Diatoms as an indicator of water quality in the Kuils River, Western Cape South Africa

by

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Declaration

I, **Leona Janet Kuturo**, declare that the contents in this dissertation represent my own unaided work, and that the dissertation has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

Signed: Leona Janet Kuturo Date: 28 August 2023

Abstract

Water is a very important resource that is vital for life. However, this resource is threatened due to anthropogenic activities such as urbanisation, agricultural runoff and industrial activities polluting open freshwater resources such as rivers. Rivers are important systems that play a significant role in nature as they are vibrant ecosystems for various plant and animal species. In addition, rivers may serve as possible sources of drinking water, while certain communities are dependent on rivers as part of their livelihoods, i.e. for fishing and agriculture.

It is therefore important to ensure that measures are put in place to manage, monitor and protect open surface water resources. Even though water quality monitoring has previously been conducted using physicochemical analysis strategies, researchers have been challenging and replacing this traditional method with biomonitoring techniques. Biomonitoring techniques allow researchers to get a time-integrated evaluation of the quality of water. In this study, four sites were selected to assess water quality along the Kuils River, Western Cape. Since physicochemical methods have traditionally been used to assess water quality, this study used both physicochemical parameters and diatoms (biomonitoring method) to determine the water quality in the Kuils River.

Historical water quality parameters (2019-2021) that were obtained from the Department of Water and Sanitation were used as a reference, to determine whether the results obtained using diatoms as biomonitors correlate with previously documented results obtained from traditionally used methods. The results obtained from the concentrations of the physicochemical parameters measured along the river indicated the presence of pollution, as changes in abundance or composition of diatoms specices were observed. The diatom species collected were counted and the diatom indices were calculated using the Omnidia 6.1 software. Ninety-eight diatom species were identified in this study.

Diatom species that are pollution-tollerant, indicators of organic pollution and eutrophication such as *Nitzchia palea, Navicula viridula, Eunotia bilunaris and Fragilaria* ulna were widely spread. Less-pollution tolerant species such as Gomphonema parvulum and Cyclotella meneghiniana were surpassed in abundance. Thus, both results from diatom indices as well as physicochemical parameters respectively, complimented the fact that water in the Kuils River is of poor quality. It is concluded that diatoms prove to be a valuable biomonitoring tool in determining the water quality of a river. Based on obtained results, it is recommended that future studies on water quality of river systems use diatoms to supplement traditional physicochemical studies to determine water quality of rivers as they have proved to support these traditional studies.

As the main aim of this study was to investigate diatoms as indicators of water quality, the index values that were obtained from the study were used to determine the ecological class into which the sampling sites fell. In addition, these indices indicated diminished water quality within the river system in both high flow and low flow seasons. Increased nutrient concentrations recorded along the river, indicated poor water quality. Additionally, increased nutrients during high flow seasons can be attributed to high rainfall levels that cause an increase in suspended solids and sediment yields. Runoff from storm water drains, sewage pipes discharging wastewater into rivers could also contribute to increased nutrient concentrations.

This study recommends the introduction of green infrastructures to reduce the amount of pollutants entering rivers. Green infrastructure techniques such as rain gardens, pervious pavements and green roofs. These techniques will slow down runoff from storm water drainages. Allowing the runoff to move slowly and be spread out onto more land whereby plants will work as filters and filter out pollutants from runoff, as well as wastewater from treatment works before it infiltrates into the ground and enters water systems.

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Dedication

To the late Michael Bhaira jr., my little brother who did not get the opportunity to see his sister finish this thesis but was always curious and fascinated by what I was doing.

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Abbreviations and explanations

1. **Bioassessment** - is an evaluation of the condition of a waterbody based on the organisms living within it (EPA,2011).
2. **Diatom** – Diatoms are a commonly occurring assemblages of algae belonging to the Bacillariophyceae group (Taylor et al., 2009).
3. **DWA** -Department of Water Affairs.
4. **DWAF** – Department of Water Affairs and Forestry.
5. **DWS** - Department of Water and Sanitation.
6. **EPA** - Environmental Protection Agency.
7. **GDI** - Generic diatom index.
8. **Index scores** - An index is a way of compiling one score from a variety of questions or statements that represents a belief, feeling, or attitude (Crossman, 2019).
9. **Macroinvertebrates** – is a term used to describe animals that lack a backbone and can be seen without the aid of a microscope, such as arthropods, snails, limpets, mussels and nematodes (Cushman and Smith, 2016).
10. **SPI** - specific pollution index.
11. **Supernatant** - clear liquid that lies above the solid residue after centrifugation (Erickson, 2015).
12. **TDI** - Trophic diatom index.
13. **Valve** - Siliceous part of the frustule containing most of the morphological features used to describe diatoms (Taylor et al., 2007).
14. **Taxa** – a group of one or more populations of an organism (Sivarajan, 1991).
15. **TWQR** - Target water quality ranges.
16. **WQM** - Water quality management.

CHAPTER ONE: INTRODUCTION

1.1 Background

Water, a very important natural resource that supports life, is also a habitat for many aquatic ecosystems. However, apart from serving as a habitat, it is also used for domestic, agricultural and industrial activities. The distribution of water on earth is uneven with 97% being found in the ocean and the remaining 3% being ascribed to freshwater. This 3% is further distributed among glaciers (2%), underground (0.9%) and less than 0.1% is in rivers, lakes and swamps (Balasubramanian, 2015). This leaves atmospheric, surface and groundwater as part of the water proportion that is available for human consumption. This makes water a limited resource as the water that is available for human consumption makes up less than 1% of the total water that covers the earth (Davies and Day, 1998).

Ramakrishnan (2003) stated that the last 10 years have seen a rise in water demand because of the rapid population growth and an increased rate of industrialisation. The high-water demand causes a decline in freshwater availability adding to the scarcity of this resource (Gqomfa et al., 2022). In addition, water quality is also deteriorating in rivers. Multiple sources of pollution such as industrial effluents, agricultural runoff, catchment land use changes, chemical contaminations, litter, freshwater flow modifications, commercial and domestic sewage, abstraction and the introduction of alien vegetation species are responsible for this deterioration (Dallas and Day, 2004).

The different types of pollution sources, therefore, releases pollutants that cause the ecological functioning of river systems to change and negatively impact the health of river systems (O’Brien et al., 2016). Another way in which these pollutants negatively impact river systems, in terms of the aquatic species found in the river, is through nutrient enrichment. Kelly et al. (1995); Bere (2007) and Dalu et al. (2016) stated that nutrient enrichment causes diversity in habitats to be reduced through eutrophication. Pollution in rivers has highlighted the need for water quality monitoring and became a topic of continued concern internationally (Nhiwatiwa et al., 2017). An advantage of water quality monitoring is that it permits the prediction of any worsening ecosystem’s health conditions. On the other hand, it provides an opportunity to evaluate existing investments in pollution control (Soininen and Ko¨no¨nen 2004; Bellinger and Sigee 2010; Nhiwatiwa et al., 2017).

Many countries are confronted with water quality challenges worldwide. These challenges are fuelled by population growth and ongoing rapid urbanisation. South Africa is not an exception to these challenges as they add to the possibility of water quality deterioration in rivers. A study conducted in 2016 looked at the national state of water and sanitation in South Africa, indicating the presence of industrial effluents, domestic and commercial sewage, acid mine drainage, agricultural runoff and litter as pollutants sources in rivers (Masindi and Dunker, 2016).

In South Africa, water is distributed unevenly as the country is semi-arid with rainfall patterns differing between provinces. The western and northern parts of South Africa receive less rainfall while the eastern and southern parts receive more rainfall per year. The Department of Water and Sanitation controls water quality monitoring in the country. The major water supply sources are rivers and dams (Matlala, 2010). Canalisation has resulted in most prevailing rivers being overused or significantly changed. According to Mwangi (2014), canalisation in the Kuils River, Diep River, Liesbeeck River, Black River and Sand River has negatively impacted the ecological aspect of these rivers. The straightening or deepening of rivers impacts the flow and sediment regimes in rivers(Mwangi, 2014). This leads to the loss of aquatic species, as their communities are disrupted by the change in flow and the type of substratum environment at the time of disruption (Mwangi, 2014). This disruption, therefore, may cause a decline in species diversity as species need specific flows or substratum conditions to survive while some might be highly intolerant of change (Admiraal et al., 1993; Petersen, 2002).

Rivers are prone to pollution which causes many rivers to experience a decline in water quality (Chiotelli, 2015). Rivers in Cape Town are exposed to many land-use impacts on the upper and lower parts in the eastern catchment (Mwangi, 2014). The Kuils River, Bottelary River, Eerste River, Kromme River, Steenbras River and Lourens River are some of the rivers that make up the eastern catchment area in Cape Town (DWAF, 2005).

Historically, the Kuils River was known as a seasonal river because it dried up in summer and experienced heavy flows in winter. However, due to the treated waste effluent that it receives from wastewater treatment works, the river now has a perennial flow (Mwangi, 2014). Scottsdene, Bellville, Zandvliet and Macassar encompass the treatment works that discharge treated effluent into the Kuils River (Li, 2005). The Kuils River is mostly channelised and paved, especially downstream. Four sampling points that are monitored by the Department of Water and Sanitation were selected and used in this study to collect diatom samples.

Different point and non-point sources of pollution are the cause of deterioration of water quality in the Kuils River. Pollution sources may include industrial effluent, effluent from wastewater treatment plants, litter, oil and other toxic substances from roads and agricultural activities (Mwangi, 2014). In 1946, the first signs of water quality degrading in the Kuils River were identified when the Department of Water and Sanitation suggested possible canalisation for irrigational purposes (Heydorn and Grindley, 1982). Canalisation would provide a measure of flood protection and could serve as a disposal site for treated effluents (Mwangi, 2014). Water quality monitoring is therefore vital in protecting water sources from anthropogenic activities.

Physicochemical monitoring and biomonitoring form part of water quality monitoring methods used in South Africa. The difference between these types of monitoring techniques is that the former describes the physical and chemical status of aquatic ecosystems based on the presence and concentration of specific variables, while the latter describes the biological status of aquatic systems (DWAF, 1996). Furthermore, biomonitoring studies the ecological condition of a resource through the examination of how organisms living in a certain environment, interact with their surroundings.

Bioindicators such as diatoms, fish and macroinvertebrates are used in river water quality monitoring. These bioindicators have different functions when used during water quality monitoring. Many studies have shown the difference between these bioindicators, as diatoms were said to be better associated with water quality while macroinvertebrates were regarded as better indicators of disturbances in river systems (Sonneman et al., 2001).Historical studies (Belore et al., 2002), discovered that diatoms and macroinvertebrates both equally predicted water quality. This made these organisms very important indicators of pollution.

After the discovery by Belore et al. (2002), researchers such as Mwangi (2014), Nhiwatiwa et al. (2017) and Tan et al. (2017), promoted the use of one or both bioindicators (diatoms or macroinvertebrates) to effectively monitor water quality. Thus, this study used diatoms to assess water quality in the Kuils River as studies that were previously conducted highlighted diatoms as good indicators of land use changes and water quality (Chessman et al., 2007; Lavoie et al., 2014; Stevenson, 2014). Additionally, diatoms are sensitive to a variety of ecological conditions. Lobo et al. (2016) stated that the tolerance and preference levels of diatoms to pH, conductivity, salinity, humidity, organic matter, trophic state, oxygen and current velocity in freshwater resources have been defined.

1.2 Research Problem

South Africa is a semi-arid country, and the deterioration quality of freshwater ecosystems continue to be a major problem. It is estimated that about 55,5% of rivers in the City of Cape Town are polluted. The sources of pollution in these rivers are associated with human activities (Kretzmann, 2019). Moreover, Cape Town is a water scarce city, hence high pollution levels in rivers places restrictions on water that is available for usage. Methods such as physicochemical and biomonitoring methods have been used to assess the quality of water in rivers (Dalu and Froneman, 2016).

These methods are both effective, with the only difference being that physicochemical methods give a partial view of water quality at the time of sampling. In other words, this method is not concerned about factors that might influence water quality such as runoff, precipitation and storm water drainage. In addition, chemical concentrations might be different daily depending on the timing of discharges, rain and water flow patterns in the river (Matlala, 2010). Although, physicochemical methods are convenient in determining contaminant sources, they do not observe the biological response to pollution because they only measure the aquatic health of ecosystems indirectly (Environmental Protection Agency, 2019).

In addition, determining or understanding river water quality requires monitoring aquatic organisms because they can cope with chemical, physical and biological influences in their habitat throughout their life cycle in the aquatic habitat (Environmental Protection Agency, 2019). Lastly, chemical methods do not look at biological threats (invasive species) to the ecosystem (Environmental Protection Agency, 2019). Therefore, biomonitoring is important because it complements traditionally used methods.

Since diatoms exists in all aquatic habitats, different environmental conditions cause their communities to change based on their response to certain conditions. Examples of some of those environmental conditions are eutrophication, changes in pH and salinisation (Matlala, 2010). This study used diatoms to bio-assess water quality in the river. In addition, this study identified possible factors affecting water quality in the Kuils River using diatoms. The effectiveness of water quality management practices in place were assessed. Lastly, measures that can be taken to improve water quality management approaches in the river were identified.

1.3 Research Questions

The main research questions that will be addressed in this study are:

1. What forms of diatoms are present in the Kuils River?

2. What role, if any, does diatoms play in determining water quality and do anthropogenic activities cause a change in the structures of diatoms?

3. Is a certain type of diatom community associated with a particular condition along the river?

1.4 Aims and Objectives

The main aim of this project was to investigate the use of diatoms as an indicator of water quality and contribute to diatom information by acting as a reference to river bio-assessment in the City of Cape Town.

In order to achieve the aim, the objectives of the study included the following:

1. To analyse the diatom community's response to change in the quality of water along the river, as well as seasonal changes.
2. To test for the presence of the documented South African river diatom indices in the Kuils River.
3. To analyse the distribution of diatoms at different points and determine on-site water quality parameters (pH, COD concentrations and PO_4^{3-}) along the river.
4. To compare diatoms information with physiochemical data.

1.5 Location of the Study area

The study was conducted on the Kuils River, in the Western Cape Province of South Africa. This river starts from the mountainous area of Durbanville and is approximately 30 km in length. It continually flows in a south-ward direction whereby it passes through residential and industrial areas of Bellville and Kuils River downstream. Moreover, the river meanders east of the residential area of Khayelitsha to Macassar and passes through the Cape flats (Thomas et al., 2010). Upstream, the Bottelary River is the main tributary of the Kuils River. Further downstream, as the river flows southwardly, it joins the Eerste River near the False Bay estuary.

Furthermore, wetlands are located in the lower parts of the river. These wetlands are of ecological importance as they play a role in the diversity of the nearby southern Cape's indigenous and endemic fauna and flora (Ayuk, 2008). Figure 1.1 shows how the river not only receives surface runoff, but how treated sewage effluent enters the river from Wastewater Treatment Works (WWTW) such as the Bellville WWTW and Zandvlei WWTW.

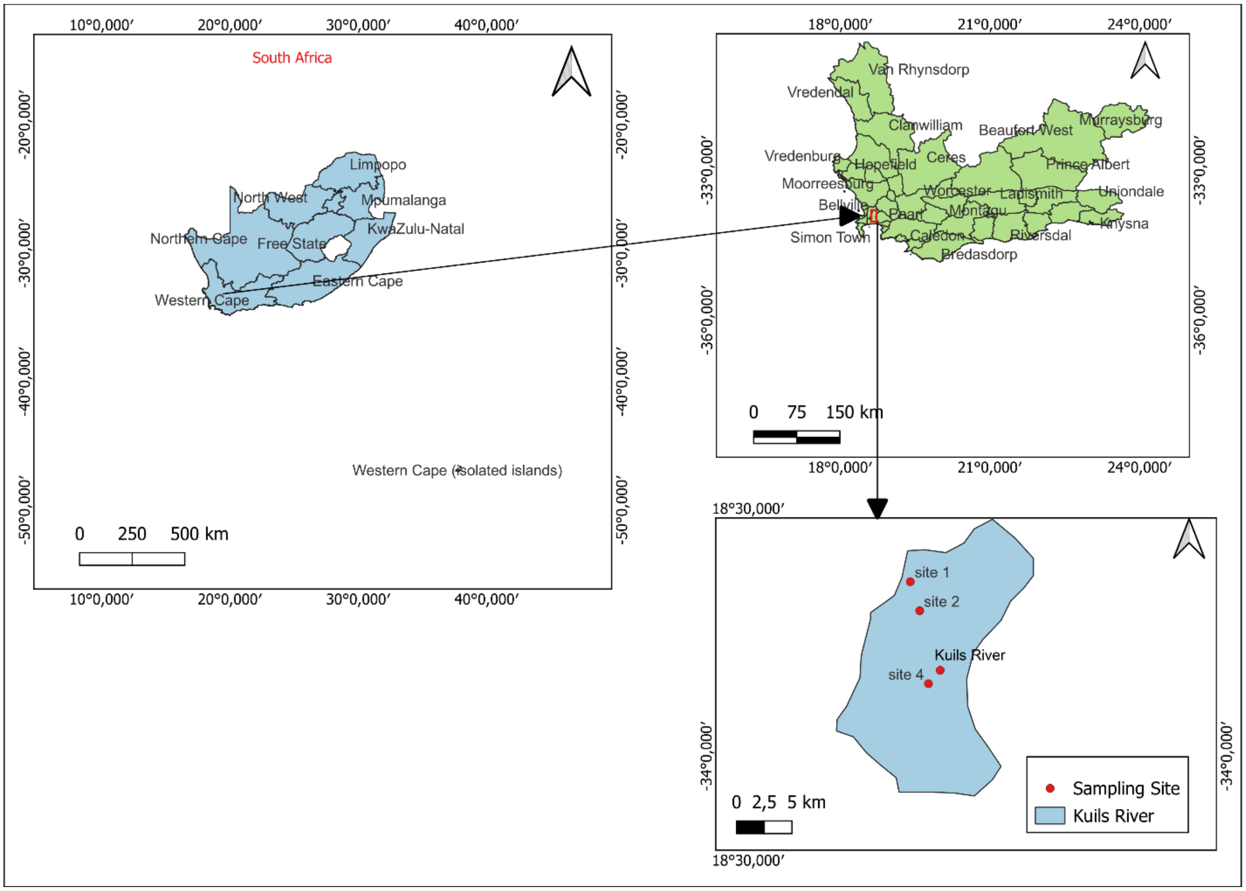

Figure 1.1 Location of the Kuils River, Western Cape, South Africa.

1.6 Climate and vegetation

The Kuils River experiences high winter and low summer flows (Li, 2005). This river is located in a Mediterranean climate that is made up of hot dry summers and cold wet winters. The mean annual precipitation of the area falls between 500 and 600mm per year (Hagen, 2022). Precipitation in the area happens between April and September. Hagen (2022) stated that the mountainous topography causes precipitation to be typically orographic.

This leads to the western part of South Africa receiving most rainfall compared to the other parts of the country. According to Petersen (2002), the climate in the area is influenced by the south Atlantic anti-cyclone which results in a south-eastly wind regime. In the summer months, temperatures may reach up to approximately 40°C while winters are cold with temperatures dropping down to approximately 10°C due to gale force north westerly winds, which sometimes leave high peak areas covered in snow (Ayuk, 2008).

Swartland Shale, Renosterveld, Cape Flats Dune Strandveld and Dune Thicket Strandveld are types of vegetation that previously existed in the Kuils River catchment. These vegetations have been affected by massive disturbances such as agricultural activities, alien vegetation and urban development (Mwangi, 2014). These disturbances have resulted in many non-natives, invasive weeds, shrubs, insects and the displacement of native biodiversity (Shand, 1994; Brown and Magoba, 2009). Port Jackson willow (*Acacia saligna*), white poplar (*Populus canescensus*), Rooikrans (*Acacia cyclops*) and Black wattle (Acacia mearnsii) are types of alien vegetation that dominate the Kuils River catchment (Heydorn and Grindley, 1982). According to the River Health Programme Report (2005), riverbanks were modified by the alien trees which confined and deepened the channel, resulting in increased erosion.

1.7 Land use activities

Agricultural, residential, commercial and industrial areas are the four factors that influence land use around the Kuils River catchment. In its surroundings, the river consists of urban development, with several informal settlements (such as the Cape Flats region) existing along the course of the river (Hagen, 2022). According to Thomas et al. (2012), vineyards cover the bigger part of land in this catchment while the remaining cultivated part of the land is used for growing fruits and lucerne. Furthermore, the other parts of land in the surrounding areas of the catchment consists of ponds, wetlands, fynbos, wetland vegetation, marshes and dams.

Areas which the river passes through, such as Bellville, Kuils River and the Cape Flats, contain some of the industrial and commercial areas that contribute to water quality in the river (Shand, 1994; Brown and Magoba, 2009; Hagen, 2022). Therefore, agricultural and industrial runoff, domestic waste from dumping in open spaces and sanitation are the pollution sources in the Kuils River. Additionally, other sources of pollution in the Kuils River are the Scottsdene, Bellville, and Zandvliet WWTWs as they discharge treated and untreated wastewater into the river (Hagen, 2022).

1.8 Geology and soils

The upper part of the Kuils River is made up of the Malmesbury groups of rocks. The formation of these rocks occurred during the pre-cambrian age (Mwangi, 2014). Rocks such as phyllite, quartzites, shale, siltstone and greywacke form part of the Malmesbury group (Heydorn and Grindley, 1982; Shand, 1994; Mwangi, 2014). According to Ninham (1979) deposits of turf and loam covers this area. Furthermore, straps of conglomerate, gravel, limestone, dolomite and turf rocks occur occasionally in this area. The bottom part of the river, after the Bottelary River joins the Kuils River, is made up of deposits of loose sands and dune formations underlain by extensive clay lenses (Mwangi, 2014).

1.9 Significance of the study

The decline in the quality of water in rivers has a negative effect on aquatic habitats, as it causes loss of habitats and aquatic life. It also destroys the capability of river systems to provide services that people need and rely upon. Water quality deterioration is also associated with many waterborne diseases that threaten human health. Therefore, the monitoring of rivers should be conducted on a regular basis in order to evaluate possible changes in the quality of water as well as the presence of any new pollutants. Furthermore, the identification of pollutant sources is also very important as it assists in tackling the origin of the problem. This allows for catchment managers to check the effectiveness of management strategies put in place to manage river systems.

This study is important because it uses two approaches (physicochemical parameters and diatoms) for monitoring water quality in rivers. The use of diatoms to monitor water quality is a cost-effective approach and this approach is not only applicable in the Kuils River as it can also be applied in other rivers across the country. Diatoms also form part of a worthy addition to a water quality monitoring programme as it is readily available and easier to use.

Physicochemical parameters play a major role in testing water quality before it is declared suitable for agricultural, industrial, recreational or domestic use. Hence, using both these approaches allows for a water quality monitoring programme that can clearly check the biological, physical and chemical aspect of water in rivers. This provides extensive data of water quality in rivers and allows for catchment managers to address each aspect according to the type of environmental problem that it presents in rivers. Furthermore, this information enables the implementation of plans to combat pollution in rivers. This study will also contribute to the management of rivers.

CHAPTER TWO: LITERATURE REVIEW

2.1. Diatoms

Aquatic plant-like organisms which are small and less complex when compared to land plants, are known as Algae. Their energy is obtained through photosynthesis (Stoermer and Smol, 1999). They also make up complex and diverse groups of organisms which are found in different types of aquatic habitats (Stoermer and Smol, 1999). According to Bate et al. (2002), blue-green algae (*Cyanophyta*), green algae (*Chlorophtya*), red algae (*Rhodophyta*) and diatoms (*Bacillariophyta*) are the most common benthic algae occurring in the freshwater habitat. This study focuses on diatoms that occur in freshwater (river) habitats.

Diatoms live in different types of aquatic habitats because of their ubiquitous nature. They are a well-defined group of algae belonging to the *Bacillariophyceae* class. Their cell walls (skeletons) are made of silica which looks like a glass house, and they are both unicellular and autotrophic organisms (Slingers, 2015). Additionally, the cell walls referred to as frustules, are constructed consisting of two halves, also called valves, which fit into each other (Van Vuuren, 2007; Karthick et al., 2010; Slingers, 2015). The diatom community is commonly used in water quality studies as it serves as a monitoring tool for past and present environmental conditions. Diatoms are also perfect organisms for biomonitoring because of characteristics such as a distinct morphology (ornamented silica cell walls). These characteristics allows for the easy identification of diatoms to species level (Holmes and Taylor, 2015). Furthermore, the frustules of diatoms can persist in the environment long after the organisms have died (Van Vuuren, 2007; Taylor et al., 2009). Therefore, this has resulted in the creation of numerous diatom-based indices for water quality.

Diatoms are ecologically important due to their autotrophic nature which enables them to globally contribute to the gross primary production (45%) and about one fifth of the earth's oxygen annually (Field et al., 1998). Additionally, diatoms are of economic significance because they are used in the production of biofuels along with diatomite (fossilised frustules) being utilised as filtering systems (Khraisheh et al., 2004).

According to Musa (2015), the condition of a system at a particular time can change, making diatom communities assemblages adapt to that environment. Therefore, the quality of the water in a river system influences the organisms that inhabit that system. It is presumable that water quality has an impact on diatom community composition because it affects diatoms negatively or positively which results in different diatom communities being found at different conditions along the river. In simpler terms, areas with similar water quality parameters will host similar diatom communities (Finlay et al., 2002). An interesting characteristic of diatoms is that they show sensitivity to changes in chemical variables like nutrient concentrations (Musa, 2015). Hence, this makes them great indicators of water quality. Table 2.1 lists the advantages of using diatoms as bioindicators.

Table 2.1 Advantages of diatoms as bioindicators. Source: (Martin et al., 2012)

1. Diatoms are ubiquitous in nature. Hence, they live in all types of habitats (aquatic, terrestrial and man-made substrates).
2. Diatoms can exist in a shallow concrete area whereby other known biomonitoring taxa do not exist (Musa, 2015).
3. They have short generation times (approximately four weeks) which enables them to reproduce rapidly.
4. Give an indication of habitat restoration or deterioration because of their fast response to changes that occur in the system (Rott, 1991).
5. Determining water quality using diatoms as a monitoring tool is a non-invasive monitoring.
6. It is observable that diatoms do not need certain conditions (such as food, habitat and water flow) to exist when compared to other biomonitoring taxa.
7. The nature of diatoms allows for the easy identification of diatom taxa in water quality monitoring which is based on the morphological structure of the frustule.
8. They require limited specialised equipment which makes water quality monitoring using diatoms cost effective (Musa, 2015).
9. Diatoms response to changes in the environment is predictable (Passy, 2007; Patrick et al., 1971). Thus, diatom species have certain favourable (optima)

conditions (such as pH, temperature ranges and nutrient concentrations) for growth, reproduction or success (Musa, 2015).

Abovementioned advantages highlight the usefulness of diatoms as indicators of water quality in water bodies. In addition, when comparing diatoms to other organisms, the advantages listed also highlight how easy it is to use diatoms as a tool in water quality monitoring. Musa (2015) therefore suggested that the use of diatoms as a monitoring tool should not be limited to rivers only but should also be applied to wetland systems as other organisms might be difficult to use as monitoring tools in wetlands. The conditions and concentrations of physicochemical parameters present in a water body control the concentrations of diatoms that will occur in that system (Passy, 2007). Thus, any change that happens in that body of water is reflected by the change in the composition of the diatom community (Musa, 2015).

It is important to understand the relationship between environmental variables that play a role in species distribution. Understanding that relationship enables the assumption that diatoms are good indicators of ecological integrity due to their ubiquitous nature. Some of the examples of those environmental variables are pH, trace elements, light, nutrient concentrations, temperature, oxygen availability and conductivity.

Different diatom indices have been developed and used to monitor water quality in rivers (Tan et al., 2017). The trophic diatom index is an example of one of the diatom indices that were developed. This index is used to monitor the trophic status of rivers based on diatom composition. Slingers (2015) stated that in practice, diatom indices entail making a list of taxa that is present in a sample as well as a measure of their abundance. In addition, the index is expressed as the mean of the optima of the taxa in the sample that is weighted by the abundance of each taxon (Slingers, 2015).

Diatoms also form part of the largest group of the epilithic algal community. Epilithic diatoms are bigger than most algae and they grow on hard substrata such as rocks and cobbles. Epilithic diatoms are excellent water quality indicators at a particular site because of their inability to move away from their environment (Holmes and Taylor, 2015).

2.2. Historical studies of diatoms

Worldwide diatoms have been used as water quality indicators on different continents. Such as Europe, North America, South America, Australia, Asia and Africa. Diatoms have also been used for paleo-environmental reconstruction (Phungula, 2018). In Central Africa, information on diatoms is limited compared to other parts of Africa (Taylor et al., 2015). Table 2.2 gives an indication of the different types of contributions made by researchers in terms of the history of diatom information in Southern Africa.

Table 2.2 Summary of diatoms historical studies (adapted from Rybak et al., 2021)

Authors	**Years**	**Summary**	**Work done**
Ehrenberg, C.G. Cleve, P.T.	1845, 1881	These two researchers investigated diatoms found in freshwater. They also provided information on new diatoms and those diatoms that had little information known about them.	Wrote journal articles
George, D.	1882	The researcher wrote about algae that is attached onto aquatic phanerogams (seed bearing plants) and found 31 diatoms out of 38 taxa discovered in the Lake Malawi.	Wrote journal articles
Müller, O.	1897,1903,1904, 1905,1910	The writer is responsible for the description of 126 new diatom taxa (species, varieties and forms) in rivers, lakes and streams.	Wrote journal articles and books.
Cholnoky, B.J.	1950 to 1960	This researcher wrote papers that investigated various diatom species found in the Southern African region.	Wrote over 40 journal articles
	1970	The researcher also discovered 3 new diatom species out of a total of	

		93 observed taxa in the Bangwelu swamps, Zambia.	
Giffen,M.H.	1966	This scientist was responsible for publishing papers that dealt with marine and estuarine diatoms. The author also published work on freshwater species that are found in Eastern Cape, South Africa.	Wrote journal articles
Schoeman, F.R. and Archibald, R.E.M.	1980	These authors contributed to the knowledge and ecology of diatoms in South Africa.	Wrote journal articles and Books
Cocquyt, C.	1993	Wrote an overview of all taxa reported in the African Great Lakes (Victoria, Malawi and Tanganyika).	Published journal articles and books.
	2006	The author also discussed the diversity of algae in lacustrine and riverine environments in the African Great Lakes.	

2.3. Use of diatoms as water quality indicators in South Africa

Cholnoky (1968) produced the most noted work on diatoms in South Africa, which dealt with the potential use of diatoms as indicators of water quality. Additionally, Cholnoky was also responsible for the application of the Thomasson community analysis in 1925 (Taylor et al., 2015). The Thomasson community analysis was adapted and applied in the determination of water quality using the benthic (attached on substrates) diatom community composition (Taylor et al., 2015). This adaptation of the Thomasson analysis enabled comparisons to be made between different sites in the same river.

Furthermore, this community analysis also allowed for changes to be tracked at a single site in the same river (Taylor et al., 2015). Therefore, two different studies that were done by Cholnoky explained how the Thomasson community compares with different sites and tracks changes as stated above. In the first study, Cholnoky used the amount of nitrogenous effluent as an aspect of water chemistry because only a single aspect of water chemistry is chosen when conducting such studies (Taylor et al., 2015). Furthermore, Cholnoky summed up all the species of the genus *Nitzschia* within a particular diatom community and calculated them as an abundance value. Some diatom species, such as *Nitzschia* depend on dissolved nitrogen compounds availability in the surrounding environment for growth and reproduction.

Thus, the relative abundance of this genus (*Nitzschia*) in a sample reflects the amount of nitrogenous pollution at the study site (Taylor et al., 2015). In the second study, Cholnoky used abundance values of the acidobiontic genus, *Eunotia*, to track a pH gradient in a river system (Taylor et al., 2015). The use of an established diatom index resulted in the outcome obtained in the second study to be significantly accurate.

In order to use the Thomasson analysis method, an in-depth knowledge of the Autecology (a branch of ecology that studies the relation of an organism to its environment) of individual diatom genera and species (Cholnoky, 1968), should be obtained. This enables scientists who use this method to obtain and report on accurate environmental information based on the diatom community composition (Cholnoky, 1968).

In the 1970's, many researchers such as Archibald (1972), Schoeman (1976) and Lange-Bertalot (1979) developed, tested and improved systems that aided in the diatom's ability to be used as indicators. A system that was capable of monitoring groups of diatoms with similar tolerances towards pollution was developed in 1979 by Lange-Bertalot (Taylor et al., 2015). The system eventually proved to be successful after several modifications. In 1983, Schoeman tested the system developed by Lange-Bertalot when the method was used in a study in the upper Hennops River, South Africa. Schoeman found a positive correlation between the species composition of the diatom communities studied and water quality (Taylor et al., 2015).

Unfortunately, this development was parallel with Europe in the study of the application of diatoms as bioindicator organisms. This led to Schoeman's 1979 work on diatom studies being terminated (Taylor et al., 2015). In 2002, an in-depth revisitation of diatom studies in South Africa by Bate et al. (2002) was established. In their study, the authors tried to relate a descriptive index based on a dataset for the environmental tolerances of diatom species that are found in the Netherlands, to the water quality in South Africa (Taylor et al., 2015).

Bate et al. (2002) adapted environmental variables of diatom species that were found in the Netherland waters which was created by Van Dam et al. (1994). Examples of these environmental variables are pH, conductivity, oxygen requirements, trophic status and saprobian status. It was concluded that benthic diatoms can be useful in water quality determination and give a time-integrated indication of specific water quality components. However, the data set tested in their study as adopted from Van Dam et al. (1994), could not be transposed directly for use under South African conditions (Bate et al., 2002; Taylor et al., 2015).

2.4. Diatom indices

For the quality of an environment in biology to be shown, a scale is used. This scale is also known as an index. It shows the quality of an environment by indicating the types of organisms as well as the abundance of a particular organism in a representative environmental sample (Snozzi, Ashbolt and Grabow, 2001). Therefore, indices are commonly used to assess water quality in aquatic ecosystems (mainly marine and freshwater). An advantage of using diatom indices is that they sum up information (ecological and hydrological) that is given by diatom assemblages (De Almeida et al., 2001).

Furthermore, calculating diatom indices has been made easier by Omnidia 6.1, a diatom database and software identification tool. The commonly used indices are centred around the Zelinka and Marvin's method (equation 1) which reflects on the weighted averages of taxa's sensitivity to nutrients, pH, organic deprivation and salinity (Dalu et al., 2016; Phungula, 2018). According to Bate et al. (2002) indices that are commonly used require the researcher to identify diatoms to species level.

$\text{Index} = \Sigma (Ai \times vi \times ji) / Aivi$………………………………Equation 1

(where ai = abundance of species j in sample, vi = indicator value and ji = pollution sensitivity of species j).

The principle on which indices are based is the fact that most diatom taxa present in waterbodies are the ones that have optimal growth conditions close to the magnitude of the variables or determinants (Musa, 2015). Nutrient concentrations and pH are examples of such variables.

In other words, when looking at each sample, the estimated size of the determinants can be deduced by looking at the average of the optima of all the taxa present in the sample with emphasis on its abundance (Musa, 2015). Therefore, results from a sample are more influenced by taxa with a higher concentration compared to those with a lower concentration in a sample. The **v (**indicator value) and **s** (taxon's pollution sensitivity value) influence results obtained from indices (Musa, 2015). In simpler terms, the v-value gives an indication of the diatom's attraction or affinity to specific water quality depending on whether it is of good or bad quality whereas the s-value reflects the strength or weakness of the relationship (Taylor et al., 2006). The production of a well-integrated method that monitors water quality trends and at the same time allow for site comparison, can only be achieved through diatoms that are centred around the richness of indicator species (Deny, 2004).

The way in which the Omnidia 6.1 database is set up, it contains over 12000 diatom taxa which are integrated into the database (Ács et al., 2004). Of the 12000 diatom taxa, approximately 1800 diatom taxa are characterised for ecological sensitivity and indicator values (Ács et al., 2004). Some of the indices that are integrated into the Omnidia 6.1 database are listed in Table 2.3 (these indices are based on the Marvin and Zelinka equation).

Table 2.3 Diatom indices integrated into Omnidia 6.1 database. Source: (Matlala, 2010)

Abbreviation	**Diatom index**	**Description**
BDI	Biological Diatom Index	Responsible for grouping taxa based on their morphology and related taxonomical groups (Lenoir and Coste, 1996).
CEE	Commission for Economical Community metric	Uses 208 species; index compiled by Descy and Coste (1991).
DES	Descy's pollution metric	Sorts 106 species into 5 sensitivity classes (Descy, 1979); Regarded as the Descy's index.
EPI-D	Eutrophication Diatom Index Diatoms	Groups data into 5 classes.
GDI	Generic Diatom Index	A unique index that utilises all freshwater taxa from the database; diatom identification is only at genus level (Coste and Ayphassoro, 1991); groups data into 5 classes of sensitivity.
IDAP	Diatom Index Artois-Picardie	Groups diatom species into 5 categories (Prygiel et al., 1996).
LandM	Leclercq and Maquet index	Groups 210 diatom species into 5 classes of sensitivity (Leclercq and Maquet, 1987).
ROT	Rott's index	Groups data into 5 classes of sensitivity based on taxa's saprobiological preferences (Rott, 1991).
SLA	Index by Sladeček	Groups 323 diatom species into 5 classes of sensitivity (Sladeček, 1986).
SPI	Specific Pollution Sensitivity index	Groups data into 5 classes of sensitivity and like GDI it makes use of all the species in the Omnidia database (Coste in Cemagref, 1982).

TDI	Trophic Diatom Index	Groups data into 5 classes of sensitivity (Kelly and Whitton, 1995). The use of this index only applies to water bodies that that experience sewage inputs. TDI is appropriate to use when quantifying heavily polluted water bodies (Musa, 2015).

Of the abovementioned indices, the TDI, SPI and GDI are the most utilised and tested indices by several researchers globally. All the developed indices have different functions. The development of TDI was to measure inorganic nutrient concentrations whereas SPI and GDI were developed as indices of organic pollution (Kelly et al., 1995).

The GDI only requires classification to genus level. This makes it an easier index to use in water quality monitoring when compared to other indices (Musa, 2015). This index is also quite resourceful in giving an indication of a polluted aquatic system (Taylor, 2004; Matlala, 2010). The GDI classification is based on 44 genera, while the SPI is a broader index as it integrates the s- and v-values of over 1300 species. Furthermore, the s- and v-values are the constants on which the performance of the index is based (Musa, 2015). Phungula (2018) stated that it is vital for indices to be used by databases such as Omnidia as they derive index scores that indicate different water quality groupings.

This therefore makes the indices and database important for interpreting data for water quality management purposes. In addition, Omnidia scores range between 0 and 20 apart from the TDI, where 0 indicates bad water quality and 20 indicates clean conditions (De la Rey et al., 2004). Table 2.4 indicates how the index scores are further divided into water classes.The TDI is different from other indices based on how it groups water quality classes. The index scores range from 0 to 100 in which 0 indicates a low nutrient concentration and 100 indicates a high nutrient concentration (Musa, 2015).

Table 2.4 Different diatom index scores indicating different water quality classes (adapted from de la Rey et al., 2004; Matlala, 2010).

Type of water quality/class	Index score
Bad	<9
Poor	9 – 12
Moderate	12 – 15
Good	15 – 17
High	>17

2.5. Evolution of water quality management in South Africa

Policies and regulations which are based on international standards control water quality, its management and how it is monitored in South Africa (Department of Water and Sanitation, 2016). Increased water demand in the country will continue to rise because of the continued population increase in the country (Department of Water and Sanitation, 2016). Therefore, it is important to ensure that sustainable development in terms of water resources is implemented and achieved. According to Walmsley et al. (2001), sustainable development can only be achieved through adequate management which is crucial. Efforts to address water quality management in South Africa have a long history, which is summarised in Table 2.5.

In order for the water services provided by government to be effective, the Water Services Act (Act 108 of 1997) makes provision for legislative framework (Department of Water and Sanitation, 2016). This framework caters for basic human needs as the national water policy is responsible for highlighting cooperative governance and putting emphasis on building capacity at all levels of government (Department of Water and Sanitation, 2016).

Additionally, the National Water Act (Act 36 of 1998) is responsible for the protection of water resources and combining or bringing together water quality and quantity. The implementation of this act was very important as it focused on having integrated resources, it allows for remediation and has a source directed approach which enabled the management of the system (Department of Water and Sanitation, 2016).

Table 2.5 Summary of the history of South African water laws. Source: (Malaza and Mabuda, 2019)

Year	Water laws
1912	Completion of the codification of the law of water rights for the Union of South Africa. A Commission of Inquiry into Water Matters was set up to report on the water needs of various secondary water users as well as their effects on water availability. The Commission of Inquiry was set up with pressure from the lobbying of the industrialists who had the support of mining and commerce industries. The report of this Commission became the basis for the new Water Act (No. 54 of 1956).
1952 to 1972	This permitted the state to use the principle of government control areas which was systematically extended to cover in some or other measure all sources of natural water. The state was thus re-invested with dominus fluminis status for all practical purposes, bearing in mind the increasing demand for water and fixed water supply.
1984	Water rights for the forestry sector controlled by an Act of Parliament after its identification as a major water user with direct effect for downstream users.
1994	The democratic transition necessitated a water legislation rationalisation and amendment process. Consultations that led to the writing of a new Water Act began.
1996	The Fundamental Principles and Objectives for a New Water Law in South Africa were approved by the Cabinet.
1997 to 1998	The National Water Act and Water Services Act were passed and published.

After the development of these acts, South Africa moved to developing operational policies and strategies that had the ability to give an effect to the main acts and policies of 1997 and 1998 (Department of Water and Sanitation, 2016).

Some of the policies and strategies are listed below:

1. **The policy and strategy for groundwater quality management in South Africa ~** This policy was developed in 2000. It had a concept that put an emphasis on an integrated strategy. It confirmed that for a strategy to be deemed integrated, it had to include integrated water quality management for all the different types of resources.
2. **The national sanitation strategy ~** It was developed in 2004 and dealt with the alignment as well as the influence of water sanitation frameworks. According to the Department of Water and Sanitation (2016), the development of the National Water Resources Strategy (NWRS) was very important because it ensured that policies, strategies, guidelines and procedures for the management of water in South Africa were arranged.
3. **Resource directed management of water quality policy ~** This policy was developed in 2006 and was very different from the others that came before it as it was only resource related and excluded a source policy and strategy.

When policies and strategies have been implemented in a country it is only a matter of time before it is assessed in order to check its effectiveness or if any changes have occurred since it has been implemented. The Department of Water and Sanitation (2016) stated that a national assessment of water quality was conducted across 330 monitoring points that were regarded as priority points in South Africa between 2010 and 2011. The results from these monitoring points showed that nutrients and salts were a problem throughout much of the country (Department of Water and Sanitation, 2016). Therefore, it was of utmost importance that new strategies which dealt with these issues were introduced.

In 2013, the National Water Resources Strategy 2 (NWRS 2) was developed. This new strategy listed a lot of concerns such as salinisation, eutrophication, microbial and metal contamination, toxicants and agrochemicals that needed to be dealt with in the country (Department of Water and Sanitation, 2016). However, this strategy was not complete as it did not include a holistic strategy on how these listed concerns would be addressed (Department of Water and Sanitation, 2016). These concerns were a result of pollution from untreated or poorly treated wastewater treatment plants, runoff from unserviced areas, industrial wastewater discharges, agricultural runoff and mining impacts (Department of Water and Sanitation, 2016).

2.6. Water quality management in South Africa

The steward for South Africa’s water resources is the Department of Water and Sanitation. Their role is to form and implement policies that govern this sector. The department also promotes effective and efficient water resources management to ensure sustainable economic and social development. According to Seago (2016), the evolution of water quality management in South Africa is proof of how the Department of Water and Sanitation have prioritised future water resource planning by placing it high on its priority list of management functions.

The aim of positioning water resources higher on the list is for the department to be able to have enough time to prepare, create and implement new techniques at the required time without causing a disruption in the supply of water to major users (Seago, 2016). Furthermore, the focus on water quality management in the country has shifted over the years. Its focus shifted from primarily infrastructure development projects to more integrated approaches whereby demand, and supply management interventions were priority. This ensured that available water not only sustained socio-economic development, but also reserved water for ecology (Seago, 2016).

In South Africa, each province receives rainfall in different seasons of the year. Water quality management will be different in each province as it will be based on the problems or crisis that each province faces. It is therefore important to have programmes or techniques that assist in the achievement of proper water quality management. These programmes are useful as they enable tracking whether the programme is effective or not. These monitoring programmes would then allow for changes to be made where the need could be identified. Currently, 11 resource quality monitoring programmes have been established in the country, namely the National Microbial Monitoring Programme (NMMP), River Health Programme (RHP), National Chemical Monitoring Programme (NCMP), National Eutrophication Monitoring Programme (NEMP), National Radioactivity Monitoring Programme (NRMP), National Toxicity Monitoring Programme (NTMP), Ecological Reserve Determination and Monitoring Programme, Hydrographic Surveys for

Sedimentation Programme, Dam walls (dam safety) Programme, Hydrological Monitoring Programme (HMP) and Geohydrological Monitoring Programme (GMP). The main purpose of these monitoring programmes is to assess the status of water resources and to track water quality trends. Each programme measures or tests for different water quality parameters (Van Niekerk, 2004).

2.7. Physiochemical parameters

Proper management of rivers and the protection of aquatic biota is achievable by measuring physicochemical parameters as the relationship between physicochemical parameters and natural conditions might regulate the growth and survival of organisms (Matlala, 2010). In other words, any change that might occur in these variables results in harmful disturbances to the ecological and physiological functions of the organisms living in that water body. The physicochemical parameters form part of the four properties that water quality can be grouped into (Table 2.6).

Table 2.6 Water quality properties into which physicochemical parameters are grouped (adapted from Pooja, 2017).

Properties	Comprises of
Aesthetic	Odour, taste, colour
Biological	Biodiversity of the system
Physical	Temperature, turbidity
Chemical	Dissolved oxygen, pH, conductivity, presence and concentration of salts and metals

Disturbances to ecological and physiological functions of aquatic organisms may cause the death of important species and ultimately the whole community structure (Matlala, 2010). Hence, to avoid the death of important species, it is crucial to monitor these variables regularly. This led the Department of Water Affairs and Forestry to develop the Target Water Quality Range (TWQR) for South African aquatic ecosystems. According to DWAF (1996) these guidelines act as a base which is responsible for notifying water users of the ranges within which water parameters should fall in order to maintain the integrity of the system. Table 2.7 provides a summary of the TWQR for freshwater systems in South Africa.

Table 2.7 A summary of the target water quality ranges for freshwater systems in South Africa (adapted from Phungula, 2018).

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%
		Conductivity	Not available	Deviation < 15%
		Temperature	5-30ºC	Deviation < 10%

CHAPTER THREE: MATERIALS AND METHODS

3.1. Introduction

This chapter discusses the methodology that was used in collecting data to achieve the objectives and address the research questions of the study. Furthermore, the chapter elaborates on the methods used to collect historical physicochemical data, sampling of diatoms and the collection of diatoms data. Essentially, this chapter addresses the analysis of how all the information in this study was gathered.

3.2. Data from the Department of Water and Sanitation

The Kuils River physicochemical data from 2019 to 2021 was obtained from the National Water Monitoring Database which is owned by the Department of Water and Sanitation. The data can be accessed on the web address below:

http://www.dwa.gov.za/iwqs/wms/data/000key.asp

3.3. Historical physicochemical data

The data received from the Department of Water and Sanitation over a period of three years was used to calculate the arithmetic mean in this study. The obtained physicochemical parameters data were pH, chemical oxygen demand and phosphorus. This data was transferred to Microsoft Excel and bar graphs were generated, making it easier to compare the change in water quality over time in the Kuils River. Furthermore, the Pearson correlation was used to correlate the relationship between physicochemical parameters and diatoms. This was done to enable the study to achieve the set aims and objectives, as well as to answer the research questions.

3.4. Sample collection

3.4.1. Diatom samples

The four sampling points used to collect diatoms were the same as those used by the Department of Water and Sanitation to collect physicochemical data. Sampling took place during the dry (low water flow) and wet (high water flow) seasons (Figures 3.1 to 3.4). Furthermore, samples for all the four sites selected were collected on the same day. Two to three rocks or cobbles were randomly collected at each sampling site and placed on a tray as shown in Figure 3.5. Diatoms were vigorously removed by scrubbing the upper surface of the substratum with a clean toothbrush to dislodge the diatom community. Only the upper side (the side most exposed to flowing water) of the rock was scrubbed to avoid contamination with sediment that might be present on the bottom part of the rock. The resulting diatom suspension, after being scrubbed, was poured into a labelled 100 mL sample bottle.

Figure 3.1 Site 1 diatom sample collection

Figure 3.2 Site 2 (a) dry season and (b) wet season

Figure 3.3 Site 3 (a) dry season and (b) wet season

Figure 3.4 Site 4 (a) dry season and (b) wet season

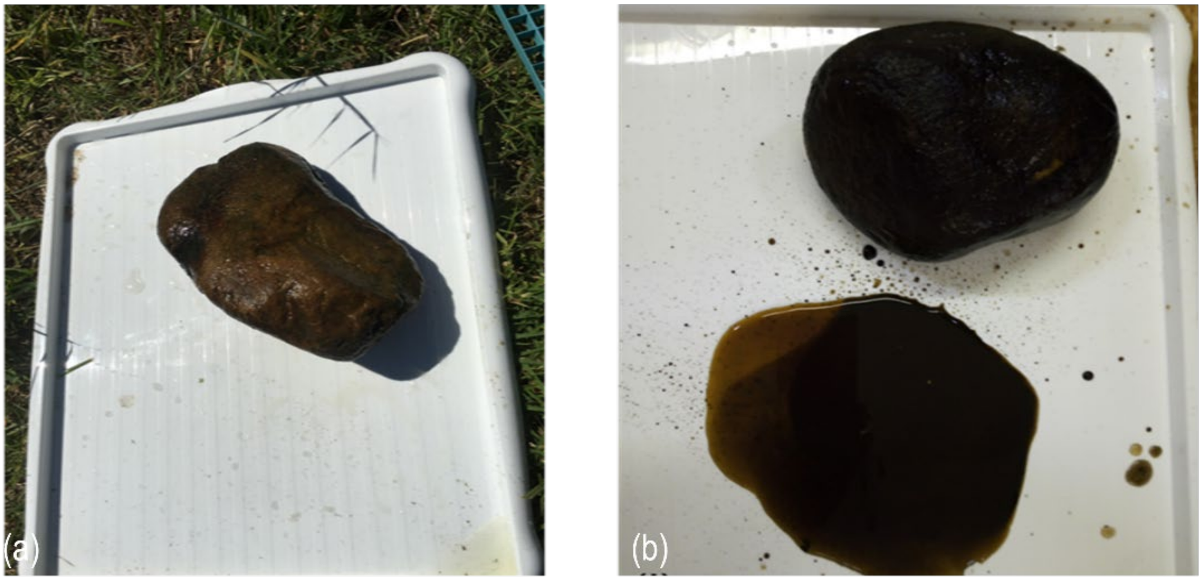

Figure 3.5 Rocks collected from the river.

Even though live or raw diatom material can be used for analysis of the diatom community, it is advantageous to use cleaned material as it can be archived and used as reference at a later stage (Matlala, 2010). Two hundred millilitres (200 mL) of ethanol (20% v/v) was added to the sample bottle with the collected diatom material. Apart from the high-water flow samples, samples were allowed to settle for 24 hours. High-water flow samples were allowed to settle for 48 hours since chemicals (Hydrochloric acid and potassium permanganate) required for processing after 24 hours, were not available. The high flow (wet season) samples were monitored each day during the 48-hour period and changes monitored.

3.4.2. Cleaning of diatom samples

The diatom cleaning process is very important as it assists in viewing diatoms clearly under the microscope without the interference of unwanted matter in the final prepared microscopic slide. Various methods have been accepted for the cleaning of diatoms. This study used the recommended cleaning method by Taylor et al. (2007) because it yielded good results in previous studies that were conducted throughout South Africa whereby, high content of organic material was detected in samples.

This study used the hot hydrochloric acid and potassium permanganate method as described and recommended by Taylor et al. (2007). An advantage to the recommended method is that there is no need to remove calcium before processing the samples compared to when using alternative accepted methods. After diatom samples were allowed to settle for 48 hours in the laboratory, the clear supernatant liquid was discarded using a pipette, taking care not to lose the diatom material. Samples were shaken well, and 10 mL of the thick diatom suspension was poured into a heat-resistant beaker, marked clearly with the sample number. An equal amount (10 mL) of saturated potassium permanganate ($KMnO_4$) solution was added to the heat-resistant beaker and the sample allowed to settle for a period of 24 hours to allow for the oxidation of the organic material present in the sample. The remaining original sample was not discarded but kept throughout the preparation stage in case the processing of more slides would be required (Kelly et al., 1998).

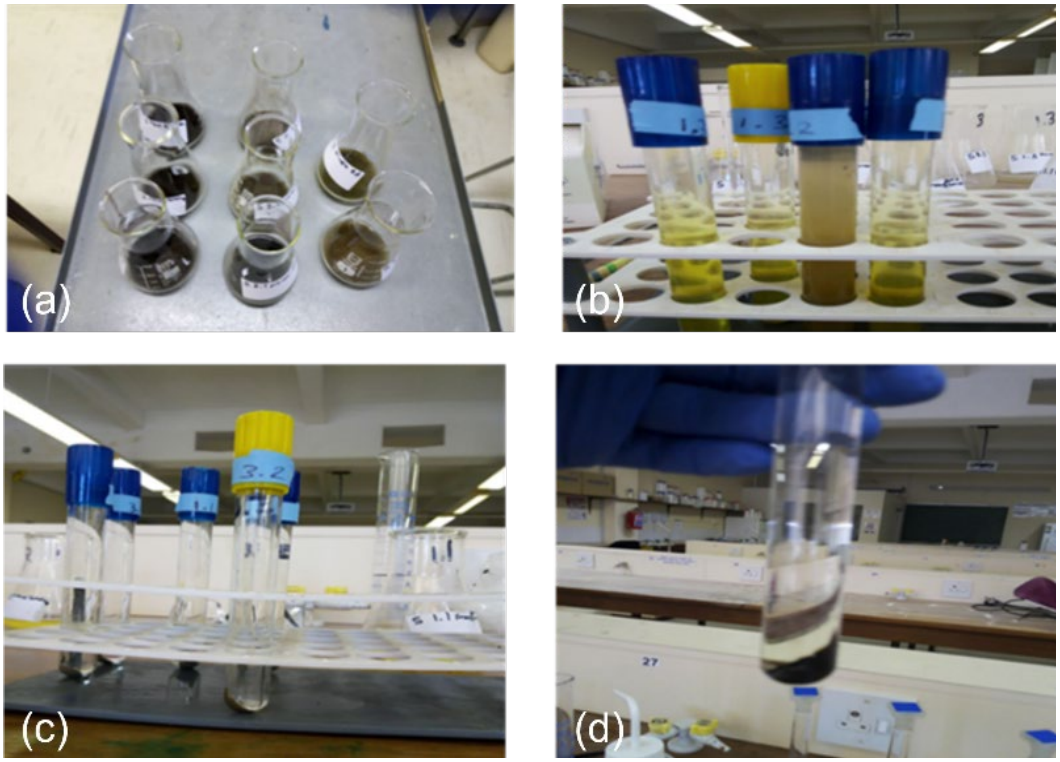

Figure 3.6 Cleaning process of diatoms (a) sample after addition of hydrochloric acid, (b) samples before centrifugation process took place, (c and d) samples after being centrifuged.

After 24 hours, 10 mL concentrated hydrochloric acid was added to the heat-resistant beaker, containing the sample (Figure 3.6 (a)). The samples were placed in a water bath at 90 ºC for two hours. The heat-resistant beakers were placed in the water bath to speed up the digestion process of potassium permanganate by the hydrochloric acid. The solutions were slowly heated until it cleared to a pale yellow (straw) colour. To check if the oxidation reaction was complete and that no organic matter remained in the sample, 1 mL of hydrogen peroxide was added.

The observation of foam upon the addition of hydrogen peroxide, showed that there was a clear indication of organic material still present within the sample, in which case the sample had to be boiled again. When oxidation was complete, the samples were left to cool to room temperature before it was centrifuged. The cooled samples were vigorously swirled and transferred into centrifuging tubes (Figure 3.6 (b)). Thereafter, the samples were rinsed by centrifuging it with distilled water at 2500 rpm for seven minutes.

After centrifugation, the supernatant was decanted, and the washing of the diatoms repeated twice until the sample became circumneutral (clear). After the rinsing process was completed, the clear supernatant (Figure 3.6 (c and d)) was removed from of the testing tubes. The diatoms and small particles that settled at the bottom of the tube were resuspended by means of a jet of distilled water from the wash bottles. More distilled water was added until the testing tubes were full.

Furthermore, after the last wash the diatoms were loosened again by distilled water and the vortex mixer was used to mix and resuspend diatom samples. The final sample solution was poured into glass cylinders bearing the necessary sample information. It is important to store diatom samples in glass as opposed to plastic vials, as glass releases silica, which counters the dissolution of diatom valves (Taylor et al., 2007). The samples were then ready for microscopic slide preparation.

3.4.3. Preparation of diatom slides

Slides and coverslips were thoroughly cleaned with detergent soap and stored in ethanol until needed. A portion of the cleaned sample was drawn from the numbered diatom suspension using a micropipette.

The cleaned diatom suspension was diluted until it appeared slightly cloudy to the naked eye. A single drop of ammonium peroxide (10% NH_4 was added) to neutralise electrostatic charges on the suspended particles and reduce aggregation. According to Taylor et al. (2007), this step is important because it ensures that diatoms and other particles do not clump together and destroy the random distribution of the cells on the slide which will influence the analysis results.

Then, using a micropipette, 1.5 mL of this cleaned diatom suspension was placed on a clean dry coverslip. The diatom suspension was allowed to air dry completely in a dust free environment. If, after 24 hours, the coverslip was not completely dried, the study used the fanning method to speed up the drying process to see whether diatoms would be visible on the slide. The coverslips were fanned, at the slowest speed, using a portable fan until it was completely dried. Thereafter, the dried diatom-coated coverslips were placed slightly over a hot plate to drive off excess moisture for approximately two minutes.

After the coverslips were cooled, it was briefly examined under the 40x objective lens (i.e. a 400x magnification of the diatom samples' actual size) to determine if the concentration of diatoms in the solution was adequate, in which case, according to Taylor et al. (2007), at least 10 but not more than 40 valves should be visible per field. This is done so that when the sample is finally viewed under the 100x objective lens (i.e. 1000x magnification), diatom valves ranging between 5 and 15 but not more than 20 should be visible per field of view (Taylor et al., 2007). Thus, if the diatom concentration was too high or too low, a new diatom slide was prepared and the whole process was repeated.

Once the concentration of diatom samples present on coverslips was found to be correct or adequate, a previously dried and cleaned slide was lowered onto the coverslips and Sellotape was used to attach the coverslip to the slide.

The completed slides were then ready for microscopic examination. When the prepared slides were viewed under the microscope no diatoms were observed. The prepared slides were left to stand for a period of 24 hours after which diatoms were observed. The temporary diatom mounting method used in this study proved to be usable as it yielded good results because diatoms were still visible on the slides after a period of three months.

3.4.4. Diatom Counts

The diatom communities were manually counted, and the results were recorded based on the amount of diatom communities that were present at each sampling site as well as their abundance. The diatom counting process is a time-consuming process as it requires focus and accuracy in identifying diatoms to their respective species level. Identified species had to be compared and verified in a manual that is recommended for the common types of diatoms that are present in South African rivers. In order to prevent the miscounting or repeat counting of already identified diatom species in the same microscopic field, horizontal lines were drawn on the numbered slides which served as a counting guide.

According to Taylor et al. (2007), the aim of counting diatom units (valves) is to produce semi-quantitative data from which ecological conclusions can be drawn. The total number of valves to be counted for each sample differs according to the purpose of the analysis and need to produce statistically good results (Taylor et al., 2007). In South Africa, Schoeman (1973) recommended that a count of 300 to 600 may be used for purposes of routine analysis. This counting range is corroborated by Prygiel et al. (2002) who found, in an inter-comparison exercise, that diatom index scores were not affected at counts of 300 and above (Taylor et al., 2007). In this study, diatom species were counted using a light microscope (Olympus CX21 LED). Diatoms were identified to the species level using the diatom guide that was developed by Taylor et al. (2007). Where possible, 300 diatoms were counted on each slide and recorded. The recorded information was then uploaded into the Omnidia 6.1 software.

3.4.5. Data analysis

Omnidia 6.1 is a database that calculates several diatom indices. It was developed by Mathieu Lecointe (IRSTEA, Bordeaux, France). Diatom data obtained from this study was taken and imported into Omnidia 6.1 software in which 17 different index values were calculated. Of the calculated diatom indices only three, Specific pollution index, Generic diatom index and Trophic diatom index, were chosen and used. These indices were chosen since it included more than 50% of the taxa which generated index values at all sites. According to Matlala (2010), values obtained from SPI and GDI ranged between 0 to 20 as they indicate water quality ranges from polluted to clean waters.

In an environment that contains different species there are certain conditions that those species require in order to survive. Certain environmental parameters such as higher pH levels might affect some species in the environment. While it may lead to the death of certain species, others may thrive and become dominant in that same environment (Phungula, 2018). The same applies to diatoms and therefore it is important to use ordination techniques such as Redundancy analysis (RDA) to determine how different species react to their environment (Phungula, 2018). It is also important to use redundancy analysis to determine which environmental variable is suitable in explaining the difference of biological assemblages. A rise in the amount of diatom species present in a sample may cause changes in diatom index scores (Leps et al., 2003; Matlala 2010; Phungula, 2018).

CHAPTER FOUR: RESULTS AND DISCUSSIONS

4.1 Introduction

This chapter presents the results of historical physicochemical parameters and the investigations of diatoms as water quality indicators in the Kuils River. The results are presented in the form of graphs and tables. As previously mentioned, historical physicochemical data from 2019 to 2021 were obtained from the Department of Water and Sanitation. The diatom data was collected during both dry (low flow) and wet (high flow) season. This chapter explains the correlation between diatoms and physicochemical parameters, in order to determine water quality.

The findings are presented in two sections: the first part (section 4.3) presents historical water quality parameters data. The pH, chemical oxygen demand (COD) and phosphorus are the water quality parameters discusses in this section. Furthermore, this section also explains how each water quality parameter affects the composition and abundance of diatoms at a particular site. The second part (section 4.4) elaborates on the importance of diatoms at the four different sampling sites by looking at their abundance, their relationship with water quality parameters through the calculation of indices and answering the research questions concerned with the study.

Diatoms are great bioindicators based on their ability to provide information on water quality and pollution levels as well as the trophic status of aquatic habitats (Hicks et al., 2006). Furthermore, they indicate any change that takes place in aquatic environments because of their sensitivity to a variety of environmental factors that are required for optimal growth (Noga et al., 2013). The only diatom indices used in this study were GDI, SPI and TDI. Diatom indices are very useful and important as they were established to enable easier management of aquatic resources (Phungula, 2016).

4.2 Species composition

During this study, the identification of diatoms was made using a light microscope. Diatom index values are derived from the statistical mean of the water quality optima of the diatom taxa in a sample that is weighted by the abundance of each taxon (Kriel, 2008; Matlala, 2010). Therefore, it is of paramount importance that diatom species are accurately counted and identified. According to Musa (2015), the dominant species in each diatom sample play a huge role in influencing the ecological category where the samples will be placed. A total of 98 diatom species (Appendix C) were observed during this study. Of the 98 diatom species identified and counted, 38 were considered dominant (>5% of the diatom community) as shown in Table 4.1.

Table 4.1 Dominant diatoms observed and counted at each site with their relative abundance (%).

Code	Taxon	Distribution at sample site	Relative abundance (%)
NVIR	*Navicula viridula (Kützing) Ehrenberg var. viridula*	site 1 and 4	30.8
NFBU	*Nitzschia frustulum (Kützing) Grunow var. bulnheimiana (Rabenhorst) Grunow*	site 4	25.5
PTRO	*Planothidium rostratum (Østrup) Round et Bukhtiyarova*	All sites	18.5
NPRP	*Nitzschia perspicua Cholnoky*	site 4	19.4
PLEN	*Planothidium engelbrechtii (Cholnoky) Round et Bukhtiyarova*	All sites	15.3
NIFR	*Nitzschia frustulum (Kützing) Grunow var. frustulum*	All sites	24.5
GPSA	*Gomphonema pseudoaugur Lange-Bertalot*	All sites	9.9
NIAR	*Nitzschia archibaldii Lange-Bertalot*	All sites	19.9
CMEN	*Cyclotella meneghiniana Kützing*	site 2 and 3	18.5
PFQS	*Planothidium frequentissimum Round and Bukhtiyarova in Zidarova and al.*	All sites	20.4
GPAR	*Gomphonema parvulum var. parvulum f. saprophilum Lange-Bertalot et Reichardt*	site 2 and 3	12.5
AOBG	*Achnanthes oblongella Østrup*	All sites	7.5

CACD	*Craticula acidoclinata Lange-Bertalot and Metzeltin*	All sites	9.0
ADEG	*Achnanthidium exiguum (Grunow) Czarnecki var. exiguum*	All sites	20.5
GDEC	*Geissleria decussis (Østrup) Lange-Bertalot et Metzeltin*	All sites	5.4
CPLA	*Cocconeis placentula Ehrenberg*	All sites	8.0
ERHO	*Eunotia rhomboidea Hustedt*	Al sites	6.8
EFOL	*Eunotia formica Ehrenberg sensu lato*	All sites	6.6
NCOM	*Nitzschia communis Rabenhorst*	All sites	8.0
NPAL	*Nitzschia palea (Kützing) W.Smith var. palea*	site 4	35.6
GYAC	*Gyrosigma acuminatum (Kützing) Rabenhorst*	site 4	5.5
CENG	*Cocconeis engelbrechtii Cholnoky*	All sites	27.8
CAMB	*Craticula ambigua (Ehrenberg) Mann*	All sites	10.5
NFIL	*Nitzschia filiformis (W.M. Smith) Van Heurck var. filiformis*	All sites	19.4
CRCU	*Craticula cuspidata (Kützing) Mann var. cuspidate*	All sites	20.4
NHEU	*Nitzschia heufleriana Grunow var. heufleriana*	All sites	22.4
CBAM	*Cymbopleura amphicephala Krammer*	All sites	10.5
EBIL	*Eunotia bilunaris (Ehrenberg) Mills var. bilunaris*	All sites	20.6
EFLE	*Eunotia flexuosa (Brébisson) Kützing var. flexuosa*	All sites	9.9
FBCP	*Fragilaria biceps (Kützing) Lange-Bertalot*	All sites	8.5
FTEN	*Fragilaria tenera (W. Smith) Lange-Bertalot var. tenera*	All sites	8.0

FULN	*Fragilaria ulna (Nitzsch.) Lange-Bertalot var. ulna*	All sites	24.5
FUAC	*Fragilaria ulna var. acus (Kützing) Lange-Bertalot*	All sites	7.2
NUMB	*Nitzschia umbonate (Ehrenberg) Lange-Bertalot*	All sites	6.3
PLFR	*Planothidium Frequentissimum (Lange-Bertalot) Lange-Bertalot var. frequentissimum*	All sites	23.4
TFAS	*Tabularia fasciculata (Agardh) Williams et Round*	All sites	12.5
TCOA	*Tryblionella coarctata (Grunow in Cl. and Grun.) D.G. Mann*	All sites	5.4
TLIT	*Tryblionella littoralis (Grunow in Cl. et Grun.) D.G. Mann var. littoralis*	All sites	6.4

4.3. Part 1: Physicochemical parameters as indicators of water quality

4.3.1. pH

The potential of hydrogen (pH) is a measure of hydrogen ion concentration in the water (Phungula, 2018). According to Davies and Day (1998), the pH of water controls the amount of chemical constituents that can be dissolved in water as well as the biological availability (amount that can be used by aquatic life) of chemical constituents such as heavy metals (lead, copper and cadmium) and nutrients like (nitrogen, phosphorus and carbon). The pH values range between zero to 14 with those values below seven representing an acidic medium and those above seven representing a basic solution, while seven is regarded as a neutral solution.Factors like geology, biotic activities, vegetation type, temperature, atmospheric influences and total dissolved salts can influence pH levels (Mwangi, 2014).

Furthermore, changes in pH results in biological assemblage changes which might end up killing sensitive taxa. Bere and Tundisi (2011), stated that the affiliation between diatoms and pH is quite strong because of a direct physiological stress that is applied by pH levels on diatoms. In addition, this relationship influences the distribution of diatoms (Matlala et al., 2010). The sensitivity of diatoms to pH makes them good water quality indicators. According to Taylor (2004), pH levels that are extreme can cause toxic results on biota in the river like diatoms especially if the diatom species have narrow pH tolerance limits.

Figures 4.1 and Figure 4.2 show the historical average pH of water along sampled points during high flows and low flows on the Kuils River from 2019 to 2021. The graph illustrates the pH levels ranging between 6 to 8, which, according to Phungula (2018), corresponds to the pH conditions in a natural water source. The graph also presents how the year 2020 recorded the highest levels of pH at site 1 which had a pH of 8.3 (during high flow) and site 4 which had a pH of 8.5 (during low flow).

The pH value during this year (2020) was above 8, which indicates that an alkaline water system is likely to develop if it is heated (Thalman and Bedessem, 2006). Furthermore, the pH level remained constant over the three-year period with 2020 having the highest recorded pH. The lowest pH was 7.5 (site 1) during high flow and 7.4 (site 1) during low flow in 2019. This low pH falls within the range of natural conditions thus indicating that the pH level across the Kuils River at all the four sites had remained constant as it ranged between 6 to 8.

Figures 4.1 and 4.2 also give an indication of how the Kuils River maintained an alkaline water column system over three years. The observed level of pH in the water is attributed to the Malmesbury group of rocks that make up this river. These rocks are carbonate-rich and are composed of calcium and magnesium carbonates that are naturally alkaline. Therefore, carbonates from these rocks are dissolved when water comes into contact with these carbonate rocks. This results in increased alkalinity in the Kuils River. Another factor contributing to alkaline water in the Kuils River is groundwater and surface water interactions. During both high flow and low flow, areas along the Kuils River whereby groundwater and surface water are interconnected, causes alkalinity to be transferred from the Malmesbury group of rocks to be transferred to surface water through seepage.

It is therefore noted that the pH levels in this study results in the dominance of diatom species that favour alkaline conditions across sample site 1 to 4. Since all four sites had alkaline waters, diatom species such as *Fragilaria ulna var. acus* were present and dominant at all the sites. This type of diatom is found all over the world and favours alkaline environments. *Planothidium frequentissimum* and *Cocconeis engelbrechtii* are examples of some of the diatom species that were in abundance at all four sites. The presence of these diatom species indicates that water conditions in the Kuils River are alkaline as these species favour such conditions.

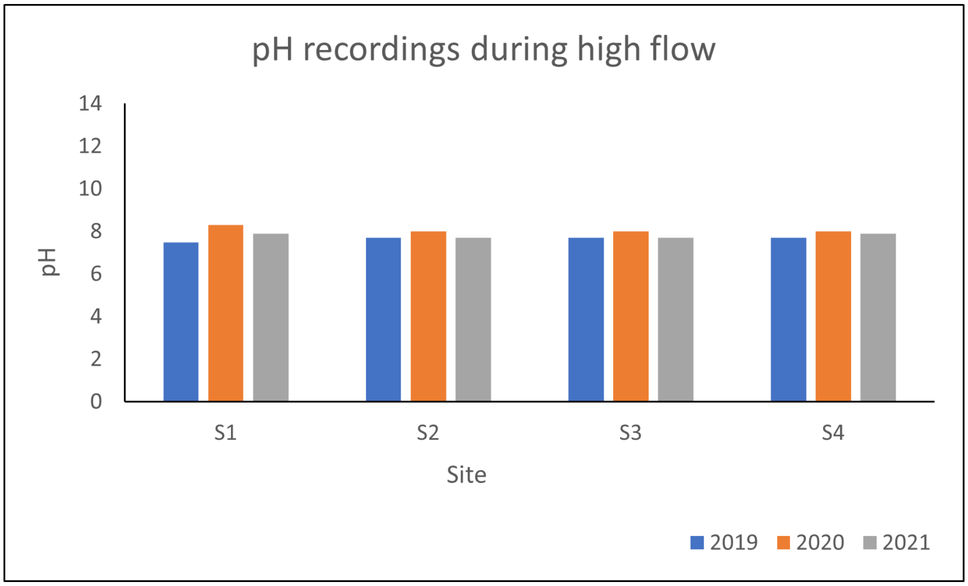

Figure 4.1 Historical pH values during high flow over a period of three years along the Kuils River.

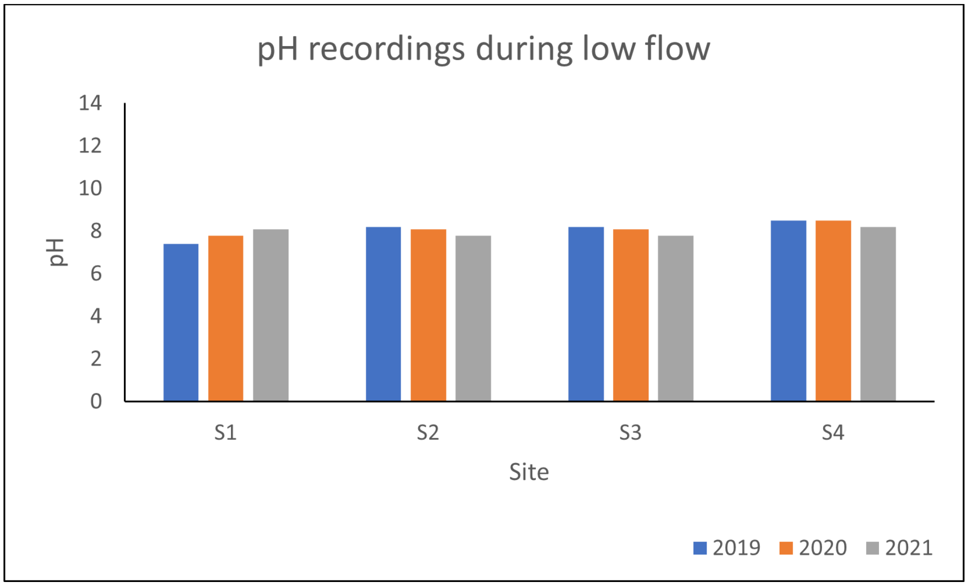

Figure 4.2 Historical pH values during low flow over a period of three years along the Kuils River.

4.3.2 Chemical oxygen demand (COD)

Chemical oxygen demand (COD) is responsible for measuring the total amount of oxygen that is needed to oxidise all organic material into carbon dioxide and water (Phungula, 2018). Furthermore, COD is a perfect tool to use in determining wastewater quality requirements that are discharged in receiving waters so as to reduce their impacts (Nkambule, 2016). Researchers have related the rise and fall in COD with inputs of organic matter and nutrients in a waterbody (Apsite and Klavins,1998). The COD values differ in waterbodies that are contaminated, uncontaminated and water that contains industrial waste. According to Chapman and Mismatch (2021), the COD in uncontaminated waters ranges from 20 mg/L or less, while water that receives effluents have a concentration of more than 200 mg/L. However, in industrial wastewaters, the values could be as high as 60 000 mg/L.

Figures 4.3 and 4.4 show the highest COD concentration of 84 mg/L recorded at site 1 in 2021. This high COD value was recorded during the low flow season. This suggests that there is a link between COD and the input of organic matter and nutrients. Phungula (2018), stated that the relationship between water flow fluctuations and nutrients proves that it is possible to use both these variables in determining the relationship with COD. Therefore, an increase in nutrients such as nitrates and phosphate are related to an increase in COD concentration (Phungula, 2018). Furthermore, a decrease in water flow can be related to higher nutrient concentrations as well as high COD concentrations (Phungula, 2018).

As previously stated, the COD of uncontaminated waters is less than 20mg/L. However, in this study, the COD levels during high flow and low flow across all the sites over the three-year period were all above 20 mg/L. This means that all the sampled sites were experiencing a moderate increase in COD concentrations during high flow at site 1 from 2019 to 2021, whereas sample site 2 and 3 remained constant. The moderate increase in COD levels in the Kuils River are as a result of the location of the river. This river is surrounded by vineyards and farms that grow fruits. As a result of these surroundings, agricultural runoff causes an increase in COD levels present in the river. The chemicals used during agricultural activities to grow the fruits (fertilizers), herbicides and pesticides contain organic compounds that are transferred into the Kuils River through runoff when precipitation occurs. Thus, the surrounding farms are contributing to the increased levels of COD in the Kuils River.

Effluent discharge from Scottsdene, Bellville and Zandvliet Wastewater Treatment Works also contribute to increased levels of COD in the Kuils River. According to Hagen (2022) these wastewater treatment plants discharge treated and untreated wastewater into the Kuils River. Untreated wastewaters contain organic compounds and once discharged into the Kuils River, it causes COD levels to rise. Furthermore, the Kuils River is surrounded by urban developments and several informal settlements along the river. The development of these settlements also contribute to increased COD levels in the Kuils River as soil erosion will occur during the construction process and cause sediment containing organic matter to enter the system.

At sampling site 2 and 3, diatom abundance was low compared to the other sites which could be ascribed to the fact that these sites were located under a bridge. The bridge thereby prevents light, required for diatoms to photosynthesise and produce energy, to reach these sites. This reduction in light at sites 2 and 3 make it difficult for diatoms to grow and produce which affects their abundance at these sites. Sites 1 and 4 had high abundance of diatoms because these sites receive adequate light.

Most diatoms are known to be sensitive to high levels of COD and organic pollution. In this study, the presence of dominant diatom species such as *Nitzchia palea* and *Fragilaria ulna* across all four sites indicate waters with high COD levels. *Nitzchia palea* and *Fragilaria ulna* are known as pollution tolerant species and commonly dominate rivers with moderate to high COD levels. Therefore, the presence of these species in the Kuils River across all the four sites indicate that water within the Kuils River contains increased levels of COD. These species also indicate organic pollution in the Kuils River as they reproduce and increase in abundance under moderate to high COD conditions.

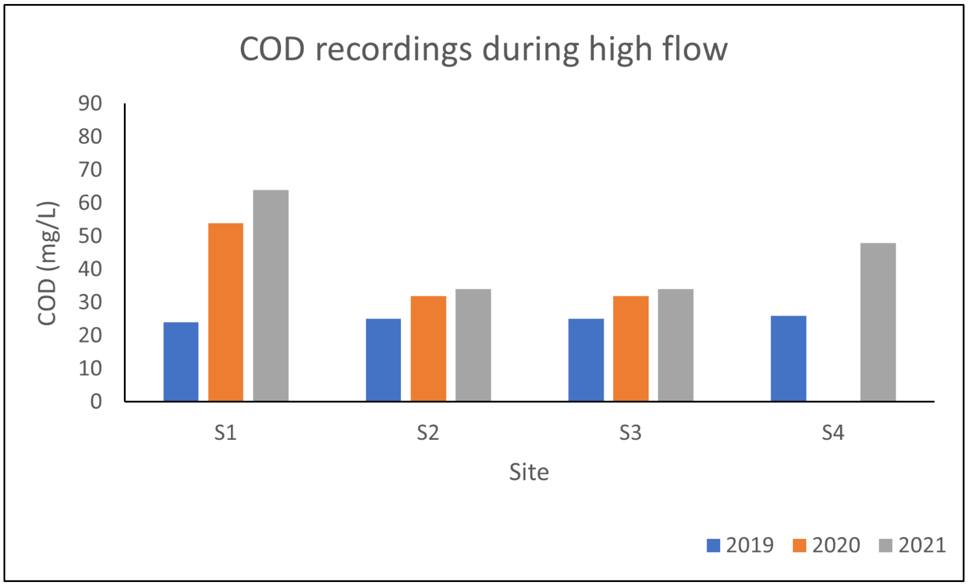

Figure 4.3 Historical COD concentrations during high flow over a period of three years along the Kuils River. (The COD value for site 4 in 2020 was not recorded because of high water levels, making the site inaccessible during time of sampling).

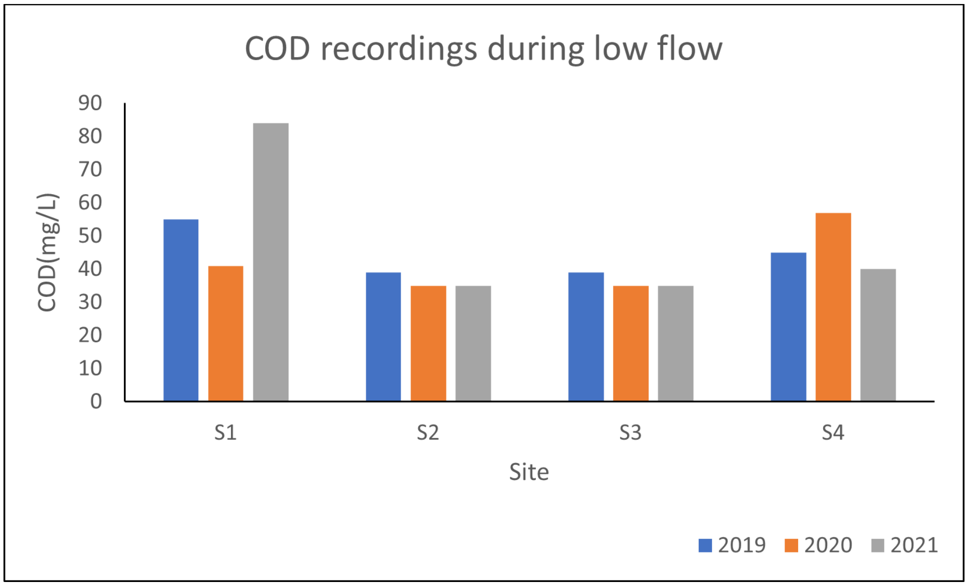

Figure 4.4 Historical COD concentrations during low flow over a period of three years along the Kuils River.

4.3.3 Phosphorus

Phosphorus plays a significant role in stimulating plant growth and animals (Davies and Day, 1998). It occurs in nature in three forms such as orthophosphates, metaphosphates and phosphate. In aquatic environments orthophosphates is the most abundant dissolved inorganic type of phosphate that is formed from the oxidation of phosphorus (DWAF, 1996). It enters the environment through natural weathering of minerals, biological decomposition and runoff from human activities (Phungula, 2018).

There is a possibility of adsorbed phosphorus being released during high flow from sediments which may result in the phosphorus entering the watercourse due to erosion of the surrounding catchment (Malan and Day, 2003, 2004; Phungula, 2018). Anthropogenic activities also play a key role in the high number of phosphates that are found in rivers. These activities may lead to and include point sources like industrial effluents and non-point sources like fertilizers (Mwangi, 2014). Increased levels of phosphorus can be detrimental to the aquatic environment because it is the principal nutrient that controls the degree of eutrophication (Phungula, 2018).

The presence of phosphorus in the Kuils River is as a result of natural processes such as the Malmesbury group of rocks that make up this catchment. Rocks like shale from the Malmesbury group contain phosphate minerals and organic matter that enter the Kuils River through weathering. Figures 4.5 and 4.6 indicate phosphate concentrations across the different sampling points over the three-year period. The phosphate concentrations were slightly consistent and lower upstream during both high flow and low flow, and slightly increased downstream. The lower phosphate concentration upstream is as a result of WWTW discharging effluent into the Kuils River being located further downstream. This means that effluent which is discharged into the river contains pollutants that will cause a rise in phosphate levels downstream (site 4).

Lower phosphate concentrations have been observed at sampling points 2 and 3. The types of bedrock that are found at the two sites might be poor in phosphorus levels which causes low phosphorus concentration at these sites. These two sites are closer to each other and experience similar conditions. Based on these results, it was observed that the location of the site plays a significant role in the number of pollutants found in the river and their source.

Cyclotella meneghiniana is a dominant diatom species that was found at sites 2 and 3. The presence of this diatom gives an indication of low phosphorus concentrations at these sites as this species favour such conditions, whereas diatoms of the genus *Nitzschia* and *Navicula* were dominant at site 4. These species flourish under high phosphorus concentrations as they prefer nutrient-rich environments. Hence their presence in abundance at site 4 gives an indication of high phosphorus concentrations.

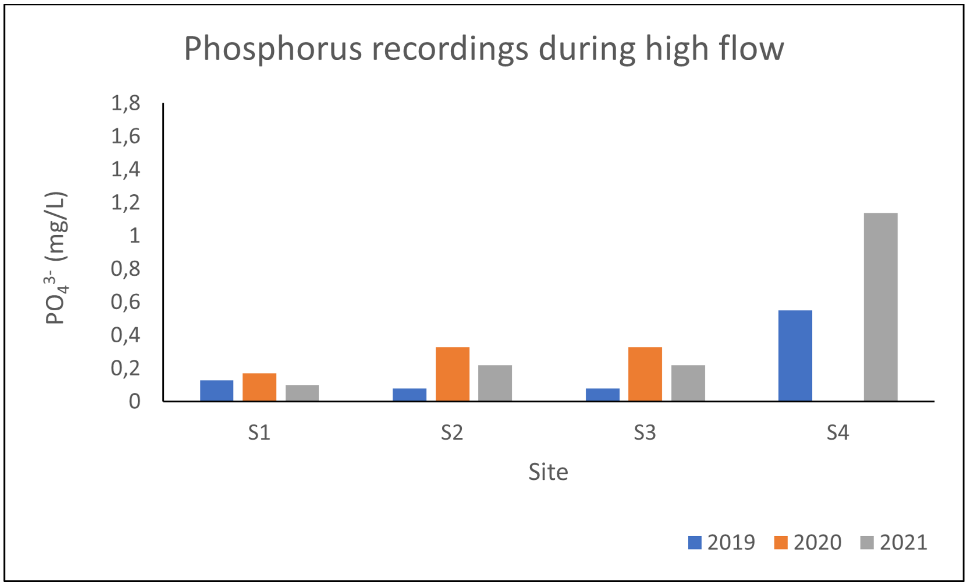

Figure 4.5 Historical PO_4^{3-} concentrations during high flow over a three-year period along the Kuils River.

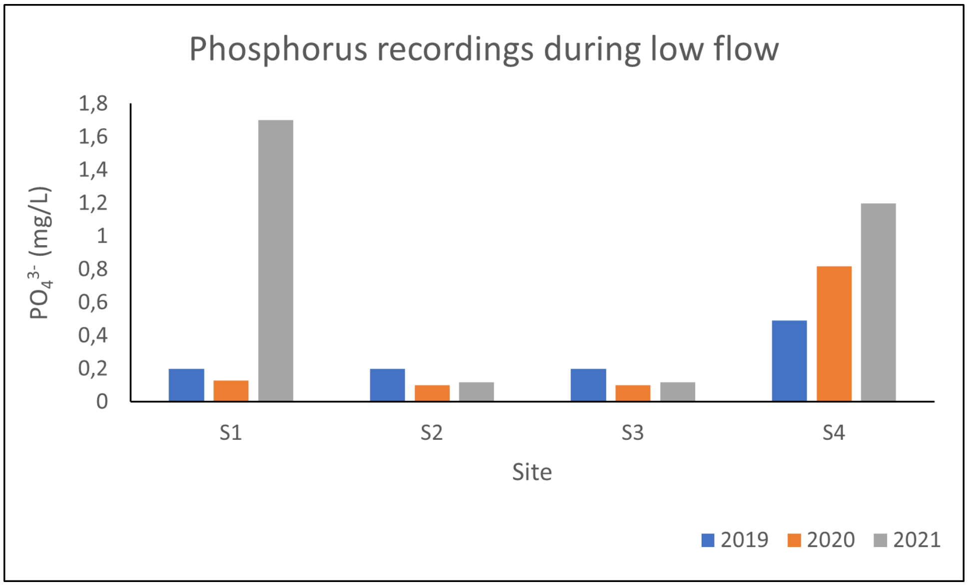

Figure 4.6 Historical PO_4^{3-} concentrations during low flow over a three-year period along the Kuils River.

4.3.4 Discussion

The results obtained indicated pH values which fell within the natural allowed freshwater system range (Figures 4.1 and 4.2) across all the sites. The carbonate-bicarbonate system is the most important buffering system in fresh waters (DWAF, 1996). South

Africa’s freshwater systems are well-buffered as the pH level ranges between 6 and 8 even though the natural range is between 4 and 11 (DWAF, 1996). In addition, even though most of South Africa's waters are well buffered, some water systems indicate pH levels below 4, making these systems acidic. A low pH affects a diatom community by altering the chemical balance of water. This makes it difficult for diatoms to absorb nutrients that are important for growth and reproduction which leads to the reduction in diatom diversity and abundance (Li et al., 2017).

The findings from this study indicated that due to the normal pH levels of natural waters that are indicative of the alkalinity of the water, diatoms favouring such waters would be found in abundance at each sampling site. This gives an indication that diatoms are able to give an indication of the condition that is present in a water body based on the type of diatom that are dominant at that particular site. Knowing the type of environment that each diatom favours allows for researchers to be able to understand the type of conditions in a water body.

This study indicates how the presence of specific types of diatoms are able to indicate acidic or alkaline conditions in a river. The only limitation with using diatoms to determine pH is that it only indicates either acidic or alkaline conditions. It does not give an exact number of the pH level present in the water body. In terms of pH, the change in season (high flow or low flow) did not have an effect on the pH levels of water as it remained alkaline which also caused the dominance of diatoms that favour such conditions. The structures of diatoms that were present and dominant under alkaline conditions were intact. This is because the diatoms present in alkaline conditions thrive and favour this environment thus, their structure will not be damaged as they are able to survive in these conditions.

The COD recorded during this study was high at site 1 and site 4 (during 2020 and 2021), which ranged between approximately 41 mg/L and 85 mg/L respectively, with 85 mg/L being the highest recorded concentration. According to Chapman et al. (1996), the COD of uncontaminated waters is less than 20 mg/L , while those contaminated with effluents have a concentration of more than 200 mg/L. All concentrations recorded during this study reflected above 20 mg/L indicating that the water is moderately contaminated at all four sites.

Regardless of phosphorus playing a significant role in stimulating plant growth and plankton (food source for aquatic organisms like fish etc.), high phosphorus concentrations or levels may lead to eutrophication (Phungula, 2018). The findings in this study indicate that organic pollutants from anthropogenic activities are entering the Kuils River system. These pollutants are causing increased levels of COD to be present in the river. Diatoms sensitivity to moderate and high COD concentration causes diatoms that are pollution tolerant (*Nitzschia palea*) to be dominant in the Kuils River.

Those diatoms that prefer low COD levels (*Gomphonema parvulum*) were present in insignificant amounts at sites 2 and 3. This suggests that the sample sites 2 and 3 had lower COD concentrations before the introduction of organic pollutants from agricultural runoff and wastewater treatment works. This introduction of organic pollutants caused COD levels to rise and affected the *Gomphonema parvulum* diatom community as they were surpassed by the diatom species, *Nitzschia palea*, that favour moderate to high COD concentrations.

The type of diatom dominant at a site in the river is able to determine which environmental conditions are present, based on the requirements for survival under high COD conditions. It is important to note that the diatom species does not indicate specific COD levels. Therefore, to accurately assess water quality in a river and the levels of organic pollution that are present, the use of methods that accommodate multiple parameters are suggested. This is because for a diatom community to be abundant, factors such as light, temperature, available nutrients, chemical and physical parameters could be considered as it might influence species abundance. Hence, using only its abundance to determine water quality will not properly give an indication of the specific levels present.

Factors such as natural processes and anthropogenic activities from surrounding areas are causing an increase in phosphate levels in the Kuils River. Sites 1, 2 and 3 have phosphorus concentration levels that are lower than 0.52 mg/L except for site 1 (2021), whereas site 4 has phosphorus concentrations that are above 0.52 mg/L. Phosphorus that is inorganic and above 0.52 mg/L might cause hypertrophic conditions that are bad for water quality but good for algal growth (DWAF, 1996). This led to site 4, in this study, having dominant diatoms in abundance that favour high phosphorus conditions. Phosphorus levels are lower upstream compared to downstream. This is due to the pollutants discharged into the river further downstream. The presence of diatoms such as *Cyclotella meneghiniana*, which favour a low phosphorus concertation, gives an indication of the ability of diatoms to determine water quality in the Kuils River at different sites. At site 4, diatoms that favour high phosphorus concentrations, like *Nitzschia*, were dominant.

These findings reveal that diatoms are able to determine water quality in the Kuils River even though, they do not give specific conditions, in this case phosphorus levels, occurring in the river. It only indicates whether phosphorus concentrations are high or low based on the dominant diatoms encountered at a site. It is important to use water quality measuring methods that give exact numbers of the condition occurring in the river.

4.4 Part 2: Diatoms as an indicator of water quality

4.4.1. Examples of Diatoms encountered during the study

Photomicrographs of different types of diatoms observed in the samples collected can be seen in Figures 4.7 to 4.10. These photomicrographs show the diatom silica structure (cell wall or frustule). Centric and pennate diatoms are two groups of diatoms commonly found in freshwater system (Taylor et al., 2007). Centric diatoms are adapted to live in the water as part of phytoplankton, while pennate diatoms live in benthic habitats (on occasion temporarily re-suspended in the water column) (Taylor et al., 2007). Figures 4.7 to 4.10 indicate diatoms belonging to the pennate group.

Cocconeis placentula var. lineata (Figure 4.7(a)) has a curved or flexed valve face. *Craticula cuspidata* (Figure 4.7(b)), which commonly occurs in eutrophic and brackish waters, is distinguished by parallel transverse striation. Additionally, *C. cuspidata* tolerates critical to heavy pollution levels (Taylor et al., 2007). *Nitzchia perspicua* (Figure 4.7(c)) contains weakly silicified frustule (cell wall), while its striae are not easily observable in light microscopy. *Navicula viridula* (Figure 4.7(d)) favours eutrophic waters and tolerates critical levels of pollution.

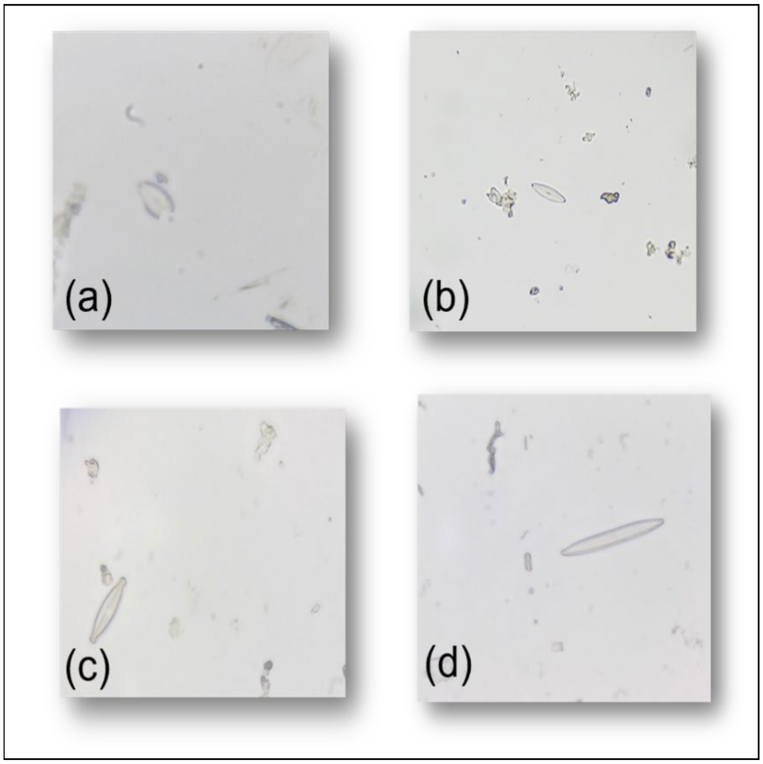

Figure 4.7: Diatom species (a) *Cocconeis placentula var. lineata*, (b) *Craticula cuspidata* (c) *Nitzchia perspicua*, (d) *Navicula viridula*

Figure 4.8(a) illustrates *Nitzschia palea,* which is a type of diatom that is found all over the world. It thrives in eutrophic, heavily polluted to extremely polluted environments (Taylor et al., 2007). *Nitzschia communis* Rabenhorst (Figure 4.8(b)) contains striae that is observable under light microscopy. It occurs in electrolyte rich as well as brackish waters and is tolerant of extremely polluted conditions (Taylor et al., 2007). *Eunotia rhomboidea* (Figure 4.8(c)), on the other hand, contains small valves and the frustule is visible in light microscopy. Figure 4.8(d) represents *Planothidium engelbrechtii* which has the ability to flourish in critical to heavily organic polluted environments.It is a cosmopolitan diatom that is found in the benthos of rivers or attached to substrates (Taylor et al., 2007).

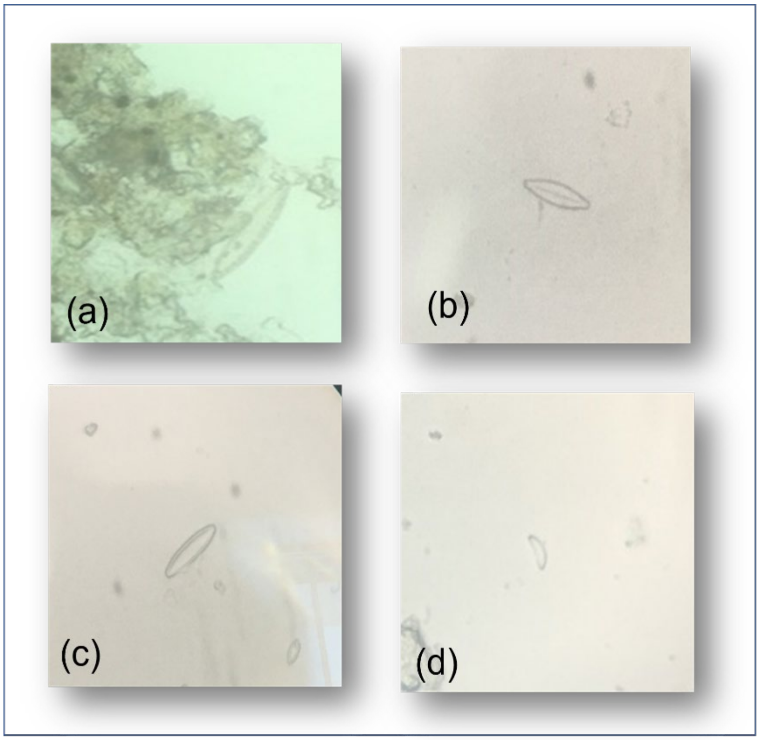

***Figure 4.8**:* Diatom species *(a) Nitzschia palea, (b) Nitzschia communis Rabenhorst, (c) Eunotia rhomboidea Hustedt, (d) Planothidium engelbrechtii*

Cocconeis engelbrechtii Cholnoky (Figure 4.9(a)) is a species that is endemic to South Africa and it occurs in alkaline inland waters (Taylor et al, 2007). *Fragilaria ulna var. acus (Kützing) Lange-Bertalot* (Figure 4.9(b)), is a cosmopolitan species that is found in the benthos of rivers and exists in alkaline freshwaters (Taylor et al, 2007). *Gomphonema pseudoaugur* (Figure 4.9(c)) has an oval shape and occurs in eutrophic waters. This diatom type is sensitive to more critical levels of pollution (Taylor et al, 2007). *Rhopalodia gibba* (Figure 4.9(d)) is found in slow flowing waters (Taylor et al, 2007).

Figure 4.10(a), *Rhoicosphenia,* contains cells that are attached to a substratum by mucilage stalks. This species flourishes in critical levels of pollution and occurs in brackish waters (Taylor et al., 2007). *Gyrosigma acuminatum* (Figure 4.10(b)), is a cosmopolitan species found in brackish waters which is capable of tolerating critical levels of organic pollution (Taylor et al., 2007). The diatom species, *Eunotia formica* (Figure 4.10(c)), occurs in an environment with slow flowing waters. *Nitzschia etoshensis* (Figure 4.10(d)), is made up of a frustule or cell wall that is weakly silicified, and striae is not observable in light microscopy (Taylor et al, 2007).

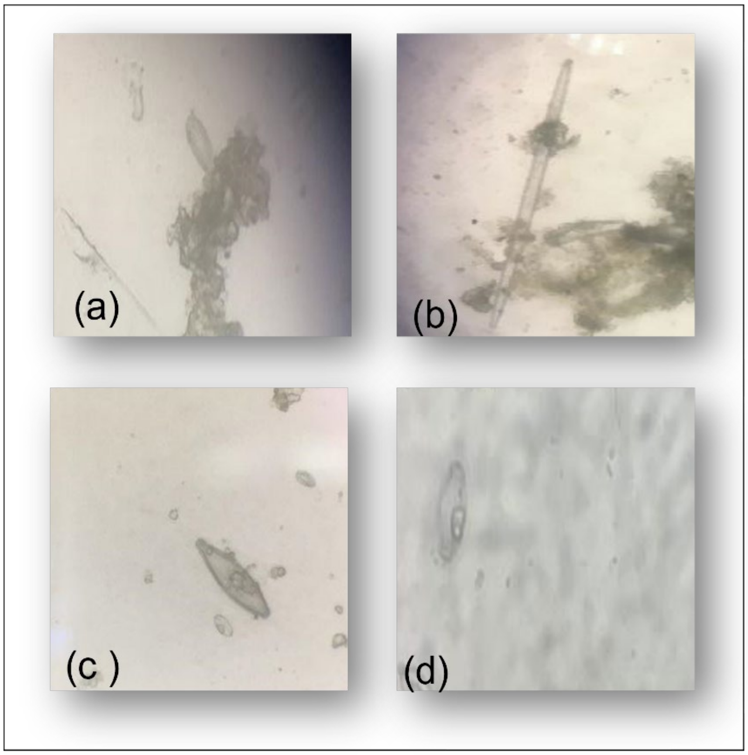

Figure 4.9: Diatom Species (a) *Cocconeis engelbrechtii Cholnoky*, (b) *Fragilaria ulna var. acus (Kützing) Lange-Bertalot,* (c) *Gomphonema pseudoaugur Lange-Bertalot*, (d) *Rhopalodia gibba*

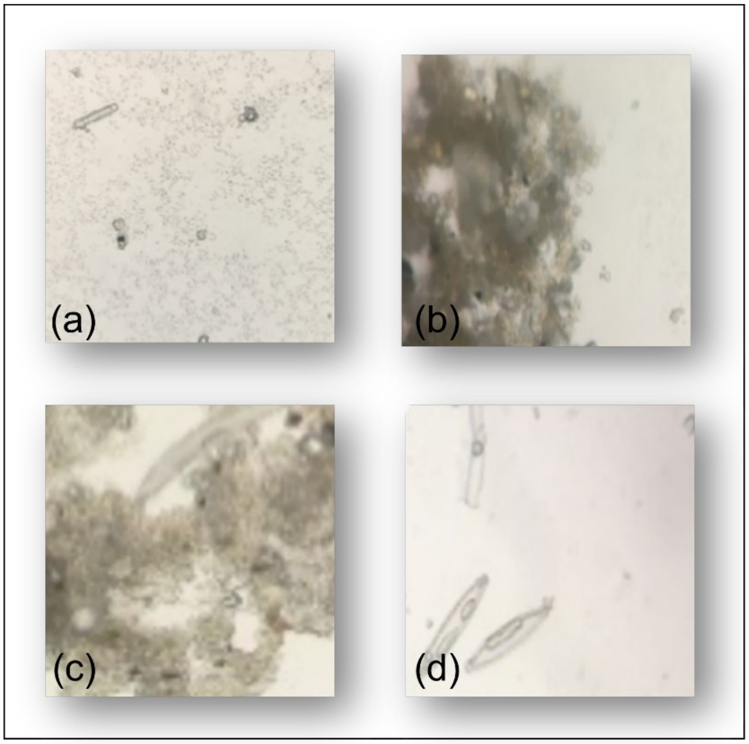

Figure 4.10: Diatom Species (a) *Rhoicosphenia*, (b) *Gyrosigma acuminatum,* (c) *Eunotia formica Ehrenberg*, (d) *Nitzschia etoshensis Cholnoky*

4.4.2. Redundancy Analysis (RDA)

In an aquatic environment, varied species have different requirements or specific optimum conditions in which they can survive. Diatoms are no exception as they have certain conditions in which they survive in aquatic waters. According to Matlala (2010), dominant species can be taken to be representative of their environment. This part of the study looks at the relationship between diatoms and the water quality parameters in the previous section (4.3).

According to Phungula (2018), the redundancy analysis is a technique which is multivariate and used in determining the response of diatoms to environmental variables. In this study the redundancy technique was used to determine the response of diatom species to pH, Chemical oxygen demand and Phosphorus. Phungula (2018) stated that the redundancy analysis can detect patterns in community composition that can be elaborated best by the environmental variables. Figure 4.11 indicates the redundancy analysis graph. The graph or plot has a central point which represents the mean or average of the variable whereas the arrows indicate the direction of maximum change in the value linked to the variable (Matlala et al., 2010).

The RDA is represented by arrows where the length of the arrow is related to the highest level of variation (Figure 4.11) (Phungula, 2018). Furthermore, there is no change in the value of the variable in the perpendicular direction (Ter Braak and Verdonschot, 1995). According to Lepš and Šmilauer (2003), a positive correlation is observed when the response variable is in the same quadrant as the water quality parameter, whereas a negative correlation can be seen when the response variable is in the opposite direction as the water quality parameter.

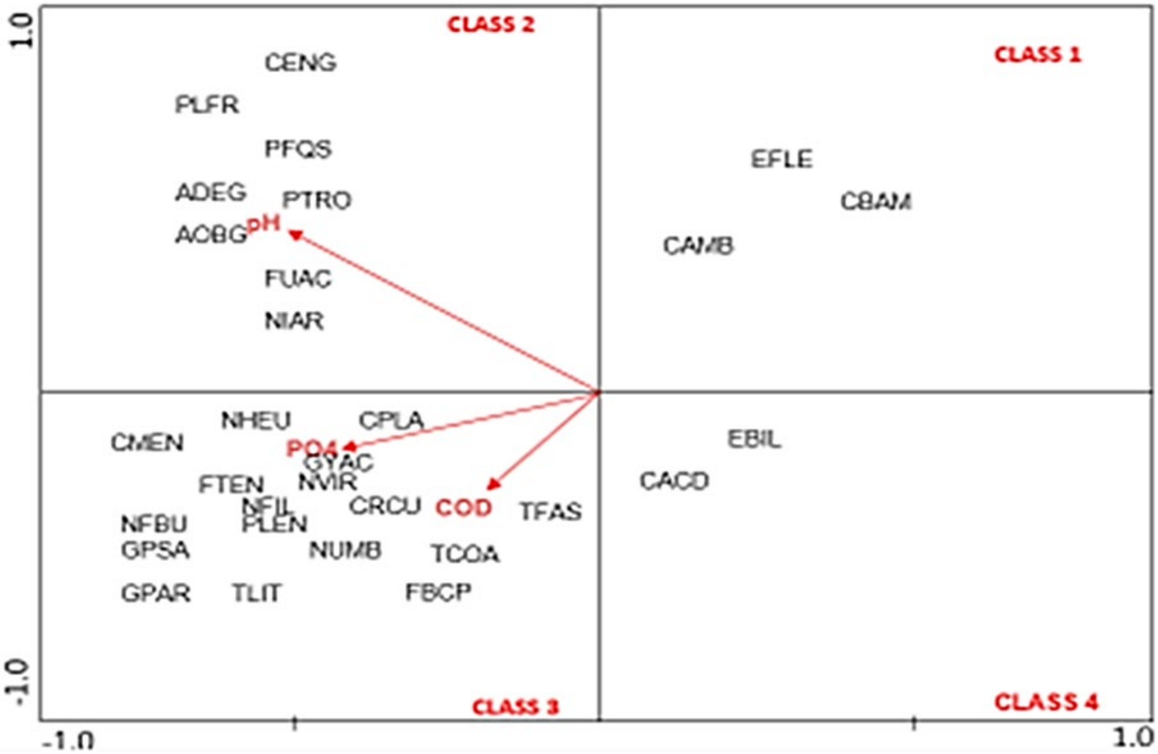

Figure 4.11: Redundancy analysis plot showing a relationship between dominant diatom taxa and environmental variables along the Kuils River.

In Figure 4.11, the red arrows indicate the environmental variables, while the classes show how dominant diatom species have been grouped into four classes/groups in each quadrant. **Class 1** (top right quadrant) shows diatoms that prefer low nutrient and COD levels, while **Class 2** (top left quadrant) represents diatoms that are related to high pH levels. **Class 3** (bottom left quadrant) displays diatom species that are linked to increased levels of nutrient concentration and COD, with **Class 4** (bottom right quadrant) indicating diatoms that have low tolerance to pH variation.

Different species have different preferences when it comes to conditions in which they survive, which is also evident in Figure 4.11. Diatoms such as *Planothidium rostratum, Achnanthes oblongella and Cocconeis engelbrechtii* found in Class 2 are associated with alkaline waters, i.e., waters with high pH levels.

Class 3 contains diatom species such *as Navicula viridula, Geissleria decussis, Fragilaria tenera* and *Cyclotella meneghiniana* which prefer increased nutrient concentrations and COD. Furthermore, these species tolerate pollution and thus makes them good indicators of pollution in water sources. *Tabularia fasciculata* is known to favour wastewater containing pollutants. This species forms part of Class 3 and is also a good indicator of pollution*. Craticula acidoclinata*, which forms part of the Class 4 diatom group, commonly appears in negative correlation to increased levels of pH. This means that *Craticula acidoclinata* prefers waters that are acidic. Therefore, on the RDA plot, diatom species are grouped according to environmental variables that they favour (Phungula, 2018).

4.4.3. Diatom indices

Seventeen different indices were generated by the Omnidia 6.1 software after counted taxa was added into the software. Phungula (2018) stated that the amount of diatom taxa that is used in the calculation of index values from the indices developed, varies. Furthermore, index values generated by the software are influenced by an amount of a particular diatom species that is present in the sample (Musa, 2015). According to Phungula (2018), the confidence level (i.e., reliability) of the changing indices is based on a section of the diatom taxa that is used when a category value is developed.

This study used three out of the seventeen indices calculated. This is as a result of the fact that the Generic Diatom Index (GDI), Specific Pollution Index (SPI) and Trophic Diatom Index (TDI) proved to be dependable as their percentage composition across all sites is greater than 50%. It is also notable in Table 4.2 that the Biological Diatom Index (BDI) mostly had values greater than 50%.

4.5. Index scores

Generic Diatom Index, SPI and TDI had the highest percentage composition of diatom species. This led to these indices being used in the study because of their consistency. The results obtained from these indices are shown in Figures 4.12 to 4.17. Tables 4.3 and 4.4 indicate categories and ranges used for SPI, GDI and TDI to determine water quality.

Table 4.2 Calculated high flow and low flow average diatom species percentage composition. The values in bold indicate more than 50% of taxa (population) used 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**
S1 LF	16,2	**56,8**	**75,7**	**59,5**	**56,8**	**59,5**	18,9	**89,2**	40,5	27	45,9	**100**	**51,4**	21,6	32,4	45,9	**78,4**
S2 LF	34,8	**56,5**	**73,9**	34,8	**56,5**	**65,2**	26,1	**100**	43,5	34,8	**56,5**	**99,6**	**52,2**	39,1	17,4	30,4	**95,7**
S3 LF	36,8	**84,2**	**89,5**	**68,4**	**73,7**	**68,4**	47,4	**100**	**78,9**	**63,2**	**68,4**	**100**	**89,5**	36,8	42,1	**63,2**	**89,5**
S4 LF	**50**	**64,3**	**92,9**	**78,6**	**71,4**	**71,4**	42,9	**100**	42,9	**57,1**	**71,4**	**100**	**78,6**	**50**	35,7	**57,1**	**85,7**
S1 HF	33,9	**62,8**	37,9	46,9	**54,8**	**61,5**	22,6	**83,9**	**55,4**	48,5	**65,2**	**98,8**	**65,4**	28	32,4	45,9	**81,9**
S2 HF	34,6	**80,4**	**82,2**	**56,9**	41,3	**74,8**	45,8	**85,9**	**73,8**	31,7	**59,8**	**100**	**77,5**	25,1	42	46,8	**84,1**
S3 HF	32,4	43	**67,6**	**50,5**	**52,2**	49,3	26,1	**78,4**	**59,6**	47,7	41,5	**100**	46	41,5	36,9	41,5	**72,1**

Table 4.3 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 4.4 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

4.5.1. SPI-Specific Pollution Index

According to the SPI graphs (Figures 4.12 and 4.13), both high flow and low flow values remained constant as they ranged between 7 and 9 in this study. A diminished water quality was observed across all the sites during all the seasons as the values obtained were less than 9 as indicated in Table 4.3. The water quality shows an improvement during the low flow season at site 2 with a value of 11.7 even though the water quality remained poor at this site.

The highest value recorded at site 2 did not meet the required range of 15 to 17, which indicates good water quality. On the other hand, bad water quality in the high flow season could be associated with the heavy rains that might have caused an increase in suspended solids and sediment yields (Whitehead et al., 2009; Phungula, 2018). Furthermore, factors which could also have contributed to poor water quality included runoff from storm water drains, as well as sewage pipes discharging wastewater into rivers.

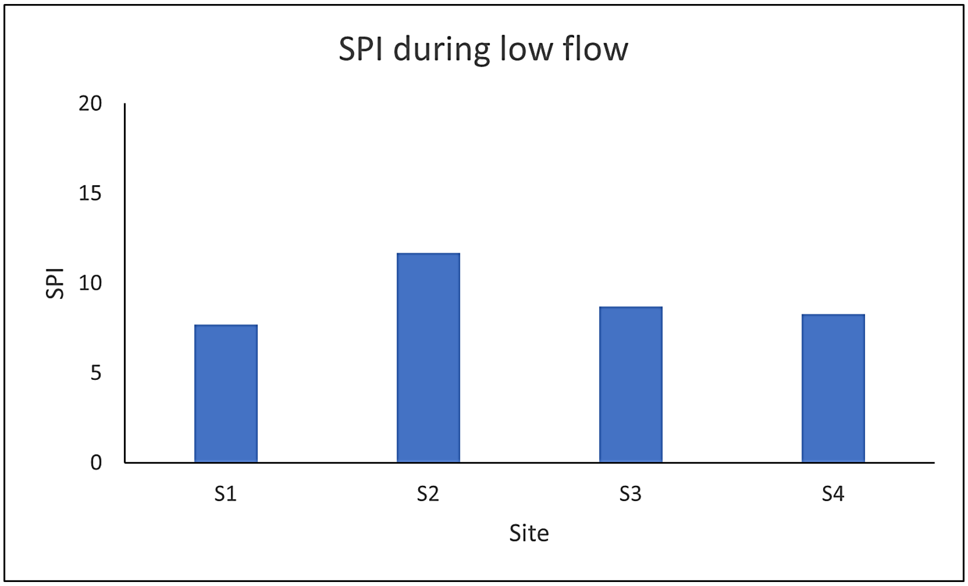

Figure 4.12 Specific pollution index (SPI) values during low flow along the sampled sites on the Kuils River.

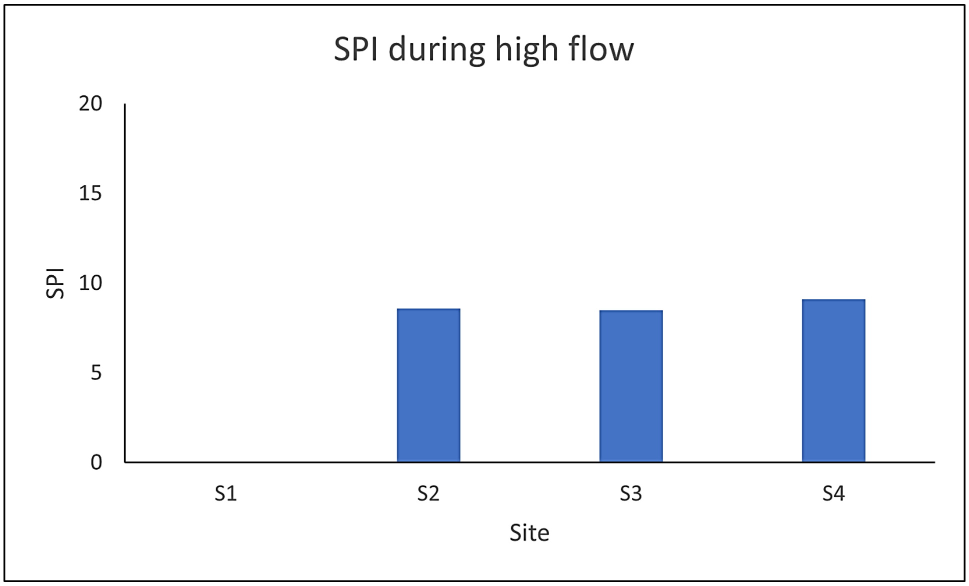

Figure 4.13 High flow Specific pollution index (SPI) values during high flow along the sampled sites on the Kuils River. (site 1 SPI value was not recorded because of high water levels at time of diatom sampling which made the site inaccessible).

4.5.2. GDI-Generic Diatom Index

The GDI values (Figures 4.14 and 4.15) indicated poor water quality at all sites across the river. The values are greater than 9, with the lowest value being 6.3 at site 1. However, there was a slight change during the low flow season in which water quality improved to moderate quality with values ranging between 12 to 15. This improvement occurred at site 2 and could have been influenced by the presence of different diatom species that were encountered during the study in the low flow season compared to those found in the high flow season. According to Phungula (2018), GDI accounts for the genera of taxa in which a genus may have different species with regards to their optimal conditions.

In other words, scores that are obtained from GDI may be due to the indication of water quality parameters shown by the dominant taxa (Phungula, 2018). The high flow season gives an indication of bad water quality in the river as the values in the graph (Figure 4.15) are not greater than 9. According to Szczepocka et al. (2009), GDI levels less than 9 are classified as bad water quality. The findings of GDI in this study corresponded to the findings from physicochemical parameters as both results are indicating the presence of pollutants in the river.

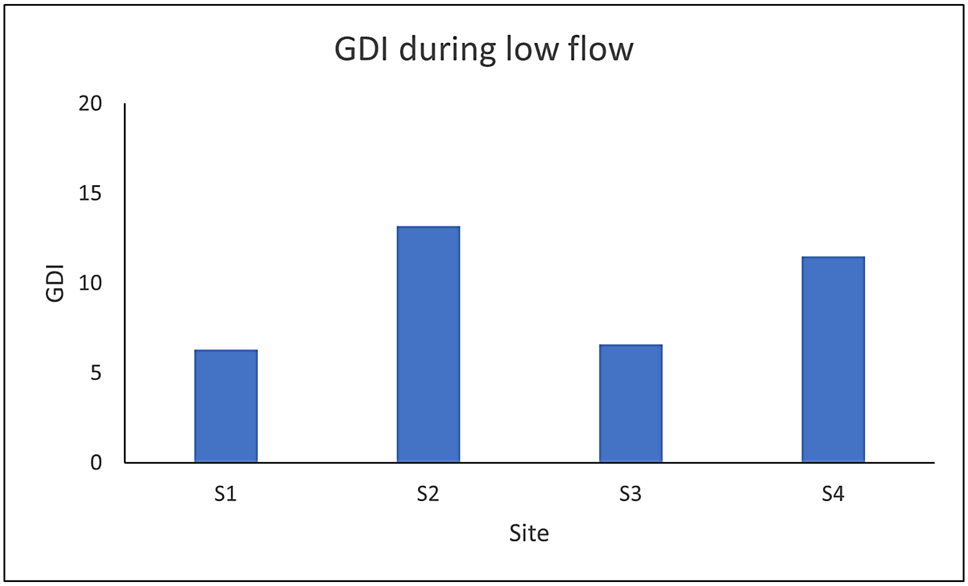

Figure 4.14. Low flow Generic diatom index (GDI) values during low flow along the sampled sites on the Kuils River.

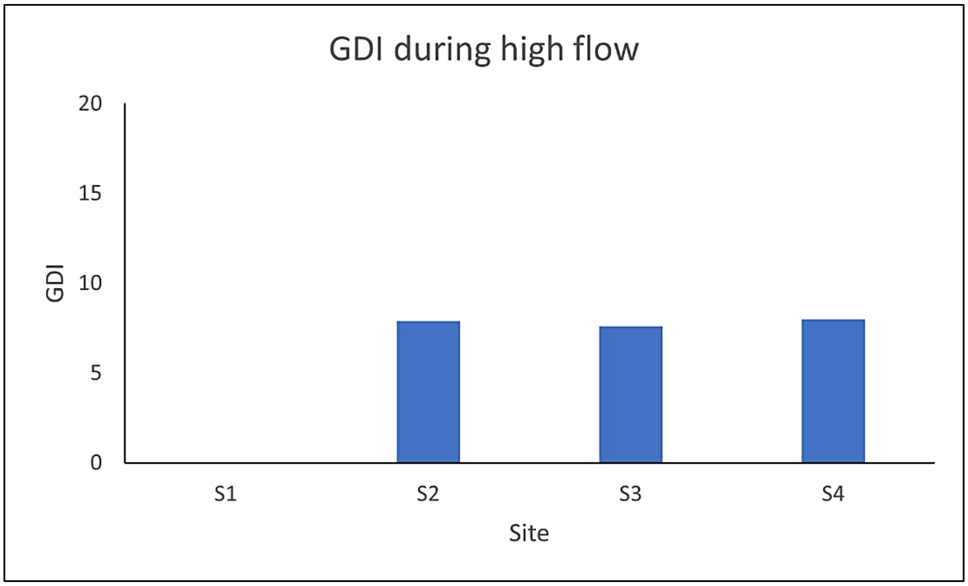

Figure 4.15 High flow Generic diatom index (GDI) values during high flow along the sampled sites on the Kuils River. (site 1 GDI value was not recorded because of high water levels at time of diatom sampling which made the site inaccessible).

4.5.3. TDI -Trophic Diatom Index

The TDI value was the lowest during low flow with a value of 55.2 at site 2. Some of the highest recorded TDI values were 71.9, 74.9, 79.6 and 89.4. All these values indicate that the water quality during both high flow and low flow ranged between mesotrophic to eutrophic conditions. A rise in the TDI concentration was also noticeable as one moved downstream from one site to the next (Figures 4.16 and 4.17).

The high concentration in TDI indicated increased pollution inputs along the river as one moved downstream. The findings from TDI when compared to those obtained from physicochemical parameters are the same. Both results, indicate polluted water quality along the Kuils River and increased pollutant concentrations downstream. Increased pollution inputs downstream can be attributed to runoff from residential areas or the dilution of diatoms in water taking place during high flow (Phungula, 2018).

Another reason for poor water quality could be nutrients entering the Kuils River through discharge from wastewater facilities, as well as farming activities. According to Matlala (2010), TDI concentrations ranged between 0 to 100, with a zero-value indicating oligotrophic conditions, while a score of 100 indicates eutrophication and poor water quality.

Therefore, the quality of water along the sampling sites chosen for this study, were regarded as poor. Matlala (2010) also stated that GDI and SPI results are given values that range from 1 to 20 with a score of zero indicating bad water quality, while a score of 20 indicates high water quality (Table 4.3). The Trophic Diatom Index (TDI) is different from SPI and GDI as its values range from 0 to 100. In TDI, the value of zero indicates low nutrient concentration, while 100 indicates high nutrient concentration and poor water quality. Table 4.4 indicates how the results obtained from trophic diatom indices are further divided into categories that indicate different classes of water quality.

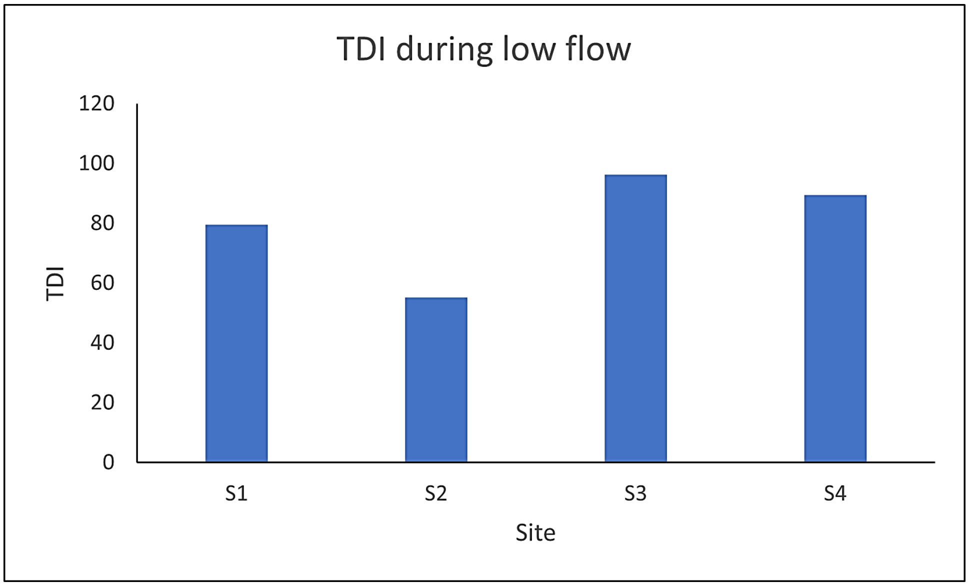

Figure 4.16 Low flow trophic diatom index (TDI) values during low flow along the sampled sites on the Kuils River.

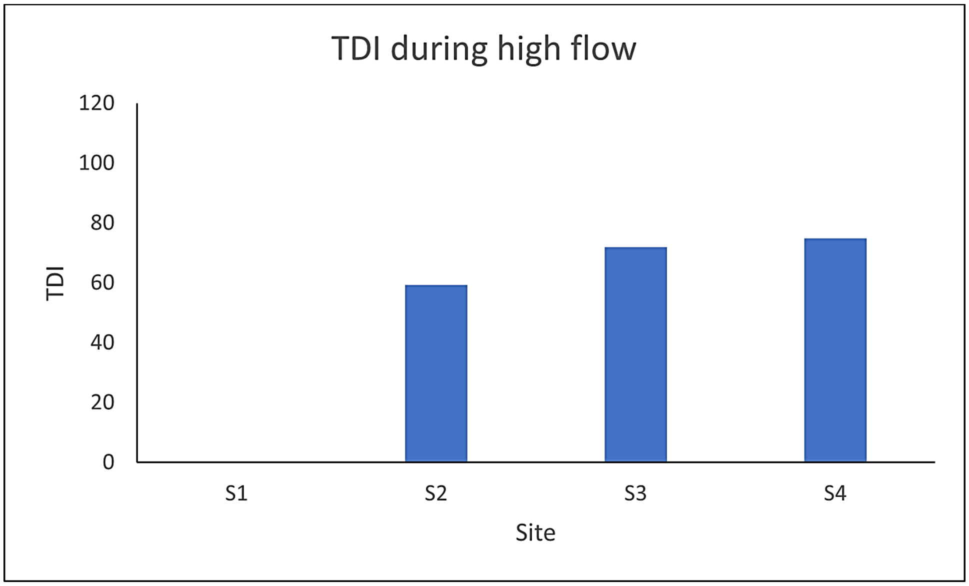

Figure 4.17 High flow Trophic diatom index (TDI) values during high flow along the sampled sites on the Kuils River. (site 1 TDI value was not recorded because of high water levels at time of diatom sampling which made the site inaccessible).

4.6. Correlation of diatom species indices to environmental variables

It is important to note that during this study the results obtained from the physicochemical parameters (4.3), the type of diatoms encountered during the study (4.4.1), results from diatom indices SPI (4.5.1), GDI (4.5.2) and TDI (4.5.3) indicated poor water quality in the Kuils River. Each of these results prove that diatoms can be used to indicate water quality as they complement the results obtained from physicochemical parameters. This section (4.6) of the study aims to investigate a possible correlation between the physicochemical parameters and diatoms indices.

In order to determine how strong the relationship between variables is, a technique or method such as the correlation method is used. According to Phungula (2018) and Matlala et al. (2010) the correlation strategy is a statistical method used in determining the strength of relationship between environmental variables. In addition, a correlation coefficient is a value which ranges from -1 to +1 measuring the strength and direction of the linear relationship between variables. Correlation coefficient values show the strength of the linear relationship between environmental variables and index numbers obtained (Phungula, 2018).

The size of the numbers shows the strength of the correlation meaning that, a value of zero indicates no linear relationship whereas a value of -1 or + 1 indicates a linear relationship where the (+/-) sign shows the direction of the correlation (Matlala et al., 2010). A positive (+) correlation coefficient indicates that as one variable increases the other variable also increases, and a negative (-) correlation coefficient indicates that when one variable increases the other variable decreases (Rummel, 1976; Matlala, 2010). For example, in Microbiology if water temperature is high, the number of microorganisms could be high which means these variables show positive correlation.

Table 4.5 showed different correlations to physicochemical parameters measured in the study. The SPI values had negative correlations to COD and PO_4. The GDI had negative correlations to pH and COD, while TDI values displayed positive correlations to PO_4 and pH. Although these results are different, it indicated that SPI and GDI values maintained a negative correlation, whereas the TDI maintained a positive correlation. The significance of the correlation strategy can only be explained once the concentration levels of the respective physicochemical parameters in a river system have been determined.

Once these concentration levels are compared to the accepted recommended levels as set out by the DEA or SABS for river systems, can a river be regarded as polluted. An increase in the levels of physicochemical parameters causes the water to be polluted and affects the behaviour and physiology of organisms living in that water (Matlala, 2010). When looking at the diatom indices, SPI and GDI indicate better water quality when they increase above 12 (Table 4.3). The TDI is different as it indicates poor water quality when it increases above 40 (Table 4.4).

Therefore, physicochemical parameters are expected to indicate a negative correlation between GDI and SPI. Furthermore, the correlation between physicochemical parameters and TDI is expected to indicate positive correlations. Using only diatoms as water quality indicators is possible, however using other methods that consider different parameters is important to produce more accurate results. the sole use of diatoms as an indicator for high or low physicochemical parameters, will not give a clear representation of water quality in the river. This is because diatoms are affected by different factors such as the availability of light, temperature, water flow and turbulence, nutrient availability and predation from other organisms such as zooplankton, small fish species and small invertebrates (Mehner, 2009).Table 4.5 presents the results of the Pearson correlation coefficients between environmental variables and diatom indices. In simpler terms, the table indicates the effectiveness of SPI, GDI and TDI diatom indices in showing changes in the water quality of environmental variables (pH, COD and PO_4).

Table 4.5 Pearson correlation coefficients between diatom indices and environmental water quality parameters.

	SPI	**GDI**	**TDI**
pH	0,1	-0,6	0,3
COD	-0,3	-0,2	-0,3
PO_4	-0,2	0,1	0,4

4.7. Discussion

The results that were obtained (Chapter 4) from the water quality parameters (section 4.3) and those that were obtained from the different diatom communities (section 4.4), indicated poor water quality along the Kuils River. The presence of diatom species such as *Achnanthidium exiguum* was evidence of polluted waters as this species is primarily found in industrial and wastewaters. Furthermore, the optimum growth of *Achnanthidium exiguum* involve alkaline water conditions (Taylor et al., 2007).

Therefore, this diatom species is an example of how diatoms are able to indicate water quality as it reflects the pH levels of water which was also evident during this study. Here, results obtained proved to correlate with those results obtained from the historical water quality parameters. Findings in this study indicate that it does not matter if the correlation between physicochemical parameters and diatom index are positive or negative. Both these correlations either being positive or negative does not change each result obtained from physicochemical parameters and diatom indices as they indicated poor water quality in the Kuils River.

Dominant diatom species that were found in this study such as *Planothidium engelbrechtii, Nitzschia frustulum, Nitzschia heufleriana* and *Craticula cuspidata* are known to be tolerant to moderate-critical pollution occurring in eutrophic waters as well as waters with high levels of pollution (Taylor et al., 2007, De Almeida et al., 2001, Phungula, 2018). The correlation table between diatom indices (SPI, GDI, and TDI) and environmental variables indicate how these two factors are usable in determining water quality. The findings from this study indicate that diatoms are able to determine water quality based on their dominance and abundance at a particular site along the river.

This information reflects the conditions present in the river. For the purposes of water quality analysis, both methods (physicochemical parameters and diatoms) are better suited to give an indication of water quality. This is because physicochemical parameters give an exact number of the conditions occurring at a particular site. For example, the physicochemical parameter method was able to record pH levels that ranged between 6 to 8 across the Kuils River which indicated alkaline water, whereas the dominance of diatom species *Fragilaria ulna var.* indicated alkaline waters as this diatom species prefers environments with moderate to high pH levels. Therefore, the information from both physicochemical parameters and diatoms complements each other as they are able to determine the quality of water along the river.

4.8. Summary

The findings in this study from both physicochemical parameters and diatoms indicate diminished water quality in the Kuils River. In certain areas the physicochemical parameters such as pH, COD or phosphorus were found in high levels. Diatom communities also existed but those that were dominant were the ones that can tolerate moderate to high water pollution levels. This means that competition between diatom communities could be observed in which dominant species survived high organic pollutant levels. Those species that were sensitive to high pollutant levels showed decreased abundance in the Kuils River.

The locality of this river contributes to the many different types of pollutant sources affecting its water quality. The Kuils River is prone to pollutants from the Bellville, Scottsdene and Zandvliet WWTW. These treatment plants discharge effluents that contain organic pollutants. Other factors contributing to pollution into the Kuils River is natural processes from the river’s bedrock, which causes surface water and ground water interaction to introduce pollutants into the river through seepage. In addition, diatom abundance is affected by the relationship between diatoms and their environment.

It was noted that the dominant species observed at the different sites had optimum growth conditions which complemented the environmental variables measured at the respective sites. It was also noted throughout the sampling period that the water quality across all the sample sites during both high flow and low flow is of concern. The results obtained provides an indication of the ability of these diatom species to be used as water quality indicators. The presence and abundance of these species also coincided with the physicochemical parameters commonly used in the water quality monitoring method. Given the ability of diatoms to be used as pollution indicators, limitations to what these indicators may present have also been identified.

Many factors affect diatom species which makes using its abundance at a site the only advantage to be used as an indicator. This means that diatoms by itself, could not fully serve as representative of the water quality in the river. Diatom species abundance at a site indicate the condition in which that diatom survive, but diatoms do not give a specific value of the level of pollutants in the river. For example, diatom species such as *Nitzschia palea* in the Kuils River only indicate moderate to high levels of COD but do not give the exact concentration of COD that is present in the river. Therefore, at this juncture, biomonitoring methods are more applicable as a complementary method to physicochemical parameters monitoring methods.

The diminished water quality in the Kuils River suggests that control measures be put in place in order to remediate this river and improve its water quality. To date, this investigation has been the only study that employed diatoms as biological indicators in the Kuils River, which indicated compromised water quality effectively. Therefore, more and similar studies with regular monitoring of this river is very important and recommended. In addition, studies focusing on diatoms as biological indicators would enable reference and changes to be noted in diatom communities found along the river.

CHAPTER FIVE: CONCLUSIONS AND RECOMMENDATIONS

The Kuils River experiences increased amounts of pollution from anthropogenic activities such as agricultural runoff, industrial effluents, informal and formal settlements (Phungula, 2018). The geology surrounding the river also contributes to pollutants entering the river through ground water and surface water interactions and erosion from the Malmesbury groups of rocks (limestone, dolomites). Therefore, water quality monitoring and management is very important in this river as it allows for the determination of contaminants in the water and the concentration of present pollutants. This enables measures to be put in place to manage, control and reduce pollution levels entering the river, which will positively affect the health of the aquatic ecosystem as well as human health and safety (Phungula, 2018).

This study was able to answer the research questions set and revealed that different diatom species are present in the Kuils River. Physicochemical parameters influence the types of diatoms that are found at each site. Furthermore, diatoms do play a role in determining water quality because the dominant taxa that are found at a site presents or provide information about the conditions which allows species to survive in, while also indicating current water quality conditions at that site. Lastly, diatom communities in the Kuils River are associated with a particular condition along the river with eutrophic conditions containing diatom communities that prefer such environments.

Physicochemical analysis is expensive and time consuming because of the lack of a standard procedure that can detect and measure all physicochemical constituents that exist in a water system (Matlala, 2010). This study indicates that physicochemical analysis and diatoms work well together in indicating water quality even though the physicochemical analyses process is expensive. The physicochemical analyses give exact levels of contaminants present in the river.

This study used historical physicochemical parameters as a reference to check whether the information on water quality that is given by diatoms correlate to those of commonly used traditional methods. This is because only a few studies on diatoms in South Africa have been conducted. In the case of this current investigation of the Kuils River, no documented reports or evidence of similar studies have been found. Hence, no reference to previous diatom species that might have been found in this river could be made. Thus, the use of historical water quality parameters as a reference was necessary.

The correlation between the physicochemical parameters and diatom index is not consistent and thus there is room for improvement with regards to their use in biomonitoring. With that said, diatoms are ubiquitous in aqueous environments where certain species that tolerate highly polluted conditions will flourish or dominate such sites, while those that survive in less affected waters will be absent or not abundant. Diatoms, therefore, do make useful bioindicators for monitoring water quality in river systems. It is also recommended that future studies use diatoms to determine water quality in river systems to supplement other water quality analysis.

The diatom indices results obtained from this study show bad water quality in the Kuils River with increased nutrient concentrations downstream, indicating a large number of pollutants entering those sites. This decline in water quality can be linked to increased rainfall causing runoff, agricultural activities and broken sewage pipes entering the water system from neighbouring communities. The discharge of effluents from the surrounding WWTWs can also be linked to the Kuils River's decline in water quality.

The findings from this study show that both methods of monitoring (physicochemical parameters and diatoms) can complement each other. Diatom analysis is important because it indicates environmental change and enables an insight into the past and present aquatic ecosystem conditions. Diatom analysis complements physicochemical data by increasing the ability of authorities to effectively assess, manage and monitor ecosystems. Furthermore, if diatom communities during analyses shift in composition this information suggests the presence of a pollutant or nutrient enrichment in the waterbody. Thus, physicochemical parameters can assist in verifying those findings.

This traditional method (physicochemical parameters) has been used for a long time with various studies available to provide a basis for reference or relevant information. However, it is recommended that regular monitoring in river systems be implemented as it is important to monitor changes in environmental variables each year during every season depending on the anthropogenic activities affecting it. This study also recommends monitoring diatoms over a period of more than a year and then using the obtained data to determine how the water quality has improved or deteriorated over the years. This study also proposes the introduction of green infrastructures to reduce the number of pollutants entering rivers. Green infrastructure techniques include rain gardens, pervious pavements and green roofs. These techniques will slow down runoff from storm water drainages, allowing the runoff to move slowly and be spread out onto more land. In this way plants would work as natural filters, filtering out pollutants from runoff, as well as wastewater from treatment works before it infiltrates into the ground and enters water systems.

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APPENDICES

Appendix A

Data from the Department of Water and Sanitation

Monitoring Point ID	Latitude	Longitude	**Season sampled** (High flow-HF/Low flow-LF**)**	pH	COD	PO_4
194181	-33.916125	18.68766667	HF	7.4	28	0.72
194181	-33.916125	18.68766667	HF	7.8	38	1.7
194181	-33.916125	18.68766667	LF	8.7	42	1.2
194181	-33.916125	18.68766667	LF	8.1	32	1.5
194181	-33.916125	18.68766667	LF	8	38	0.96
194181	-33.916125	18.68766667	LF	8.6	34	1.1
194181	-33.916125	18.68766667	HF	9.1	49	1.4
194181	-33.916125	18.68766667	LF	8.3	32	0.44
194181	-33.916125	18.68766667	LF	9.4	36	0.73
194181	-33.916125	18.68766667	LF	7.9	48	2.3
194181	-33.916125	18.68766667	LF	8	49	1.9
194181	-33.916125	18.68766667	LF	8.2	40	1.8
194181	-33.916125	18.68766667	LF	8.4	45	1.6
194181	-33.916125	18.68766667	HF	7.9	41	1.6
194181	-33.916125	18.68766667	HF	8.2	39	1.4
194181	-33.916125	18.68766667	HF	7.5	42	1.6
194184	-33.85793056	18.66760833	HF	7.3	25	0.11
194184	-33.85793056	18.66760833	HF	8.1	26	0.05
194184	-33.85793056	18.66760833	LF	8.2	38	0.05
194184	-33.85793056	18.66760833	LF	8.2	38	0.05
194184	-33.85793056	18.66760833	LF	8.5	52	0.05
194184	-33.85793056	18.66760833	LF	8	31	0.22

194184	-33.85793056	18.66760833	LF	8.2	39	0.17
194184	-33.85793056	18.66760833	LF	8	32	0.33
194184	-33.85793056	18.66760833	HF	8	33	0.05
194184	-33.85793056	18.66760833	LF	7.9	37	0.14
194184	-33.85793056	18.66760833	LF	8.1	34	0.05
194184	-33.85793056	18.66760833	LF	8.3	35	0.12
194184	-33.85793056	18.66760833	LF	7.8	35	0.12
194184	-33.85793056	18.66760833	HF	7.9	35	0.39
194184	-33.85793056	18.66760833	HF	7.5	33	0.05
194187	-33.82949167	18.65839167	HF	7.5	32	0.22
194187	-33.82949167	18.65839167	HF	7.6	16	0.05
194187	-33.82949167	18.65839167	HF	8	30	0.05
194187	-33.82949167	18.65839167	LF	7.9	28	0.05
194187	-33.82949167	18.65839167	LF	6.1	122	0.41
194187	-33.82949167	18.65839167	LF	7.6	39	0.29
194187	-33.82949167	18.65839167	LF	7.7	43	0.05
194187	-33.82949167	18.65839167	HF	8.3	54	0.17
194187	-33.82949167	18.65839167	LF	8	31	0.05
194187	-33.82949167	18.65839167	LF	7.7	48	0.12
194187	-33.82949167	18.65839167	LF	7.9	37	0.05
194187	-33.82949167	18.65839167	LF	8.1	47	0.39
194187	-33.82949167	18.65839167	LF	8.1	84	1.7
194187	-33.82949167	18.65839167	HF	8.2	41	0.16
194187	-33.82949167	18.65839167	HF	7.6	87	0.05
194189	-33.92924167	18.67618611	HF	7.3	27	0.12
194189	-33.92924167	18.67618611	HF	8.2	26	0.98
194189	-33.92924167	18.67618611	LF	7.8	42	0.66
194189	-33.92924167	18.67618611	LF	8.6	45	0.58
194189	-33.92924167	18.67618611	LF	8.9	56	0.3
194189	-33.92924167	18.67618611	LF	8.7	40	0.44

194189	-33.92924167	18.67618611	LF	9.2	43	0.44
194189	-33.92924167	18.67618611	LF	8.1	116	1.4
194189	-33.92924167	18.67618611	LF	8.1	46	0.92
194189	-33.92924167	18.67618611	LF	8.6	38	0.65
194189	-33.92924167	18.67618611	LF	8.9	42	0.71
194189	-33.92924167	18.67618611	LF	8.2	40	1.2
194189	-33.92924167	18.67618611	HF	8.1	35	0.79
194189	-33.92924167	18.67618611	HF	7.7	61	1.5

Appendix B

Averaged physicochemical parameters for the different sites during high flow (HF) and low flow (LF).

Sites	pH			COD			PO4		
	2019	**2020**	**2021**	**2019**	**2020**	**2021**	**2019**	**2020**	**2021**
Site 1 LF	7,4	7,8	8,1	55	41	84	0,2	0,13	1,7
Site 2 LF	8,2	8,1	7,8	39	35	35	0,2	0,1	0,12
Site 3 LF	8,2	8,1	7,8	39	35	35	0,2	0,1	0,12
Site 4 LF	8,5	8,5	8,2	45	57	40	0,49	0,82	1,2
Site 1 HF	7,5	8,3	7,9	24	54	64	0,13	0,17	0,1
Site 2 HF	7,7	8	7,7	25	32	34	0,08	0,33	0,22
Site 3 HF	7,7	8	7,7	25	32	34	0,08	0,33	0,22
Site 4 HF	7,7	8	7,9	26	0	48	0,55	0	1,14

APPENDIX C

Diatoms encountered in the study (highlighted diatoms are dominant)

Code	Taxon	Code	Taxon
CKPP	*Cymbella kappii (Cholnoky) Cholnoky*	ESLE	*Encyonema silesiacum (Bleisch in Rabh.) D.G. Mann var. silesiacum*
NLIB	*Navicula libonensis Schoeman*	RABB	*Rhoicosphenia abbreviata (C.Agardh) Lange-Bertalot*
PGIB	*Pinnularia gibba Ehrenberg var. gibba*	NAUR	*Nitzschia aurariae Cholnoky*
GLGN	*Gomphonema lagenula Kützing*	EADN	*Epithemia adnata (Kützing) Brébisson var. adnata*
NIPR	*Nitzschia pura Hustedt*	NAMP	*Nitzschia amphibia f. amphibia Grunow var. amphibia*
RGBA	*Rhopalodia gibberula (Ehr.) O.Müller var. argentina (Brun in Brun et Tempère) Frenguelli*	GGRA	*Gomphonema gracile Ehrenberg var. gracile*
FCAP	*Fragilaria capucina Desmazieres var. capucina*	SSTM	*Sellaphora stroemii (Hustedt) Kobayasi in Mayama Idei Osada and Nagumo*
NRCH	*Navicula reichardtiana Lange-Bertalot var. reichardtiana in LBK*	GPLA	*Gomphonema parvulum var. lagenula (Kützing) Frenguelli*

NPAE	*Nitzschia paleacea (Grunow) Grunow in Van Heurck var. paleacea*	NROS	*Navicula rostellata Kützing var. rostellata*
NDES	*Nitzschia desertorum Hustedt*	NCPL	*Nitzschia capitellata Hustedt in A. Schmidt et al. var. capitellata*
NVLC	*Nitzschia valdecostata Lange-Bertalot et Simonsen*	NRIE	*Navicula riediana Lange-Bertalot and Rumrich*
NETO	*Nitzschia etoshensis Cholnoky*	NCOM	*Nitzschia communis Rabenhorst*
SELI	*Staurosira elliptica (Schumann) Williams and Round*	NVIR	*Navicula viridula (Kützing) Ehrenberg var. viridula*
NFBU	*Nitzschia frustulum (Kützing) Grunow var. bulnheimiana (Rabenhorst) Grunow*	CPLI	*Cocconeis placentula var. lineata (Ehrenberg)Van Heurck*
PTRO	*Planothidium rostratum (Østrup) Round et Bukhtiyarova*	CRAC	*Craticula accomoda (Hustedt) D.G. Mann in Round et al.*
PVFI	*Pinnularia viridiformis Krammer var. viridiformis morphotype 5*	NIFR	*Nitzschia frustulum (Kützing) Grunow var. frustulum*
MAAT	*Mayamaea atomus (Kützing) Lange-Bertalot var. atomus*	NANT	*Navicula antonii Lange-Bertalot*

TFAS	*Tabularia fasciculata (Agardh) Williams et Round*	NPAL	*Nitzschia palea (Kützing) W.Smith var. palea*
NPRP	*Nitzschia perspicua Cholnoky*	NCPL	*Nitzschia capitellata Hustedt in A. Schmidt et al. var. capitellata*
EINC	*Eunotia incisa Gregory var. incisa*	NVEN	*Navicula veneta Kützing*
ERHO	*Eunotia rhomboidea Hustedt*	HCAP	*Hippodonta capitata (Ehr.)Lange-BertalotMetzeltin and Witkowski*
NAFR	*Nitzschia amphibia f. frauenfeldii (Grunow) Lange-Bertalot*	GPSA	*Gomphonema pseudoaugur Lange-Bertalot*
PVFM	*Pinnularia viridiformis var. minor Krammer*	NIAR	*Nitzschia archibaldii Lange-Bertalot*
DSBO	*Diploneis subovalis Cleve var. subovalis*	CMEN	*Cyclotella meneghiniana Kützing*
PLEN	*Planothidium engelbrechtii (Cholnoky) Round et Bukhtiyarova*	CAQT	*Caloneis aequatorialis Hustedt var. aequatorialis*
SPUP	*Sellaphora pupula (Kützing) Mereschkowksy var. pupula*	GRAU	*Gyrosigma rautenbachiae Cholnoky*
PFQS	*Planothidium frequentissimum Round and*	CPLA	*Cocconeis placentula Ehrenberg*

	Bukhtiyarova in Zidarova and al.		
GPAR	*Gomphonema parvulum var. parvulum f. saprophilum Lange-Bertalot et Reichardt*	GYAC	*Gyrosigma acuminatum (Kützing)Rabenhorst*
PRST	*Planothidium rostratum (Østrup) Lange-Bertalot*	CPLI	*Cocconeis placentula var. lineata (Ehrenberg)Van Heurck*
ACEC	*Achnanthidium exiguum var.constrictum (Grunow) Andresen. Stoermer and Kreis*	MVAR	*Melosira varians Agardh*
AOBG	*Achnanthes oblongella Østrup*	CENG	*Cocconeis engelbrechtii Cholnoky*
CACD	*Craticula acidoclinata Lange-Bertalot and Metzeltin*	NDRA	*Nitzschia draveillensis Coste and Ricard*
ADEG	*Achnanthidium exiguum (Grunow) Czarnecki var. exiguum*	CAMB	*Craticula ambigua (Ehrenberg) Mann*
GDEC	*Geissleria decussis (Østrup) Lange-Bertalot et Metzeltin*	NFIL	*Nitzschia filiformis (W.M.Smith) Van Heurck var. filiformis*

CBAM	*Cymbopleura amphicephala Krammer*	CRCU	*Craticula cuspidata (Kützing) Mann var. cuspidata*
NLBT	*Nitzschia liebethruthii Rabenhorst var. liebethruthii*	NHEU	*Nitzschia heufleriana Grunow var. heufleriana*
NREC	*Nitzschia recta Hantzsch in Rabenhorst var. recta*	CVIX	*Craticula vixnegligenda Lange-Bertalot*
ENCM	*Encyonopsis microcephala (Grunow) Krammer var. microcephala*	NIRM	*Nitzschia irremissa Cholnoky*
EBIL	*Eunotia bilunaris (Ehrenberg) Mills var. bilunaris*	FSAX	*Frustulia saxonica Rabenhorst var. saxonica*
EFLE	*Eunotia flexuosa (Brébisson) Kützing var. flexuosa*	FUAC	*Fragilaria ulna var. acus (Kützing) Lange-Bertalot*
EFOL	*Eunotia formica Ehrenberg sensu lato*	FVUL	*Frustulia vulgaris (Thwaites) De Toni var. vulgaris*
EMIN	*Eunotia minor (Kützing) Grunow in Van Heurck*	NLIN	*Nitzschia linearis (Agardh) W.M.Smith var. linearis*
FBCP	*Fragilaria biceps (Kützing) Lange-Bertalot*	NLSU	*Nitzschia linearis var. subtilis (Grunow) Hustedt*
FTEN	*Fragilaria tenera (W. Smith) Lange-Bertalot var. tenera*	NRPR	*Nitzschia recta Hantzsch in Rabenhorst f. producta Manguin*

FULN	*Fragilaria ulna (Nitzsch.) Lange-Bertalot var. ulna*	NZSU	*Nitzschia supralitorea Lange-Bertalot var. supralitorea*
TFAS	*Tabularia fasciculata (Agardh) Williams et Round*	NUMB	*Nitzschia umbonata(Ehrenberg)Lange-Bertalot*
TCOA	*Tryblionella coarctata (Grunow in Cl. and Grun.) D.G. Mann*	PLFR	*Planothidium Frequentissimum (Lange-Bertalot)Lange-Bertalot var. frequentissimum*
TLIT	*Tryblionella littoralis (Grunow in Cl. et Grun.) D.G. Mann var. littoralis*	PLFQ	*Planothidium frequentissimum (Lange-Bertalot) Round and Bukhtiyarova*
STDE	*Stenopterobia delicatissima (F.W.Lewis) Brébisson ex van Heurck var. delicatissima*	PSAL	*Pleurosigma salinarum (Grunow) Cleve and Grunow*

Appendix D

Diatom indices obtained from diatom index used in the study as produced by Omnidia

Sites	SPI	GDI	TDI
S1 LF	7.7	6.3	79.6
S2 LF	11.7	13.2	55.2
S3 LF	8.7	6.6	96.3
S4 LF	8.3	11.5	89.4
S1 HF	0	0	0
S2 HF	8.6	7.9	59.2
S3 HF	8.5	7.6	71.9
S4 HF	9.1	8	74.9

APPENDIX E

Diatom identification key