The use of diatoms in assessing water quality in the Nyl and Mogalakwena River system, in the Limpopo, South Africa

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LIST OF ABBREVIATIONS

BDI Biological Diatom Index

CMA Catchment Management Agency

COD Chemical Oxygen Demand

DEAT Department of Environmental Affairs

DWAF Department of Water Affairs and Forestry

DWS Department of Water and Sanitation

EC Electrical Conductivity

GDI Generic Diatom Index

GPS Global Positioning System

HF High Flow

LF Low Flow

NH_3-N Ammonia

NH_4-N Ammonium

NO_2-N Nitrite

NO_3-N Nitrate

NWA National Water Act

PO_4-P Phosphates

RDA Redundancy Analysis

RQO Resource Quality Objectives

SPI Specific Pollution Index

STW Sewage Treatment Works

TDI Trophic Diatom Index

TDS Total Dissolved Solids

TWQR Target Water Quality Range

%PT Percent Pollution Tolerant taxa

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SUMMARY

Water is an important natural resource but undervalued. Urbanisation has resulted in an increase in water use and resulted in water being polluted. To manage water and to protect this natural resource monitoring programmes have been developed. Anthropogenic activities have resulted in a decline in water quality and ecosystem biota in the Nyl and Mogalakwena River system.

Traditionally water quality monitoring focused on physico-chemical analysis. Recently water quality has shifted towards biological monitoring which allows researchers to get a time integrated assessment of the water quality. Biomonitoring techniques are used since chemical monitoring gives a glimpse of water quality at a specific moment in time and it is time-consuming and expensive.

Nine sampling sites were selected to assess water quality of the Nyl and Mogalakwena rivers in the Limpopo Province. Physico-chemical parameters and diatom assemblages were used to assess water quality during high and low flow conditions.

The *in situ* results indicate that temperature, electric conductivity and chemical oxygen demand increased downstream from the source. The pH remained more or less stable with the highest peak at Glen Alpine Dam. The nutrient concentrations increased at the sewage treatment works indicating that effluents from anthropogenic activities have an influence on the water quality. Omnidia software was used to calculate the diatom indices. These index values were subsequently used to determine which ecological category the sites fell in. Diatom categories with respect to ecological classification were compared to the water quality to get a time-integrated assessment of the river system. The diatom indices indicated a decline in water quality from the source of the Klein Nyl River along its course to the confluence with the Limpopo River. There was an increase in pollution tolerant taxa (%PT) from the sewage treatment works to the Limpopo River confluence indicating a negative effect on water quality, and organic matter pollution. The diatom indices indicated poor water quality during high flow conditions which can be related with the dilution effect associated with high flow conditions, as studies indicate that high rainfall will cause an increase in nutrient loading from land to rivers.

CHAPTER 1

GENERAL INTRODUCTION

1.1 Water an important resource

Water is a scarce resource; nonetheless, it is an important natural resource for all economies. There has been an accelerated use of water for food and energy production to sustain an increasing population and to improve social welfare (DEAT, 1999). Rivers play a crucial role in water supply (Harpe, 1998) and is important for economic and social development (Barnhoorn and van Vuren, 2004). Water resources are susceptible to pollution and overexploitation and many areas are therefore suffering from a decline in water quality (Chiotelli, 2015). The quality and availability of water has an effect on every single aspect of human life, as humans depend on water for their daily survival. Water quality is an important factor that will eventually safeguard or unpleasantly have an effect on ensuring the survival of aquatic organisms. Since water is an essential element for human existence, it is important to conserve, manage and develop water sources such as rivers (Barnhoorn and Van Vuren, 2004).

Most water resources are threatened by salinization and eutrophication (Slabbert, 2007). Eutrophication is one of the growing environmental problems in freshwater and marine ecosystems worldwide (Carpenter *et al.*, 1999; Selman and Greenhalgh., 2010), which is a process of extreme nutrient over-enrichment and related extreme plant growth (van Ginkel, 2011; Selman and Greenhalgh, 2010). Eutrophication is indicated by extreme plant and algal growth resulting from the enhanced accessibility of one or more restrictive elements required for photosynthesis (Schindler, 2006; Chislock *et al.*, 2013) such as sunlight, carbon dioxide and nutrients (Chislock *et al.*, 2013).

Eutrophication is caused by nutrients such as phosphorus and nitrogen. These nutrients are of utmost importance for biological processes in aquatic ecosystems, the accelerated runoff of these nutrients to aquatic ecosystems can cause an increase in biomass production, disturbing the natural balance of these ecosystems (Selman and Greenhalgh, 2010).

Salinisation is a rise in the concentration of total dissolved solids in inland waters (Slabbert, 2007). According to Koch *et al* (1990), salinisation is a common impact derived from urban, agricultural, mining and industrial activities and results from most activities involving the use of water (Slabbert, 2007). Salinisation has an effect on the usefulness of water in that increased salt content decreases water quality (Slabbert, 2007).

1.1.1 South African situation

South Africa has a very low conversion of mean annual rainfall to mean annual runoff. The mean annual rainfall for the subcontinent is 452 mm which is about 60% of the world's average, and South Africa is therefore considered a semi-arid country (DWAF, 1994; Musa, 2015).

The supply of rainfall in the country is very irregular with the eastern and southern parts receiving considerably more rain than the northern and western parts. The chemical suitability of the water is dependent on the source of the water, geology of the area, ecology of the area and anthropogenic activities. The major sources of water supply in South Africa are rivers and dams. Groundwater is vital as it contributes to base flow in perennial rivers along the eastern escarpment and wetter north-eastern parts of the country, it is significant in areas where surface water is less available (Cessford and Burke, 2005).

Much of the country's prevailing water resources have been over-utilized or changed substantially. Many areas of the country experience water shortages, where water needs exceed reliable supply of water, putting a strain on the environment (Harpe, 1998). Water quality deterioration in rivers is problematic in South Africa (Mwangi, 2014). South Africa's freshwater resources are under enormous pressure from the growing population and developing economy therefore most of the surface water has been assigned, so future growth in storage and supply will need to come from groundwater sources (WWF SA, 2017; du Plessis, 2010). In terms of the present and expected future population growth rates, South Africa does not have enough freshwater resources at hand for sustaining present water usage and water discharge patterns (WWF SA, 2017; du Plessis, 2010). It is predicted that by 2030 South Africa will face a water shortage of 17% as the country's water resources will be fully utilized and will not meet the growing demand of the country's population. It is important for the country to make improvements to its treatment technologies and water quality, so as to decrease the costs related with alleviating water quality challenges, and at the same time escalate the profits related with alleviating its freshwater resource problems (WWF SA, 2017; du Plessis, 2010). The recent droughts that have occurred in the country impede socio-economic development. The demand for water has had a steep increase with three major sectors driving this demand. The highest is the agricultural sector at 63%, municipal sector at 26% and industrial sector at 11% (WWF SA, 2017). Since there is a growing demand for water it is important to manage water resources. In order to manage water resource, the National Water Act (NWA) prescribed measures to ensure their management . Catchment Management Agencies (CMAs) were developed to guarantee coordinated planning for water security (WWF SA, 2016). Within the NWA, Resource Quality Objectives (RQOs) were

developed to institute comprehensive water quality management targets. The RQOs can also be formed to initiate and strengthen the guidelines or prohibition of in-stream or land-based activities, which may have an effect on the quality and quantity of the water resource (Mosoa, 2013).

1.1.2 Suitability of water for use

The suitability of water to be used by either humans or aquatic organisms not only depends on the accessibility, however it also depends on the physical, chemical, and biological properties of water. Different users have different water quality prerequisites, which can be influenced by natural processes, diffuse and point source discharge or by inter-catchment transfer of water (Cessford and Burke, 2005). Suitability of water for use can result naturally due to the underlying geology, biological processes, atmospheric deposition and evaporation. Water pollution occurs in the form of point and non-point sources. Contamination of surface water is easier to notice than that of groundwater, resulting in groundwater contamination being more difficult to detect and to improve than surface water contamination (Cessford and Burke, 2005).

1.1.3 Impacts of anthropogenic activities on water resources

Human activities exact pressure on the environment resulting in the decrease of surface water quality (Xiao-long *et al.*, 2006). The growing human population places greater demands f on water quality (Chapman, 1996). Human activities that pollute water resources include, urban, industrial and agricultural activities and the discharge of sewage effluent (Adeousu *et al.*, 2016). Consuming polluted water may result in waterborne diseases (Deshmukh, 2013). Sewage and industrial waste discharged into rivers have the highest possibility of causing water pollution, as industrial effluent contains mainly heavy metals. Fertilizers from agriculture also causes water pollution (Adeousu *et al.*, 2016). Increased urbanization has resulted in poor standards of waste water management, as some areas of the country have little or no treatment of waste water, and some of the treatment systems are poorly managed, resulting in the release of the effluent into the rivers. High organic and nutrient loads in urban runoff can cause an increase in nutrient and organic loads and microbial contamination (Cessford and Burke, 2005). Increases in land use has increased the amount of impermeable surfaces in urban areas therefore reducing groundwater recharge (Cessford and Burke, 2005). Land use change also has a key effect on the groundwater system, because land development typically causes large alterations in flood peak and infiltration properties (Albhaisi *et al.*, 2013; Jinno *et*

al., 2009). Human activities such as mining and urbanization have caused major changes in the landscape, therefore impacting on the water balance of surface and groundwater systems (Albhaisi *et al*., 2013) Urbanization and land use change drastically increases the flow of water to rivers making its travel time to be shorter, as a result groundwater recharge to shallow unconfined aquifers decreases by the same amount (Jinno *et al*., 2009). Overgrazing has caused a risk of erosion where the quantity of sediments entering surface waters increases (Cessford and Burke, 2005). Water pollution have negative cost effect implications to municipalities, as some pollutants cannot be easily removed (Shuuya, 2008). Declining water quality threatens to weaken economic growth and development. Factors that have been associated with the decline in water quality involve poor governance, poor intergovernmental co-operation, lack of technical capacity, poor water quality management and lack of compliance to license conditions by some of the water users (Mosoa, 2013). . Providing clean water and safe disposal of wastewater for the towns of developing countries turns out to be gradually more difficult and crucial (Mwangi, 2014). Anthropogenic activities such as riparian removal have interrupted the pristine conditions of the river continuum resulting in a net loss of aquatic habitat, which will have an effect on biodiversity and ecological functions (Mwangi, 2014). In South Africa, the abuse caused by humans on water sources has caused the deterioration of organisms since some organisms cannot survive in polluted water (Mwangi, 2014)

1.2 Water quality and management

The South African Constitution section 24 states that "*everyone has a right to an environment that is not harmful to human health or well-being, and to have the environment protected, for the benefit of present and future generations, through reasonable legislative and other measures that prevent pollution and ecological degradation; promote conservation; and secure ecologically sustainable development and use of natural resources while promoting justifiable economic and social development*" (Constitution of the Republic of South Africa, 1996). Water quality is the term used in describing the biological, chemical, physical and aesthetic properties of the water (Musa, 2015). The Department of Water and Sanitation is the custodian of the water resources in the country, and it manages water resources to guarantee consistent and rightful provision of water for sustainable and social development; to guarantee that the resource is protected and also ensuring the development of effective water management institutions (DWA, 2013). In order for the resource to be managed correctly, the Department of Water and Sanitation formally known as the Department of Water Affairs and Forestry (DWAF) developed the South African National Water Act (Act No. 36 of 1998) (Musa,

2015). This act provides the legal foundation for understanding South Africa's water quality management policy and also provides the basis for a new and essentially distinct methods of administering the nations water (Pollard and duToit, 2008).

In order to assess water quality, different tests are used. The physico-chemical test, which measures the present physical and chemical characteristics of the water and biomonitoring which uses bio-indicators (Musa, 2015).

1.2.1 Chemical monitoring versus biomonitoring

Since natural and anthropogenic factors play parts influencing the chemical constituents of contaminated waters, it is important to test the water for chemical parameters so as to determine their constituents and also concentration (Musa, 2015). Initially water quality assessment focused on chemical parameter concentration and toxicity, however it was realized that surface water quality degradation and biotic integrity are affected by multiple factors, and it provides a glimpse of the system being studied (Karr and Dudley 1981; Karr *et al.*, 1986; Musa, 2015).

Chemical monitoring is time consuming and relatively costly and may give a partial representation of actual water quality because of the difficulty in assessing every chemical parameter in the ecosystem. Chemical constituents in a river may be exposed to dilution from inflows of rainwater, or increased from runoff from point and non-point sources, or become concentrated during times of drought and low flow. As a result, it becomes challenging to supply anything other than a fragmented indication of the status of the river along its complete length using conventional chemical monitoring techniques (Taylor *et al.*, 2006).

1.2.2 Biomonitoring

Biomonitoring is a more efficient tool used to evaluate the exposure and effects of environmental pollution (Bere *et al.*, 2014). Since chemical and physical parameters can easily be altered by an immediate pollution event and are often insufficient in presenting the long-term effect on the water environment, monitoring of biological organisms have been used (Dayıoğlu and Tokatli, 2014). Biomonitoring uses living organisms as biological indicators, as these organisms can give a long-term integrated indication of water quality and quantity, habitat quality and other environmental conditions (River Health Programme in the North West, 2009).

The use of biomonitoring methods has some several advantages and disadvantages over chemical monitoring. 1) biomonitoring can detect changes in water quality, 2) detect the effect of changes in water flow 3) biomonitoring methods are more precise and more sensitive than chemical analysis in detecting unfavorable conditions in the environment (River Health Program in North West Province, 2009). The disadvantages of biomonitoring are 1) a fair amount of training is needed, 2) it is open to subjective interpretation, 3) does not provide an exact figure of water quality parameters and 4) cannot pinpoint the exact cause of water quality problems (River Health Program in North West Province, 2009).

1.3 Nylsvley - A Ramsar accredited site

According to Cowan (1995), wetlands are identified as one of the most important life support systems on earth. Nylsvley is a floodplain situated in the Limpopo Province of South Africa, between latitudes 24º15'S and 24º50'S, and longitudes 28º10'E and 29º05'E, and is situated on the Nyl River between the towns of Modimolle and Mokopane. In 1998 Nylsvley was recognized by the Ramsar Convention on Wetlands as an internationally important wetland. Ramsar defines wetlands as "*areas of marsh, peatland or water, be it natural or artificial, permanent or temporary with flowing or static water, fresh, brackish or salt including areas of marine water lower than six metres at low tide*" and might include riparian and coastal zones adjacent to wetlands (Koester, 1989).

Nylsvley provides habitat for large numbers of inland waterbird species and a large variety of flora and fauna including many red data species (Musa 2015). It also plays a vital role in retaining and purifying water and mitigation of flood and drought events (Musa, 2015). Increasing pressure on the water resource in the upstream catchments is putting the ecological status under threat, affecting the volume and timing of water supplied in the floodplain (Kleynhans *et al.*, 2007).

Wetlands also provide the following goods and services; 1) improving water quality and hydrology because wetlands greatly influence the flow and quality of water, 2) flood control because they act as buffers to store excessive rainfall and release runoff gradually, 3) retention of sediments as wetland vegetation also acts as sieves that retain sediments in water, 4) nutrient retention and retention of pollutants because wetland vegetation absorb nutrients and toxicants, the nutrients retained in wetlands support the growth of other wetland organisms,5) biomass export as wetland plants fix inorganic carbon into organic matter in turn feeding into the wetland ecosystem, 6) recreation and tourism because of their scenery and products (Veeravaitaya, 2008).

1.4 Diatoms

Diatoms (Bacillariophyceae) are unicellular microscopic algae (Dayıoğlu and Tokatli, 2014) found in all waterbodies. They have become a primary focus in monitoring studies due to their mass reaction to stress, and the frustules in sediments can be used for historical reconstruction (Round, 1991). Diatoms are broadly distributed with many cosmopolitan species allowing cross-system assessment to be easily made (Bellinger *et al.*, 2006). They play a vital role in food webs and biogeochemical cycles (Bate *et al.*, 2002), as they are the main primary producers in waterbodies (Pandey *et al.*, 2017). Of all the organisms (fish, macroinvertebrates etc.) used in biomonitoring, diatoms are highly suitable for evaluating chemical status of a waterbody, due to their sensitivity to nutrient and organic pollution, whereas fish and macroinvertebrates are more sensitive to hydrological changes in aquatic ecosystems (Pandey *et al.*, 2017). Diatoms are the key primary producers and chemical modulators in freshwater aquatic ecosystems and represent an important carbon and energy source for secondary consumers (Dalu *et al.*, 2016).

Diatoms are exceptional in responding to fluctuations taking place in aquatic ecosystems, especially eutrophication, increased pollution and acidification, for the reason that they are sensitive to a range of environmental factors required for optimal growth (Noga *et al.*, 2013).

1.4.1 Diatoms as biological indicators

Diatoms are used for the following reasons:

- Diatoms are ubiquitous
- Easy to collect, preserve and prepare for observation
- Highly reactive to changes in environmental conditions (Schoeman and Hayworth, 1986)
- Respond rapidly to environmental variations and decline in water quality (Descy, 1979; Kelly and Whitton, 1995; Stoermer and Smol, 1999; Hirst *et al.,* 2002).
- Cooperatively display a comprehensive variety of tolerance along a gradient of aquatic productivity
- Possess the smallest generation times (~2 weeks)
- Appropriate for diversity analysis
- Accessibility to interpretive software packages (OMNIDIA) (Taylor *et al.*, 2005)

- Assemblages are typically species-rich
- Diatoms can be sampled at most times of the year and even precisely display recent or historical conditions (Taylor *et al.*, 2005)
- Can be sampled at most times of the year, because they can be found on substrata in stream beds even when dry (Stevenson *et al.*,1999).

1.4.2 History of diatom research in South Africa

Regular use of diatoms has been recognized worldwide (Dalu *et al.,* 2016). Studies of diatom flora extend as far back as the middle of the 19th century with efforts from Ehrenberg (1845) and Cleve (1881). In South Africa diatom research began as early as the 1950's commencing with DR BJ Cholnoky. Over many years Cholnoky collected significant information, played a role in the fields of taxonomy and ecology of diatoms (Musa, 2015), producing over 40 papers dealing with numerous diatom species found in Southern Africa (e.g. Cholnoky, 1960). Cholnoky believed that diatoms can be useful in determining water quality more precisely compared to chemical testing alone (Musa, 2015). Adding to the aforementioned he was one of the first people to infer the pH of water based on the diatom community (Taylor *et al.*, 2005).

Resulting from Cholnoky research, Dr REM Archibald and Dr F Schoeman took over with the research in freshwater diatoms, making huge progress in the field of taxonomy and started developing a guide to Southern African diatom floras (Musa, 2015).

Diatom research was again explored deeply by Bate *et al.* (2002) in South Africa, and are now being applied with growing consistency as indicators of water quality (Taylor and Harding, 2014).

1.4.3 Diatom indices

In order to sum up ecological and hydrological information given by diatom assemblages diatom indices are utilized (de Almeida *et al.*, 2014). The availability of software programmes such as Omnidia have made index calculation quick and easy (Martín and de los Reyes Fernandez, 2012). Widely used indices are centered on Zelinka and Marvan's (1961) method, which reflects weighted averages of taxa's sensitivity to nutrients and organic deprivation, as well as pH and salinity (Dalu *et al.*, 2016). Commonly used indices require the identification of diatoms to species level (Bate *et al.*, 2002).

$$\text{Index} = \Sigma (Ai \times vi \times ji) / Aivi$$

"where a_j = abundance of species j in sample, v_j = indicator value and i_j = pollution sensitivity of species j"The principle of diatom indices is that: In a sample from a waterbody having a specific level or concentration of determinant, diatom taxa with their optimum near to that level of determinant will be plentiful. Thus it can be possible to estimate the level of determinant in the sample from the average of the optima of the pollution sensitivity ('s') each weighted by its abundance ('a'). In simple terms a commonly found taxon will have more effect on the outcome than the uncommon one. An 'indicator value' ('v') is incorporated to provide better weight to those taxa that are efficient indicators of certain environmental conditions. Applying diatom indices consists of making a list of the available taxa in a sample together with the degree of their abundance. The index is expressed as the average of the pollution sensitivity of the taxa in a sample weighted by the abundance of each taxon. The indicator value works to boost the effect of particular species (De la Rey,2007).

The functioning of the indices depends on the values assigned to the constants s and v for each taxon and the index values range from 1 to an upper limit equal to the highest values of s. Diatom indices vary in the number of species utilised and in the values of s and v (Taylor *et al.*, 2005).

The index of Zelinka and Marvan acted as a base for numerous indices:

- TDI = Trophic Diatom Index used in detecting eutrophication in rivers caused by sewage inputs. Categorises data into 5 classes of sensitivity and 3 classes of reliability (Martín and de los Reyes Fernandez, 2012; Musa, 2015)
- DES = Descy's Index categorises data into 5 classes of sensitivity and uses 106 data species
- SLA = Sladecek index categorises data into 5 classes of sensitivity and uses 323 data species
- GDI = Generic Diatom Index identifies diatoms to a genus level and categorises data into 5 classes of sensitivity (Musa, 2015)
- BDI = Biological Diatom Index groups taxa based on morphological classification and taxonomical groups (Musa, 2015)
- SPI = Specific Pollution sensitivity Index categorises data into 5 classes of sensitivity using all the taxa included into OMNIDIA
- L&M = Leclercq and Maquet index categorises 210 diatom species into 5 classes of sensitivity

- IDAP = Diatom Index Artois-Picardie classifies diatom species into 5 categories
- ROT = Rott's Index catergories taxa into 5 classes of sensitivity based on saprobiological inclinations
- CEE = Descy and Coste index uses 208 species of the database
- EPI-D = Eutrophication Pollution Index Diatoms catergorises data into 5 classes (Musa, 2015).

Researchers worldwide have tested and utilized the TDI, SPI and GDI indices. The establishment of SPI and GDI were primarily for organic pollution, whereas the TDI was utilized for inorganic nutrient concentration. It is easier to make use of the GDI as it involves classification only to the genus level, therefore valuable in giving an initial indication of the condition of the system, whereas the SPI is the broadest index as it incorporates the s and v values of over 1300 species. The s and v being the constants in which the performance of the index is depended upon. The classification in the GDI is based on 44 genera (Musa, 2015).

For interpreting data for water quality management purpose, it is important for indices to be utilized by databases such as Omnidia, for deriving index scores that will give an indication of the different water quality categories. Omnidia scores extend between 0 and 20 except for TDI, where 0 signifies bad water quality and 20 signifies immaculate conditions (De la Rey *et al.*, 2004)

The scores are further divided into water classes (Eloranta and Soininen, 2002; De la Rey *et al.,* 2004), whereby:

- Bad water quality = < 9
- Poor water quality = 9-12
- Moderate water quality = 12-15
- Good water quality = 15-17
- High quality = >17

The TDI scores range from 0 to 100, where 0 indicates low nutrient concentration and 100 high nutrient concentration (Musa, 2015).

1.5 Hypothesis, Aims and Objectives

1.5.1 Hypothesis

For the purpose of this study, two hypotheses were set:

1) Anthropogenic activities have caused a decline in the water quality of the Nyl River. This will be compared to the previous studies conducted in the Nyl River.

2) The sewage treatment plant and mining activities are major contributors of pollution in the Nyl/ Mogalakwena River, causing a change in diatom community structure.

1.5.2 Aims

- Determine human impacts on the physico-chemical constituents of water on the Nyl/Mogalakwena River System
- Determine the diatoms community response to different pollution levels

1.5.3 Objectives

- Determine the *in situ* water quality parameters e.g. temperature, electric conductivity, pH and oxygen content
- Determine the concentration of nutrients e.g. nitrite, nitrate, ammonium and phosphate in the water over the sampling periods
- Estimate the dependence of index response on variation in habitat and seasonal changes
- Use diatom metrics to measure the diatom community response to pollution levels at different sampling points along a pollution gradient
- Examine epiphytic diatom community assemblages found on aquatic macrophytes

1.6 Thesis Outline

Chapter 1: Introduction

This chapter provides an overview on the water crisis faced in the world, how to manage water resources. It lists the importance of water resources and problems associated with water resources. It compares physico-chemical monitoring and biomonitoring, also introduces diatoms with their advantages for water quality monitoring. This chapter also mentions the hypothesis with the aims and objectives.

Chapter 2: Site Selection

This chapter gives a description of the study area and the sampling site selected.

Chapter 3: Materials and Methods

This chapter gives an in-depth look on the equipment used and methods followed in determining *in situ* water quality parameters, nutrient concentration and diatom sampling. It also looks at the laboratory methodologies, *in situ* water parameter analysis together with nutrients, and preparations of diatom slides.

Chapter 4: Water Quality Parameters

This chapter gives an overview of the water quality parameters measured and the possible sources of pollution. It gives results on the water quality parameters measured and compares the water quality parameters to the Target Water Quality Ranges Proposed by the Department of Water Affairs (DWAF, 1996).

Chapter 5: Diatom Indices

This chapter reports on the use of diatoms, and how they are applied in water quality assessment. It discusses the results obtained from different indices and the correlation between environmental variables, diatom taxa, sites and water quality parameters. A diversity index is used to show a change in species composition along the sites.

Chapter 6: Conclusion and Recommendations

This chapter gives concluding remarks of the study, and also provides recommendations to contribute to the effective use on how to monitor and manage water resources sustainable.

Appendices: Raw data are given in this section

- Appendix A - Physico-chemical parameters
- Appendix B - Diatom Indices
- Appendix C - Relative abundances
- Appendix D -Micrographs

CHAPTER 2

SITE SELECTION AND DESCRIPTION

2.1 Study background

The study was conducted in the Waterberg Catchment in the Limpopo Province, which is South Africa's northern-most province, located within the great curve of the Limpopo River (Vermeulen and Bester, 2009). The Limpopo Province covers an area of 123 910 km^2, falling in the summer rainfall region, with the western parts being semi-arid and the eastern part largely subtropical. The province has inadequate surface and ground water resources. As a result of the limit to the resource, communities in the Limpopo Province have limited access to sufficient water resource (Limpopo DFED, 2004). Water has a significant part in the distribution of industries relying on stable provision of water, and in the Limpopo Province this is predominantly applicable to the mining and agricultural sectors. Potential health risks occur from drinking polluted water. A report by EcoAfrica in 2015 stated that the levels of water stored in the Limpopo Province have diminished by 13% between October 2014 and October 2015. The province experiences a diverse rainfall pattern, with the northern part receiving between 201 to 400 mm^2 a year, however the greater part of the province receives between 401 to 600 mm^2 a year, and a minor portion in the east receives over 1000 mm^2 a year (EcoAfrica, 2015).

The study follows the course of the Klein Nyl River from its origin at the Klein Nyl Oog, in Modimolle to Moodrif Dam (MOOR) near Mokopane. From there the river changes its name to the Mogalakwena River, which is a tributary of the Limpopo River (Greenfield, 2001). The area is influenced by formal and informal settlements, agricultural practices and mining.

The Nyl River system is situated in the bushveld-savanna of South Africa, and it drains into the Mogalakwena River, acting as part of the broader Limpopo drainage system. The Nyl River has a 24 000 ha floodplain (Higgins *et al.*, 1996), being one of the largest floodplain wetlands in South Africa (Dahms, 2015), and is characterized by periodically inundated reed beds and grasslands. The floodplain is of significant value, both as an agricultural and as a conservation asset, and supports a growing eco-tourism industry, which is underlain by a diverse community of rare water-birds and antelope species (Higgins *et al.*, 1996).

The Nyl River systems faces extensive pressure from an increase in urbanization and growth of informal and formal settlements resulting in further water resource exploitation (Higgins *et al.*, 1996).

The Nyl River is renamed the Mogalakwena River near Mokopane (Dahms, 2015), covering an area of 19 327 km (Waterberg district environmental Management Framework, undated). There are two major dams located in this river catchment; the Glen Alpine Dam and the Doringdraai Dam. Mokopane receives water from the Doringdraai Dam. The Glen Alpine Dam

has a limited yield due to its small size and supplies water for primary use and irrigation to the downstream area. The Mogalakwena River runs north east towards the Botswana border subsequently meeting the Limpopo River (Dahms, 2015).

Previous studies on the Nyl River focused on metal contamination (Vlok *et al*.,2006),(Greenfield *et al*., 2007; Greenfield *et al*.,2010; Greenfield *et al.,* 2012), (Dahms and Greenfield, 2015),(Dahms, 2015), (Musa *et al*.,2017),(Dahms *et al.*,2017) and Musa (2015) was the first to conduct a study on the diatom flora of the system.

2.2 Study Sites

Nine sampling sites were selected under high and low flow conditions (Figure 2.1). Six sites are located in the upper reaches of the Nyl River, two in the Mogalakwena River and one in the Limpopo River after the Mogalakwena River confluence. The sites selected are based on their position in relation to possible sources of pollution.

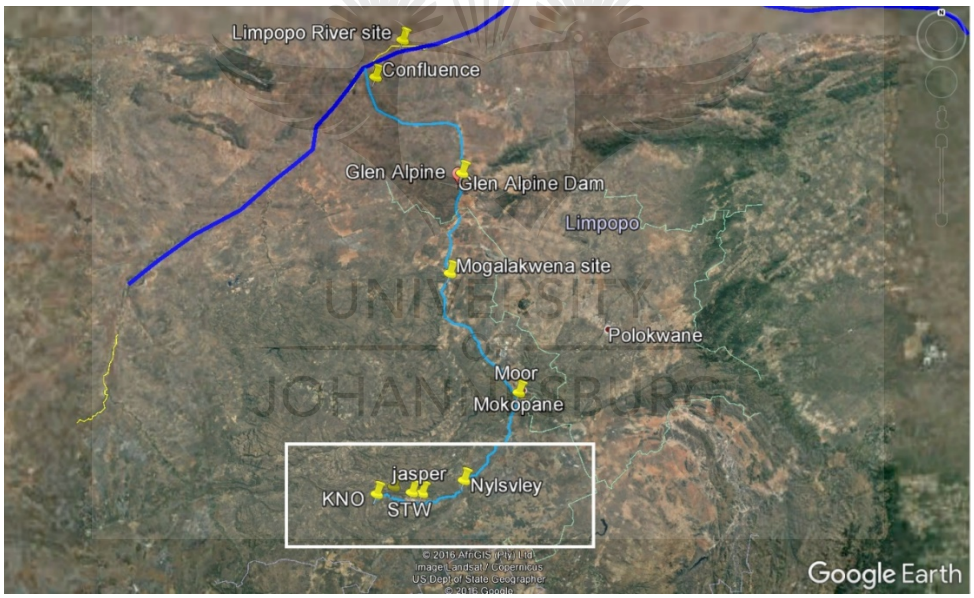

Figure 2.1 Google Earth image of the sampling sites along the course of the Nyl/Mogalakwena River system. A: map of entire length of the study area. B: Inlay indicating spatial differentiation between sites in the upper reaches of the Nyl River (Google Earth, 2017).

Table 2.1 Location, GPS co-ordinates of selected study sites, including site numbers and abbreviations (courtesy N Baker, 2017)

SITE NUMBER	SITE NAME	ABBREVIATION	LATITUDE	LONGITUDE
1	Klein Nyl Oog	KNO	-24.6959033	28.2533066
2	Donkerpoort Dam	DPD	-24.6780083	28.3356247
3	Modimolle Sewage Treatment Works	STW	-24.706085	28.4277766
4	Jasper	JASP	-24.7097017	28.47950333
5	Nylsvley Nature Reserve	NYL	-24.6444817	28.69697166
6	Glen Alpine Dam	GlEN	-23.1910233	28.69766333
7	Piet's Farm	PIET	-22.590188	28.900041
8	Mogalakwena – Limpopo Confluence	ECHO 15	-22.4575233	28.923919999
9	Limpopo River	ECHO 14	-22.3851067	28.970865

Site 1 Klein Nyl Oog (KNO) - is located close to the Klein Nyl River Eye on a private farm. The farm is a cattle farm which grows lucerne (*Medicago sativa*). There are limited impacts at this point and they are from the geological make-up of the area, and some from the cattle. There is unusual sedimentation resulting from the addition of a weir. The macrophytes in the river are dominated by *Carex austro-africana, Cladium marsiscus, Phragmites* spp and *Cyperus* spp (Dahms, 2015; Musa, 2015).

Figure 2.2 Indicates how the sites looked like during high and low flow conditions. Photographs of site one, Klein Nyl Oog (KNO) (photographs courtesy N Baker, 2017).

Site 2 Donkerpoort Dam (DPD) - is located downstream of a man-made impoundment, about one kilometer downstream of KNO. In this site the riparian and floodplain vegetation is marsh vegetation with indigenous grasses and sedges being found (Greenfield, 2004). The dominant vegetation is *Cyprus* spp and macrophytes such as *Carex austro-africana* (Dahms, 2015; Musa, 2015). There are agricultural and livestock farms between KNO and this site.

Figure 2.3 Indicates how the sites looked like during high and low flow conditions. Photographs of site two, Donkerpoort Dam (DPD) (photographs courtesy N Baker, 2017).

Site 3 is located at the discharge stream of the Modimolle Sewage Treatment Works (STW) - The site is a shallow stream characterized by scarce vegetation due to the thick canopy provided by the riparian vegetation (Dahms, 2015; Musa, 2015). An abundance of alien invasive trees can be observed at the site.

Figure 2.4 Indicates how the site looked like during high flow conditions. Photograph of site three, Sewage Treatment Works (STW) (photograph courtesy N Baker, 2017).

Site 4 Jasper (JASP) - is located downstream of the STW with a wetland between the two sites. Concrete pipes are used to channel the river under a dirt road resulting in a damming effect upstream. The site may be influenced by run-off from a cattle farm as it is located adjacent to it (Dahms, 2015; Musa, 2015). The effluent from STW and from Phaganang, which is an informal settlement have an impact on this site (Greenfield, 2004). Macrophytes that are abundant are *Cyperus sexangularis* and *Phragmites maurianum* (Dahms, 2015; Musa, 2015). There are seasonally variations in water levels and flow regime.

Figure 2.5 Indicates how the sites looked like during high and low flow conditions. Photographs of site 4, Jasper (JASP) (photographs courtesy N Baker, 2017).

Site 5 Nylsvley Nature Reserve (Nyl) - is located in the Nylsvley Nature Reserve which, is a Ramsar accredited wetland of international importance. The nature reserve supports a large number of waterbird species, wide variety of mammals, reptiles, fish and insects and the

endangered roan antelope (*Hippotragus equinox*) and various waterfowl species (Higgins *et al.*, 1996). There is an abundance of submerged and emergent vegetation with the riparian zone characterized by reeds and sedges. Seasonal floods drive the ecological functioning of the floodplain (Higgins *et al.*, 1996). The ecological functioning has been affected by water resource development within the Nyl catchment, which changed the flooding regime (Kleynhans *et al*., 2007).

Figure 2.6 Indicates how the site looked like during high flow conditions. Photograph of site five, Nylsvley (NYL) (photograph courtesy N Baker, 2017).

Site 6 Glen Alpine Dam (GLEN) is located in the Mogalakwena River. Sampling took place downstream of the Glen Alpine Dam. The Glen Alpine Dam has limited yield due to its small size, the ephemeral nature of the run-off into the dam and high evaporation rates in the area.

Figure 2.7 Indicates how the site looked like during high and low flow conditions. Photographs for site six, Glen Alpine Dam (GLEN) (photographs courtesy N Baker, 2017).

Site 7 Piet Farm (PIET) is a site in the Mogalakwena River just before the Limpopo River confluence and has agricultural impacts. It is characterized by bedrock and has little or no riparian zone.

Figure 2.8 Indicates how the site looked like during high flow conditions. Photograph of site seven, Piet Farm (PIET) (photograph courtesy N Baker, 2017).

Site 8 Mogalakwena Limpopo Confluence (Echo 15) is located at the confluence of the Mopgalakwena and Limpopo rivers. It is characterized by bedrock and has little or no riparian zone.

Figure 2.9 Indicates how the site looked like during high flow condition. Photograph of site eight, Mogalokwena Limpopo River confluence (Echo 15) (photographs courtesy N Baker, 2017).

Site 9 Limpopo River (Echo 14), sampling was done 10 km downstream of the Mogalakwena and Limpopo River confluence. It is characterized by bedrock and has little or no riparian zone.

Figure 2.10 Indicates how the site looked like during high flow condition. Photograph of site nine, Limpopo River confluence (Echo 14) HF (photographs courtesy N Baker, 2017).

CHAPTER 3

MATERIALS AND METHODS

3.1 Field Protocol

The study was conducted along the course of the Nyl/Mogalakwena River until the confluence with the Limpopo River. The sampling sites begin at the source of the Klein Nyl River at Klein Nyl Oog to the confluence with the Limpopo River. The river flows through the Nyl River floodplain which contains the Ramsar accredited Nylsvley Nature Reserve. Epiphytic diatoms were chosen because of the presence of submerged vegetation (*Phragmites sp*) at all sites. The choice of substrate (epiphytes) has no impact on the results gathered as the aim of the project is to determine the impacts of anthropogenic activities on the study area rather than conduct a comprehensive diatom biodiversity study (Musa, 2015).

3.1.1 *In situ* water quality

Sampling was conducted during both high (27th February-2nd March 2017) and low flow (16-18 July 2016) seasons. The physico- chemical parameters of the water were determined *in situ* at each site using portable water quality meters during each sampling trip. The parameters measured were pH, temperature (^{o}C), and Electrical Conductivity (µS/cm).

3.1.2 Water sample collection

Water samples were collected at each site during each sampling trip for nutrient analysis. Clean 1 litre polyethylene sampling bottles were used to collect water (Musa 2015, Dahms, 2015). Water samples were frozen to stop microbial activity and reaction changes during transportation to the laboratory and remained frozen until they were ready to be analysed (Musa, 2015).

3.1.3 Collection of diatom samples

Diatoms were sampled from the biofilm present on the submerged parts of macrophytes according to the method stipulated by Taylor *et al.*, 2007. Presence of diatoms notable by brown film existing on submerged macrophytes was an essential factor when deciding which plants to use. As far as possible it was ensured that the substrate was not dominated by filamentous algae and was located in the main flow channel (or close to) that was not sheltered by overlying canopy.

Plant material of a certain morphological type was placed into clean zip-lock bags along with some of the water from the system. Care was taken to ensure that the water collected was not contaminated with particles that might have been lifted up from the benthos due to disturbance. The bag was sealed and shaken vigorously to remove the biofilm of the plans. The bag was opened, and water with the brown suspension was poured into a clean plastic bottle.

3.1.4 Preservation of diatoms

The diatom samples were preserved in 20% ethanol. The volume of the water and diatoms was measured and 95% ethanol was added to make a final ethanol volume of 20%. The bottles were then stored in a cool dark place until sample preparation and mounting on slides could take place.

3.2 Laboratory procedure

3.2.1 Nutrient Analysis

Frozen water samples were thawed to room temperature before the testing procedure commenced. The nutrients under consideration included nitrites (NO_2-N), nitrates (NO_3-N), ammonia (NH_3-N), ammonium (NH_4-N) and phosphates (PO_4-P). The water samples were treated according to the method protocol stipulated by the respective test kits manuals developed by Merck. The catalogue numbers for nutrients are as follows NO_2-1.14776.0001; NO_3 - 1.14773.0001; NH_3 and NH_4- 1.14752.0001; PO_4-1.14848.0001. A Spectroquant Pharo-100 (Merck) was used for the nutrient analysis.

3.2.2 Diatom processing

3.2.2.1 Cleaning of Diatoms

The protocol stipulated by Taylor *et al.*, 2007 was followed during the cleaning process for the different diatom samples. The cleaning process is vital as to remove the presence of any matter which will prevent the observer in having a clear view of diatoms in the sample. In order to identify the diatom accurately, no undesirable organic matter should be present on the final slide mount.

The cleaning method used for this study uses potassium permanganate and hot hydrochloric acid, as it has been proven to be effective in South African waters containing high amounts of organic material (Taylor *et al.*, 2007).

The samples were allowed to settle in the sampling bottles, after which the supernatant was discarded without any loss of the material. The remaining sample was concentrated to ensure a random distribution of diatoms in the bottle. Five millilitres of sample was pipetted into a small clean glass beaker. Five ml of saturated potassium permanganate was added into the beaker and mixed thoroughly. Potassium permanganate oxidises the organic matter. The solute was left for

24 to 36 hours to ensure sufficient oxidation. The reaction time depends on the amount of organic matter present. Five to ten ml of concentrated hydrochloric acid (32%) was then added to the samples. The beakers were heated on a hot plate until the solution became yellow or clear to digest the potassium permanganate mixture.

Once clear, two drops of hydrogen peroxide were added to the beaker to determine if there was any remaining organic matter. If organic matter was present, the solution foamed. If foaming occurred the samples were heated again till oxidation was complete, and samples were allowed to cool to room temperature.

Ten ml of the cleared solution containing diatom valves was transferred into glass centrifuge tubes and centrifuged at 2500rpm for 10 minutes. After centrifuging the supernatant was decanted in one go making sure that diatoms were not lost. The pellet was re suspended using 10 ml MilliQ water. The centrifugation process was repeated four times to ensure that the valves were clean and the acid was removed. After the cleaning process, each sample was stored in individual glass vials making up a stock solution.

3.2.2.2 Preparation of slides

The stock solution was diluted until it appeared slightly cloudy to prevent overcrowding on the slide. A drop of 10% ammonium chloride was added to each sample to neutralise the negative charge of diatoms as to avoid clattering of diatom valves in the solution. Coverslips and microscope slides were cleaned with 95% ethanol. Half a millilitre of diluted diatom solution was placed into cover slips and allowed to dry.

After the cover slips were dry, they were placed on a hot plate at 350^0C to sublimate the ammonium chloride. A drop of pleurax (r.i. 1.73) was placed on the cover slips and the microscope slide was lowered onto the cover slips. The slides were placed on a hot plate for about two minutes so as to boil the solvents off the pleurax. The slides were cooled and labelled.

3.2.2.3 Archiving

Archiving is important for future verification and cross referencing. Clean samples were stored in ethanol to prevent the growth of bacteria. The samples were stored in a dark, dry environment.

3.2.2.4 Identification and Enumeration

Diatom cells were counted under a Zeiss Axioplan II microscope, using 60-100 times objective magnification. The identification was done to species level using a diatom key developed by

Taylor *et al*., (2007). On each slide four hundred diatoms were counted and recorded in opticount. The data was then uploaded into OMNIDIA, a diatom database.

3.3 Statistical Analysis

Raw data was processed and a number of analyses were performed from the data obtained from *in situ* water quality, nutrient tests and diatom indices.

Ominidia was used and 17 different index values were derived classifying sites into differing categories of health. For this study only four indices were used (SPI, GDI, TDI, %PT) since they included more than 80% of the taxa in generating the index values.

Individuals within a population have distinctive requirements or ideal conditions in order to survive. Any disturbances in the natural living conditions, will result in sensitive species dying first. Thus the resilient species would thrive and become dominant as competition for resources will be minimal. In studying differences within communities throughout distinct environmental conditions huge differences in the composition of species can be observed (Lepš and Šmilauer, 2003). This may possibly be related to different but occasionally overlapping requirements of the individual species for specific environmental conditions such as certain pH and nutrient concentration resulting from the capability of particular species to compete for light, nutrients and other crucial resources (Lepš and Šmilauer, 2003). Diatom communities are not excluded as different species having distinct needs, with some species being sensitive to certain environmental variables compared to others. Therefore, an ordination technique (RDA) was used to see how different species react to their environment.

Redundancy Analysis (RDA) was used in this study to determine which environmental variable best explains the differences of biological assemblages (Lepš and Šmilauer, 2003), seen as fluctuations in the diatom community may cause changes in diatom index scores (Matlala *et al*., 2011). RDA is a linear constrained ordination method of two sets of variables which assesses the manner in which the difference in one set of variables describes the difference in another set (Gugger, 2012). In a RDA environmental variables such as temperature and nutrient concentration are presented as arrows, with the length of the arrow representing an increase in the environmental variables (Lepš and Šmilauer, 2003). RDA was applied to determine principal patterns of diatom species distribution relative to the assessed environmental variables (Matlala *et al*., 2011).

Correlation is a bi-variate method, which determines the strength of the relationship amongst two variables and the direction of the relationship. Correlation coefficient values vary between +1 and -1, as the values lie around ± 1 there's a perfect relationship amongst the two variables. As the values moves towards 0, there will be a weaker relationship. A positive correlation (>0 to +1) implies that the variables increase together and a negative correlation (<0 to -1) implies that when one variable increases the other one decreases. When the coefficient value is nearer to -1 or +1, the stronger the negative and positive correlation (Lepš and Šmilauer,2003).

A Pearson correlation was done on the data since the data was parametric. It was done to determine the strength in relationship between the environmental variables to the diatom indices. The Pearson correlation was done to see the extent of changes in environmental variables results in a linear change in the index value.

A Shannon-Wiener diversity index was performed on the diatom list attained, which is a species richness index. The index was used to measure the species diversity along the course of the river, and possibly determine anthropogenic effects on the diversity of diatom assemblages. The index accounts for both abundance and evenness of the species in a community. It is denoted by the following formula:

$$H' = -\, pi \sum lnpi$$

Pi= the proportion of individuals found in species i

The index values relate to the degree of difficulty with which the next predictable specie sampled is identified (Hill *et al*.,2003) and values are generally between 1 and 4 increases as richness and evenness increases.

The RDA was drawn using Canoco v5. The diatom indices and Shannon-Wiener index graphs were drawn using Excel 2016.

CHAPTER 4

RESULTS AND DISCUSSION: WATER CHEMISTRY

4.1 Introduction

In this chapter the focus will be on the results obtained from measuring water quality variables, physico-chemical parameters and nutrient concentrations. It is important to measure the physico-chemical parameters in order to properly manage surface water and protect aquatic biota (Matlala *et al.*, 2011).

Relations between physico-chemical parameters and natural conditions may regulate the growth and survival of organisms, therefore changes in these variables can result in serious disturbances to the ecological and physiological functions of aquatic organisms (DWAF, 1996), which may lead to the death of important species and eventually the whole community structure (Matlala *et al.*, 2011).

Water quality can be divided into four groups: 1) aesthetic properties comprise of odour, taste and litter, 2) biological properties refer to the biodiversity of the system, 3) physical properties comprises of temperature and turbidity and 4) chemical properties comprises of dissolved oxygen, conductivity, pH, metals and salts (DWAF,1996). Monitoring these variables frequently is therefore vital to avoid physiological distractions and loss of crucial biological groupings. The Department of Water and Forestry (DWAF) therefore developed the Target Water Quality Range (TWQR) for South African aquatic ecosystems (DWAF, 1996). These guidelines serve as a basis to notify water users of the ranges within which water parameters should fall so as to maintain the over-all integrity of the system (DWAF,1996).

Table 4.1 Summary of TWQR for freshwater systems in South Africa (DWAF, 1996).

Water quality variables			**Natural values**	**TWQR**
Nutrients	**Nitrogen**	**Ammonium, ammonia, nitrites and nitrates**	<0.5mg/l	Deviation < 15%
	Phosphorus	**Orthophosphates**	<50mg/l	Deviation < 15%
Physico-chemical variables		**pH**	4-11	Deviation < 5%
		Temperature	5-30^{o}C	Deviation < 10%
		Conductivity	Not available	Deviation < 15%

4.2 Water quality parameters

4.2.1 *In situ* parameters

4.2.1.1 pH

pH is the measure of hydrogen ion activity in the water, and is expressed as negative $\log_{10}$ of the hydrogen ion activity (Dallas and Day, 2004).

$$pH=-\log_{10}[H+]$$

Where [H+] denotes the hydrogen ion concentration (Dallas and Day, 2004)

The pH of water controls the solubility and biological availability of chemical constituents such as nutrients and heavy metals (Davies and Day, 1998). The pH values range from 0-14 with 7 representing neutral conditions, pH <7 (acidic) and pH>7 (basic). pH can be influenced by geology, biotic activities, vegetation type and atmospheric influences, biological activities, total dissolved salts and temperature (Mwangi, 2014). These influences may change pH, therefore change biological assemblages killing of sensitive taxa. The pH of rivers un-impacted by anthropogenic effects range from 6.5-8 and in order for most freshwater organisms to survive the pH range from 4.5-9 (Dallas and Day, 2004), however extensive differences might occur as a result of catchment geology (Faniran *et al.,* 2001). Acid pollution is more common in rivers than alkaline pollution. Acidification in rivers is usually the result of industrial effluents, mine drainage and waste water, whereas alkaline pollution may occur from specific industrial effluents such as food canning as well as anthropogenic eutrophication (Dallas and Day, 2004). pH values can have an effect on other pollutants in the water for example solubility of heavy metals increases at low pH therefore resulting in higher concentrations of heavy metals in the water column, whereas extremes in pH can have an effect in the palatability of the water (Nkambule, 2016).

There is a strong relationship between diatoms and pH because pH exerts a direct physiological stress on diatoms (Bere and Tundisi, 2011) and also influences the distribution of diatoms (Matlala *et al*., 2011). Diatoms respond to extremes in pH because of their sensitivity to pH and in some instances narrow tolerance limits to pH. Extremes in pH can cause toxic results on river biota including diatoms particularly if the diatom species have narrow pH tolerance limits (Taylor, 2004).

From the graph (Figure 4.1) it can be observed that the highest recorded pH was during low flow at Glen Alpine 10.73, this exceeded the range of pH of natural conditions, which is 6-8. pH values greater than 8.5 for example can show that alkaline water system will be likely to develop scale when heated (Thalman and Bedessem, 2006). The lowest pH value is recorded at Jasper during

the low flow 4.4. Low pH values particularly those less than 4 are likely to signal the corrosive nature of the water and be likely to dissolve metals and other substances that it could have been in contact with (Thalman and Bedessem, 2006). At the other sites the pH was almost constant ranging between 6-8. The lowest pH at Jasper can be attributed to the discharge of untreated sewage released to the system as it is the site below the sewage treatment works. The pH values are within the natural values for freshwater systems proposed by DWAF (1996).

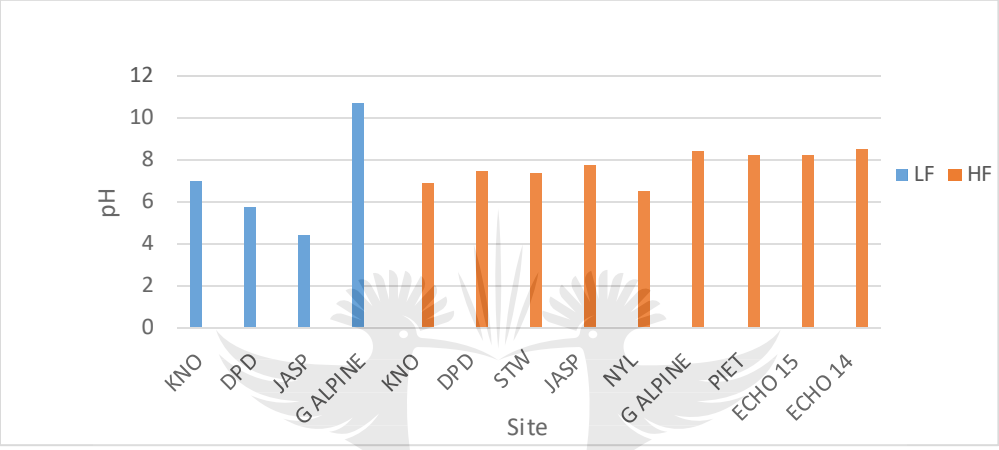

Figure 4.1 Bar graph representing pH values along the Nyl/Mogalakwena River, Limpopo Province

High precipitation periods can result in a change in hydrological patterns (Sternberg, 2003) whereas low precipitation periods characterised by low flow can cause nutrients and other contaminants to become more concentrated (Adams and Peck, 2008). A rise of flow through the organic soil horizon of saturated soils can occur if rain persists (Sternberg, 2003), resulting in changes in chemical composition of dissolved natural organic matter and fluxes (Ivarsson and Jansson, 1994, Laudon *et al.*, 1999, Correl *et al.*, 2001). Decomposing of organic matter releases carbon dioxide, which combines with water to form carbonic acid thereby lowering the pH.

4.2.1.2 Temperature

Temperature is an important environmental variable (Musa, 2015), as it affects the speed of chemical reactions, therefore affecting the metabolic rates of organisms in water (Matlala *et al.*, 2011). Temperature can cause mortality and can have an effect on the solubility of dissolved oxygen (Carr and Nearly, 2008). Temperature is influenced by latitude, altitude, seasons, time of

day, air circulation, cloud cover as well as the flow depth of the waterbody (Chapman and Kimstach, 1996).

In a natural river, water temperature rises as discharge drops (Hockey *et al.*, 1982; Webb *et al*., 2003) and the magnitude of diurnal variations is inversely proportional to flow rate (Dallas, 2008).

Temperature can have an influence on the reproductive rate of diatoms (Matlala *et al.*, 2011). Human activities changing the temperature of aquatic systems may have an impact on the community structure of the organism living in the system (Dallas and Day, 2004). Anthropogenic factors causing a change in temperature include heated industrial and agricultural effluents, removal of riparian vegetation and release of water from impoundments. High temperatures reduces the solubility of dissolved oxygen therefore decreasing its availability to aquatic organisms. Increased temperatures also have an effect on the metabolic rate of aquatic organisms (DWAF, 1996).

From the graph (Figure 4.2) it can be seen that the lowest recorded temperatures were during low flow periods (Ranging from11.4-15.6) with the temperature values almost constant from KNO to Jasp with a slight increase in Glen Alpine.

The high temperatures occurred during high flow conditions with the highest recorded temperature being at Echo 15 and Echo 14 at 27^{o}c and 27.1^{o}c respectively. This could be expected because the sampling was done in summer. There is no riparian vegetation in these sites which can cause an increase in temperature, because a lack of a riparian zone exposes water to increased direct solar radiation, leading to higher temperature and greater temperature ranges and fluctuations (Dallas, 2008).

Irrigation practices and return of agricultural drainage causes an increase in temperature (Dallas and Day, 2004). The temperature values fall within the TWQR shown in table 4.

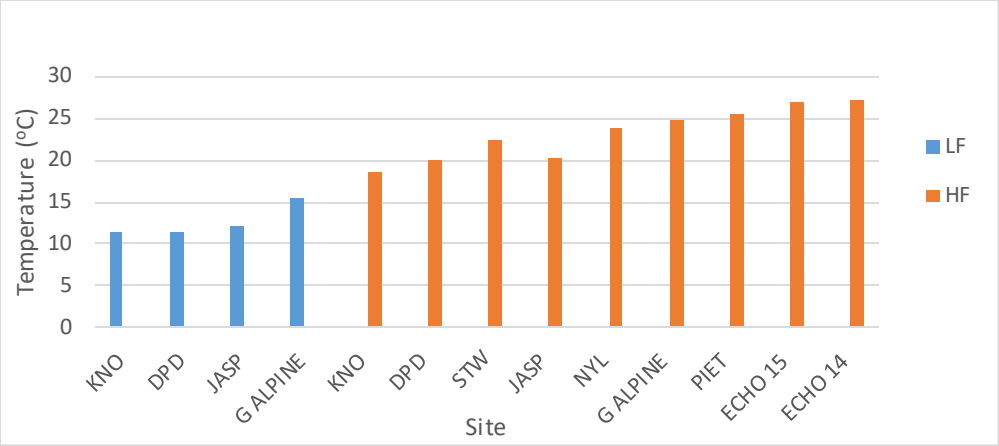

Figure 4.2 Bar graph represent temperature ranges along the course of the Nyl/Mogalakwena River, Limpopo Province.

Temperature is essential in the growth rate of diatoms (Matlala *et al*., 2011) and therefore completely affects diatom metabolism, and thus the specific composition of the assemblages (Rimet, 2012), since some taxa prefer a specific temperature range (DeNicola,1996).

Mounting water temperatures will cause a rise in primary production, organic matter decomposition and nutrient cycling rates causing lower dissolved oxygen levels (Adams and Peck, 2008). The optimum temperature for benthic diatoms ranges from 25-30^{0}c with a decline in diversity at temperatures higher than 30^{0}c (Jakovljević *et al.*, 2016)

4.2.1.3 Electrical Conductivity (EC)

Electrical conductivity is the measure of the waters ability to conduct an electric current (DWAF, 1996, Mwangi, 2014), it is closely correlated with total dissolve salts (TDS) and is frequently utilised for estimating the amount of TDS (Poe, 2015). An increase in temperature can cause an increase in EC (Nkambule, 2016). Naturally conductivity depends on the geology of the area and degree of dissociated ions. High conductivity may occur as a result of discharging saline domestic and industrial effluents in the river (Mwangi, 2014). The EC values of most freshwater range between 10 and 1000 µS/cm, whereas that polluted waters or waters receiving large amount of land runoff can have values higher than 1000 µS/cm (Chapman and Kimstach, 1996).

The higher the conductivity, the greater the number of ions in solution will be (Dallas and Day, 2004).

Figure 4.3 indicates the lowest recorded EC is at Piet farm 25.6 µS/cm and the highest being at Jasp low flow 700 µS/cm, Jasp high flow 686 µS/cm and STW high flow with a value of 597 µS/cm. As conductivity indicates ions which enters the river from sewage works, industrial runoff, urban runoff, mining and agricultural runoff, the higher conductivity values at Jasp can be due to sewage from Modimolle sewage works (Dugarpesad, 2002). Therefore, the highest conductivity values are at Jasp, which is below the STW can be due to high dissolved salts entering the water from sewage runoff, urban runoff from informal settlements and industrial effluents.

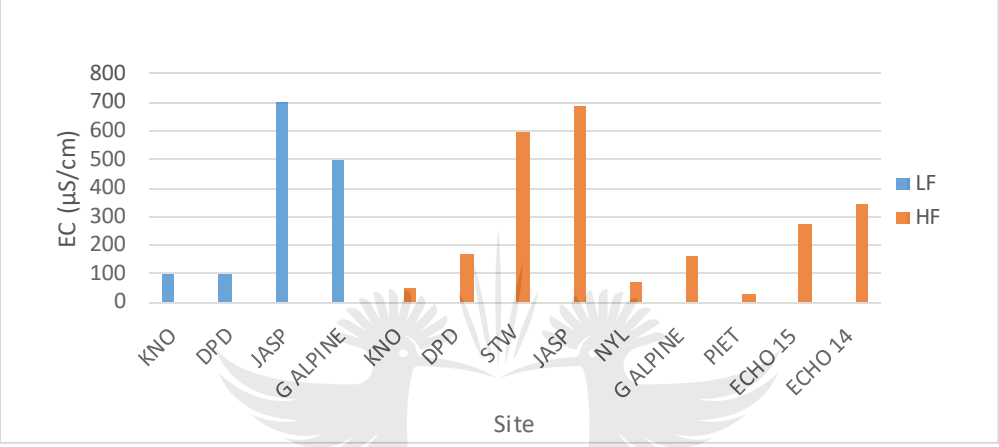

Figure 4.3 Bar graph representing Electrical Conductivity (EC) along the Nyl/Mogalakwena River, Limpopo Province.

A study conducted by Dinka *et al* (2004), indicate that conductivity decreases with water level increase, nonetheless other scientific studies came to the conclusion that with a decline in water flow the EC will increase as a result of an increment in the number of particulates and nutrients. In conclusion relationship between EC and water flow can be described to be site specific (du Plessis, 2010)

4.2.1.4 Chemical Oxygen Demand (COD)

COD measures the total amount of oxygen needed to oxidize all organic material into carbon dioxide and water. COD is suitable for determining wastewater quality requirements discharged in receiving waters so as to reduce their impacts (Nkambule, 2016). Fluctuations in COD are stated to be precisely related with inputs of organic matter and nutrients in the waterbody (Apsite and Klavins, 1998). COD can assess vulnerability to oxidation of organic and inorganic matter currently in water and in the effluents from sewage and industrial works (Chapman and Kimstach,

1996). The COD of uncontaminated water range from 20mg/l O_2 or less, whereas water receiving effluents have a concentration more than 200mg/l, and in industrial waste water the values could be as high as 60 000mg/l O_2 (Chapman and Kimstach, 1996).

Since there is a direct association of COD with input of organic matter and nutrients, scientific studies that have been conducted on the relationship between water flow fluctuations and nutrients can be utilised in determining the possible relationship with COD. Therefore, a rise in nutrients such as nitrate and phosphates is related with a rise in COD concentration. A decline in water flow can be related with higher nutrients and therefore high COD concentrations (Sanchez-Carrilo and Alverez- Cobelas, 2001; Geraldes and Boavida,2005; Dinka *et al.*,2004; Naselli-flores and Barone, 2005; Baldwin *et.al*, 2008; White *et al.*, 2008).

From the graph (Figure 4.4) it can be seen that the lowest COD was during the low flow at KNO and DPD (4.600; 9.200, respectively). The highest COD concentration is observed during high flow at NYL. This indicates that there is a high amount of oxygen consumed. It then drops downstream of the NYL.

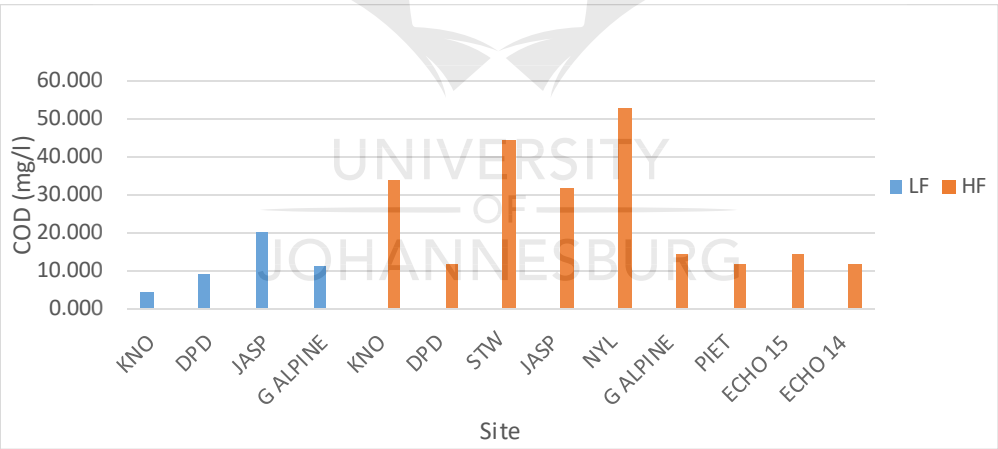

Figure 4.4 Bar graph representing Chemical Oxygen Demand (COD), along the Nyl/Mogalakwena River, Limpopo Province.

4.2.2 Nutrient Concentration

Nutrients are crucial elements to life because they are needed for growth and reproduction (Addiscott *et al*., 1991; Dallas and Day, 2004). In aquatic ecosystems nitrogen and phosphorus

are two nutrients that usually reduce maximum biomass of algae (Carr and Neary, 2008). An increase in nutrient enhancement may be as a result of effluent from non- functional sewage works and un-sewered human settlements (de Villiers and Thiart, 2007), causing a high risk to water quality and biodiversity (de Villiers and Thiart, 2007). There has been an increase in nutrient levels of many freshwaters due to widespread agricultural intensification and increased discharge of domestic waste (Vitousek *et al.*, 1997; Galloway and Cowling, 2002).

4.2.2.1 Nitrogen

Nitrogen is one of the most profuse elements in nature and is a vital constituent of most biological and biochemical processes (Matlala *et al.*, 2011). In aquatic systems nitrogen may occur in an organic and inorganic form (Carr and Nearly, 2008). Organic nitrogen is vital for protein synthesis whereas inorganic nitrogen contains all the main inorganic nitrogen such as Nitrites (NO_2-N), Nitrates (NO_3-N), Ammonia (NH_3-N), Ammonium (NH_4-N) (DWAF, 1996). Here the focus will be on inorganic forms of nitrogen. In natural systems levels of nitrogen are occasionally high because they are consumed by plants for protein synthesis, usually as nitrates (Musa, 2015). Nitrate is the most completely oxidized state of nitrogen found in water. Increased nitrate concentrations can happen naturally, and may give an indication of biological waste in water, runoff from fertilizers, animal waste, land application of manure, municipal and industrial wastewater (Dugapersad, 2002; Zucker and Brown, 1998, du Plessis, 2010).

Total ammonia (NH_3 + NH_4) is the reduced form of nitrogen, occurring naturally in water due to the breakdown of nitrogenous organic and inorganic matter found in the soil and water (Chapman and Kimstarch,1996; Lester and Birjet,1999; Van Loon and Duffy, 2005), and is regulated by water temperature and pH. Under alkaline conditions, ammonia is more toxic as compared to neutral conditions, whereas the toxicity is low in acidic conditions (DWAF 1996a). In non-contaminated waters ammonia levels are usually lower than 0.1 mg/l N (du Plessis, 2010). High levels of ammonia are an indication of organic contamination from domestic sewage or agricultural contamination due to fertilizers (Dugapersad, 2002). Ammonia can contribute to eutrophication (DWAF, 1996). Seasonal variations in ammonia levels develop from the death and decay of aquatic organisms such as phytoplankton and bacterial in waters high in nutrients (Chapman and Kimstarch, 1996; Davis and Mccuen, 2005).

From graphs (Figures 4.5, 4.6, 4.7, and 4.8) it can be observed that nitrogen concentrations vary significantly at the expected impacted sites with the highest concentrations at SWT and Jasp. The nitrogen concentration is increased by wastewater from urbanised area as seen in STW high flow, which was non-functional during the study period discharging raw sewage. The untreated sewage is then carried downstream to JASP. After JASP the nitrogen concentrations remain constant because of the NYL wetland which reduces the nitrogen concentration drastically. The highest NO_2-N are at JASP (0.1 mg/l) and JASP (0.130 mg/l) respectively. The highest NO_3-N were at JASP during low flow (4.7 mg/l). The highest NH_3-N were at JASP during low flow (6.360 mg/l) and at STW during high flow (7.00 mg/l) due to the effluent from STW, this can also be observed with the concentrations of NH_4-N where the highest are at JASP during low flow (8.2 mg/l) and STW (7.00 mg/l) respectively. The high concentration of these nutrients at these sites indicate eutrophic conditions.

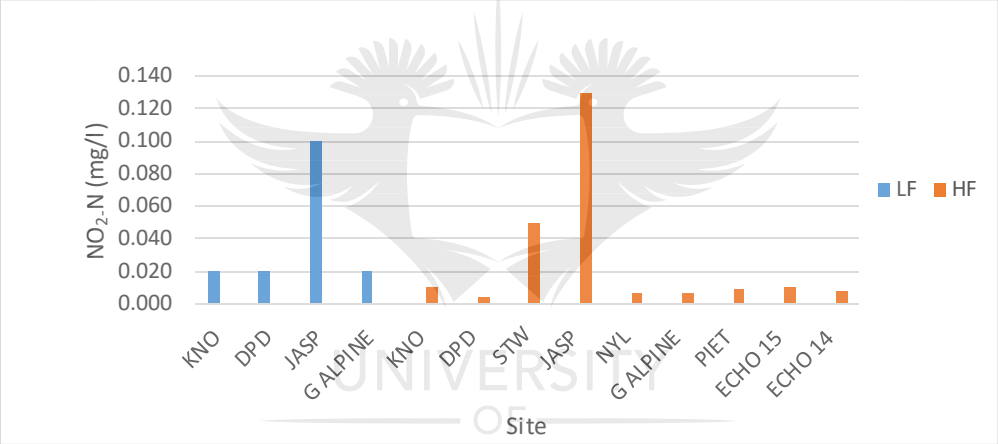

Figure 4.5 Bar graph representing NO_2-N concentrations (mg/l) along the Nyl/Mogalakwena River, Limpopo Province.

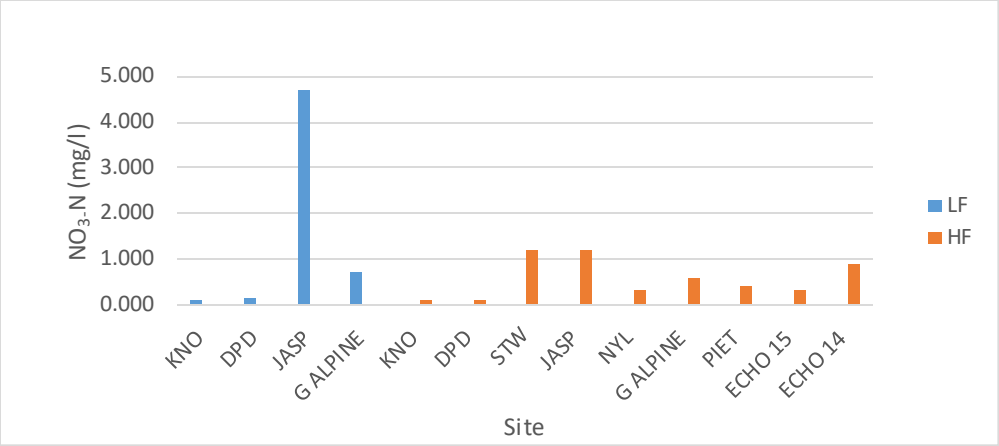

Figure 4.6 Bar graph representing NO_3-N concentrations (mg/l) along the Nyl/Mogalakwena River, Limpopo Province.

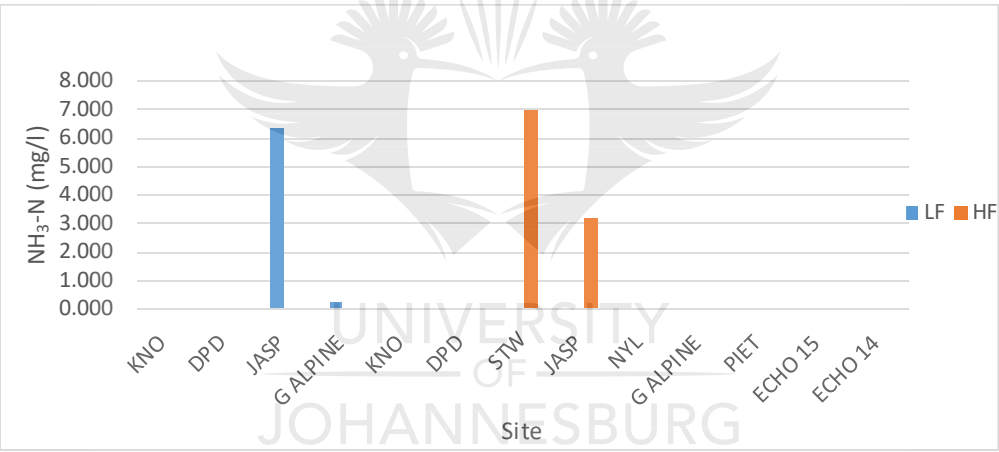

Figure 4.7 Bar graph representing NH_3-N concentrations (mg/l) along the Nyl/Mogalakwena River, Limpopo Province.

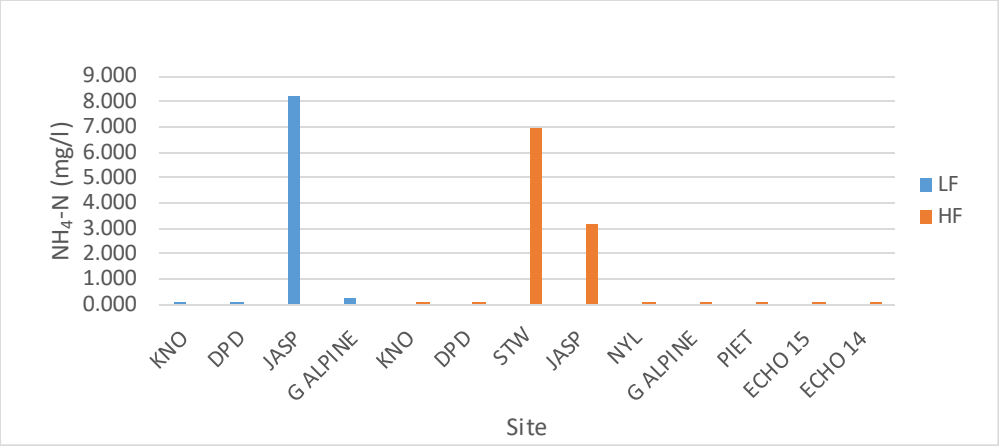

Figure 4.8 Bar graph representing NH_4-N concentrations (mg/l) along the Nyl/Mogalakwena River, Limpopo Province.

4.2.2.2 Phosphorus

Phosphorous is vital for plant growth stimulation, and a crucial micronutrient with an important function in the structure of nucleic acids and in the storage and production of energy in cells (Nkambule, 2016, Malan and Day ,2005). Phosphates enter the aquatic environment from natural weathering of minerals, biological decomposition, and runoff from human activities. Orthophosphates (PO_4-P) are the most important phosphate species and it is bioavailable to aquatic biota and normally found in sewage waters (Carr and Nearly, 2008; du Plessis, 2010).

An increase in flow can cause an increase in total phosphorus because a huge quantity of its load is bound to sediments with small amounts dissolved in water (Malan and Day, 2003). During high flow adsorbed phosphorus might be released from sediments and enter the watercourse due to erosion of the surrounding catchment (Malan and Day, 2003; Dallas and Day, 2004).

Human activities can contribute to high phosphates in water through point sources such as industrial effluents or non-point sources such as fertilizer runoff (Mwangi, 2014). High levels of phosphorus can be harmful to aquatic ecosystems as it is the principle nutrient controlling the degree of eutrophication. Increasing levels of phosphates can be linked to low pH conditions (DWAF, 1996).

From the graph (Figure 4.9) showing phosphate concentrations along the study area for different sampling periods. The phosphate concentrations upstream of STW and downstream of NYL are consistent and low with an increase at JASP and a peak observed at the STW showing extreme fluxes in the PO_4-P concentration in the system. The high phosphate concentration shows that STW, which receives wastewater from nearby areas gets increasing loads of phosphate in the effluent (Musa, 2015). The highest concentration during the sampling period are at STW high flow (0.81 mg/l). The high phosphate levels signal pollution and are responsible for eutrophication (du Plessis, 2010).

Figure 4.9 Bar graph representing PO_4-P concentrations (mg/l) along the Nyl/Mogalakwena River, Limpopo Province.

4.3 Discussion

The capability of the aquatic environment to maintain healthy systems is influenced by the physical, chemical and biological constituents of water, therefore a drop in water quality and quantity of a system will have a detrimental effect on the organisms living in that system (Matlala *et al.*, 2011).

The TWQR were used in this study as they serve to inform the public of the ranges within which no measurable undesirable outcome is anticipated on the general health of the ecosystem (DWAF, 1996). The measured parameters excluding pH and temperature it is recommended that the deviation from natural values should be less than 15% (DWAF, 1996). From the results

attained, it can be observed that the pH and temperature fall within the proposed TWQR for freshwater systems in South Africa (see Table 4.1)

pH regulates the accessibility and toxicity of chemical constituents (Matlala *et al.*, 2011), therefore affecting water chemistry, together with the types of organisms occurring in aquatic ecosystems. It is therefore a vital water quality variable (DWAF,1996). The temperature in the study area are able to maintain aquatic organism since they fall within the TWQR ranges. Temperature is also an important parameter, vital for regulating many physiological processes in aquatic organisms, in addition it plays a vital role in the concentration of dissolved oxygen because elevated temperature ranges decreases the solubility of dissolved oxygen in water therefore reducing its concentration and its accessibility to aquatic organisms (Matlala *et al.*, 2011).

The EC in the study was high at STW and at JASP, which ranged from (686 µS/cm and 700 µS/cm) respectively. The highest readings (685 µS/cm) were also recorded in Greenfield (2004) in the Nyl River wetland with the highest values at STW. The elevated EC values in these sites can indicate pollution. .

Nitrite can be converted promptly to nitrate, and nitrate to nitrite by bacterial methods. Nitrite is oxidized to nitrate by nitrifying bacteria under aerobic conditions, while under anaerobic conditions NO_3 is reduced to NH_4 by denitrifying bacteria (DWAF, 1996). Nitrate is the steadier of the two forms, therefore vital forms of dissolved inorganic nitrogen occurring in natural water are ammonium and nitrates (DWAF,1996).

The concentration of inorganic nitrogen found in this study exceeded the recommended TWQR values with the highest at STW and JASP. This may be the result of wastewater effluent from the sewage treatment works indicating high anthropogenic impacts.

As mentioned in section 4.2.2.2 phosphorus is vital for plant growth stimulation and plankton which can be food sources for fish and other aquatic organisms, therefore may result in a rise in the fish population and increase the overall water quality. Nonetheless high phosphorus concentration may cause eutrophication. According to DWAF (1996) inorganic phosphorus above 0.52 mg/l can cause hypertrophic conditions which are bad for water quality but good for algal growth as evident in figure 4.9 at STW. The phosphate levels in the study sites were all below the recommended levels.

4.4 Conclusion

The results show that there are limited impacts upstream of STW. Extreme changes at STW for all variables measured indicate its impact on the system breaking the TWQR. NYL meets the properties of a wetland by purifying water quality (Musa, 2015). Temperature is relatively stable during low flow with increases taking place during high flow this can be because in high flow sampling was done in summer. The nutrient concentrations are stable with peaks being observed at STW and JASP this can be related to effluents realised from STW, agricultural runoff, animal and human excrement. It can be established that every environmental variable determined in the study are significant, as every single variable has an effect on the next (Matlala *et al.*, 2011).

CHAPTER 5

RESULTS AND DISCUSSION: DIATOM INDICES

5.1 Introduction

Diatoms are widely used as bio-indicators, as they can be used in determining water pollution and the trophic status of aquatic habitats (Hicks *et al.,* 2006, Dayıoğlu and Tokatli*,* 2014). They are also good indicators of changes taking place in aquatic systems because they are sensitive to a range of environmental factors required for optimal growth (Noga *et al.,* 2013).

This chapter will discuss the importance of diatoms collected at different sampling sites by examining their richness, how they relate to water quality parameters, by calculating diatom indices and correlating these indices with environmental parameters examined in Chapter 4.

The diatom indices were developed to manage aquatic resources. In the case of GDI or SPI, which were used in the study, a score of 19 denotes pristine water, while a score of 5 will denote unsatisfactory water quality (Szczepocka and Szulc, 2009). The index scores in section 5.4.2 were generated from diatom species composition using Omnidia where only three indices were selected as they used >80% of taxa incorporated when generating the index values.

The indices used in this study were the GDI, SPI and TDI. The GDI only accounts for the genera of the taxa and a species within genera might vary with respects to their optimal conditions, so other indices should be used to complement or confirm results obtained from the GDI (Musa, 2015). The SPI was developed to indicate general water quality and requires taxa to be identified to specie level (Taylor, 2004). It gives a reliable indication of water quality as it was designed to incorporate the specific requirements of every single species in a particular sample (Matlala *et al.*, 2011). The TDI was developed for monitoring trophic status with scores ranging from 0-100. Increasing scores indicate eutrophication and decreasing scores indicating oligotrophic conditions. The %PT is used to estimate the dependability of the TDI with values above 20 indicating organic pollution.

Within a waterbody a rise in the level or concentration of environmental variables can cause pollution, therefore resulting in behavioral and physiological changes of organisms within that waterbody. An increase in SPI and GDI denotes good water quality, while an increase in TDI denotes a decrease in water quality, and as a result, environmental variables are likely to display negative correlations with the GDI and SPI, while a positive correlation is expected between the measured environmental variables and TDI (Matlala *et al.*, 2011).

5.2 Specie composition

In order to generate diatom indices, it is important to accurately identify and count the diatom species, since the index values are built on the weighted abundance of species, which occur due to the average optima of diatom taxa in the sample. The dominant species play a significant role in influencing the ecological category where the samples will be placed (Musa, 2015).

A total of 146 diatom species were counted during the identification process and used in the generation of the diatom index values. Out of the total species counted 12 were considered as dominant (>15% of a community). Omnidia software was used to generate 17 indices and of these 17 indices generated three indices were selected based on the basis of the percentage of taxa used to generate the index values. The indices selected were: The Generic Diatom Index (GDI), the Specific Pollution Index (SPI) and the Trophic Diatom Index (TDI). The indices were used because they appeared to be highly dependable because they gave the utmost percentage composition of diatom species in almost all sampling sites (>80%). The list of the species is listed in Table 5.1 with the dominant species highlighted in yellow.

Table 5.1 The list of diatom taxa encountered in the study with dominant taxa highlighted in yellow. The codes correspond to their identity in Omnidia. The associated name belongs to the person who identified the taxon.

CODE	DIATOM SPECIES
ADMI	*Achnanthidium minutissimum* (Kützing) Czarnecki
ADEU	*Achnanthidium eutrophilum* (Lange-Bertalot)Lange-Bertalot
ADEG	*Achnanthidium exiguum* (Grunow) Czarnecki
ADSA	*Achnanthidium saprophilum* (Kobayasi et Mayama) Round & Bukhtiyarova
ACAF	*Achnanthidium affine* (Grun) Czarnecki
ADSB	*Achnanthidium straubianum* (Lange-Bertalot)Lange-Bertalot
ADMA	*Achnanthidium macrocephalum* (Hust.)Round & Bukhtiyarova
ADBT	*Achnanthidium biasolettianum*(Grunow)Lange-Bertalot abnormal form
AUGA	*Aulacoseira granulata* (Ehr.) Simonsen var.angustissima (O.M.)Simonsen
AUGR	*Aulacoseira granulata* (Ehr.) Simonsen
APED	*Amphora pediculus* (Kützing) Grunow
ACS1	*Achnanthes sp.1*
BPAR	*Bacillaria paradoxa* Gmelin

BNEO	*Brachysira neoexilis* Lange-Bertalot
CBAC	*Caloneis bacillum* (Grunow) Cleve
CHYA	*Caloneis hyalina* Hustedt
CPTG	*Cocconeis placentula* Ehrenberg abnormal form
CDUB	*Cyclostephanos dubius* (Fricke) Round
CMLF	*Craticula molestiformis* (Hustedt) Lange-Bertalot
CRAC	*Craticula accomoda* (Hustedt) Mann
CRBU	*Craticula buderi* (Hustedt) Lange-Bertalot
CRCU	*Craticula cuspidata* (Kützing) Mann
CAMB	*Craticula ambigua* (Ehrenberg) Mann
CMEN	*Cyclotella meneghiniana* Kützing
CATO	*Cyclotella atomus* Hustedt
CASP	*Cymbella aspera*(Ehrenberg) H.Peragallo
CCYM	*Cymbella cymbiformis* Agardh
CKPP	*Cymbella kappii*(Cholnoky) Cholnoky
CTGL	*Cymbella turgidula* Grunow 1875 in A.Schmidt & al. *var. turgidula*
CBNA	*Cymbopleura naviculiformis* (Auerswald) Krammer *var. naviculiformis*
DCOT	*Diadesmis contenta* (Grunow ex V. Heurck) Mann
DCTG	*Diadesmis confervacea* Kützing abnormal form
DPST	*Discostella pseudostelligera* (Hustedt) Houk et Klee
DSBO	*Diploneis subovalis* Cleve
ENMI	*Encyonema minutum* (Hilse in Rabh.) D.G. Mann
ESLE	*Encyonema silesiacum* (Bleisch in Rabh.) D.G. Mann
ENNG	*Encyonema neogracile* Krammer
EDID	*Eunotia didyma* Grunow var.didyma
ERHO	*Eunotia rhomboidea* Hustedt
EMIN	*Eunotia minor* (Kützing) Grunow in Van Heurck
EBIL	*Eunotia bilunaris* (Ehr.) Mills *var. bilunaris*
ESBM	*Eolimna subminuscula* (Manguin) Moser Lange-Bertalot & Metzeltin
EPUN	*Eunotia pectinalis*(Kütz.)Rabenhorst var.undulata (Ralfs) Rabenhorst
ESUM	*Encyonopsis subminuta* Krammer & Reichardt
ECPM	*Encyonopsis minuta* Krammer & Reichardt
ENCM	*Encyonopsis microcephala* (Grunow) Krammer
FUMP	*Fallacia umpatica* (Cholnoky) Mann in Round et al.
FPYG	*Fallacia pygmaea* (Kützing) Stickle & Mann ssp.pygmaea Lange-Bertalot
FSAP	*Fistulifera saprophila* (Lange-Bertalot & Bonik) Lange-Bertalot
FCRS	*Frustulia crassinervia* (Breb.) Lange-Bertalot et Krammer

FVUL	*Frustulia vulgaris* (Thwaites) De Toni
FUAC	*Fragilaria ulna* (Nitzsch.)Lange-Bertalot var.acus (Kütz.) Lange-Bertalot
FRUT	*Fragilaria capucina var. rumpens* (Kütz)Lange-Bert.ex Bukht.abnormal form
GPAR	*Gomphonema parvulum* (Kützing) Kützing var. parvulum f. parvulum
GPVL	*Gomphonema parvulius* Lange-Bertalot & Reichardt
GAFF	*Gomphonema affine* Kützing
GINS	*Gomphonema insigne* Gregory
GLGN	*Gomphonema lagenula* Kützing
GGRA	*Gomphonema gracile* Ehrenberg
GPLA	*Gomphonema parvulum var.lagenula* (Kütz.)Frenguelli
GGRT	Gomphonema aff gracile
GEXL	*Gomphonema exilissimum*(Grun.) Lange-Bertalot & Reichardt
GMIN	*Gomphonema minutum*(Ag.)Agardh f. minutum
GANG	*Gomphonema angustatum* (Kützing) Rabenhorst
GPSA	*Gomphonema pseudoaugur* Lange-Bertalot
GSCA	*Gyrosigma scalproides* (Rabenhorst)Cleve
HAFC	*Hantzschia amphioxys* (Ehr.) Grunow fo.capitata O. Muller
LHUN	*Lemnicola hungarica* (Grunow) Round & Basson
LMUT	*Luticola mutica* (Kützing) D.G. Mann
LKOT	*Luticola kotschyi* (Grunow) in TDI3 Kelly
MAAT	*Mayamaea atomus* (Kützing) Lange-Bertalot
MAPE	*Mayamaea atomus var. permitis* (Hustedt) Lange-Bertalot
NCTE	*Navicula cryptotenella* Lange-Bertalot
NAMA	*Navicula arvensis* Hustedt *var.maior* Lange-Bertalot
NMCA	*Navicula microcari* Lange-Bertalot
NNOT	*Navicula notha* Wallace
NVDA	*Navicula vandamii* Schoeman & Archibald var. vandamii
NZAN	*Navicula zanoni* Hustedt
NGER	*Navicula germainii* Wallace
NVEN	*Navicula veneta* Kützing
NTRV	*Navicula trivialis* Lange-Bertalot var. trivialis
NSYM	*Navicula symmetrica* Patrick
NSHR	*Navicula schroeteri* Meister var. schroeteri
NERI	*Navicula erifuga* Lange-Bertalot
NROS	*Navicula rostellata* Kützing
NGRE	*Navicula gregaria* Donkin
NCRY	*Navicula cryptocephala* Kützing
NRCH	*Navicula reichardtiana* Lange-Bertalot var. reichardtiana
NRAD	*Navicula radiosa* Kützing

NCTO	*Navicula cryptotenelloides* Lange-Bertalot
NSRH	*Navicula subrhynchocephala* Hustedt
NCPU	*Navicymbula pusilla* Krammer *var pusilla*
NEPR	*Neidium productum* (W.M.Smith)Cleve
NPAL	*Nitzschia palea* (Kützing) W.Smith
NFON	*Nitzschia fonticola* Grunow in Cleve et Möller
NACD	*Nitzschia acidoclinata* Lange-Bertalot
NIFR	*Nitzschia frustulum*(Kützing)Grunow var.frustulum
NDES	*Nitzschia desertorum* Hustedt
NAGN	*Nitzschia agnita* Hustedt
NIGR	*Nitzschia gracilis* Hantzsch
NIRM	*Nitzschia irremissa* Cholnoky
NAMP	*Nitzschia amphibia* Grunow f.amphibia
NZRA	*Nitzschia radicula* Hustedt
NSIG	*Nitzschia sigma*(Kützing)W.M.Smith
NINT	*Nitzschia intermedia* Hantzsch ex Cleve & Grunow
NIPR	*Nitzschia pura* Hustedt
NUMB	*Nitzschia umbonata*(Ehrenberg)Lange-Bertalot
NAGW	*Nitzschia agnewii* Cholnoky
NREV	*Nitzschia reversa* W.Smith
NIAR	*Nitzschia archibaldii* Lange-Bertalot
NLBT	*Nitzschia liebetruthii* Rabenhorst var.liebetruthii
NLIN	*Nitzschia linearis*(Agardh) W.M.Smith *var.linearis*
NREC	*Nitzschia recta* Hantzsch in Rabenhorst
NBCL	*Nitzschia bacillum* Hustedt
NACI	*Nitzschia acicularis (Kützing) W.Smith*
NFIL	*Nitzschia filiformis* (W.M.Smith) Van Heurck var. filiformis
NETO	*Nitzschia etoshensis* Cholnoky
PACR	*Pinnularia acrospheria* W. Smith var. acrospheria
PMRO	*Pinnularia microstauron* (Ehr.) Cleve var. rostrata Krammer
PGIB	*Pinnularia gibba* Ehrenberg
PSCA	*Pinnularia subcapitata* Gregory var. subcapitata
PVIR	*Pinnularia viridis* (Nitzsch) Ehrenberg *var.viridis morphotype* 1
PDIV	*Pinnularia divergens* W.M.Smith *var. divergens*
PSBV	*Pinnularia subbrevistriata* Krammer
PLFR	*Planothidium frequentissimum*(Lange-Bertalot)Lange-Bertalot
PTRO	*Planothidium rostratum* (Oestrup) Round & Bukhtiyarova
PDIC	*Placoneis dicephala* (W.Smith) Mereschkowsky
PSAL	*Pleurosigma salinarum* (Grunow) Cleve & Grunow
ROPE	*Rhopalodia operculata* (Agardh) Hakansson
RMUS	*Rhopalodia musculus* (Kützing) O.Muller

SSEM	*Sellaphora seminulum* (Grunow) D.G. Mann
SPUP	*Sellaphora pupula* (Kützing) Mereschkowksy
SELI	*Staurosira elliptica* (Schumann) Williams & Round
SANG	*Surirella angusta* Kützing
SSTM	*Sellaphora stroemii* (Hustedt) Mann
SMST	*Seminavis strigosa* (Hustedt) Danieledis & Economou-Amilli
STMI	*Stephanodiscus minutulus* (Kützing) Cleve & Moller
SOVI	*Surirella ovalis* Brebisson
STKR	*Stauroneis kriegeri* Patrick
STAN	*Stauroneis anceps* Ehrenberg
SPIN	*Staurosirella pinnata* (Ehr.) Williams & Round
TFAS	*Tabularia fasciculata* (Agardh) Williams et Round
THUN	*Tryblionella hungarica* (Grunow) D.G. Mann
TLEV	*Tryblionella levidensis* Wm. Smith
TCAL	*Tryblionella calida* (grunow in Cl. & Grun.) D.G. Mann
TAPI	*Tryblionella apiculata* Gregory
TCOA	*Tryblionella coarctata* (Grunow in Cl. & Grun.) D.G. Mann

5.3 Redundancy analysis (RDA)

Redundancy analysis is a multivariate technique used to determine the response of diatoms to environmental variables, where taxa are directly related to the environmental variables. A redundancy analysis was used in this study to determine the response of diatom species to each set of environmental variables. The RDA can detect the patterns in community composition that can be explained best by the environmental variables (De De Almeida and Gill 2001). Redundancy Analysis offers a combined explanation of species-environment relationships as it assumes a response model communal to all species, and existence of a distinct group of principal environmental gradients to which all the species react (ter Braak, 1986).

In the RDA the centre of the plot shows the mean of the variable, while the arrows indicate the direction of maximum change in the value linked to the variable (Matlala *et al.*, 2011).

The RDA is represented by arrows where a length of the arrow is related to the highest level of variation. There is no alteration in the value of the variable in the perpendicular direction (ter Braak and Verdonschot, 1995). A positive correlation shows when the response variable is in the same quadrant as the driver, whereas a negative correlation can be seen when the response variable is in the opposite direction as the driver (Lepš and Šmilauer, 2003).

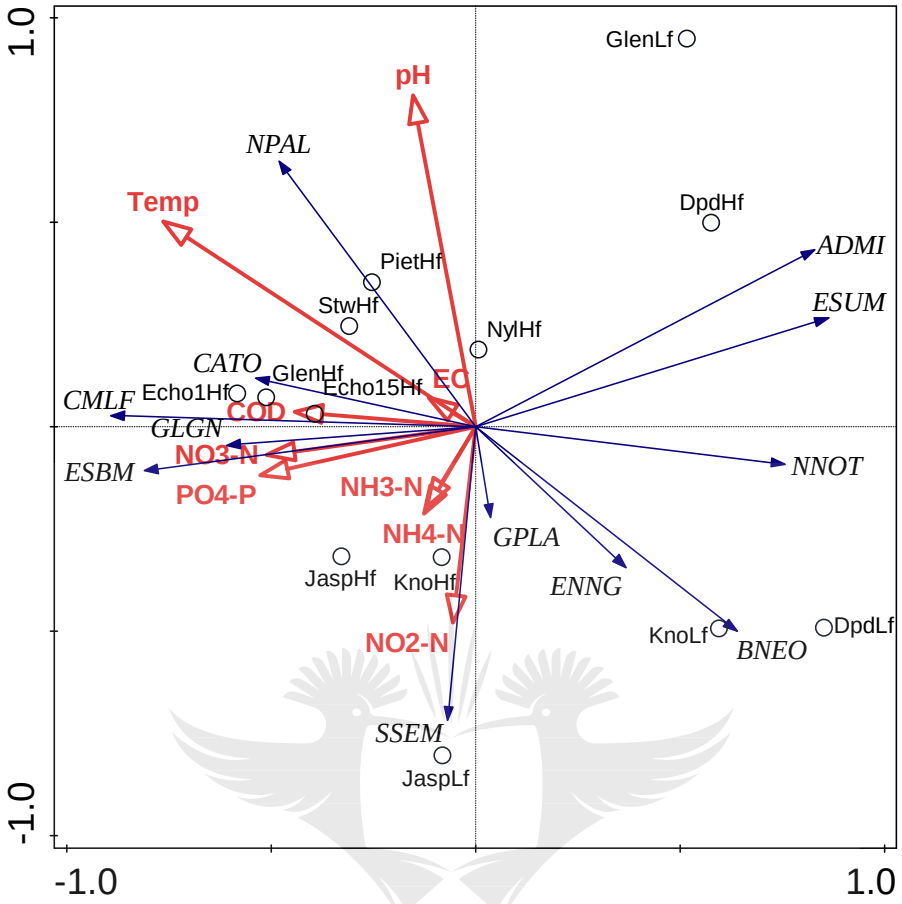

Figure 5.1 Redundancy Analysis plot showing a relationship between dominant diatom taxa and environmental variables in the Nyl/Mogalakwena River system. Open circles indicate locality with the LF and HF indicating high and low flow.

The red arrows in the figure represent the drivers, which are the environmental variables with the blue arrows representing diatom species. Figure 5.1 shows that diatom species found in the study area are divided into the four quadrants. The first quadrant (top right quadrant) represents diatoms that have preference to low levels of nutrients. The second quadrant (top left quadrant) indicates diatom species that are linked to temperature, EC, pH and COD. The third quadrant (bottom left quadrant) indicates diatom species that are associated with high levels of nutrient concentration, with the fourth quadrant (bottom right quadrant) representing diatom species that have a low tolerance to pH, temperature, EC and COD.

The first group of species found in the first quadrant, which prefer low level of nutrients are *Achnanthidium minitissimum* and *Encyonopsis subminuta*. These species denote an association to DPD during the high flow sampling period.

Group two species found in the second quadrant are associated with high temperature, increased pH, increased COD and low EC are *Craticula molestiformis, Cyclotella atomus,* and *Nitzschia palae*

The third quadrant shows a group of diatom species, which prefer increased nutrient concentrations, this quadrant, comprises of species such as *Sellaphora seminulum* and *Eolimna, subminiscula*.

Group four species located in the fourth quadrant are species, which have a negative correlation to pH, temperature, COD and EC and are *Gomphonema aff lagenule, Navicula notha, Encyonema neogracile* and *Brachysira neoexilis.*

From the RDA plot it can be seen that *Achnithidium minutissimum* and *Brachysira neoexilis* were abundant in sites (KNO and DPD) where there was a negative correlation with nutrients and other environmental variables, occurring in good quality waters (Taylor *et al.*, 2007; de De De Almeida *et al.*, 2014). Species such as *Nitzschia palae, Eolimna subminiscula* are tolerant to pollution making those good indicators of pollution (Sabater, 2000; Taylor *et al.*, 2007; de De De Almeida *et al.*, 2014). They therefore group with their associated environmental drivers.

5.4 Diatom indices

5.4.1 Percentage of taxa calculated

The list of diatom taxa counted was imported into the Omnidia database and 17 different indices were generated. There is a variation in terms of the amount of taxa included in the calculation of the index values from the indices developed. An amount of a particular taxa found in the sample as an influence on the index values generated (Musa, 2015).

The confidence level of the changing indices is centred on the fraction of the taxa included in developing a category value. The greater the species composition used by an index, the more trustworthy the correlation between the index value and water quality will be.

As mentioned in section 5.2, only three indices will be used as they showed to be dependable because of their utmost percentage composition in almost all sampling sites (>80%),with only in one site where they showed (<80%) these indices are the Trophic Diatom Index (TDI), the Generic Diatom Index (GDI) and the Specific Pollution Index (SPI). Even though the Biological Diatom Index (BDI) included more than 80% of the taxa it was not used because in two sites the

percentage was lower than 80% at NYL and ECHO 15 as compared to other indices where only one site showed a lower percentage.

Table 5.2 The average percentage of diatom species utilized in the calculation during the high flow and low flow period. Bold values shows greater than 80% of taxa incorporated in the calculation of index values

SITES	IDAP	EPI-D	DBI	SHE	SID	TID	WAT	SPI	SLA	DES	IDSE	GDI	CEE	LOBO	IDP	DI-CH	TDI
KNO LF	27,78	47,22	**91,67**	58,33	58,33	58,33	33,33	**97,22**	63,89	47,22	55,56	**100**	69,44	58,33	33,33	50	**100**
DPD LF	27,59	37,93	**86,21**	58,62	41,38	41,38	31,03	**93,1**	51,72	31,03	41,38	**100**	55,17	34,48	24,14	37,93	**89,66**
JASP LF	48,15	66,67	**88,89**	70,37	59,26	70,37	33,33	**92,59**	77,78	37,04	62,96	**96,3**	74,07	59,26	51,85	48,15	**92,59**
GLEN LF	33,33	53,33	**100**	60	53,33	53,33	33,33	**100**	53,33	40	46,67	**100**	53,33	20	40	60	**100**
KNO HF	21,95	51,22	**80,49**	53,66	56,1	60,98	24,39	**97,56**	56,1	31,71	58,54	**97,6**	58,54	41,46	31,71	36,59	**97,56**
DPD HF	29,41	50	**88,24**	61,76	52,94	58,82	35,29	**97,06**	52,94	26,47	41,18	**100**	58,82	41,18	35,29	50	**100**
STW HF	36,67	60	**83,33**	56,67	56,67	66,67	30	**93,33**	66,67	26,67	56,67	**93,3**	60	50	40	43,33	**90**
JASP HF	46,15	57,69	**88,46**	61,54	61,54	61,54	30,77	**92,31**	65,38	38,46	61,54	**96,2**	61,54	46,11	50	46,15	**92,31**
NYL HF	20,83	62,5	79,17	50	58,33	58,33	29,17	79,17	62,5	45,83	62,5	**95,8**	70,83	41,67	45,83	45,83	**87,5**
GLEN HF	32,26	58,06	**87,1**	58,06	51,61	61,29	32,26	**93,55**	61,29	29,03	58,06	**96,8**	64,52	32,26	38,71	41,94	**83,87**
PIET HF	31,58	47,37	**81,58**	50	42,11	47,32	23,68	**92,11**	52,63	26,32	44,74	**100**	55,26	42,11	34,21	31,58	**89,47**
ECHO 15 HF	33,33	58,33	75	50	58,33	58,33	41,67	**83,33**	58,33	25	58,33	**91,7**	58,33	50	50	31,03	**83,33**
ECHO 14 HF	37,93	44,83	**86,21**	44,83	34,48	41,38	31,03	**89,66**	55,17	20,69	41,38	**96,6**	51,72	41,38	37,93	50	75,86

5.4.2 Diatom index scores

The GDI, SPI, and TDI were utilized in the study area as they proved to be more consistent indices, since they showed the highest percentage composition of diatom species in the study area. The results of these indices are represented in Figures 5.2, 5.3, and 5.4 respectively. The scores are further classified into five classes of sensitivity. The results will be interpreted based on table 5.3 and 5.4 respectively. The results for the GDI and SPI are assigned values in the range of 1-20 with a score of zero indicating bad water quality with a score of 20 indicating high quality (Matlala *et al.*, 2011)

The TDI gives values ranging from 0-100, where a value of zero indicates a low nutrient concentration, whereas a score of 100 indicating a high nutrient concentration and poor water quality. The resultant %PT values will be interpreted using Table 5.5

Table 5.3: Water quality categories indicating different classes of water quality based on the GDI and SPI (adapted from Szczepocka and Szulc, 2009).

CLASS	INDEX SCORE
BAD	< 9
POOR	9-12
SATISFACTORY	12-15
GOOD	15-17
VERY GOOD	>17

Table 5.4 TDI scores and their corresponding trophic status (adapted from: Kelly and Whitton, 1995)

TROPHIC CATEGORY OF WATER	INDEX VALUE RANGES
OLIGOTROPHIC	0-20
OLIGO-MESOTROPHIC	21-40
MESOTROPHIC	41-60
MESO-EUTROPHIC	61-80
EUTROPHIC	>80

Table 5.5 interpretation of % PT score (Adapted from: Kelly and Whitton, 1995)

WATER QUALITY CONDITIONS	%PT VALUES
NO EVIDENCE OF ORGANIC POLLUTANTS	<20%
MINOR INDICATION OF ORGANIC POLLUTION	21-40%
SIGNIFICANT CONTRIBUTION OF ORGANIC POLLUTION TO EUTROPHICATION	41-60%
HEAVILY CONTAMINATED WITH ORGANIC POLLUTION	>61%

5.4.2.1 Generic Diatom Index (GDI)

The GDI generates values based on the genera of the taxa, and gives a good assessment of water quality because it includes 174 taxa when the index is produced.

The results for a GDI values are shown in Figure 5.2

In Figure 5.2 it can be noted that water quality decreases as the river flows downstream from the source. From the graph it can be seen that KNO, DPD and Glen Alpine have good water quality during low flow with GDI scores of 16.3, 17.1 and 16.8 respectively. During high flow conditions moderate water quality was at DPD, NYL and Glen Alpine with GDI scores of 13.3, 12.4 and 12.3 respectively the variations from high flow to low flow may be attributed to the dilution effect which comes with high flow conditions, because studies high rainfall can increase nutrient loading from

land to rivers(Weyhenmeyer, 2001, Mckee *et al*., 2003, Malmaeus *et al*.,2006, Pierson *et al.*,2010), and increased rainfall could cause more frequent incidence of combined sewer overflows, discharging highly polluted water into receiving waterbodies (Whitehead *et al*., 2008).

The lowest GDI values during low flow were at JASP with a GDI score of 7 whereas during high flow the lowest GDI scores were at KNO, STW, Piet, Echo 15 and Echo 14 with values of 6.5, 3.4, 8.9, 6.6 and 6.7, respectively, these indicate bad water quality. Since the GDI accounts for the genera of the taxa and in a genus there may be different species with regards to their optimal conditions. The generated scores may be as a result of the indication of water quality parameters shown by the dominant genera. The use of other diatom indices is important to complement or validate results obtained from the GDI (Musa, 2015)

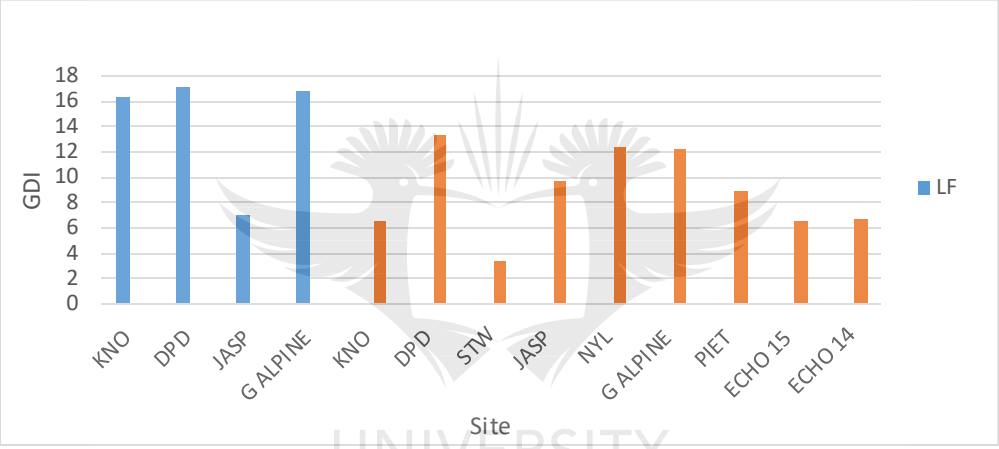

Figure 5.2 Graph showing the Generic Diatom Index (GDI) along the course of the study area, with values obtained from Omnidia.

5.4.2.2 Specific Pollution Index

The SPI accounts for both organic and inorganic pollution, on the bases of the formula used in deriving the Descy's method, but it differs with respect to the sensitivity and indicator values (de Almeida, 2001). The Descy's method classes 106 species into 5 classes of sensitivity (Matlala *et al*, 2011) In order for this index to be reliable, the identification needed to generate the index value must be at the maximum taxonomical determination with some taxa required at subspecies level (Musa, 2015).

The SPI values are shown in Figure 5.3

From Figure 5.3 it can be observed that water quality decreases as it moves downstream with the highest values experienced during low flow conditions, upstream of the STW and downstream of

the NYL wetland, the highest SPI values are at DPD during low flow and high flow with values of 18.4 and 12.4 respectively. The lowest SPI values are observed during high flow at STW with a value of 2.2. The water quality should show a huge improvement at NYL since it is a wetland, which should play a role in water purification, but it showed little improvement. The water is still of bad quality this may be due to the fact that during sampling trips the NYL was flooded. As flooding events could cause a rise in suspended solids, sediment yields and associated metal fluxes (Whitehead *et al*., 2008). The bad water quality can be observed from NYL up till the Mogalakwena/ Limpopo River confluence. The bad water quality can be attributed to the development of a major platinum mine and some small mines in the Mogalakwena River catchment and irrigation from commercial farms (van Vuuren *et al*., 2003)

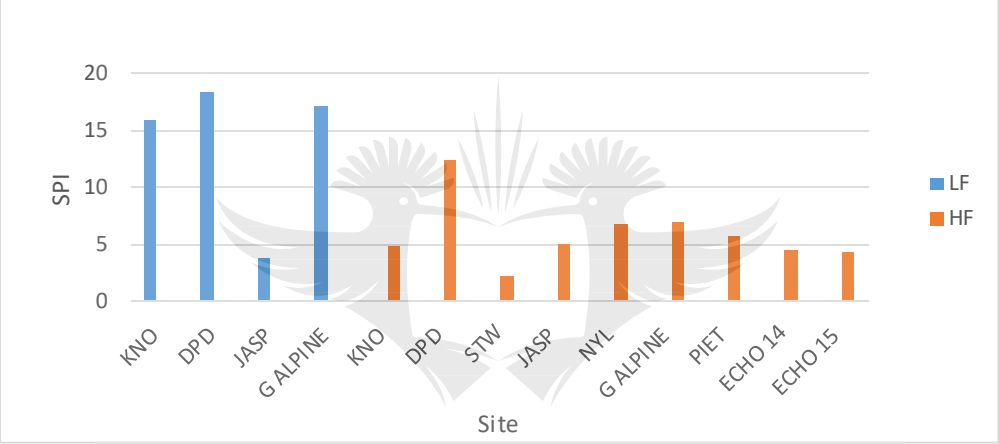

Figure 5.3 Graph showing the Specific Pollution Index (SPI) values along the course of the study period, with values obtained from Omnidia.

5.4.2.3 Trophic Diatom Index

Trophic Diatom Index (TDI) is a method utilised to differentiate between organically polluted water and water high in nutrient concentrations. The TDI is able to monitor eutrophication (Kelly and Whitton, 1995). The Trophic Diatom Index was developed to assess organic pollution because in some instances there will be organic pollution rather than high nutrient concentrations that have a major effect on diatom assemblages (Musa, 2015). The index value is generated by linking the abundance of species to phosphate concentrations. The %PT was developed to indicate the dependability of the TDI (Kelly, 1998). The TDI provides a measure of eutrophication (Matlala *et al.*, 2011).

The TDI values are illustrated in Figure 5.4 and the %PT results are shown in Figure 5.5

The lowest TDI values were at KNO during low flow with a value of 28.4 and at DPD low flow and high flow with values of 23 and 10 respectively. The highest TDI values occurred during high flow at STW, JASP, NYL, PIET, Echo 15 and Echo 14 with values of 93, 95, 89.6, 84.8, 98.5 and 92.4 respectively. Using Table 5.4 it can be seen that KNO, DPD and Glen Alpine during the low flow period shows oligotrophic to meso-oligotrophic conditions. KNO high flow shows (68) meso-eutrophic conditions. This may be due to dilution of diatoms in water taking place during high flows and may also be as a result of runoff. STW plays a significant role in rising the eutrophication of the system. The sites downstream of STW up till the Mogalakwena/Limpopo River confluence shows eutrophic conditions with the exception of Glen Alpine high flow which indicated meso-eutrophic conditions, indicating additional inputs to the system. Another reason could be because during the low flow sampling trips, Glen Alpine Dam was affected by drought conditions and the dam was not 100% full as compared to the high flow conditions.

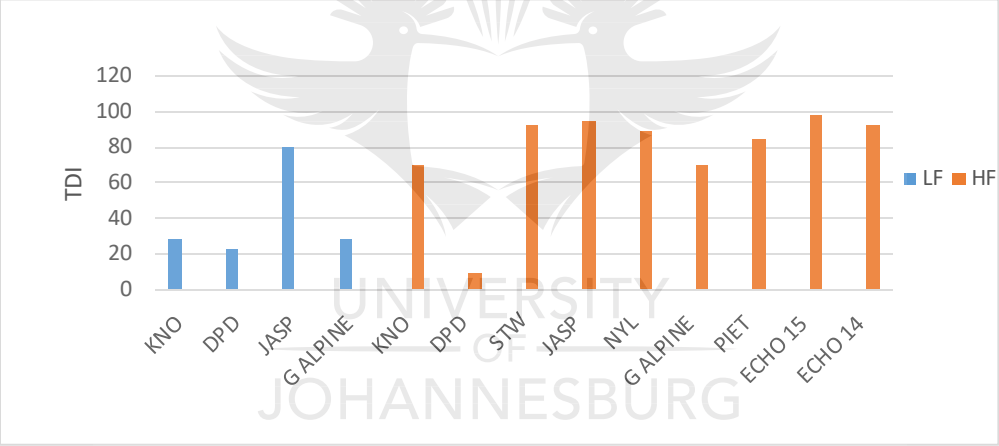

Figure 5.4 Graph showing Trophic Diatom Index (TDI) values along the course of the study period, with values obtained from Omnidia

Relating the data in Figure 5.5 with Table 5.5 it can be observed that during the low flow at KNO, DPD, and Glen Alpine Dam there is no evidence of organic pollution (%PT values < 20%). During the high flow at DPD and the Glen Alpine Dam there is no evidence of organic pollution, whereas KNO, STW, JASP and Echo 14 (67.2, 78.1, 65.1 and 65.5 respectively) show that the sites are heavily polluted with organic pollution (% PT > 61 %). The increase in organic pollution at STW and JASP can be attributed to high organic loads from the effluents and at NYL high flow there is no evidence of organic pollution which can be attributed to the functioning of the wetland. The

increase in %PT downstream of NYL to the Mogalakwena/Limpopo River confluence indicates additional organic waste inputs to the system.

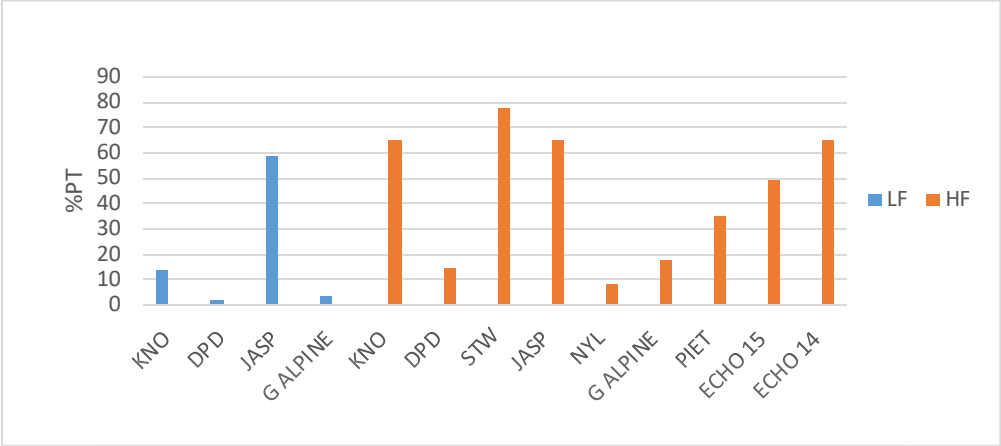

Figure 5.5 *graph showing %PT values along the course of the study period, with values obtained from Omnidia*

5.5 Correlation of indices to environmental variables

Correlation is a statistical method used to determine the strength of the relationship between variables and a correlation coefficient is a value which range from -1 to + 1 measuring the strength and direction of the linear relationship between variables (Matlala *et al.*, 2011)

Correlation values can be used to indicate the strength of linear relationship between an environmental variable and index value obtained. The degree of the value indicates the strength of the correlation, therefore a value of zero indicates no linear relationship, while a value between -1 or + 1 indicates a linear relationship where (+/-) sign show a direction of the correlation (Matlala *et al.*, 2011). A positive (+) correlation coefficient indicates that as one variable increases the other one also increases, and a negative (-) correlation coefficient indicates that when one variable increases the other one decreases (Rummel, 1976).

The results of the Pearson's correlation between environmental variables and diatom indices are shown in Table 5.6. Significant correlations are denoted by the superscript asterisk symbols. Pearson's correlation was used because the data was parametric.

Table.*5.6 Pearson correlation coefficients between the diatom indices and environmental variables. Values marked with* indicates (p<0.05) and ** indicates (p<0.01)*

	GDI	**SPI**	**TDI**
TEMP	-0.526	-0.637*	0.751**
EC	-0.348	-0.309	0.290
PH	0.145	0.140	-0.043
COD	-0.444	-0.533	0.538
NO_2-N	-0.251	-0.294	0.273
NO_3-N	-0.362	-0.371	0.263
NH_3-N	-0.535	-0.465	0.355
NH_4-N	-0.514	-0.454	0.334
PO_4-P	-0.569*	-0.446	0.350

* indicates significance at 0.05 (p<0.05)

** indicates significance at 0.01 (p<0.01)

Table 5.6 shows that the GDI had a significant negative correlation to PO_4-P, with no significant correlation between other environmental variables. SPI showed a significant negative correlation to temperature. The TDI showed a significant positive correlation to temperature. In order to understand the correlation, considerations need to be made in the way in which the GDI and SPI and TDI indicate water quality. The GDI and SPI have values ranging from 0-20 showing poor water quality with decreasing index scores, therefore showing a negative correlation. The TDI values range from 0-100 with increasing values indicating a decrease in water quality therefore showing a positive correlation, indicating increased nutrient concentrations thus decreasing water quality (Musa, 2015).

5.6 Shannon Wiener diversity index

Diversity indices are used in water pollution research as a means of evaluating the impacts of pollution on species composition (Archibald,1972). Species diversity reacts to fluctuations in particular, to pressures and regulating influences, therefore indicating a lot of interactions which may characterize communities (Khuantrairong and Traichaiyapon, 2008).

Diversity will be affected by fluctuations in environmental conditions (Washington, 1984), if adaptation is scarce or does not exist, or gene movement from non-adaptation regions

is high. A decrease in the amount of species and a growh in the amount of individuals that symbolizes contaminated regions leads to a major decline in values of diversity (Khuantrairong and Traichaiyapon, 2008).

Shannon Wiener diversity index is used to signify variations in community assemblages with regard to species richness and evenness.

Dallas and Day (2004) stated that in pollution studies Shannon diversity index is centered on the principles that species diversity declines as human impacts rise. They further stated that it is not always the case, nonetheless a decline in diversity may occur as a result of physical stress for example in instances where contamination may be disguised by variation in species constituents that would not be shown in any diversity index.

The results for the Shannon Wiener index are represented in Figure 5.6

The values of the Shannon Wiener index are generally between 1 and 4, with increasing values indicating an increase in both species richness and evenness. In Figure 5.6, KNO, DPD indicate good species diversity, as compared to STW, NYL and Eco 15, which shows a decline in species diversity. A decline in species diversity indicates a deteriorating ecosystem (Khuantrairong and Traichaiyapon, 2008).

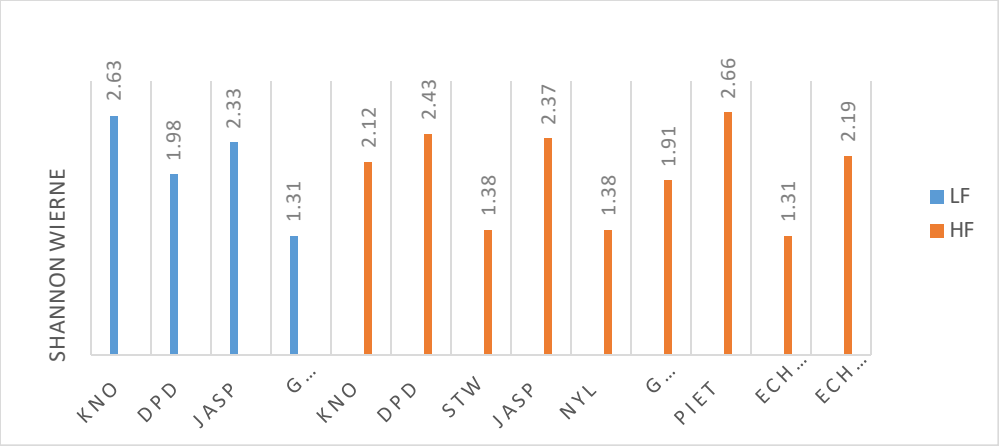

Figure 5.6 Graph showing Shannon Wiener diversity index along the study sites during low flow and high flow periods, with values indicating species richness and evenness.

5.7 Discussion

Species such as *Achnanthidium minutissimum*, and *Brachysira neoexilis* were dominant at KNO and DPD indicating generally unpolluted waters. Species such as *Nitzschia palea*, *Sellaphora Seminulum*, *Eolimna subminuta* and *Craticula molestiformis*, which are species regarded to be tolerant to some form of pollution usually occurring in eutrophic waters and highly polluted waters, indicated poor water quality at STW and JASP (Sabater, 2000; Taylor *et al.*, 2007; de Almeida *et al.*, 2014).

From Table 5.6 it can be seen that the indices showed different correlations to environmental variables, with GDI and SPI showing negative correlations and TDI showing positive correlations. The GDI and SPI showed a negative correlation because of poor water quality and indicated by the values of these indices had decreased. The TDI had a positive correlation indicating the increase in nutrient concentration thus decreasing water quality.

Figure 5.6, which shows the diversity index, indicates that KNO and DPD have higher species diversity than STW, NYL and Echo 15.

5.8 Conclusion

It can be concluded that diatom communities' assemblages are affected by the relationship with the environment with dominant taxa having their optima near the environmental variables measured at different sites. From the diatom indices calculated it can be seen that good water quality was during low flow, so seasonal variation does play a role in water quality. As mentioned high flow conditions can cause sewer overflows and increase in nutrients loads. This is the second study using diatoms as biological indicators in the Nyl/Mogalakwena river system. A study conducted by Musa (2015) on the Nyl river showed good water quality KNO and DPD during high flow whereas in this study it shows bad to moderate water quality in the sites. This indicates that it is important to conduct regular monitoring as the system is subjected to continuous and regular pollution.

The Table 5.7 gives a summary of the results from the diatom indices generated.

Table 5.7 Summary of water quality conditions during high flow in the Nyl/Mogalakwena river system as shown by diatom indices

HIGH FLOW	GDI	SPI	TDI
KNO	BAD	BAD	MESO-EUTROPHIC
DPD	MODERATE	MODERATE	MESOTROPHIC
STW	BAD	BAD	EUTROPHIC
JASP	POOR	BAD	EUTRPHIC
NYL	MODERATE	BAD	EUTROPHIC
GLEN ALPINE	MODERATE	BAD	MESO-EUTROPHIC
PIET	BAD	BAD	EUTROPHIC
ECHO 15	BAD	BAD	EUTROPHIC
ECHO 14	BAD	BAD	EUTROPHIC

Table 5.8 Summary of water quality conditions during low flow in the Nyl/Mogalakwena river system as shown by diatom indices

LOW FLOW	GDI	SPI	TDI
KNO	GOOD	GOOD	MESO-OLIGOTROPHIC
DPD	HIGH	HIGH	MESO-OLIGOTROPHIC
JASP	BAD	BAD	MESO-EUTROPHIC
GLEN ALPINE	GOOD	HIGH	MESO-OLIGOTROPHIC

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1 Concluding Remarks

The growing population and socio-economic development trends have put a major strain on the quality of water and its availability (Ashton *et al*., 2008). Anthropogenic stressors such as intensive agriculture, large industrial development and urbanization result in a significant decline of surface water quality of aquatic systems in watershed (Xiao-long *et al.*, 2006). Since rivers are mainly susceptible to land use change and ubiquitous exploitation it is important to understand the relationship between land use and water quality in order to identify primary threats to water quality so as to institute relevant measures to reduce pollutant loading (Ding *et al*., 2015).
The Nyl River floodplain is an important wetland, which supports a large number of rare and endangered bird species and animal populations. It is also a Ramsar accredited wetland (Higgens *et al.*, 1996). The Nyl River and Mogalakwena River system faces large amounts of pollution from formal and informal settlements, agricultural runoff, industrial effluents and mining activities.

The quality of water is important for aquatic organisms and for human consumption. It is important to monitor water in order to determine the type of contaminant, concentration of pollutants, so as to develop a contingency plan, which will have a good effect on human health and safety.

Traditional water monitoring focused on the physico- chemical and chemical constituents. Physico-chemical and chemical monitoring gives a reflection of rapid measurements and may not give sufficient information on the ecological status of the system (Kriel, 2008), whereas biotic parameters give better evaluation of environmental changes because community development integrates a time period, reflecting conditions that might no longer be present at the time of sampling. Aquatic organisms incorporate and indicate the impacts caused by environmental disturbances over long periods of time, therefore giving a holistic and integrated measure of health (Chutter, 1998; Ndiritu *et al*., 2006).

Diatoms are important in aquatic ecosystems because they are primary producers and have precise environmental requirements and respond promptly to fluctuations in environmental circumstances (Kelly and Whitton, 1995).

Chemical constituents of the water in the study area show that the STW has a major influence on the system as the peaks in chemical constituents are observed at this site and the site below the STW (JASP). STW introduces the nutrients in the system thereby affecting the pH and EC. The nutrient levels also decrease downstream of JASP. The high levels of nutrient concentrations at STW is because the wastewater treatment plant was not functioning properly therefore cannot treat pollutants introduced into the system.

The GDI and SPI showed declining water quality along the river systems. The declining water quality is observed during the high flow periods. This can be the result of the effect of rainfall on the hydrological patterns causing them to change (Sternberg, 2003). Increased rainfall will affect the physical and chemical parameters of surface waters. The first hypothesis was that anthropogenic activities have caused a decline in the water quality of the Nyl River and Mogalakwena River and changed the physico-chemical parameters. Based on the results we can accept this hypothesis as nutrient and *in situ* parameters have changed from natural conditions

The second hypothesis was that the sewage treatment works and agriculture are the major contributors of pollution in the river, causing a change in diatom community. This hypothesis can be accepted as the diatom indices show bad water quality at the sewage treatment works. The SPI and GDI show poor water quality at Piet indicating that the site is impacted by agriculture, it is also evident from changes in diversity and richness at these sites.

6.2 Recommendations

It is recommended that there should be continuous monitoring on the system has a whole since environmental conditions vary seasonally and with increased urbanisation, agricultural runoff.

Other microorganisms should be integrated in the study of water quality, such has fish, and also conduct toxicity studies on the system.

The necessity for user participation in preserving water quality and looking at other aspects like hygiene, environment sanitation, storage and disposal are critical elements to maintain the quality of water resources.

CHAPTER 7

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Appendix A

Water Chemistry

Table A Physico-chemical parameters for the different sites and sampling periods. Low Flow (LF) and High Flow (HF)

SITES	TEMP	EC	PH	COD	NO2-N	NO3-N	NH3-N	NH4-N	PO4-P
KNO LF	11.4	100	7	4.6	0.02	0.1	0.03	0.03	0.005
DPD LF	11.4	100	5.77	9.2	0.02	0.15	0.04	0.04	0.005
JASP LF	12.2	700	4.4	20.4	0.1	4.7	6.36	8.2	0.26
G ALPINE LF	15.6	500	10.73	11.5	0.02	0.7	0.23	0.23	0.005
KNO HF	18.5	47	6.9	33.9	0.01	0.1	0.015	0.015	0.02
DPD HF	20	167	7.5	11.6	0.005	0.1	0.028	0.023	0.05
SWT HF	22.3	597	7.4	44.6	0.05	1.2	7	7	0.81
JASP HF	20.2	686	7.8	31.6	0.13	1.2	3.2	3.2	0.13
NYL HF	23.8	69	6.5	52.8	0.007	0.3	0.035	0.035	0.031
G ALPINE HF	24.8	165	8.4	14.3	0.007	0.6	0.023	0.023	0.024
PIET HF	25.6	25.6	8.2	11.6	0.009	0.4	0.021	0.021	0.02
ECHO 15 HF	27	275	8.2	14.3	0.011	0.3	0.027	0.027	0.02
ECHO 14 HF	27.1	341	8.5	11.7	0.008	0.9	0.014	0.014	0.04

Appendix B

Diatom Indices

Table B Values obtained from the diatom indices used in the study as produced by Omnidia

SITES	TDI	SPI	GDI	%PT
KNO LF	28.4	16	16.3	14
DPD LF	21.5	18.4	17.1	2
JASP LF	79.9	3.9	7	59
G ALPINE LF	28.5	17.1	16.8	4
KNO HF	69.5	4.9	6.5	65.5
DPD HF	52.4	12.4	13.3	14.5
SWT HF	93	2.2	3.4	78.1
JASP HF	95	5	9.7	65.1
NYL HF	89.6	6.9	12.4	8.3
G ALPINE HF	70	7	12.3	18.2
PIET HF	84.8	5.7	8.9	35.3
ECHO 15 HF	98.5	4.3	6.6	49.3
ECHO 14 HF	92.4	4.5	6.7	65.5

Appendix C

Relative Abundance

Table C Relative abundance of diatom species counted in the different sampling sites.

CODE	DIATOM SPECIES	KNO LF	KNO HF	DPD LF	DPD HF	STW	JASP HF	JASP LF	NYL	PIET	GLEN HF	GLEN LF	ECHO 14	ECHO 15
ADMI	*Achnanthidium minutissimum* (Kützing) Czarnecki	4	-	51	128	-	-	-	-	-	1	225	-	-
ADEU	*Achnanthidium eutrophilum* (Lange-Bertalot)Lange-Bertalot	-	-	1	4	-	-	-	-	-	-	20	-	-
ADEG	*Achnanthidium exiguum* (Grunow) Czarnecki	-	1	-	-	1	-	-	-	-	-	-	-	-
ADSA	*Achnanthidium saprophilum* (Kobayasi et Mayama) Round & Bukhtiyarova	1	-	-	2	-	-	-	-	-	-	12	-	-
ACAF	*Achnanthidium affine* (Grun) Czarnecki	-	-	1	-	-	-	-	-	-	-	1	-	-
ADSB	*Achnanthidium straubianum* (Lange-Bertalot)Lange-Bertalot	-	-	4	-	3	-	-	-	-	-	-	-	-
ADMA	*Achnanthidium macrocephalum*(Hust.)Round & Bukhtiyarova	23	1	3	5	-	-	-	-	-	-	-	1	-
ADBT	*Achnanthidium biasolettianum*(Grunow)Lange-Bertalot abnormal form	3	-	1	10	-	-	-	-	1	-	-	-	-
AUGA	*Aulacoseira granulata* (Ehr.) Simonsen var.angustissima (O.M.)Simonsen	-	-	-	-	-	-	-	-	-	-	-	5	-
AUGR	*Aulacoseira granulata* (Ehr.) Simonsen	-	-	-	-	-	-	-	-	2	-	-	-	-
APED	*Amphora pediculus* (Kützing) Grunow	-	-	-	-	-	-	-	-	-	-	6	-	-
ACS1	*Achnanthes sp.1*	-	-	1	2	-	-	-	-	-	-	-	-	-
BPAR	*Bacillaria paradoxa* Gmelin	-	-	-	3	-	-	1	-	-	-	1	-	-
BNEO	*Brachysira neoexilis* Lange-Bertalot	107	8	136	-	-	-	-	-	-	1	-	-	-
CBAC	*Caloneis bacillum* (Grunow) Cleve	-	2	-	-	-	-	-	-	-	1	-	-	-
CHYA	*Caloneis hyalina* Hustedt	-	4	-	-	-	-	-	-	-	-	-	-	-
CPTG	*Cocconeis placentula* Ehrenberg abnormal form	-	-	-	-	3	-	-	-	-	-	--	2	-
CDUB	*Cyclostephanos dubius* (Fricke) Round	-	-	-	-	-	-	-	-	-	-	-	3	-

CMLF	*Craticula molestiformis* (Hustedt) Lange-Bertalot	-	6	-	-	12	12	1	3	30	4	-	28	191
CRAC	*Craticula accomoda* (Hustedt) Mann	-	-	-	-	-	-	1	-	-	1	-	-	-
CRBU	*Craticula buderi* (Hustedt) Lange-Bertalot	1	-	-	-	-	-	-	-	2	-	-	-	-
CRCU	*Craticula cuspidata* (Kützing) Mann	-	-	-	-	-	-	-	1	-	-	-	-	-
CAMB	*Craticula ambigua* (Ehrenberg) Mann	1	-	-	-	-	-	-	1	-	-	-	-	-
CMEN	*Cyclotella meneghiniana* Kützing	-	-	1	-	7	32	10	4	6	2	-	30	-
CATO	*Cyclotella atomus* Hustedt	-	-	-	-	-	-	-	-	80	2	-	3	3
CASP	*Cymbella aspera*(Ehrenberg) H.Peragallo	-	2	-	-	-	-	-	-	-	-	-	-	-
CCYM	*Cymbella cymbiformis* Agardh	-	-	2	-	-	-	-	-	-	-	-	-	-
CKPP	*Cymbella kappii*(Cholnoky) Cholnoky	-	-	3	-	-	-	-	-	-	-	-	-	-
CTGL	*Cymbella turgidula* Grunow 1875 in A.Schmidt & al. *var. turgidula*	-	-	5	11	-	-	-	-	-	-	-	-	-
CBNA	*Cymbopleura naviculiformis* (Auerswald) Krammer *var. naviculiformis*	28	-	-	2	-	-	-	-	-	-	-	-	-
DCOT	*Diadesmis contenta* (Grunow ex V. Heurck) Mann	-	2	-	-	-	-	-	-	-	-	-	-	-
DCTG	*Diadesmis confervacea* Kützing abnormal form	-	1	-	-	1	1	1	-	-	-	-	1	-
DPST	*Discostella pseudostelligera* (Hustedt) Houk et Klee	-	-	2	-	-	-	-	-	-	1	-	2	-
DSBO	*Diploneis subovalis* Cleve	-	-	-	-	-	-	-	-	2	-	-	-	-
ENMI	*Encyonema minutum* (Hilse in Rabh.) D.G. Mann	30	7	15	3	-	-	-	-	14	16	-	-	1
ESLE	*Encyonema silesiacum* (Bleisch in Rabh.) D.G. Mann	-	2	-	-	-	-	-	-	-	-	-	-	-
ENNG	*Encyonema neogracile* Krammer	67	1	-	-	-	-	-	-	-	-	-	-	-
EDID	*Eunotia didyma* Grunow var.didyma	-	-	-	-	-	-	-	3	-	-	-	-	-
ERHO	*Eunotia rhomboidea* Hustedt	-	-	-	-	-	-	-	-	-	1	-	-	-
EMIN	*Eunotia minor* (Kützing) Grunow in Van Heurck	-	-	-	-	-	-	-	1	-	-	-	-	-

EBIL	*Eunotia bilunaris* (Ehr.) Mills *var. bilunaris*	2	7	-	-	-	-	-	9	-	-	-	-	-
ESBM	*Eolimna subminuscula* (Manguin) Moser Lange-Bertalot & Metzeltin	-	1	-	-	7	28	5	-	42	1	-	117	122
EPUN	*Eunotia pectinalis*(Kütz.)Rabenhorst var.undulata (Ralfs) Rabenhorst	-	-	-	-	-	-	-	3	-	-	-	-	-
ESUM	*Encyonopsis subminuta* Krammer & Reichardt	7	-	53	8	-	-	-	-	-	-	108	-	-
ECPM	*Encyonopsis minuta* Krammer & Reichardt	-	-	-	-	-	-	-	-	1	-	2	-	--
ENCM	*Encyonopsis microcephala* (Grunow) Krammer	-	-	-	--	-	-	-	-	-	-	2	-	-
FUMP	*Fallacia umpatica* (Cholnoky) Mann in Round et al.	-	-	-	-	-	-	-	-	-	-	-	-	-
FPYG	*Fallacia pygmaea* (Kützing) Stickle & Mann ssp.pygmaea Lange-Bertalot	-	-	-	-	1	-	-	-	-	-	-	-	-
FSAP	*Fistulifera saprophila* (Lange-Bertalot & Bonik) Lange-Bertalot	-	-	-	-	-	-	-	-	-	-	-	-	2
FCRS	*Frustulia crassinervia* (Breb.) Lange-Bertalot et Krammer	1	24	-	-	-	-	-	-	-	-	-	-	-
FVUL	*Frustulia vulgaris* (Thwaites) De Toni	1	3	-	-	-	-	-	-	-	-	-	-	-
FUAC	*Fragilaria ulna* (Nitzsch.)Lange-Bertalot var.acus (Kütz.) Lange-Bertalot	-	-	-	1	-	-	-	-	1	35	-	-	-
FRUT	*Fragilaria capucina var. rumpens* (Kütz)Lange-Bert.ex Bukht.abnormal form	-	-	-	-	-	-	-	-	1	-	-	-	-
GPAR	*Gomphonema parvulum* (Kützing) Kützing var. parvulum f. parvulum	2	1	-	4	4	49	25	2	5	41	-	2	-
GPVL	*Gomphonema parvulius* Lange-Bertalot & Reichardt	2	4	-	4	-	-	-	-	-	-	-	-	-
GAFF	*Gomphonema affine* Kützing	-	-	-	1	-	-	4	-	-	-	-	-	-
GINS	*Gomphonema insigne* Gregory	-	-	-	-	1	-	-	43	-	-	-	-	-
GLGN	*Gomphonema lagenula* Kützing	-	1	-	-	7	5	21		19	208	-	13	
GGRA	*Gomphonema gracile* Ehrenberg	8	2	1	4	-	2	-	5	-	-	-	-	-

GPLA	*Gomphonema parvulum var.lagenula* (Kütz.)Frenguelli	12	8	-	5	-	6	15	270	6	3	-	-	-
GGRT	Gomphonema aff gracile	-	-	-	-	-	-	1	1	-	-	-	-	-
GEXL	*Gomphonema exilissimum*(Grun.) Lange-Bertalot & Reichardt	20	15	-	-	-	-	-	-	-	-	-	-	--
GMIN	*Gomphonema minutum*(Ag.)Agardh f. minutum	-	6	-	-	-	-	-	-	-	-	-	-	-
GANG	*Gomphonema angustatum* (Kützing) Rabenhorst	1	-	-	-	-	-	2	-	-	-	-	-	-
GPSA	*Gomphonema pseudoaugur* Lange-Bertalot	-	-	-	1	-	5	23	10	-	-	-	-	-
GSCA	*Gyrosigma scalproides* (Rabenhorst)Cleve	-	-	-	-	-	-	-	-	6	-	-	-	-
HAFC	*Hantzschia amphioxys* (Ehr.) Grunow fo.capitata O. Muller	-	-	-	-	-	-	-	-	1	1	-	-	-
LHUN	*Lemnicola hungarica* (Grunow) Round & Basson	-	-	-	-	3	8	22	-	-	-	-	-	-
LMUT	*Luticola mutica* (Kützing) D.G. Mann	-	-	-	-	-	-	-	-	-	-	-	-	-
LKOT	*Luticola kotschyi* (Grunow) in TDI3 Kelly	-	1	-	-	-	-	-	-	4	-	-	-	--
MAAT	*Mayamaea atomus* (Kützing) Lange-Bertalot	-	-	-	-	-	2	-	-	-	-	-	1	-
MAPE	*Mayamaea atomus var. permitis* (Hustedt) Lange-Bertalot	-	-	-	1	2	-	-	-	-	-	-	-	-
NCTE	*Navicula cryptotenella* Lange-Bertalot	8	9	7	24	8	54	28	-	9	6	-	-	-
NAMA	*Navicula arvensis* Hustedt *var.maior* Lange-Bertalot	-	13	-	--	5	22	1	-	1	-	-	-	1
NMCA	*Navicula microcari* Lange-Bertalot	-	-	1	56	-	1	-	-	2	-	1	-	-
NNOT	*Navicula notha* Wallace	4	-	96	57	-	-	-	-	1	-	-	-	-
NVDA	*Navicula vandamii* Schoeman & Archibald var. vandamii	2	-	-	-	4	-	-	-	-	2	-	8	-
NZAN	*Navicula zanoni* Hustedt	1	-	-	-	-	-	-	-	-	-	-	-	1
NGER	*Navicula germainii* Wallace	1	-	2	-	-	-	-	-	-	-	-	-	-
NVEN	*Navicula veneta* Kützing	-	-	-	-	1	-	-	-	5	-	-	-	-
NTRV	*Navicula trivialis* Lange-Bertalot var. trivialis	-	-	-	-	1	-	-	-	-	-	-	-	-

NSYM	*Navicula symmetrica* Patrick	-	-	-	-	-	-	-	-	43	10	-	32	3
NSHR	*Navicula schroeteri* Meister var. schroeteri	-	-	-	-	-	-	-	-	-	4	-	-	-
NERI	*Navicula erifuga* Lange-Bertalot	-	-	-	-	-	-	-	-	-	-	-	-	-
NROS	*Navicula rostellata* Kützing	-	-	-	2	-	-	1	-	-	-	-	1	-
NGRE	*Navicula gregaria* Donkin	10	-	2	5	-	-	-	-	-	-	-	-	-
NCRY	*Navicula cryptocephala* Kützing	-	-		3	-	-	-	-	-	-	-	-	-
NRCH	*Navicula reichardtiana* Lange-Bertalot var. reichardtiana	-	-	-	1	1	-	-	-	-	-	-	-	-
NRAD	*Navicula radiosa* Kützing	1	-	2	-	-	1	2	-	-	-	1	-	-
NCTO	*Navicula cryptotenelloides* Lange-Bertalot	-	-	-	-	-	-	-	-	-		4	-	2
NSRH	*Navicula subrhynchocephala* Hustedt	-	-	-	-	-	-	-	-	5	22	-	1	-
NCPU	*Navicymbula pusilla* Krammer *var pusilla*	-	-	-	-	-	-	-	-	-	4	-	-	-
NEPR	*Neidium productum* (W.M.Smith)Cleve	-	1	1	-	-	-	-	-	-	-	-	-	-
NPAL	*Nitzschia palea* (Kützing) W.Smith	1	220	-	35	287	46	160	26	77	20	14	108	60
NFON	*Nitzschia fonticola* Grunow in Cleve et Möller	24	10	-	-	-	-	-	-	-	-	-	-	-
NACD	*Nitzschia acidoclinata* Lange-Bertalot	3	2	-	-	-	-	-	-	-	-	-	-	-
NIFR	*Nitzschia frustulum*(Kützing)Grunow var.frustulum	-	-	-	1	-	4	-	-	-	1	2	7	11
NDES	*Nitzschia desertorum* Hustedt	-	-	-	-	-	-	-	-	1	-	-	-	-
NAGN	*Nitzschia agnita* Hustedt	-	-	-	-	-	-	-	-	-	1	-	-	-
NIGR	*Nitzschia gracilis* Hantzsch	1	-	-	-	-	-	-	-	-	-	-	19	3
NIRM	*Nitzschia irremissa* Cholnoky	-	-	-	-	-	-	-	1	4	-	-	1	-
NAMP	*Nitzschia amphibia* Grunow f.amphibia	-	-	-	-	-	-	-	-	1	-	-	2	-
NZRA	*Nitzschia radicula* Hustedt	1	1	-	6	-	-	-	-		-	-	-	-
NSIG	*Nitzschia sigma*(Kützing)W.M.Smith	-	-	-	-	-	-	-	-	1	-	-	-	-
NINT	*Nitzschia intermedia* Hantzsch ex Cleve & Grunow	-	-	-	-	-	-	1	-	-	-	-	-	-
NIPR	*Nitzschia pura* Hustedt	-	-	-	-	-	-	-	-	-	1	-	-	-
NUMB	*Nitzschia umbonata*(Ehrenberg)Lange-Bertalot	-	-	-	1	-	-	-	1		1	-	-	-

NAGW	*Nitzschia agnewii* Cholnoky	-	-	-	-	-	-	-	-	7	-	-	2	-
NREV	*Nitzschia reversa* W.Smith	-	-	-	4	1	-	-	-	-	-	-	3	-
NIAR	*Nitzschia archibaldii* Lange-Bertalot	-	-	1	1						7	-	-	-
NLBT	*Nitzschia liebetruthii* Rabenhorst var.liebetruthii	4	1	-	-	-	2	-	-	1	-	-	-	-
NLIN	*Nitzschia linearis*(Agardh) W.M.Smith *var.linearis*	-	-	-	-	4	-	10	-	-	-	-	-	-
NREC	*Nitzschia recta* Hantzsch in Rabenhorst	-	-		-	-	-	3	4	-	-	-	-	-
NBCL	*Nitzschia bacillum* Hustedt		-	2	-	-	-	-		-	-	-	-	-
NACI	*Nitzschia acicularis (Kützing) W.Smith*	1	-	-	-	-	-	-	-	-	-	-	-	-
NFIL	*Nitzschia filiformis* (W.M.Smith) Van Heurck var. filiformis	-	-	-	-	-	-	-	-	1		-	-	-
NETO	*Nitzschia etoshensis* Cholnoky	-	-	-	-	-	-	-	-	-	1	-	-	-
PACR	*Pinnularia acrospheria* W. Smith var. acrospheria	-	-	-	-	-	-	-	-	1	-	-	-	-
PMRO	*Pinnularia microstauron* (Ehr.) Cleve var. rostrata Krammer	-	1	-	-	-	-	-	-	-	-	-	-	-
PGIB	*Pinnularia gibba* Ehrenberg	1	1	-	-	1	-	-	1	-	-	-	-	-
PSCA	*Pinnularia subcapitata* Gregory var. subcapitata	-	2	-	-	1	1	-	6	-	1	-	-	-
PVIR	*Pinnularia viridis* (Nitzsch) Ehrenberg *var.viridis morphotype* 1	-	1	-	-	1	-	-	-	-	-	-	-	-
PDIV	*Pinnularia divergens* W.M.Smith *var. divergens*	-	-	-	-	1	-	-	-	-	-	-	-	-
PSBV	*Pinnularia subbrevistriata* Krammer	-	-	-	-	-	4	20	1	-	-	-	-	-
PLFR	*Planothidium frequentissimum*(Lange-Bertalot)Lange-Bertalot	-	-	-	-	-	3	-	-	-	-	-	-	-
PTRO	*Planothidium rostratum* (Oestrup) Round & Bukhtiyarova	-	-	-	-	-	1	-	-	-	-	1	-	-
PDIC	*Placoneis dicephala* (W.Smith) Mereschkowsky	-	-	1	3	-	-	-	-	-	-	-	-	-

PSAL	*Pleurosigma salinarum* (Grunow) Cleve & Grunow	-	-	-	-	-	-	-	-	2	-	-	-	-
ROPE	*Rhopalodia operculata* (Agardh) Hakansson	-	2	-	-	-	-	-	-	-	-	-	-	-
RMUS	*Rhopalodia musculus* (Kützing) O.Muller	-	2	-	-	-	-	-	-	-	-	-	-	-
SSEM	*Sellaphora seminulum* (Grunow) D.G. Mann	8	12	3	-	3	107	25		-	-	-	2	-
SPUP	*Sellaphora pupula* (Kützing) Mereschkowksy	7	11	-	-	28	3	4	2	1	-	-	1	-
SELI	*Staurosira elliptica* (Schumann) Williams & Round	-	-	-	2	-	-	-	-	-	-	-	-	-
SANG	*Surirella angusta* Kützing	-	-	-	-	-	-	7	-	-	-	-	-	-
SSTM	*Sellaphora stroemii* (Hustedt) Mann	-	-	-	-	-	-	-	1	-	-	-	-	-
SMST	*Seminavis strigosa* (Hustedt) Danieledis & Economou-Amilli	-	-	-	-	-	-	-	-	-	-	-	1	-
STMI	*Stephanodiscus minutulus* (Kützing) Cleve & Moller	-	-	-	-	1	-	-	-	-	-	-	-	-
SOVI	*Surirella ovalis* Brebisson	-	-	-	-	-	-	-	-	1	-	-	-	-
STKR	*Stauroneis kriegeri* Patrick	1	-	-	-	-	-	-	-	-	-	-	-	-
STAN	*Stauroneis anceps* Ehrenberg	-	-	-	-	-	-	-	1	-	-	-	-	-
SPIN	*Staurosirella pinnata* (Ehr.) Williams & Round	-		2	-	-	-	-	-	-	-	-	-	-
TFAS	*Tabularia fasciculata* (Agardh)Williams et Round	-	1	-	-	-	-	-	-	-	-	-	-	-
THUN	*Tryblionella hungarica* (Grunow) D.G. Mann	-	-	-	-	-	1	6	-	-	-	-	-	-
TLEV	*Tryblionella levidensis* Wm. Smith	-	-	-	-	-	-	-	-	-	-	-	-	-
TCAL	*Tryblionella calida* (grunow in Cl. & Grun.) D.G. Mann	-	-	-	-	-	-	-	-	8	-	-	3	-
TAPI	*Tryblionella apiculata* Gregory		-	-	-	-	-	-	-	5	-	-	-	-
TCOA	*Tryblionella coarctata* (Grunow in Cl. & Grun.) D.G. Mann	-	-	-	-	-	-	-	-	-	-	-	1	-

APPENDIX D

MICROGRAPHS

Micrographs

Some species examples encountered during the study are given in the figure

Figure D1: Gomphonema pseudoaugur

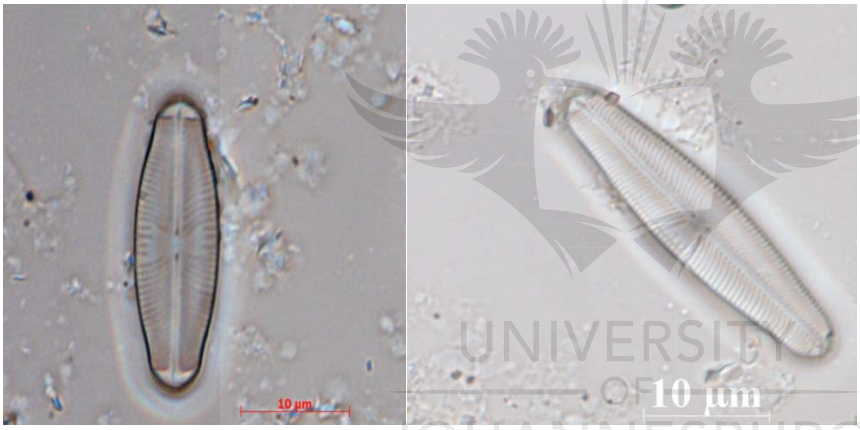

Figure D2: Selaphora pupula

Figure D3: Gyrosigma scalproides

Figure D4: Hantzschia amphioxys

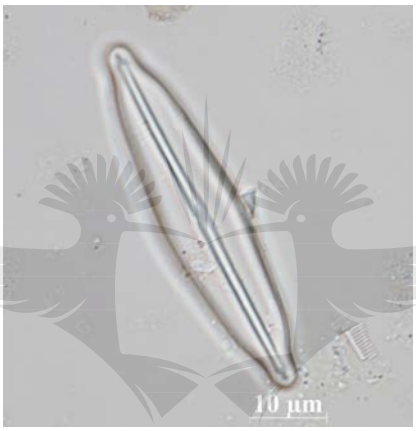

Figure D5: Frustululia crassinervia

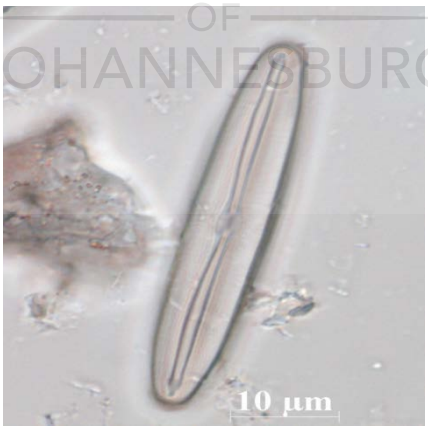

Figure D6: Frustulia vulgaris

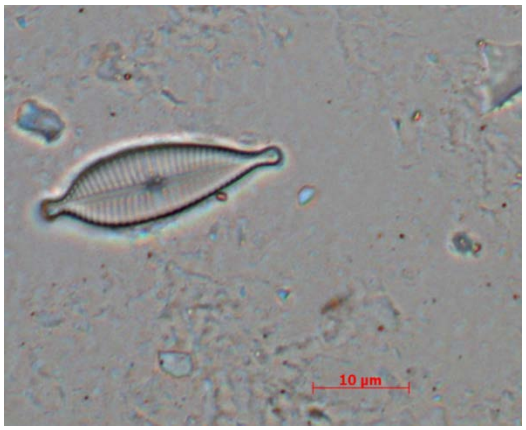

Figure D7:1 Gomphonema aff lagenule

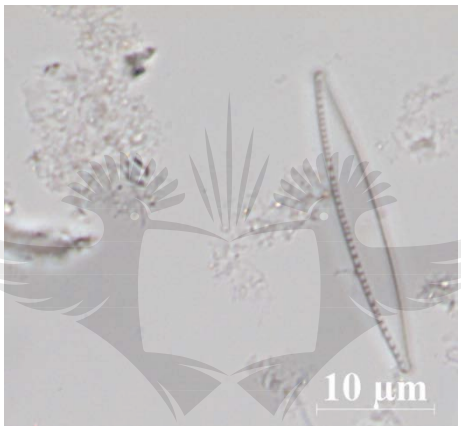

Figure D8: Nitzschia palae

Figure D9: Nitzschia sigma

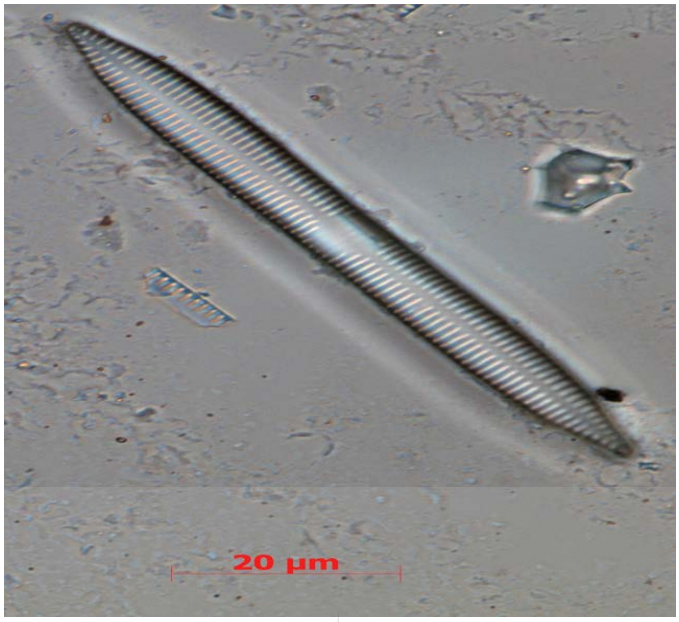

Figure D10: Fragilaria ulna