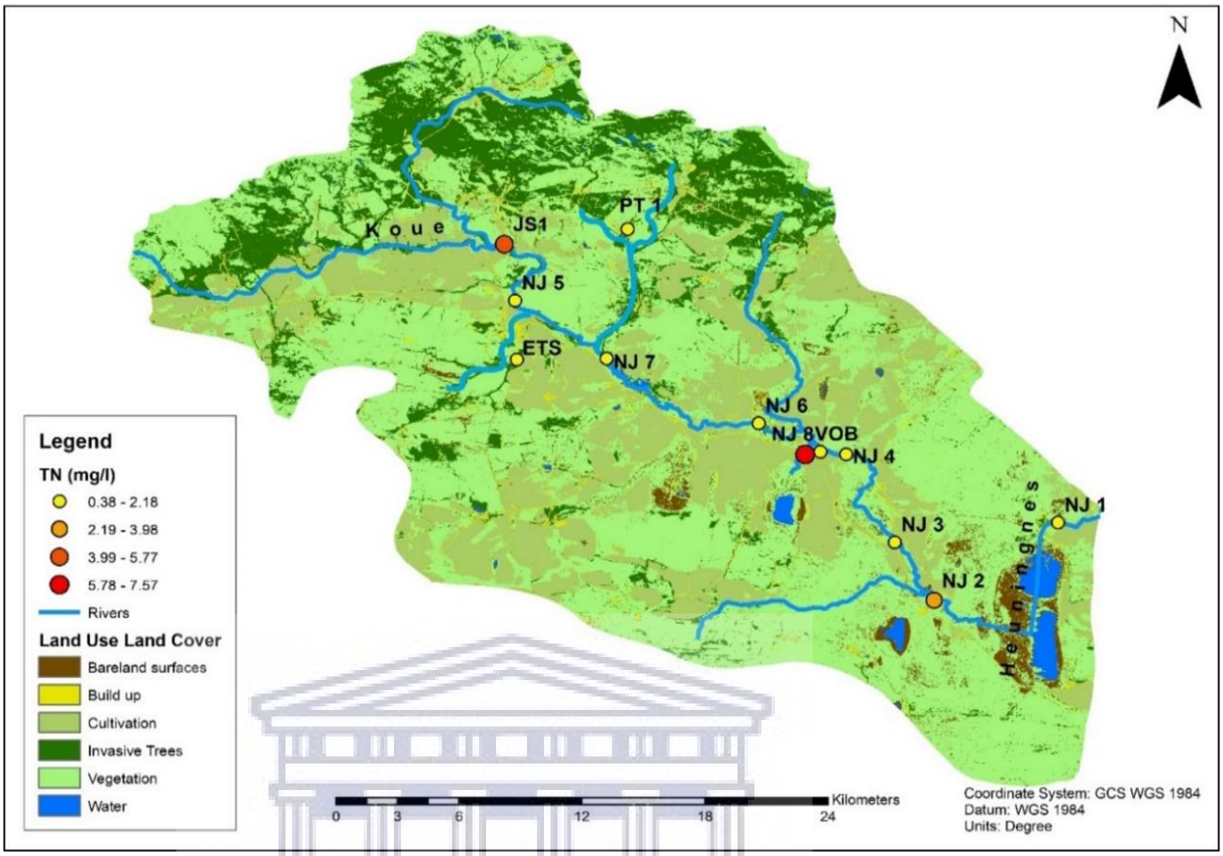


Figure 13: Spatial distribution of average total nitrogen concentration in surface water in relation to land use and land cover in the Nuwejaars Catchment.

4.3.2 *Phosphate and Total Phosphorus*

Phosphate concentrations in surface water ranged between 0.1 and 18.6 mg/l, with the highest concentrations occurring during the dry season in March 2018 and October 2019. A high concentration of 9.7 mg/l was also found in February 2019. It was observed that the highest phosphate concentrations were measured in the dry season, whereas during the wet season concentrations were mostly low.

Total Phosphorus (TP) concentrations in surface water ranged between 0.21 and 20 mg/l during the sampling period. As is the case with phosphate concentrations, TP concentrations were high during the dry season and low during the wet season. The lower TP concentrations in the wet season can be attributed to the effects of the dilution process that takes place during the wet season when more rainfall is received. It can also be attributed to adsorption to particulate matter and large organic molecules available in the river. Water samples from two sites (NJ2 and VOB) had particularly high concentrations of TP in March 2018, attributable to reduced river flow and a lack of dilution.

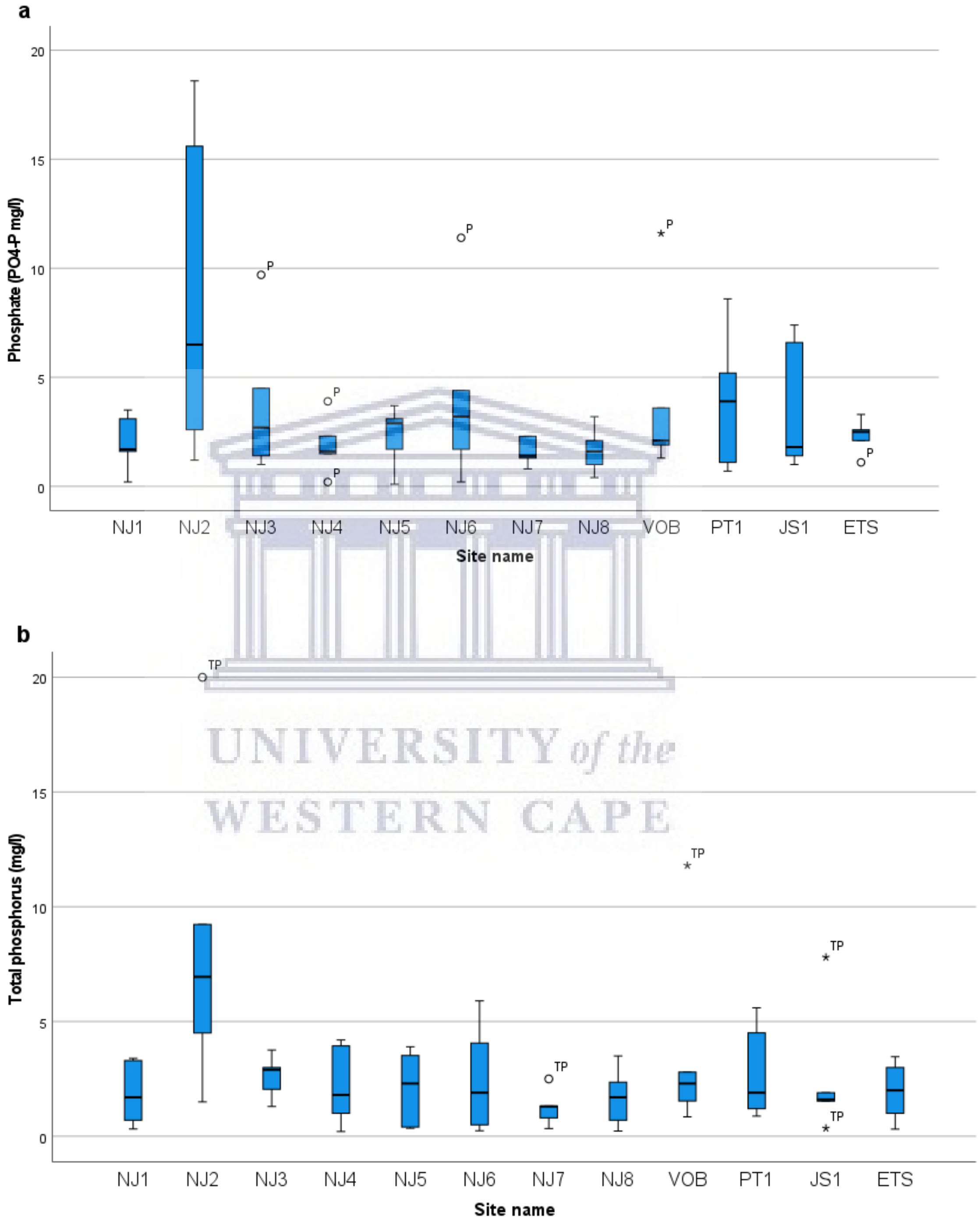


Figure 14: Boxplots for average PO_4^{-3}-P (a) and total phosphorus (b) concentrations at the sampling sites (n=12) during the study period, July 2017- October 2019.

It is important to note that site NJ2 is located directly downstream of the dairy farm which acts as a non-point source of PO_4^{-3}-P hence the observed high concentrations of the nutrients.

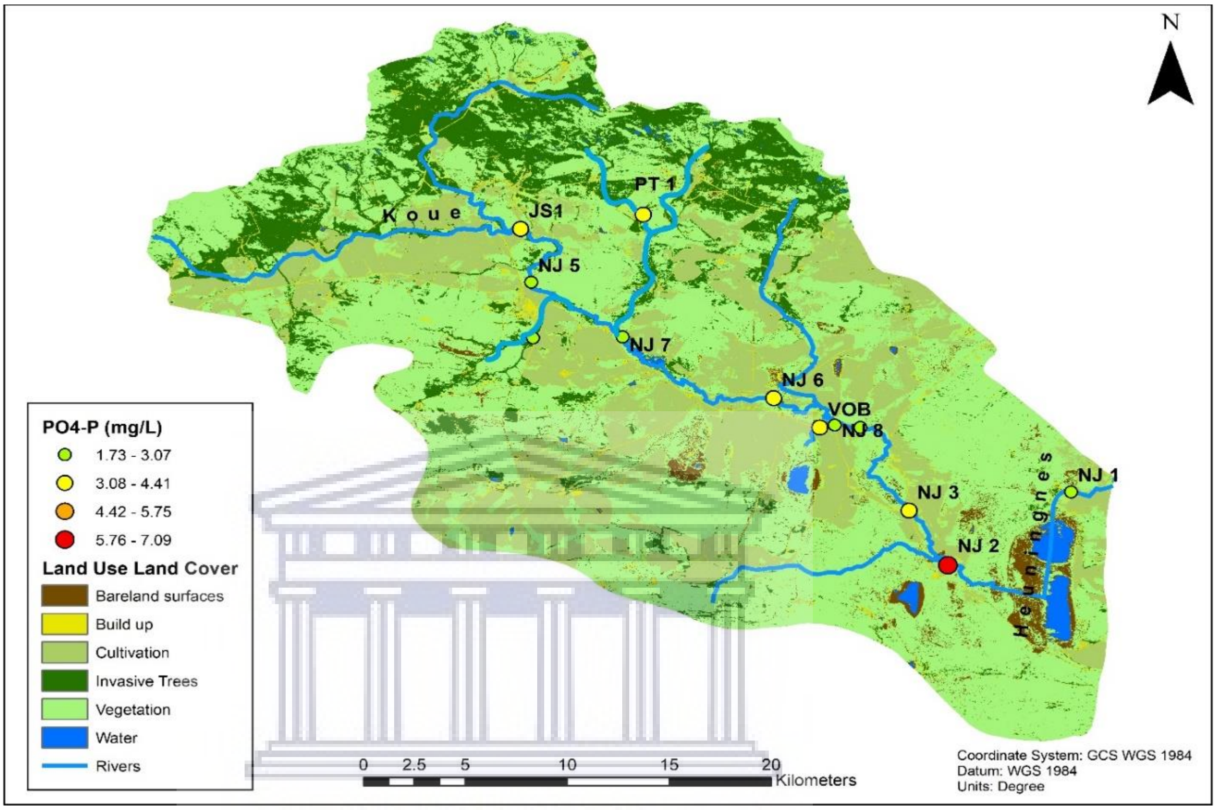


Figure 15: Spatial distribution of average PO_4^{-3}-P concentrations in surface water in relation to land use and land cover in the Nuwejaars Catchment.

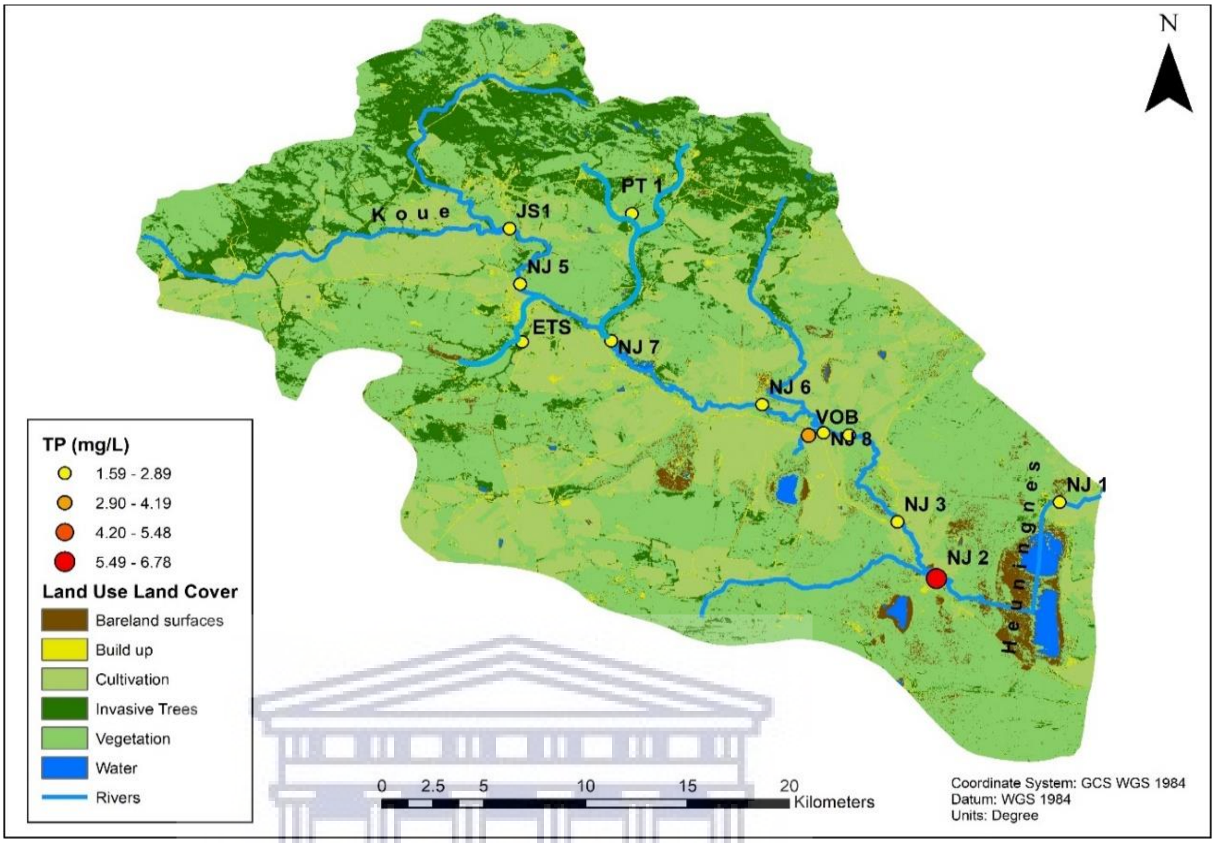


Figure 16: Spatial distribution of average total phosphorus concentrations in surface water in relation to land use and land cover in the Nuwejaars Catchment.

4.3.3 *Chlorophyll a and Phycocyanin*

Chlorophyll a (chl a) concentrations were measured from March 2018 to October 2019. Chlorophyll a values ranged between 0.01 and 139.43 mg/l. The chlorophyll a concentration in March 2018 was extremely low with the maximum value measured being 1.35 mg/l, the lowest maximum value measured for chlorophyll a during the study period.

The highest measured chlorophyll a concentration was 139.43 mg/l, measured in February 2019 at site NJ2. Phycocyanin concentrations ranged from 0.1 to 8.6 mg/l during the duration of the study, with the highest concentrations being measured as site NJ2, which is located directly below the dairy farm.

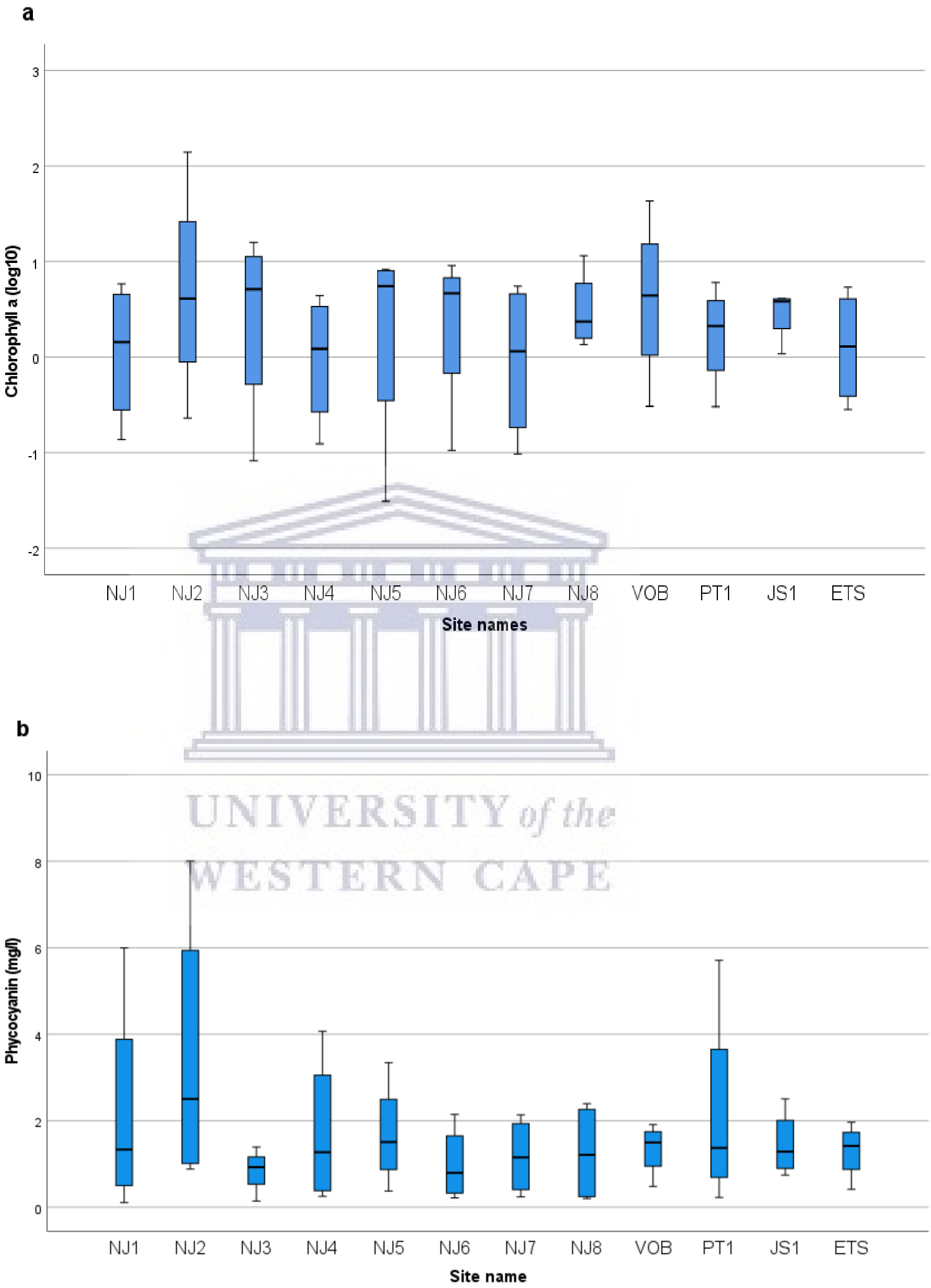


Figure 17: Boxplots of chlorophyll a (a) and phycocyanin (b) at the sampling sites (n=12) during the study period July 2017-October 2019.

Site NJ2 exhibited the highest concentrations of both chlorophyll a and phycocyanin, as can be seen in both the boxplots (Figure 17) and the spatial distribution maps (Figure 18 and 19). The high concentration of chlorophyll a at this particular site can be a reflection of the high TP and TN concentrations also measured at this site.

Other sites that exhibited high concentrations are VOB, PT1 and NJ1; VOB had high concentrations of chlorophyll a, while sites NJ1 and PT1 had high concentrations of phycocyanin. It is important to note that sites VOB and PT1 are tributary rivers to the Nuwejaars River, while site NJ1 is located downstream at the outlet of Soetendalsvlei. Thus it can be assumed that these high concentrations observed at these sites were a result of other factors such as the redox status, temperature, light intensity or the pH occurring within the tributaries and the wetland. This is because when sediment surfaces turn anoxic during summer stratification, phosphorus concentrations may increase dramatically and high pH (> 9.8) may strongly enhance phosphorus release resulting in high photosynthetic activity (Christoffersen and Kaas, 2000).

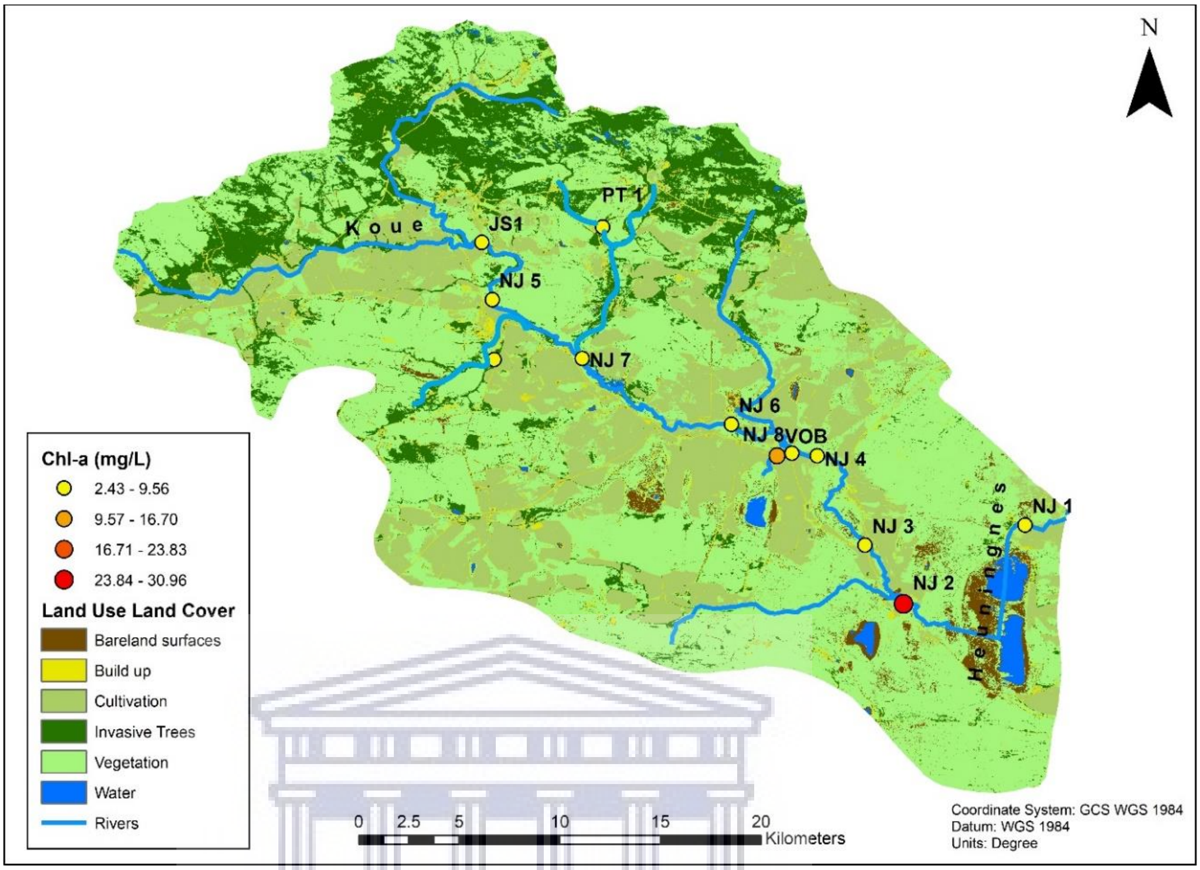


Figure 18: Spatial distribution of average chlorophyll a concentrations in surface water in relation to land use and land cover in the Nuwejaars Catchment.

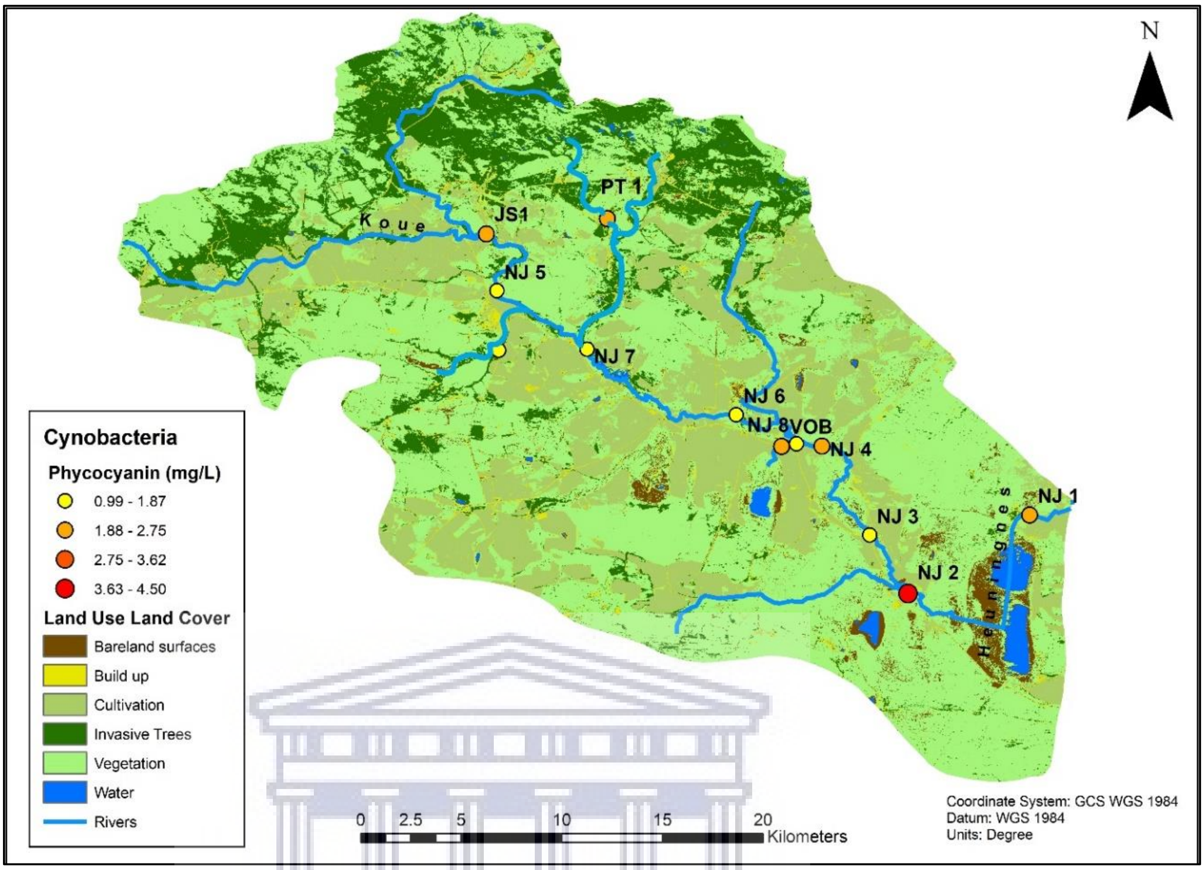


Figure 19: Spatial distribution of average phycocyanin concentrations in surface water in relation to land use and land cover in the Nuwejaars Catchment.

4.4 Statistical Analysis

To further evaluate the relationships between the chemical constituents of the surface waters, multivariate statistical methods (i.e. multiple regression, Hierarchical Cluster Analysis and Principal Components Analysis) were conducted. The statistical methods and procedures are discussed in detail in Chapter 3. The statistical analyses was performed on the variables that are implicated in nutrient enrichment/eutrophication (nitrate, total nitrogen, phosphorus, total phosphorus, chlorophyll a and phycocyanin).

4.4.1 Multiple regression

Two multiple regression models were run to determine which independent variables (total nitrogen, nitrate, total phosphorus, phosphorus) might be predictors of phycocyanin and chlorophyll a concentrations. Regression results for the prediction of chlorophyll a concentrations indicated that the three remaining independent variables significantly predict chlorophyll a concentration (R^2= 0.963, F (3, 8) = 69.884, p<0.001). The model accounted for 96.3% of the variance in chlorophyll concentrations in the catchment.

The regression results for the prediction of phycocyanin indicate that the predictors significantly predict phycocyanin (R^2= 0.846, F (3.8) = 14.703, p<0.001). The model accounted for 84.6% of the variance in phycocyanin concentrations showing that these parameters to some extent play a role in phycocyanin concentrations. Summary tables of the regression models are presented in Tables 6 and 7.

Table 6: Multiple regression model results for chlorophyll a with independent variables nitrate, phosphate, total phosphorus and total nitrogen.

	df	*SS*	*MS*	*F*	*Significance F*	R^2
Regression	3	678.0671	226.0224	69.88496	4.4249E-06	0.963244566
Residual	8	25.87365	3.234206			
Total	11	703.9407				

Table 7: Multiple regression model results for phycocyanin with independent variables nitrate, phosphate, total phosphorus and total nitrogen.

	df	*SS*	*MS*	*F*	*Significance F*	R^2
Regression	3	7.662717	2.554239	14.70357	0.00128013	0.846480723
Residual	8	1.389724	0.173716			
Total	11	9.052441				

4.4.2 Pearson Correlation

The correlation analysis results of the magnitudes of total phosphorus, total nitrogen, nitrate, phosphate, chlorophyll a, phycocyanin, electrical conductivity, temperature, pH and dissolved oxygen values are represented in Table 8.

Table 8: Correlation analysis of total nitrogen (TN), nitrate (N), total phosphorus (TP), phosphorus (P) phycocyanin (Phyco), chlorophyll a (chl a) and physicochemical parameters relationship in the Nuwejaars River.

	TN	N	TP	P	Chl a	Phyco	EC	Temp	pH	DO
TN	1									
N	0.29	1								
TP	0.33	0.49	1							
P	0.33	0.63*	0.95**	1						
Chl a	0.28	0.37	0.98**	0.91**	1					
Phyco	0.24	0.59*	0.89**	0.84**	0.83**	1				
EC	0.14	0.23	0.26	0.39	0.19	-0.02	1			
Temp	0.12	-0.08	0.39	0.27	0.32	0.42	0.08	1		
pH	0.04	0.1	0.64*	0.47	0.61*	0.58*	0.27	0.68**	1	
DO	-0.01	0.16	0.55*	0.39	0.52*	0.67**	-0.33	0.49*	0.44	1

*. Correlation is significant at the 0.05 level (1-tailed).

**. Correlation is significant at the 0.01 level (1-tailed).

Significant correlations are marked with an asterisk. Amongst the parameters that determine nutrient enrichment in rivers and often result in eutrophication, only total phosphorus and PO_4^{-3}-P correlated with both phycocyanin and chlorophyll a. It is important to note that chlorophyll a correlated with phosphate and phycocyanin. The correlation between phycocyanin and chlorophyll can be attributed to the fact that both phycocyanin and chlorophyll are pigments that photosynthesise.

There are positive correlations between the nutrients, with phosphorus correlating with TP and with NO_3^{-3}-N; the correlation between PO_4^{-3}-P and TP is stronger than the one between PO_4^{-3}-P and NO_3^{-3}-N (p values 0.95 and 0.63 respectively). This is expected as PO_4^{-3}-P is included in TP. TN is the only nutrient measurement that did not have a significant correlation with any of the other nutrients, it also did not correlate with any of the physicochemical parameters as well as with phycocyanin and chlorophyll.

Phycocyanin correlated well with four out of the five other parameters related to eutrophication. It had a significant correlation with TP at p = 0.89, chlorophyll a also had a significant correlation with TP at 0.98. With TP being the only nutrient that has significant correlations with both phycocyanin and chlorophyll a it can be inferred that TP plays a significant role in the concentrations of phycocyanin and chlorophyll a in the Nuwejaars River.

Physicochemical parameters significantly correlating with nutrient concentrations are DO and pH, both of which correlated with TP, phycocyanin and chlorophyll a. The correlation of these physicochemical parameters with phycocyanin and chlorophyll a can be attributed to the fact that the concentrations of DO and pH change diurnally and their concentrations are affected by photosynthesis. DO and pH are also significantly correlated with temperature, this can be attributed to the relationship that temperature has with these parameters which is that temperature has an inverse relationship with both DO and pH. However, this may also be incidental as spot temperatures only read at one point in the day.

4.4.3 Cluster Analysis (CA)

The dendrogram (Figure 20) consists of two distinct clusters that are grouped due to their similarity in NO_3-N, PO_4-P, TP, TN, phycocyanin and chlorophyll a concentrations. The hierarchical agglomerative method average (between groups) linkage method was used, and the distance between two clusters is calculated as the average distance between all pairs of subjects in the clusters, which is considered a fairly robust method (Cornish, 2007). The clusters represent the sampling points based on nutrients and are also illustrated in the PCA plot (Figure 22).

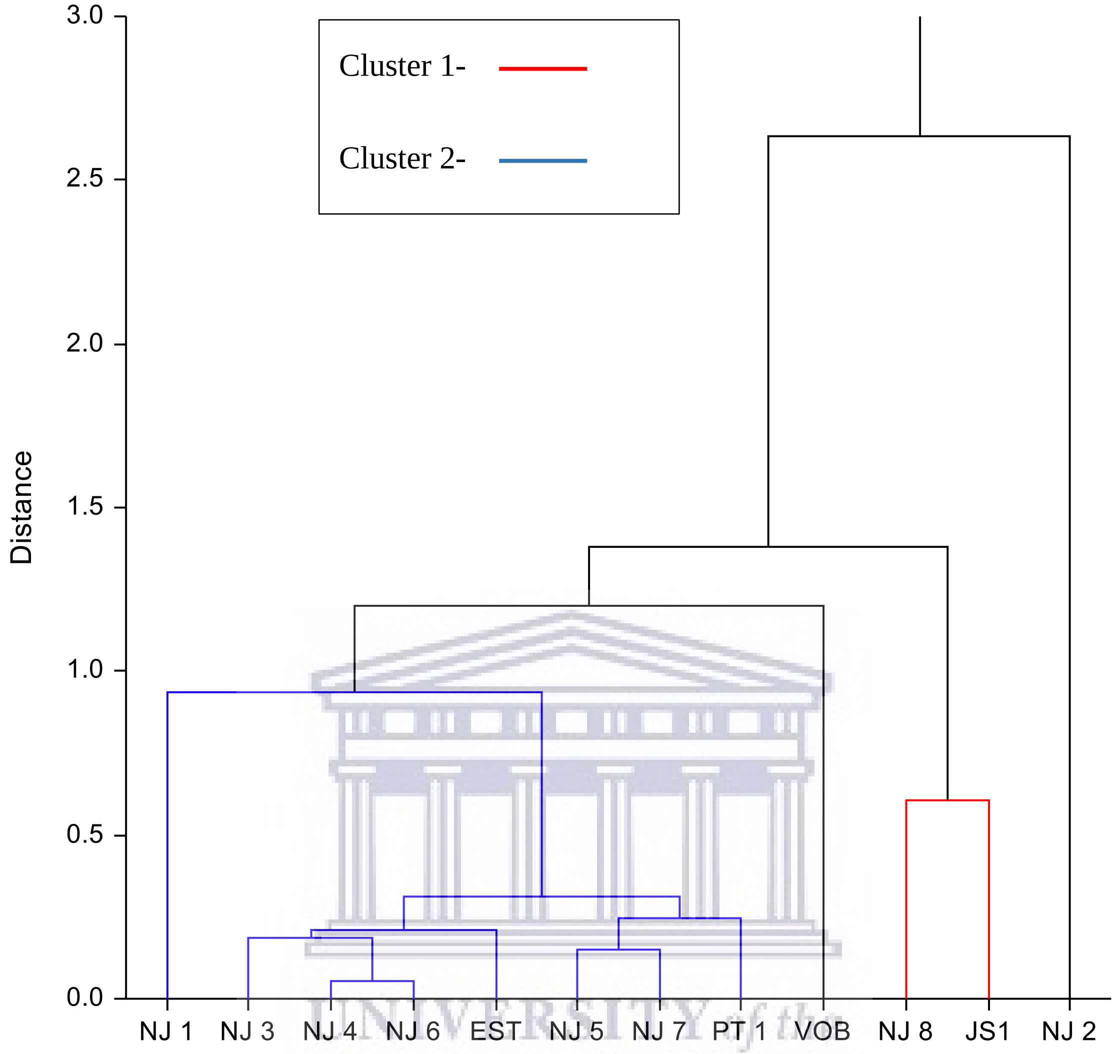


Figure 20: Cluster analysis results of nutrient, chlorophyll a and phycocyanin concentrations in the Nuwejaars River.

Cluster 1 consists of only two sites and Cluster 2 is represented by the large cluster consisting of eight sites. Two sites i.e. NJ2 and VOB did not cluster into any group.

Cluster 1 consists of samples from two sites, NJ8 and JS1. NJ8 is located in the mid-section of the river whilst JS1 is a tributary located further upstream. Cluster 1 consists of samples with similarities in chlorophyll a and phycocyanin concentrations but with contradictory nutrient concentrations. These sampling sites have distinctively high concentrations of both chlorophyll a and phycocyanin, while their nutrient concentrations are relatively low. It may be that the concentration of chlorophyll a and phycocyanin were affected by factors such as temperature, pH and DO that were not included in the Cluster Analysis (Chen *et al.*, 2018).

Cluster 2 consists of water samples collected from eight sites i.e. NJ1, NJ3, NJ4, NJ6, EST, NJ5, NJ7 and PT1 which were collected from both downstream and upstream the river. This cluster consists of samples with relatively low nutrient concentrations, resulting also in low chlorophyll a and phycocyanin concentrations. The clustering of these particular sites can be taken as an indication that these sites receive a similar amount of nutrient enrichment. It is therefore likely that the nutrients are from non-point or diffuse sources, resulting in the unevenness in nutrient concentrations in the sites.

The sites NJ2 and VOB did not cluster in any group. Site NJ2 is located downstream of the dairy farm and site VOB is located upstream of the farm on a tributary of the Nuwejaars River. These outliers possess some similarities and differences from the clustered sites as well as each other. Firstly, site VOB is similar to cluster 2 in that they all have low concentrations of chlorophyll a and phycocyanin, but they are dissimilar in their nutrient concentrations, VOB has higher nutrient concentrations than the sites in cluster 2.

Secondly, site NJ2 is similar to cluster 1, in that they both have distinctively high concentrations of phycocyanin, but there are differences in their nutrient concentrations. NJ2 has the highest nutrient concentration compared to cluster 1. Lastly, site VOB is similar to site NJ2 in that they both have high concentrations of nutrients but of all the measurements, NJ2 has the highest concentration of phycocyanin.

Site NJ2 had the highest concentration of nutrients and this can be attributed to its location, which is it is downstream of the dairy farm, a potential source of nutrient enrichment, resulting in the observed concentrations of nutrients and phycocyanin at the site. From the high concentrations of both chlorophyll a and phycocyanin at this site along with the high concentrations of NO3-3-N, TP and PO4-3-P, it can be inferred that these nutrients play a role in the high concentrations at site NJ2.

Site VOB, on the other hand, has high nutrient concentrations but low chlorophyll a and phycocyanin concentrations, perhaps attributed to factors that were not part of the analysis but that also affect the concentration of chlorophyll a and phycocyanin -pH, temperature, DO, light intensity and flow regime (Morgan *et al.*, 2006).

4.4.3.1 Cluster Analysis with physicochemical parameters

Figure 21 shows two distinct clusters that are grouped according to their similarity in water quality parameters, the parameters are total nitrogen, total phosphorus, nitrate, phosphate, chlorophyll a, phycocyanin, electrical conductivity, temperature, dissolved oxygen and pH. The clusters are also represented in the PCA plot, Figure 23.

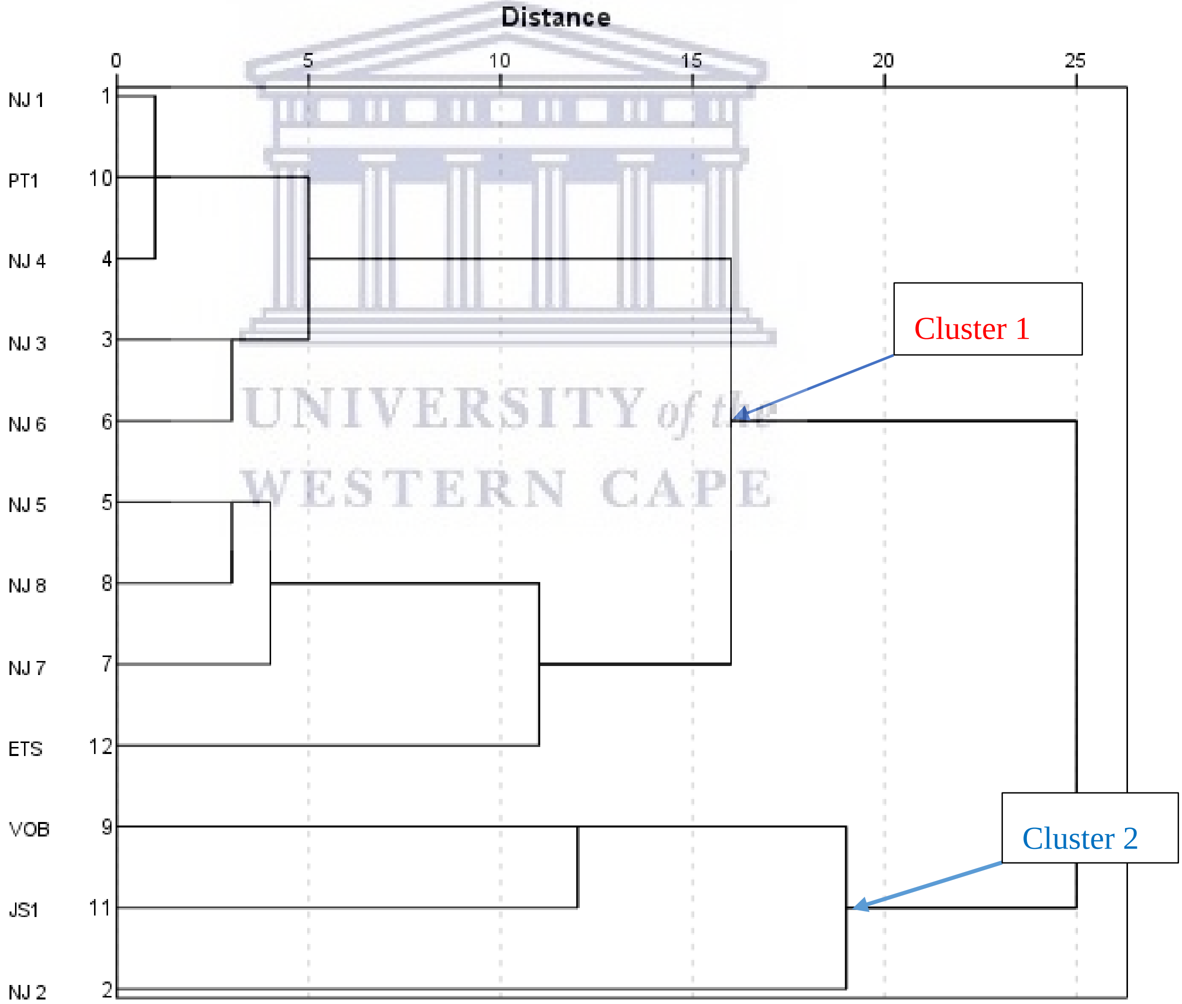


Figure 21: Cluster analysis results between the physicochemical parameters, nutrients, phycocyanin and chlorophyll a in the Nuwejaars River.

Cluster 1 consists of sites NJ1, PT1, NJ4, NJ3, NJ6, NJ5, NJ8, NJ7 AND ETS, these sites have fairly low nutrient concentrations and low phycocyanin and chlorophyll a concentrations. This applies to all the sites. Site ETS is dissimilar from the other sites in that it has the lowest readings of electrical conductivity, temperature and pH compared to the other sites in cluster 1.

Cluster 2 consists of sites VOB, JS1 and NJ2, these sites have distinctively high concentrations of chlorophyll a, phycocyanin, phosphate, total phosphorus and total nitrogen. Site NJ2 is slightly dissimilar to VOB and JS1 in that it has a higher concentration of chlorophyll a, phycocyanin and phosphate compared to VOB and JS1.

In conclusion, cluster 1 was grouped according to the similarity of the site in low concentrations of nutrients, phycocyanin and chlorophyll a; while cluster 2 was grouped according to higher concentrations of nutrients, phycocyanin and chlorophyll a.

4.4.4 Principal Component Analysis (PCA)

The Principal Component Analysis conducted included a total of six variables measured in the surface water of the Nuwejaars River i.e. nitrate, phosphate, total phosphorus, total nitrogen, chlorophyll a and phycocyanin. The output from the PCA includes eigenvalues and the cumulative percentage of the variance of each component is represented in Table 4.5.

Table 9: Principal component analysis results of nutrients, chlorophyll a and phycocyanin: eigenvalues and percentage of variation.

Component	Initial Eigenvalues		
	Total	% of Variance	Cumulative %
1	3.717	61.950	61.950
2	1.543	25.713	87.663
3	0.636	10.600	98.262
4	0.086	1.441	99.703
5	0.015	0.253	99.956
6	0.003	0.044	100.000

	Component	
	1	2
N	0.603	-0.443
TN	0.990	-0.078
P	0.955	-0.039
TP	0.960	-0.063
Phyco	-0.016	0.974
Chl a	0.735	0.621

The PCA extracted two components, which together account for 87.66% of the total percentage of variance. PC1 accounts for a 61.95% percentage of variance, with an eigenvalue of 3.72 correlated with most of the parameters included in the analysis except for chlorophyll a.

In PC1 all loadings were above 0.5, except for chlorophyll a. Total nitrogen, phosphorus and total phosphorus, which are indicators of nutrient enrichment, yielded similar loadings, this may suggest an anthropogenic source of nutrients that may exist in the catchment.

PC2, which accounts for 25.71% of the variance with an eigenvalue of 1.54, only yielded positive loadings for chlorophyll a and phycocyanin. As these are a result of nutrient enrichment, this can be taken as an indication that land-use activities such as the dairy farm influence the water quality.

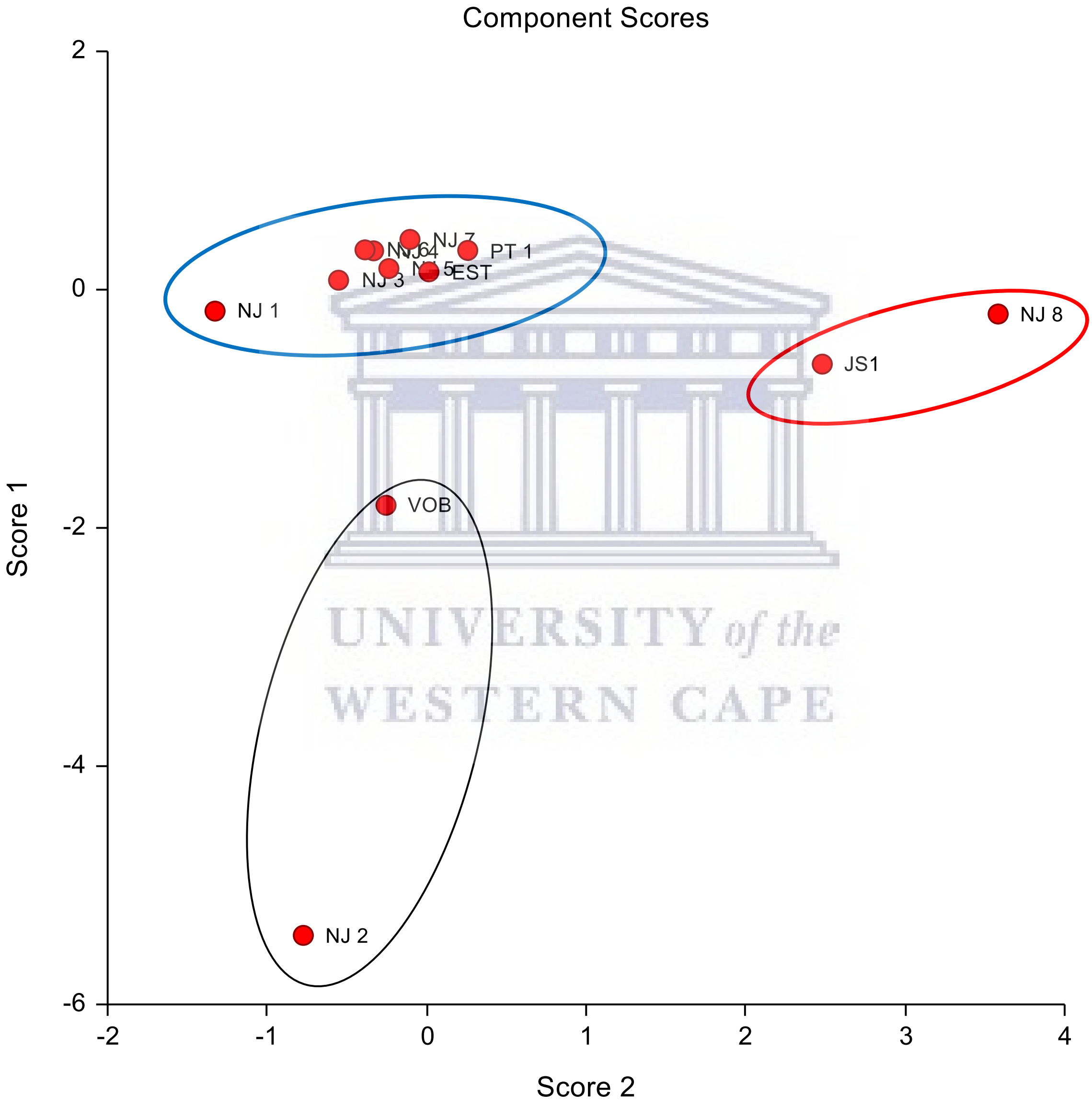


Figure 22: Principal component scores plot of nitrate, phosphate, total phosphorus, total nitrogen, phycocyanin and chlorophyll a in the Nuwejaars River.

4.4.4.1 Principal Component Analysis with physicochemical results

The PCA conducted included a total of ten variables measured in the Nuwejaars River i.e. total phosphorus, total nitrogen, nitrate, phosphate, chlorophyll a, phycocyanin, temperature, dissolved oxygen, pH and electrical conductivity. The output from the PCA includes eigenvalues and the cumulative percentage of the variance of each component, represented in Table 10.

Table 10: Principal component results of nutrients, chl a, phycocyanin and physicochemical properties: eigenvalues and cumulative percentage.

Component	Initial Eigenvalues		
	Total	% of Variance	Cumulative %
1	5.155	51.550	51.550
2	1.723	17.229	68.779
3	1.441	14.408	83.187
4	0.648	6.484	89.671
5	0.549	5.488	95.159
6	0.230	2.300	97.459
7	0.149	1.494	98.953
8	0.075	0.753	99.706
9	0.026	0.259	99.965
10	0.003	0.035	100.000

	Component		
	1	2	3
TN	0.621	-0.485	0.075
TP	0.976	-0.069	-0.015
N	0.636	-0.504	-0.190
P	0.885	-0.366	0.024
Chl a	0.833	-0.112	-0.010
Phyco	0.842	0.157	-0.398
EC	0.495	-0.044	0.821
Temp	0.514	0.706	0.241
pH	0.658	0.562	0.331
DO	0.536	0.491	-0.631

The PCA extracted three components, which together account for 83.187% of the total variance. PC1 accounts for 51.55%, which correlated with all the parameters included in the analysis.

In PC1 all loadings were above 0.5 except EC, which had a loading of 0.495. All nutrient-enrichment-related parameters had loadings above 0.6, pH being the only physicochemical parameter with a loading above 0.6.

PC2 accounts for 17.22% of the variance with an eigenvalue of 1.72, it only yielded positive loadings with phycocyanin, temperature, dissolved oxygen and pH. PC3 accounts for 14.41% of the variance with an eigenvalue of 1.44, it yielded positive loadings with total nitrogen, phosphate, electrical conductivity, temperature and pH.

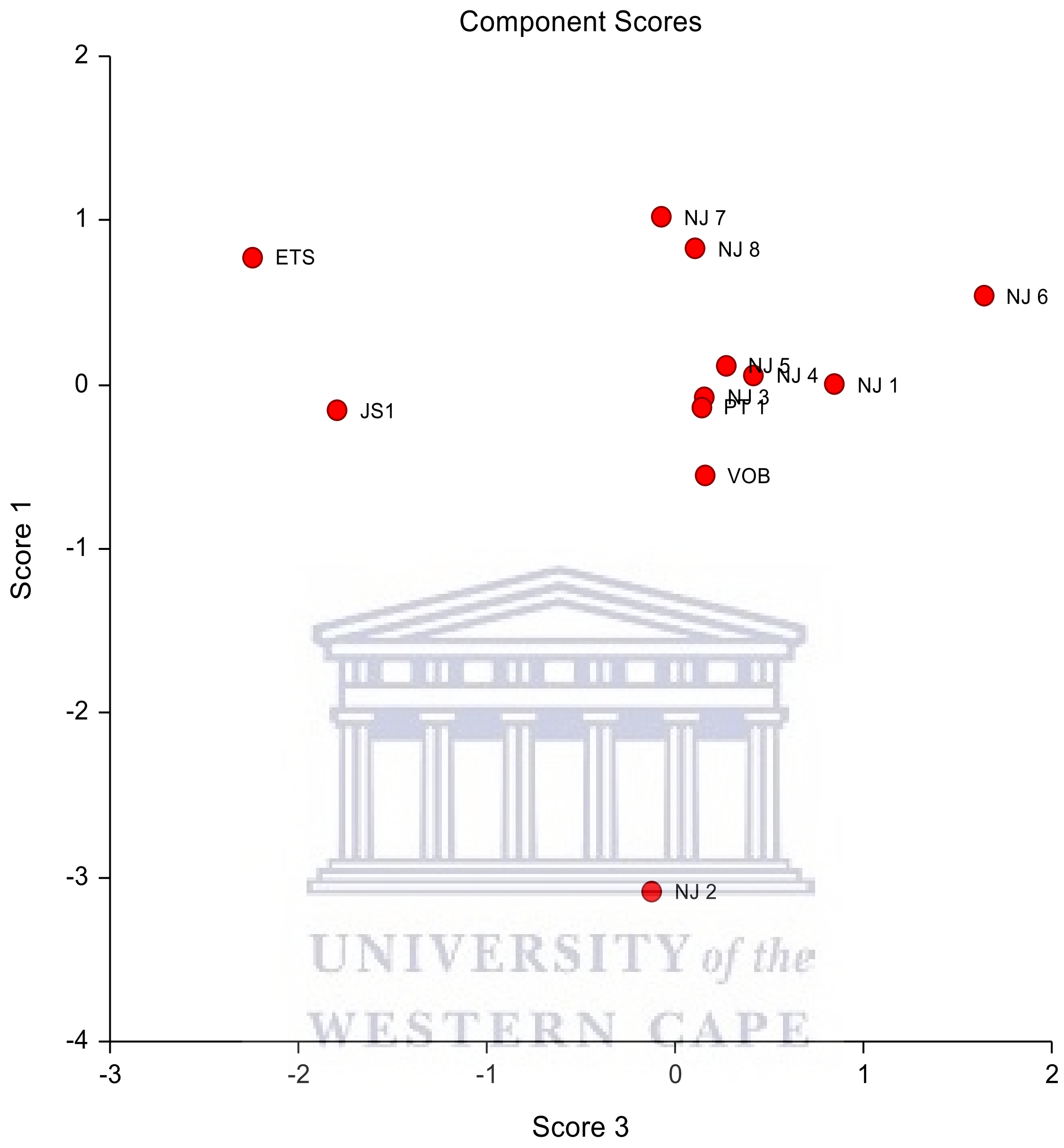


Figure 23: Principal component scores plot of nitrate, phosphate, total phosphorus, total nitrogen, DO, EC, pH, temperature, phycocyanin and chlorophyll a in the Nuwejaars River.

CHAPTER FIVE - CONCLUSIONS AND RECOMMENDATIONS

5.1 Introduction

This study focused on investigating the relationship between concentrations of nutrients, phycocyanin and chlorophyll a in the Nuwejaars River, by firstly assessing temporal and spatial variations in physico-chemical parameters as well as nutrients, phycocyanin and chlorophyll a. This was done by conducting seven sampling campaigns, where i*n situ* parameters were measured and water samples were collected for nutrient analysis in the laboratory.

Secondly, to assess the relationship between nutrients, phycocyanin and chlorophyll a, the multivariate statistical analyses Principle Components Analysis and Cluster Analysis, were conducted to highlight the similarities and differences between the parameters at the sampling sites.

5.2 Relationship between nutrients and chlorophyll a concentration

Previous studies by Dodds et al, (2002; 2006) found that TN and TP correlated well with chlorophyll a in temperate streams in the United States and New Zealand but only to a point, which is the correlation was less significant when TP exceeded TN and vice versa i.e. TP and TN were both limiting nutrients for chlorophyll a. The results presented in the previous studies provide evidence that both NO_3^{-3}-N and PO_4^{-3}-P play a role in the productivity of the algal biomass in streams (Dodds and Smith, 2016b). More recently, Dodds and Smith (2016), investigated nitrogen, phosphorus and eutrophication in streams, their study concluded that a strong statistical link existed between N, P and algal biomass in streams. The current study sought to investigate this link in the Nuwejaars River.

In a study conducted by Atique and An, (2019) in South Korea chlorophyll a was shown to have a positive linear relationship with TP in phosphorus-limited waters and to have a linear relationship with TN in nitrogen-limited aquatic systems. In this current study chlorophyll a only had a positive linear relationship with TP and PO_4^{-3}-P, it can thus be concluded that the Nuwejaars River is a phosphorus-limited aquatic system - i.e. phosphorus has a greater effect on chlorophyll a production than nitrogen has. The highest measured chl a concentration was 139.43 mg/l, measured in February 2019 at site NJ2; this high concentration correlated with the high nutrient concentrations measured at this site. Although the measured chl a concentration correlates with the nutrient concentrations, the measured concentration of chl a at this site was very high compared to other chl a values found at the site and that are found globally (Sylvan *et al.*, 2007; Bbalali *et al.*, 2013; Filstrup and Downing, 2017; Mamun, Lee and An, 2018; Prayitno and Afdal, 2019), thus it can be inferred that the measured value is an error or an anomaly.

Biggs (2000) examined data on chlorophyll a and soluble nutrient concentrations in run-off-affected rivers and streams and discovered that an increase in nutrients led to an increase in chlorophyll a and benthic algae biomass. Qiao et al. (2017) investigated nutrient and chlorophyll a concentrations in Bohai Bay, China, and discovered a negative relationship between dissolved inorganic nitrogen and chlorophyll a concentration. The researchers concluded that phosphorus inhibited phytoplankton growth.

The results of the bivariate correlation conducted in this study showed that phycocyanin had a significant correlation with total phosphorus at p = 0.89, chlorophyll a also had a significant correlation with total phosphorus at p = 0.98. With total phosphorus being the only nutrient that has significant correlations with both phycocyanin and chlorophyll a it can be inferred that total phosphorus plays a significant role in the concentrations of phycocyanin and chlorophyll a in the Nuwejaars River. Also, phycocyanin concentrations significantly correlated with chlorophyll a at p= 0.83; this was expected as phycocyanin is an accessory pigment found in cyanobacteria that can also photosynthesise.

5.3 In-situ fluorometry for measuring chlorophyll a

One of the research questions of this study was to determine if the in-situ fluorometric method for measuring chlorophyll a is a suitable method of measurement compared to the traditional spectrometric measurement.

The concentrations of both phycocyanin and chlorophyll a measured by the Aqua-Fluor handheld fluorometer co-varied consistently throughout the sampling period. Statistical analysis was conducted using the results and the results from the analysis concurred with a previous study conducted by Atique and An, (2019), where the concentration of chl-a was measured using a multi-parameter water quality sensors. Also, bivariate correlation analysis showed positive linear relationships between phycocyanin and chlorophyll a, reinforcing the results found in a previous study (i.e. Atique and An, 2019).

It can thus be concluded that the in-situ fluorometric method of measuring chlorophyll is a suitable surrogate for the traditional spectrometric method. It was able to yield results that allowed for statistical analysis to be conducted as well as produced informative results on the relationship between nutrients, phycocyanin and chlorophyll a.

5.4 Recommendations.

- Further investigations on the Pearson correlation results, specifically of total nitrogen concentrations with other nutrients and with phycocyanin and chlorophyll a
- Further investigations sampling site NJ2 that produced an extremely high chlorophyll concentration only once during the sampling period should be done.
- Further investigations should be done to examine the relationship between the nutrients, phycocyanin and chlorophyll a to determine the trophic status of the Nuwejaars River.
- Further investigations should be done on the correlation results, specifically the significant correlation of phycocyanin and chlorophyll a with phosphorus levels but not with nitrate levels.
- A comparative study between the handheld fluorometer and the spectrophotometric methods of measuring phycocyanin and chlorophyll should be conducted.

- Control strategies for anthropogenic activities other than agriculture are required to continuously monitor environmental degradation caused by nutrient enrichment. Establishment of a national water quality database that contains seasonal monitoring data for nutrient and chlorophyll a concentrations.

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APPENDICES

Appendix 1: Physicochemical parameters of the Nuwejaars River (n=12), 2017. (Units: Temperature in °C, EC in mS/m, DO in mg/l).

	July (2017)				October (2017)			
Site name	**EC**	**Temp**	**pH**	**DO**	**EC**	**Temp**	**pH**	**DO**
NJ 1	1069.00	16.00	7.86	7.78	998.00	24.50	7.90	8.40
NJ 2	1056.00	19.70	8.41	12.75	106.90	21.00	7.79	9.48
NJ 3	3740.00	17.30	8.05	9.87	103.80	20.20	7.60	8.32
NJ 4	379.00	14.30	7.66	7.46	83.30	21.30	7.13	7.99
NJ 5	214.10	12.80	6.99	8.80	68.30	16.40	6.68	8.06
NJ 6	144.70	18.10	6.86	4.67	78.70	19.00	6.39	5.73
NJ 7	61.90	12.20	6.53	4.93	59.80	17.70	6.23	3.80
NJ 8	1239.00	16.00	7.27	6.19	87.60	18.30	7.67	6.91
VOB	452.00	14.90	7.81	10.64	135.20	19.80	6.82	9.51
PT 1	54.80	17.90	6.44		56.10	22.00	7.90	9.04
JS1					50.10	13.60	6.58	6.94
ETS								

Appendix 2: Physicochemical parameters of the Nuwejaars River (n=12), 2018. (Units: Temperature is in °C, EC in mS/m, DO in mg/l).

	March (2018)				July (2018)			
Site name	**EC**	**Temp**	**pH**	**DO**	**EC**	**Temp**	**pH**	**DO**
NJ 1	2200.00	25.70	7.93	6.93	2179.90	16.60	8.08	7.14
NJ 2	2590.00	24.20	8.84	11.93	1796.60	17.50	7.68	7.12
NJ 3	538.00	23.50	8.17	8.74	2744.40	17.20	7.79	6.42
NJ 4	197.50	22.80	8.24	7.51	323.90		7.02	6.29
NJ 5	822.00	24.30	7.42	9.56	376.00	15.70	6.54	7.13
NJ 6	153.80	23.70	7.09	5.71	97.70	14.40	6.87	3.56
NJ 7	94.70	23.40	6.69	4.01	68.90	12.10	8.07	3.31
NJ 8	308.00	22.10	8.04	8.12	182.20	14.50	6.15	6.34
VOB	1311.00	22.90	8.02	4.78	244.20	14.70	7.41	6.10
PT 1	64.60	26.00	6.11	7.58	45.15	19.30	5.74	8.64
JS1	90.90	22.90	6.25	3.63	97.80	12.40	5.56	3.36
ETS	35.50	19.40	7.70	8.53	26.50	11.40	5.65	10.23

November (2018)

Site name	EC	Temp	pH	DO
NJ 1	43.13	19.43	7.94	
NJ 2	104.37	21.90	8.30	
NJ 3	59.20	20.90	8.30	
NJ 4	463.93	21.23	8.24	5.90
NJ 5	58.10	26.70	8.24	
NJ 6	122.10	18.57	6.14	
NJ 7	422.53	22.07	7.63	6.92
NJ 8	99.57	22.30	7.92	5.46
VOB	84.47	24.60	6.10	5.84
PT 1	2091.20	21.35	7.34	2.36
JS1	167.20	24.63	6.46	
ETS	117.03	22.45	6.17	4.31

Appendix 3: Physicochemical parameters of the Nuwejaars River (n=12), 2019, (Units: Temperature is in °C, EC in mS/m, DO in mg/l).

	February (2019)				October (2019)			
Site name	EC	Temp	pH	DO	EC	Temp	pH	DO
NJ 1	51.67	23.17	7.22	5.64	41.27	23.17	7.22	5.64
NJ 2	392.57	23.17	8.38	6.44	106.20	23.17	8.38	6.44
NJ 3	76.60	19.27	6.01	0.42	51.40	19.27	6.01	0.42
NJ 4	1113.60	23.03	8.48	8.98	361.53	23.03	8.48	8.98
NJ 5	63.87	30.47	7.85	6.24	32.90	30.47	7.85	6.24
NJ 6	12724.37	20.43	8.18	1.86	57.10	20.43	8.18	1.86
NJ 7	211.20	23.23	7.77	5.62	325.45	23.23	7.77	5.62
NJ 8	153.33	24.20	6.47	2.84	99.00	24.20	6.47	2.84
VOB	4290.55	24.75	7.97	4.39	235.33	24.75	7.97	4.39
PT 1	1485.07	21.73	8.47	8.87	1961.73	21.73	8.47	8.87
JS1	746.83	23.03	8.22	7.64	164.53	23.03	8.22	7.64
ETS					1.49			

Appendix 4: Nutrient concentrations (mg/l) of the Nuwejaars River (n=12), July 2017.

Site Name	Nitrate	Total Nitrogen	Phosphate	Total Phosphorus
NJ 1	0.1	0.5	2.3	2.5
NJ 2	0.2	1.2	1.7	2.3
NJ 3	0.7	1.1	2.1	2.7
NJ 4	0.3	0.4	1.8	2.0
NJ 5	0.2	0.2	2.3	2.5
NJ 6	0.1	0.5	1.5	2.0
NJ 7	0.1	0.5	1.6	2.0
NJ 8	0.1	0.3	1.4	1.9
VOB	0.3	0.5	0.3	0.7
PT1	0.2	2.0	2.3	2.5
JS1				
ETS				

Appendix 5: Nutrient concentrations (mg/l) of the Nuwejaars River (n=12), October 2017.

Site Name	Nitrate	Total Nitrogen	Phosphate	Total Phosphorus
NJ 1	0.3	0.5	3.0	3.2
NJ 2	0.0	0.4	3.4	3.0
NJ 3	0.1	0.5	2.3	2.5
NJ 4	0.0	0.4	2.8	3.4
NJ 5	0.2	0.5	2.9	3.5
NJ 6	0.1	0.5	1.9	2.1
NJ 7	0.2	0.5	2.5	2.9
NJ 8	0.0	0.4	2.4	2.7
VOB	0.0	0.3	3.6	3.8
PT1	0.3	0.5	1.4	1.9
JS1	0.0	0.4	2.7	2.8
ETS				

Appendix 6: Nutrient concentrations (mg/l) of the Nuwejaars River (n=12), March 2018.

Site Name	**Nitrate**	**Total Nitrate**	**Phosphate**	**Total Phosphorus**
NJ 1	0.6	0.9	0.2	0.7
NJ 2	0.6	4.4	18.6	20
NJ 3	0.3	0.5	1.4	3
NJ 4	0.3	0.4	0.2	1
NJ 5	0.2	0.5	1.7	2.3
NJ 6	0.3	0.5	0.2	0.5
NJ 7	0.2	0.4	0.8	0.8
NJ 8	0.1	0.5	0.4	0.7
VOB	0.2	1.6	11.6	11.8
PT1	0.2	0.5	0.7	1.2
JS1	0.3	0.5	1.8	1.9
ETS	0.3	0.5	1.1	1

Appendix 7: Nutrient concentrations (mg/l) of the Nuwejaars River (n=12), July 2018.

Site Name	**Nitrate**	Total Nitrogen	Phosphate	Total Phosphorus
NJ 1	0.7	0.7	1.6	1.7
NJ 2	0.1	0.2	1.2	1.5
NJ 3	0.1	0.5	1	1.3
NJ 4	0.1	0.1	1.6	1.8
NJ 5	0	0.4	3.7	3.9
NJ 6	0.1	2.9	1.7	1.9
NJ 7	0.2	0.4	1.3	1.3
NJ 8	0.1	1.7	1.6	1.7
VOB	0.2	0.5	2.1	2.8
PT1	0.1	0.4	1.1	1.9
JS1	4.2	18.9	1.4	1.6
ETS	0.2	0.4	2.1	2

Appendix 8: Nutrient concentrations (mg/l) of the Nuwejaars River (n=12), November 2018.

Site Name	Nitrate	Total Nitrogen	Phosphate	Total Phosphorus
NJ 1	0.1	0.5	3.1	3.30
NJ 2	3.5	4.3	2.6	4.50
NJ 3	0.1	0.5	2.7	2.90
NJ 4	1.3	1.8	2.3	4.20
NJ 5	0.1	0.3	0.1	0.40
NJ 6	0.1	0.2	4.4	5.90
NJ 7	0.1	0.4	2.3	2.50
NJ 8	0.1	0.3	3.2	3.50
VOB	1	1.1	1.3	2.30
PT1	0.2	0.5	5.2	5.60
JS1	0.4	0.4	7.4	7.80
ETS	0.1	0.8	2.6	3.00

Appendix 9: Nutrient concentrations (mg/l) of the Nuwejaars River (n=12), February 2019.

Site Name	Nitrate	Total Nitrogen	Phosphate	Total Phosphorus
NJ 1	2.3	0.9	3.5	3.4
NJ 2	1.1	2.9	6.5	6.95
NJ 3	4.2	5.8	9.7	3.76
NJ 4	0.6	2.1	3.9	3.94
NJ 5	0.9	1.2	2.9	3.52
NJ 6	0.8	1.4	3.2	4.06
NJ 7	0.1	0.1	1.4	1.32
NJ 8	1	3.8	2.1	2.36
VOB	1.4	41.4	1.9	1.54
PT1	0.8	2.4	3.9	4.51
JS1	0.5	0.3	1	1.53
ETS	1.3	1	2.5	3.47

Appendix 10: Nutrient concentrations (mg/l) of the Nuwejaars River (n=12), October 2019.

Site Name	Nitrate	Total Nitrogen	Phosphate	Total Phosphorus
NJ 1	0.2		1.7	0.32
NJ 2	0.7	6.1	15.6	9.23
NJ 3	1		4.5	2.05
NJ 4	0.5		1.5	0.21
NJ 5	0.5	7.6	3.1	0.35
NJ 6	2.3		11.4	0.24
NJ 7	0.5		2.3	0.34
NJ 8	0.1		1	0.23
VOB	0.2		3.6	0.85
PT1	1.7		8.6	0.88
JS1	0.9	4.7	6.6	0.35
ETS	1		3.3	0.31

	Feb-19		Oct-19	
Site Name	**Chlorophyll a**	**Phycocyanin**	**Chlorophyll a**	**Phycocyanin**
NJ 1	0.58	1.68	3.55	6.00
NJ 2	139.43	8.60	3.43	8.01
NJ 3	15.86	5.61	8.04	1.39
NJ 4	0.58	2.53	4.42	2.03
NJ 5	7.76		3.93	1.65
NJ 6	9.07		5.05	2.15
NJ 7	0.35	2.39	5.53	1.73
NJ 8	11.57		3.01	2.13
VOB	43.22	4.16	3.61	1.58
PT1	1.77	2.89	2.51	5.71
JS1	4.06	7.57	3.60	1.51
ETS	0.54	2.79	3.08	1.34

Appendix 11: Phycocyanin and chlorophyll a concentrations (mg/l) in the Nuwejaars River (n=12), 2018.

	MARCH 2018 SW		JULY 2018 SW		Nov-18	
Site Name	**Chlorophyll a**	**Phycocyanin**	**Chlorophyll a**	**Phycocyanin**	**Chlorophyll a**	**Phycocyanin**
NJ 1	0.14	0.11	2.64	0.90	5.83	1.77
NJ 2	0.23	3.87	6.78	1.14	4.92	0.88
NJ 3	0.08	0.14	2.21	0.92	3.27	0.93
NJ 4	0.12	0.25	5.88	4.07	2.58	0.51
NJ 5	0.03	0.37	7.27	3.34	8.28	1.37
NJ 6	0.11	0.22	2.21	1.15	4.34	0.44
NJ 7	0.10	0.24	2.35	0.57	3.84	2.14
NJ 8	1.35	2.40	1.30	0.29	1.85	0.20
VOB	0.31	0.48	0.00	1.42	5.40	1.91
PT1	0.30	0.23	4.93	1.59	6.04	1.15
JS1	1.09	2.51	4.58	1.06	4.10	0.74
ETS	0.28	0.42	8.69	1.97	5.39	1.50

Appendix 12: Phycocyanin and chlorophyll a concentrations (mg/l) in the Nuwejaars River (n=12), 2019.

	Feb-19		Oct-19	
Site Name	**Chlorophyll-a**	**Phycocyanin**	**Chlorophyll-a**	**Phycocyanin**
NJ 1	0.58	1.68	3.55	6.00
NJ 2	139.43	8.60	3.43	8.01
NJ 3	15.86	5.61	8.04	1.39
NJ 4	0.58	2.53	4.42	2.03
NJ 5	7.76		3.93	1.65
NJ 6	9.07		5.05	2.15
NJ 7	0.35	2.39	5.53	1.73
NJ 8	11.57		3.01	2.13
VOB	43.22	4.16	3.61	1.58
PT1	1.77	2.89	2.51	5.71
JS1	4.06	7.57	3.60	1.51
ETS	0.54	2.79	3.08	1.34