Physico-chemical and microbiological data of the Mooi River: A historical perspective

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DECLARATION

I declare that the dissertation submitted by me for the degree Magister Scientiae in Environmental studies at the North-West University (Potchefstroom Campus), Potchefstroom, North-West, South Africa, is my own independent work and has not previously been submitted by me at another university.

Signed in Potchefstroom, South Africa

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ABSTRACT

Increased urbanisation and anthropogenic disturbances have caused water quality of many freshwater systems to deteriorate over the years in South Africa. This is due to domestic, industrial, and agricultural waste being disposed of into surface waters and the surrounding environment. To meet growing water requirements a monitoring program needs to be applied nationally. Government agencies set forth this initiative by creating water management areas to meet integrated water resource management needs. Applying data mining techniques, this study attempts to determine the water quality status by analysing historical and current data of one of these management areas, specifically the Mooi River and Wonderfonteinspruit. It focuses on mining microbiological, physico-chemical and geographic information systems (GIS) data to explore relationships between bacterial communities and physico-chemical changes and the correlation between industrial pollution, agriculture and urbanisation on water quality. The results demonstrated that the Mooi River has water usable for all purposes, while the Wonderfonteinspruit's water is highly polluted with PO_4^{3-}, SO_4^{2-} and NO_3-NO_2. The Wonderfonteinspruit sites also show high EC values. The bacterial community composition of the Mooi River and Wonderfonteinspruit seemed mostly similar. *Bacteriodetes, Proteobacteria, Actinobacteria* and *Cyanobateria* are the four most dominant phyla identified spatially and temporally, but the Wonderfonteinspruit had a higher abundance of *Cyanobateria.* The major land-use activities that influenced physico-chemical parameters and bacterial communities were identified as mining and agriculture, with erosion also playing a role.

Keywords: Mooi River; Wonderfonteinspruit; geographic information systems (GIS) data; physico-chemical; bacterial community structure; data mining; physico-chemical changes; industrial pollution; agriculture; urbanisation.

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ABBREVIATIONS

Acid mine drainage	AMD
Ammonium	NH_4^+
Artificial neural networks	ANN
Bacterial community composition	BCC
Barite	$BaSO_4$
Calcium	Ca^{2+}
Carbonate	CO_3^{2-}
Catchment Management Strategy	CMS
Chloride	Cl^-
Data mining	DM
Department of Water Affairs	DWA
Dissolved organic carbon	DOC
Electrical Conductivity	EC
Evolutionary Algorithms	EA
Geographic information system	GIS
Gypsum	$CaSO_4{\cdot}2H_2O$
Integrated Water Quality Management	IWQM
Integrated Water Resource Management	IWRM
KwaZulu-Natal	KZN
Magnesium	Mg^{2+}
Management Agency	CMA
Multi criterial decision making	MCDM
National Water Act	NWA
National Water Policy	NWP
Neural networks	NN
Nitrates	NO_3^{2-}
Nitrites	NO_2^{2-}
Phosphates	PO_4^{3-}
Phosphorus	P
Phthalate ester	PE
Potassium	K^+
Principal Component Analysis	PCA
Resource Water Quality Objectives	RWQO
Resource-directed measures	RDM
Resources Quality Objectives	RQO
Sodium	Na^+
Sulphate	SO_4^{2-}
Sulphate reducing bacteria	SRB

Total Alkalinity	TAL
Wastewater Treatment Plants	WWTP
Water Management Areas	WMA.
Water quality index/indices	WQI
Water Research Commission	WRC
Wonderfonteinspruit	WFS
World Health Organisation	WHO

CHAPTER 1 – INTRODUCTION

1.1 Background

Water in South Africa is scarce and valuable and is used for several purposes ranging from irrigation to domestic use. It is a crucial resource for all, especially the poor people relying on water to survive. In South Africa about 80% of the population rely on surface water as the main source for their daily water needs (Venter, 2001). Approximately 54% lack basic sanitation and 17% of population does not have access to potable water (Zamxaka *et al*., 2004; Nevondo & Cloete, 1999). This leads to people utilising untreated surface water for their domestic purposes. In the year 2000, the estimated number of South African people dying each year of diarrhoeal diseases caused by inadequate drinking water was approximately 43 000. This number most probably rose sharply over the last two decades due to rapidly growing urbanisation and industrialisation (Zamxaka *et al*., 2004). Water-borne pathogens are subject to geographical factors. The surrounding environment and land-use activities near water systems have an influence on the incidence and prevalence of these disease-causing organisms (Obi *et al., 2002*). One easily explained example of this is human informal settlements that lack the necessary sanitary infrastructure to effectively deal with their waste water, which then ends up in surrounding water systems (Fatoki *et al*., 2001). Thus, to critically monitor the microbial quality of water and to protect our water sources from excessive pollution and unwanted physico-chemical changes is of the utmost importance (Taylor *et al*., 2005).

South Africa's National Water Policy (NWP), adopted by Cabinet in 1997, epitomised three main goals regarding water resource management, namely equity, efficiency and sustainability of rivers, estuaries, wetlands and groundwater (DWAF, 2008). By setting up 'The Reserve', they aimed to provide good quality water for all users. In the interest of all water users a framework for managing the quality of water resources—such as 'The Reserve'—as well as drinking water alike must be developed. Such a management plan cannot be implemented without the primary focus of monitoring (Boyd *et al*., 2011). However, monitoring alone means little if the necessary steps to improve water quality are not implemented (Boyd & Tompkins, 2011). South Africa set in motion the Integrated Water Quality Management (IWQM) strategy which involves monitoring to ensure sustainability and good quality water systems (Boyd *et al*., 2010). The philosophy of IWQM is "everyone is downstream". This simply means that everyone's use of water impacts someone else's use of water. This philosophy forces every water user to manage their own water usage to not negatively impact the water for the next user. A benefit of this model is that smaller geographical areas can be held accountable for pollution (Boyd *et al*., 2010).

Developing such a strategy involves multiple approaches. It is thus essential to make decisions based on the given information, historical problems and present short comings. Data mining becomes a valuable tool in the decision making process as it allows for handling big data sets that contain the answers to the problem at hand. By analysing big data sets it is possible to uncover the trends of the past, determine the current societal patterns and predict future problems using the available data. Water research can especially benefit from decision-making and data-mining processes as this field consists of multiple criteria that influence one another. Physico-chemical parameters, geospatial activities and microbial communities form the backbone of water research. When combined, these interlinking aspects can form one complete picture and provide a broader understanding of the shortcomings certain water systems face on a daily basis. Tools such as geographic information systems (GIS) can be incorporated to analyse geospatial data and identify the land-use activities affecting the surrounding aquatic environment. Metagenomics is another tool used for bacterial community identification. By analysing the bacterial community structure, it is possible to assess the quality of water. This technique is the more advanced method of total coliform and faecal-coliform identification and gives insight into the entire bacterial community present in a certain water source.

This study was conducted to show the potential decision making and data mining have in water quality research. By using large data sets that include historical data, meaningful information could be extracted that could be used to assess the water quality of specific sites and the downstream impacts they have. This is done by setting up water quality indices from physico-chemical data, using various research articles and international, as well as South African limits set by agencies to ensure water quality remains stable. It includes evaluating geospatial data to identify certain land-use activities and their effect on water systems. In addition, identifying bacterial community structures of selected sites. Linking the information could allow the researcher to determine (a) whether the selected Mooi River catchment area is affected by pollution, (b) if this is true, what type of pollution is causing the biggest problem, and (c) in what why the pollution is affecting the bacterial community.

1.2 Problem statement

Agriculture, industries such as mining, increased urbanisation, informal settlements and other anthropogenic activities have resulted in deterioration of water in river catchments globally (Vollmer *et al*., 2018). In many cases, GIS data for river catchment areas and water quality is available. Water quality is mainly based on physico-chemical parameters, but some microbiological data has also become available recently. Combining these data sets could provide important new tools, useful for predicting the effects land-use changes had on the quality of water available for various uses and how this impacts the bacterial community composition (BCC). To implement such a tool, data gathered from the Mooi River catchment in the North West Province, consisting of the two sub-catchments of importance for this study—Mooi River and Wonderfonteinspruit River—will be analysed. Van der Walt et al. (2002) and Hamman (2012) reported on the water quality deterioration of the Mooi River catchment area. They used available data gathered since the early 1960's and blamed the deterioration on anthropogenic contamination. Increasing electrical conductivity and sulphate concentrations have been observed by Van der Walt *et al*. (2002) and Hamman (2012) expressed concern about increasing heavy metal contamination (Hamman, 2012). The Wonderfonteinspruit River is centred between multiple mining industries and informal settlements, increasing potential contamination by organic and inorganic pollution of the surrounding water sources (Jordaan & Bezuidenhout, 2013). This adds to potential downstream contamination of key water sources, including the Boskop and Potchefstroom Dams. These are the main drinking water resources for Potchefstroom (Barnard *et al*., 2013) and contamination could have detrimental long-term health effects if left unchallenged. The Wonderfonteinspruit River area has been the subject of various studies by Coetzee (2004), Coetzee *et al*. (2006), Hamman (2012), Van der Walt *et al*. (2002) and Winde (2010) to name a few. These mostly focused on water quality determination of the Mooi River and Wonderfonteinspruit River area, without combining physico-chemical, microbiological and land-use data. This study addresses this problem by interlacing microbiological data, land-use practices (mines, informal settlements, industries, etc.), physico-chemical water quality data (EC, pH, Ca, SO_4^{2-} etc.) and other anthropogenic data.

1.3 Aim and Objectives

The aim of this study was to create an overview of historical water quality data using the gathered GIS, physico-chemical parameter, as well as microbiological data, interlacing every aspect affecting water quality in a river system.

The specific objectives are:

(i) To analyse historical and recent physico-chemical and microbiological water quality of the Mooi River catchment area to determine temporal and spatial variables.

(ii) To develop a water quality index for the Mooi River and Wonderfonteinspruit River, using available historical and recent physico-chemical data.

(iii) To compare this data to GIS (land-use) data.

(iv) To link the recent water quality and land-use data to bacterial community structures.

CHAPTER 2 – LITERATURE REVIEW

2.1 Aquatic ecosystems

Aquatic ecosystems cover 73% of the Earth's surface and are diverse habitats that support highly productive food-webs (Duarte & Prairie, 2005), as well as a variety of life including reptiles, fish, macroinvertebrates and a large number of microbial communities. These organisms all differ in abundance, chemical composition, growth rates and metabolic functions as environmental conditions such as oxygen availability, temperature, salinity, pH, light, nutrients and dissolved gases may vary. This is due to changes in the surrounding landscape and contaminants entering aquatic systems via point source and non-point source pollution. These aquatic environments are home to intense anabolism and catabolism of chemical elements, such as organic carbon that is internally produced by destruction of organic matter and externally added from land materials (Duarte & Prairie, 2005; Schlesinger & Melack, 1981). These ecosystems exchange CO_2 with the atmosphere, making them key metabolism components of the biosphere and have even been described as early indicators of both regional and global environmental change (Newton *et al*., 2011)

Worldwide, aquatic ecosystems are experiencing water quality problems, originating from human population growth leading to urbanization, mining and other anthropogenic activities that generates effluents polluting water, sediment and soil (Babovic *et al*., 2002). Population growth also contributes to increased agricultural activities to feed the growing population, causing more agricultural run-off containing considerable amounts of potentially harmful substances. Industries, mining and agriculture are the main culprits contaminating water and the entire ecological food chain (Patil *et al*., 2012) with soluble salts, nitrogen compounds (Burgin & Hamilton, 2007) and metals like Fe^{2+}, Cu^{2+}, Zn^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+}. Contaminants and nutrients like these pose significant threats to the water quality and health of the aquatic systems. Pollution increases the naturally present suspended solids within aquatic systems, which impacts all living organisms as it can lead to physical, chemical and biological changes within the waterbody (Bilotta & Brazier, 2008). Eutrophication has become a major concern due to an increase in suspended solids (Adams & Greeley, 2000). Therefore, aquatic ecosystems are predicted to suffer a greater loss in biodiversity than terrestrial ecosystems if the current pollution trend continues, highlighting the importance of sustained monitoring of water quality (Patil *et al*., 2012). Variables impacting water quality include factors such as geological backgrounds, hydrological systems, anthropogenic activities (mines, informal settlements, industries, etc.) and transformations of water characteristics by micro-organisms (Ayoko *et al*., 2007; Einax *et al*., 1997). These variable all require careful evaluation, interpretation, meaningful predictions and pattern recognition to devise a plan for future treatments (Ayoko *et al*., 2007).

2.2 Freshwater ecosystems

Figure 2.1: Water availability on the earth's surface (adapted from Duarte & Prairie, 2005)

Fresh water has been crucial for survival since the beginning of human civilization (Adesuyi *et al*., 2015), by sustaining daily tasks and socio-economic development (Debroas *et al*., 2009). Of the 73% of water sources available on earth, only 3% consists of fresh water, of which only 0.3% to 0.5% is available for human consumption (Figure 2.1). Most of the fresh water is found in glaciers and mountains with ice caps, mainly in Greenland and Antarctica, making fresh water extremely valuable (Thorsteinsson *et al*., 2013). Therefore, the presence of contaminants in natural fresh water resources continues to be one of the world's most important environmental issues (Ayoko *et al*., 2007). Population growth, combined with climate change, groundwater depletion and pollution, will impose even greater pressure on an already scarce resource. Degradation of fresh-water ecosystems threatens biodiversity, raising the need for integrated solutions to fresh-water management (Vollmer *et al*., 2018). The present global challenge is to lower contaminant concentrations within freshwater environments to a point which reserves the functional attributes of the freshwater system being contaminated and protects the species diversity within that system while maintaining good quality water. For this to be possible, monitoring is needed to identify the freshwater systems at risk (Maltby *et al*., 2005).

The field of water quality monitoring gains new impetus as widespread implications arise due to the aforementioned problems. The study of water quality has increased markedly, interweaving all aspects of life—from landscape interactions to economic welfare—into water quality models (Allan & Johnson, 1997). Microscale (e.g. individual, households) and macroscale (e.g. industries such as mining), and interaction between humans and nature over space and time influence each other, culminating in patterns ready to be analysed. These patterns pose a significant challenge to water quality monitoring as they are difficult to interpret, but open a door to compelling and powerful results if linked together (House-Peters & Chang, 2011).

2.3 Water quality

2.3.1 Africa and developing countries

Fresh water ultimately becomes drinking water and millions of lives are lost yearly from water-borne diseases arising from industrialisation and informal settlement run-off (Adefemi et al., 2007). Worldwide, approximately 2.4 billion people suffer from diseases linked to polluted water, mostly in developing countries (Asonye *et al*., 2006). This is especially true in Africa, the world's second largest continent, where clean drinking water remains the most important issue. Rural Africa has reached a point where less than 50% of people have access to potable water and sanitation (WHO, 2015). Lack of piping systems and electricity prohibits the continuous pumping of treated water, increasing the demand of potable water in these areas. However, the potable water problem is also becoming the biggest global issue as many of the Earth's major rivers and groundwater supplies are either over-exploited or polluted (Ayoko *et al*., 2007). Furthermore, the African continent suffered extreme droughts that add to the water distribution problems. The more water quantity decreases due to drought, the more available water resources will get re-used, which increases potential pollution. In addition to drought, Africa's water resources comprise large river basins shared by multiple countries. Sharing water sources further complicates water monitoring and developing sustainable solutions to supply cleaner water. This is due to differences between the level of economic, social and political development within each country (Ashton, 2002). A dramatic increase in population in virtually every African country over the past century has led to an increase in the demand for water. In 1989, Falkenmark (1989) already concluded that the scarcity of water effectively limits further development.

2.3.2 South Africa

South Africa finds itself in an arid to semi-arid region on the African continent, and facing a multitude of environmental issues. These include a lack of clean water (the biggest problem) as pollution intensifies due to anthropogenic factors including urbanisation, mining and informal settlements (Van Heerden *et a*l., 2006). Unevenly distributed water is only adding to the problem as 65% of the country receives less than 500 mm in annual rainfall. This is far below the world average of 860 mm/annum (Bezuidenhout *et al.*, 2013). Approximately 20% of the country receives less than 200 mm/annum (Annandale & Nealer, 2011). This, together with a very high evaporation rate, causes water to be a very scarce resource in parts of the country (Van Heerden *et al.,* 2006). In 2011, the Department of Water Affairs (DWA) stated that the water quality of South African rivers are deteriorating (Bezuidenhout *et al.*, 2013). At the time it was predicted that if management of the quality and quantity did not improve, the demand for water will exceed the rate at which it can be supplied before 2025 (Oberholster *et al*., 2008).

The North-West Province shares its border with the Northern Cape, Free State, Gauteng and Limpopo. It has clear seasonal weather patterns with a rainy season from September/October to April/May (DWAF, 2004). Dolomitic eyes or springs feed most major rivers, making the ground and surface water interdependent as groundwater quality impacts surface water quality. Deterioration of water quality is a pressing issue in North-West as industrial areas, mining, intensive agriculture, informal settlements and population growth are causing effluent discharges that end up in aquatic ecosystems (e.g. rivers, dams, streams and wetlands). A combination of hazardous chemicals, untreated waste, pesticides and fertilisers at various scales and in different regions are all contributing to pollution and may ultimately impact human health in the North-West area. At this point water quality begins to play a dominant role in how water is used. It is essential to initiate a management plan to ensure good quality water, starting with identifying the problem. It is clear that this is not only an environmental problem, but also a development issue. To address the deterioration of the water quality, evolving challenges need to be identified and investigated, and existing policies updated (DWAF, 2009; DWS, 2017).

2.4 Identifying the problem

Socio-economic growth has been prioritised by all developing countries. This strengthens their world rank and ensures money entering countries through tourism and exportation. Gaining economic status can lead to losing environmental stability and, in the case of South Africa, is exactly what has happened. Mining and industrial processes increased dramatically—especially in the West-Rand area upstream of the Wonderfonteinspruit River (Lusilao-Makiese *et al*., 2013), which attracted workers who settle around their new working area. These settlements, mines and other increased industrial activities all cause point source and non-point source discharge entering water sources. Growing economy allows for faster urbanisation, which leads to over loading the municipal Waste Water Treatment Plants (WWTP), adding to the pollution (DWAF, 2008). If the problem is not addressed urgently, it can, combined with global warming and droughts, cause irreversible damage to South Africa’s water resources. Reduced water availability, increased water cost, reduced economic productivity, negative impact on human health and other environmental implications in the near future are predicted (DWS, 2016).

2.5 Water legislation in SA

South Africa was in desperate need of improved water management and laws to reinforce necessary changes. With the new democratic Constitution came new water laws. South Africa’s National Water Policy (NWP), adopted by Cabinet in 1997, epitomised three main goals regarding water resource management: equity (fairly distributed economic benefits), efficiency (maximising economic returns) and sustainability (securing future use of aquatic systems) of rivers, estuaries, wetlands and groundwater (DWAF, 2008). These goals are only achievable with healthy aquatic ecosystems that meet national and international biodiversity conservation obligations over the short and long term. The Water Act of 1956 gave way to the new National Water Act (NWA) (36 of 1998) under the newly implemented policy. Palmer *et al*. (2000) described the National Water Act as one of the most advanced water laws globally. The Act specifies that the protection, development, conservation and management of water must be ensured by the government, in a sustainable, as well as equitable manner. The act also includes riparian rights to use water, and declares water a common asset. Finally, protective measures were put in place to provide good quality water to the public for developmental and basic needs. These measures are referred to as “The Reserve” (DWA, 2010).

2.5.1 Ecological Reserve

The National Water Act defines "The Reserve" as an unallocated portion of water not subjected to competition with other water uses (DWA, 2010). The Reserve has two components:

- Basic Human Need Reserve: the amount of water for drinking, food and personal hygiene; and
- Ecological reserve: classified as an objective to ensure the protection of water resources by maintaining healthy ecosystem functioning (quality and quantity) of aquatic and groundwater-dependent ecosystems.

Managing each resource varies as they are all unique. "The Reserve" classification differs from resource to resource and is determined on the basis of the ecological class of the resource in question. Six ecological classes have been identified from A to F. Categories A to D are within the desired range, whereas E and F are not (DWA, 2010). To keep "The Reserve" acceptable requires implementation of resource-directed measures (RDM). By setting quality goals (Resources Quality Objectives [RQO]), desired levels of protection for specific resources can be defined (DWA, 2010; Rossouw, 2011). RQO are clear numerical or descriptive statements resources can be compared to when evaluating quality, which can include indicators such as biological and physical characteristics of the desired resource (DWAF, 2003; DWS, 2017). RQO then become Resource Water Quality Objectives (RWQO) (DWAF, 2005; DWAF, 2009). RWQO are the water quality components of the Resource Quality Objectives (RQO) defined by the National Water Act as clear goals relating to water quality. RWQO are descriptive or quantitative components with spatial or temporal resolutions set to visualise a healthy aquatic system, and to aid in identifying deterioration in water quality. This helps authorities to maintain a desired state of quality water in which no pathogenic potential arises. It is clear that water has a limited capacity to absorb and degrade pollutants before deterioration of water quality takes place, making RWQO part of the mechanism to define pollution (DWAF, 2009).

2.5.2 Integrated Water Resource Management (IWRM)

Shifting from a reactive to a proactive framework is the only way to sustain already declining water resources (Buysse & Verbeke, 2003). To achieve all the goals set by the NWP and NWA of South Africa, smaller groups need to lend a helping hand. Drinking water quality regulation cannot take place at a national level as there are far too many variables for even one very large group to solve (Boyd *et al.*, 2011). Thus, the implementation of new management strategies had to be developed.

Integrated water resource management (IWRM) promotes guiding principles for South Africans to use water resources sustainably and equitably. It is not only defined as a strategy, but a way of living to ensure that local, regional, national and international catchments serve their optimum socio-economic benefits without deteriorating the aquatic ecosystem in use. IWRM also ensures sustainable use for all future generations equal if not more dependent on it, as promoted by the NWA (DWAF, 1998; DWAF, 2009). IWRM enables the Department of Water Affairs and Forestry to ensure the establishment of statutorily directed Water Management Areas (WMA). There used to be 19 WMA, but a decision was made to lower the number to only 9 for convenience sake. Each WMA has its own catchment management agency (CMA) with their own catchment management strategy (CMS). These strategies aim to improve water quality and quantity within a catchment area. The main focus of the strategies is to promote and ensure equitable and sustainable water use within a catchment area. IWRM incorporates social, economic and ecological dimensions when developing a strategy. The need for IWRM is essential and increasingly important as deterioration of freshwater ecosystem continue to increase (Vollmer *et al*., 2018). The IWRM is incorporated into all national legal and policy frameworks. The strategy aims to provide an overview assessment of all the issues within a WMA that impact water quality and even grants permission for water to be transferred between water-rich and water-poor catchments (DWAF, 2009).

It is thus important for the people depending on the resource to get involved to achieve integrated management. Land-use, run-off, rainfall, informal settlements, mining etc. are interdependent, burdening the Department of Water and Sanitation as they alone cannot oversee all the aspects linked to good quality water. The department has no jurisdiction over land-use planning and regulation, making them reliant on other government departments and local authorities, as well as stakeholders in the catchment who are well-equipped to ensure implementation of these strategies (DWAF, 1998; DWAF, 2009).

2.5.3 Integrated Water Quality Management (IWQM)

CMAs strive to achieve specific quality objectives in various WMAs. IWQM is the process of managing water quality by taking into consideration the economic and social backgrounds of the WMA being analysed, the geological region and the impacts associated with the surrounding area (Boyd *et al.*, 2011). Figure 2.2 shows how IWQM aims to be implemented.

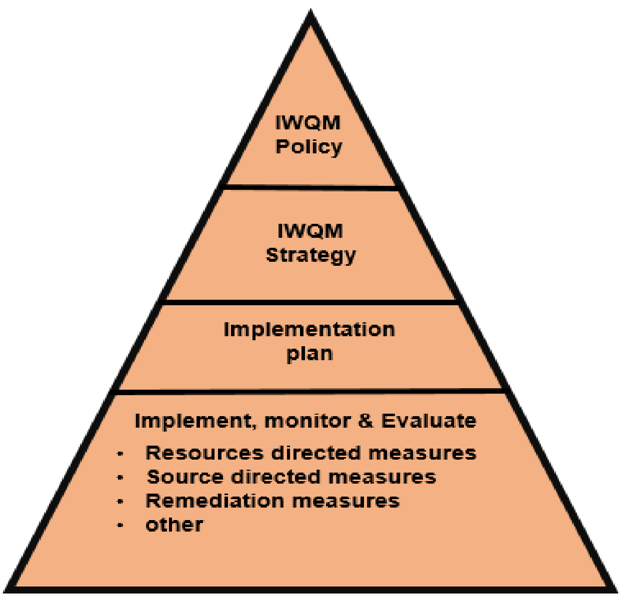

Figure 2.2: IWQM steps for success (adapted from DWAF, 2009)

2.5.3.1 IWQM policy

The IWQM policy (figure 2.2) is the first step that sets out to improve water quality in South Africa. The policy recognises that managing water quality is a complex problem and lessons from international and local management plans needs to be taken into account while focussing on issues impacting water quality across the country. This requires a joint effort by government, civil society and the private sector. The policy has to be flexible as development and other unexpected events may cause the need for correction or change (DWAF, 2009). To meet the standards set by the IWQM policy, aims must be set to ensure progress and act as a checklist of what needs to be done in the future. The aims are as follows:

- Reviewing current management plans and building on existing strengths.
- Identifying weaknesses and addressing them as they appear.
- Setting realistic timeframes supported by sustainable financing.
- Address key operational aspects (e.g. adopting an integrated approach, improving knowledge and information).
- Provide guidance on sustainable water use.

2.5.3.2 Implementing a strategy

Setting up a strategy has to begin with a simple question: *What do I need to achieve*? There has been substantial work conducted in South Africa over water, its scarce natural resource, and the answer is simple: *Good quality water*. The integrated water quality management plan (IWQMP) is the best strategy to achieve this, but all implementers and stakeholders (government, civil society and the private sector) need to agree on present issues affecting South Africa first before developing the most applicable IWQMP (DWAF, 2009). This strategy is a national document and considers the short-, medium- and long-term impacts, actions, goals and priorities of South African water improvement. It serves as a basis for other strategy development and implementation of different scales in South Africa. The strategy to improve water quality does not only apply to the environmental sector, but to all who plays a role in South Africa's water quality deterioration (DWAF, 2005). It is necessary to abide by this strategy as it provides a framework for activities which ensures the sustainability of freshwater environments. The IWQM strategy includes the following aspects:

- Analysing existing information (Previous documents, recommendations, expectations and stakeholder opinions).
- Consideration of all the aspects, e.g. community impacts, co-operation, pragmatic, finances, time and water quality deterioration due to agriculture, domestic, industrial.
- Creating a strategy that solves water quality deterioration at specific sites.
- Turning the strategy into objectives and actions to reach the goal of sustainable water resources.

- The new strategy should align with original policies (IWQM policy and the NWRS).

Successful IWRM and IWQM rely upon data mining to provide historical and current information on the identified problem. Data mining is of utter importance in water management to ensure past mistakes are not repeated, present problems are correctly identified and enough information is available to set in motion actions that will ensure success and sustainability. Water quality assessment of the Mooi River catchment area provides the opportunity to mine and analyse physico-chemical data, microbiological data and GIS data to determine urbanisation impacts on the physico-chemical parameters of selected sites along the Mooi River and Wonderfonteinspruit River, in turn affecting the bacterial composition of the water.

2.6 Land-use

Freshwater ecosystems can be influenced by land use activities at regional or broad geographic scales. A significant relationship exists between land use and water quality parameters at a catchment level (Namugize *et al*., 2018). Land-use changes have various negative impacts on the water quality, as they lead to both increases and de-clines in the concentration of water quality variables (de Mello *et al*., 2018). For this reason, water problems cannot ignore land use activities and continue to be treated in isolation (Mitchell, 2005). An increasing need to understand land use activities is necessary for the maintaining and improving of water quality (Meador & Goldstein, 2003). The growing population leads to the conversion of natural habitats into anthropogenic landscapes. These landscapes are covered in agricultural lands, mining and urban areas, which has been described as the most influential contributors of increased nutrients, sediments, salts, acids and other contaminants within freshwater ecosystems worldwide (de Mello *et al*., 2018). Complex interactions between water and land patterns complicates the determination of the precise non-point pollution source. However, there is increasing recognition that agricultural lands, mining, erosion and urban areas have negative impacts on the surrounding water systems (Monaghan *et al*., 2007). Land-use changes are key drivers affecting catchment hydrology in South Africa. Water quality deterioration took place in the uMngeni Catchment in the KwaZulu-Natal (KZN) Province due to agriculture and industries (Namugize *et al*., 2018).

2.6.1 Urbanisation

Urbanisation is the process of growth. It is an economic, demographic and ecological phenomenon that increases urban areas (Cobbinah *et al*., 2015). Cities grow due to industrialization and economic development, and this in turn leads to more growth (Uttara *et al*., 2012). Population increases around the world results in the requirement of more living space, which is addressed by increasing urban areas, which results in environmental destruction and land-use changes (Zasada *et al*., 2011). For safeguarding of the environment, sustainable development should be implemented. This results in human development with sustainable use of natural and environmental resources (Duh *et al*., 2008). Although, urbanisation is occurring at an uncontrollable rate that the environment is not able to adapt. The current rate of urbanisation is already impacting Africa in terms of urban poverty, and unsustainable exploitation of resources including land (Cobbinah *et al*., 2015). The destruction of natural land is required for the creation of agricultural lands. With rapid population increases, comes rapid changes in food demands (Young *et al*., 1998). Agricultural, combined with mines, industries, informal settlements and increasing urban areas are subjecting aquatic resources to increased stress, giving rise to water pollution (Suthar *et al*., 2010). Natural vegetation and undisturbed soil are replaced with concrete, brick and other impermeable surfaces. This means that, when it rains, water is less likely to be absorbed into the ground and, instead, flows directly into the surrounding aquatic systems (Parnell, S. & Walawege, R., 2011). Wastewater treatment plant effluent, mine effluent, agricultural runoff and industrial runoff are all contributing to water pollution (Nhapi *et al*., 2004)

2.6.2 Agriculture

Agriculture is of fundamental importance around the world. Factors such as climate, landscape topography and parent material have broad effects on regions. These factors characterize the ecosystems based on the similarity of inputs, and establish the type of agricultural practices that are possible (Zalidis *et al*., 2002). It aims to provide food to humans and animals alike and succeeds in job creation. Agriculture is one of the biggest economic drivers of present day life (Godfray *et al*., 2010; Gebbers & Adamchuk, 2010). Among all the positive impacts agriculture seem to have, there are still negative associations. In recent years more attention has been given concerning agricultural impacts on water quality. It has come to light that agriculture contributes to high phosphorus (P) and nitrogen in water systems around the world (Sharpley *et al*., 2001). This has been a concern for more than 30 years (Sims *et al*., 1998). Phosphorus is an essential nutrient for crops and animal production and is used in multiple pesticides. Nitrogen on the other hand is used in fertilizers. When agricultural runoff ends up in water systems it increases P in the surface waters causing eutrophication (Verhoeven *et al*., 2006). Agricultural lands produce the highest nutrient concentration (Tong and Chen, 2002) and the US Environmental Protection Agency and US Geological Survey identified eutrophication due to agricultural runoff as the most ubiquitous water impairment in the US. This is understandable as agriculture underwent evolution and started using P in feed and mineral fertilizers (Dabrowski *et al*., 2009). Studies conducted in Mexico during the 2000's found that water quality problems were attributed to agriculture for 18% of rivers studied. These problems were mainly caused by plant nutrients, especially nitrogen in the form of nitrate (NO_3) that has been identified as the major contaminant of surface water (Greenan *et al*., 2006). Another study done on the Sangamon River in the United States found that NO_3-NO_2 and NH_4^+ were also exceeding limits. The sources feeding these substances into the rivers were identified as erosion and cropping (Kohl *et al*., 1971). Agricultural lands and excess amount of fertilizer and manure applications are the leading sources of nonpoint source pollution in waterways, causing elevated PO_4^{3-} levels (Poudel *et al*., 2013). Agricultural lands can get swept away to other locations through erosion and can ultimately end up in the surrounding rivers. Omernik and McDowell (1979) reported that total N and PO_4^{3-} concentrations are much greater downstream from agricultural lands than downstream from forested areas. In a study done by Smith *et al*. (1994) in the United States, it was found that Nitrate concentrations in rivers close to agricultural areas were at an all-time high in the late 1970's. South Africa is especially vulnerable as agriculture is a large part of the economy (London *et al*., 2005). In the uMngeni River in the (KZN) Province of South Africa, high N and PO_4^{3-} were recorded and was attributed to agriculture run-off and sewage spilling from the surrounding area (Namugize *et al*., 2018)

2.6.3 Erosion

Erosion is a mechanical wear process that gradually removes material, usually land, by continuously repeating actions of removal. Various forms of erosion exist, sheet erosion being one of the most common forms. Sheet erosion takes place when rain or shallow running water induces the removal of a thin layer of the upper soil horizon and is recognised as a major threat to the sustainability of natural ecosystems (Dlamini *et al*., 2011). This form of erosion can increase eutrophication in surface waters and water pollution by heavy metals and pesticides. Huge amounts of money are spend yearly dealing with problems caused by erosion. Thus, erosion has drawn significant attention in research fields to provide an understanding of nutrient losses, ground properties, movement of pesticides and environmental change associated with erosion (Islam & Farhat, 2014).

Soil erosion plays an important role in aquatic ecosystems dynamics. It not only affects the area of erosion, but also the productivity of the environment downstream. Heavy rainfall is one culprit that allows nutrients, like nitrogen and phosphorus, and other substances to flow from its present eroded area into surrounding water systems (Pavlík *et al*., 2013). The presence of phosphorus and nitrogen in aquatic systems may be of concern as they can cause severe eutrophication and poisoning of aquatic organisms among other problems (Mihara & Ueno, 2000; Kim & Gilley, 2008). The most common form of N, NH_4^+, may result from breakdown of manure ending up in water due to eroding runoff (Kim & Gilley, 2008; Barger *et al*., 2006). NH_4^+ alone can cause eutrophication, but can also be converted to NH_3, which is more toxic, in the environment (Jeong *et al*., 2013). This is one of Japan's major problems; 68% and 81% of the total annual loads from different erosion sites cause runoff, composed of nitrogen and total phosphorus respectively leading directly to water systems (Mihara & Ueno, 2000). Malaysia seems to have the same problems. During 2009, 577 water bodies were tested and 46% were found to be polluted with high recorded values of PO_4^{3-}, NO_2, NO_3 and NH_4^+. Most of the suspended sediment including the nutrients present in these water bodies came from runoff and erosion (Zakeyuddin *et al*., 2016). It is estimated that 85% of South Africa's terrestrial area is threatened by land degradation and desertification (Dlamini *et al*., 2011). In South Africa annual soil losses by water erosion have also been estimated at approximately 400 million tons.

2.6.4 Mining

Mining is a process where precious materials are harvested form the ground. Agriculture and mining ranked together as the primary industries of early civilization (Tufano, 1996). The mining industry forms massive networks and contribute a significant amount to pollution around the world. One of the most important problems affecting mining companies is the treatment of their AMD that end up in the surrounding environment (Garcia *et al*., 2001).

AMD is characterised by its high concentrations of metals and dissolved sulphates causing high acidity, with pH lower than 3 and sulphate concentration higher than 3000 mg/L. This leads to a reduction in dissolved oxygen concentration when entering water systems (Ashton *et al*., 2001). Potential sources of AMD include seepage from leach ponds, runoff from residue dumps, surface runoff from open cast mining areas and drainage from underground workings (Ashton *et al*., 2001). Mining industries aim to neutralise water and remove the dissolved metals and sulphates, but this is still a work in progress globally. AMD are of concern as they end up in the surrounding water systems via non-point source and point source pollution mentioned above and increase sulphate concentrations. Sulphates and other sulphide breakdown products can lead to increasing suspended solids and dissolved solids, and thus to salinization (Zhao *et al*., 2018). Carlos *et al*. (2011) studied three rivers in Morizini River Basin in Brazil and the impacts of the surrounding coal mine on water quality. High levels of SO_4^{2-}, Ca, Mg, K and Fe were recorded. Swer and Singh (2004) studied the Damodar River basin in India that flows throw the country's richest coal mining belt found that water samples collected from mining and effluent disposal sites had high concentrations of SO_4^{2-} and Cl^-. SO_4^{2-} was also the dominant anion in the pond water samples collected near the mining sites, with concentration ranging as high as 624 mg/L. Here SO_4^{2-} was also the dominant ion in the mine water itself. Sing *et al.* (2008) repeated the study four years later and found the same results. South Africa also struggles with mining pollution and its aquatic ecosystems are threatened (Ochieng *et al*., 2010). The Wonderfonteinspruit River is impacted by gold mining, and has been since the early 1900s. Thus huge amounts of water are used and large quantities of effluent, including acid mine drainage is produced

2.7 Data mining

The 21st century provides huge technological resources capable of analysing data and information to identify degrading environmental health. Sophisticated databases store untouched data waiting to be sorted, transformed, and processed both statistically and analytically (Kropp & Caulfield, 2004). Computer science has developed drastically leading to a relatively new field in data analysis called data mining (DM). DM is included in a larger process called knowledge discovery in databases (KDD, computer-aided instruction virtually independent of a specific location or hardware platform). KDD involves retrieving data from large data warehouses, selecting target data and storing it in usable formats (Babovic *et al*., 2002). DM then attempts to extract knowledge and analyse the stored data to identify potentially useful and understandable patterns, finding existing associations, identifying anomalies, recognising trends and predicting potential outcomes (Jiménez *et al*., 2018). Large amounts of data are available at any given moment, although only small amounts have been used for analysis or processed in ways humans can understand. A key aspect of DM is pre-processing: data selection, converting data into suitable formats and combing different data sets. DM techniques alone will not yield significant results and should thus be combined with classical statistical techniques to discover significant coherencies in researched data and strengthen DM results (Lausch *et al*., 2015).

Environmental research consists of enormous variations both spatially and temporally (spatial data and time series data). Spatial DM is the process of discovering compelling and previously unknown, but potentially useful patterns from spatial data (Vatsavai *et al*., 2012). It is much harder to extract useful information form spatial data than traditional numeric and categorical data (Shekhar *et al.,* 2002). Spatial data is of the utmost importance when analysing environmental data. Predicting the spatial extent of an impacting variable, e.g. agricultural or industrial run-off, or pollutant concentration at different connecting sites, is recognised as one of the most challenging problems in environmental science (Shekhar *et al.,* 2002). Everything is related to everything else, although nearby things are more related to one another than distant things. This law of geography leads to approaching spatial data by auto correlating and identifying the affects neighbouring sites have on each other (Shekhar *et al.,* 2003).

Time series data is a collection of observations made chronologically, like daily temperatures or weekly rainfall (Fu, 2011). The aim of time series data is to study past observations and develop appropriate models which describe the structure and patterns of the observed data series (Adhikari & Agrawal, 2013). Time series data is never looked at as individual data points, but always as a whole. Time series data is also useful when aiming to predict future values, based on past and present data—in other words predicting the future by understanding the past. Time series data is usually large in size, highly dimensional and updates continuously. One benefit of time series representation is reducing the dimensions (number of data points) of the original data set and moving forward with weekly or yearly means of each segment of data. This simplifies the creation of indices using time series data and declutters the original data set (Keogh & Kasetty, 2003).

The field of environmental science holds true potential for data mining as pattern recognition and trend analysis is essential, as well as predicting outcomes to ensure sustainability. Numerical and categorical data of large and complex data sets is common in environmental research and various data mining techniques can be combined to formulate the perfect result (figure 2.3).

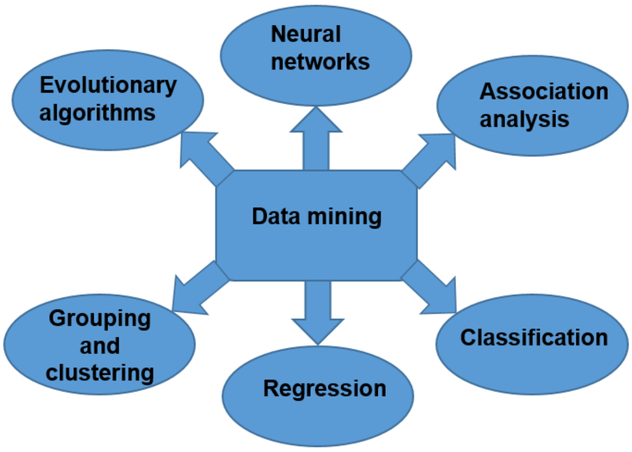

Figure 1.3: Disciplines in the data mining processes (Adapted from Lausch et al., 2015).

2.8 Data mining techniques

DM consists of numerous techniques that can be implemented to obtain a desirable result. Each data set will require a different set of techniques and approaches as the data are available in different formats (Goswami *et al*., 2018).

2.8.1 Neural networks

Neural networks (NN) are complicated processors that have a natural propensity for analysing complex data sets, storing knowledge and generating output available for human use. The network receives information from its surrounding environment and learns to process the data in a meaningful manner, increasing accuracy as more knowledge is acquired (Połap *et al*., 2018). It uses interneuron connections, known as synaptic weights, to store and analyse the acquired knowledge (Haykin, 2009). Studies on animal and human brain functions are opening the door to new computational thinking and software design. The human brain works in a very complex, nonlinear way. It can structure neurons to perform computations like pattern recognition, perception and prediction (like calculating the trajectory of a ball thrown at you in real time and analysing every variable working in on it to predict where it will be to catch it) (Zamirpour & Mosleh, 2018). Prediction has long been one of the main environmental challenges as it needs efficient software tools accurate enough to provide credible and understandable estimates of future pollution, river levels, and urbanisation impacts. Rather than complex physical models, solutions such as conceptual-and-black-box modelling are fast becoming attractive alternatives as they are easy to verify and train in a flexible context (Brath *et al.,* 2002).

Especially in hydrological research, the application of artificial neural networks (ANN) have become popular as they try to mimic the human brain by forming networks between input variables and generating output (Abrahart & See, 2000). Hydrological modelling has four guiding principles:

parsimony (low complexity), modesty (should not pretend to do too much), accuracy and verifiability (must be designed to be validated) (Corwin *et al.,* 1999). ANN can be developed to meet all these requirements while forecasting or predicting even from small data sets. ANN are also flexible. For instance, data such as urbanisation rates can be added or excluded so the modelling procedure could be reproduced on alternative catchments where additional data might be or may not be available. ANN are trained to represent the implicit relationships and processes that are inherent in each data set and accepts different inputs with different scales or resolutions that can be combined to generate more accurate output (Feng & Hong, 2008). There are weights on each of the interconnections that can be altered during the training process to ensure that the inputs produce an output that is close to the desired value. In the process, an appropriate "training rule" is used to adjust the weights in accordance with the data presented to the network (Abrahart & See, 2000). These networks come in multiple shapes and sizes. Feedforward multi-layered perception (information flows in one direction) is presently at the top due to its basic structure. It consists of a number of simple processing units (commonly called nodes or neurons) arranged in a number of different layers to form a network. Data entering the network comes from the input units (input layer), passes through successive layers in the middle (hidden layer), where calculations take place, and emerge from the output layers for our interpretation.

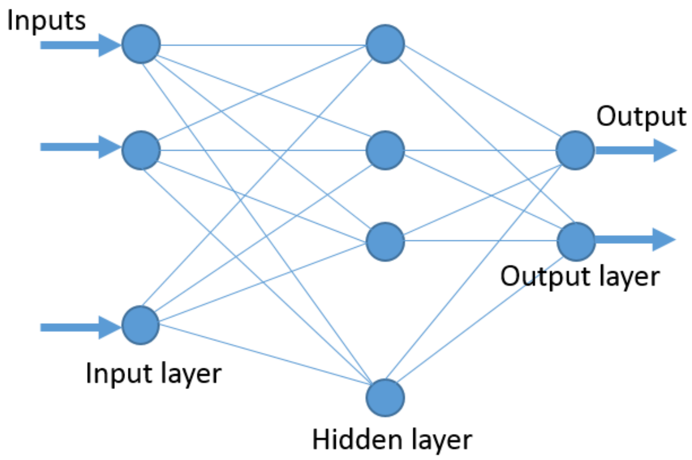

Figure 2.4: The structure of a neural network used in environmental prediction (adapted from Oprea & Matei, 2010).

2.7.1.1 Benefits of neural networks

Different factors make Neural networks an attractive tool when analysing environmental data. NN needs to comply with numerous criteria when used for analysing environmental data. It also opens the door for other researchers to improve upon an already set model (Tang *et al*., 1991). The criteria include:

- **Adaptability** Neural networks have the extraordinary capability to adapt to the surrounding environment by adapting their synaptic weights. The neural network can be retrained allowing it to readjust to new variables and can even change its synaptic weights in real time when operating in a nonstationary environment (statistics change over time).

- **Input-output mapping**: Input-output mapping can be seen as the neural network teacher. To understand and predict the outcomes, it first has to learn how to manoeuvre towards the desired outcome. Analysts present the network with a random sample from the data set. Modifying the synaptic weights of the network with an appropriate statistical criterion to reduce significant differences between the actual outcome and the desired outcome. Repeating this step ensures that the network reaches a steady state between actual and desired synaptic weight changes. This allows the network to learn and relearn based on the input and output given, constructing its own input-output mapping for the specific problem.
- **Evidential response:** By analysing the outcomes and classifying the patterns, a neural network can confirm the certainty of particular patterns and decisions made. By setting specific certainties the neural network improves its classifications by focussing on the highest certainty rates, thus improving performance.
- **Fault tolerance:** Complete breakdown of a neural network is unlikely due to the information stored in different neurons. If one neuron gets damaged or is not able to function properly, the performance of the neural network will only degrade slightly as it still receives information from other neurons.
- **Uniformity of analysis and design:** Neural networks share a basic design. Neurons, input, output. This makes it possible to share theories and learning algorithms with anyone seeking your information and techniques (Haykin, 2009).

Before data can be used as input for evolutionary algorithms and artificial neural networks, they need to be sorted by extracting the most useful information needed for the task at hand. Different data types will require different data for meaningful analysis and choosing the correct data types is one of the major steps in DM. Methods like association analysis, grouping and clustering, regression and classification are mostly used for gathering useful information (Bharati & Ramageri, 2010).

2.8.2 Evolutionary Algorithms

Evolutionary algorithms (EA) are stochastic search methods. They are algorithms that provide endless potential solutions (known as individuals) to complex problems. They analyse each problem and try to solve them by applying one solution at a time, manipulated competitively by applying some variation operators (Bäck, 2000). The individual resembling the desired outcome is processed, leaving the analyst with a suitable solution to a complex problem. These algorithms mimic Darwinian evolution (survival of the fittest), like ants finding the shortest route to food or birds finding their destination during migration. They learn, adapt and constantly evolve to be as efficient as possible (Elbeltagi *et al*., 2005). EA can function and generate output from little problem-specific knowledge and can be applied to most problems where data is available. When more information becomes available, it can easily be added to the EA heuristic to improve its performance and yield more accurate results. EA methods can also be applied to complex problems where humans find the answer unobtainable (Du *et al*., 2018). Additionally, EA are easy to use for very different problems without the need for special tuning or expert knowledge, because they handle added parameters very well without disrupting the EA. There are multiple EA methods suited for different specific problems and data types, making it easy to select an EA most suitable for the problem at hand (Bosman & Thierens, 2002)

2.8.3 Association analysis

Association analysis help identify patterns in data over time by associating specific results with specific external factors happening at the same time (Rajak & Gupta, 2008). Association analysis quantifies relationships between objects using specific indicators present for all objects. In environmental studies these indicators can be a variety of factors such as physico-chemical parameters, microbiological data and/or land-usage. More corresponding factors strengthens the association and increases the accuracy of the analysis.

2.8.4 Grouping and clustering

Grouping and clustering refers to identifying similarities and grouping objects based on analogies, grouping objects that are similar together to be analysed as one group. Having contrasting groups can pinpoint differences in the data set and aid in identifying the influences contributing to that unexpected result (Bijuraj, 2013). Grouping and clustering is a common technique for statistical data mining and aids in pattern recognition and bioinformatics. The groups and clusters depend solely on the individual analysing the data set, as well as the available information within the data set. It involves trail and failure to find the best groups that complement one another and gives the best results or desired properties when put together.

2.8.5 Regression

Regression is one of the most fundamental statistical techniques to solve problems where one feature depends on other measured features. Regression analysis determines functional dependencies among variables (Shen *et al*., 2018). It can be used to model the relationships between independent (attributes already known) and dependent variables (result needed). Models such as linear regression, multivariate linear regression, nonlinear regression, multivariate nonlinear regression can also be used to determine the statistical significance between the variables and using past and present data from these models to predict outcomes based on trends over time.

2.8.6 Classification

Classification discovers the class values of test datasets, aiming to predict unseen objectives to one of their set classes. For instance, setting your own classes when conducting an experiment with certain criteria which data must obey to form a specific class and matching data from raw datasets to those classes to simplify future pattern recognition or time series analysis (Hadi *et al.,* 2018). Classification can identify existing models and use their information or baselines to explain your own findings. Comparing your data to known models or classes set for a specific experiment can be done by procedures like neuronal networking and time series analysis and various algorithms (Gola *et al*., 2018).

2.9 Physico-chemical parameters

Before water can be used for drinking, domestic, agriculture or industrial purposes it is essential to test the water using different parameters. Parameter selection depends on the usage purpose of the water and to what extent quality and purity is needed. (Patil *et al*., 2012). Water quality can be defined in terms of chemical, physical and biological contents and also change with the seasons, even if no pollution occurs (Lawson, 2011). Water quality guidelines provide basic scientific information about water quality parameters and ecological relevant toxicological threshold values to protect specific water uses and serve as standard values for healthy aquatic systems. Water quality is influenced by various factors such as electrical conductivity (EC) (Patil *et al*., 2012), dissolved organic matter, temperature (Adams and Greeley, 2000), pH (Bhandari & Nayal, 2008), sulphates (Greenwood & Earnshaw, 1984), nitrates/nitrites (Adesuyi *et al*., 2015), phosphates (Mekonnen & Hoekstra, 2018) and rainfall (Lawson, 2011). These are the key parameters contributing to the survival of aquatic ecosystems (flora and fauna) (Lawson, 2011). Changes in water quality have already been documented, intensifying the need for physical, chemical and biological monitoring of degrading water sources (Patil *et al*., 2012).

Physico-chemical parameters are key components to ensure that the water quality of “The Reserve” stays useable. These parameters fall under the resources water quality objectives (RWQO) that describe the desired levels of certain parameters for specific resources (DWA, 2010).

2.9.1. Physical-Chemical Parameters: Temperature

Water temperature changes with the seasons as solar radiation heats water during the summer and cold air decreases water temperature during the winter. Water temperature is seen as a crucial physical environmental variable as it controls all chemical and biological reactions within a water system. This includes growth, reproduction, feeding and metabolic activities and affects the solubility of gasses in water (Lawson, 2011). Chemical and biological reaction rates increase with increasing temperature. Aquatic organisms prefer specific temperature ranges. If temperatures are too far outside of a specific range, organisms will die. Higher temperatures can lead to oxygen stress as it decreases the maximum amount able to dissolve in higher temperature if there is too much organic matter in the water. Thermal pollution (artificial temperature change) can thus be seen as a serious problem. Most thermal pollution occurs when water is used as a cooling agent in power, manufacturing and industrial plants and is then returned to water systems (DWAF, 1996).

2.9.2 Physical-Chemical Parameters: pH (Acidity and Alkalinity)

Hydrogen ion concentration or pH is a key factor in the physiology survival, growth and metabolism of aquatic organisms. Like temperature, pH varies seasonally as hydrogen ion concentration decreases due to photosynthesis in the summer allowing pH to increase. The opposite happens in winter. Temperature affects pH slightly, but not enough to disturb the natural ecosystem. pH also changes as acids or bases enter a water body disturbing the H^+ and OH^- equilibrium. Acidity is the measure of water's ability to neutralise a base, and alkalinity is the measure of water's ability to neutralise an acid. Water acidity is known to influence the solubility, availability and toxicity of metals in the aquatic ecosystems (Ochieng *et al*., 2010). The ideal pH range for biological productivity is 6.5–9 (DWAF, 1996). Changes in pH beyond these limits rarely occurred within South Africa in the past, but recently the numbers have drastically increased (Hamman, 2012). Atmospheric pollution (industrial gasses) causing acid rain, acid mine drainage and urban-and-agricultural runoff are all culprits causing acidification within aquatic environments (Berezina, 2001).

2.9.3 Physical-Chemical Parameters: EC (Electrical Conductivity)

Electrical conductivity (EC) can be described as the ability of water to conduct an electrical current. Dissolved salts that breaks into positively and negatively charged ions are capable of conducting an electrical current and can influence EC (DWAF, 1996). The more salts entering an aquatic system, the higher the electrical conductivity will be. Salt can enter these systems naturally during rainfall, or unnaturally during industrial spill or effluent released. Sudden increases or decreases in the EC within a site, during a certain time period is a good indicator of possible pollution and water quality changes (Jonnagalgada & Mhere, 2001). Major positive ions are sodium (Na^+), calcium (Ca^{2+}), potassium (K^+) and magnesium (Mg^{2+}) whereas major negative ions are chloride (Cl^-), sulphate (SO_4^{2--}), carbonate (CO_3^{2-}), and bicarbonate (HCO^{3-}), Nitrates (NO_3^{2-}) and phosphates (PO_4^{3-}). Using these dissolved salts, water can then conduct an electrical current which can be measured. Patil *et al*. (2012) found that EC showed significant correlation with ten parameters. The parameters are pH, temperature, total hardness, chemical oxygen demand, calcium, total dissolved solids, alkalinity, and the chloride and iron concentration in water (Patil *et al*., 2012). Measuring EC is a useful screening tool for detecting pollution and, can provide unambiguous information about anthropogenic sources of contaminant discharges. Land-use activities such as mining increases EC within aquatic systems when mine effluent enters these systems. EC is measured by an EC meter which measures the resistance offered by the water between two platinized electrodes (de Sousa *et al*., 2014).

Physical characteristics of water, such as the colour of the water, the taste, temperature, odour emanating from water etc. are determined by the senses of touch, smell, sight and taste. The above-mentioned parameters can affect the taste and odour of water if concentrations reach a certain point. Physical characteristics can be altered on various ways including (DWS, 2016):

- Increasing suspended sediment loads may change the clarity of the water.
- Chemical run-off may alter the taste.
- Chemical run-off can cause blue-green algae breakout or breakdown, altering taste.
- Blue-green algae may impact oxygen usage by other species.
- Certain discharges can raise or lower water temperature.
- Temperature fluxes can also cause dissolved oxygen fluxes.
- The most obvious physical problem is urban litter (e.g. plastic bottles, food wrappers) entering aquatic systems.

2.9.4 Physical-Chemical Parameters: Sulphate

Sulphate (SO_4^{2-}) concentrations in fresh-water systems are generally lower than that of marine and brackish systems. Sulphates are natural elements that occurs in various forms and within multiple minerals. They are widely used industrially and domestically and minerals such as gypsum ($CaSO_4 \cdot 2H_2O$) are used in fertilisers and is also commonly mined. Barite ($BaSO_4$) is another mineral used industrially. Sulphates can be also used domestically and is found in soap, insecticides, glass and paper (Greenwood & Earnshaw, 1984). Sulphate concentrations are increasing in fresh-water ecosystems throughout the world and find their way into water systems via mining discharge, agricultural and urban run-off, as well as industrial effluents. Increasing sulphate concentrations have been identified as a major issue in the management of fresh-water ecosystems. Enhanced inputs of sulphates into aquatic systems may stimulate sulphate reduction which in turn alters the cycling of elements such as carbon, nitrogen, phosphorus and iron in these exposed water systems (Holmer & Storkholm, 2001). This causes physico-chemical changes which can disrupt the entire aquatic ecosystem as sulphide is toxic to plants (Kelly *et al*., 1995). In addition, sulphate can lead to increased mobilisation of phosphate, which may enhance eutrophication and change bacterial community structures—especially of sulphate reducing bacteria (SRB), such as *Proteobacteria* and *Firmicutes* (Caraco *et al*., 1993). Concentration no higher than 500 mg/L should be found in aquatic environment (WHO, 2011). This is a major problem in the Netherlands, USA, China and South Africa (Coetzee, 2004; Geurts *et al*., 2009; Chen *et al.,* 2016). Sulphate can be measured by the nephelometric method in which the concentration of turbidity is measured against the known concentration of synthetically prepared sulphate solution.

2.9.5 Physical-Chemical Parameters: Nitrate/Nitrite

Nitrogen is the most abundant chemical element of the Earth's atmosphere (almost 80%). The most ionic forms of inorganic nitrogen in aquatic ecosystems are ammonium (NH_4^+), nitrite (NO_2) and nitrate (NO_3; one of the most common pollutants in surface water) (Adesuyi *et al*., 2015). These ions occur naturally due to nitrogen-rich geological deposits, N_2 fixation by certain prokaryotes (like *cyanobacteria*), biological degradation of organic matter and even surface and groundwater run-off (Fan and Steinberg, 1996). Over the last five decades, humans have increased both the availability and the mobility of nitrogen across the earth via addition of inorganic nitrogen by point and nonpoint sources (Camargo and Alonso, 2006). Urban development, industrial activities, municipal wastewater, septic tanks and agriculture have increased nitrogen discharge into aquatic systems over the years (Patella *et al*., 2017). The breakdown of organic matter first forms ammonia (NH_4^+), then nitrites (NO_2), and finally nitrates (NO_3). Schlesinger (2009) estimated that about 150 Tg of anthropogenic nitrogen is annually produced. Of this, 23% ends up in aquatic systems. Nitrogen pollution causes significant effects and ultimately degradation of many aquatic organisms. Inorganic nitrogen pollution can (Wick *et al.*, 2012; Harker, 2015):

- Result in acidification due to increased hydrogen ion concentrations;
- Result in eutrophication by stimulating and proliferating primary producers;
- Can cause death to aquatic animals; and
- Can lead to adverse effects on human health.

Anthropogenic activities have increased nitrate concentration in the coastal waters of the north-eastern USA by 6–8-fold (Carpenter *et al*., 1998), into the coastal waters of the Gulf of Mexico by 4–5-fold, the European rivers draining to the North Sea region by 6–20-fold and most water bodies in Nigeria, linking these increased N fluxes to increased numbers of algal blooms (e.g., *Cyanobacteria*) over the last four decades (Yoon *et al*., 2015; Adesuyi *et al*., 2015; Sharpley *et al*, 2001). Levels no higher than 4 mg/L should be found in aquatic environments (WHO, 2011).

2.9.6 Physical-Chemical Parameters: Phosphate

Phosphorus (P) plays an important role in all life forms. It is an integral part of the metabolism as the key component of nucleic acids and intermediary metabolites like sugar phosphates and adenosine phosphates (Correll, 1998). Phosphorus only occurs in aquatic systems in the form of orthophosphates (PO_4^{3-}, the only form of P that can be assimilated by bacteria, algae and plants), organic phosphate esters ($O{=}P(OR)_3$), pyrophosphates or other pentavalent forms (Carpenter *et al*., 1998). Phosphate is derived from anthropogenic sources, such as agriculture, industry and sewage. This nutrient is regarded as one of the key drivers of aquatic biodiversity loss and bacterial community changes (Geurts *et al*., 2008). Excessive inputs of phosphate in freshwater environments causes eutrophication, changes in biological diversity and ecosystem services (Mekonnen & Hoekstra, 2018). Phosphorus loads entering freshwater systems are becoming a global concern. Studies in Nigeria have shown high loads of phosphorus in most water bodies linked to industrial effluents, mining and urbanisation (Adesuyi *et al*., 2015). South Africa has since 1980 started to focus on phosphorus-concentration management, with the implementation of a 1 mg/l P effluent standard in 1985. Phosphate loading in South Africa has been associated with urban run-off, mining, agricultural activities (mineral fertilisers and manure), discharge of waste water effluent and even phosphate-containing detergents (Griffin, 2017). Eutrophication is a major problem in South Africa (De Villiers, 2007).

2.9.7 Physical-Chemical Parameters: Calcium

Calcium plays a vital role in the human body and is seen as a key structural element. Water low in calcium can cause inadequate levels within the human body, which can lead to osteoporosis, kidney stones, insulin resistance and even strokes. Thus, drinking water may be intentionally supplemented with additional Ca^{2+} (WHO, 2009). It is the third most abundant alkaline metal and is also highly abundant within the Earth's crust. Calcium carbonate found in limestone is the most common calcium compound found on Earth. The hardness and EC of freshwater environments are directly affected by calcium and it ends up in fresh water by the disintegrating of limestone and gypsum (Kožíšek, 2003). A buffering effect can be caused by calcium in fresh water that can maintain pH values even when external impacts cause acidic effluent. It is measured by complexometric titration with standard solution of ETDA using Patton's and Reeder's indicator under the pH conditions of more than 12.0 (Patil *et al.*, 2012).

2.9.8 Physical-Chemical Parameters: Magnesium

Magnesium is commonly found within the environment. Numerous minerals contain magnesium and it is also an important cation within the human body. Magnesium deficiency can cause hypertension and blood pressure problems (WHO, 2009). Rock contains high amounts of magnesium that subsequently ends up in water, which elevated magnesium concentrations within aquatic environments (Kožíšek, 2003). The Mooi River catchment is rich in dolomite—magnesium containing mineral—that can increase magnesium concentrations. Industries also elevate magnesium levels within aquatic environments by using plastics with added magnesium, which serve as a fire protection measure. Agriculture is another culprit that uses fertilisers and feed for cattle rich in magnesium. Calcium is usually more abundant within water as it is found in higher amounts within the Earth's crust. Magnesium and calcium combined can create water with a high buffering capacity (Geeza *et al*., 2018). This is true for the Mooi River catchment. It is measured by complexometric titration with standard solution of EDTA using Eriochrome black T as indicator under the buffer conditions of pH 10 (Patil *et al*., 2012).

2.9.9 Physical-Chemical Parameters: Sodium

Sodium is represented by the Na symbol and is commonly associated with chloride as sodium chloride is known as common salt. It is an important element within the human body to maintain blood pressure and muscle function and sodium deficiency can cause hyponatremia (WHO, 2011). Sodium is an alkali metal and is the sixth most common element within the Earth's crust. Sodium has many industrial purposes, including as a cooling agent in nuclear reactors, applied as a synthetic fertiliser, forms part of the ingredients in soaps and baking soda and is also used as a preservative or flavouring agent. Thus, Industrial and domestic run-off may contain considerable amounts of sodium that end up in water sources. Sodium concentrations above 200 mg/L will give water a salty taste (WHO, 2011). It is measured with the help of flame photometer. The instrument is standardised with the known concentration of sodium ion (1–100 mg/L) (Patil *et al.*, 2012).

2.9.10 Physical-Chemical Parameters: Fluoride

Global attention has been drawn to the occurrence of fluoride in ground water since it has severe human health implications (WHO, 2011). India is experiencing a geo-environmental issue relating to abnormal levels of fluoride in their ground water. Most drinking-waters contain some fluoride. Concentrations of fluoride in groundwater range from 0–67 mg/L and surface water contains concentrations as low as 0.1 mg/L or less. Excessively high levels of fluoride intake cause crippling skeletal fluorosis, increased bone-fracture risk and even dental fluorosis. Children are most susceptible (Bhagavan & Raghu, 2005). The WHO drinking-water guideline value for fluoride is <1.5 mg/L, but this value is not considered by all as dangerous. Fluoride within water depends on the type of rock present and this can cause fluoride levels to increase. The fluoride limit set within the Mooi River is <3 mg/L. Fluoride can be determined by the ion selective electrode method (Patil *et al.*, 2012).

2.9.11 Physical-Chemical Parameters: Chloride

The chloride content of water can be an indication of pollution (WHO, 2011). Low levels of chloride is not toxic to humans and concentrations must exceed about 200 mg/L before chloride becomes a concern. Chloride is used in the water purification and a concentration that exceeds 600 mg/L can start to impair drinking water (WHO, 2003). Aquatic environments receive chloride naturally through sources such as calcium chloride ($CaCl_2$), potassium chloride (KCl) and sodium chloride (NaCl) already present within the natural environment. Contamination with NaCl creates higher water densities and prevent oxygen distribution throughout the aquatic system. Anthropogenic activities like industrial effluents, sewage, fertilisers, animal feed run-off and irrigation drainage (WHO, 2011).

Changes in the chemical characteristics of water can alter the entire metabolism of the ecosystem. Degrading water quality, causing a decrease in species diversity in aquatic systems. Some impacts to keep in mind may be:

- Industrial effluent increasing the salinity of the water body upon entering.
- Balance disruption due to nutrient-rich agricultural effluent or untreated domestic effluent increasing sulphate and chloride contents in a water body.
- Mines close to aquatic systems can cause chemical disruption in water bodies when acid mine drainage flows into these systems.
- Mines also discharge radioactive material into water bodies such as radioactive sodium (DWS, 2016).

2.10 Microbial monitoring and community composition

Microbiological data can help us understand water quality, serving as another puzzle piece in freshwater quality monitoring. Microbial water quality monitoring is essential. According to the National Water Act, responsibility for setting up such a monitoring system is the responsibility of the Minister of Water and Environmental Affairs. The monitoring of microbial water quality applies to the National Microbial Monitoring Programme of surface water (NMMP) and was developed based on research by du Preez *et al*. (2001). The programme aims to create water quality databases from microbial monitoring data (Luyt *et al*., 2012; WHO, 2011). This is crucial in South Africa as the country is forced to supply inadequate microbial quality water for drinking purposes due to infrastructural problems (Luyt *et al*., 2012).

Micro-organisms form communities, comprised of multiple species and each with their own function (Handelsman, 2007). Chemicals entering freshwater ecosystems may alter these bacterial community compositions as micro-organisms possess the ability to adapt to the ever-changing environmental conditions for insured survival, which explains why different environments consists of diverse microbial communities (Jordaan & Bezuidenhout, 2015). Bacteria play a prominent role in freshwater ecosystem processes like organic matter breakdown, and greatly impact water quality. Evidence suggests that many bacteria are freshwater specific. Analysing the bacterial community compositions can give insight into physical and chemical stresses, as changes in bacterial community compositions affects freshwater ecosystem dynamics (Jordaan & Bezuidenhout, 2015; Newton *et al*., 2011). Within these communities it is possible to identify indicator organisms serving their purpose to identify faecal pollution and the integrity and cleanliness of a water source. Total coliform bacteria can be seen as an indicator group, which includes *E. coli*. Faeces that is excreted by animals and human alike contain *E. coli*, making it a suitable indicator for faecal pollution within the environment. A clear relationship has been established between *E. coli* and contaminated drinking water. The water-quality guidelines for domestic use and drinking water regulations require that the concentration of *E. coli* or faecal coliforms is below 0 CFUs or cells/100cm3 (Luyt *et al*., 2012). Other groups include intestinal *Enterococci*, *Clostridium*, coliphages and *Bacteroides fragilis* phages (WHO, 2011). These indicators serve a specific purpose, but are not suited for all experiments or research purposes when larger communities are needed.

Even though microbes are present within all environmental systems and are the centre of the functional elements driving environmental metabolisms, they are often overlooked by scientist that study environmental ecosystems (Balser & Firestone, 2005). For a more in-depth look at the environment and especially water quality, more than just faecal coliforms need to be identified. Identifying the microbial community composition can give far more valuable information about the inner workings of water systems and impact these microbes have. However, studying and gathering information on entire microbial communities can be a daunting challenge, as only 0.01-0.1% of micro-organisms can be cultured in a laboratory (Handelsman, 2007). One approach called metagenomics solves this problem. Metagenomics is a direct genetic analysis of genomes contained within an environmental sample without the need for cultivating clonal cultures. Metagenomics grants access to compare community biodiversity profiles via genomic sequences and associate unknown organism lineages to known lineages previously deposited into publicly available databases such as GENBANK, SILVA or EMBL (Oulas *et al.,* 2015; Handelsman, 2007). These environmental DNA libraries enable analysis of metabolic and phylogenetic diverse microbes identified in a given environmental sample. Phylogenetic information can be obtained by screening libraries for 16s rRNA genes to explore the metabolic potential of targeted bacterial groups (Cottrell *et al*., 2005). It is widely accepted that sequencing the 16s rRNA gene reflects eubacterial groups (Oulas *et al.,* 2015).

2.11 Decision making

When approaching the development of strategies and policies multiple courses of action each leading to alternative conclusions should be considered. Too make the appropriate choice, an appropriate tool for water quality interpreting is necessary. Multi-criterial decision making (MCDM) involves combining every feasible piece of information and striving to solve problems involving multiple criteria (Shooshtarian *et al*., 2018). The purpose of MCDM is to support decision makers facing problems involving huge data sets where an optimal solution typically does not exist, but aids decision makers in choosing the alterative best (Majumder, 2015). Multiple criteria in water research includes physical-chemical parameters, rainfall, microbiological community structure, urbanisation, mining, agriculture and industries. MCDM is shown to benefit water quality research in general such as the assessment of agricultural impacts on water quality (Khan & Maity, 2016), water quality monitoring (Hernandez & Uddameri, 2010), health-risk assessment due to water quality problems (Li *et al*., 2016) and validating water quality index classes (Zahedi *et al*., 2017). MCDM has been applied to climate change research affecting water resource allocation and management by predicting droughts using monthly temperature and precipitation sequences (Yan *et al*., 2017).

MCDM is a trial and error method, which involves a different approach every time it is utilised. It depends on data and location, as each new location will include different variables. Using physico-chemical parameters for index setup, analysing bacterial community composition (BCC), and incorporating GIS to identify land-usage and its associated water quality impacts using multiple programs and literacies are the basic decisions made to assess water quality in the Mooi River catchment area.

2.11.1 Water Quality index/indices

Water quality indices (WQI) serve as another tool in the decision-making process. A water quality index is the basis of water pollution control and can be applied in both surface and groundwater quality assessment (Yang *et al.,* 2012). It promotes understanding of the water quality status, which reflects the composite influences of physical, chemical and biological parameters, and helps to formulate management plans for the aquatic sources to be assessed (Bora & Goswami, 2016). WQI receives complex sets of water quality data and transforms these into a single value of exploitable information that provides extensive interpretation of water quality for various purposes like drinking and irrigation (Liou *et al*., 2004). Each variable contributing to the index receives its own unique rating curve based on standards from the World Health Organisation (WHO), RQO and various other quality indices. The WQI is calculated as the weighted average of all the parameters, showing the degree to which the water quality was effected by anthropogenic activities, and usually assigning a numerical value out of a hundred. This makes WQI the most effective tool to present deterioration of water quality on a visual scale (Wanda *et al.,* 2016).

2.11.2 GIS

The necessity of integrating GIS have recently been identified due to its increased availability and affordability. GIS is a computer-based tool for mapping and analysing spatial data. A fundamental part of GIS is the ability to store, analyse, and combine large, diverse datasets within databases for use in models. GIS can also aid in predicting outcomes, explaining events and planning strategies (Sala & Vighi, 2008). Spatial data is crucial for GIS planning and management and without spatial data it is impossible for models to be developed (Wang *et al*., 2005). GIS will integrate spatial data with numerous other data resources and database systems. GIS has become a key factor in water quality assessment as it allows for identification of temporal and spatial changes in landscape, mining activities, industrial start-ups, informal settlements and agriculture. It helps to explain on a visual scale why certain water quality changes took place within a certain area's water systems and adds another criterion in water quality monitoring, which contributes to more accurate results (Shooshtarian *et al*., 2018). Integrating GIS for zoning purposes further contributes to the decision-making process. Numerous papers integrate GIS as a modelling tool for finding patterns and impacts fundamental to the research. Shakya and Tiwari (2014) used GIS data around the Bagmati River in Nepal from 1979-2012 to identify land-use changes impacting the water quality of the river. Coming to the conclusion that increasing run-off from growing industrial and urban areas intensified polluting in the river.

A similar study was done in Punjab, India, where Pons *et al*. (2012) analysed GIS data from 1989-2016 and found that changing industrial land-use led to groundwater quality declining. Verro *et al*. (2002) developed a GIS-based procedure for site-specific pesticide risk assessment in surface water. Rozario *et al*. (2016), who focused on housing density, forest cover, agricultural land, erosion and road density in North Dakota in the U.S., used GIS to conclude that water treatment costs in the area changed due to forest cover affecting water quality. Europe developed the GIS-assisted model Geo-Referenced Regional Exposure Assessment Tool for European Rivers (GREAT-ER) to simulate chemical movement as it travels from the point source into the waste-water treatment plant, ultimately ending up in a river (Sala & Vighi, 2008). The number of studies improved by GIS shows its capability in water quality assessment and emphasises the importance of adding GIS to the decision-making process. Although, without data, none of this would be possible. It is thus crucial to combine GIS and the entire decision-making process with DM.

2.12 Summary

Water deterioration globally needs to be addressed. During this study the monitoring of water quality of South African Rivers, specifically the Mooi River and Wonderfonteinspruit River, was done to bring us one step closer to the global vision where water becomes a sustainable resource. By identifying the RQO limits set for our WMA and comparing physico-chemical data—obtained through the process of DM—to these limits, it is possible to determine the water quality within our CMA—the Mooi River catchment. By implementing MCDM and combining information, one can analyse the water quality of the Mooi River catchment by creating water quality indices to compare the quality of sites to one another and to sites classified as healthy. Linking GIS and bacterial community structures to physico-chemical data can give insight into the geospatial factors impacting the quality of water and how this affects the bacterial community structure within selected sites.

CHAPTER 3 – MATERIALS AND METHODS

The study was conducted on the Mooi River and Wonderfonteinspruit River within the Mooi River catchment. Sites were selected—five Mooi River sites and six Wonderfonteinspruit River sites—and physico-chemical data from around 1965 to 2017 were gathered through DM. Microbiological data were also available for sites close to the selected physico-chemical data sites for the years 2015 and 2016. The available microbiological data gave insight into the bacterial community structure within those sites. Using the physico-chemical data, water quality indices were created. The microbiological data were separately analysed to reveal the bacterial communities and afterwards used in multivariate analysis with the physico-chemical data. Physico-chemical sites closest to microbiological sites were matched and analysed followed by the geospatial analysis, which aimed to explain the impacts land-use activities had on the various physico-chemical parameters within each site.

3.1 Study sites

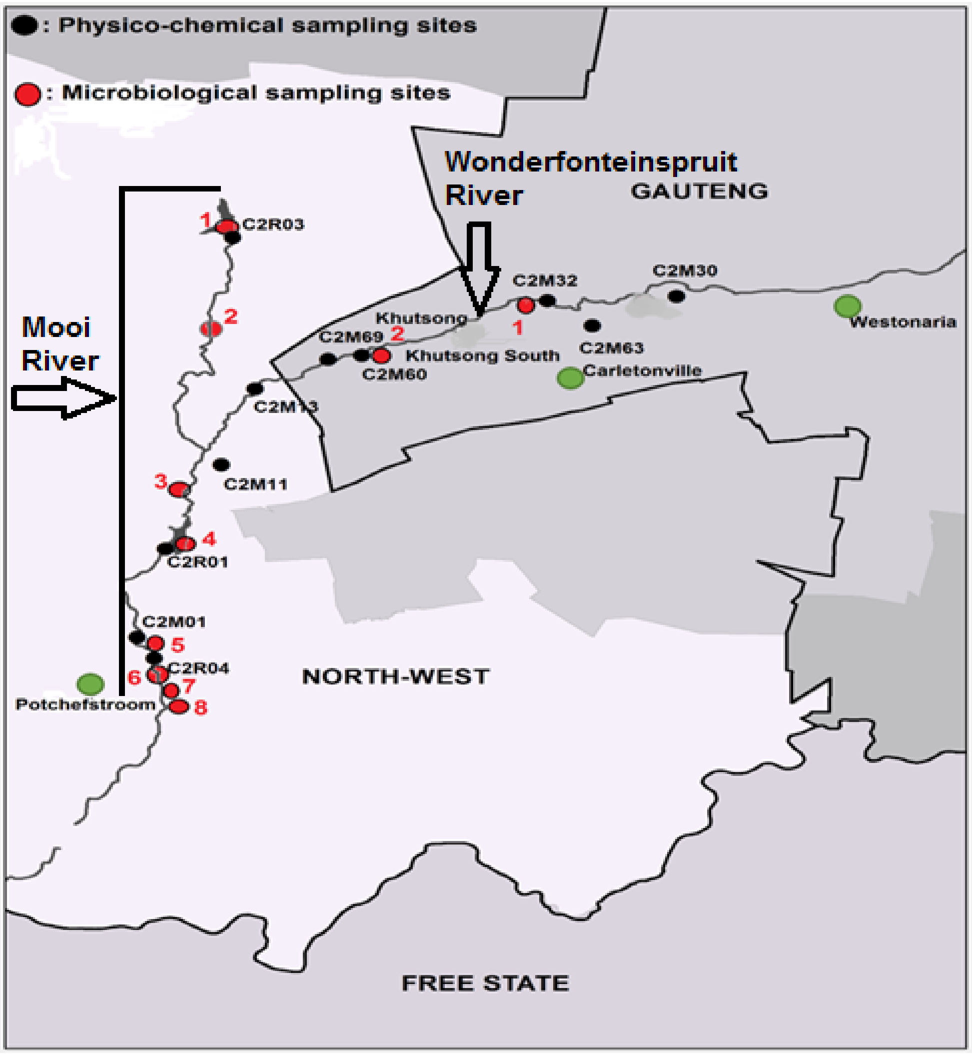

Figure 3.1: Location of sites of the historical physic-chemical and microbiological sampling collection

3.1.1 Mooi River

The Mooi River is situated within the North-West province and the Wonderfonteinspruit River crosses over from the Gauteng province. The Mooi river area within North-West province has four major dams: Klipdrift, Boskop, Klerkskraal and Potchefstroom (Van der Walt *et al.*, 2002). The Klerkskraal, Boskop and Potchefstroom dams are the largest and are used to store water to be treated for human consumption for the town of Potchefstroom. The Klipdrift dam was excluded during the study because little data was available for this site. Mining, agriculture and urbanisation are present within this catchment area (Coetzee *et al*., 2006; Hamman, 2012; Jordaan and Bezuidenhout, 2016; Van der Walt *et al.*, 2002; Wade *et al*., 2002)

The Mooi River originates at the Bovenste Eye, a dolomitic spring near Ventersdorp (Mathopestad). The river flows into the Klerkskraal Dam, through the Boskop Dam and then into the Potchefstroom Dam. Before entering Boskop, it confluences with the Wonderfonteinspruit River. The Mooi River flows through the town of Potchefstroom, then on the southern edge of the town the river confluences with the Loopspruit River before it flows into the Vaal River (Hamman, 2012). Further details of the three major dams are described in Table 3.1.

3.1.2 Wonderfonteinspruit River

The Wonderfonteinspruit River (WFS) consists of two sections: The Upper- and the Lower-Wonderfonteinspruit segments. The Upper WFS originates south of Krugersdorp at the Tudor Dam, and ends near Westonaria in the Donaldson Dam. This area has been impacted by mining since 1887 and although most mines have been closed or abandoned, they left behind rehabilitated rock-and-sand dumps as well as slime dams (Coetzee *et al*., 2006; Hamman, 2012). Numerous studies identified the Wonderfonteinspruit River as an area of significant pollution due to various factors including mining, agriculture and erosion (Coetzee *et al*., 2006; Winde, 2016). The Lower WFS starts below the Donaldson Dam and continues until it meets the Mooi River (Coetzee *et al*., 2006, Winde, 2016). The confluences of the Wonderfonteinspruit with the Mooi River takes place just above site C2M11.

With the aim at preventing recirculation, water from underground mining activities in the Wonderfonteinspruit area is discharged into the Wonderfonteinspruit River via canals and pipelines, and sometimes into adjacent catchments. The origins of the discharged water lead to varied consequences for the receiving water bodies. This includes increasing sediment loads and contamination with dissolved pollutants such as sulphates and metals. At some gold mines along the Wonderfonteinspruit River, discharging of fissure water had to be stopped, as it exceeded ionising radiation limits and contained elevated concentrations of sulphates (Coetzee *et al*., 2006).

Table 3.1: Details of dams in the Mooi River catchment (Annandale, E. and Nealer, E., 2011)

Klerkskraal dam (C2R03)	The Klerkskraal Dam (full capacity of 8 million m^3 has the largest surface area (383 ha) in the Mooi River catchment. It's situated to the north of the confluence of the Wonderfonteinspruit, 30 km east of Ventersdorp. The Klerkskraal Dam is considered as fairly unpolluted as it is not impacted upon by any mining activities. This dam can be used to describe an unpolluted site.
Boskop dam (C2R01)	The Boskop Dam area is a proclaimed nature reserve ± 12 km north of Potchefstroom. It has a surface area of 373 ha and a catchment area of 3287 km^2. Potchefstroom's potable water is collected, stored and released from the Boskop Dam (capacity of 21 million m^3) to the purification works of the city. The water travels via two 12 km-long cement canals (Boskop right bank and left bank canals) and these are open to pollution. The dam is fed by the Mooi River, but also by underground water from the Gerhard Minnebron Eye.
Potchefstroom dam (C2R04)	The Potchefstroom Dam has a catchment area of 3632 km^2, a capacity of 2 million m^3, and a surface area of 77.3 ha. Situated downstream of the Boskop Dam, it offers an accumulation point for all upstream impacts (mining, agriculture, etc.). The Potchefstroom Dam is also one of the main water reservoirs for drinking water production for Potchefstroom.

3.2 Physico-chemical data

To conduct this study, historical physico-chemical data provided by Dr Andre Esterhuyzen, a retired senior lecturer in Microbiology at the North-West University in Potchefstroom, was used. The data was collected from around 1965 to 2017 as part of an informal monitoring programme and consisted of water quality parameter data for all the sites shown in black within Figure 3.1. The data was recorded in hard copy and had to be converted to electronic format. This was done using Microsoft Excel (2013).

Additional physico-chemical data used for analysis was obtained from the Department of Water Affairs (DWA). The water quality parameters for analysis were selected and included: electrical conductivity (EC), total dissolved salts (TDS), pH, sodium (Na), magnesium (Mg^{2+}), calcium (Ca^{2+}), silicon (Si), fluoride (F^-), chloride (Cl^-), nitrate/nitrite (NO_3-NO_2), ammonium (NH_4^+), sulphate (SO_4^{2-}) and phosphate (PO_4^{3-}). For the RQO limits, only EC, pH, SO_4^{2-}, PO_4^{3-}, NO_3-NO_2 were used to draw graphs as these parameters had the most variation (decreases and increases) and RQO limit breaking values during analysis period from around 1965 to 2017. They impacted the quality of water the most.

3.3 Simplified index

Water quality indices (WQI) were prepared for all Wonderfonteinspruit River and Mooi River sites for which time series data were available. Yearly, monthly and daily data were used in the creation of the WQI. Afterwards, the data were condensed into yearly index averages for all the studied sites. The score for each site was recorded and plotted. The scores range from 0–100, where higher scores represent higher water quality. The IWRM process for the Mooi River catchment set RQO limits to which water sources must abide. These limits aid in the identification of polluted water sources by identifying the physico-chemical parameters of concern and matching them to the guidelines or limits set by the RQO (DWA, 2016; Labuschagne, 2017). When the values exceeded the highest or lowest prescribed RQO limits, those specific sites were classified as polluted and given a corresponding score. Several elements and nutrients were selected for the WQI based on numerous articles of other indices created for freshwater systems by various researchers (DWA, 2016; Labuschagne, 2017; Kawo & Karuppannan, 2018; Soleimani *et* al. 2018; Ewaid *et al*. 2018; Vasanthavigar *et al*. 2010, Liou *et al.* 2004; Cude, 2001; Ramakrishnaiah *et al*. 2009). PO_4^{3-}, SO_4^{2-}, pH, NO_3-NO_2, EC, F, Mg^{2+}, Cl^- and NH_4^+ were the variables of highest concern when compared to most identified studies. In Taiwan, Liou *et al.* (2004) used pH, NO_3-NO_2, NH_4^+ and Mg^{2+} in their index. Cude (2001) used pH, NH_4^+ and NO_3-NO_2 in the United States. Ramakrishnaiah *et al*. (2009) used pH, calcium, magnesium, NH_4^+, NO_3-NO_2, SO_4^2, PO_4^{3-} and F in India. The limits set for these variables are contained in Table 2.

Table 3.2: RQO limits for environmental health of different parameters of the Mooi River and Wonderfonteinspruit River

Parameter	Abbreviation	Units	Non-compulsory (1)	Intermediate (0.5)	Compulsory (0)
Phosphate	PO_4^{3-}	mg/L	≥ 0.125	≥ 0.09 but ≤ 0.125	≤ 0.09
Sulphate	SO_4^{2-}	mg/L	≥ 500	≥ 250 but ≤ 500	≤ 250
Nitrate-Nitrite	NO_3-NO_2	mg/L	≥ 4	≥ 3 but ≤ 4	≤ 3
Electrical conductivity	EC	mS/m	≥ 111	≥ 90 but ≤ 111	≤ 90
pH	pH	pH units	≤ 5.8 but ≥ 8.8	*	*
Magnesium	Mg^{2+}	mg/L	≥ 60	≥ 50 but ≤ 60	≤ 50
Fluorine	F^-	mg/L	≥ 3	≥ 2.54 but ≤ 3	≤ 2.54
Chloride	Cl^-	mg/L	≥ 400	≥ 280 but ≤ 400	≤ 280
Ammonium	NH_4^+	mg/L	≥ 5	≥ 4 but ≤ 5	≤ 4
Sodium	Na^+	mg/L	≥ 200	≥ 180 but ≤ 200	≤ 180

Table 3.3: RQO limits for environmental health of different parameters of the Boskop dam (C2R01), Potchefstroom dam (C2R04) and Klerkskraal dam (C2R03)

Parameter	Abbreviation	Units	Non-compulsory (1)	Intermediate (0.5)	Compulsory (0)
Phosphate	PO_4^{3-}	mg/L	≥ 0.025	≥ 0.015 but ≤ 0.025	≤ 0.015
Sulphate	SO_4^{2-}	mg/L	≥ 150	≥ 125 but ≤ 150	≤ 125
Nitrate-Nitrite	NO_3-NO_2	mg/L	≥ 1	≥ 0.5 but ≤ 1	≤ 0.5
Electrical conductivity	EC	mS/m	≥ 85	≥ 70 but ≤ 85	≤ 70
pH	pH	pH units	≤ 5.8 but ≥ 8.8	*	*

Magnesium	Mg^{2+}	mg/L	≥ 60	≥ 50 but ≤ 60	≤ 50
Fluorine	F^-	mg/L	≥ 3	≥ 2.54 but ≤ 3	≤ 2.54
Chloride	Cl^-	mg/L	≥ 75	≥ 60 but ≤ 75	≤ 60
Ammonium	NH_4^+	mg/L	≥ 5	≥ 4 but ≤ 5	≤ 4
Sodium	Na^+	mg/L	≥ 200	≥ 180 but ≤ 200	≤ 180

These limits represent the highest concentration values for a specific parameter. Different limits were set for sites C2R01, C2R03 and C2R04. This was done due to the fact that river and impoundment characteristics differ from one another. In the cases where no parameter limits were available for a specific impoundment site, the river parameter limit was used. This is easily interchangeable and as soon as a RQO limit for the missing parameter is available, it can be substituted with the river limit. When the parameters exceed these limits, they are seen as a polluting factor within the specific aquatic system. When the parameters exceeded these values, they were assigned a value of 1. Intermediate pollution concentrations were also considered, they were assigned a value of 0.5 (in some cases 0.25 when sub pollution limits were available) and when the parameters were comfortably below these limits and considered compulsory, they were assigned a value of 0. By combining and summing the assigned values for each parameter (which was done by Excel 2013), a value out of 10 was generated. This value was converted to a value out of a 100 by multiplying the value by 10 and subtracting it from 100.

WQIv= water quality index value

i= value out of 10

WQIv= 100- (i*10)

Table 3.4: Example of water quality index calculations for one day in 2014.

Site	**C2M32**									
Parameter	SO_4^{2-}	PO_4^{3-}	NO_3-NO_2	**EC**	**pH**	Mg^{2+}	F^-	Cl^-	NH_4^+	Na^+
Value (2014)	284.43 mg/L	0.89 mg/L	1.99 mg/L	115.21 mS/m	8,435 pH units	28,526 mg/L	0,331 mg/L	49,316 mg/L	0,289 mg/L	69,700 mg/L
Assigned values	0.5	1	0	1	0	0	0	0	0	0

WQIv= water quality index value

i= 2.5

WQIv= 100- (2.5*10)

WQIv=75 out of 100

3.4 Microbiological data

Water samples for DNA extraction (for 16S microbiome analysis) were collected at ten different sites in the Mooi River during 2015 and 2016 for a Water Research Commission (WRC) project (Bezuidenhout *et al*., 2017). Eight sites were in the Mooi River and two sites in the Wonderfonteinspruit River as shown in red within Figure 3.1. This data originates from the abovementioned project, and was not sampled. The sequencing results were obtained and processed. Methodology for DNA extraction and sequencing is summarised in 3.2.1 below.

3.4.1 16S rRNA gene amplification and MiSeq sequencing

DNA was extracted directly from water and quantified as described in Bezuidenhout *et al*. (2017). Illumina 16S metagenomics sequencing was performed on all water samples collected across all ten sites in the Mooi River catchment area. Locus-specific primer sequences were used to target the 16S V3-V4 region of the bacterial 16S rRNA gene (≈460bp). Gene-specific primers, 341F (Forward) and 805R (Reverse) (Klindworth *et al*.,2013), attached to the Illumina forward and reverse overhang adapters, were used for amplification:

- 16S amplicon forward primer 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3' and
- 16S amplicon reverse primer 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3' (Illumina).

All PCR components and protocols were exactly as reported in the library preparation guide (Illumina) and were conducted by Dr C Mienie. The workflow that was used entailed: a first-stage 'amplicon PCR' aimed at amplifying the region of interest by using 12.5 ng genomic DNA, 2x KAPA HiFi HotStart ReadyMix and 5 µM of forward and reverse primers. PCR was carried out in a 25 µl final volume by denaturing for 3 min at 95°C followed by 25 cycles of 95°C for 30 sec, 55°C for 30 sec and 72°C for 30 sec. A final elongation step of 72°C for 5 min was included. Amplicons were then subjected to a 'PCR clean-up' step using Agencourt AMPure XP beads (Beckman Coulter Genomics, California, USA). Following clean-up, index PCR attaching dual indexes (Nextera XT Index Kit) was performed using 5 µl of amplicon PCR product DNA, 5 µl of Illumina Nextera XT Index Primer 1 (N7xx), 5 µl of Nextera XT Index Primer 2 (S5xx), 25 µl of 2x KAPA HiFi HotStart Ready Mix, and 10 µl of PCR-grade water. The thermocycling conditions were 95°C for 3 minutes followed by 8 cycles of 95°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds and a final elongation step of 72°C for 5 minutes. Index PCR products were subjected to a second 'PCR clean-up' step. The partial 16S rDNA libraries were then quantified using a Qubit fluorometer (Qubit 3.0, Life Technologies, Malaysia), normalized, pooled to a final concentration of 20 pM, and denatured in 0.2 N NaOH. The pooled library was diluted to a final concentration of 3 pM, spiked with 5% PhiX control and heat denatured for 2 min prior to loading samples on the MiSeq V3 reagent cartridge (Illumina, San Diego, CA, USA). Following completion of a 2x300 bp paired-end reads sequencing run on the Illumina MiSeq, de-multiplexing and secondary analyses of the reads were performed using the MiSeq reporter software (Illumina, San Diego, CA, USA).

PCR amplification was done with KAPA HiFi Hotstart ReadyMix (KAPA Biosystems) according to the Illumina protocol. Amplicons were purified with Agencourt AMPure XP beads (Beckman Coulter Genomics, California, USA). Dual indexes (Nextera XT Index Kit) were attached with 9 cycles of PCR, followed by a final clean-up step. Amplicons were quantified using a Qubit fluorometer (Qubit 3.0, Life Technologies, Malaysia), normalized and pooled. A final concentration of 3 pM, spiked with 5% PhiX was analysed on a 2 x 300 bp MiSeq V3 reagent cartridge (Illumina, San Diego, CA, USA). Paired-end reads were de-multiplexed with MiSeq reporter software and further analysed.

3.4.2 Data processing

Amplicon sequencing (explained above) resulted in two FASTQ files, a forward and reverse read. The FASTQ files contained the sequences as well as quality information for base identification. After receiving the FASTQ files, the FastQC tool (v0.0.13) (Andrews, 2010) was incorporated for quality determination of the raw reads. The amplicon analysis was done using quantitative insights into microbial ecology (QIIME) (Caporaso *et al.,* 2010). Using one of QIIME's built-in paired-end DNA sequence assemblers (PANDAseq), the forward and reverse reads were merged according to a simple Bayesian algorithm with a minimum specified sequence length of 420 (Masella *et al.,* 2012). A single FASTA file with QIIME labels was then generated by combining the merged sequences. Making use of pick_open_reference_otus.py lead to operational taxonomic units (OTU)-picking at 97%-sequence identity threshold which corrected errors, filtered noisy reads and removed chimeras. Using usearch61 with SILVA 128 set as database reference. Alpha diversity (Faith's Phylogenetic Diversity index "PD_Whole_Tree", Chao1, Simpson and Shannon's index) and beta diversity (Weighted/Unweighted UniFrac, and Bray-Curtis) were then calculated (Kuczynski *et al*., 2012). The BIOM files generated by QIIME was then converted into text files and the OTU tables were further processed by R programming to modify, sort and organize the OTU data to fit the format of Excel 2016, which was used for simplified visual analysis. Bar charts were drawn up to analyse and compare different phyla and families of the organisms identified in all samples from each site based on relative abundance.

3.5 Statistical analysis

For statistical analysis annual levels of EC, pH, SO_4^{2-}, PO_4^{3-}, NO_3-NO_2, NH_4^+ and index score was used. These parameters had the most impact on water quality in the Mooi River catchment and can be linked to agriculture, mining, industries and factors such as erosion. Where necessary, Microsoft Excel (2013) was used to calculate averages, maximums and minimums. The gathered data for this project was analysed using IBM SPSS Statistics software (version 25) (IBM Corp, 2017). Linear regression was done on physico-chemical time series data, with year being the independent variable and the different parameters being the dependent variables. The Durbin-Watson test was used to identify autocorrelation across all years for a specific site to determine whether previous years had an impact on the following year. A Durbin-Watson value of 2 or -2 shows no autocorrelation and values moving further away from 2 or -2 show more autocorrelation. In other words, the closer a Durbin–Watson value is to 2, the less variation is found across time. The closer the value is to 0, the stronger the positive correlation gets and the closer the value is to 4, the stronger the negative correlation becomes.

After linear regression the data was also put through a univariate general linear model which ran ANOVA for year and parameters, with Bonferroni as the post-hoc test to identify statistical significance between years when the p-value is lower than 0.05. Durbin-Watson goes hand in hand with statistical significance, as Durbin-Watson shows whether there was any variation and statistical significance shows whether the variation was significant.

Principal Component Analyses (PCA) and redundancy analyses were conducted using CANOCO for Windows Version 4.5 (Ter Braak & Smilauer, 1997) on all the parameters. This was done to indicate the relationships between physico-chemical parameters and different site interactions over time in the Mooi River and Wonderfonteinspruit River.

Redundancy analyses (RDA) were done using CANOCO for Windows Version 4.5 (Ter Braak & Smilauer, 1997) to indicate the relationship between physico-chemical parameters and microbiological compositions for each site, as well as response curves to identify positive and negative microbial correlations to certain parameters. The 10 most abundant bacterial phylums were selected for the RDA and response curve analysis.

3.6 Geospatial analysis

Land-use data was provided by Mr T de Klerk from the GIS facility of the North-West University. Some of the data came from Bezuidenhout *et al*. (2017). Graphs were prepared in Microsoft Excel. Analyses to relate the impact of land-use on physico-chemical parameters were done using the neural network Alyuda ForecasterXL (Alyuda, 2001), which is an add-on in Microsoft Excel (2013). Land-use data was used as the first variable and physico-chemical data was used as the second variable. The physico-chemical data was used in different combinations against the land use data, and Alyuda ForecasterXL identified patterns, where various land-use activities impacted specific physico-chemical parameters. The water quality index score was also used as a variable on its own and used in a combination where land-use and physico-chemical parameters were grouped together. Alyuda ForecasterXL aimed to calculate how the land-use activities and the physico-chemical parameters impact water quality.

CHAPTER 4 – RESULTS

Within this chapter the individual sites were analysed by identifying the concentrations of PO_4^{3-}, SO_4^{2-}, NO_3-NO_2, pH and EC within each site. The selected parameters were compared with the acceptable RQO limits set by the DWS. Statistics were done for each site to describe yearly increases or decreases of selected parameters and whether they were statistically significant from year to year. Multivariate analysis was done to explain the relationships between each year and parameter. Water quality indices were created using the physico-chemical data for each site. The bacterial community structure was identified for the Mooi River and Wonderfonteinspruit River and multivariate analysis was done to show the relationships between bacterial phyla and families from different sites with physico-chemical parameters. Afterwards, neural network analysis was done to determine the geospatial activities impacting the water quality within the Mooi River area.

4.1 Characteristics of the water quality at the individual sites

4.1.1 Site C2M11

C2M11 is found at the furthest downstream sample point of the Wonderfonteinspruit River just before the Wonderfonteinspruit River enters the Mooi River. It is situated between C2R03 and C2R01, just below C2M13 as seen in Figure 3.1. The data trends from 1969 to 2017 as seen in Figure 4.1. There were only two data points between 1969 and 1979. The phosphate levels measured in this period were high. This could be a biased result. However, there were elevated levels during 2002 and 2012.

Site C2M11 had a pH average of 8.02 during the analysis period. The pH exceeded the RQO limit once during 1980 with a measured value of 9.62. The EC values for C2M11 increased slightly over the analysis period and never exceeded the RQO limit of 111 mS/m. C2M11 had the highest Si average of all sites. The measured PO_4^{3-} average were the lowest of all river sites and only exceeded the RQO limit twice in 2002 (0.277 mg/L) and 2012 (0.393 mg/L) (Figure 4.1A). The SO_4^{2-} also never exceed the RQO limit, although an increase in the levels over time were observed (Figure 4.1B). C2M11 had the lowest NH_4^+ average of all river sites. C2M11 did not exceed the RQO levels for NO_3-NO_2. However, the levels were elevated (Figure 4.1C). The measured physico-chemical parameter averages were within the RQO limits and the water at this site could be classified as good quality.

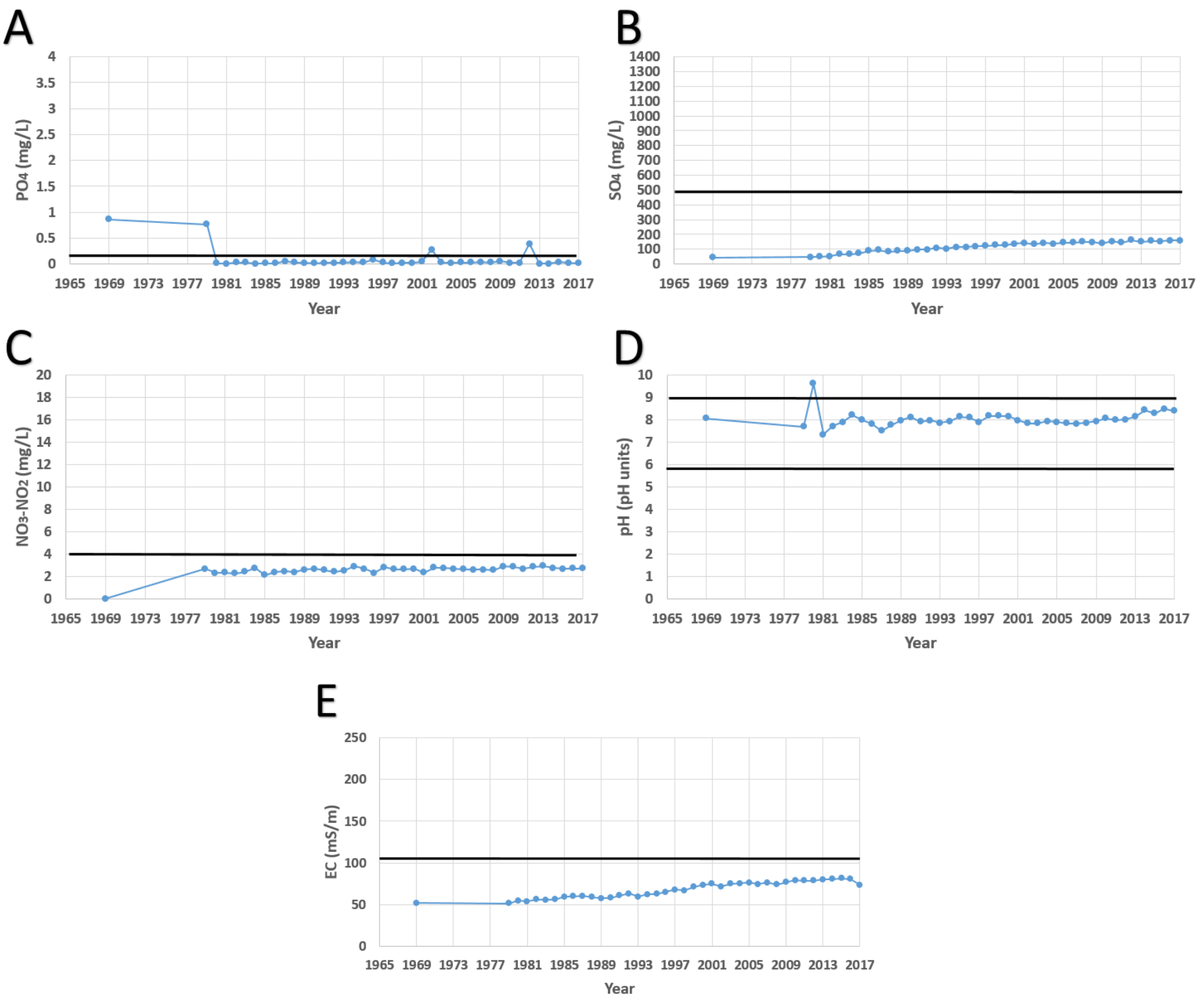

Figure 4.1: Physico chemical parameters and limits of site C2M11

Table 4.1: Yearly statistics of site C2M11

Dependent variable	Independent variable	Unstandardised coefficients		Unstandardised coefficients	t	Sig.	Durbin-Watson
		B	Std. Error	Beta			
EC	Year	0.789	0.017	0.891	47.67	0.000	1.675
pH	Year	-0.001	0.01	-0.006	-0.14	0.889	2
SO_4^{2-}	Year	3.008	0.072	0.863	41.6	0.000	1.727
PO_4^{3-}	Year	-0.006	0.005	-0.053	-1.24	0.214	2
NO_3-NO_2	Year	0.013	0.003	0.177	4.25	0.000	1.191
NH_4^+	Year	0.001	0.001	0.065	1.54	0.125	1.954
IDX	Year	-0.155	0.016	-0.371	-9.75	0.000	1.734

Site C2M11 (Table 4.1) showed autocorrelation for EC, SO_4^{2-}, IDX, NO_3-NO_2 and NH_4^+. Where pH, PO_4^{3-} showed no autocorrelation. Statistical significance for all except three parameters (pH, PO_4^{3-} and NH_4^+) were obeserved. The unstandardized coefficients B showed decreasing mean values for pH,

PO_4^{3-} and IDX as time went on, where EC, SO_4^{2-}, NO_3-NO_2 and NH_4^+ had increasing mean values over time. Here SO_4^{2-} had high annual increases of 3.008 mg/L, as can also be seen in Figure 4.1B.

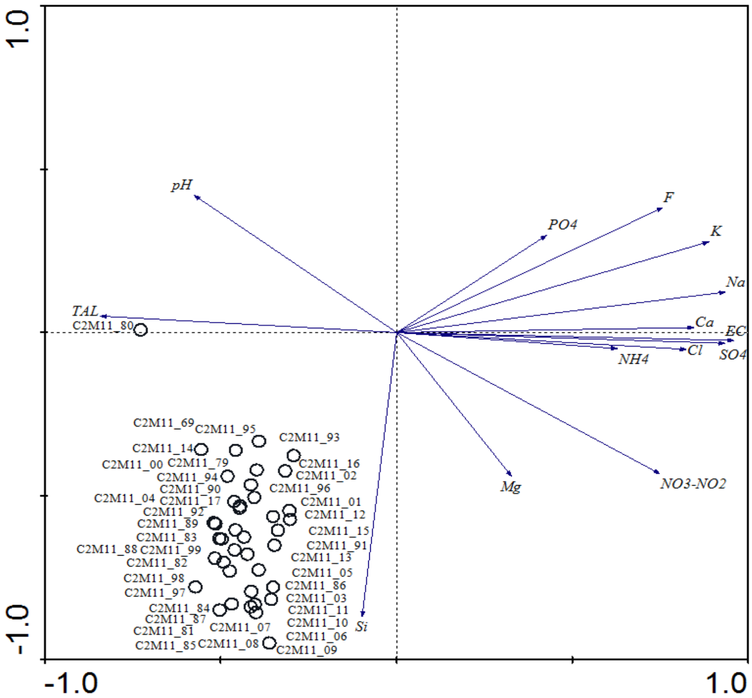

Figure 4.2: Physico-chemical PCA of site C2M11

The PCA of C2M11 (Figure 4.2) showed high levels of Si. The recorded values of PO_4^{3-}, SO_4^{2-}, EC and NO_3-NO_2 were low during the analysis period as seen in Figure 4.1. This trend was also apparent in the PCA (Figure 4.2) with the majority of the samples clustering at the lower end of the PO_4^{3-}, SO_4^{2-}, EC and NO_3-NO_2 gradients. During 1980 the pH exceeded the RQO limit of 9 by 0.62 as seen in Figure 4.1.

4.1.2 Site C2M01

C2M01 is situated between C2R01 and C2R04, but closer to C2R04, along the Mooi River as seen in Figure 3.1. The data trends from 1979 to 2017 as seen in Figure 4.3.

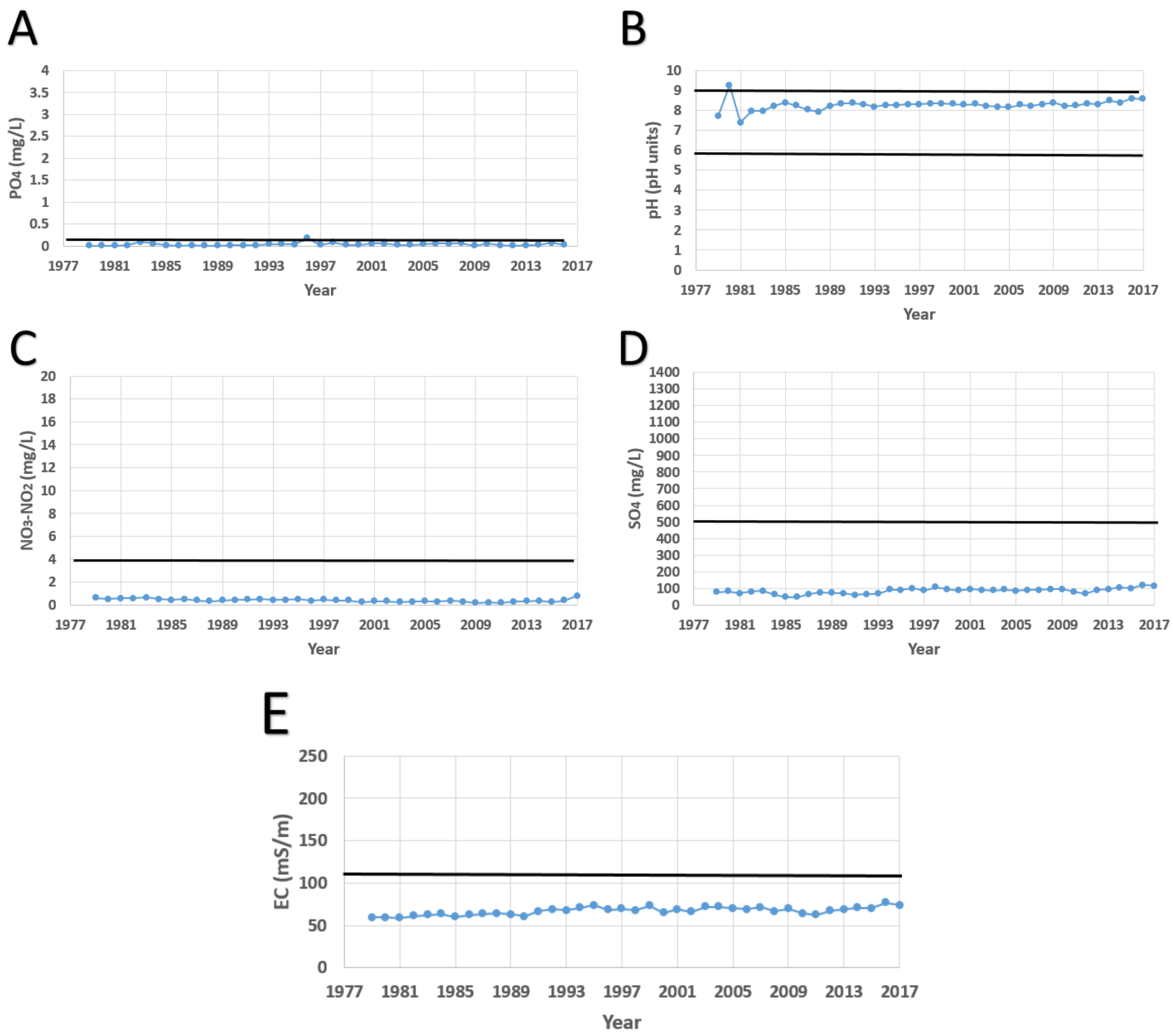

Figure 4.3: Physico chemical parameters and limits of site C2M01

All the parameters for C2M01 remained relatively constant. C2M01 had constant pH values, except during 1980 when the pH increased to 9.25 (Figure 4.3B). The average pH for the analysis period were 8.25. The data showed that 1978 to 1984 had pH < 8, but increases to 8.6 in 2017. High TAL values were measured. Even though the EC values are low, they also slightly increased over time (Figure 4.3E). C2M01 had low NO_3-NO_2 values during the analysis period. The lowest average of all river sites (Figure 4.3C). The measured NH_4^+ was low. This was also true for PO_4^{3-} and SO_4^{2-} (Figure 4.3A and D), although PO_4^{3-} slightly exceeded the RQO limit in 1996. The measured physico-chemical parameter averages were within the RQO limits and the water at this site could be classified as good quality.

Table 4.2: Yearly statistics of site C2M01

Dependent variable	Independent Variable	Unstandardised coefficients		Unstandardised coefficients	t	Sig.	Durbin-Watson
		B	Std. Error	Beta			
EC	Year	0.266	0.015	0.386	17.26	0.000	1.107
pH	Year	-0.057	0.042	-0.033	-1.36	0.173	2
SO_4^{2-}	Year	0.904	0.063	0.329	14.36	0.000	1.273
PO_4^{3-}	Year	0.000	0.00	-0.026	1.06	0.291	1.385
NO_3-NO_2	Year	-0.008	0.001	-0.031	-14.4	0.000	1.277
NH_4^+	Year	0.000	0.001	0.013	0.554	0.58	1.975
IDX	Year	-0.067	0.01	-0.153	-6.41	0.000	1.207

Site C2M01 (Table 4.2) showed autocorrelation for all parameters except pH and NH_4^+ and statistical significance for all except three parameters (pH, PO_4^{3-} and NH_4^+). The unstandardized coefficients B showed pH, NO_3-NO_2 and IDX had decreasing mean values over the years, where EC and SO_4^{2-} had increasing mean values. PO_4^{3-} and NH_4^+ also had increasing mean values, however they were smaller than 0.0001 mg/L per year.

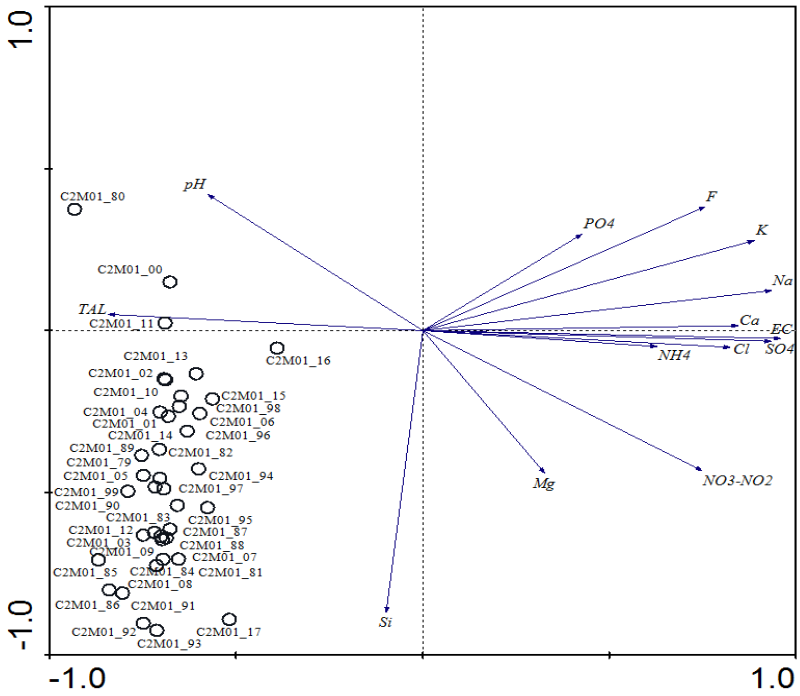

Figure 4.4: Physico-chemical PCA of site C2M01

The PCA of C2M01 (Figure 4.4) showed high levels of Si. The recorded values of PO_4^{3-}, SO_4^{2-}, EC and NO_3-NO_2 were low during the analysis period as seen in Figure 4.3. During 1980 the pH exceeded the RQO limit of 9 by 0.25 as seen in Figure 4.3B. The TAL was high during 2000 and 2011. Overall the trends that can be observed were very similar to that of site C2M11 (Figure 4.1).

4.1.3 Site C2R01

C2R01 is situated between C2M11 and C2M01 along the Mooi River as seen in Figure 3.1. The data trends from 1968 to 2017 as seen in Figure 4.5.

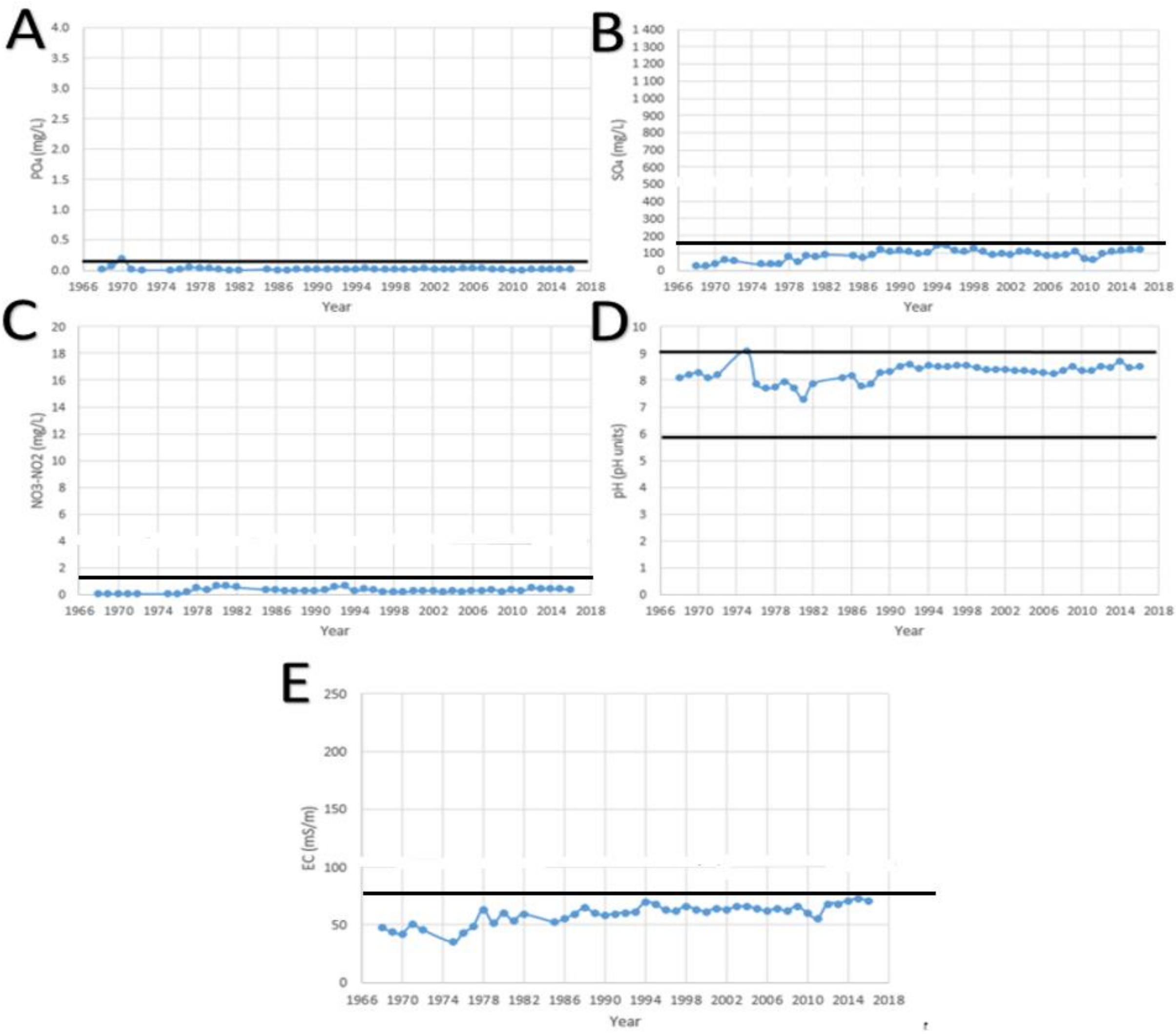

Figure 4.5: Physico chemical parameters and limits of site C2R01

C2R01 showed low PO_4^{3-}, SO_4^{2-}, NO_3-NO_2 and EC values. The SO_4^{2-} (Figure 4.5B), pH (Figure 4.5D) and EC (Figure 4.5E) showed increases during the analysis period. During 1975 the pH value exceeded the RQO limit by 0.11. The PO_4^{3-} values remained constant, except during 1970 where it exceeded the RQO limit by 0.051 mg/L (Figure 4.5A). The EC decreased during 1975 and then started increasing again. The measured physico-chemical parameter averages were within the RQO limits and the water at this site could be classified as good quality.

Table 4.3: Yearly statistics of site C2R01

Dependent variable	Independent Variable	Unstandardised coefficients		Unstandardised coefficients	t	Sig.	Durbin-Watson
		B	Std. Error	Beta			
EC	Year	0.341	0.024	0.395	14.25	0.000	0.918
pH	Year	0.013	0.001	0.361	12.83	0.000	0.67
SO_4^{2-}	Year	0.391	0.09	0.13	4.366	0.000	0.619
PO_4^{3-}	Year	0.000	0.000	0.043	1.426	0.154	1.789
NO_3-NO_2	Year	-0.001	0.001	-0.052	-1.733	0.083	0.739
NH_4^+	Year	0.000	0.000	-0.05	-1.651	0.099	1.235
IDX	Year	-0.029	0.007	-0.123	-4.109	0.000	1.642

Site C2R01 (Table 4.3) showed autocorrelation for all parameters. Statistical significance were not present for PO_4^{3-}, NO_3-NO_2 and NH_4^+. The unstandardized coefficients B showed decreasing mean values for NO_3-NO_2 and IDX. Having increasing mean values for PO_4^{3-}, SO_4^{2-}, NH_4^+, pH and EC. The SO_4^{2-} increased by an average of 0.391 mg/L per year. The EC increased by an average of 0.341 mS/m per year. IDX decreased by 0.029 per year.

4.1.4 Site C2R04

C2R04 situated beneath C2M01. This was the furthest downstream site in the Mooi River as seen in Figure 3.1. The data trends from 1979 to 2017 as seen in figure 4.6.

Site C2R04 showed low PO_4^{3-}, SO_4^{2-}, NO_3-NO_2 and EC values. The EC (Figure 4.6E), pH (Figure 4.6D) and SO_4^{2-} (Figure 4.6B) increased over time. All measured physico-chemical parameters were within the RQO limits and the water at this site could be classified as good quality.

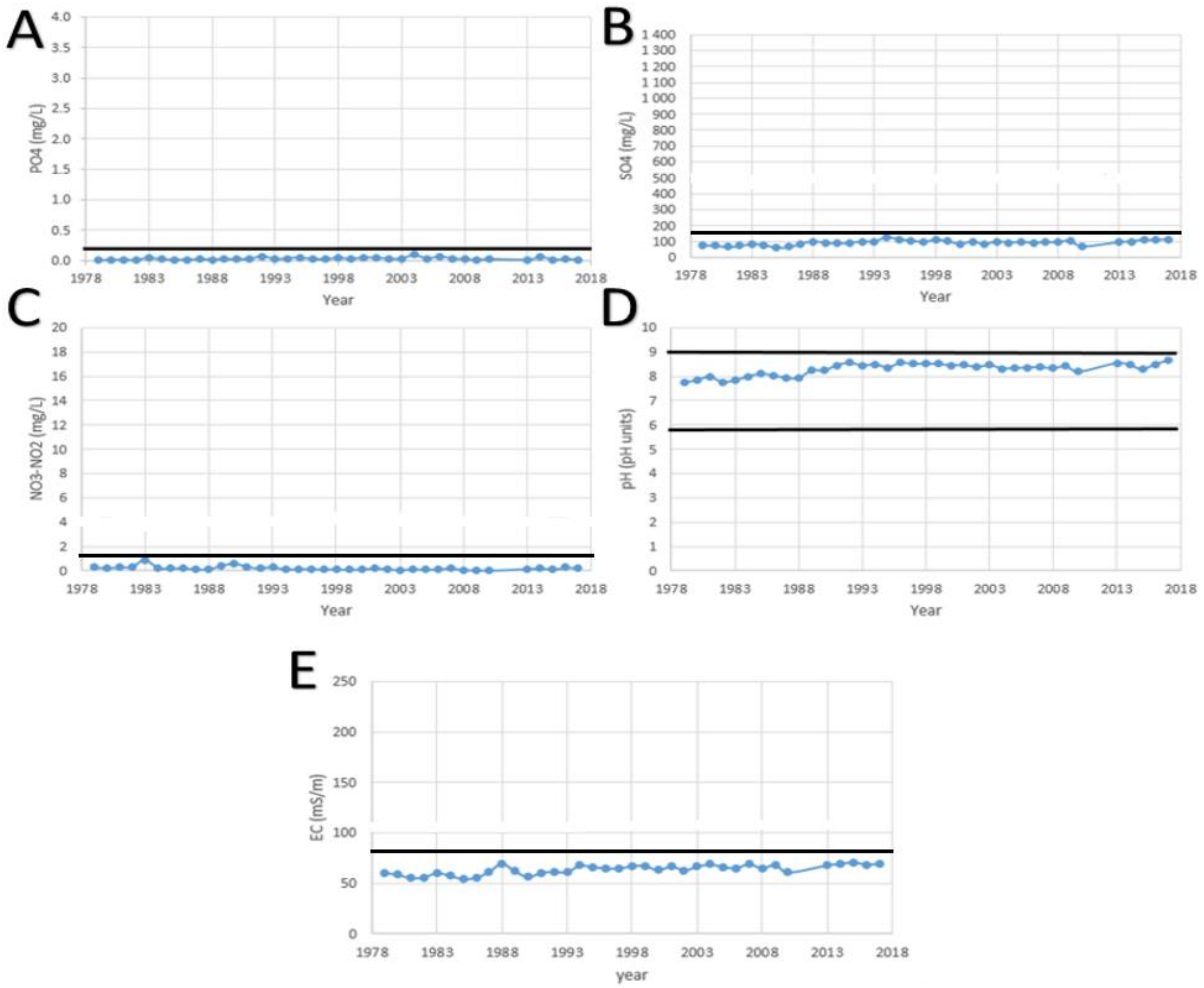

Figure 4.6: Physico chemical parameters and limits of site C2R04.

Table 4.4: Yearly statistics of site C2R04

Dependent variable	Independent Variable	Unstandardised coefficients		Unstandardised coefficients	t	Sig.	Durbin-Watson
		B	Std. Error	Beta			
EC	Year	0.315	0.025	0.506	12.74	0.000	1.219
pH	Year	0.024	0.002	0.569	15.06	0.000	1.16
SO_4^{2-}	Year	0.769	0.071	0.448	10.85	0.000	0.905
PO_4^{3-}	Year	0.001	0.000	0.138	3.01	0.003	1.796
NO_3-NO_2	Year	-0.005	0.002	-0.155	-3.37	0.001	1.411
NH_4^+	Year	0.002	0.002	0.054	1.18	0.241	2
IDX	Year	-0.061	0.012	-0.234	-5.24	0.000	1.682

Site C2R04 (Table 4.4) showed autocorrelation for all parameters except NH_4^+. This was also true for statistical significance. The unstandardized coefficients B showed decreasing mean values for NO_3-NO_2 and IDX. Having increasing mean values for PO_4^{3-}, SO_4^{2-}, NH_4^+, pH and EC. The SO_4^{2-} increased by an average of 0.769 mg/L per year. The EC increased by an avergae of 0.315 mS/m per year. IDX decreased by 0.061 per year.

4.1.5 Site C2R03

C2R03 was the most upstream site analysed in the Mooi River as seen in Figure 3.1. The data trends from 1972 to 2010 as seen in figure 4.7. Data available for this site was only recorded every other year, creating gaps during the beginning of the analysis period. This did not affect the trends identified.

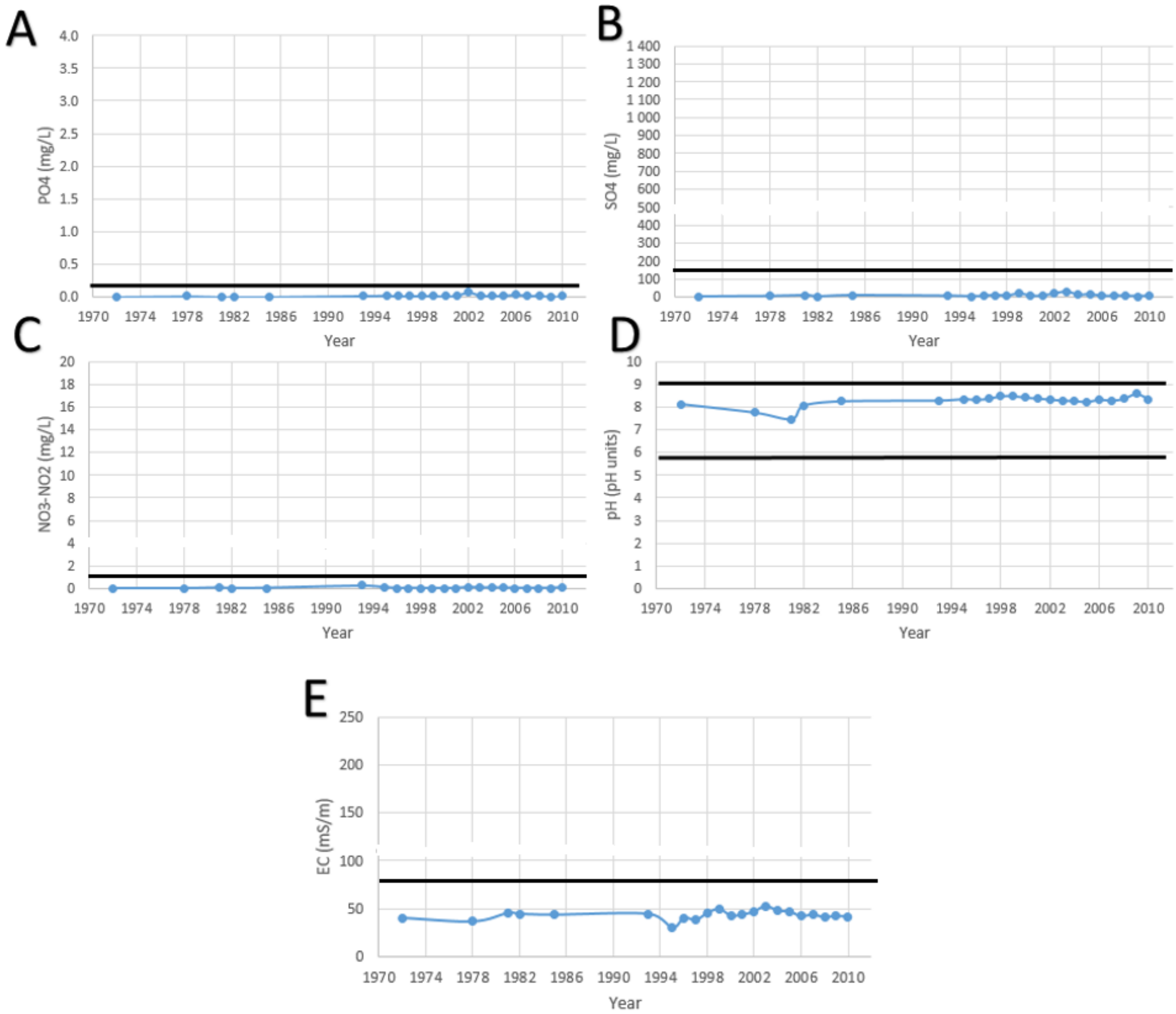

Figure 4.7: Physico chemical parameters and limits of site C2R03

Site C2R03 showed low PO_4^{3-}, SO_4^{2-}, NO_3-NO_2 and EC values. The EC (Figure 4.7E), pH (Figure 4.7D) and SO_4^{2-} (Figure 4.7B) increased over time. All measured physico-chemical parameters were within the RQO limits and the water at this site could be classified as good quality.

Table 4.5: Yearly statistics of site C2R03

Dependent variable	Independent Variable	Unstandardised coefficients		Unstandardised coefficients	t	Sig.	Durbin-Watson
		B	Std. Error	Beta			
EC	Year	0.205	0.068	0.223	3.011	0.003	1.156
pH	Year	0.004	0.002	0.167	2.24	0.026	1.554
SO_4^{2-}	Year	0.183	0.188	0.074	0.974	0.331	1.867
PO_4^{3-}	Year	0.001	0.000	0.141	1.858	0.65	1.989
NO_3-NO_2	Year	0.002	0.001	0.191	2.504	0.013	1.366
NH_4^+	Year	0.000	0.000	0.106	1.396	0.164	1.254
IDX	Year	0.095	0.021	0.331	4.624	0.000	2.28

Site C2R03 (Table 4.5) showed negative autocorrelation for IDX, with positive autocorrelation for pH, EC, NO_3-NO_2, NH_4^+, SO_4^{2-}, PO_4^{3-} (slight autocorrelation) and IDX. Showing statistical significance for all except three parameters (SO_4^{2-}, PO_4^{3-} and NH_4^+). These parameters showed no variation over time (Figure 4.7A and B) resulting in non statistical significant values. The unstandardized coefficients B showed increasing yearly averages for pH, PO_4^{3-}, SO_4^{2-}, NO_3-NO_2, NH_4^+, IDX and EC.

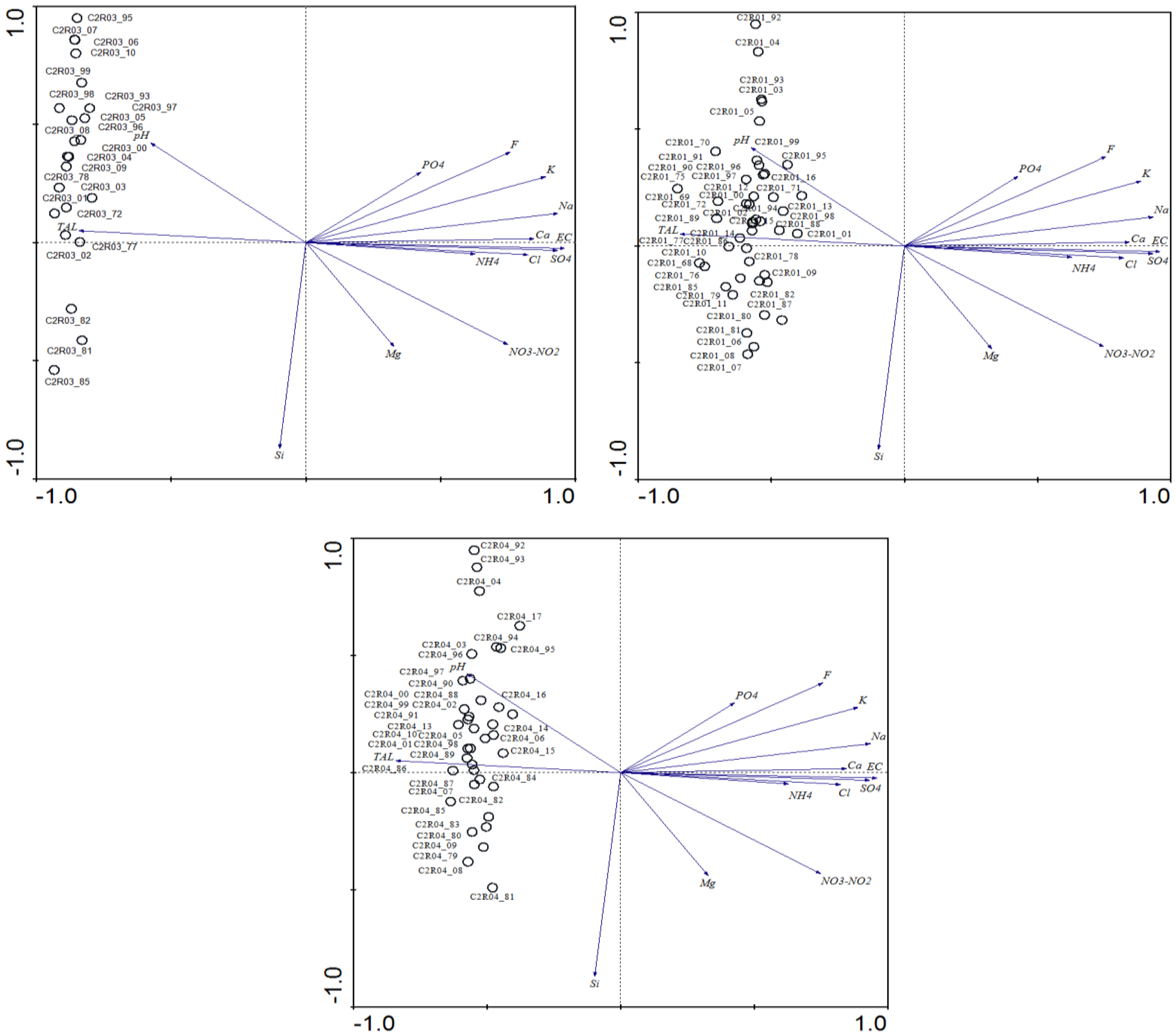

Figure 4.8: Physico-chemical PCA of site C2R01, C2R03 and C2R04

The PCA's for C2R01, C2R03 and C2R04 (Figure 4.8) all clusters around pH and TAL. These sites showed high TAL and pH levels. These three sites had an average recorded pH value of 8.3 during the analysis period. As seen in Figures 4.5, 4.6 and 4.7 the PO_4^{3-}, SO_4^{2-}, NO_3-NO_2 and EC values were low. This was also apparent in the PCA (Figure 4.8) with the majority of the samples being at the lower end of the PO_4^{3-}, SO_4^{2-}, EC and NO_3-NO_2 gradients.

4.1.6 Site C2M30

C2M30 was the most upstream site analysed within the Wonderfonteinspruit River. It is situated downstream of the West Rand area and close to Carletonville and Westonaria as seen in Figure 3.1. The data trends from 1978 to 2012 as seen in Figure 4.9.

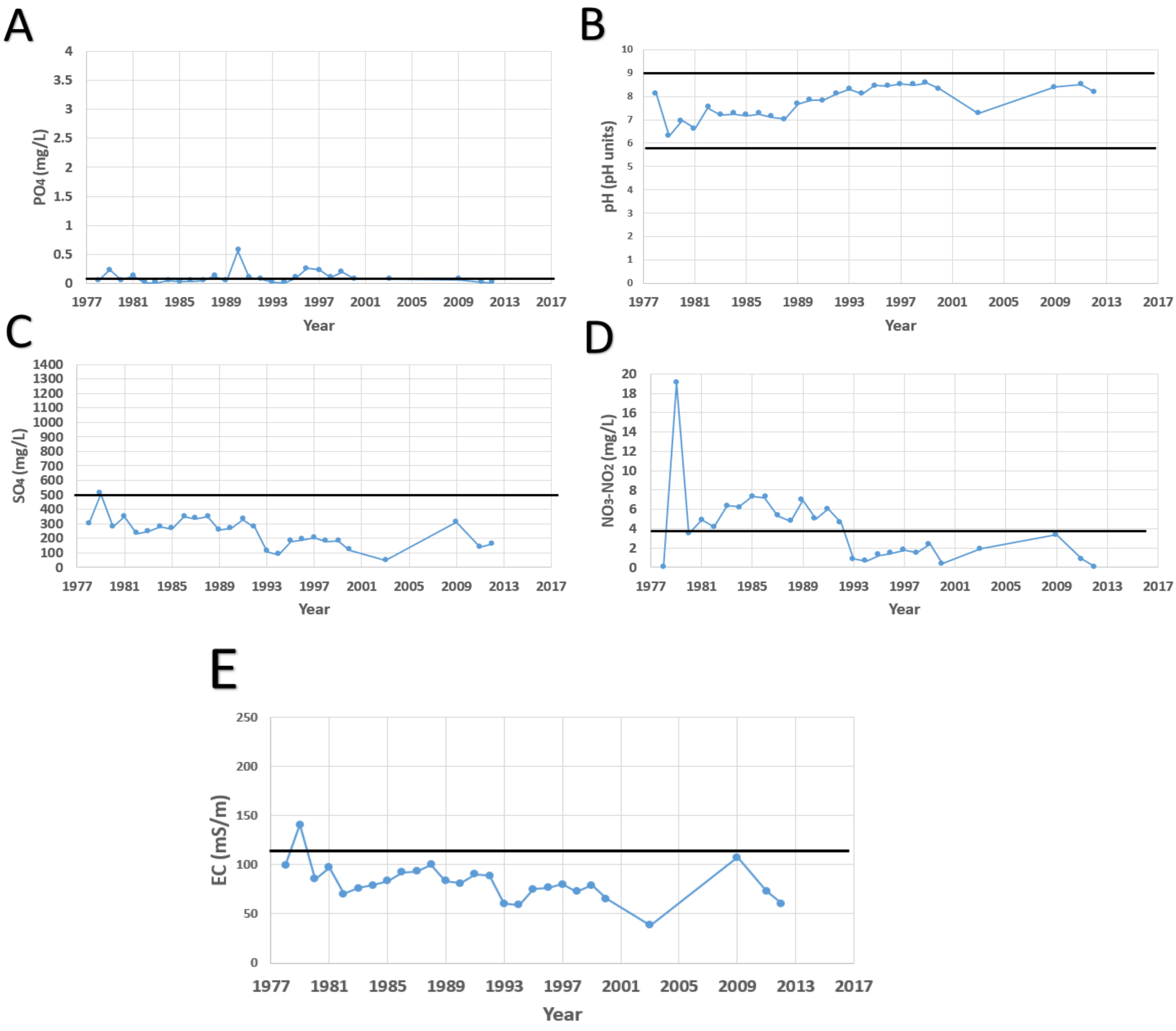

Figure 4.9: Physico chemical parameters and limits of site C2M30

The pH values never exceeded the RQO limit (Figure 4.9B). The 1980's had lower pH values (lowest of 6.3 and highest of 7.6) than the 1990's (lowest of 7.8 and highest of 8.5). This showed an increase in pH over the years (Figure 4.9B). This pattern is exactly repeated with TAL. The NO_3-NO_2 values were lowest in 1978 (0.025 mg/L) and highest only a year later in 1979 (19.1 mg/L). It exceeded the RQO limit from 1979 to 1992 with an average of 6.539 mg/L before decreasing. The lowest EC value of 38.7 mS/m were recorded in 2003 and the highest value of 141.03 mS/m in 1979, which exceeded the RQO limit by 30.03 mS/m (Figure 4.9E). The year 1979 had massive increases in NO_3-NO_2, SO_4^{2-}, PO_4^{3-}, EC, K and Na and very low TAL. The year 1979 was also the only time the SO_4^{2-} limit (508.13 mg/L) and EC limit (141.03 mS/m) was higher than the prescribe RQO limit of 500 mg/L and 111 mS/m, respectively (Figure 4.9C and E). The PO_4^{3-} limit was exceeded in 1979 by 0.105 mg/L, 1981 by 0.003 mg/L, 1988 by 0.007 mg/L, 1990 by 0.437 mg/L (which is the highest recorded value), 1996 by 0.135 mg/L, 1997 by 0.116 mg/L and 1999 by 0.07 mg/L. This showed that the 1990s had the most limit breaking PO_4^{3-} values.

Table 4.6: Yearly statistics of site C2M30

Dependent variable	Independent variable	Unstandardised coefficients		Unstandardised coefficients	t	Sig.	Durbin-Watson
		B	Std. Error	Beta			
EC	**Year**	**0.023**	**0.152**	**0.009**	**0.152**	**0.879**	**1.439**
pH	**Year**	**0.083**	**0.004**	**0.785**	**21.26**	**0.000**	**0.858**
SO_4^{2-}	**Year**	**-2.325**	**0.705**	**-0.193**	**-3.29**	**0.001**	**1.414**
PO_4^{3-}	**Year**	**0.017**	**0.006**	**0.177**	**3.012**	**0.003**	**1.972**
NO_3-NO_2	**Year**	**-0.12**	**0.027**	**-0.252**	**-4.37**	**0.000**	**0.837**
NH_4^+	**Year**	**-0.006**	**0.008**	**-0.046**	**-0.77**	**0.443**	**2**
IDX	**Year**	**-0.394**	**0.099**	**-0.231**	**-3.98**	**0.000**	**1.655**

Site C2M30 (Table 4.6) showed autocorrelation for all except NH_4^+ and statistical significance for all except two parameters (EC and NH_4^+). The unstandardized coefficients B showed increasing mean values for EC, pH and PO_4^{3-} . Where NO_3-NO_2, NH_4^+ and IDX had decreasing mean values over time, especailly SO_4^{2-} which decreased with an average of 2.325 mg/L per year.

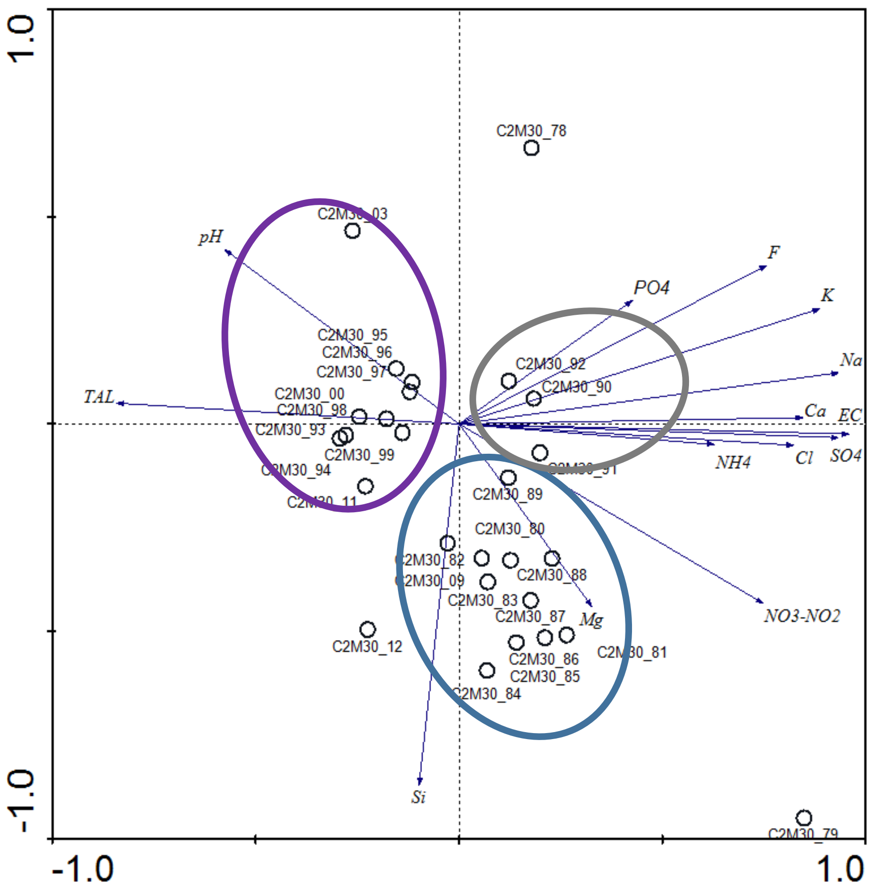

Figure 4.10: Physico-chemical PCA of site C2M30

From the PCA ordination 3 distinct groupings were apparent (Figure 4.10). Grouping 1 (blue) represent the timeframe of the 1980 to 1989, as well as 2009. During this time NO_3-NO_2 levels were high and pH low as can be seen in Figure 4.9B and D. Grouping 2 (purple) represents the period 1993 to 2000, as well as the year 2011. During this period the pH levels were high, but the NO_3-NO_2 and SO_4^{2-} low as seen in Figure 4.9B and D. Lastly group 3 (green) represents 1990 to 1992. During 1990 the PO_4^{3-} were the highest (Figure 4.9A)

4.1.7 Site C2M32

C2M32 is downstream of C2M30 within the Wonderfonteinspruit River as seen in Figure 3.1. It receives water flowing down from C2M30. The data trends from 1979 to 2017 as seen in Figure 4.11.

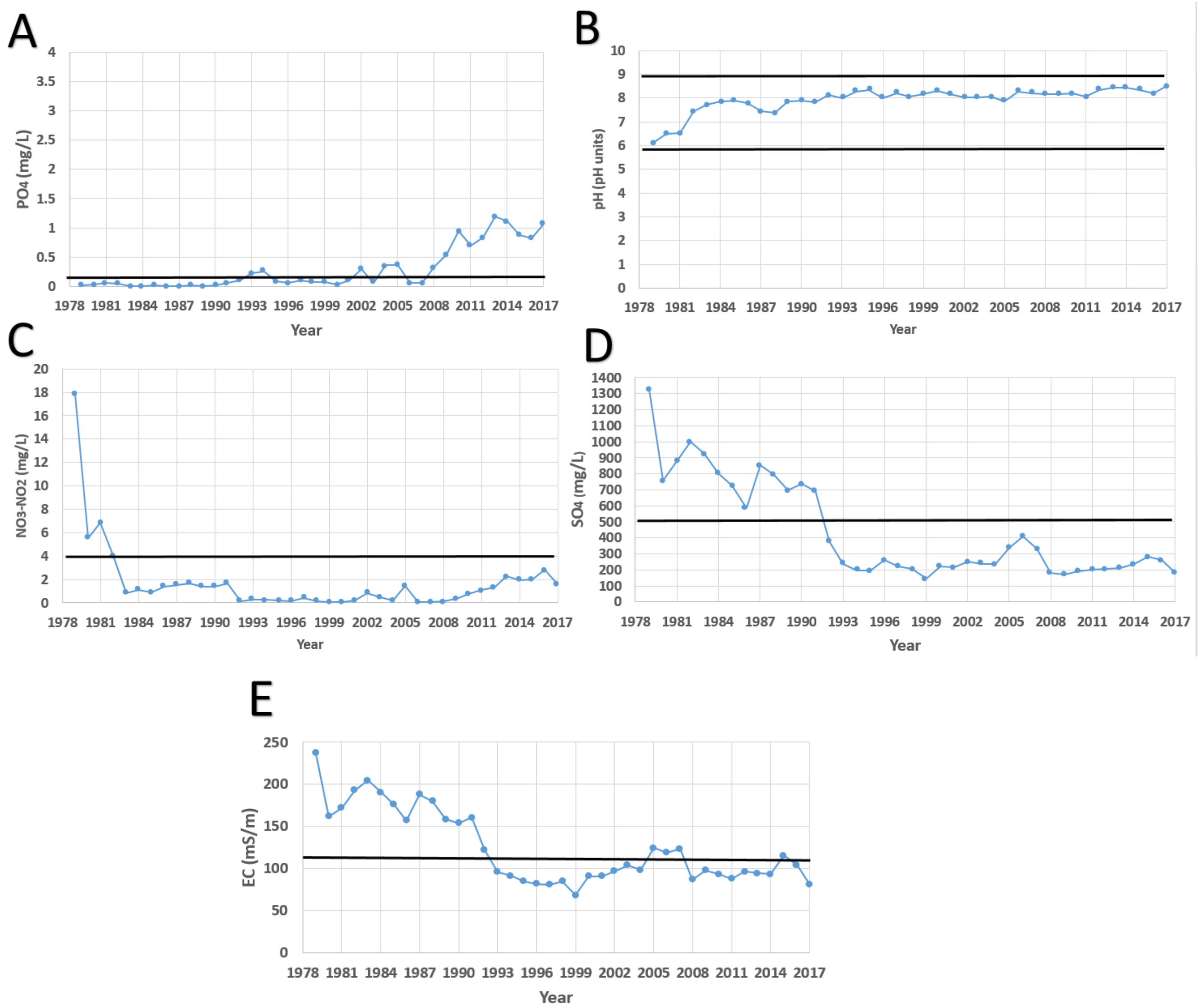

Figure 4.11: Physico chemical parameters and limits of site C2M32

The pH of C2M32 increased over the analysis period with no limit breaking values. The pH was low during 1979 with a value of 6.1, gradually increasing to 7.8 in 1989 (Figure 4.11B). The pH increased further in the 1990's reaching as high as 8.3 in 1995. Similar results were obtained for TAL. The PO_4^{3-} values fluctuated over time, but rapidly started to increase in 2001 and continued to increase throughout the 2000's reaching a high of 1.19 mg/L in 2013, the second highest value in 2014 (1.11 mg/L) and the third highest in 2017 (1.072 mg/L). The 2000's had an average PO_4^{3-} value of 0.605 mg/L, which is much higher than the prescribed RQO limit of 0.125mg/L (Figure 4.11A). The NO_3-NO_2 started with extremely high values in 1979 (17.81 mg/L), 1980 (5.56 mg/L), 1981 (6.84 mg/L) and 1982 (4 mg/L), which are all limit breaking RQO values. After 1983 the NO_3-NO_2 has no limit breaking RQO values and stays mostly constant, although it definitely showed higher values during the 1980's (Figure 4.11C). C2M30 had a decreasing EC trend, starting with limit breaking RQO EC values of 237.8 mS/m in 1979. The values decreased, but still remained high till 1992 with a value of 121.8 mS/m which exceeded the RQO limit by 10.8 mS/m. It decreased further after 1992, no longer exceeding the RQO limit (Figure 4.11E). The SO_4^{2-} values followed the same pattern as EC, having limit breaking RQO values from 1979 to 1991, averaging 801.45 mg/L in the 1980's; 300 mg/L higher than the prescribed RQO limit (Figure 4.11D).

Table 4.7: Yearly statistics of site C2M32

Dependent variable	Independent variable	Unstandardised coefficients		Unstandardised coefficients	t	Sig.	Durbin-Watson
		B	Std. Error	Beta			
EC	**Year**	**-3.578**	**0.116**	**-0.605**	**-30.7**	**0.000**	**1.326**
pH	**Year**	**0.046**	**0.002**	**0.545**	**26.44**	**0.000**	**1.223**
SO_4^{2-}	**Year**	**-26.68**	**0.685**	**-0.693**	**-39**	**0.000**	**1.187**
PO_4^{3-}	**Year**	**0.02**	**0.001**	**0.588**	**29.32**	**0.000**	**1.295**
NO_3-NO_2	**Year**	**-0.15**	**0.011**	**-0.325**	**13.9**	**0.000**	**1.226**
NH_4^+	**Year**	**-0.004**	**0.003**	**-0.032**	**-1.28**	**0.2**	**1.856**
IDX	**Year**	**0.824**	**0.029**	**0.568**	**28.04**	**0.000**	**1.337**

Site C2M32 (Table 4.7) showed autocorrelation for all parameters and statistical significance for all parameters except NH_4^+. The unstandardized coefficients B showed decreasing mean values for EC, SO_4^{2-}, NO_3-NO_2 and NH_4^+ as time went on, where pH, PO_4^{3-} and IDX had increasing mean values. The SO_4^{2-} decreased by 26.68 mg/L per year. The EC decreased by 3.578 mS/m per year. The IDX increased by 0.824 per year.

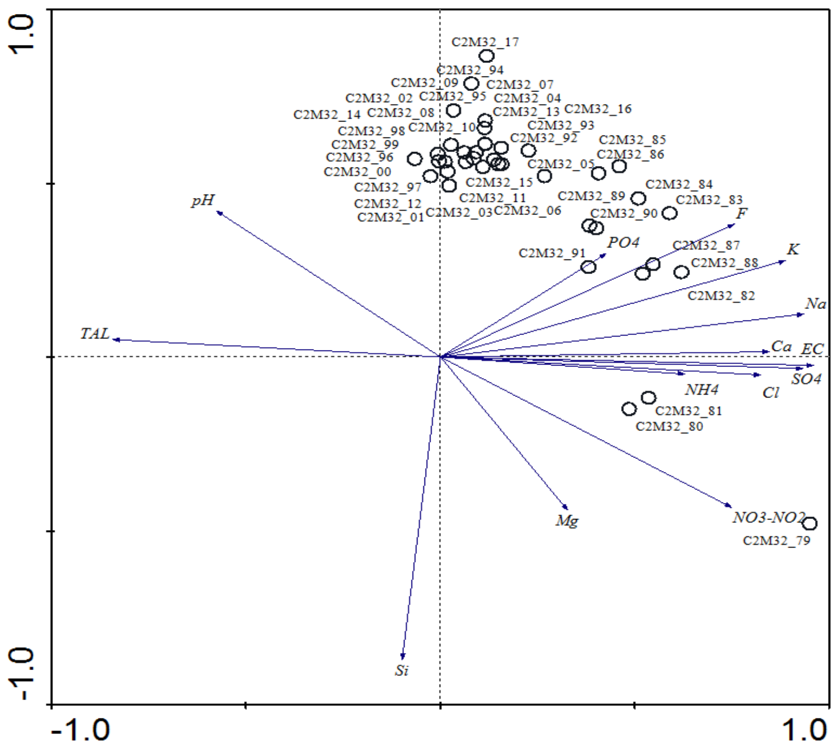

Figure 4.12: Physico-chemical PCA of site C2M32

The PCA of C2M32 (Figure 4.12) showed high levels of NO_3-NO_2 during 1979, 1980 and 1981 as seen in Figure 4.11C. The 1970's and 1980's showed high SO_4^{2-} and EC values with low PO_4^{3-}. This trend can also be seen in Figure 4.11A, D and E. From the PCA it was clearly visible that SO_4^{2-} and EC started decreasing and PO_4^{3-} started increasing during the 1990's and following this trend to the 2000's. This can also be seen in Figure 4.11A, D and E.

4.1.8 Site C2M63

C2M63 is situated between C2M30 and C2M32, although it is not within the Wonderfonteinspruit River (Figure 3.1). The data trends from 1980 to 2017 as seen in Figure 4.13.

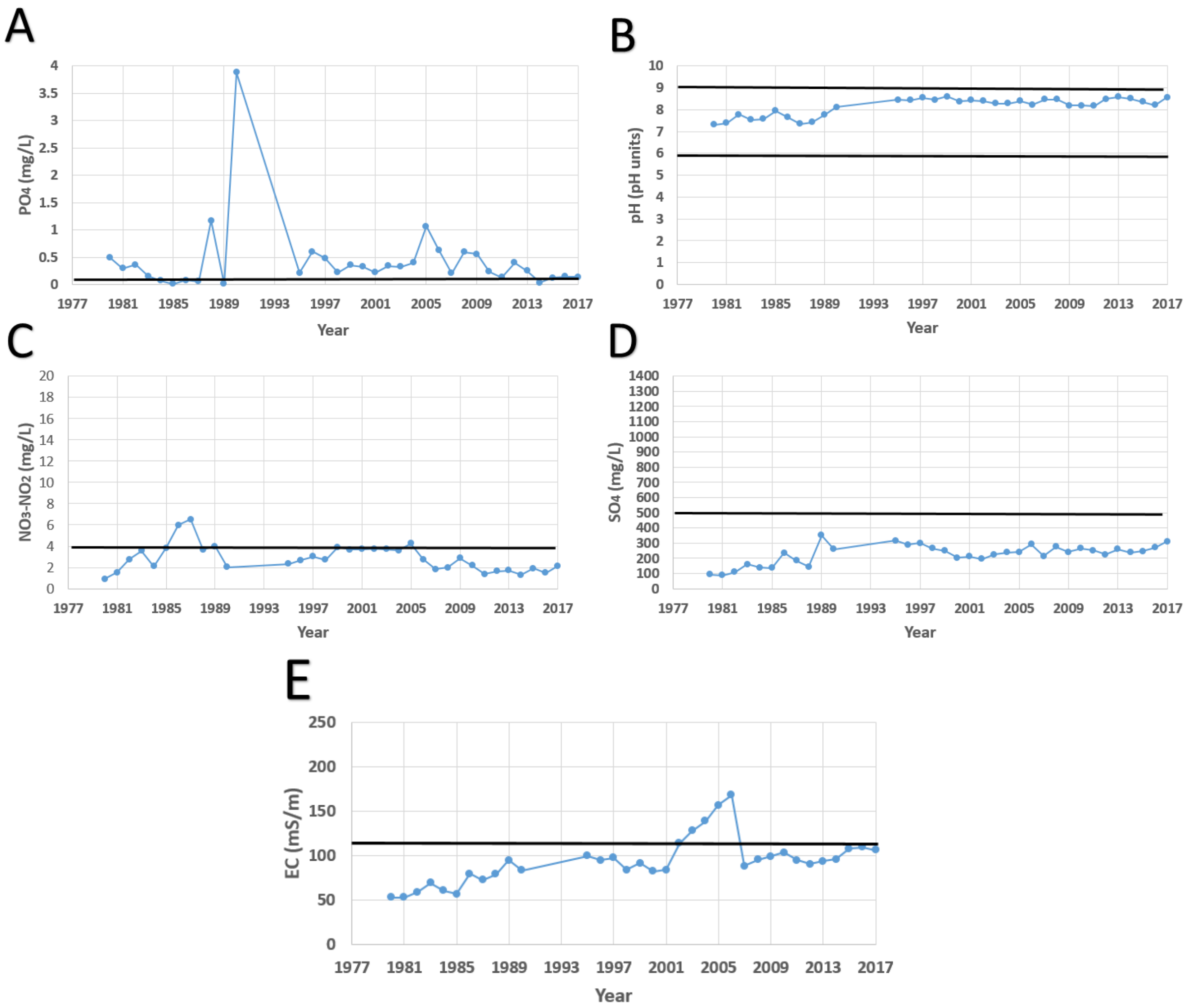

Figure 4.13: Physico chemical parameters and limits of site C2M63

Site C2M63 had increasing pH, with no limit breaking RQO values (Figure 4.13B). During 1980 to 1989 pH values were between 7.3 (which is the lowest in 1980) and 7.9. After 1989 the pH started increasing and alternated between 8.1 and 8.6 (highest value in 1999). The 2000's also consisted of high pH values ranging between 8 and 8.5 (Figure 4.13B). The measured EC for C2M63 were low in 1980 with a value of 52.86 mS/m and steadily increased, having limit breaking RQO values from 2002 to 2006, with the highest value in 2006 at 168.04 mS/m (Figure 4.13E). During 1990 extremely high NH_4^+, K and PO_4^{3-} values were recorded. The PO_4^{3-} showed constant high values from 1980 to 2017. Exceeding the RQO limit almost every year, except for 1984 to 1987 and 1989 (Figure 4.13A). During the analysis period the average value of SO_4^{2-} were 227.29 mg/L, never exceeding RQO limit, but increasing over time (Figure 4.13D). The NO_3-NO_2 exceeded the RQO limits by 1.99 mg/L in 1986, 2.52 mg/L in 1987 and 0.25 mg/L in 2005. The NO_3-NO_2 showed the lowest values from 2006 to 2017 (Figure 4.13C). The water quality index at this site is markedly lower than C2R01, C2R03 and C2R04, indicating severe impacts.

Table 4.8: Yearly statistics of site C2M63

Dependent variable	Independent variable	Unstandardised coefficients		Unstandardised coefficients	t	Sig.	Durbin-Watson
		B	Std. Error	Beta			
EC	**Year**	**1.634**	**0.093**	**0.608**	**17.58**	**0.000**	**0.824**
pH	**Year**	**0.030**	**0.001**	**0.712**	**23.39**	**0.000**	**1.412**
SO_4^{2-}	**Year**	**4.659**	**0.224**	**0.67**	**20.79**	**0.000**	**1.374**
PO_4^{3-}	**Year**	**-0.002**	**0.003**	**-0.027**	**-0.594**	**0.553**	**1.814**
NO_3-NO_2	**Year**	**-0.006**	**0.009**	**-0.033**	**-0.728**	**0.467**	**1.505**
NH_4^+	**Year**	**0.006**	**0.004**	**0.070**	**1.579**	**0.121**	**1.88**
IDX	**Year**	**-0.422**	**0.041**	**-0.411**	**-10.38**	**0.000**	**1.035**

Site C2M63 (Table 4.8) showed autocorrelation for all parameters and statistical significance for all parameters except PO_4^{3-}, NO_3-NO_2 and NH_4^+. The unstandardized coefficients B showed decreasing mean values for PO_4^{3-}, NO_3-NO_2 and IDX, where EC, pH and NH_4^+ had increasing mean values over time. The SO_4^{2-} increased by 4.659 mg/L per year. The EC increased by 1.634 mS/m per year. The IDX decreased by 0.422 per year.

The PCA of C2M63 (Figure 4.14) showed that the 1980's had high levels of Si and high NO_3-NO_2 during 1985, 1986 and 1987. Low SO_4^{2-} and EC were present during the 1980's. High levels of Na were measured during 2001, 2002, 2003, 2004 and 2005. This is also true for NO_3-NO_2 (Figure 4.13C) and EC (Figure 4.13E). As seen in the PCA (Figure 4.14) and Figure 4.13E, EC had an increasing trend. The PO_4^{3-} were high during the entire analysis period, especially 1990 as seen in Figure 4.13A.

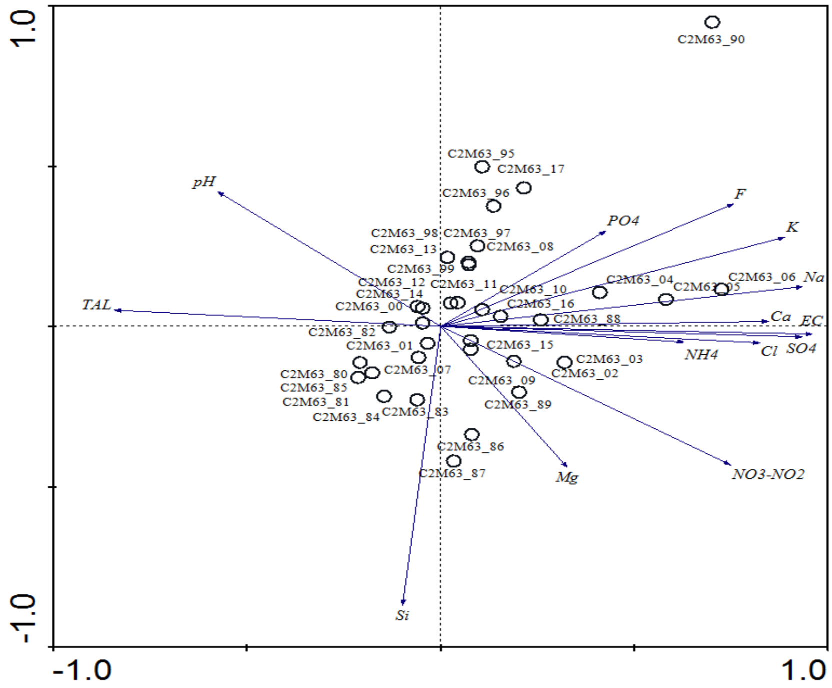

Figure 4.14: Physico-chemical PCA of site C2M63

4.1.9 Site C2M60

C2M60 is downstream of C2M32 and upstream of C2M69. It is situated between Khutsong and Khutsong South as seen in Figure 3.1. The data trends from 1969 to 2017 as seen in Figure 4.15. There were only two data points between 1969 and 1979. The EC, NO_3-NO_2 and SO_4^{2-} measured in this period were low. This could be a biased result as it seemed to break the pattern of high concentrations that follow.

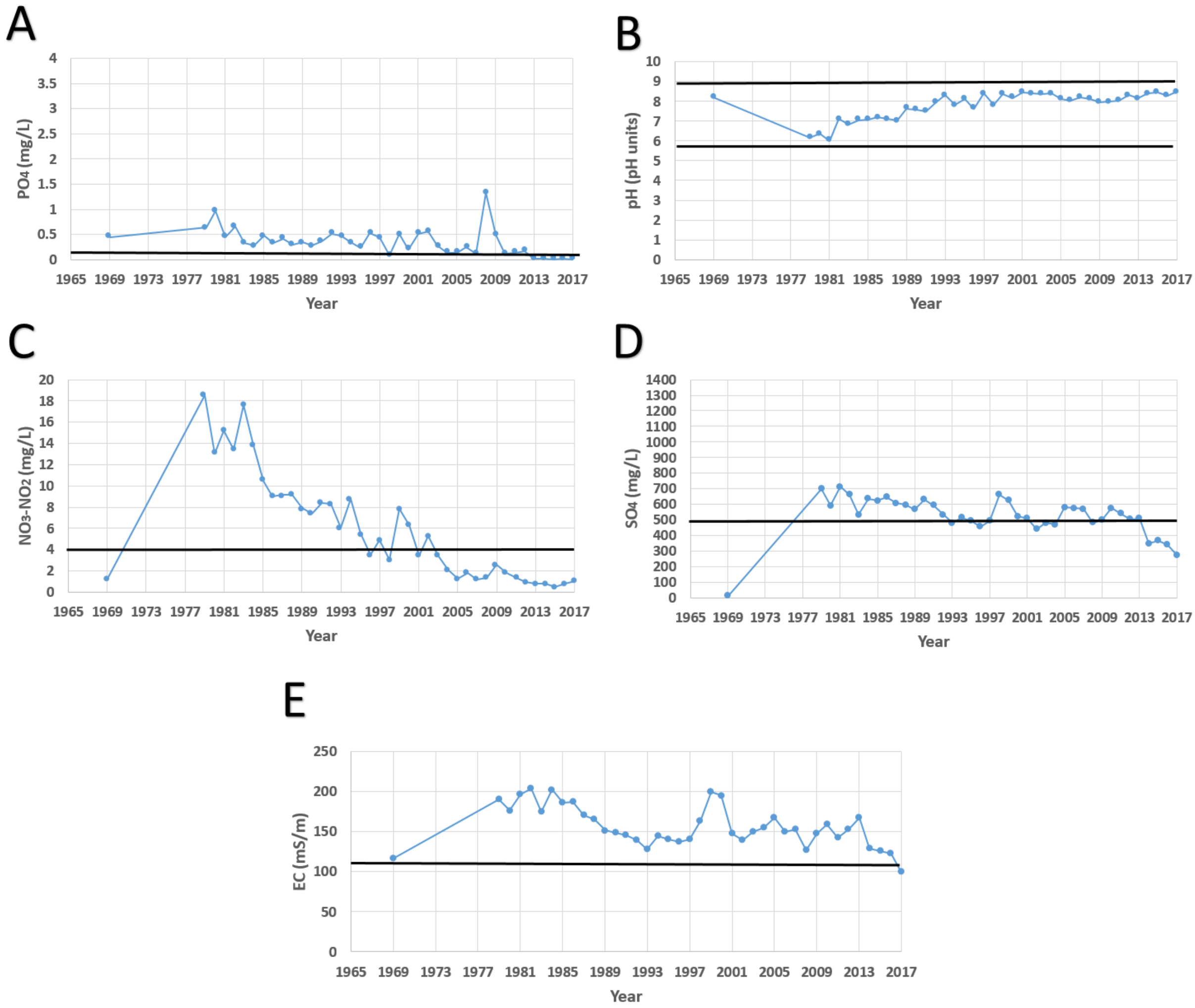

Figure 4.15: Physico chemical parameters and limits of site C2M60

The pH of C2M60 (Figure 4.15B) increased during the analysis period, but never exceeded the RQO limit. During the analysis period the pH started with low values in 1979 of 6.16 and slowly increased to 7.62 in 1989. The 1990's had higher pH values than the 1980's. At the end of 2017 the pH reached its highest recorded value of 8.5. Site C2M60 showed limit breaking RQO values for NO_3-NO_2 during the first two decades, reaching a high of 18.536 mg/L in 1979, before decreasing in the 1990's and reaching a low of 0.49 mg/L in 2015 (Figure 4.15C). The EC (Figure 4.15E) broke the RQO limit every year except 2017, but still consisted of a decreasing trend. A similar pattern emerged for PO_4^{3-}, again breaking the RQO limit almost every year except in the late 2000's (Figure 4.15A). The SO_4^{2-} exceeded the RQO limit from 1979 to 1992 and again in 1994, 1998–2001, 2005–2007 and 2010–2013 (Figure 4.15D). All measured physico-chemical parameters except pH were higher than the RQO limits during most of the analysis period and the water quality index at this site is markedly lower than C2R01, C2R03 and C2R04, indicating severe impacts.

Table 4.9: Yearly statistics of site C2M60

Dependent variable	Independent variable	Unstandardised coefficients		Unstandardised coefficients	t	Sig.	Durbin-Watson
		B	Std. Error	Beta			
EC	**Year**	**-1.172**	**0.084**	**-0.311**	**-13.9**	**0.000**	**1.427**
pH	**Year**	**0.05**	**0.002**	**0.614**	**33.11**	**0.000**	**1.113**
SO_4^{2-}	**Year**	**-5.295**	**0.373**	**-0.353**	**-14.2**	**0.000**	**1.450**
PO_4^{3-}	**Year**	**-0.01**	**0.002**	**-0.18**	**-6.85**	**0.000**	**1.738**
NO_3-NO_2	**Year**	**-0.42**	**0.015**	**-0.591**	**-27.6**	**0.000**	**0.787**
NH_4^+	**Year**	**-0.075**	**0.006**	**-0.318**	**-12.6**	**0.000**	**1.59**
IDX	**Year**	**1.156**	**0.032**	**0.649**	**36.41**	**0.000**	**1.607**

Table 4.9 of site C2M60 showed autocorrelation and statistical significance for all parameters. The unstandardized coefficients B showed decreasing mean vlaues for EC, PO_4^{3-} NO_3-NO_2 and NH_4^+ during the analysis period. SO_4^{2-} decreased by 5.295 mg/L yearly. IDX and pH increased.

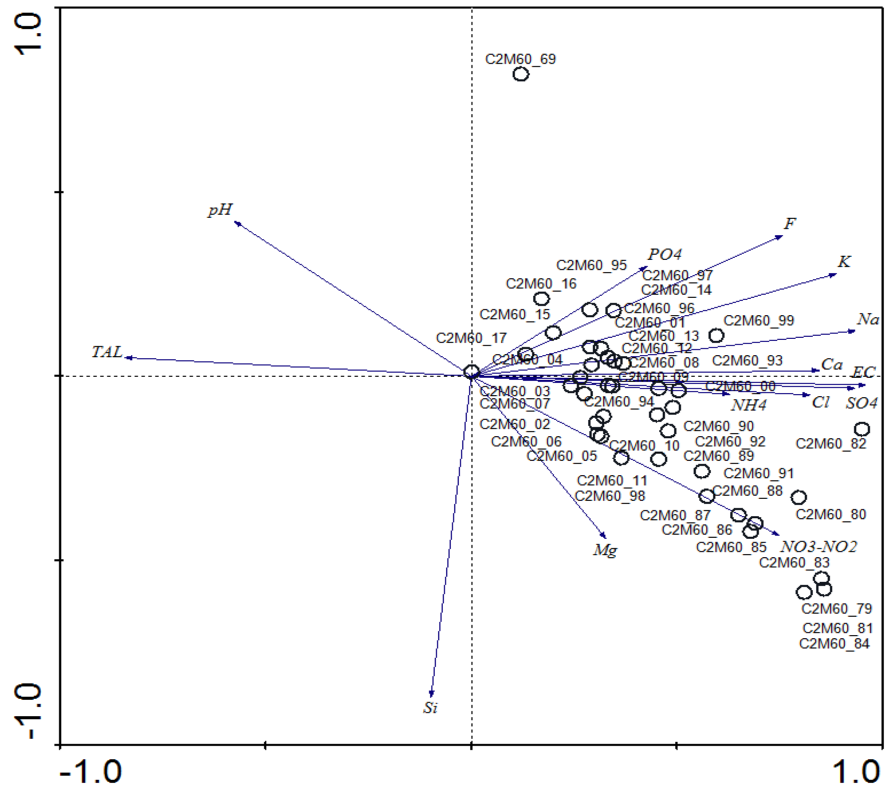

Figure 4.16: Physico-chemical PCA of site C2M60

The PCA of C2M60 showed high PO_4^{3-}, NO_3-NO_2, SO_4^{2-} and EC values during the analysis period (Figure 4.15A, C, D and E). The NO_3-NO_2 show limit breaking RQO values during 1979, 1980's and early 1990's as seen in Figure 4.15C. This was also apparent form the clustering within the PCA (Figure 4.16). The 2000's showed high Mg levels. The EC values of C2M60 were high during the entire analysis period, except 2017 (Figure 4.15E). The PO_4^{3-} were also high during the entire analysis period, except 2014, 2015, 2016 and 2017 (Figure 4.15A). This is also true for NH_4^+. The SO_4^{2-} exceeds the RQO limit from 1979 to 1992 and again in 1994, 1998–2001, 2005–2007 and 2010–2013 as mentioned above and seen in Figure 4.15D. The isolated 1969 data point may be a biased result as only two data point were available between 1969 and 1979.

4.1.10 Site C2M69

C2M69 is found downstream of C2M60. It is situated between Khutsong and Khutsong South as seen in Figure 3.1. The data trends from 1979 to 2016 as seen in Figure 4.17.

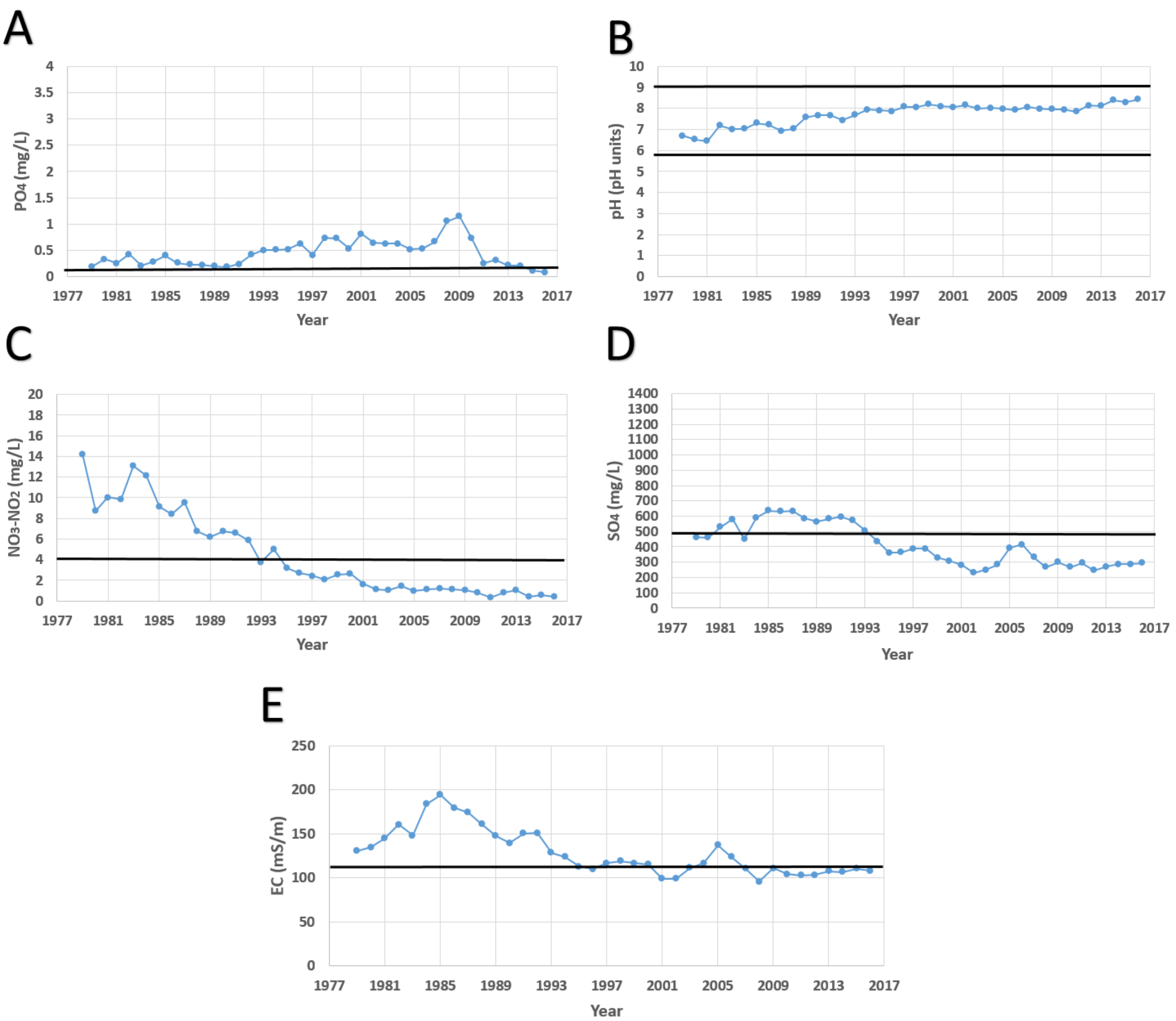

Figure 4.17: Physico chemical parameters and limits of site C2M69

Between 1979 and 1993, low pH values were recorded. With 1979 at 6.7, 1980 at 6.5 and 1981 at 6.4. From 1981 it steadily increased, reaching 8.4 in 2016 (Figure 4.17B). Site C2M69 showed high values for NO_3-NO_2 from 1979 to 1994 exceeding the RQO limit of 4 mg/L with an average 8.48 mg/L and a high of 14.16 mg/L in 1979, before decreasing in 1995, having no further limit breaking values (Figure

4.17C). Between 1979 and1994 high NH_4^+ and EC values were recorded. The NH_4^+ decreased after 1994 to acceptable levels. The data showed EC breaking RQO limits from 1979 to 2006, only excluding 1996 and 2001–2002. Then 2007 to 2017 showed lower and acceptable EC values (Figure 4.17E). The SO_4^{2-} exceeded the RQO limit of 500 mg/L during 1981 to 1993 with an average of 573.37 mg/L. During 1985, SO_4^{2-} had its highest value of 636.36 mg/L, which exceeded the RQO limit by 136.36 mg/L. From 1994 it returned to acceptable values (Figure 4.17D). Increases in the PO_4^{3-} values started in 1993. Every year except 2015 and 2016 exceeded the RQO limit of 0.125 mg/L. The PO_4^{3-} reached a high at 2009 with a value of 1.14 mg/L, which exceeded the RQO limit by 1.015 mg/L (Figure 4.17A). The water quality index at this site is markedly lower than C2R01, C2R03 and C2R04, indicating severe impacts.

Table 4.10: Yearly statistics of site C2M69

Dependent variable	**Independent variable**	**Unstandardised coefficients**		**Unstandardised coefficients**	**t**	**Sig.**	**Durbin-Watson**
		B	**Std. Error**	**Beta**			
EC	**Year**	**-2.007**	**0.057**	**0.611**	**-35.08**	**0.000**	**1.006**
pH	**Year**	**0.04**	**0.001**	**0.645**	**38.45**	**0.000**	**1.215**
SO_4^{2-}	**Year**	**-10.86**	**0.255**	**0.685**	**-42.59**	**0.000**	**0.915**
PO_4^{3-}	**Year**	**0.009**	**0.002**	**-0.117**	**5.33**	**0.000**	**1.755**
NO_3-NO_2	**Year**	**-0.337**	**0.008**	**-0.688**	**-40.28**	**0.000**	**0.864**
NH_4^+	**Year**	**-0.039**	**0.003**	**-0.274**	**-12.92**	**0.000**	**1.605**
IDX	**Year**	**1.089**	**0.028**	**0.646**	**38.53**	**0.000**	**1.084**

Site C2M69 (Table 4.10) showed autocorrelation and statistical significance for all parameters. The unstandardized coefficients B showed increasing mean values for pH, PO_4^{3-}, and IDX as time went on, where EC, SO_4^{2-}, NO_3-NO_2 and NH_4^+ had decreasing mean values over time. The SO_4^{2-} levels decreased by 10.86 mg/L per year. The EC decreased by 2.007 mS/m per year. The NO_3-NO_2 decreased by 0.337 mg/L per year and the IDX increased by 1.089 per year.

From the PCA ordination 2 distinct groupings were apparent (Figure 4.18). Grouping 1 (blue) represent the timeframe of 1979 to 1993. During this time NO_3-NO_2, SO_4^{2-} and EC were high and pH low as can be seen in Figure 4.17B and C. Grouping 2 (purple) represents the period 1994 to 2017. During this period the pH and PO_4^{3-} levels were higher, but the NO_3-NO_2, SO_4^{2-} and EC were lower as seen in Figure 4.17A, B, C, D and E.

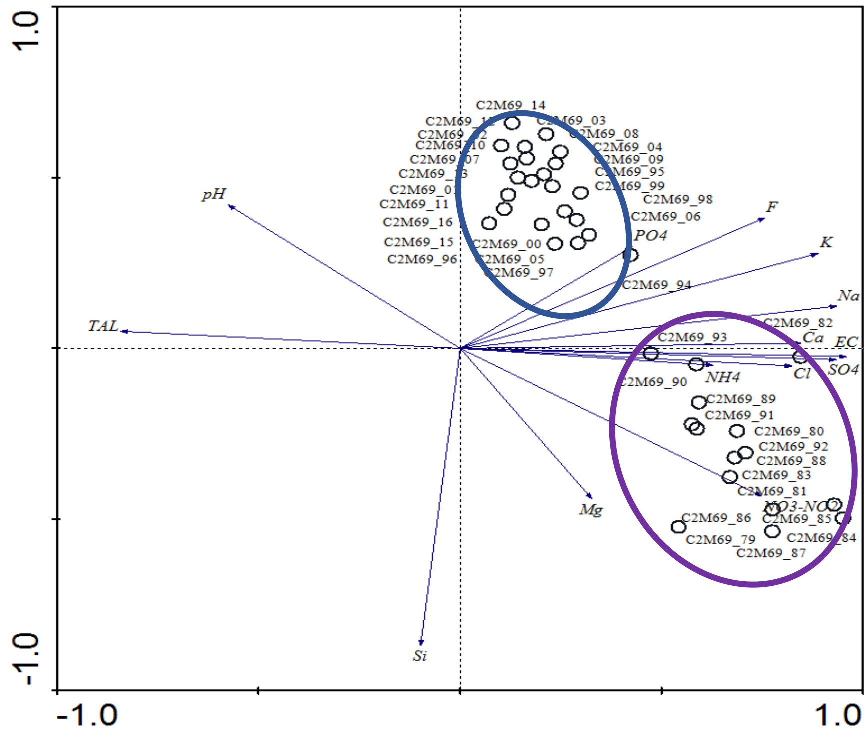

Figure 4.18: Physico-chemical PCA of site C2M69

4.1.11 Site C2M13

C2M13 is situated downstream of C2M69 and upstream of C2M11 as seen in Figure 3.1. Between C2M13 and C2M69 there is a wetland. The data trends from 1969 to 2017 as seen in Figure 4.19. There were only two data points between 1969 and 1979. This could have caused biased results.

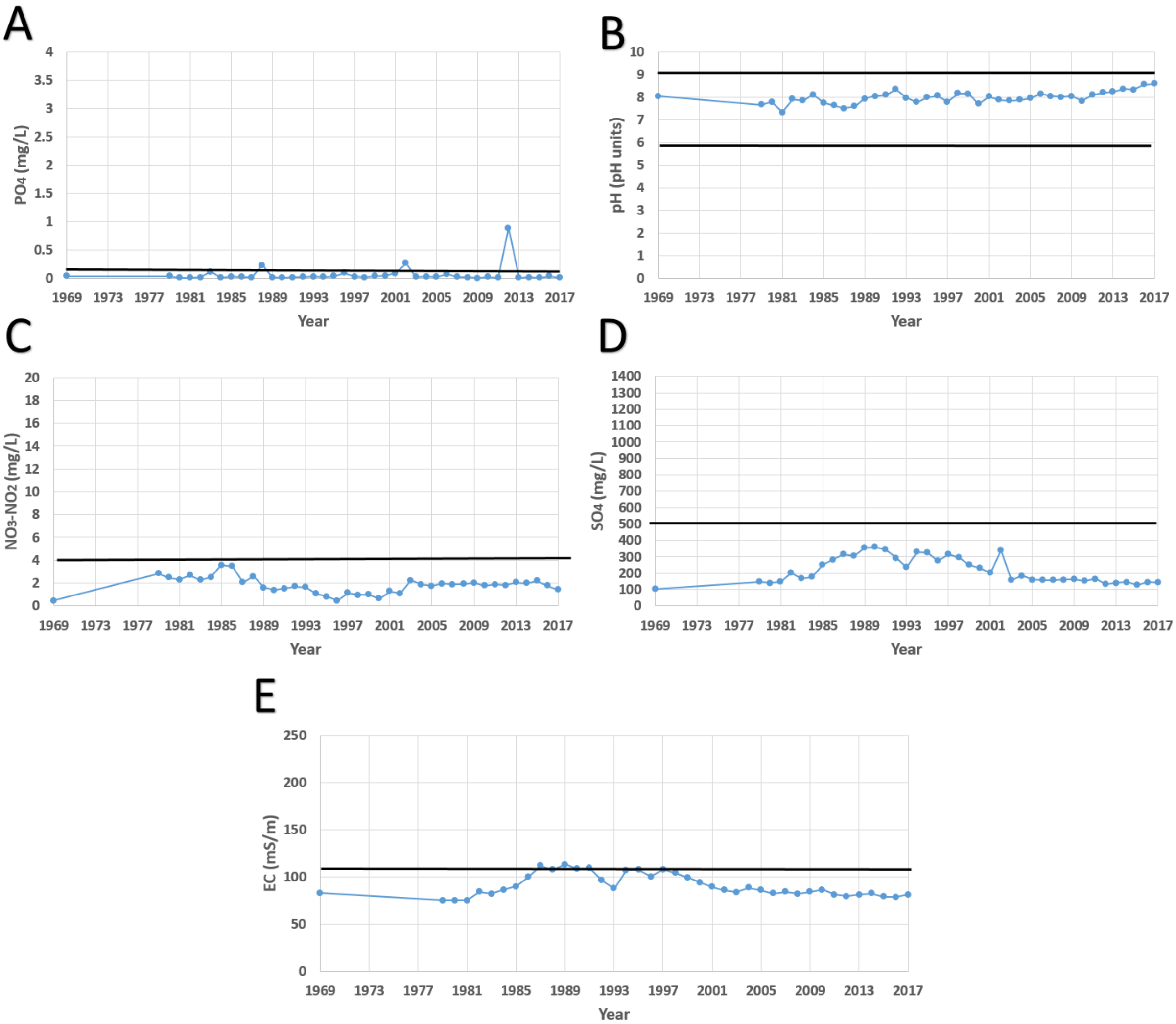

Figure 4.19: Physico chemical parameters and limits of site C2M13

The pH value during 1979 was 7.6 and increased to 8.6 2017 (Figure 4.19B). The EC had an increased from 1979 to 1998, only exceeding the RQO limit twice during 1987 by 0.8 mS/m and 1989 by 22.25 mS/m. From 1998 it decreased (Figure 4.19E). The 1980's had the highest NO_3-NO_2 values, with the highest in 1985 (3.57 mg/L). The lowest values were between 1994 and 2000. Having no RQO limit breaking values during the analysis period (Figure 4.19C). The SO_4^{2-} values were all inside the allowed range and averaged 216.67 mg/L during the analysis period. Having the highest recorded values between 1989 and 2002, averaging 297.1 mg/L (Figure 4.19D). The PO_4^{3-} were also acceptable across all years except 1988 (0.23 mg/L), 2002 (0.27 mg/L) and 2012 (0.89 mg/L) (Figure 4.19A). Low NH_4^+ values were recorded for this site.

Table 4.11: Yearly statistics of site C2M13

Dependent variable	Independent variable	Unstandardised coefficients		Unstandardised coefficients	t	Sig.	Durbin-Watson
		B	Std. Error	Beta			
EC	**Year**	**0.025**	**0.051**	**0.02**	**0.49**	**0.624**	**0.629**
pH	**Year**	**0.015**	**0.001**	**0.474**	**13.1**	**0.000**	**1.443**
SO_4^{2-}	**Year**	**-0.763**	**0.415**	**-0.076**	**-1.84**	**0.066**	**1.312**
PO_4^{3-}	**Year**	**0.002**	**0.001**	**0.071**	**1.659**	**0.098**	**1.798**
NO_3-NO_2	**Year**	**-0.024**	**0.005**	**-0.217**	**-5.2**	**0.000**	**0.979**
NH_4^+	**Year**	**0.001**	**0.000**	**0.169**	**4.03**	**0.000**	**1.653**
IDX	**Year**	**-0.014**	**0.035**	**-0.016**	**-0.4**	**0.699**	**0.749**

Site C2M13 (Table 4.11) showed autocorrelation between years for all parameters and statistical significance for only three parameters (pH, NO_3-NO_2 and NH_4^+). The unstandardized coefficients B showed decreasing mean vlaues for SO_4^{2-}, NO_3-NO_2 and IDX, where EC, pH, PO_4^{3-} and NH_4^+ had increasing mean values.

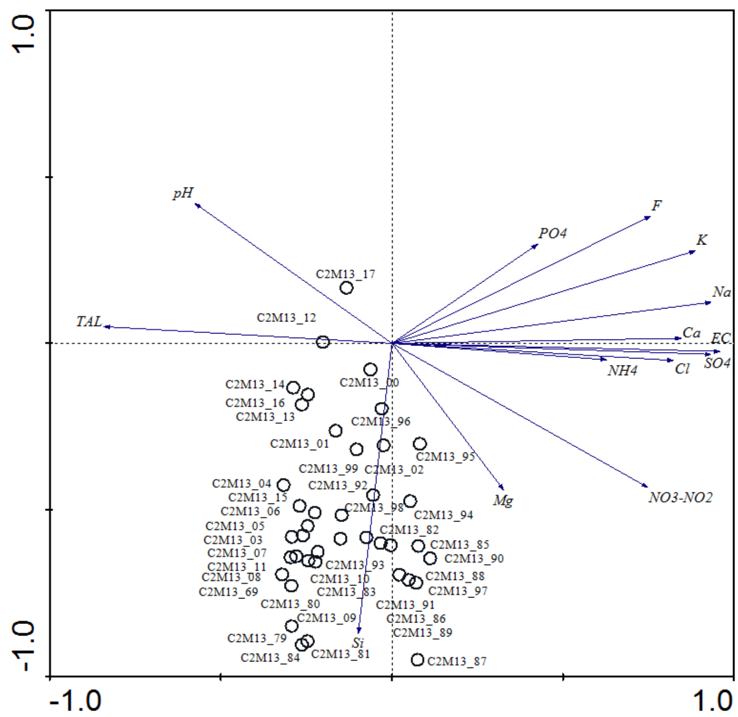

Figure 4.20: Physico-chemical PCA of site C2M13

The PCA of C2M13 (Figure 4.20) showed high levels of Si. The recorded values of PO_4^{3-}, SO_4^{2-}, EC and NO_3-NO_2 were relatively low during the analysis period as seen in Figure 4.19. This trend was also apparent in the PCA (Figure 4.20) with the majority of the samples clustering at the lower end of the PO_4^{3-}, SO_4^{2-}, EC and NO_3-NO_2 gradients. During 2017 the pH was highest during the analysis period and during 2012 the PO_4^{3-} were highest. The Mg^{2+} levels were high during the 1980's and 1990's. During 2002 to 2017, the TAL levels were high.

4.2 Simplified index

4.2.1 Water quality of the Mooi River

For ease of discussion the following combination of sites are grouped together:

- The Mooi River section (C2R03, C2M11, C2R01, C2M01, and C2R04)

Table 4.12: Mooi River section

Site name	Description
Mooi River at Witrand	C2M01
Gerhard Minnebronn eye	C2M11
Boskop dam	C2R01
Klerkskraal dam	C2R03
Potchefstroom dam	C2R04

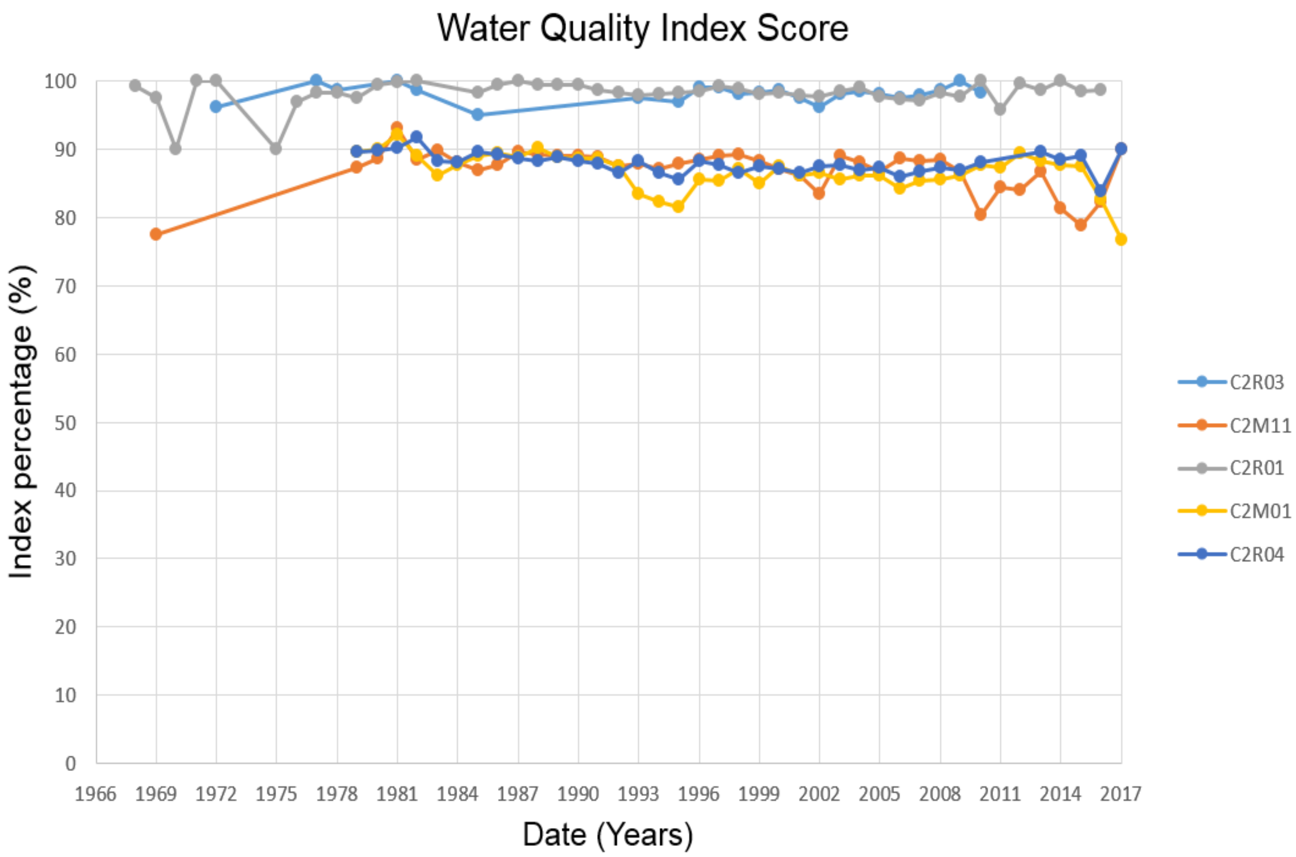

Figure 4.21: The Mooi River water quality index (C2R03, C2M11, C2R01, C2M01, and C2R04).

The Mooi River and the Wonderfonteinspruit River were the 2 major river systems studied. The Mooi River section starts off at site C2R03 and ends at C2R04 (Figure 3.1). Water that is used for potable water production for Potchefstroom is obtained from this region. The figures show the quality indices from a range of studied variables and their score values for water quality for the Mooi River catchment.

Identification of the parameters were done to create the water quality index. The parameters included: PO_4^{3-}, SO_4^{2-}, pH, NO_3-NO_2, EC, F^-, Mg^{2+}, Cl^- and NH_4^+. Data sets for the Mooi River and Wonderfonteinspruit River were analysed to identify the spatial and temporal trends of these parameters within the specific sites from around 1965 to 2017 and compared them to the RQO limits. Quality scores were given to the specific parameters depending on the concentrations present within a specific site. High scores were given when the identified parameters were low within a specific site and lower scores were given when they exceeded the RQO limit. The highest possible score being 100 and the lowest 0. Lower scores indicated higher concentration of specific parameters, which indicated pollution.

Site C2R03, was classified as relatively unpolluted and had an average water quality score of 98.13 over the four-decade period of the available data. When there were water quality challenges, the index was 95 during 1985 but the quality improved over the next years scoring index values of 100 on several occasions (Figure 4.21). Similar challenges were observed for C2R01. In the period 1967 to 1976, water quality challenges indicated lower water quality indices (90 to<95) for this site. The quality since 1980 was more consistent and also improved to quality indices above 95. Sampling points C2M11, C2M01, C2R01 and C2R04 resides downstream of C2R03. Site C2M11 and C2M01 had an average water quality score of 86.97 and 86.74 respectively. Site C2R04 can be seen as the furthest downstream sampling point from C2R03 and had an average water quality score of 87.94. Site C2R01 had an impressive water quality score averaging 98.21 over the studied period and is also the highest value recorded of the 5 sites (Figure 4.21). It reached a low of 90 in 1970 and 1975 and had multiple 100 values over the years. C2M01 had the lowest average of all sampling points in this area and the value in 2017 was 76.7. As a whole, the studied area and sites consist of good quality water useable for all purposes.

4.2.2 Water quality of the Wonderfonteinspruit River

For ease of discussion the following combination of sites are grouped together:

- The Wonderfonteinspruit River section (C2M30, C2M32, C2M63, C2M60, C2M69 and C2M13)

Table 4.13: Wonderfonteinspruit River section

Site name	Description
Eye from Wonderfontein	C2M30
Mooirivierloop at Wonderfontein	C2M32
Doornfonteinkanaal at Blaawbank	C2M60
Wes-driefontein at rooiport	C2M63
Mooirivierloop at Blaauwbank	C2M69
Turffontein eye	C2M13

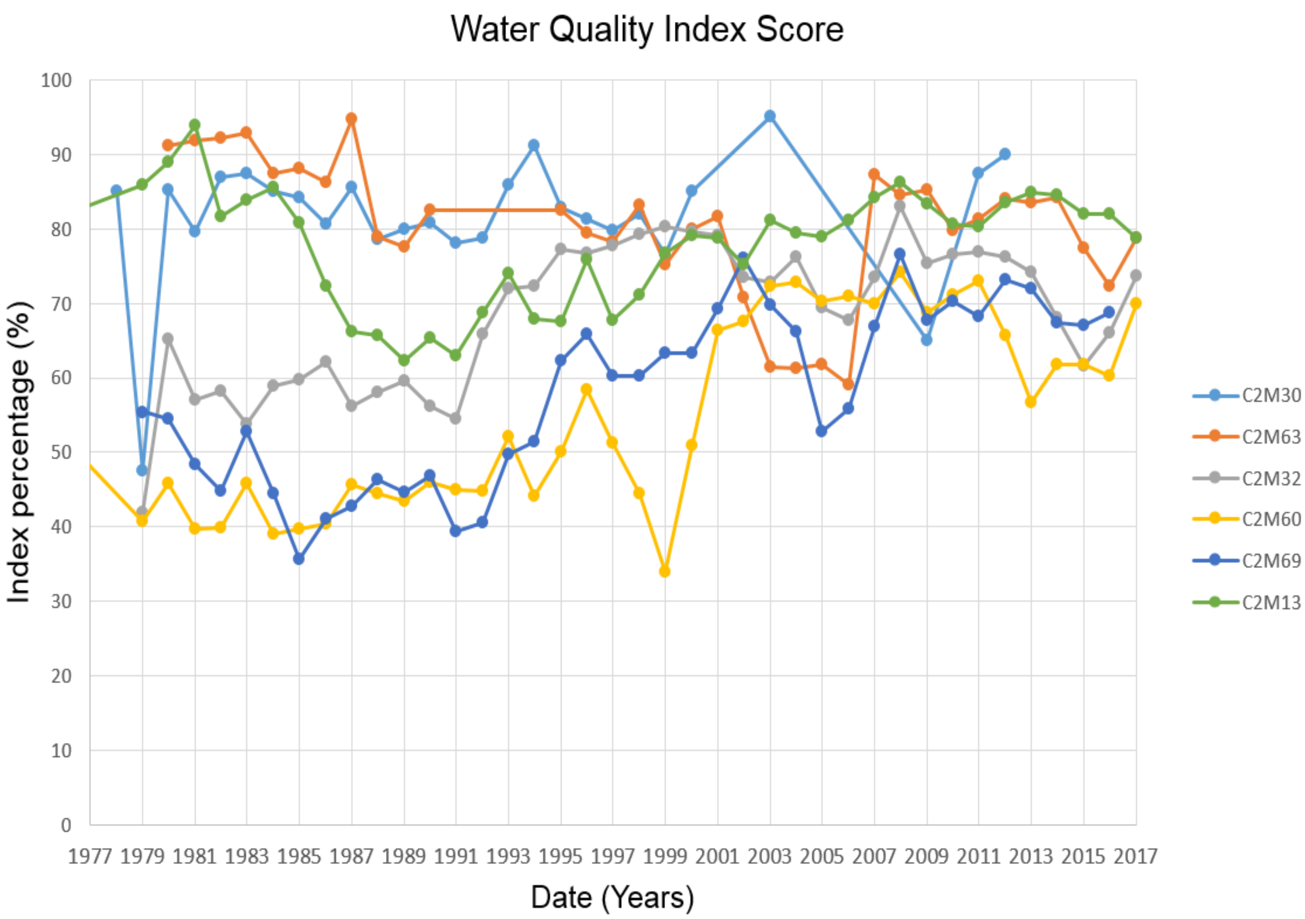

Figure 4.22: The Wonderfonteinspruit River water quality index (C2M30, C2M32, C2M63, C2M60, C2M69 and C2M13).

The study area of the Wonderfonteinspruit River, flowing into the Mooi River further downstream, starts off at C2M30, flowing into downstream sites C2M32, C2M60, C2M69, C2M13 and finally meeting up with C2M11 as the Mooi River and Wonderfonteinspruit River confluences (Figure 3.1). The Wonderfonteinspruit River section had a much lower overall water quality score compared to the Mooi River section with index scores for at least three of the six sites ranging from <40 to 60 for the period 1979 to approximately 1991 (Figure 4.22). These three sites (C2M32, C2M60, C2M69) are down-stream from mines as well as urban (formal and informal) developments. The latter developments expanded rapidly over the period in question. An improvement in the water quality scores for the three sites were observed for the period 1991 to 1996. The improvement in water quality continued for C2M32 until 2001. However, the water quality, as depicted by the water quality scores, decreased in the cases of both C2M60 and C2M69. The impact at C2M60 was more profound and the data for 1999 resulted in the lowest quality score at this site (C2M60; water quality index score 35). The only other water quality score that was lower than 40 was for site C2M69 and this was in 1985 (Figure 4.22). Water quality scores for sites C2M60 and C2M69 generally followed similar patterns potentially indicating similar impacts. In general, the water quality scores for these three sites (C2M32, C2M60, C2M69) were better in the years 2009 to 2017 when compared to the period. However, the index for the period 2011 to 2016 decreased from between ±70 and ±78 to between ± 60 and ±70 as seen in Figure 4.22.

The water quality index scores for site C2M32 was greater than 70 for the period 1993 to 2005 and consistently in the order of +75 during 1995 to 2001, approaching a value of 80. After a consistent increase in the water quality values from 68 in 2006, it increased to 85 in 2008 and then the values declined to ±75 for the period 2009 to 2013. Impacts between 2013 and 2015 was such that the water quality index was lower than 70 (Figure 4.22).

Site C2M30 is the most upstream sampling point (just before the town of Carletonville) in the Wonderfonteinspruit River. This point is downstream from Westonaria and Randfontein (Figure 3.1). The C2M30 water quality scores averaged 81.69 on the created index during the study period and was higher compared to the previously described sites. The data shows that 1979 to 1991 had the lowest recorded index scores, averaging 79.98, due to a decrease in quality in 1979 of 47.5 (only three readings during October, November and December were available and could have impacted the calculations). An increase in water quality score (83.14) was determined for the period from 1991 to 2012.

The water quality index for Site C2M30 generally had a score in the order of 80, except for 1979, 1991 to 1993, 1999 and 2009, when the scores were very low (below 50 to about 78). There was only one data point between 2003 and 2011. The values for this point was such that the score was approximately 65 (Figure 4.22). Only on two data points (1994 and 2003) had water quality scores exceeding 90. Even so, the water quality indices for this site was higher than sites C2M32, C2M60 and C2M69.

Site C2M63, a site that was not directly in the Wonderfonteinspruit River but some kilometres south of the river had a water quality index average of 80.48. This site was downstream from mining activities. Water quality index in the 1980's was higher compared to the 1990's. However, it was during 1999 that impacts at or upstream from this site had a dramatic impact on the water quality. The water quality index dropped to just above 60 and was constantly at that level from 2003 to 2008. The water quality was better (between 80 and 90) for the period 2007 to 2013 (Figure 4.22). As the case was with all the other sites, the water quality was lower in the period 2014 to 2016.

Site C2M13 is the site that is the furthest downstream in the Wonderfonteinspruit River (Figure 3.1). It is just downstream of site C2M69 and site C2M60. This sampling point is just before the Mooi River and the Wonderfonteinspruit River confluence. The water quality index between 1977 and 1985 was above 80. However, the impacts on the water quality was such that the index were lower than 70, in the order of lower 60's during 1987 to 1991. During 1992 to 1997, the water quality index remained around 70 with two periods (1993 and 1996) when higher water scores were recorded. The water quality scores increased and scores >80 were generally recorded for the period 2003 to 2017 (Figure 4.22).

4.3 Bacterial community composition of the Mooi River and the Wonderfonteinspruit River

After Qiime and R analysis, MS Excel was used to draw various graphs (Figures 26, 27 and 28) indicating the bacterial Phyla composition in the Mooi River and Wonderfonteinspruit River during 2015 and 2016. Both 2015 and 2016 shows similarities in bacterial composition as *Bacteriodetes, Proteobacteria, Actinobacteria* and *Cyanobateria* are the four most dominant phyla identified spatially and temporally. Numerous studies had identical results, implying that certain bacteria are indigenous to freshwater ecosystems via 16s rRNA sequencing.

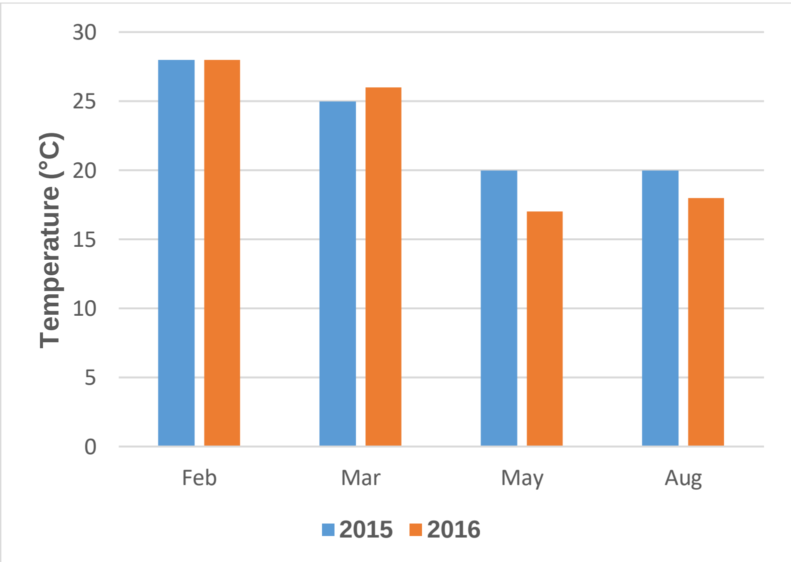

Figure 4.23: Average reported temperature for sampling periods (worldweatheronline, 2019)

Figure 4.23 represents the ambient temperatures from the Potchefstroom and Carletonville area during 2015 and 2016. During February and March, the temperature was around, and even exceeded, 25°C compared to May and August where temperatures were 20°C and lower. Water temperature data for the specific studied sites were not available, although ambient temperatures impact water temperatures.

4.3.1 Bacterial community composition of the Mooi River based on phyla

Bacteroidetes dominated the microbial community during 2015 as seen in Figure 4.24 and Table 4.14. It was the dominating phylum during August (32.45%) and second most abundant during the March (29.37) and May (33.71%). The high abundancy was expected as literature suggests that *Bacteroidetes* is one of the most dominant phyla within freshwater, this will be further discussed in Chapter 5. Site 3 (just upstream of Boskop Dam (C2R01), close to the confluence of the Mooi and Wonderfonteinspruit Rivers) exhibited the highest *Bacteroidetes* numbers for all months. The highest number of *Bacteroidetes* sequences were detected during August. Site 5 (Thabo Mbeki Bridge just below Potchefstroom dam (C2R04)) showed the lowest numbers of *Bacteroidetes* during May, March and August, and the lowest numbers of sequences were detected in March as seen in Figure 4.24.

Proteobacteria was second most abundant overall within the Mooi River during 2015. *Proteobacteria* was considerably lower in August (13.25%) compare to its dominating numbers during March (35.37%) and May (40.85%) (Table 4.14). Site 4 (Just downstream of Boskop Dam (C2R01)) had the highest numbers (16.19%) of *Proteobacteria* during May and site 3 (just upstream of Boskop Dam (C2R01)) had the lowest number (1.11%) during August as seen in Figure 4.24.

Actinobacteria was the third most abundant phylum overall in the Mooi River during 2015. It was the second most abundant during August with an average of 22.03%, but were the least abundant during March (10.4%) and May (7.49%) as seen in Table 4.14. The lowest number (0.15%) of *Actinobacteria* sequences were detected at site 3 (just above Boskop Dam (C2R01)) during May followed by the second lowest value again at site 3 in August (0.38%). This was surprising as August seemed to favour *Actinobacteria* during 2015, as well as the fact that the highest number (18.95%) of *Actinobacteria* also occurred in August at site 7 as seen in Figure 4.24. There were thus potentially other forces driving the observed levels of *Actinobacteria*.

Cyanobacteria was the fourth most abundant phylum overall during 2015. The *Cyanobacteria* levels were higher during August (15.26%) compared to the average of March (12.72%) and May (7.16%) (Table 4.14). Site 3 during March had the lowest *Cyanobacteria* numbers (0.29%), with the highest at site 6 during August (13.7%) as seen in Figure 4.24.

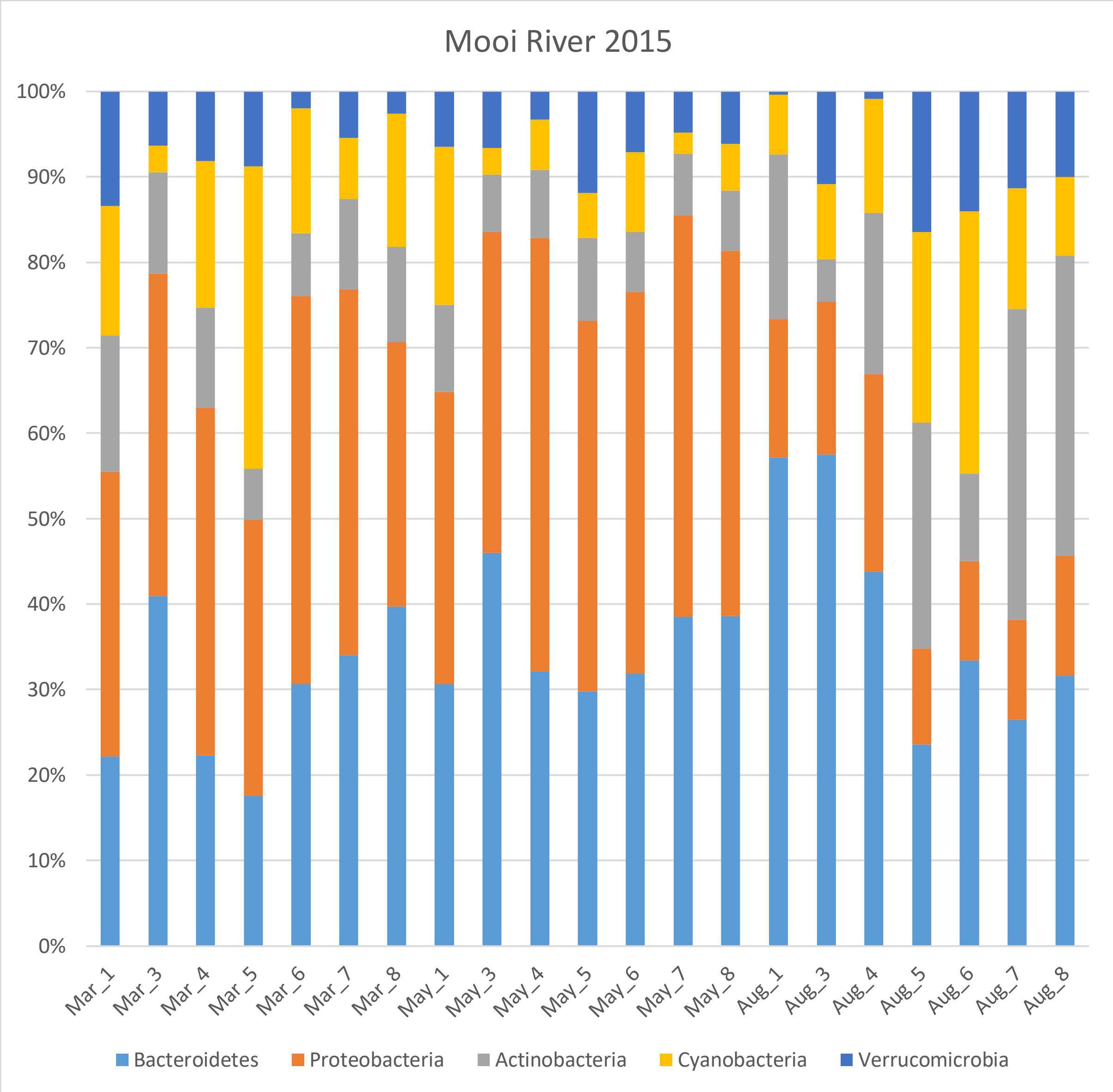

Figure 4.24: Bacterial community structure of the Mooi River during 2015 (Legend: Mar = March, May = May, Aug = August; _x = Site x)

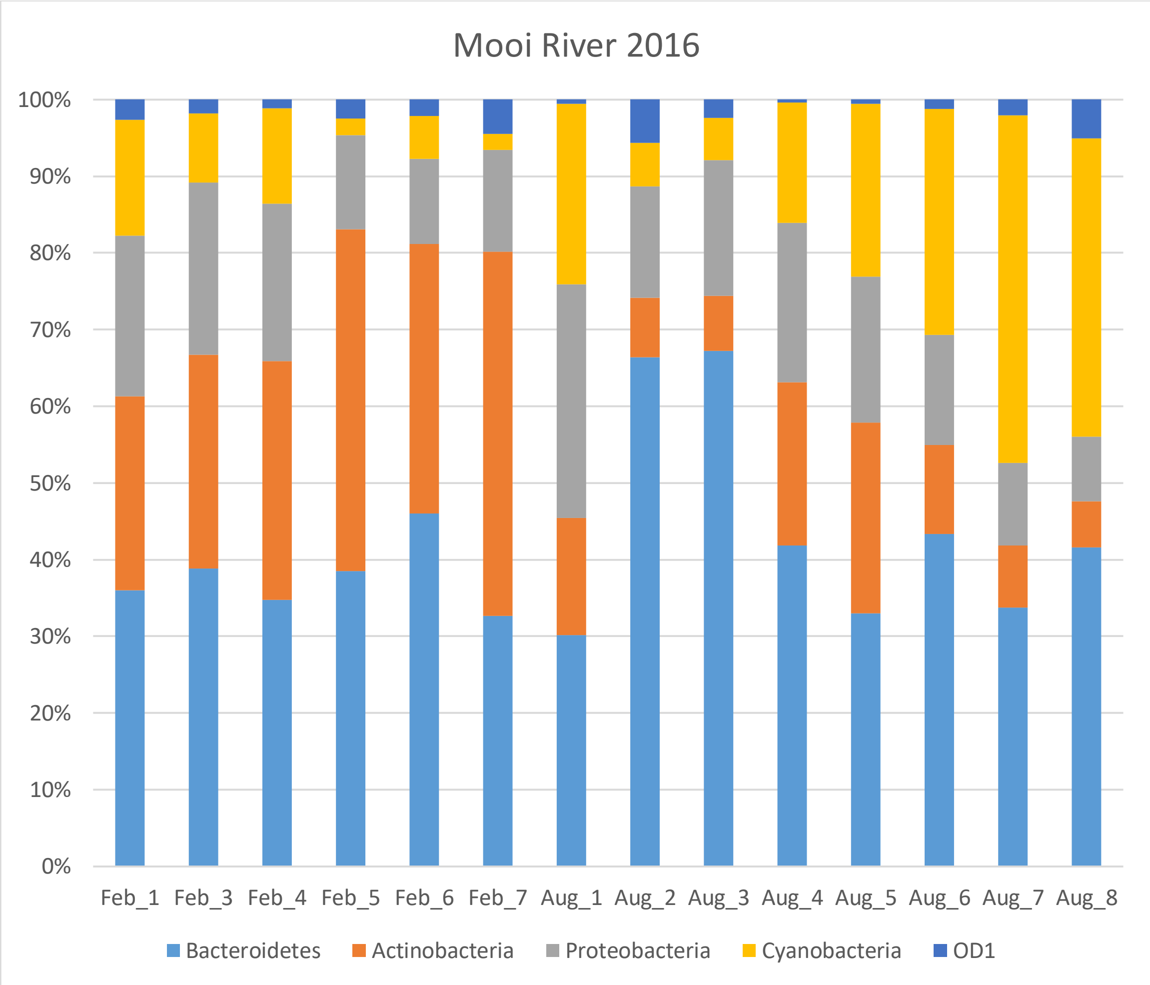

Figure 4.25: Bacterial community structure of the Mooi River during 2016 (Legend: Feb = February, Aug = August; _x = Site x)

Bacteroidetes dominated the Mooi River during 2016. It was the dominant phylum during August (45.41%). *Bacteroidetes* also dominated February with an average of 32.65% (Table 4.14). Site 3 had the highest number of *Bacteroidetes* during August and the lowest number was at site 1, also during August. August seemed to favour *Bacteroidetes* during 2015 and 2016, which makes the low amount at site 1 unexpected.

Actinobacteria was the second most abundant phylum overall within the Mooi River during 2016. It followed *Bacteroidetes* closely during February with an average of 30.84% of the total bacterial composition, but was much lower (8.61%) in August (Table 4.14). This is the opposite pattern identified during 2015, where the highest amount of sequences were present during August. *Actinobacteria* had the highest number (20.24%) of sequences at site 7 during February. Site 5 also had high number (19.77%) of sequences during February, with the lowest value (3.6%) at site 8 during August (Figure 4.25). This again shows the opposite pattern from 2015.

Proteobacteria was the third most abundant phylum overall within the Mooi River during 2016. Sequences were evenly distributed in abundancy during February (15.56%) and August (12.87%). August of 2015 had an average of 13.25%, showing similarities between 2015 and 2016 for *Proteobacteria* sequences (Table 4.14). Site 1 had the highest *Proteobacteria* numbers (18.5%) during August and the lowest percentage (1.1%) at site 8 also during August (Figure 4.25).

Cyanobacteria was the fourth most abundant phylum overall within the Mooi River during 2016. It had a higher abundancy during the August (24.47%) compared to the February (7.2%) (Table 4.14). Site 7 had the highest percentage (42.67%) of *Cyanobacteria* sequences during August and the lowest number (0.88%) at site 7 during February (Figure 4.25).

Table 4.14: Abundancy of Bacterial phyla of the Mooi River during 2015 and 2016

Phylum	2015			2016	
	March (%)	May (%)	August (%)	February (%)	August (%)
Bacteroidetes	29.37	33.71	32.45	32.65	45.41
Actinobacteria	10.4	7.49	22.03	30.84	8.61
Proteobacteria	35.37	40.85	13.25	15.55	12.87
Cyanobacteria	12.72	7.16	15.26	7.18	24.47
Other	12.17	10.79	17.01	13.78	8.64

4.3.2 Bacterial community composition of the Wonderfonteinspruit River based on phyla

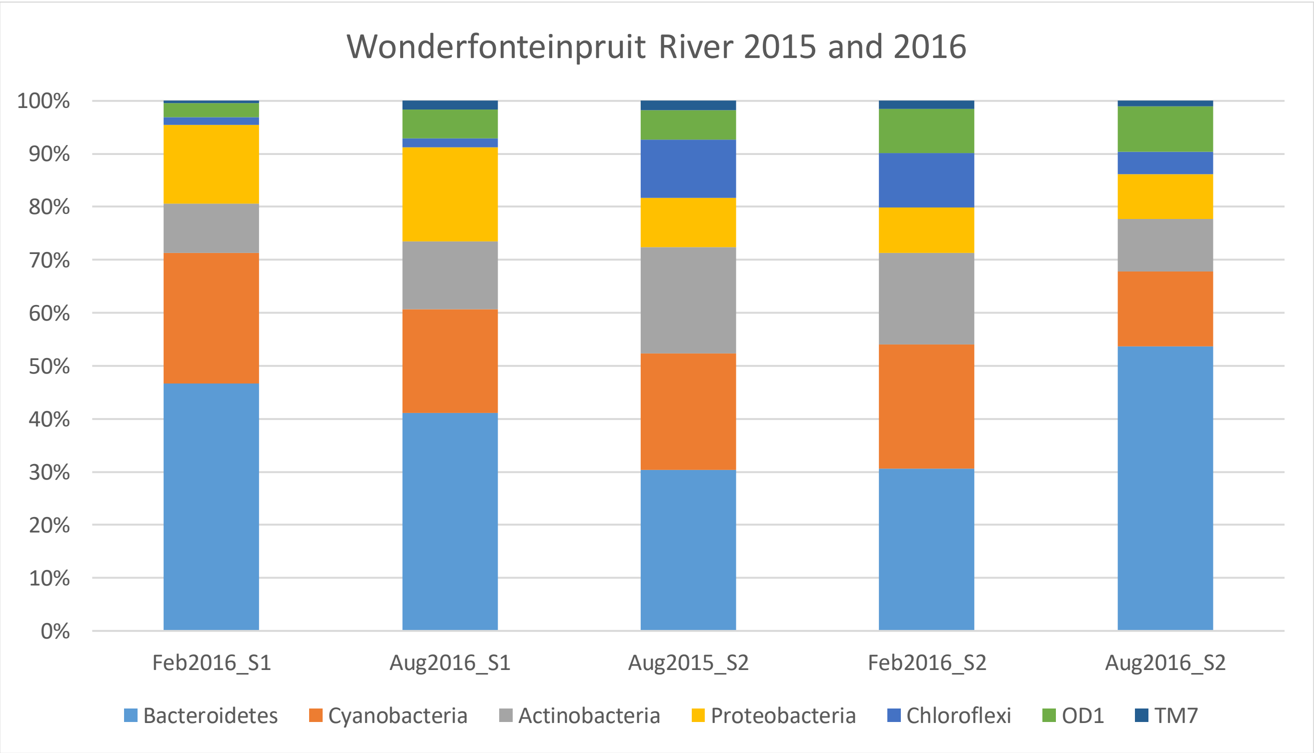

Figure 4.26: Bacterial community structure of the Wonderfonteinspruit during 2015 and 2016

Table 4.15: Abundancy of Bacterial phyla of the Wonderfonteinspruit during 2015 and 2016

Phylum	2015	2016	
	August (%)	February (%)	August (%)
Bacteroidetes	26.77	32.16	38.03
Actinobacteria	17.61	13.83	11.05
Proteobacteria	8.24	9.43	14.67
Cyanobacteria	19.47	21.82	16.77
Other	27.91	22.76	19.48

Overall *Bacteroidetes* dominated the microbial community within the Wonderfonteinspruit River during 2015 and 2016 as seen in Figure 4.26 and Table 4.15, similar to the Mooi River. The highest abundancy was recorded during August (38.03%) of 2016, compared to February (32.16%) of 2016 and August (26.77%) of 2015. The lowest number (6.31%) of *Bacteroidetes* sequences were detected in August 2015 at site 2, only to become the highest (31.82%) abundance a year later at the same place and time (August 2016 at site 2). This showed an increase in *Bacteroidetes* from 2015 to 2016 at site 2 as seen in Figure 4.25.

Cyanobacteria was overall the second most abundant within the Wonderfonteinspruit River. It was most abundant during February (21.82%) and the least abundant during August (16.77) during 2016. Exactly the opposite trend was recorded within the Mooi River during 2016. *Cyanobacteria* had the highest number of sequences (35.54%) during February 2016 at site 1 and the lowest (2.77%) in August 2016 at site 2 (Figure 4.26).

Actinobacteria was third most abundant overall within the Wonderfonteinspruit River and had the highest number of sequences during August of 2015 (17.61%) as seen in Table 4.15. The highest number (43.61%) of *Actinobacteria* sequences were detected in August 2015 at site 2 and the lowest number (2.6%) of sequences were detected in February 2016 at site 1 (Figure 4.26).

Proteobacteria was the least abundant of the four major phyla within the Wonderfonteinspruit River. It was the most abundant during August (14.67%) of 2016 and least abundant during August (8.24%) during 2015. This showed an increase in *Proteobacteria* from 2015 to 2016 during August. Site 1 had the highest abundance (41.19%) of *Proteobacteria* during August 2016 compared to site 2, which had the lowest abundance (3.02%) during August 2016. This can all be seen in Table 4.15.

The RDA (Figure 4.27) of the microbial sampling sites with all combined factors showed Wonderfonteinspruit River sites were grouped near PO_4^{3-}, SO_4^{2-}, NO_3-NO_2, EC and all ions contributing to EC, whereas the Mooi River sites grouped around pH and TAL and year 2015 grouped around rainfall (slightly dryer year). The Wonderfonteinspruit sites were grouped (blue) close to *OD1* and *Chloroflexi*, these phyla showed positive interactions regarding PO_4^{3-} and EC as can be seen in Figure 4.28. *Cyanobacteria*, *Actinobacteria* and *Firmicutes* were grouped (green) together and showed positive interactions with PO_4^{3-} and tolerance toward EC. *Bacteriodetes*, *Proteobacteria*, *Verrucomicrobia*, *Planctomycetes* and *Armatimonadetes* grouped (orange) within the vicinity of index (Figure 4.28), which indicated they prefer better water quality and also showed a general association with rainfall and silica.

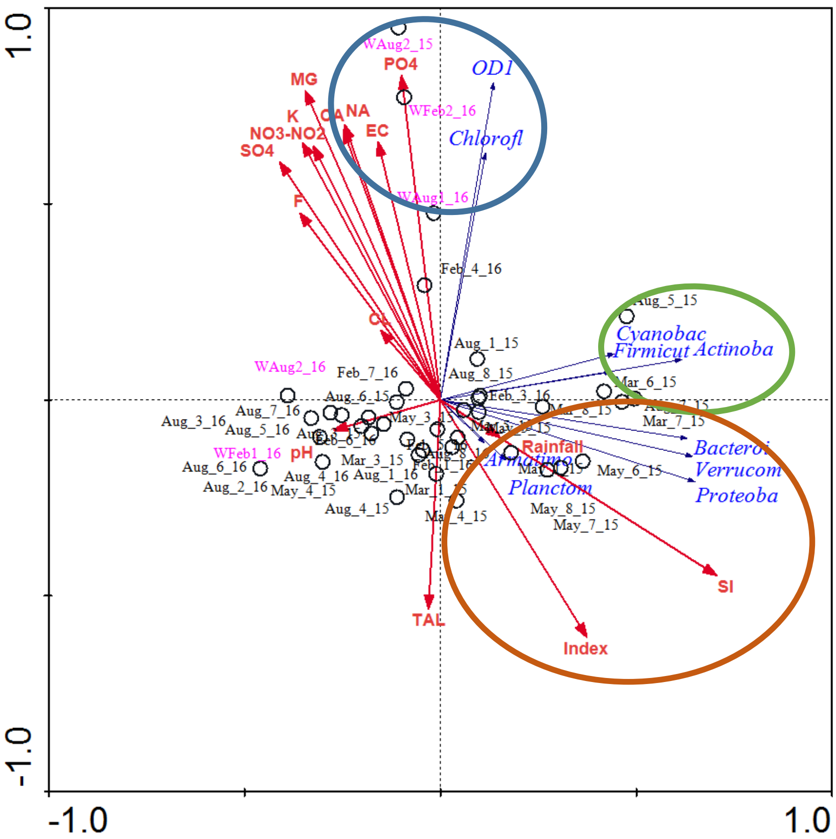

Figure 4.27: RDA of the microbial sampling sites with all combined factors based on phyla

4.3.3 Response curves for bacterial phyla

Figure 4.28 provides response curves for the various bacterial phyla when compared to selected water quality parameters and the overall water quality index. Most bacterial phyla seem to prefer better quality water as seen by the response in the curve labelled index in Figure 4.28. This is especially true for *Bacteriodetes* and *Proteobacteria*. It was however, not the case when the individual parameters were considered (Figure 4.28)

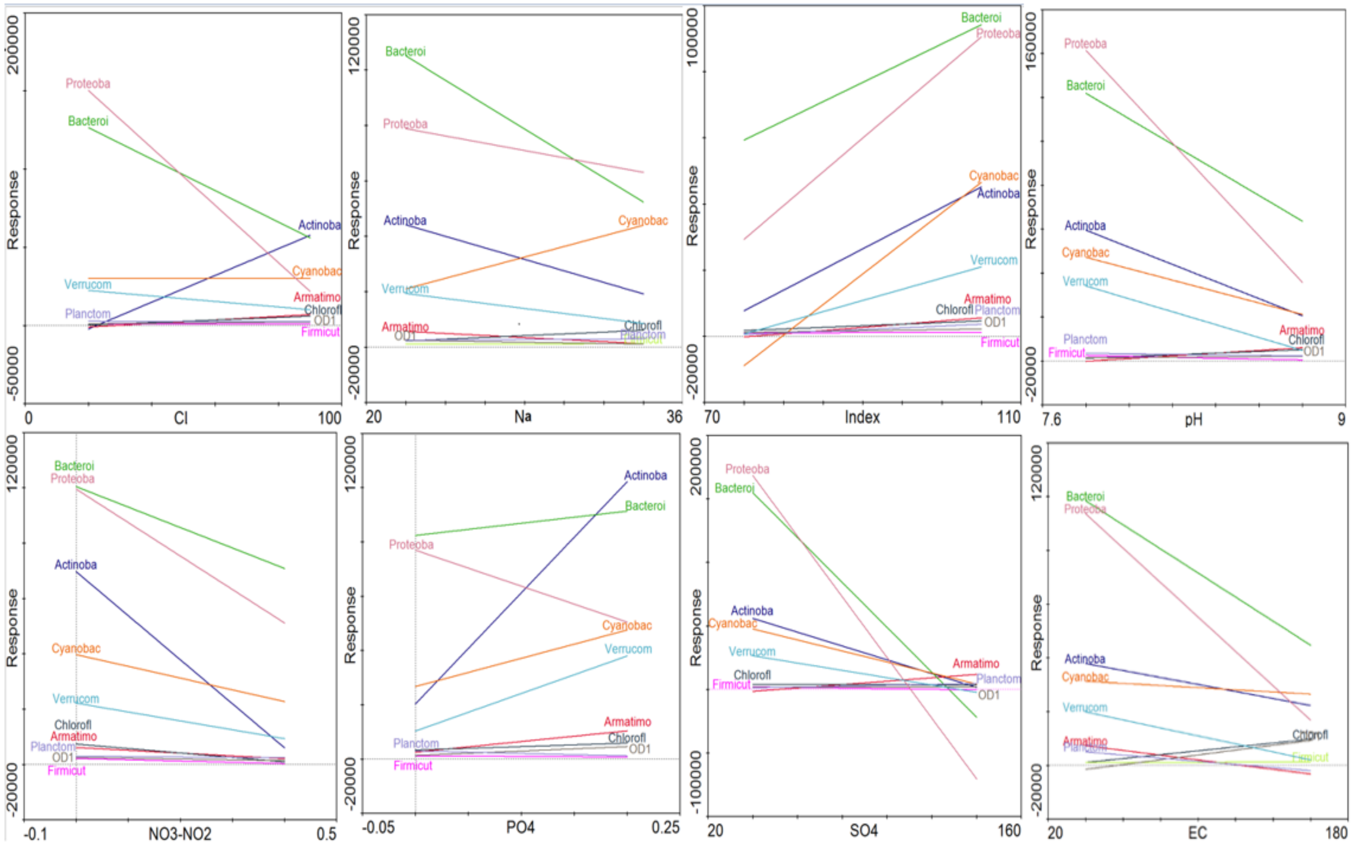

Figure 4.28: Bacterial response curves in relation to physico-chemical parameters based on phyla

When one considers chlorine *Cyanobacteria* and *Actinobacteria* was positively impacted by this parameter. *Cyanobacteria* was also positively impacted by the sodium levels. The SO_4^{2-} concentrations negatively impacted the levels of all the phyla (Figure 4.28).

4.3.4 Bacterial community composition of the Mooi River based on Family

The most abundant family within the Mooi River during 2015 was *Comamonadaceae*, which is part of the *Proteobacteria* phylum. It had the highest number of sequences (23.35%) during August and was also the most dominant during March (19.58%) and May (19.75%) as seen in Table 4.16. Site 3 during August had the lowest number (0.3%) of *Comamonadaceae* sequences and site 1 had the highest number (16.54%) during May as seen in Figure 4.29.

The second most abundant family within the Mooi River was *Flavobacteriaceae*, which is part of the *Bacteroidetes* phylum. It was most abundant during May (17.93%), closely followed by August (16.35%) (Table 4.16). Site 1 had the highest number (14.61%) of *Flavobacteriaceae* sequences during August and the lowest (0.19%) during March at site 1 (Figure 4.29).

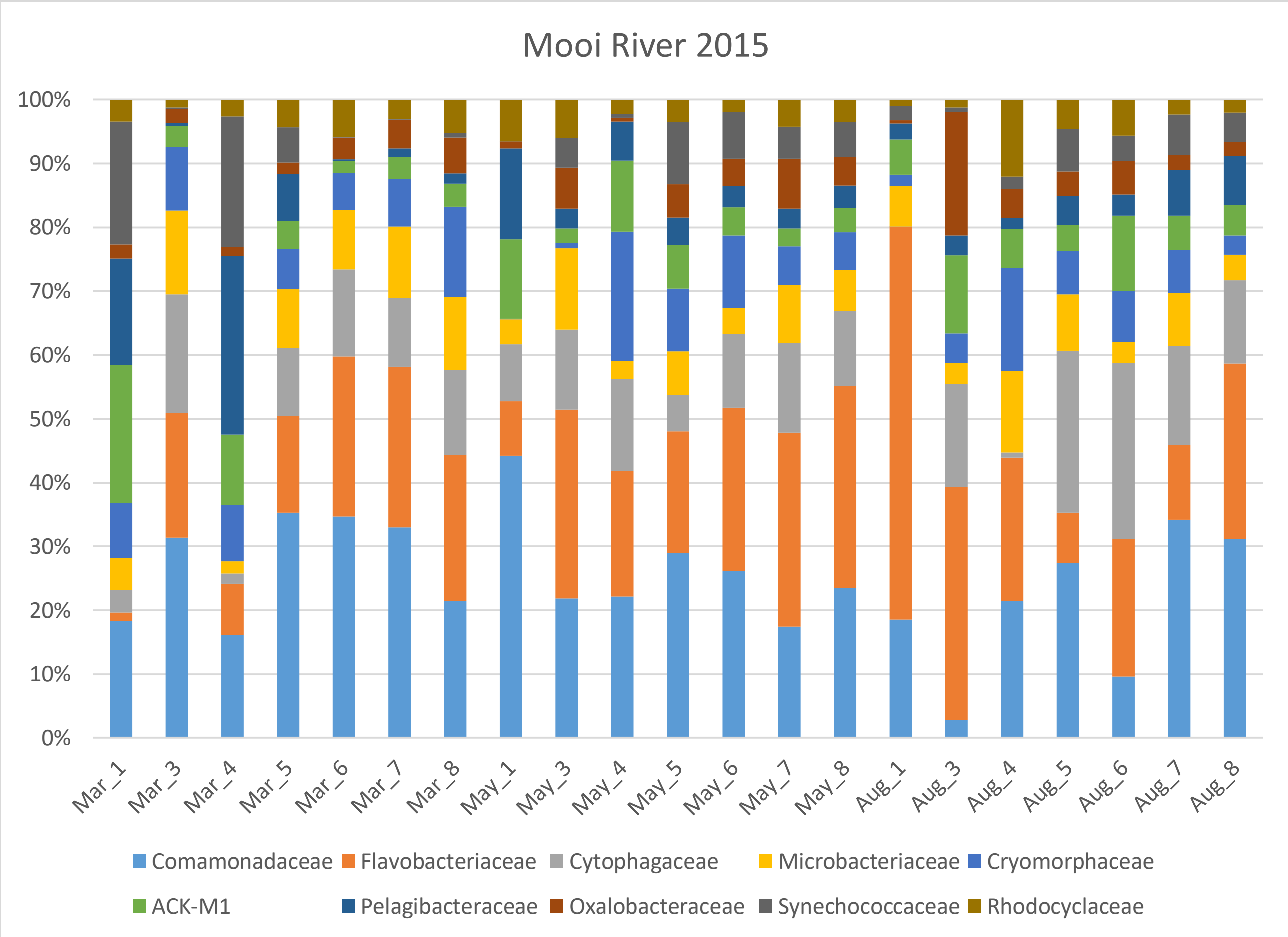

Figure 4.29: Bacterial community structure of the Mooi River during 2015

Cytophagaceae was the third most abundant within the Mooi River. It is another representative of the *Bacteroidetes* phyla. It consisted of 7.48% during March, 8.56% during May and 12.62% during August (Table 4.16). The highest number (11.38%) of *Cytophagaceae* sequences were detected at site 6 during August and the lowest (0.008%) at site 1 during August. There were thus potentially other forces decreasing the observed levels of *Cytophagaceae* at site 1 (Figure 4.29).

The fourth most abundant family within the Mooi River was *Microbacteriaceae*, which is part of the *Actinobacteria* phylum. *Microbacteriaceae* had the highest number of sequences (6.98%) during March. Followed by August (5.66%) and May (4.76%) (Table 4.16). It had the highest (18.97%) abundancy within site 3 during March and the lowest (0.55%) within site 4 during March (Figure 4.29).

The less abundant families followed in order of *Cryomorphaceae* (*Bacteroidetes*), *ACK-M1* (*Actinobacteria*), *Pelagibacteraceae* (*Proteobacteria*), *Oxalobacteraceae* (*Proteobacteria*). The ninth most abundant family within the Mooi River was the first representative of the *Cyanobacteria* phylum, being *Synechococcaceae*. Tenth most abundant were the family member *Rhodocyclaceae* of the *Proteobacteria* phylum (Figure 4.29).

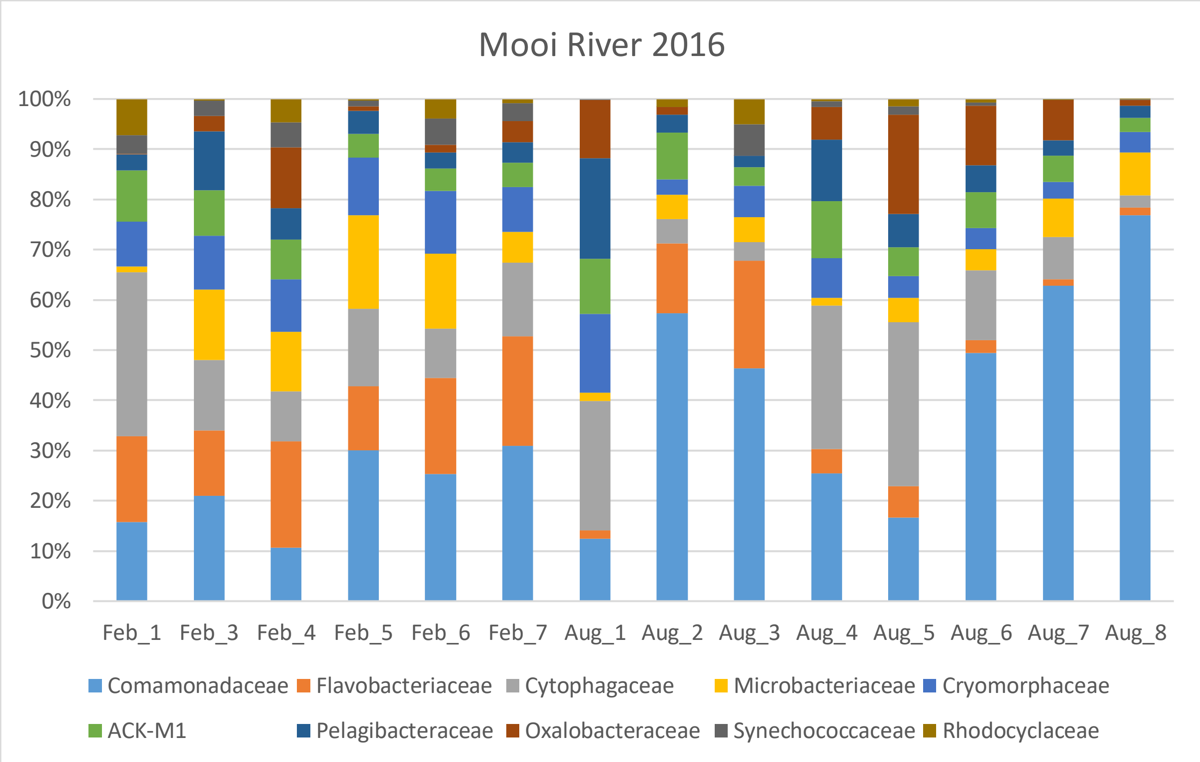

Figure 4.30: Bacterial community structure of the Mooi River during 2016

The most abundant family within the Mooi River during 2016 was *Comamonadaceae* (*Proteobacteria)*. It had much higher abundance during August (47.67%) compared to February (17.82%). This was also much higher than 2015 (19.86%) as seen in Table 4.14. Site 5 during February had the lowest number (0.44%) of *Comamonadaceae* sequences and site 8 had the highest number (26.45%) during August as seen in Figure 4.30.

The second most abundant family within the Mooi River was *Flavobacteriaceae (Bacteroidetes)*. It had lower abundancy (8.19%) in August during 2016 compared to 2015 (16.35%) and had an average of 15.10% during February (Table 4.16). Site 5 had the highest number (19.91%) of *Flavobacteriaceae* sequences during February and the lowest (0.08%) during August at site 7 (Figure 4.30).

Cytophagaceae (*Bacteroidetes*) was the third most abundant within the Mooi River. It consisted of 6.71% during August and 12.86% during February, which is an opposite trend to 2015 (Table 4.16). The highest number (27.15%) of *Cytophagaceae* sequences were detected at site 1 during February and the lowest (0.72%) at site 8 during August (Figure 4.30).

The fourth most abundant family within the Mooi River was *Microbacteriaceae* (*Actinobacteria*). *Microbacteriaceae* showed similar results during 2016 compared to 2015. It consisted of 5.24% during August and 8.96% during February as seen in Table 4.16. The highest number (22.76%) of sequences were detected at site 6 during February and the lowest (0.2%) within site 1 during August as seen in Figure 4.30.

The families following the major four were similar than that of 2015 and followed in order of *Cryomorphaceae* (*Bacteroidetes*), *ACK-M1* (*Actinobacteria*), *Pelagibacteraceae* (*Proteobacteria*), *Oxalobacteraceae* (*Proteobacteria*). The ninth most abundant family within the Mooi River was again the first representative of the *Cyanobacteria* phylum, being *Synechococcaceae*. Tenth most abundant were the family member *Rhodocyclaceae (Proteobacteria)* as seen in Figure 4.30.

Table 4.16: Abundancy of Bacterial families of the Mooi River during 2015 and 2016

Family	2015			2016	
	March (%)	May (%)	August (%)	February (%)	Augusts (%)
Comamonadaceae	19.58	19.75	23.35	17.82	47.67
Flavobacteriaceae	14.40	17.93	16.35	15.10	8.19
Cytophagaceae	7.48	8.56	12.62	12.86	6.71
Microbacteriaceae	6.98	4.76	5.66	8.96	5.24
Cryomorphaceae	6.66	4.73	6.25	8.75	4.64
Other	44.9	44.27	35.77	36.51	27.55

4.3.5 Bacterial community composition of the Wonderfonteinspruit River based on Family

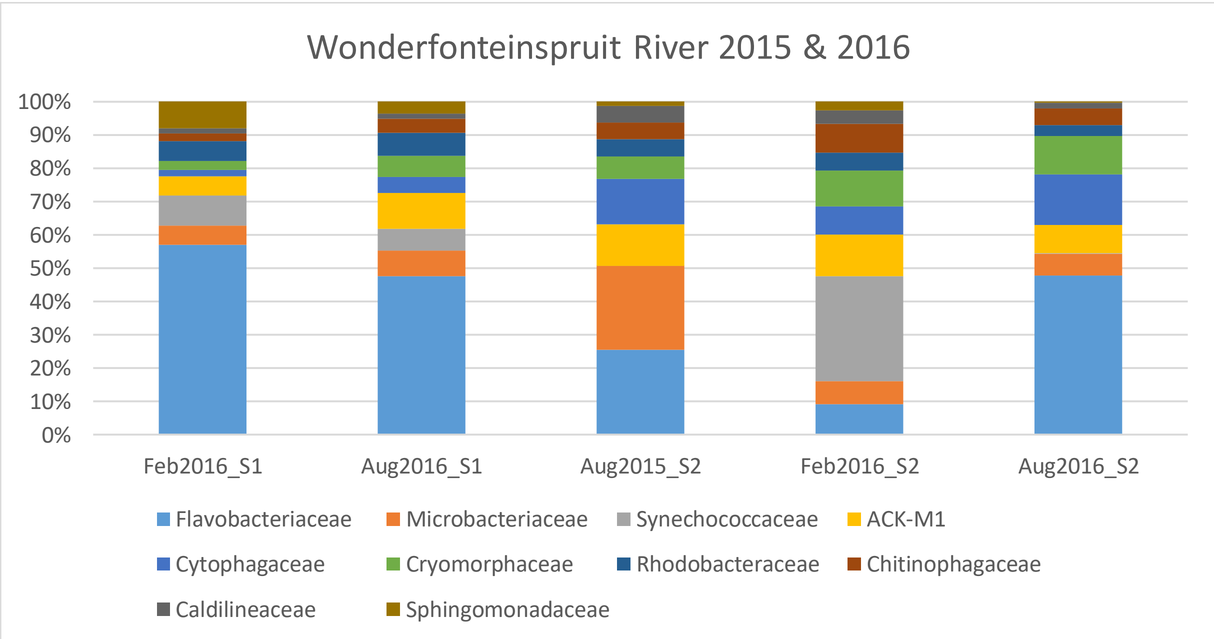

Figure 4.31: Bacterial community structure of the Wonderfonteinspruit River during 2016

The family *Flavobacteriaceae* (*Bacteroidetes*) dominated the Wonderfonteinspruit River. The abundancy of *Flavobacteriaceae* during August of 2015 was 18.56%. A year later *Flavobacteriaceae* increased to 34.33% (Table 4.17). There were thus potentially other external impacts causing *Flavobacteriaceae* to increase. February had an abundancy of 17.02 %. The highest number of sequences were detected within site 1 during February and the lowest at site 2 during February (Figure 4.31).

The second most abundant family member within the Wonderfonteinspruit River was *Microbacteriaceae* (*Actinobacteria*) with an average of 18.56% during August of 2015 and decreased in abundance a year later during August (5.45%), followed by February (5.16%) as seen in Table 4.17. Site 2 during August showed the highest abundancy and February site 2 the lowest (Figure 4.31).

Synechococcaceae (*Cyanobacteria*) was the third most abundant family member within the Wonderfonteinspruit River, especially during February (19.77%) during 2016. The Wonderfonteinspruit River showed a much higher parentage of *Synechococcaceae* compared to the Mooi River. Although sample site 2 showed very low amounts of *Synechococcaceae* during August 2015 (0.09%) and 2016 (0.014%).

ACK-M1 were the fourth most abundant family within the Wonderfonteinspruit River with mostly constant averages during August of 2015 (9.06%), August of 2016 (7.58%) and February of 2016 (8.31%) (Table 4.17).

The abundancy order followed with *Cytophagaceae* (*Bacteroidetes*) and *Cryomorphaceae* (*Bacteroidetes*). The seventh and eight most abundant families were *Rhodobacteraceae* (*Proteobacteria*) and *Chitinophagaceae* (*Bacteroidetes*) and this was followed by *Caldilineaceae. Sphingomonadaceae* being the tenth most abundant family within the Wonderfonteinspruit River (Figure 4.31).

Table 4.17: Abundancy of Bacterial families of the Wonderfonteinspruit River during 2015 and 2016

Family	2015	2016	
	August (%)	February (%)	August (%)
Flavobacteriaceae	18.56	17.02	34.33
Microbacteriaceae	18.39	5.16	5.45
Synechococcaceae	0.01	19.77	4.02
ACK-M1	9.06	8.31	7.58
Cytophagaceae	9.88	5.20	4.57
Other	44.1	44.54	44.05

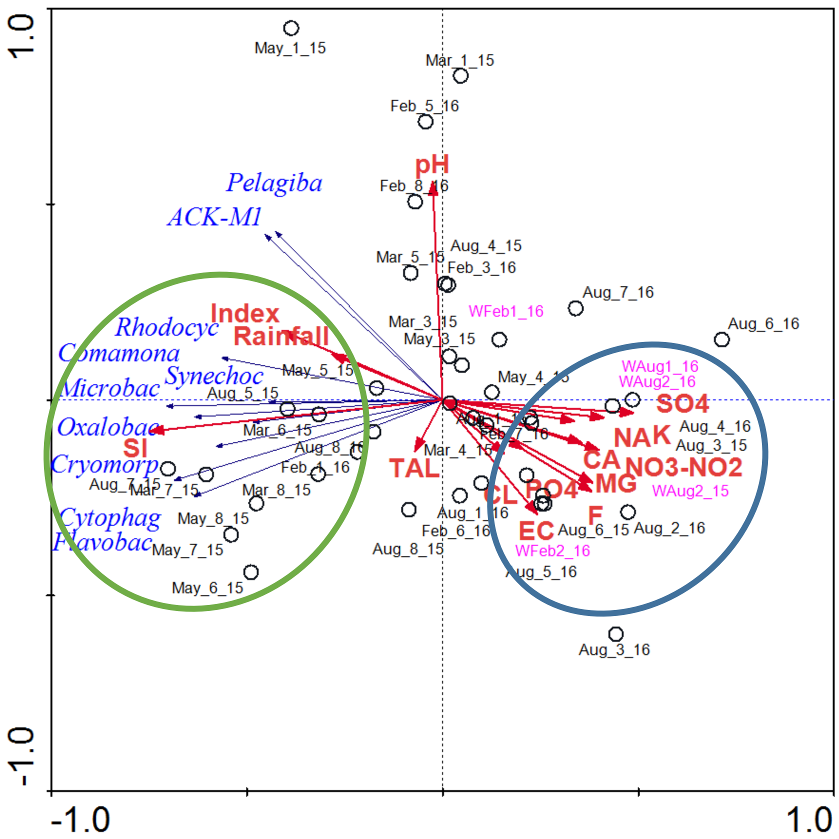

Figure 4.32: RDA of the microbial sampling sites with all combined factors based on families

The RDA (Figure 4.32) of the microbial sampling sites with all combined factors showed Wonderfonteinspruit River sites were grouped (blue) near PO_4^{3-}, SO_4^{2-}, NO_3-NO_2, EC and all ions contributing to EC, whereas the Mooi River sites grouped around pH, Si, index and TAL. *Flavobacteriaceae*, *Microbacteriaceae*, *Synechococcaceae*, *Cytophagaceae*, *Comamonadaceae*, *Oxalobacteraceae*, *Rhodocyclaceae*, *Cryomorphaceae* grouped around index which indicated they prefer better water quality and this was supported by the response curve in Figure 4.33 and also showed a general association with rainfall and silica.

4.3.6 Response curves for bacterial families

Figure 4.33 provides response curves for the various bacterial families when compared to selected water quality parameters and the overall water quality index. Most bacterial families seem to prefer better quality water as seen by the response in the curve labelled index in Figure 4.33. This is especially true for *Flavobacteriaceae* and *Comamonadaceae*. It was however, not the case when the individual parameters were considered (Figure 4.33)

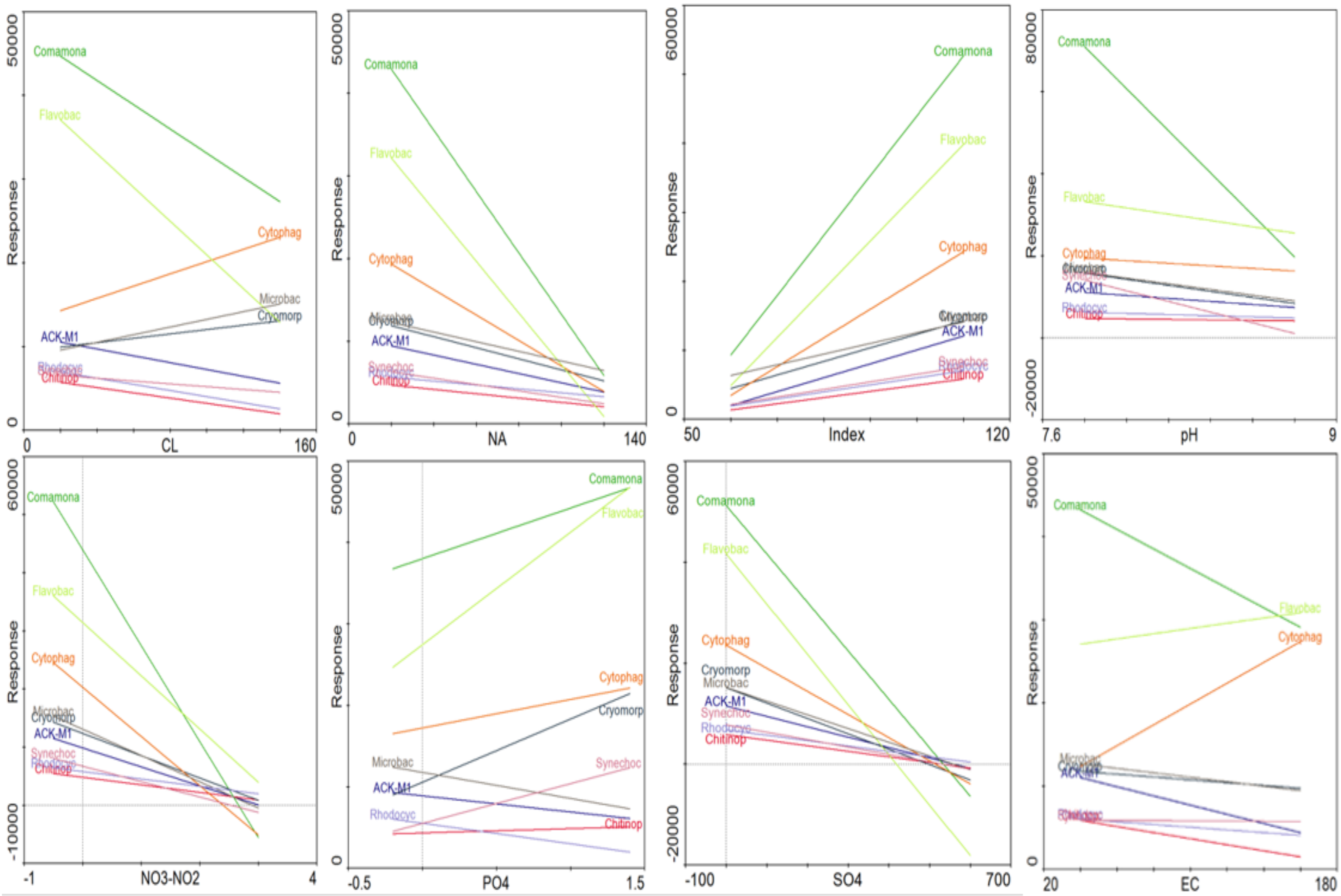

Figure 4.33: Bacterial response curves in relation to physico-chemical parameters based on families

The SO_4^{2-} concentrations negatively impacted the levels of all the families (Figure 4.28). This was also true for Na. PO_4^{3-} had a positive impact on *Flavobacteriaceae, Cytophagaceae*, *Comamonadaceae* and *Cryomorphaceae.* EC on the other had had a negative impact on *Comamonadaceae* and *Cryomorphaceae*, but again a positive impact on *Flavobacteriaceae* and *Cytophagaceae.*

4.4 Geospatial analysis of the Mooi River catchment area

Geospatial data providing an overview of land-use changes over a 10 period were obtained from databases. These were plotted to visualy represent the land-use changes over time within the North-West and Gauteng area. This particular approach is very crude but provided some insight into potential effects that could have impacted water quality at various sites.

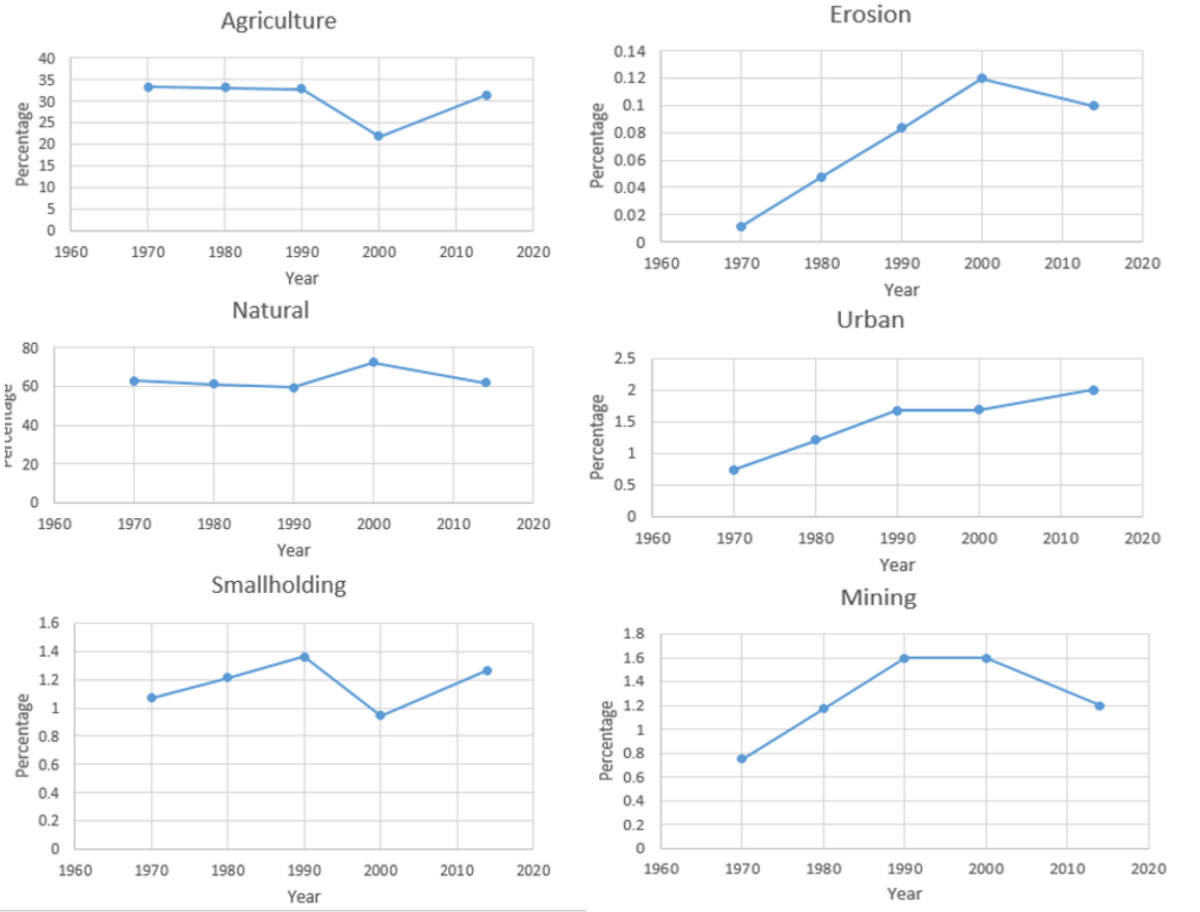

Figure 4.34: Land-use changes over the course of ±50years

The Graphs in Figure 4.34 show there was an increase in mining and smallholding occupation from the 1970s to 1990s. Mining stayed constant between the 1990s and 2000s, then decreased. Smallholdings decreased during the 1990s to 2000s and then increased again. Erosion increased from the 1970s to the 2000s and then started to decrease. Agricultural land-use were high and remained relatively constant during the 1970s to the 1990s. Agriculture decreased from the 1990s to the 2000s and the started to increase again. Similar to agriculture, natural lands stayed constant during the 1970s to the 1990s. As agriculture decreased, natural lands started increasing during the 1990s, but started decreasing again when agriculture started increasing. Urbanization generally increased over the entire period, which was expected.

4.4.1 Linking geospatial and physico-chemical data for the Mooi River

During this analysis the neural network Alyuda ForecasterXL was used to analyse the available physico-chemical and land-use data. Even though the land-use data consisted of low resolution, ForecasterXL was still used in an attempt to predict the most influential variables impacting the water quality within the Mooi River and Wonderfonteinspruit River. Land-use data and physico-chemical data were combined to predict certain impacting variables. As seen in Figure 4.35 and 4.36 the forecasted data fit almost perfectly to the actual data. The water quality score was determined using the physico-chemical data used, and explains why the forecasted values had an almost perfect fit. This allowed ForecasterXL to evaluate the physico-chemical data and land use data, against the water quality index scores and generate an input importance table showing the most impactful variables altering the quality of water within the Mooi River and Wonderfonteinspruit River. When ForecasterXL was only given land-use data, it kept on giving repeating step like output that does not fit the actual data at all. This is a result of the small amount of land-use data, one data value every ten years, available over 50 years. Land-use data resolution needs to increase before meaningful predictions can be made on variable importance using only land-use data. An evolutionary algorithm was also used to evaluate the land-use data and its impacts on water quality, but the same repeating step like output were received (Results not shown). This is understandable as the amount of land-use data input is not enough. The land-use data were still implemented within this study to show the potential that geospatial data may have in the understanding in water quality and as a pilot study that can be improved on. The results obtained from ForecasterXL seems to make sense, even though so little land-use data was available. Again, the more geospatial data there are available the better neural networks and evolutionary algorithms can perform.

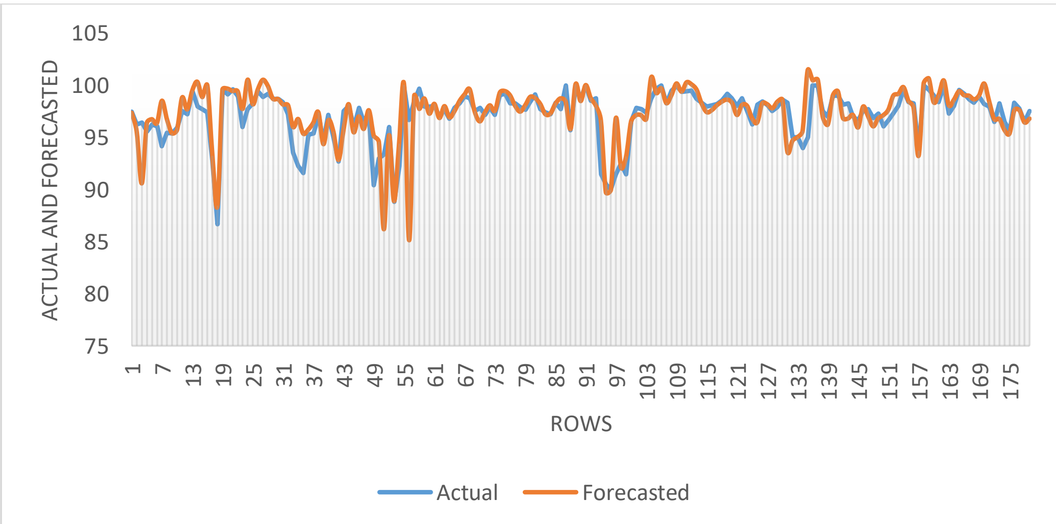

Figure 4.35: Neural network results using ForecasterXL for water quality with physico-chemical and land-use data for the Mooi River. (Rows refer to data points used in the neural network)

Table 4.18: Input importance for the Mooi River

Input	Value (%)
EC	14.36
NH_4^+	0.08
NO_3-NO_2	2.274
pH	0.006
PO_4^{3-}	12.83
SO_4^{2-}	0.775
Agriculture	29.14
Industrial	3.52
Mining	10.452
Urban	4.613
Erosion	20.04
Smallholding	1.91

The variables impacting water quality the most in the Mooi River was as expected. The major influences were EC, agriculture, PO_4^{3-} and erosion. As was already mentioned and will also follow in the discussion; agriculture is one of the main land-use activities present in the Mooi River area. This explains the neural network output linking agriculture to the changes in water quality. As water flows into the Mooi River from the Wonderfonteinspruit River, mining can have an impact on the water downstream and this may explain the 14.36% value of EC. Again the output in Table 4.18 is justified by literature and will further be discussed in chapter 5.

4.4.2 Linking geospatial and physico-chemical data for the Wonderfonteinspruit

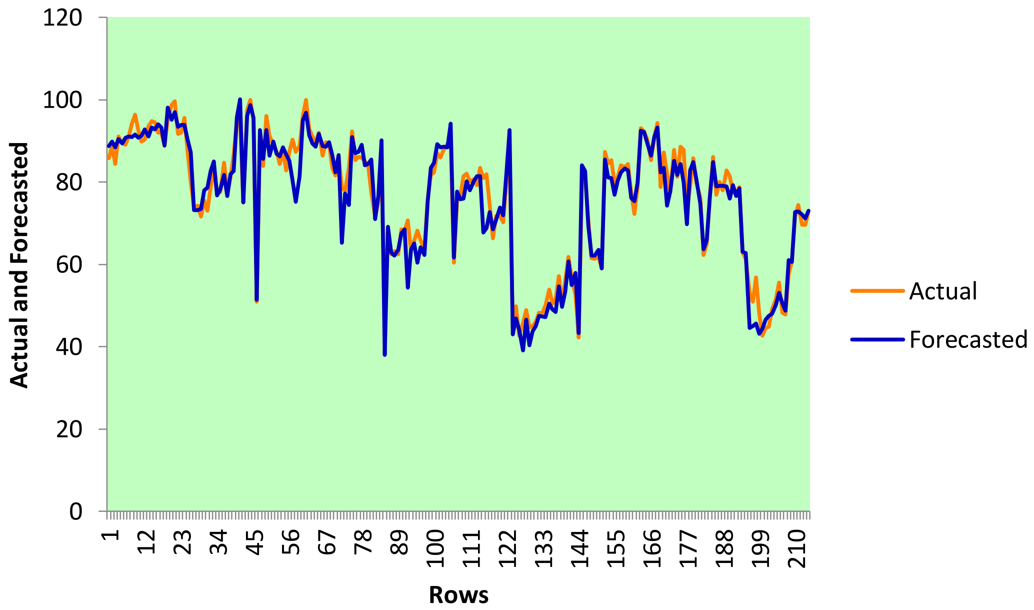

Figure 4.36: Neural network results using ForecasterXL for water quality with physico-chemical and land-use data for the Wonderfonteinspruit (Rows refer to data points used in the neural network)

Table 4.19: Input importance for the Wonderfonteinspruit

Input	Value (%)
EC	12.87
NH_4^+	0.334
NO_3-NO_2	13.19
pH	1.718
PO_4^{3-}	1.145
SO_4^{2-}	16.57
Agriculture	18.004
Industrial	1.138
Mining	17.577
Urban	1.953
Erosion	9.304
Smallholding	6.197

The dominant influences in the Wonderfonteinspruit River were EC, NO_3-NO_2, SO_4^{2-}, mining and erosion Theses variables were as expected. As previously seen in chapter 4 section 4.1, the EC, NO_3-NO_2, SO_4^{2-} of the Wonderfonteinspruit River are highly elevated. This is also true for PO_4^{3-}. The EC and SO_4^{2-} parameters are linked with mining, whereas NO_3-NO_2 and PO_4^{3-} are linked with agriculture, erosion and urbanisation. This will be further discussed in chapter 5.

4.4.3 Land-use parameter impacts in the Mooi River catchment

Table 4.20: Importance and quality training data for the Mooi River catchment

Predictor	Water quality	PO_4^{3-}	SO_4^{2-}	pH	NH_4^+	NO_3-NO_2	EC
Agriculture	16.84	84.85	23.40	22.43	5.206	11.225	31.76
Industrial	8.87	6.46	23.71	15.24	22.011	36.06	22.18
Mining	15.36	1.13	30.90	13.28	27.81	5.69	33.13
Urban	9.6	2.91	12.84	11.17	3.023	14.61	9.89
Erosion	28.53	4.45	3.20	8.43	41.44	32.41	1.84
Smallholding	20.8	0.20	5.95	29.45	0.51	0.005	1.2
Good FC_Train %	93	13	44	94	13	32	77
Bad FC_Train %	7	87	56	6	87	68	23
Good FC_Test %	90	4	19	94	4	12	42
Bad FC_Test %	10	96	81	6	96	88	58

Legend: FC = forecast

Output received from neural network with land-use data only, shows how each land-use property impacts certain parameters. Agriculture shows 84.85% importance with PO_4^{3-} changes and 31.76 % for EC. Industrial plays a role in NO_3-NO_2 values, mining in SO_4^{2-}, erosion in NH_4^+ and NO_3-NO_2 and smallholding in the pH values. Most parameters yielded bad training results making the data less trustworthy, although literature supports agricultural, mining and erosion findings. With more land-use data, it would be possible to predict these land-use impacts with more certainty. It is important to note that although urban type land use reflects a small contribution to the overall land use both in terms of overall land use, and percentage contribution to the neural network models, one cannot interpret the effect of urban land use as merely a percentage of overall land use. The impact of urbanization is more noticeable in an end of pipe scenario.

CHAPTER 5 –DISCUSSION

Within this chapter the results will be discussed and supporting literature will be given that support the finding of this study and aim to explain the results obtained.

5.1 Physico-chemical parameters, Water quality index and Geospatial analysis

The water quality of the Mooi River showed little degradation over the four-decade period. The water quality scores never decreased to a score lower than 75. Site C3R03, which had the highest water quality score is situated upstream of most land-use activities and urban areas and receives little to no effluent, which explains why C2R03 showed such high quality scores (Winde, 2009; Coetzee *et al*., 2006; Hamman, 2012). Site C2R01, also showed high water quality scores. The water quality index for the Mooi River sites were slightly impacted by PO_4^{3-} throughout the analysis period. The predominant land-use activities for the Mooi River area are crop farming and grazing (Van der Walt et al., 2002). This may have contributed to the PO_4^{3-}, especially for site C2M11 and C2M01, which caused a slight decrease in the water quality score. The neural network results also indicated that agriculture and PO_4^{3-} impacted water quality, as seen in figure 4.18 and 4.20.

The yearly PO_4^{3-} data in the Mooi River catchment were as follow: The Mooi River area showed significantly lower PO_4^{3-} levels compared to the Wonderfonteinspruit River. There was no annual increase of PO_4^{3-} at any of the Mooi River sites according to the statistics in Chapter 4. PO_4^{3-} levels were thus not a concern for most sites within the Mooi River as the combined average for all the sites were 0.041 mg/L. Site C2M11 (Figure 4.1A) had limit breaking PO_4^{3-} values during 1966 (0.838 mg/L) and again during 1979 (0.768 mg/L). This was higher than the prescribed 0.125 mg/L recommended levels suitable for healthy aquatic life. Only two data points were available within this time period. However, the limits stayed mostly constant from 1966 to 1979, which may indicate that the limits would have been similar during the late 1960s and 1970s as seen in Figure 4.1A.

The yearly NO_3-NO_2 data in the Mooi River catchment were as follow: The Mooi River again showed significantly lower Nitrate/Nitrite levels than that of the Wonderfonteinspruit River. There was no annual increase of NO_3-NO_2 at any of the Mooi River sites, the exception being site C2M11 (Figure 4.1C). This site had much higher NO_3-NO_2 concentrations compared to all other Mooi River sites, even though no limit breaking (≥ 4 mg/L) values were recorded. C2M11 had an average value of 2.55 mg/L across all years and an annual increase of 0.013 mg/L. Site C2M11 is situated where the Wonderfonteinspruit River and Mooi River confluence. Crop farming and grazing (Van der Walt et al., 2002) combined with potential runoff from the Wonderfonteinspruit River may have impacted this site and can potentially explain the elevated PO_4^{3-} and NO_3-NO_2 levels for this site.

The yearly SO_4^{2-} data in the Mooi River catchment were as follow: Similar to PO_4^{3-}, the Mooi River area showed lower sulphate levels than that of the Wonderfonteinspruit River. However, all the sites situated in the Mooi River area experienced yearly average SO_4^{2-} increases. This is especially true for the river sites (C2M11 and C2M01), which had average yearly increases of 3.008 mg/L and 0.904 mg/L respectively (Tables 4.1 and 4.2). Due to the yearly average SO_4^{2-} increases, EC also increased annually. Naturally the dams had the lowest average EC levels. Average EC for sites C2R01, C2R03 and C2R04 during the entire analysis period were 59.12 mS/m, 43.50 mS/m and 63.70 mS/m, respectively. Site C2M11 and C2M01 had 67.40 mS/m and 66.86 mS/m, respectively.

The Wonderfonteinspruit River on the other hand had much lower water quality compared to the Mooi River. The Wonderfonteinspruit River is impacted by numerous land-use activities. The Wonderfonteinspruit area alone has multiple point and non-point discharges from sewage works, mines, agriculture, settlements and industry, which results in physico-chemical changes, degradation of water quality (Hamman, 2012) and decreased water quality index scores. The water quality scores were impacted by SO_4^{2-}, EC, NO_3-NO_2 and PO_4^{3-}. These parameters degraded the water quality within the Wonderfonteinspruit River.

High PO_4^{3-} levels were recorded for all sites during the analysis period. The combined PO_4^{3-} average in the Wonderfonteinspruit River was 0.32 mg/L. This is 0.28 mg/L higher compared to the Mooi River and also 0.155 mg/L higher than the prescribed RQO limit. Site C2M30 (Figure 4.9A) and C2M32 (Figure 4.11A) had numerous limit breaking values during the analysis period. C3M30 showed and increase during 1990 (0.562 mg/L), but decreased after the year 2000. Site C2M32 had the opposite pattern and started increasing during the year 2000 and continued this trend until 2017. Sites C2M63, C2M60 and C2M69 (Figure 4.17A) had the highest PO_4^{3-} average values with 0.42 mg/L, 0.35 mg/L and 0.44 mg/L, respectively.

The Wonderfonteinspruit River had high SO_4^{2-} levels for all recorded sites during the analysis period compared to the Mooi River. The combined SO_4^{2-} average in the Wonderfonteinspruit River was 368.1 mg/L. This is 290.53 mg/L higher compared to the Mooi River, but still 209.47 mg/L lower than the prescribed RQO limit for environmental health. The SO_4^{2-} and NO_3-NO_2 levels were much higher during the 1970s and 1980s in the Wonderfonteinspruit River. It remained high after 1990, but not so much as during the two previous decades. Sites C2M32, C2M60 and C2M69 had the highest SO_4^{2-} average values during the analysis period with 434.99 mg/L, 524.84 mg/L and 411.89 mg/L, respectively. The Wonderfonteinspruit River showed higher EC levels compared to the Mooi River sites. This is understandable due to higher SO_4^{2-} levels in the Wonderfonteinspruit. The combined EC average in the Wonderfonteinspruit River was 116.81 mS/m. This is 56.7 mS/m higher compared to the Mooi River and also 5.81 mS/m higher than the prescribed RQO limit for environmental health. Sites C2M32, C2M60 and C2M69 had the highest Electrical conductivity average values with 123.92 mS/m, 156.08 mS/m and 128.63 mS/m, respectively. Again, not surprising as these were the sites with the highest SO_4^{2-} values.

The Wonderfonteinspruit River sites are downstream of the West Rand (Figure 3.1), which includes Westonaria, Witwatersrand, Randfontein and Carletonville (Figure 3.1). Randfontein, which is approximately 40 km west of Johannesburg, is situated next to Westonaria and upstream of the Wonderfonteinspruit River. During the late 1970s and 1980s, when the SO_4^{2-} and NO_3-NO_2 concentrations were the highest within the Wonderfonteinspruit River, there was an increase in mining activity on the West Rand. This lead to urbanisation, which in turn lead to population size increases, building of mines and informal settlements and overworked wastewater treatment plants (Lusilao-Makiese *et al*., 2013; Ochieng *et al*., 2010). This was also recorded by the geospatial trends, which indicated that mining activities increased from the 1970s to the 1990s in the North-West and Gauteng area (Figure 4.34). Urbanisation also increased during the 1970s and 1990s as seen in Figure 4.34 and continues to increase, which is an expected urbanisation effect. The effluent from the West Rand area has been found to pollute surrounding water systems with SO_4^{2-} and NO_3-NO_2, also impacting sites downstream of tailings dams and dense mining and urban areas (Oelofse *et al*., 2007; Naicker *et al*., 2003). McCarthy (2011) demonstrated that SO_4^{2-} was a concern in the Vaal River (east of Johannesburg) and attributed the increase to urbanisation and industrialisation. The Jukskei River catchment located within the Witwatersrand area also experience high levels of NO_3-NO_2 in their aquatic systems (Dudula, 2008). This is also linked to urbanisation, industries and municipal wastewater. This corresponds to trends observed in the Northern Cape, which experienced high nitrate levels in the water systems surrounding rural and urban areas. This pattern seems to be connected to accelerated urbanisation (Tredoux *et al*., 2000). The urbanisation and industrial expansion in the West Rand region could be responsible for the observed increases in SO_4^{2-} and NO_3-NO_2 within the Wonderfonteinspruit River during this period, which could have potentially flowed downstream from the West Rand area into the Wonderfonteinspruit River. Urbanisation and mining within the Wonderfonteinspruit area has also been an ongoing concern since the 1960s (Hamman, 2012) and may also explain the high SO_4^{2-} and NO_3-NO_2 concentrations within the Wonderfonteinspruit River during the 1970s and 1980s. Jordaan and Bezuidenhout (2015) found SO_4^{2-} concentration within the Wonderfonteinspruit River were consistently higher than the prescribed RQO levels, due to mining and industrial activities, which support the findings of this study (Winde, 2009; Winde & Stoch, 2010; Coetzee *et al*., 2006; Hamman, 2012).

The high PO_4^{3-} concentrations within the Wonderfonteinspruit River can potentially be linked to the agricultural activities within the Wonderfonteinspruit area combined with upstream pollution ending up in the Wonderfonteinspruit River. A study done by Schulz *et al*. (2001) found that agricultural runoff clearly increased PO_4^{3-} levels in the Lourens River. This was also the case in the Umtata, Buffalo, Keiskamma and Tyume Rivers in the Eastern Cape (Fatoki and Awofolu, 2003). The agricultural activities, particularly irrigation, within the Wonderfonteinspruit area have existed for decades and is an important financial aspect within this area (Winde, 2009; Coetzee *et al*., 2006; Hamman, 2012). The runoff from these agricultural lands may be a key factor impacting the PO_4^{3-} concentration. The usage of fertilizers also increased dramatically globally in 1974-1982 (Poudel *et al*., 2013) and can possibly also explain the high PO_4^{3-}, as well as the NO_3-NO_2 concentrations during this time, due to agricultural runoff in the Wonderfonteinspruit area. The geospatial trends in section 4.4 shows high agricultural activity, as well as increasing erosion within the North-West and Gauteng area during the 1970s to 1990s (Figure 4.34). The Wonderfonteinspruit River undergoes gully erosion, as well as subsurface erosion which transports contaminants into adjacent watercourses (Winde, 2009; Coetzee et al., 2006; Hamman, 2012). Agriculture, erosion and sewage runoff can combine to increase NO_3-NO_2 and PO_4^{3-} in aquatic systems. This was reported by Igbinosa and Okoh (2009) and Fatoki and Awofolu (2003) who studied the Keiskamma River—which receives discharge from the Keiskammahoek sewage treatment plant—in the Eastern Cape of South Africa. They found that the levels of NO_3-NO_2 and PO_4^{3-} were above the South Africa guideline values at the downstream sites. This was also true for the Berg River in South Africa (De Villiers, 2007). In the West Rand area, urban complexes generate large amounts of sewage. This gives rise to effluents that, even if treated, are high in PO_4^{3-} that ultimately ends up in aquatic systems causing deterioration (Oberholster *et al*., 2005). A study was done by Roux *et al*. (2010) on the Upper Crocodile-West Marico water management area, which includes parts of Johannesburg and Pretoria where the wastewater includes contributions from mining activities, industries as well as urban and agricultural areas. It was found that the PO_4^{3-} and SO_4^{2-} load in the Crocodile River—which receives effluent from Johannesburg and Pretoria—continued to increase annually. This strengthens the observation that the high levels of PO_4^{3-}, SO_4^{2-} and NO_3-NO_2 reported within the Wonderfonteinspruit River may partly be due to the upstream area pollution, combined with the mining and industrial activities within the Wonderfonteinspruit area itself. The increase in EC is directly correlated with the increase in SO_4^{2-} within the Wonderfonteinspruit River.

The yearly pH data in the Mooi River and Wonderfonteinspruit River were as follow: The Mooi River showed higher pH levels than that of the Wonderfonteinspruit River, considering the SO_4^{2-} levels were lower in the Mooi River. The pH stayed mostly constant for all Mooi River sites. The Mooi River had an average pH of 8.21 pH units. This falls within the recommended levels suitable for healthy aquatic life. The Wonderfonteinspruit River had a combined average of 7.86 pH units, which is 0.35 pH units lower than the Mooi River. All sites within the Wonderfonteinspruit River showed lower pH during the 1970's and 1980's. This again would be due to the higher SO_4^{2-} levels during that time period. Site C2M30, C2M60 and C2M69 had the lowest pH values. They also showed high SO_4^{2-} levels.

The Wonderfonteinspruit area is known to be home to numerous mines, which cause AMD that leaches into surface and groundwater (Coetzee *et al*., 2006). These impacts seemed to have no effect on the pH of sites within the Wonderfonteinspruit River. The Wonderfonteinspruit area has alkaline surface water due to the dolomite that consists of calcium carbonate and magnesium carbonate within the water that causes a buffering capacity (Hamman, 2012). These carbonates can explain why pH seemed to have stayed mostly constant, even though SO_4^{2-} was so high in this area. Ochieng *et al*. (2010) also concluded that pH mostly stayed within the recommended limits when impacted by urban and industrial effluent, although a decrease in pH did occur when SO_4^{2-} increased. This explains the lower pH values during the 1970's and 1980's within the Wonderfonteinspruit area—the same period of high SO_4^{2-} values.

Overall in summary the WQI represented a convenient and simple tool to evaluate water quality by reducing key parameters into a single index. This allows a user to rapidly get an overview over large datasets, where significant changes in the WQI can guide the user to the relevant data point to investigate what parameter(s) contribute to the shift in the WQI.

5.2 Bacterial community composition

Newton *et al. (*2011), reviewed 69 published papers and described *Bacteriodetes, Proteobacteria, Actinobacteria, Cyanobacteria* as the most common freshwater bacterial phyla, reporting that *Actinobacteria and Proteobacteria* are the most abundant bacterial phyla in lakes and rivers. Zwart *et al*. (2002) analysed databases of 16S rDNA sequences, including 24 sequences from Parker River (Massachusetts, USA), 42 from Lake Soyang (South Korea) and 148 from Lake IJssel (The Netherlands) and also found *Bacteriodetes, Proteobacteria, Actinobacteria, Cyanobacteria* as the most dominant freshwater bacteria. de Oliveira and Margis (2015) found that the Sinos River in Brazil were dominated by *Proteobacteria*, *Bacteroidetes*, *Actinobacteria* and *Cyanobacteria.* Cottrell *et al*. (2005) noted that *Proteobacteria* and *Actinobacteria* are of the largest fractions of bacteria in the Delaware River and Crump *et al*. (1999) also found that the majority of freshwater bacteria in the Columbia River are *Proteobacteria.* A study done by Jordaan & Bezuidenhout (2013) on the bacterial diversity of the Vaal river in South Africa also found *Bacteriodetes, Proteobacteria, Actinobacteria, Cyanobacteria* to be the most abundant. Another study done by Jordaan and Bezuidenhout (2015) found *Bacteriodetes, Proteobacteria, Actinobacteria, Cyanobacteria* as the most abundant phyla within the Mooi River. This correlates with the findings of the present study as *Bacteroidetes* was identified as the most common Phylum in the Mooi River and Wonderfonteinspruit. This was followed by *Proteobacteria, Actinobacteria, and Cyanobacteria* as seen in Figure 4.24, 4.25 and 4.26*.* The fifth most common phylum during 2015 was Verrucomicrobia, with OD1 being the fifth most common in 2016 for the Mooi River and Chloroflexi for the Wonderfonteinspruit.

5.2.1 Actinobacteria

Actinobacteria were the third most abundant phylum overall within the Mooi River and Wonderfonteinspruit River during 2015 and 2016 (Figures 4.24, 4.25 and 4.26). During 2016 *Actinobacteria* became the second most abundant phylum within the Mooi River, which showed an increase of *Actinobacteria* from 2015 to 2016. A clear pattern was not observed seasonally as *Actinobacteria* showed abundancy during August and February (Tables 4.14 and 4.15). *Microbacteriaceae* were the fourth most abundant family member within the Mooi River during 2015 and 2016. Within the Wonderfonteinspruit River it was second most abundant during 2015 and 2016. *ACK-M1* were the fourth most abundant family member within the Wonderfonteinspruit River during 2015 and 2016. The abundancy of *ACK-M1* were less within the Mooi River, being the sixth most abundant family (Figures 4.29, 4.30 and 4.31). These *Actinobacteria* family members showed no seasonal patterns.

Actinobacteria is highly abundant in freshwater systems around the world including Asia, South America, Europe, Africa, Antarctica and Australia (Ghai *et al*., 2011). The phylum *Actinobacteria* are Gram-positive bacteria with a high G+C DNA composition. *Actinobacteria* abundancy decreases with decreasing oxygen concentrations, and are often the numerically dominant phylum, containing several of the recognized freshwater cosmopolitan bacterial groups and comprising an integral part of freshwater bacterioplankton (Newton *et al*., 2007; Hahn, 2009). They are important decomposers of plant material into simple sugars using cellulolytic enzymes and play a role in cycling a variety of carbon sources, including chitin, the structural polymer in the cell wall of fungi and the exoskeletons of invertebrates. Additionally, some *Actinobacteria* are able to degrade hydrocarbons and organic contaminants, including aromatic hydrocarbons, sulfonated azo dyes, nitroaromatics and pesticides. *Actinobacteria* also have large pathways for nucleic and amino acid metabolism (Lewin *et al*., 2016).

The reasons for their survival success includes having the capability of growing in a range of conditions and are characterized by remarkably small cells and thin cell walls making them less susceptible to grazing mortality caused by flagellates. Observations also indicate that planktonic *Actinobacteria* are less vulnerable to protistan predation than other taxa of freshwater bacterioplankton and it has been postulated that *Actinobacteria* has UV stress resistance. They appear to be more tolerant of conditions with low organic carbon concentrations, and may be replaced by *Proteobacteria* during algal blooms causing increased carbon levels (Tarao *et al.*, 2009; Newton *et al*., 2011; Jordaan & Bezuidenhout, 2013; 2015). Minor fluctuations in the abundance of *Actinobacteria* during seasonal changes points to a consistent source of energy generation for these organisms (Lewin *et al*., 2016). This pattern was also identified within the Mooi River and Wonderfonteinspruit River. They have been revealed in more recent studies as widespread symbionts of eukaryotes; serving as nutritional-and-defence mutualists producing natural defence products and helping herbivores gain access to plant biomass (Lewin *et al*., 2016). Also, pH has been identified as one of the major drivers of *Actinobacteria* distribution (Newton *et al.,* 2011). The family members *Microbacteriaceae* and *ACK-M1* are part of the *Actinobacteria* phylum. Park *et* al. (1993) first proposed the *Microbacteriaceae* family. Three years later Stackebrandt et al. (1997) emended this family. It was isolated from diverse environments, including freshwater, groundwater, seawater, sediment, sewage and ice samples. It has since been found in numerous freshwater environments. The *Microbacteriaceae* family has high GC content and is irregularly shaped. It is aerobic, non-spore-forming, non-motile and Gram-positive (Hahn *et al*., 2014). *ACK-M1* has a similar structure and is mostly found within freshwater environments. They have been detected in almost all freshwater habitats investigated for them so far (Zwart *et al*., 2002). *Microbacteriaceae* was found in Canada at Point Pleasant Park pond in Halifax (Sharma *et al*., 2009) and have been isolated from five freshwater habitats in Europe and Asia (Hahn *et al*., 2003). *Microbacteriaceae* and *ACK-M1* was present within the Sinos River in Brazil (de Oliveira and Margis, 2015). Li *et al*. (2015) found that *ACK-M1* was the dominant family of the *Actinobacteria* phylum within Lake Taihu.

The response curve (Figures 4.28 and 4.33) showed negative correlation with pH and revealed that, for the Mooi River catchment at least, *Actinobacteria* thrived in slightly lower pH conditions. They seemed to also prefer low NO_3-NO_2 limits (Figure 4.28), making the Wonderfonteinspruit River slightly less attractive for *Actinobacteria*. Although the response curves showed positive correlation with PO_4^{3-} (Figure 4.28). These conditions were present within the Mooi River and Wonderfonteinspruit River during 2015 and 2016, which may explain the abundancy of *Actinobacteria.*

5.2.2 Bacteroidetes

Bacteroidetes were the most abundant phylum overall within the Mooi River during 2015 and third most abundant within the Wonderfonteinspruit River during 2015 and 2016 (Figures 4.24, 4.25 and 4.26). A clear pattern was not observed seasonally as *Bacteroidetes* showed abundancy during March, May and August (Tables 4.14 and 4.15). *Flavobacteriaceae* were the most abundant family member within the Wonderfonteinspruit River during 2015 and 2016. Within the Mooi River it was second most abundant during 2015 and 2016. *Cytophagaceae* were the third most abundant family member within the Mooi River during 2015 and 2016. The abundancy of *Cytophagaceae* were less within the Wonderfonteinspruit River, being the fifth most abundant family. *Cryomorphaceae* were fifth most abundant within the Mooi River and sixth most abundant within the Wonderfonteinspruit River (Figures 4.29, 4.30 and 4.31). These *Bacteroidetes* family members showed no seasonal patterns.

The members of the *Bacteroidetes* phylum occurs in soil, aquatic environments, or as symbionts in plants, animals and humans. They exhibit enormous phenotypic and metabolic diversity (Newton *et al.,* 2011). Based on analysis of 16S rRNA gene sequencing, they are highly abundant in aquatic environments (O'Sullivan *et al.,* 2004; Jordaan & Bezuidenhout, 2013; 2015). They are non-flagellated, Gram-negative rod-shaped cells. Most *Bacteroidetes* isolates are described as chemoorganotrophs and comprise a large proportion of particle-associated bacteria. Some species of *Bacteroidetes* like *Fluviicola taffensis* does not grow in the presence of sodium in freshwater ecosystems (O'Sullivan *et al., 2005*). The response curve generated (Figure 4.28) supports this statement as it showed a negative response of *Bacteroidetes* with increasing Na. *Bacteroidetes* seem to play an important role in degrading complex biopolymers, humic matter and exhibits an increase in propensity in the presence of high external dissolved organic carbon (DOC) indicating strong dependency to organic matter. Newton *et* al. (2011) noticed increasing abundance of *Bacteroidetes* after cyanobacterial blooms in freshwater systems. *Cyanobacteria* and *Bacteroidetes* are the two most abundant phyla in the Wonderfonteinspruit River (Figure 4.28). This may indicate that *Bacteroidetes* were aided by *Cyanobacteria* blooms in the Wonderfonteinspruit River.

Bacteroidetes was also highly abundant in the Mooi River (Figures 4.24 and 4.25). Unlike common freshwater groups, *Bacteroidetes* does not seem to exhibit perceivable seasonal patterns as seen by their dominance in Tables 4.14 and 4.15 across all months. *Bacteroidetes* are important constituents of particle associated bacterial assemblages, possibly due to their ability to degrade macromolecules (Riemann & Winding, 2001). Members from this phylum *Bacteroidetes* can be used to detect faecal pollution in freshwater through 16S rRNA gene markers (Dick & Field, 2004). The family members *Flavobacteriaceae, Cytophagaceae* and *Cryomorphaceae* are part of the *Bacteroidetes* phylum. The family *Cytophagaceae* are one of the largest families in the *Bacteroidetes* phylum and are widely distributed within freshwater ecosystems. They are able to degrade many organic compounds such as cellulose, chitin and starch (Joung *et al*., 2014). All known members of this family are heterotrophic and most are aerobic, having a primarily respiratory metabolism. This distinguishes them from physiologically similar bacteria from the family *Flavobacteriaceae* (Liu *et al*., 2016). The family *Flavobacteriaceae* are mostly found in freshwater and marine environments and also prefer higher temperatures. They appear to always be present in water purification systems and can represent up to 60% of the bacterial community within activated sludge plants. Jordaan & Bezuidenhout (2013) found *Flavobacteriaceae* to be the most abundant family member from the *Bacteroidetes* phylum within the Mooi River. The *Cryomorphaceae* family was first described by Bowman et al. (2003) stating that it closely resembles *Flavobacteriaceae*. *Cryomorphaceae* occurs in a range of aquatic habitats including rivers, seawater, marine sediments, and endorheic water bodies. Their cells are Gram-negative and they are nonsporulating, rodlike and sometimes filamentous, and multiply by binary fission. Data suggests that *Cryomorphaceae* have secondary production roles in aquatic ecosystem (Lee *et al*., 2010). *Cryomorphaceae* was found to be abundant within various Rivers in Western Japan (Nakai *et al*., 2015).

The response curves (Figures 4.28 and 4.33) indicated *Bacteroidetes* preferred better quality water, as well as lower pH and SO_4^{2-} values. This makes the Mooi River area an especially flourishing environment for *Bacteroidetes* and thus supports the high levels found at the Mooi River sites. *Bacteroidetes* also responded with slight negativity to NO_3-NO_2 which was also found to be the case with the study of Jordaan & Bezuidenhout (2013) conducted in South Africa on the Vaal River.

5.2.3 Cyanobacteria

Cyanobacteria were the fourth most abundant phylum overall within the Mooi River during 2015 and 2016. It was the second most abundant within the Wonderfonteinspruit River during 2015 and 2016 (Figures 4.24, 4.25 and 4.26). Within the Mooi River *Cyanobacteria* seemed to favour the month of August, although this pattern was not observed within the Wonderfonteinspruit River (Tables 4.14 and 4.15). *Synechococcaceae* were the third most abundant family member within the Wonderfonteinspruit River during 2015 and 2016. The Mooi River showed a lower abundancy of Synechococcaceae, being the ninth most abundant during 2015 and 2016 (Figures 4.29, 4.30 and 4.31). This *Cyanobacteria* family member showed no seasonal patterns as no complete seasonal cycle was included in the monitoring programme.

Members from the phylum *Cyanobacteria* are the photosynthetic prokaryotes and are able, to produce oxygen and remove carbon dioxide (Yannarell & Kent, 2009). Their life processes only require water, carbon dioxide, inorganic substances and light. Ultraviolet absorbing sheath pigments increases their fitness in exposed land environments. They are often classified as nuisance organisms, because they release toxins into the aquatic system and when they perish after a bloom they cause anoxic conditions. Worldwide, *Cyanobacteria* have been related to several human influences, such as agricultural nutrient loadings, industrial discharges and urban pollution (Newton *et al*., 2011). Such conditions prevail in the Worndenfonteinspruit and Mooi Rivers.

Evidence show that rising temperatures, nutrient loads and enhanced stratification favour the dominance of *Cyanobacteria* (Cao *et al*., 2016). *Cyanobacteria* are able to grow at low rates of dissolved nitrogen and are able to fix atmospheric nitrogen (N_2) making them good indicators for N-limited sites. Certain *Cyanobacteria* are able to live at phosphorus limitation due to phosphatase activity and others are capable of degrading phthalate ester (PE) intracellularly (Monteagudo & Moreno, 2016). Numerous freshwater *Cyanobacteria* species can withstand high concentrations of sodium chloride and have the ability to survive extreme temperature changes (Chorus & Bartram, 1999). Increasing eutrophication results in increasing Cyanobacteria (Van Ginkel, 2011). Like mentioned by Yoon *et al*. (2015); Adesuyi *et al*. (2015) and Sharpley *et al*, (2001), *Cyanobacteria* thrive in water systems with high levels of N and P. A study conducted on the Sinos River in Brazil by de Oliveira and Margis (2015) found that *Synechococcaceae* were the most abundant family within the *Cyanobacteria* phylum. Muir and Perissinotto (2011) did a study on Lake St Lucia in South Africa, which is impacted by agricultural effluent, and found that the *Synechococcaceae* (*Cyanobacteria*) were the family responsible for persistent phytoplankton blooms within the lake. This may also become a concern within the Wonderfonteinspruit River as *Synechococcaceae* is so abundant in this area. Harland *et al*. (2014) found *Synechococcaceae* to be the dominant family of the *Cyanobacteria* phylum in Selwyn River, New Zealand causing the same problems. Chopyk *et al*. (2018) collected water samples during October 2016, November 2016 and December 2016 from a large freshwater agricultural pond in central Maryland, United States. They found that *Synechococcaceae* was present in high abundance.

The response curves (Figures 4.28 and 4.33) indicated that *Cyanobacteria* reacted positively to higher PO_4^{3-} values and was not affected by changes in NO_3-NO_2 as some members of this group already possess the ability to fix nitrogen themselves. In the present study (Figures 4.28 and 4.33) *Cyanobacteria* was not affected nearly as much by Cl and SO_4^{2-} as *Proteobacteria* and *Bacteroidetes*. On the other hand, sodium had a positive effect on the *Cyanobacteria* population.

Reviewing literature and interpreting the results of this study it is understandable why *Cyanobacteria* is second most abundant in the Wonderfonteinspruit. Both Wonderfonteinspruit and Mooi Rivers are impacted by agricultural and erosion, resulting in elevated PO_4^{3-} and NO_3-NO_2.

5.2.4 Proteobacteria

Proteobacteria were the second most abundant phylum overall within the Mooi River during 2015 and third most abundant during 2016. It was the fourth most abundant within the Wonderfonteinspruit River during 2015 and 2016 (Figures 4.24, 4.25 and 4.26). Within the Mooi River *Proteobacteria* seemed to favour the wet season, although this pattern was not observed within the Wonderfonteinspruit River (Tables 4.14 and 4.15). *Comamonadaceae* were the most abundant family member within the Mooi River during 2015 and 2016. The Wonderfonteinspruit River showed a much lower abundancy of

Comamonadaceae, not even being the top 10 most abundant families during 2015 and 2016 (Figures 4.29, 4.30 and 4.31). This *Proteobacteria* family member seemed to prefer the month of August more (Table 4.16).

The *Proteobacteria* are Gram-negative bacteria consisting of phototrophs, chemolithotrophs and chemoorganotrophs and can be found in both oxic and anoxic environments (Yannarell & Kent, 2009; Kent *et al.,* 2006). They are sulphate reducing bacteria and are also recognised as industrially, agricultural and medically relevant organisms that consists of six classes. *Alphaproteobacteria, Betaproteobacteria* and *Gammaproteobacteria* are the major classes found in aquatic systems (Lefort and Gasol, 2013). *Alpha*- and *Gammaproteobacteria* dominates marine costal water (still ubiquitous in freshwater), whereas *Betaproteobacteria* can be found more abundantly in freshwater (Newton, 2006; Yannarell and Kent, 2009). *Alphaproteobacteria* play a significant role in freshwater by degrading complex organic compounds (Newton *et al*., 2011). *Gammaproteobacteria* on the other hand, are copiotrophs (adapted to high-nutrient conditions) and members of this class can be used in the source tracking of faecal pollutants (Stoeckel & Harwood, 2007). The class *Betaproteobacteria* are rarely grazed upon and favours high nutrient conditions, using these nutrients for rapid growth, and is often associated with algae and carbon-based particulate matter, but prefers relatively clean water (Newton, 2006; Newton *et al* 2011). Members of this class are involved in the nitrogen cycle by providing fixed nitrogen to plants via the oxidation of ammonium to nitrate (Newton, 2006). (Newton, 2006; Yannarell & Kent, 2009). The family *Comamonadaceae* falls within *Betaproteobacteria* and are characterized by a relatively high genomic GC content. Members of this family are prevalent within freshwater and activated sludge. They are capable of degrading organic and inorganic compounds, including amino acids (Ge *et al*., 2015). They are aerobic, while some species use nitrate as a last acceptor of electrons and they also prefer higher temperature (Jara *et al*., 2018). Moon *et al*. (2018) stated that the *Comamonadaceae* family is found ubiquitously in freshwater environments as one of the dominant bacterial groups. Ntougias *et al*. (2016) sampled stream and lake surface water to determine the bacterial community structures in Svalbard. They found that bacterial communities mostly comprised of *Comamonadaceae*. Cheng and Foght (2007) found that *Comamonadaceae* and *Flavobacteriaceae* were highly abundant in oligotrophic freshwater environments, which included the Canadian High Arctic glaciers. Jordaan & Bezuidenhout (2013) also found *Comamonadaceae* to be the highly abundant within the Mooi River.

Proteobacteria showed negative correlation with PO_4^{3-}, SO_4^{2-}, EC and NO_3-NO_2 (Figure 4.28). This was also the case with the Jordaan & Bezuidenhout (2013) study on the Vaal River. These parameters are all present with high levels in the Wonderfonteinspruit area and explains why *Proteobacteria* is abundant here.

CHAPTER 6 –CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

6.1.1 Analysing historical and recent physico-chemical and microbiological water quality of the Mooi River catchment to determine temporal and spatial variables.

The analysis of physico-chemical and microbiological water quality was successful. The analysis took place after converting historical hardcopy data into softcopies using Microsoft Excel (2013). Physico-chemical trends were apparent during the analysis period for both spatial and temporal variables. The parameters PO_4^{3-}, SO_4^{2-}, EC and NO_3-NO_2 were the most influential during this study. Higher PO_4^{3-}, SO_4^{2-}, EC and NO_3-NO_2 were present within the Wonderfonteinspruit River compared to the Mooi River. The microbiological results also varied spatially. *Bacteroidetes* were the most abundant phyla within the Mooi River and Wonderfonteinspruit River. More *Cyanobacteria* were identified within the Wonderfonteinspruit River compared to the Mooi River. More *Actinobacteria* were present within the Mooi River compared to the Wonderfonteinspruit River. When comparing the families, *Comamonadaceae* were most abundant within the Mooi River and *Flavobacteriaceae* within the Wonderfonteinspruit River. The family member Synechococcaceae, belonging to the Cyanobacteria phylum were more abundant within the Wonderfonteinspruit River compared to the Mooi River.

6.1.2 Developing a water quality index for the Mooi River and Wonderfonteinspruit River, using the historical and recent physico- chemical data.

The developing of a water quality index for the Mooi River and Wonderfonteinspruit River were successful. From the water quality index, it was clear that the Mooi River had better water quality compared to the Wonderfonteinspruit River. Sites C2R01, C2R03 and C2R04, which are situated within the Mooi River area, were the best quality sites overall. The sites that were most concerning were C2M32, C2M60 and C2M69 within the Wonderfonteinspruit River. The quality of these sites were lowest from 1979 to around 1995. During the early 2000s the water quality of the sites improved. Site C2M13, which is downstream of sites C2M60 and C2M69 showed better quality. This may be due to the wetland situated between these sites.

6.1.3 Comparing water quality index data to geospatial information systems (land-use) data

Comparing water quality and land-use data were successful. Agriculture and urbanisation mostly affected the water quality within the Mooi River. Agriculture, erosion, urbanisation and mining were the major land-use contributors affecting the water quality within the Wonderfonteinspruit River. The most concerning water quality periods were between years when agriculture, erosion, urbanisation and mining were increasing. This was mostly during the 1970s to 1990s

6.1.4 Link the recent water quality and land-use data to bacterial community structures.

Linking the bacterial community structures to water quality and land-use data were successful. Most bacterial phyla and families preferred better water quality. The SO_4^{2-} concentrations negatively impacted the levels of all phyla and families, although some were more tolerant. This was also true for EC, except in the case of *Flavobacteriaceae* and *Cytophagaceae,* which had a positive association with EC. The PO_4^{3-} had positive impacts on the phyla (*Actinobacteria*, *Cyanobacteria* and *Bacteroidetes*), except *Proteobacteria* and on the family members *Comamonadaceae*, *Cryomorphaceae*, *Flavobacteriaceae* and *Cytophagaceae*. NO_3-NO_2 had the opposite impact on these bacterial communities and negatively affected them, although the *Cyanobacteria* phylum was not as negatively impacted. Mining, agriculture, urbanisation and erosion were the land-use activities that impacted the bacterial phyla and families the most, as they are the contributors of EC, SO_4^{2-}, PO_4^{3-} and NO_3-NO_2.

6.2 Recommendations

The following recommendations are suggested based on the results of this project:

- Though not possible with the historical data, it is highly recommended that the specific land-use data used for future modelling be representative of the area directly affecting the sampling site. The current land-use data was a total summary for the entire North-west and Gauteng region and as such lacked resolution.
- Larger land-use data sets are highly recommended.
- Microbiological data sites and physical-chemical data sites should, for future studies, be perfectly aligned with one another. This was not possible with the available data for this study.
- More historical microbiological data is recommended to compare with the historical physico-chemical data. This will give more resolution and yield better results. Additionally, a complete season cycle also need to be covered to give a more detailed and complete picture of the bacterial phyla present.
- Expansion on the parameter limits for impoundments and rivers will be required to improve on the water quality index.

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