Water quality of the Mooi River North-West Province: A supporting study for the determination of resource quality objectives

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Dissertation submitted in fulfilment of the requirements for the degree *Magister Scientiae* in Environmental Sciences at the Potchefstroom Campus of the North-West University

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May 2017

ABSTRACT

South Africa, a semi-arid country, is currently facing an increasing water scarcity. Therefore a need exists for the management of water resources. A balance must however exist between the need to protect and maintain our water resources and the need to utilize it. As a means to ensure a desired level of protection, resource quality objectives (RQO) have to be determined for all significant water resources. The purpose of the RQO is to provide numerical and narrative descriptors of quality, quantity, habitat and biotic conditions as a basis from which management actions can be implemented for the sustainable use of all water resources. The Mooi River, located in the North West Province, is a significant water resource that forms part of the Upper Vaal catchment region. Potable water for the City of Potchefstroom is gathered from the Mooi River catchment, specifically the Boskop Dam, from where it is transported to the purification plant.

During this study the water quality of the Mooi River were determined by means of algal indices, physico-chemical analyses and microbiological analyses. Samples were taken at eight sites along the Mooi River, including three reservoirs. The Mooi River, regularly form part of the news due to the Wes Rand mining activities and the impact thereof on the Mooi River via the Wonderfonteinspruit. The main uses of the Mooi River include abstraction for drinking water and irrigation, agricultural activities and recreation. The physico-chemical and microbiological data is therefor expected to exhibit results indicative of aforementioned activities. The variables for this study were chosen with these activities in mind in order to achieve the objectives set out for this study.

Due to their high reproductive rates, algae respond rapidly to natural and/or anthropogenic changes in their environmental conditions. During this study four algal indices and the overall algal group abundance was used to aid in determining the water quality of the Mooi River. The Palmer index, indicative of organic pollution, the Shannon-Weaver index, indicative of inorganic pollution, and the Margalef- and Pielou index indicative of species richness and evenness respectively.

The Palmer index showed that the Mooi River currently experiences high levels of organic pollution with index scores higher than 20. Possible sources of organic pollution include

livestock, sewage effluent from informal settlements, agricultural runoff and abattoirs. The Palmer Index identified genera that contributed most to the high scores are *Euglena* spp, *Scenedesmus* spp and *Chlamydomonas* spp, present at all sites. On investigation the high species richness and diversity identified by the Margalef and Pielou indices, showed that it was contributed by mostly Palmer Index recognised species. It was found that the Mooi River water quality deteriorated from an oligotrophic state to a mesotrophic - eutrophic state in the current study. The trophic state is further confirmed as Mesotrophic by the mean nitrate and nitrite concentration of 0.877mg/l and the mean orthophosphate concentration of 0.163mg/l determined for the whole Mooi River. The abundance of the Cyanophyceae and Bacillariophyceae algal groups, characteristic of mesotrophic to eutrophic water, were found to have increased when compared to previous studies. This change is most probably brought on by the agricultural activities surrounding the Mooi River. Problematic Cyanophyceae genera identified at Site 2: KKD and Site 5: BKD were *Microcystis* sp. and *Oscillatoria* sp. *Microcystis* is known for producing cyanotoxins, which pose a health risk for both humans and animals. *Oscillatoria* is known to be a taste and odour causing culprit, and was also identified at Site 3: BWFS, Site 6: PD and Site 7: WWTP. The results obtained during the evaluation of the algal community corresponds to the class III classification of the Mooi River, stating that the river is heavily impacted on by human activity but is still ecologically sustainable.

Significant differences in the levels of the physico-chemical parameters: electrical conductivity, magnesium, calcium, total dissolved solids and sulphate were seen, after the confluence of the Mooi River with the Wonderfonteinspruit. Highlighting the effect of the mining activities. The magnesium and calcium levels are most probably contributed by not only the dolomitic lithology of the region but also the West Rand mining activities via the Wonderfonteinspruit. The dissociation of the dolomitic lithology has a buffering effect and contributes to higher pH.

A significant correlation, ($p<0.05$), exists between the sulphate concentration and the cell concentration of sulphate reducing bacteria in the Mooi River. Even though the sulphate levels are currently not a threat when considering the RQO, the activity of Sulphate Reducing bacteria may pose a threat due to the formation of H_2S. This phenomenon once again highlights the impact of the mining activities on the electrical conductivity, magnesium-, calcium concentration and total dissolved solids on the river. During this

study the need for RQOs to manage and improve the water quality of the Mooi River is evident.

Compared to previous studies the uranium concentration decreased at Site 5: BKD where water is abstracted for drinking water purposes.

The average *E.coli* counts determined for the Mooi River were 828cfu/100ml. The sites displaying high count were mainly Site 4: AWFS, where cattle grazing were evident, and Site 8: EBR, influenced by agricultural activities and the runoff from a piggery.

Results for which the 95% percentile exceeded the set RQO for the Upper Vaal were pH, orthophosphate, magnesium and *E.coli*. Variables measured that were below the set ROQs for the Upper Vaal were: nitrate and nitrite, electrical conductivity, sulphate, dissolved manganese and dissolved uranium.

Considering the physico-chemical, phytoplankton and biological levels measured it can be concluded that the Mooi River system has high levels of organic pollution with a high faecal pollution load. The nutrient pollution needs intervention as it is rapidly contributing to an eutrophic system. It is also found that the Mooi River is a productive system with high species diversity. Blooms of nuisance algae can however be expected.

The implementation of resource quality objectives are thus of need and must be continuously reconsidered and monitored as the quality of the Mooi River changes.

Keywords: water quality, phytoplankton assemblages, physico-chemical variables, Resource Quality Objectives, indices, Mooi River

OPSOMMING

Suid Afrika is a semi-ariede land. Die bestuur van Suid Afrika se waterbronne is dus van uiterse belang om te verseker dat die waterkwaliteit van hierdie bronne geskik bly vir die verskeidenheid van gebruike. Daar moet egter ’n balans gehandhaaf word tussen die benutting en die beskerming van hierdie waterbronne. Die Hulpbron Kwaliteitsdoelwitte (HBD) is juis vir hierdie doel daargestel deur die Department van Waterwese en Sanitasie. Die doel van die HBD is om kwantitatiewe en kwalitatiewe beskrywende aspekte rakende die waterkwaliteit, hoeveelheid, habitat en biotiese toestande daar te stel om sodoende ’n basis te vorm vir die bestuur van ’n betrokke waterbron. Voor die instelling van hierdie HBD is dit egter belangrik om die waterkwaliteit te bepaal. Die Mooirivier is geleë in die Noordwes provinsie en vorm deel van die Boonste Vaal opvangsgebied. Drinkwater vir die dorp Potchefstroom word onttrek vanuit die Mooirivier, meer spesifiek die Boskop Dam, vanwaar dit vervoer word na die watersuiweringsaanleg.

Tydens hierdie studie is die waterkwaliteit van die Mooirivier bepaal deur te kyk na alg indekse, die fisies-chemiese analises en mikrobiologiese analises. Monsters is geneem by agt punte langs die Mooirivier wat drie reservoirs insluit. Die Mooirivier is gereeld in die nuus a.g.v die Wesrand se mynaktiwiteite en die impak daarvan op die Mooirivier via die Wonderfonteinspruit. Gebruike van die Mooirivier sluit in: onttrekking vir drinkwater, ontspanning en besproeiing. Daar word verwag dat die fisies-chemiese eienskappe, fitoplankton- en mikrobiologiese resultate hierdie aktiwiteite sal weerspieël en die veranderlikes is juis gekies met hierdie aktiwiteite in gedagte. As gevolg van alge se hoë vermeerderingstempo, reageer dit vinnig op natuurlike en antropogeniese omgewingstoestande. Tydens hierdie studie was vier alg indekse sowel as die algemene alg groep samestelling gebruik in die bepaling van die waterkwaliteit van die Mooirivier. Die volgende alg indekse is saamgestel: die Palmer indeks, aanduidend van organiese besoedeling, die Shannon-Weaver indeks, aanduidend van anorganiese besoedeling en die Margalef en Pielou indeks aanduidend van spesie rykheid en verspreiding onderskeidelik.

Hoë vlakke van organiese besoedeling is aangedui deur die Palmer indeks met 'n Palmer telling van bo 20. Moontlike bronne van organiese besoedeling is riool afvloei vanaf informele nedersettings, landbou afloop en abattoirs. Die Palmer genera wat bygedra het

tot die hoë Palmer indeks telling is *Euglena* spp, *Scenedesmus* spp en *Chlamydomonas* spp wat teenwoordig was by al die versamelpunte. By verdere ondersoek is ook gevind dat die Palmer indeks genera bydra tot die hoë spesie rykheid en spesie diversiteitstelling van die Margalef en Pielou indekse onderskeidelik. Tydens hierdie studie is daar bevind dat die waterkwaliteit van die Mooirivier afgeneem het vanaf 'n oligotrofiese vlak na 'n meso- tot eutrofiese vlak. Die gemiddelde nitraat en nitriet konsentrasie van 0.877mg/l en gemiddelde ortofosfaat konsentrasies van 0.163mg/l bepaal vir die Mooirivier as geheel is aanduidend van ‘n mesotrofiese stelsel. Hierdie verskynsel kan moontlik verklaar word deur die landbou aktiwiteite omliggend van die Mooirivier. Die Cyanophyceae en Bacillariophyceae fitoplankton groepe, kenmerkend van 'n meso- tot eutrofiese stelsel, het toegeneem in vergelyking met vorige studies. Hierdie veranderinge is heel moontlik veroorsaak deur die landbouaktiwiteite en afvloei wat die Mooirivier omring. Probleem fitoplankton wat geïdentifiseer is, is *Microcystis* sp. en *Oscillatoria* sp. by punt 2: KKD en punt 5: BKD. *Microcystis* sp. produseer sianotoksiene, wat ’n gesondheidsrisiko inhou vir beide mense en diere. *Oscillatoria* sp. veroorsaak ook smake en reuke en is ook gevind by punt 3: BWFS, Punt 6: PD en Punt 7:WWTP. Die fitoplankton resultate stem ooreen met die klas III klassifikasie van die Mooirivier, wat bevestig dat die Mooirivier ernstig beïnvloed is deur menslike aktiwiteite maar steeds ekologies onderhoubaar is.

’n Merkbare verskil is gesien in die fisies-chemiese veranderlikes nl.: elektriese geleiding, magnesium, kalsium, totale opgeloste stowwe en sulfaat, na die invloei van die Wonderfonteinspruit. Hierdie verskynsel lig weereens die impak van die Wes Rand se mynbou aktiwiteite via die Wonderfonteinspruit op die Mooirivier uit. Die magnesium en kalsium konsentrasies word egter heel moontlik ook verder bygedra deur die dissosiëring van die dolomitiese gesteentes. Hierdie verskynsel dra ook by tot die verhoging van die pH.

Alhoewel die sulfaat konsentrasie laer as die gestelde HBD is, het die sulfaat reduserende bakterieë ’n statisties betekenisvolle verwantskap ($p<0.05$) getoon met die sulfaatkonsentrasie in die oppervlakwater. Hierdie verskynsel lig weereens die impak van die mynbouaktiwiteite uit op die elektriese geleiding, magnesium, kalsium, en totale opgeloste stowwe in die rivier. Tydens hierdie studie het dit duidelik na vore gekom dat HBD nodig is vir die bestuur en voortdurende verbetering van die rivier se waterkwaliteit.

Die gemiddelde *E.coli* tellings bepaal vir die Mooirivier was 828kve/100ml. Die versamelpunte wat hierdie hoë tellings toon is punt 4: AWFS waar vee teenwoordig is, en punt 8: EBR, hoofsaaklik beïnvloed deur landbouaktiwiteite en afloop vanaf 'n varkplaas.

Resultate wat die 95% persentiel van die HBD oorskry is pH, ortofosfaat, magnesium en *E.coli*. Veranderlikes gemeet wat laer as die HBD gevind is, is nitraat en nitriet, elektriese geleiding, sulfaat, opgeloste mangaan en opgeloste uraan.

Die fisies-chemiese, fitoplankton en mikrobiologiese tydens hierdie studie toon aan dat die Mooiriviersisteem hoë organiese besoedeling teenwoordig het met ‘n hoë fekale besoedelingslading. Ingryping en bestuur van die nutrient-besoedeling is egter nodig om te verhoed dat die sisteem eutrofies word. Opbloeie van probleem alge kan egter verwag word. Daar is ook gevind dat die Mooirivier ‘n produktiewe stelsel is, met hoë vlakke van spesie diversiteit.

Die implementering van hulpbron kwaliteitsdoelwitte is dus noodsaaklik vir die bestuur van die Mooirivier se waterkwaliteit en moet voordurend heroorweeg word soos die waterkwaliteit van die Mooirivier verander.

Sleutelwoorde: waterkwaliteit, fitoplankton bevolkings, fisies-chemiese veranderlikes, Hulpbron kwaliteitshulpbron, indekse, Mooirivier.

ACKNOWLEDGEMENTS

I would like to thank the following people without which this would not have been possible:

God, without which nothing is possible. Thank you for your mercy and undying love. For being just a prayer away. "For I know the plans I have for you," declares the LORD, "plans to prosper you and not to harm you, plans to give you hope and a future." Jer. 11:29

Professor Sandra Barnard. Thank you for making this possible! Thank you for your time, patience, wisdom and guidance. You are an amazing promoter that always leaves me inspired. Words cannot express my gratitude.

Midvaal Water Company Scientific Services. A special thanks to Jan Pietersen and Marina Kruger for the resources made available to test my water samples. Thank you for granting me opportunities.

Serlina Venter, Daleen von Möllendorf, Phatheka Koli, Germarie de Kock you are more than just colleagues. Thank you for the support.

Shalene Janse van Rensburg. You are an inspiration to me. Thank you for your support and love through every step. Your enthusiasm is contagious. I treasure your friendship.

Marna Butler. Thank you for your willing ear and support. Your hugs made the load lighter to carry. It would not have been the same without you. I am grateful to have you as my supervisor and friend.

Nicola Gouws. Thank you for every message of encouragement and your interest in my research.

Lehanri Mulder. Thank you for your interest and support. You are a special friend which I love dearly.

A. Erasmus, for the phytoplankton identification and enumeration.

My family, thank you for the unconditional love and daily support.

My grandparents, thank you for your love and guidance and always being there.

Gimli, my trusty companion. Thank you for the comfort during late nights.

Adriaan Marais, my husband. Thank you for every word of encouragement and your unselfish love. Thank you for always being there and making me laugh. I love you. Now all the papers lying around can finally be put away. Thank you for your assistance during sample collection.

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ACRONYMS AND SHORT FORMS

AWFS	After Wonderfonteinspruit
BKD	Boskop Dam
BVO	Bovenste oog
BWFS	Before Wonderfonteinspruit
COA	Certificate of Analysis
DNA	Deoxyribonucleic acid
DWA	Department of Water Affairs
DWS	Department of Water and Sanitation
EBR	Elbrixen Bridge River
EC	Electrical Conductivity
GPS	Global Positioning System
ISO	International Organization for Standardization
KKD	Klerkskraal Dam
MPN	Most Probable number
MUG	4-methylumbelliferyl-β-D- glucuronide
NMMP	National Microbial Monitoring Programme
NWA	National Water Act
NWU	North West University
ONPG	o-nitrophenyl-β-D-galactopyranoside
PCR	Polymerase Chain Reaction
PD	Potchefstroom Dam
RNA	Ribonucleic acid
RQO	Resource Quality Objectives

RWQO	Resource Water Quality Objective
SANAS	South African National Accreditation System
SD	Standard Deviation
SE	Standard Error
SOP	Standard Operating Procedure
SRB	Sulphate Reducing Bacteria
TDS	Total dissolved solids
WFS	Wonderfonteinspruit
WWF	World Wildlife Fund
WWTP	Waste water treatment plant

Please note our acknowledgement of DWA that is currently known as DWS Department of Water and Sanitation

CHAPTER 1: INTRODUCTION

"*The wars of the twenty-first century will be fought over water*" ~ Ismail Serageldin (1995)

Water is a very complex resource. On a molecular level the bond between oxygen and hydrogen is perhaps the most prolific bond in the universe. These two elements come together in such a unique structure that it paves the way for what we describe as life.

Compared to land, a steady resource, water occurs in an active cycle of rain, runoff and evaporation, with time-based and spatial variations. Water quality is the largest contributing factor to the usefulness and value of water for both people and ecosystems (Rijsberman, 2004).

Water might appear to be an abundant resource as it covers 70% of our planet. However, only 3% is considered fresh water, with a mere 1% being easily accessible. The World Wildlife Fund (WWF) reports that 1.1 billion people don't have access to clean water and a total of 2.7 billion experiences extreme water scarcity (WWF, 2016).

As can be seen from the map (Figure 1.1) depicting the average water scarcity experienced by water users in each country, South Africa falls under the areas experiencing high stress.

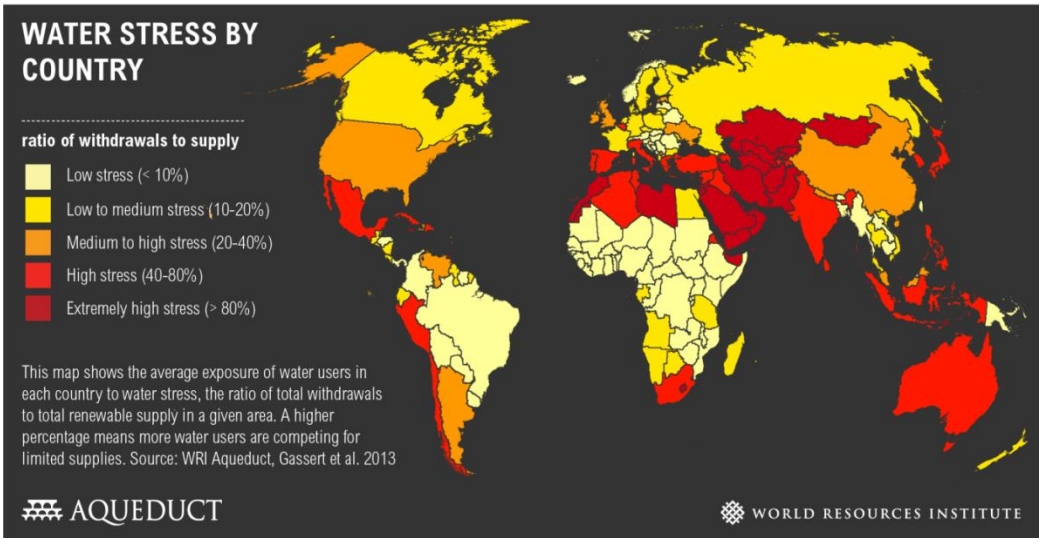


Figure 1-1: A map depicting water scarcity experienced on average by water users in each country (Gassert *et al*, 2013).

South Africa, a semi-arid country, is no exception. South Africans are already using 98% of their available water supply (Thelwell, 2014). This phenomenon of increasing water scarcity can be attributed to both natural and anthropogenic causes.

Due to the low rainfall experienced currently, the levels of our important water resources are quickly depleting. Figure 1.2 compares the current levels of some of the main reservoirs for 2016, to that of 2015. It is clear that the management of our water resources is essential for our future survival as a water stressed country.

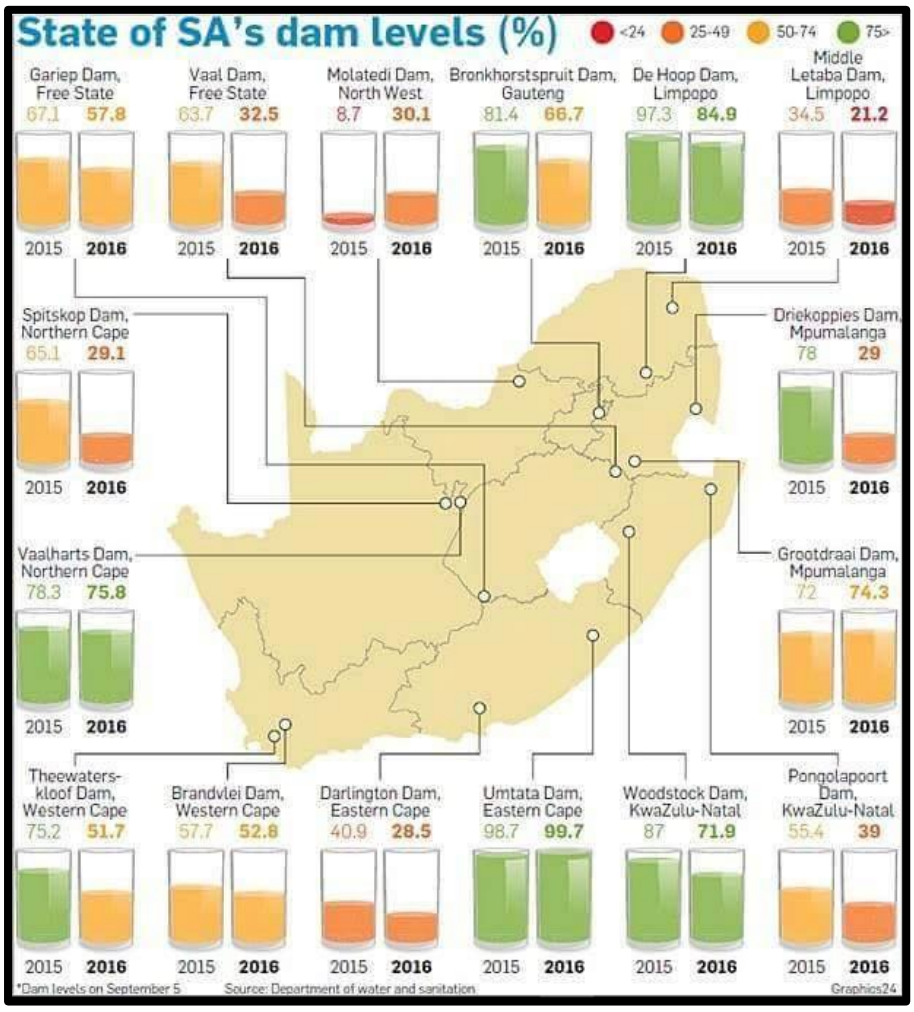


Figure 1-2: A map depicting the reservoir levels of 2016, compared to 2015. (Department of Water and Sanitation, 2016)

The National Water Act (NWA) (Act No 36 of 1998) provides for the protection of water resources, ultimately aiming to achieve the sustainable use of these water resources

to the benefit of all users. An equilibrium must however exist between protecting and maintaining our water resources and the utilization thereof.

As a means to ensure a desired level of protection, resource quality objectives (RQO) have to be determined for all significant water resources. RQO are defined by the National Water Act as “clear goals relating to the quality of the relevant water resources”. These goals are scientifically derived criteria (Dickens *et al,* 2011).

The purpose of the RQO is to provide qualitative and quantitative information regarding the quality, size, habitat and living conditions as a basis from which management actions can be implemented for the sustainable use of all water resources (Dickens *et al,* 2011).

Managing a water body by means of the RQO approach is advantageous as it focuses on managing problems caused due to various demands placed on a waterbody. This approach focuses not only on the effect of individual discharges, but on the total effects of a range of multiple discharges. Overall limits of pollution variables are set in accordance with the required water use (Dickens *et al*, 2011)

The RQO forms an important part of water resource management as the protection of water resources can only become a reality once managers of these water resources have a clear set objectives to work towards.

South Africa is currently split up into 9 water management areas, down from the originally proposed 19 (Figure 1.3).

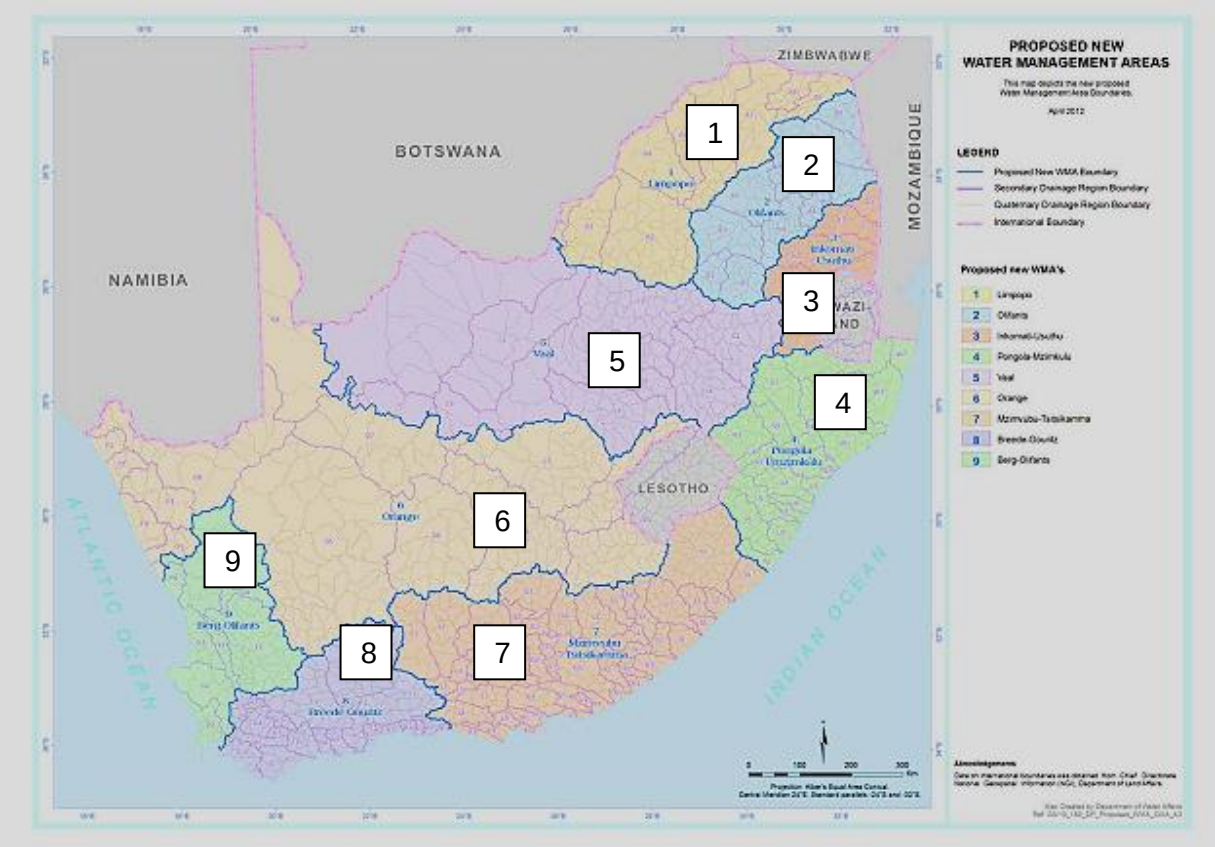


Figure 1-3: The 9 proposed catchment management areas. 1: Limpopo, 2: Olifants, 3: Inkomati-Usuthu, 4: Pongola-Mzimkulu, 5: Vaal, 6: Orange, 7: Mzimvubu-Tsitsikamma, 8: Breedt-Gouritz, 9: Berg-Olifants (McDonald, 2014).

The RQO for the Vaal catchment (5) is currently under review. The Vaal catchment management area is divided into the Upper Vaal, Middle Vaal and Lower Vaal region (Figure 1.4).

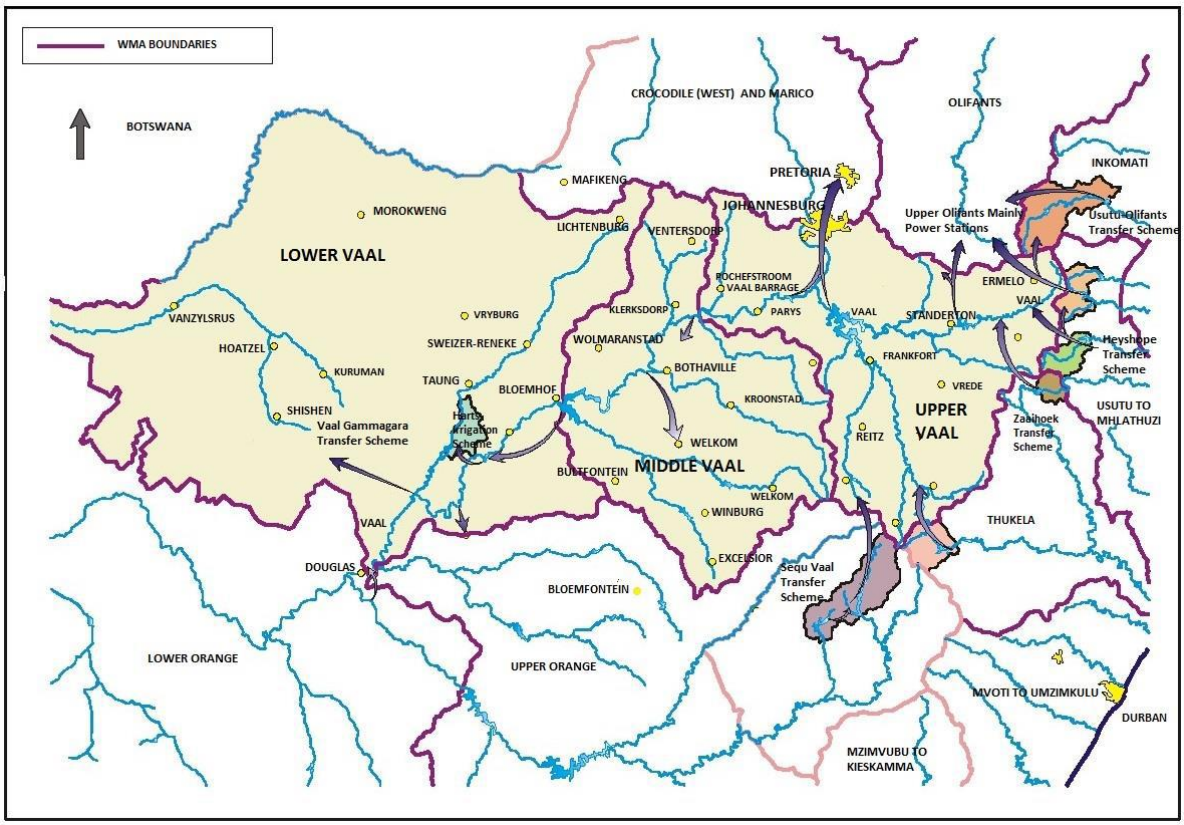


Figure 1-4: **A map of the Vaal Catchment currently under review. The 3 management areas, namely: Lower-, Middle – and Upper Vaal are clearly visible (McDonald, 2014).**

The Upper Vaal is located in the middle of the country (Figure 1.4) and covers four provinces. The industrial, metropolitan and mining sectors accounts for 80% of the water use in the Upper Vaal region, while 9% is used for irrigation purposes and 7% for power generation, with the rest being attributed to rural area water supply (McDonald, 2014).

Located in the North West Province, the Mooi River is a significant water resource that forms part of the Upper Vaal catchment region. Drinking water for the City of Potchefstroom is abstracted from the Mooi River catchment, specifically the Boskop Dam, and transported to the purification plant (Annandale & Nealer, 2011).

Due to the many mining activities of the West Rand and the far West Rand regions, the Mooi River has been subjected to high volumes of mining pollution (Coetzee *et al*, 2006) see section 2.2. These mines were first established in 1887, only one year after

the discovery of gold on the Witwatersrand. The far West Rand is the richest of the seven active goldfields (Coetzee *et al,* 2006). These mined reefs contained not only gold but uranium too. Uranium mining contributes up to 5.8% of the mining activities in this region (Coetzee *et al,* 2006). All of these mining activities are negatively impacting the Mooi River, regarding aspects such as acid mine drainage, closure of mines and the natural rewatered gold mines.

Information obtained at a biological level contributes greatly to the determination of Resource Quality Objectives (RQO). Due to high sulphate concentrations (Barnard *et al,* 2013), caused by the mining effluent, favourable conditions for sulphate reducing bacteria (SRB) are created. SRB's, associated with mining pollution; use both organic and inorganic energy sources for the anaerobic respiration of sulphate (Luptakova, 2007). Other microbiological activity considered during this study is that of *E.coli* and Total coliforms. *E.coli* is non-pathogenic indicator organism associated with faecal pollution and forms part of the coliform group. Phytoplankton is also excellent bio-indicators, as they rapidly respond to changes in water chemistry, reflecting the overall ecological integrity of a water body (Venter *et al,* 2013).

The purpose of this study is to contribute to the proposed RQO the Upper Vaal catchment by determining the water quality of the Mooi River, with the use of both biological and physico-chemical analyses.

The objectives of this study are to:
1. Measure the physical and chemical attributes of the water in the river;
2. Determine the bacteriological water quality of the river;
3. Determine spatial changes in the abundance of algal assemblages as well as algal biotic indices.

Dickens *et al*: 2011, states that existing information should be used where possible as criteria for the indicators of the RQO. The data obtained from this study and the statistical analysis thereof can thus be used to provide information relating to the Mooi River water quality that contributes to the composition of the proposed Resource Quality Objectives for the Upper Vaal.

CHAPTER 2: LITERATURE REVIEW

2.1 RESOURCE QUALITY OBJECTIVE (RQO)

Resource quality objectives (RQO) provide qualitative and quantitative information of quality, size, habitat and living conditions of a water resource, from which management actions can be implemented for the balanced sustainable use of all water resources (Dickens *et al,* 2011). Water utilisation includes domestic, agricultural and industrial uses.

A seven step process for the establishment of the RQO has been applied. These steps are illustrated in Figure 2.1.

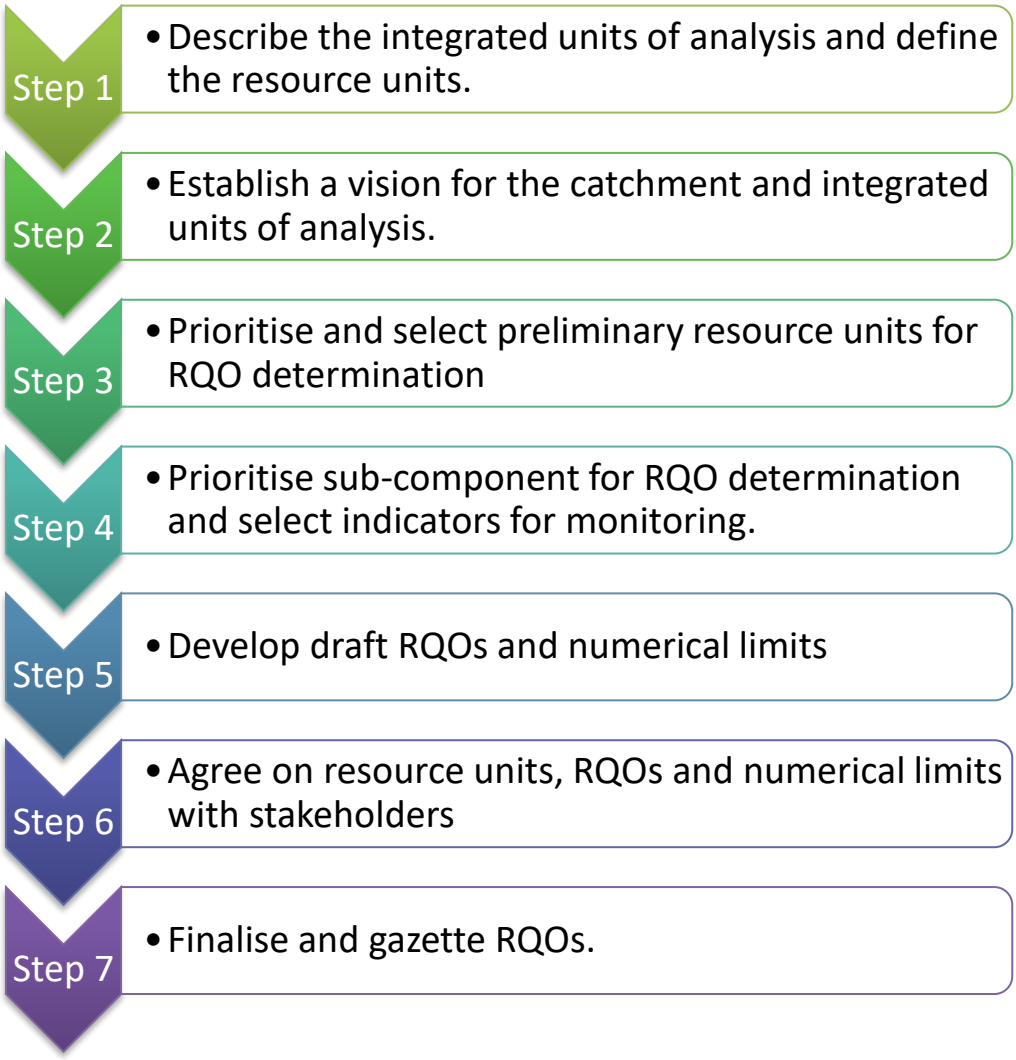


Figure 2-1: The seven steps in determining RQO (Dickens *et al*, 2011).

Three extra steps are added by Dickens *et al*, (2011) (Figure 2.2). These steps are added to complete the adaptive management cycle of a resource.

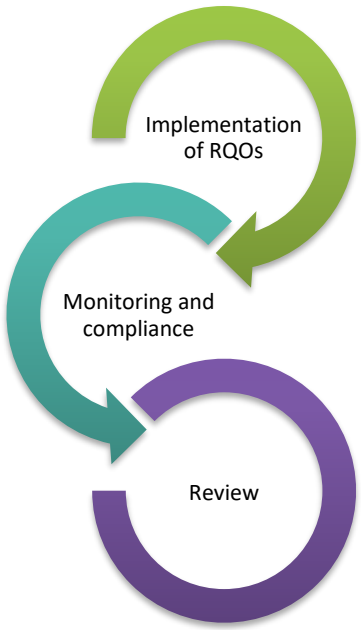


Figure 2-2: The three additional steps added for RQO determination for the implementation of adaptive management (adapted from Dickens *et al*, 2011).

Implementation involves the decision making on the assignment of water resources to various users to support and implement the RQO. The **Monitoring and compliance** entails the measuring and overseeing of the implementation and management of the RQO within a waterbody. Lastly **Review** refers to the regular assessment of whether set RQO goals are being achieved or at least moving in that direction. Reviewing the process (Figure 2.1) will repeat, with a re-evaluation of RQO and numerical limits set for the resource unit.

Other documents to be considered during the determination of RQO

Dickens *et al*, 2011 states that it is also important to examine the following documents in order to determine the origin of the RQO reasoning and for the detail given on how RQO fit into water resource management:

a) The National Water act (1998);
b) RDM Integration Manual (1999);
c) The National Water Resource Strategy;
d) Resource Quality Objectives;
e) Resource Water Quality Objectives
f) Ecological Reserve, Eco-classification, Eco-status and Eco-specs.

a) The National Water act (1998) (Dickens *et al*, 2011):

The purpose of the RQO according to the National Water Act (No. 36 of 1998) is to set distinct goals with regards to the quality of water resources. The act highlights the importance of a balance between the need to protect and utilize a water resource.

The act also states that: "once the class of a water resource and the Resource Quality Objectives have been determined they are binding on all authorities and institutions when exercising any power or performing any duty under this act."

b) RDM Integration Manual (1999) (Dickens *et al*, 2011):

The publication of the 1999 guidelines for resource directed measures states that RQO are a scientifically derived numerical and descriptive statement of the conditions to be met in receiving water to ensure water resource protection.

In short the RDM manual describes the purpose and application of RQO as:

- Representing a goal towards which management can be directed to achieve desired protection of a resource;

- Clearly stating the acceptance or unacceptance of impacts and activities on a water resource (point sources, non-point sources, land use, water abstraction etc.).

- It is a tool from which the success and effectiveness of management of source directed control and regulating activities can be evaluated and reviewed.

- Providing a steady time frame for decision making and planning.

c) The National Water Resource Strategy (Dickens *et al*, 2011):

The national water resource strategy, section 3.1.2.3 states that RQO provides descriptive an numerical accounts regarding the chemical, biological and physical attributes taking into account the class and user requirements of a resource. RQO might describe, among other things, the condition and character of both the habitat and aquatic biota, the quantity, pattern and timing of instream flow and the water quality.

d) Resource Quality Objectives (Dickens *et al*, 2011).:

Developing a system to classify a water resource is described by the national water act as the first step in preserving and managing a water resource. The management classes developed by the classification system, which directs the setting of the RQO, are listed in Table 2.1

Table 2-1: The summary of the management classes developed by the classification system as listed by Dickens *et al*, 2011.

Management class	Description
Class I	Natural - Minimal impact of humans, natural water quality and safe for most uses, of high significance. Other classes are defined in terms of degree of deviation from the natural class.
Class II	Moderately used/impacted - slightly altered from natural due to human activity.
Class III	Heavily impacted/used - significantly changed from natural due to human activity but nevertheless ecologically sustainable.
Class IV	Unacceptable degraded resources - due to overexploitation. The Management class is set higher in order to rehabilitate.

e) Resource Water Quality Objectives:

Resource water quality objectives (RWQOs) are a component of RQO and are set in greater detail. RQO must provide the framework for RWQOs.

f) Ecological Reserve, Eco-classification, Eco-status and Eco-specs (Dickens *et al*, 2011):

The ecological reserve's focus does not fall on the protecting but also on maintaining aquatic ecosystems to continue to provide goods and services required. Eco-specs are clear and measurable specifications of ecological characteristics and serves as an input to the RQO. During the process of eco-classification the present ecological state and factors influencing this state is determined. These classifications are summarised in Table 2.2 (DWA, 2016). The eco-status refers to all the features and characteristics of a resource influencing its ability to both support natural fauna and flora and produce goods and services.

Table 2-2: Ecological status described in terms of ecological categories. These categories are further depicted on a continuum. (DWA, 2016).

Description of the ecological status	
A	Near natural
B	Largely natural
C	Moderately modified
D	Largely modified
E	Seriously modified
F	Critically modified

These documents are of importance as they provide information and inputs towards the compilation of RQO.

Indicators used as RQO

The indicators used for RQO may include chemical and physico-chemical, biological and hydro-geomorphological characteristics. The choice of indicator is important as it needs to give information regarding the bigger picture, being able to track a measureable change over time, without having to measure everything. Dickens *et al*, 2011, lists criteria to consider when choosing an indicator for use as RQO. These criteria are summarised in Table 2.3.

Table 2-3: A summary of the criteria used when considering an indicator for use as RQO (adapted from Dickens *et al*, 2011).

Criteria for indicators	
1. Simple, easy measurements, understood and applied	The more complex an indicator, the less useful it is.
	An indicator must be: - measurable with standard techniques, - the data must be easily understood and fit for analytical use, - explained by use of established principles.
2. As few as necessary	Financial and human resource limitations must be taken into account.
	Indicators give an exact description of the situation with fewer parameters and measurements than usually needed.
3. Existing information must be used where possible	Assisting in cost effectiveness, it is preferred that the information can be derived or collected through existing data sources and monitoring programs.

4. Relate to an appropriate scale	An indicator should represent the information required from the specific situation and measurable both temporarily and geographically.
5. Detect change	The progress and management of a system must be depicted by the indicator.
6. Comparable, repeatable and sustainable between sites and times.	Indictor must be comparable between river basins even countries, improving transboundary water resource management.
7. Need to reflect both the ecosystem and user requirements.	
8. Seasonal and annual variabilities must be considered.	
9. RQO need to be site specific.	

It is important to note that RQO have certain limitations, and is not a "catch all" for resource management. RQO are determined as a whole for a resource unit and can thus not be part of licenses issued for any one user. RQO are in no way a replacement for other monitoring programmes following their own objectives. All the resource quality variables of interest are not included in the RQO but only those necessary to manage and protect a resource. Limits set by the RQO are not to be seen as indisputable or the "absolute truth" as the RQO-system is a product of a flawed science trying to quantify an unknown and ever changing environment.

2.2 STUDY SITE

Located in the North West province (Winde, 2010), the Mooi River (Figure 2.3) and its tributaries run through the district of Tlokwe, Westonaria, Oberholzer, Fochville and Carletonville and forms part of the Upper Vaal catchment area (Figure 1.4) (McDonald, 2014).

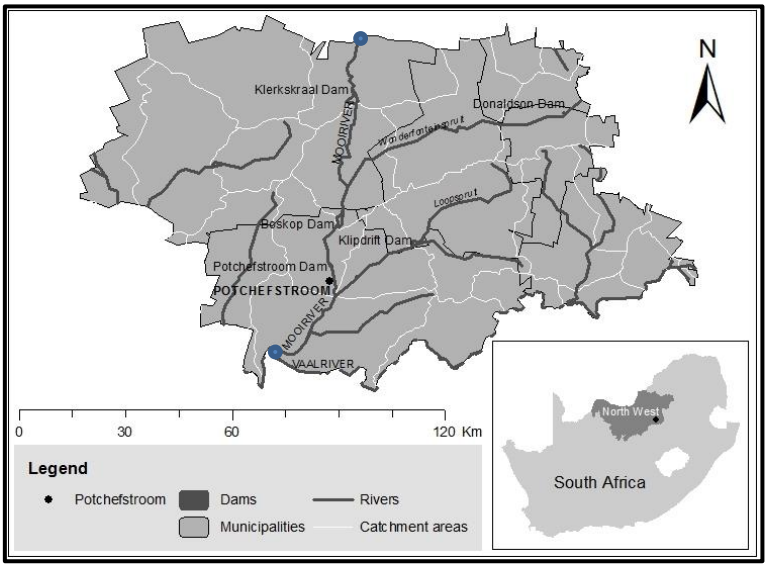


Figure 2-3: Map indicating the location of the Mooi River from source to confluence with the Vaal River (Barnard *et al,* 2013).

With an average rainfall of 507mm per annum, mainly during mid-summer, only 44.2% of the catchment yields a significant runoff due to extensive dolomite outcrops (Winde & van der Walt, 2004).

The Mooi River and its tributaries are recharged through several dolomitic eyes by the precipitation that ends up as ground water recharge (Winde & van der Walt, 2004) (Figure 2.4). The Bovenste Oog as well as surface water from the Wonderfonteinspruit (WFS) feeds the Mooi River. The Boskop-Turffontein compartment and Gerhard Minnebron eye supplements the Mooi River through underground dolomitic compartments (Annandale and Nealer, 2011).

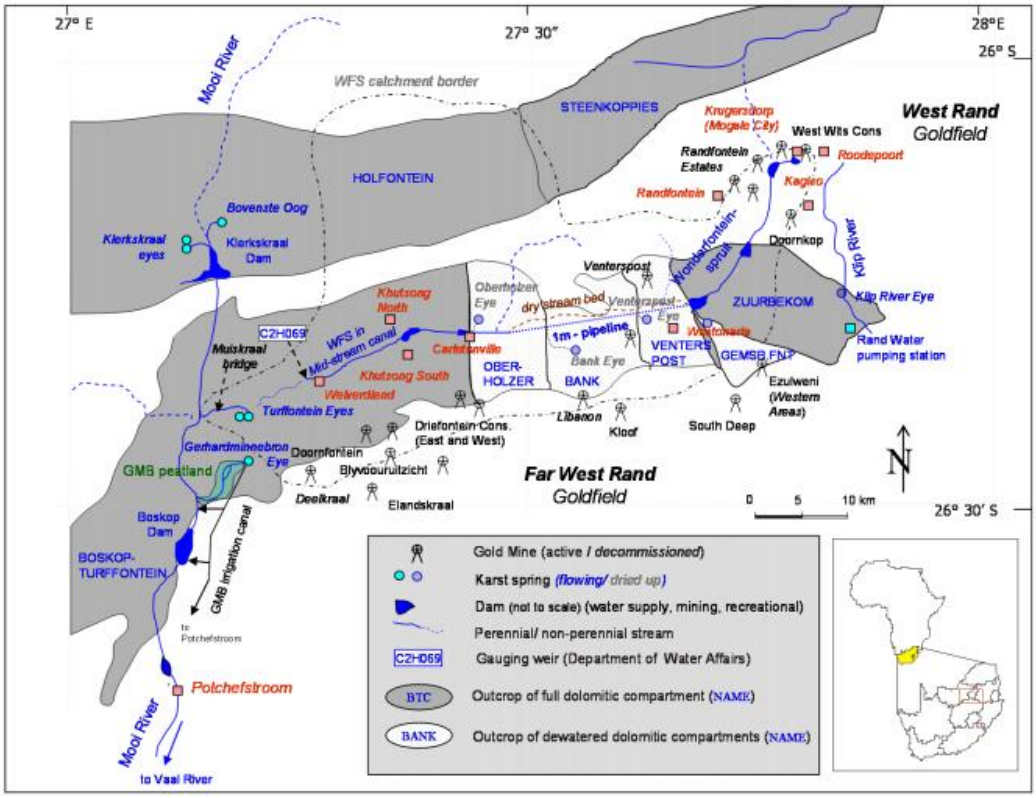


Figure 2-4: Location of the dolomitic compartments feeding the Mooi River (Winde & van der Walt, 2004). The dolomitic compartments mentioned feeding the Mooi River is circled in yellow.

The Mooi River comprises of three major sub-catchments namely: the Wonderfonteinspruit (WFS) (north-eastern reach); the Mooi River proper (northern reach) and the Loopspruit (eastern reach). The upper and middle catchment of the Wonderfontein Spruit and the upper reaches of the Loopspruit are negatively affected by large-scale mining in the far West Rand and Carletonville areas. Large scale mining in the WFS sub-catchment already commenced in the 1930's (Winde & van der Walt, 2004). The confluence of the Wonderfonteinspruit and the Mooi River is situated just upstream of the Boskop Dam and forms part of this study.

Four major reservoirs are present along the Mooi River namely: the Klerkskraal Dam, the Boskop Dam, the Klipdrift Dam and the Potchefstroom Dam (Winde & van der Walt, 2004) (Figure 2.3). The Klipdrift Dam does not form part of this study.

Potchefstroom Dam, completed in 1910, was built to meet the growing number of Potchefstroom residents' water needs (Annandale and Nealer, 2011). It covers a

catchment area of 3 632 km^2 and has a capacity of 2 Mℓ (Barnard *et al*, 2013). Even though the Potchefstroom Dam was built mainly for irrigation purposes, it has now become an important recreational spot (Barnard *et al*. 2013). By 1959 Boskop Dam was built to address the increasing water demand with cement water-transporting canals on both sides (Annandale and Nealer, 2011). It has a catchment area of 3 287 km^2 and has a capacity 20 Mℓ (Barnard *et al*, 2013). Boskop Dam is fed indirectly by the WFS, as the WFS feed the underlying karst aquifer of the Boskop-Turffontein compartment (Barnard *et al,* 2013). Klerkskraal Dam with a catchment area of 1 324 km^2 and a capacity of 8 Mℓ (Barnard *et al*, 2013), is situated north of the Ventersdorp-Krugersdorp provincial road. Klerkskraal Dam was built to effectively manage the surface water in the Mooi River valley during 1 971 (Annandale and Nealer, 2011).

The major land use practise in the Northern sub-catchment of the Mooi River mainly consists of crop farming and grazing. Dryland maize and sunflower cultivation and cattle ranching being the principle land use (Barnard *et al,* 2013) Small scale diamond diggings are present between the Klerkskraal Dam and the Boskop Dam in the Mooi River stream channel (Winde & van der Walt, 2004).

A peat mine is situated close to Gerrit Minnebron. Peat is mainly used as growing substrate for mushrooms and as a pot soil mix (Grundlingh & Retief, 2005). Peat is extracted either by draining the peatland and removing the peat by means of a mini excavation, or less destructively by means of the peat flotation peat mining method (Grundlingh & Retief, 2005). These mining activities contribute as non-point source pollution. The peat is of ecological importance for the Mooi River area as it is able to remove uranium from mine polluted water (Winde, 2010).

The Mooi River State Water Scheme is situated between the Boskop Dam and Potchefstroom Dam where water is extracted for irrigation purposes, livestock watering and domestic use (Van der Walt *et al,* 2002).

Growing informal settlements are located in the Mooi River catchment area, having a negative impact as possible non-point source pollution (Anon, 2012).

Industrial use of water from the Mooi River is concentrated in and around the Potchefstroom area (Anon., 2012). Further uses of the Mooi River include angling and recreational purposes.

The Mooi River is currently classified as a class III water resource, meaning it is heavily impacted by human activity but nevertheless ecologically sustainable. The recommended ecological category for the Mooi River is C/D, indicating it is moderately to largely modified (DWA, 2016).

During this study surface water samples were taken along the gradient of the Mooi River, once a month over a 20 month sampling period. The study was conducted at 8 sites along the gradient of the Mooi River (Figure 2.5). These 8 sampling site were chosen after an Honours study conducted in 2013 by the author, exhibited clearly an increase in electrical conductivity from Klerkskraal Dam towards the Vaal River (Figure 2.6). These stepwise increases where seen as significant as they can be connected to specific land uses influencing the Mooi River's water quality.

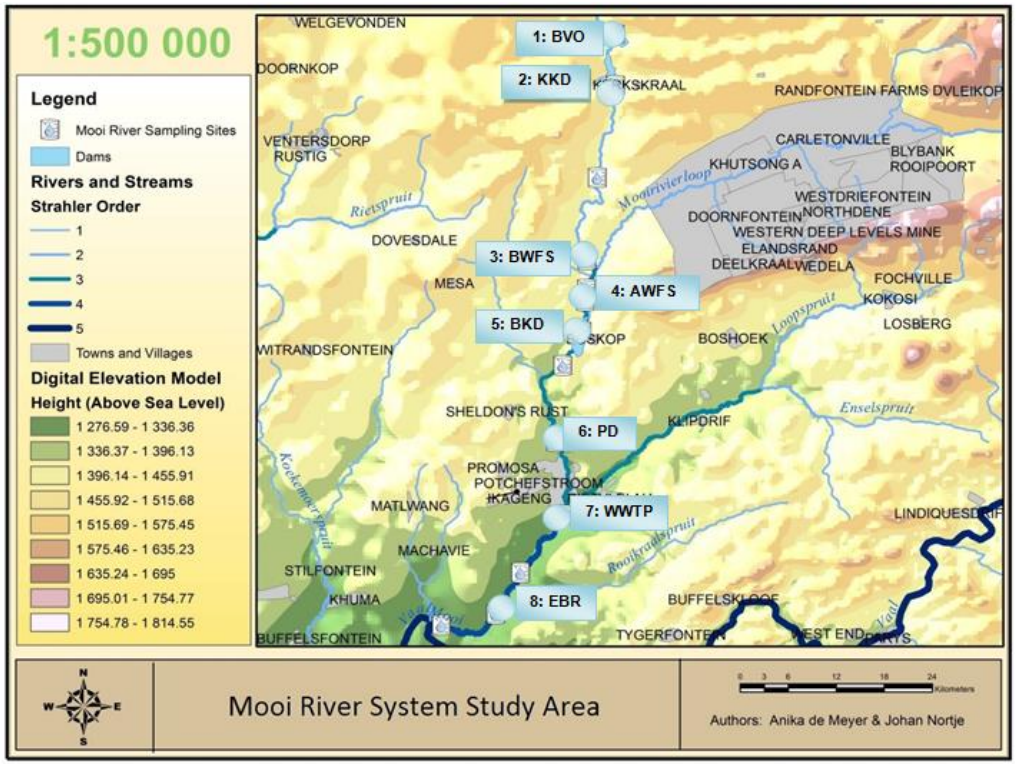


Figure 2-5: **A map illustrating the sampling points along the Mooi River gradient** ***Site 1: BVO, Site 2: KKD, Site 3: BWFS, Site 4: AWFS, Site 5: BKD, Site 6: PD, Site 7: WWTP, Site 8: EBR***

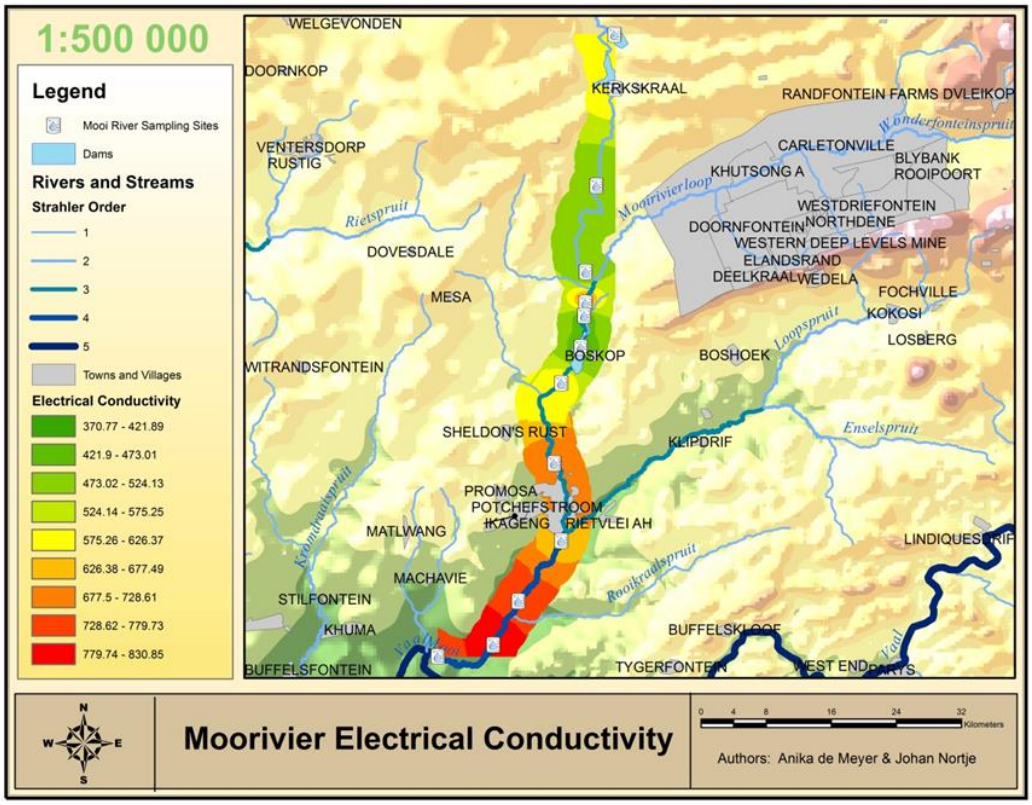


Figure 2-6: A map indicating the stepwise increase in the electrical conductivity along the Mooi River.

The 8 sampling sites, with their GPS coordinates and descriptions are summarized in Table 2.4.

Table 2-4: A summary of the eight sampling sites depicting each site's number, name and GPS coordinates with a short description of each site.

Site nr.	Site name	GPS Coordinates	Description
1	**BVO**: Bovenste oog	S 26.19813 E 27.16477	This is the eye where the head waters of the Mooi River rises at an altitude of approximately 1600m above sea level. Mainly dolomitic lithology is present. Grazing cattle are often present.
2	**KKD**: Klerkskraal Dam	S 26.25256 E 27.15948	Located below the eye, Klerkskraal Dam is situated north of the Ventersdorp-Krugersdorp provincial road, 30km East of Ventersdorp. It is not directly impacted on by mining activities, with the major non-point pollution impact from farming activities.
3	**BWFS**: Before-Wonderfontein-spruit	S 26.45518 E 27.12716	This area is believed to be un-impacted by mining activities, the presence of mining related contaminants are the result of atmospheric depositions.
4	**AWFS**: After Wonderfontein-spruit	S 26.48949 E 27.12684	AWFS is located after the surface water contribution of the Wonderfonteinspruit, but above the contribution of the Gerhard Minnebron eye. Peat mining is situated close to the Gerhard Minnebron area.
5	**BKD**: Boskop Dam	S 26.57958 E 27.10058	The main source water originates from dolomitic underground compartments. Boskop Dam is recharged indirectly via the Boskop-Turffontein compartment and Gerhard Minnebron eye. The Boskop Dam is founded on fairly complex lithology consisting of a quartzite ridge, shale, lava, dolomitic limestone, a number of faults and a diabase dyke.

Table 2-4: Continued

6	**PD**: Potchefstroom Dam	S 26.66688 E 27.09214	Mainly build for irrigation purposes, Potchefstroom Dam has become an important recreational venue (Annandale and Nealer, 2011).
7	**WWTP**: Waste Water Treatment Plant	S 26.75248 E 27.10023	Purified sewage effluent is released, creating possible point source pollution. A wetland is situated at the site of sampling.
8	**EBR**: Elbrinxen Bridge River	S 26.86730 E 27.02492	Deep water with slow flowing waters present amongst heavily irrigated fields.

CHAPTER 3: METHODOLOGY

3.1 SAMPLING

Physico-Chemical, microbiological and chlorophyll a sampling occurred on a monthly basis from January 2014 to October 2015.

Phytoplankton analysis occurred on a monthly basis from January 2014 to October 2015.

3.1.1 PHYSICO-CHEMICAL SAMPLING

Physico-Chemical sampling was performed in accordance with SOP-Sampling-3.7A of Midvaal Water Company.

Clean 2 litre screw cap polyethylene sampling bottles were obtained from Midvaal Water Company. Each sample bottle was labelled with a permanent marker with the sampling site name and date.

Once at the site, the bottle was rinsed with the sample to be taken. The bottle was then submerged in the direction of the water flow. After the sample has been taken the bottle was capped and placed in a cooler box and transported to the Midvaal Water Company laboratory where analyses commenced.

3.1.2 MICROBIOLOGICAL SAMPLING

Samples were collected in sterile Whirl-Pak bacteriological sampling bags in accordance to SOP-Sampling-3.7A of Midvaal Water Company.

The date and site name were recorded on the Sampling bag with a permanent marker before the sample was taken.

The Whirl-Pak bags (Figure 3.1) were aseptically opened by pulling the white tabs away from one another. Care was taken to not contaminate the inside of the bag. The bag was then lowered into the river, with the mouth of the bag directed towards the current. Once the bag was three quarters full it was sealed by pulling on the yellow tabs and swinging it in a circular motion, while still holding the yellow tabs. Once the bag was sealed, the two yellow tabs were twisted together to prevent the bag from opening.

All sampling bags were transported in an ice filled cooler box to the Midvaal Water Company laboratory, where analyses commenced within 24hours of sampling.

Figure 3-1: Sterile Whirl-Pak sampling bags used for microbiological sampling.

3.1.3 PHYTOPLAKTON AND CHLOROPHYLL *a* SAMPLING

Clean 1Liter screw cap polyethylene sampling bottles were labelled with the corresponding sample site name and date. Surface water samples were taken (0-5cm below the surface) for both the phytoplankton and chlorophyll *a* analysis in their respective 1Liter bottles.

Chlorophyll *a* samples were transported to Midvaal Water Company laboratory where analysis commenced.

Phytoplankton samples were transported to NWU where analyses were performed.

Samples were kept in a dark cooling room and processed within 24hours of sampling.

3.2 PHYSICO-CHEMICAL ANALYSES

Samples were analysed by Midvaal Water Company, a SANAS accredited laboratory. Methods used are listed in Table 3.1

Table 3-1: Physico-Chemical analysis methods performed by Midvaal Water Company

Analysis	Working Range (mg/l)	Method number	Method title	Instrument
Dissolved Calcium (Ca)	≥2	ICP 1	Determination of dissolved and total metals by Inductively Coupled Plasma (Simultaneous) -ICP Prodigy.	ICP prodigy
Dissolved Magnesium (Mg)	≥2			
Dissolved Iron (Fe)	≥0.1			
Dissolved Manganese (Mn)	≥0.1			
Dissolved Uranium (U)	≥0.01			
Nitrate and Nitrite (NO_3 & NO_2)	≥0.5	GL7-2	Determination of Total Oxidized Nitrogen (TON) as N by the Colorimetric Vanadium Chloride method.	Gallery Plus Automated Chemistry Analyser
Sulphate (SO_4)	≥0.5	GL7-4	Determination of the Sulphate ion by the Colorimetric method.	Gallery Plus Automated Chemistry Analyser
Orthophosphate(PO_4-P)	≥0.05	N2	Determination of orthophosphate as phosphorus (PO_4-P).	DU 800 Spectrophotometer
Dissolved cyanide (CN)	≥0.01	CFA-1D	Method for determination of Free and Total Cyanide	Continuous flow analyser

Physico-Chemical parameters measured *in situ* are listed in Table 3.2. Turbidity and alkalinity was determined at the NWU laboratory and is also listed in Table 3.2.

Table 3-2: Physico-Chemical analysis performed in situ and at the NWU laboratory.

Analysis	Method	Working range
Temperature (T)	HI 9813-6 pH/EC/TDS/°C meter	0 - 60°C
pH	HI 9813-6 pH/EC/TDS/°C meter	0 – 14 pH
Electrical Conductivity (EC)	HI 9813-6 pH/EC/TDS/°C meter	0 – 400 mS/m
Total Dissolved Solids (TDS)	HI 9813-6 pH/EC/TDS/°C meter	0 – 1999 mg/l
Turbidity (NTU)	HACH PORTABLE TURBIDIMETER Model 2100P ISO	0 – 1000 NTU
Alkalinity (Alkal)	HI 755 Marine Alkalinity	0 – 300 mg/l

3.2.1 DISSOLVED CALCIUM (Ca), DISSOLVED MAGNESIUM (Mg), DISSOLVED IRON (Fe), DISSOLOVED MANGANESE (Mn) AND DISSOLVED URANIUM (U) DETERMINATION

An adequate volume of sample was filtered through a 0.45 µm cellulose filter paper and acidified with nitric acid (HNO_3). Two and a half millilitres (2.5ml) of Scandium (Sc) was added to a 25ml volumetric flask. Scandium is the internal standard. The 25ml volumetric flask was then filled to the mark with the filtered, acidified sample (22.5ml). The contents of the 25ml volumetric was shaken, transferred to plastic tubes and measured on the ICP in accordance with method ICP 1 (Table 3.1).

3.2.2 NITRATE and NITRITE (NO_3 & NO_2) DETERMINATION

An adequate volume of sample was filtered through a 0.45 µm cellulose filter paper.

A positive control sample with a known concentration of nitrate and nitrite was added after every 20 samples. This value is plotted on a quality control chart.

The filtered samples were transferred to Decacell cuvettes and loaded into the Gallery plus Automated Chemistry Analyser and analysed in accordance with method GL 7 – 2 (Table 3.1).

3.2.3 SULPHATE (SO_4) DETERMINATION

An adequate volume of sample was filtered through a 0.45 µm cellulose filter paper.

A positive control sample with a known concentration of sulphate was added after every 20 samples.

The filtered samples were transferred to Decacell cuvettes and loaded into the Gallery plus Automated Chemistry Analyser and analysed in accordance with method GL 7 – 4 (Table 3.1).

3.2.4 ORTHOPHOSPHATE(PO_4-P) DETERMINATION.

The samples were filtered through a 0.45µm membrane into glass containers and analysed as soon as possible, preferably within 48 hours.

The necessary solutions were prepared for the analyses as follows:

- **Sulfuric Acid (5N)**: 112ml concentrated H_2SO_4 were added to 800ml de-ionized water and allowed to cool. It was then made up to 1000ml.
- **Potassium Antimony Tartrate**: 2.66g $K(SbO)C_4H_4O6.½H_2O$ were dissolved in 800ml de-ionized water and diluted to 1000ml. Reagent was stored in a dark reagent bottle.
- **Ammonium molybdate**: 9.6g $(NH_4)_6Mo_7O_{24}.4H_2O$ was dissolved in about 800ml de-ionized water and left to gently dissolve. Reagent was then diluted to 1000ml.
- **Ascorbic Acid**: 10g ascorbic acid was dissolved in 80ml de-ionized water. Mix and dilute to 100ml. This reagent was used within 4 hours.

The following volumes of the above mentioned solutions were then added together to a final volume of 140ml of the combined reagent:

- 50ml of the 5N Sulfuric acid,
- 20ml of the Potassium antimony tartrate solution,
- 50ml of the Ammonium molybdate solution,
- 20ml of the Ascorbic acid solution.

The solution was well mixed after each addition.

The calibration range of standards used was between 0.01 and 3.0mg/l PO_4-P.

These standards were then used for the calibration of the spectrophotometer and the construction of a multipoint standard curve. The concentration of each sample was then calculated from the standard curve and reported directly in mg/l PO_4-P. Samples were analysed with a DU 800 Spectrophotometer at wavelength of 890nm, in accordance with method N2 (Table 3.1).

All analytical data was calculated using the software program provided with the instrument.

3.2.5 DISSOLVED CYANIDE (CN) DETERMINATION

An adequate volume of sample was filtered through a 0.45µm cellulose filter paper.

The hydrogen cyanide present at a pH of 3.8 is separated by in-line distillation at 125°C under vacuum. The hydrogen cyanide is then determined spectrophotometrically. The spectrophotometric determination is based on the reaction of cyanide with chloramine-T under the formation of cyanogen chloride. This reacts with 4-pyridine carboxylic acid and 1, 3-dimethylbarbituric acid to give a red colour. The absorbance was measured at 600nm.

All analytical data is calculated using the software program provided for the instrument.

3.2.6 TEMPERATURE (T), pH, ELECTRICAL CONDUCTIVITY (EC), TOTAL DISSOLVED SOLIDS (TDS) DETERMINATION

A HI 9813-6 pH/EC/TDS/°C portable meter (Hanna Instruments) was used (Table 3.2) for temperature, pH, EC and TDS determination. At each sampling point

the probe was lowered into the first 200 to 300mm of the surface water. Parameters were read once the meter has stabilised.

3.2.7 TURBIDITY (NTU) DETERMINATION

Turbidity was determined as per Table 3.2 on a HACH portable turbidimeter Model 2100P. The sample cell was thoroughly cleaned before analysis commenced.

The sample was shaken gently by inverting several times. A sample cell was rinsed out with the sample, filled with the sample and placed into the meter. Turbidity result was recorded when the reading stabilised and the lamp symbol turned off.

3.2.8 ALKALINITY DETERMINATION

Alkalinity was determent as per Table 3.2. The sample was shaken gently by inverting several times. Ten millilitres (10ml) of sample is added to a cuvette and placed into the meter. The meter was then zeroed.

One millilitre of HI 755S reagent was subsequently added to the sample in the cuvette and gently inverted five times. The cuvette was then placed back into the meter. Once the button was pressed the instrument displayed the alkalinity as ppm of $CaCO_3$.

3.3 BACTERIOLOGICAL ANALYSES

3.3.1 ENUMERATION OF *E.COLI* and TOTAL COLIFORMS

All microbiological methods were performed at Midvaal Water Company. Colilert-18® was used for the enumeration of *E.coli* and total coliforms. Colilert-18® is a most probable number (MPN) method incorporating a defined substrate medium containing o-nitrophenyl-β-D-galactopyranoside (ONPG) and 4-methylumbelliferyl-β-D-glucuronide (MUG).

All analyses were performed in a laminar flow cabinet to avoid air contamination. One hundred millilitre of each sample was aseptically added to a 120ml Colilert® vessel with antifoam. A single snap pack of Colilert-18® reagent was then added to the vessel,

after which the vessel was capped and gently mixed. After the reagent has dissolved, the sample was poured into a quantitray-2000. Care was taken to avoid any contamination when transferring the sample. The quantitray-2000 was sealed by means of a quantitray sealer. The sealed quantitray-2000 was then incubated for 12–18 hours at 35 ± 0.5°C, with the wells facing upwards. Temperature of the incubator was monitored by means of a calibrated thermometer. Positive and negative reference cultures together with sterile quality control samples were incubated with the samples analysed to ensure the integrity of the results in accordance with Midvaal Water Company quality control procedures.

After the incubation period coliforms produced a yellow colour due to the production of β-galactosidase. *E.coli* produces a yellow colour that fluoresces as a result of the action of the β-glucuronidase (Figure3.2 (b)). A MPN was then calculated from the number of positive wells using the table supplied by the supplier. Results are expressed as colony forming units per 100ml (cfu/100ml).

(a) **(b)**

Figure 3-2: **(a) Colilert-18® reagents and consumables used for enumeration of *E.coli* and total coliforms. (b) Yellow wells indicating the presence of total coliforms, fluorescent wells indicating the presence of *E.coli.* (Paruch, 2010)**

3.3.2 ENUMERATION OF SULPHATE REDUCING BACTERIA

A pour plate method using Merck Sulphite Iron Agar with 20ml 7% Iron sulphate per one litre (1Litre) of agar was used. Positive bacterial spores reduce sulphate in the sample to sulphide, which reacts with iron to form black iron sulphide. This stains the concerning colonies black and weakly-positives brown. In an anaerobic environment sulphur reducing bacteria form black colonies under these conditions.

One millilitre of sample was aseptically pipetted into a sterile petri dish. A 1/100 dilution was also made. Cooled agar was then poured into each petri dish, gently swirling the plate and leaving it to solidify. Plates were inverted and placed in an aerobic jar with an AnaeroPack-Anaero sachet. The AnaeroPack-Anaero sachet absorbs the oxygen and generates carbon dioxide, creating an anaerobic environment. Plates were then incubated at 35°C ± 2°C for 2-4 days.

Black colonies in and on the agar was counted (Figure 3.3). Latex gloves were used when handling plates after incubation.

Figure 3-3: Black SRB colonies present on agar plate.

3.3.3 BACTERIOLOGICAL QUALITY CONTROL

The following quality control procedures were in place to ensure the integrity and accuracy of microbiological results:

- Both positive and negative controls as well as sterile distilled water samples were incubated simultaneously with each batch of samples (Table 3.4).

Table 3-4: Reference cultures used for positive and negative quality control.

Reference Culture used		
Escherichia coli	ATCC11775 +	*E.coli* positive
Klebsiella pneumonia	ATCC31488 +	Total coliform positive
Pseudomonas aeruginosa	ATCC10145 -	Total coliform negative

- River samples were analysed in duplicate. The results were logged and the difference was plotted on a quality control chart.
- Incubator temperatures were monitored daily by means of a minimum and maximum thermometer. These temperatures were noted in a quality control logbook and checked for compliance in accordance with the method specifications.
- Temperatures of media and consumable storage were monitored daily to comply with the manufacturers criteria.
- All volumetric equipment used were calibrated externally by a SANAS service provider and verified once a month to comply to a %CV <1.
- Laminar flow cabinets were validated twice a year.
- Before analysis, a laminar flow cabinet was decontaminated with 70% ethanol.
- All consumables were tested to comply with the method criteria before taken into use. Positive-, negative controls and sterility were tested, as well as the volume criteria of Colilert-18® vessels were verified.
- Air plates were performed once a month. Results should be >15cfu per 15 minutes.

3.4 CHLOROPHYLL *a* ANALYSIS

The chlorophyll *a* determination method was an accredited in-house Midvaal Water Company method.

Two to three hundred millilitres of sample was filtered through GF/C filter paper. All samples were filtered in duplicate sets. The volume of the sample filtered was dependent on the turbidity of the sample.

The filter paper was rolled up and placed into a 10ml glass vial. Ten millilitres of 96% ethanol was then added to the vials and the caps screwed on loosely. The vials were placed in a water bath at 78°C for 5 minutes. The vials were removed, the caps screwed on tightly and the vials were allowed to cool in a dark place. After cooling to room temperature, the vials were inverted. Three to five drops of 0.3M hydrochloric acid were added to one vial of each the duplicate set. The vials were centrifuged for 5

minutes at 3000 rpm. The absorbance of the samples was then measured at 666nm and 750nm on a Beckman DU® 650 spectrophotometer.

The following calculation was used to determine chlorophyll *a* concentrations:

$$\text{Chlorophyll } a \text{ (ug/l)} = \frac{[(A666 - A750) - (A666a - A750a)] \times 28.66 \times v}{V}$$

Where:

A666	=	Absorbance of sample t 666nm without acid
A750	=	Absorbance of sample at 750nm without acid
A666a	=	Absorbance of sample at 666nm with acid
A750a	=	Absorbance of sample at 750nm with acid
v	=	Volume of extract used (10ml 96% ethanol)
V	=	Volume of sample filtered (ml)

3.4.1 QUALITY CONTROL

- A river sample was analysed in duplicate and plotted on a quality control chart.
- Certificate of Analysis (COA) was present for all chemicals used.
- Spectrophotometer was calibrated annually. Weekly checks on absorbance were performed.
- A blank sample of ethanol was measured on the spectrophotometry before samples were read.

3.5 PHYTOPLAKTON ANALYSIS

Phytoplankton sample preparation and enumeration method used was “The Inverted Microscope Method of Estimating Algal Numbers”. This method was first described by Utermöhl (1931; 1958), and later adjusted by Lund *et al.* (1958).

3.6 BIOTIC INDICES

The phytoplankton data was used to determine the following biotic indices:

3.6.1 SHANNON-WIENER DIVERSITY INDEX (*H*)

(Aslam, 2009 and Lad, 2015).

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

Formula 3.6.1: Shannon-Wiener

$\boldsymbol{Pi}$ = S/N

S = Number of individuals of one genus

N = Total number of all individuals in the sample

ln = Natural logsrithm

The Shannon-Wiener index score was interpreted according to Table 3.4. Colour keys are allocated to each level of pollution.

Table 3-4: The Shannon-Wiener Diversity index score interpretation (adapted from Lad, 2015).

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

3.6.2 MARGALEF SPECIES RICHNESS INDEX (Aslam, 2009).

Margalef index was used as a measure of species richness.

$$e = (S - 1) / In\ N$$

Formula 3.6.2: Margalef Richness

S = Total number of genera

N = Total number of individuals in the sample

ln = Natural logarithm

3.6.3 PIELOU EVENNESS INDEX (Aslam, 2009).

The Pielou Index was used for calculating the evenness of species.

$$e = H/In\ S$$

Formula 3.6.3 Pielou evenness

H = Shannon-Wiener Diversity Index

S = Total number of genera in the sample

ln = Natural logarithm

3.6.4 PALMER ALGAL GENUS POLLUTION INDEX (Krhirsagar, 2013).

Twenty phytoplankton genera most tolerant to organic pollution are each assigned a pollution factor index. These assigned index scores are presented in Table 3.5, with 1 being less tolerant and 5 representing the genera most tolerant to organic pollution (Table 3.5).

Table 3-5: Palmer's Algal Genus Pollution Index in order of decreasing tolerance to organic pollution (Palmer, 1969)

Genus	Assigned Index Score
Euglena	5
Oscillatoria	5
Chlamydomonas	4
Scenedesmus	4
Chlorella	3
Nitzschia	3
Navicula	3
Stigeoclonium	2
Fragilaria	2
Ankistrodesmus	2
Phacus	2
Phormidium	1
Melosira	1
Gomphonema	1
Cyclotella	1
Closterium	1
Micractinium	1
Pandorina	1
Anacystis	1
Lepocinclis	1

The sum of these scores was calculated per site, and the total indicates the pollution level (Krhirsagar, 2013). The interpretation of the Palmer Index score depicting the organic pollution levels are listed in Table 3.6. Colour keys are allocated to each level of organic pollution.

Table 3-6: The Palmer Index score interpretation (adapted from Aslam, 2009).

Palmer index score	Pollution level
0-10:	Lack of organic pollution
10-15:	Moderate pollution
15-20:	Probable high organic pollution
20 or more:	Confirms high organic pollution

3.7 STATISTICAL ANALYSES

STATISTICA 13 (StatSoft Inc ©, 2016) software was used for the statistical analyses of the data. Descriptive statistics were performed to determine the valid N, mean, minimum, maximum and standard deviation. The Kolmogorov-Smirnov and Lilliefors tests for normality were used to determine the normality of the data. Most of the data did not meet the assumption of normality; therefore non-parametric statistics were applied. The Kruskal-Wallis ANOVA was used for comparing multiple independent variables. The Spearman Rank Order Correlation test was used to determine whether significant correlations existed (Appendix B).

The percentile was calculated by means of the statistical function in Excel.

CHAPTER 4: RESULTS

4.1 PHYTOPLANKTON ASSEMBLAGES OF THE MOOI RIVER

During this study Chlorophyll *a* was measured for the study period from January 2014 to October 2015 (Figure 4.1). Chlorophyll *a* also forms part of the RQO and gives important information with regards to the amount of algal growing in a water body and trophic condition thereof. All phytoplankton genera were identified and enumerated to genus level. Phytoplankton identification guides such as Croasdale and Flint (1986, 1988), Croasdale *et al.* (1994), Entwisle *et al.* (1996), Gell *et al*. (1999), Guiry *et al*. (2007), Hindák (2008), Janse van Vuuren *et al*. (2006), John *et al*. (2002), Joska and Bolton (1993), Prescott (1983), Taylor *et al*. (2007) and Wehr and Sheath (2002) were used. The abundance of algal genera is depicted in Figure 4.2. This information was used for the compilation of the different biotic indices (Table 4.3), all contributing to the overall objective of the study to determine the Mooi River water quality.

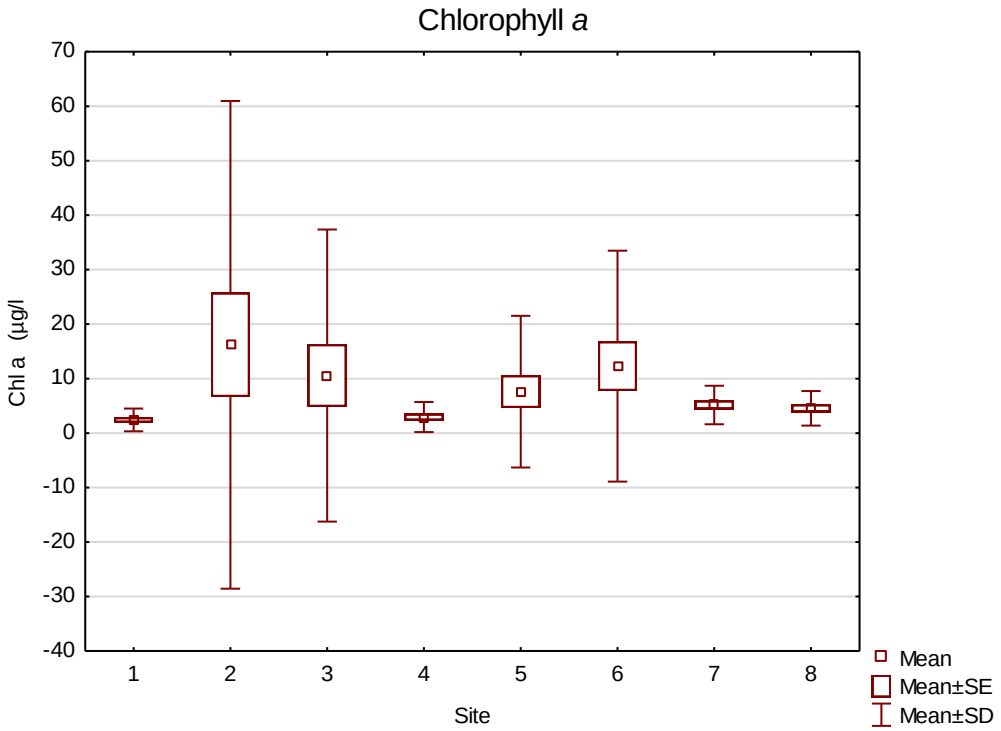


Figure 4-1: **Box and whiskers plot illustrating the Chlorophyll *a* concentration measured along the Mooi River during the study period January 2014 - October 2015.**
±SE (Standard Error); ±SD (Standard Deviation); Site1: BVO, Site 2: KKD, Site 3: BWFS, Site 4: AWFS, Site 5: BKD, Site 6: PD, Site 7: WWTP, Site 8: EBR

An average chlorophyll *a* concentration of 7.768µg/l was measured for the Mooi River system (Table 4.4). The highest average chlorophyll *a* concentration was measured at Site 2: KKD, 16.214µg/l, with the lowest concentration being at Site 1: BVO, 2.419µg/l.

Low algal counts were observed at Site 1: BVO. This was confirmed by the low chlorophyll *a* concentration of 2.419µg/l measured at Site 1: BVO. Site 1: BVO was therefore omitted from the algal indices and the algal counts were only applied from Site 2: KKD onwards.

A genus list of all the genera identified is shown in Table 4.1. The largest amounts of different genera (23) were present at Site 8: EBR (Table 4.1). During this study the algae genera identified were grouped into the following classes: Cyanophyceae, Bacillariophyceae, Chlorophyceae, Cryptophyceae, Chrysophyceae, and Dinophyceae. According to Venter *et al* (2013) Bacillariophyceae are characteristic of oligotrophic to hypereutrophic water. The least amount of Bacillariophyceae genera was present at Site 5: BKD, with only 11 Bacillariophyceae genera present. The Chlorophyceae were most abundant at Site 3: BWFS with a total of 25 genera identified. Only two Cyanophyceae genera were identified at Site 4: AWFS with a total of 10 genera at Site 2: KKD and Site 6: PD. Site 5: BKD had 9 genera of the class Cyanophyceae (Table 4.3). It can be seen from Table 4.3 that the highest number of Cyanophyceae genera was present in the three reservoirs. The classes with the least amount of species present were Cryptophyceae, Chrysophyceae, Dinophyceae and Euglenophyceae (Table 4.4). No Cryptophyceae or Chrysophyceae were identified at Site 8: EBR.

Table 4-1: Species list of identified at each site for each algal class.

BACILLARIOPHYCEAE							
Genus	**Site 2 KKD**	**Site 3 BWFS**	**Site 4 AWFS**	**Site 5 BKD**	**Site 6 PD**	**Site 7 WWTP**	**Site 8 EBR**
Achnanthes sp. (Ach)		x	x		x		
Achnanthidium sp. (Achn)		x	x	x			
Amphora sp. (Amp)		x	x		x	x	
Aulacoseira sp. (Aul)	x	x	x	x	x	x	x
Cocconeis sp. (Coc)	x	x	x	x	x	x	x
Craticula sp. (Cra)	x	x	x		x	x	x
Cyclotella sp. (Cyc)		x	x		x	x	x
Cymatopleura sp. (Cyma)		x	x			x	x
Cymbella sp. (Cymb)	x	x	x	x	x	x	x
Diadesmus sp. (Diad)	x			x	x		
Diatoma sp. (Diat)		x	x		x	x	x
Eunotia sp. (Eun)	x		x				
Fragilaria sp. (Frag)	x	x	x	x	x	x	x
Frustulia sp. (Fru)	x	x	x		x	x	x
Gomphonema sp. (Gom)	x	x	x	x	x	x	x
Gyrosigma sp. (Gyr)		x	x		x	x	x
Luticola sp. (Lut)					x		x
Melosira sp. (Mel)	x	x	x			x	x
Navicula sp. (Nav)	x	x	x	x	x	x	x
Nitzschia sp. (Nit)	x	x	x	x	x	x	x
Pennate diatoms (Pen)	x	x	x	x	x	x	x
Pinnularia sp. (Pin)	x	x	x		x	x	x
Rhoicosphenia sp. (Rhoi)							x
Rhopaloidia sp. (Rhop)	x	x			x		x

Table 4-1: Continued

Sellaphora sp. (Sel)		x	x		x	x	x
Staurosira sp. (Sta)	x	x	x	x	x	x	x
Surirellas sp. (Sur)						x	x
Tryblionella sp. (Try)					x	x	x
Total genera of the class Bacillariophyceae	**16**	**22**	**22**	**11**	**22**	**21**	**23**
CHLOROPHYCEAE							
Genus	**Site 2 KKD**	**Site 3 BWFS**	**Site 4 AWFS**	**Site 5 BKD**	**Site 6 PD**	**Site 7 WWTP**	**Site 8 EBR**
Actinastrum sp. (Act)	x	x	x			x	x
Ankistrodesmus sp. (Anki)	x	x		x	x	x	
Carteria sp. (Car)	x	x		x	x	x	x
Chlamydomonas sp. (Chla)	x	x	x	x	x	x	x
Chlorella sp. (Chlo)	x	x	x	x	x	x	x
Chlorococcum sp. (Chloro)	x	x	x	x	x	x	x
Coelastrum sp. (Coe)	x	x		x	x	x	x
Cosmarium sp. (Cos)	x	x		x	x		x
Crucigenia sp. (Cruc)	x	x	x	x	x	x	
Crucigeniella sp. (Cruci)	x	x	x		x	x	
Desmodesmus sp. (Des)	x	x	x	x	x	x	x
Dictyosphaerium sp. (Dic)	x	x		x	x		x
Elakotothrix sp. (Ela)	x	x		x	x		
Geminella sp. (Gem)				x	x		x
Gonatozygon sp. (Gon)	x	x	x		x	x	x
Monoraphidium sp. (Mon)	x	x	x	x	x	x	x
Mougeotia sp. (Mou)	x	x	x	x	x	x	
Nephrocetium sp. (Nep)	x	x	x				

Table 4-1 Continued

Oocystis sp. (Ooc)	x	x	x	x	x	x	x
Pediastrum sp. (Ped)	x	x	x		x	x	
Scenedesmus sp. (Sce)	x	x	x	x	x	x	x
Sphaerocystis sp. (Sph)			x	x	x		
Staurastrum sp. (Stau)	x	x		x	x		
Tetraedron sp. (Tet)	x	x		x	x	x	x
Tetrastrum sp. (Tetr)		x			x		
Treubaria sp. (Tre)		x				x	x
Total genera of the class Chlorophyceae	**23**	**25**	**16**	**20**	**24**	**19**	**17**
CYANOPHYCEAE							
Genus	**Site 2 KKD**	**Site 3 BWFS**	**Site 4 AWFS**	**Site 5 BKD**	**Site 6 PD**	**Site 7 WWTP**	**Site 8 EBR**
Anabaena sp. (Ana)	x	x			x		
Aphanocapsa sp. (Aph)	x	x		x	x	x	
Aphanothece sp. (Apha)	x				x		x
Geitlerinema sp. (Gei)	x			x	x		
Leptolyngbya sp. (Lep)	x	x	x	x	x	x	x
Merismopedia sp. (Mer)	x	x		x	x	x	
Microcystis sp. (Mic)	x			x			
Oscillatoria sp. (Osc)		x			x	x	
Phormidium sp. (Pho)	x	x	x	x	x	x	x
Pseudanabaena sp. (Pse)	x	x		x	x	x	x
Synechococcus sp. (Syn)		x		x			
Synechocystis sp. (Syne)	x	x		x	x	x	
Total genera of the class Cyanophyceae	**10**	**9**	**2**	**9**	**10**	**7**	**4**

Table 4-1 Continued

CRYPTOPHYCEAE							
Genus	**Site 2 KKD**	**Site 3 BWFS**	**Site 4 AWFS**	**Site 5 BKD**	**Site 6 PD**	**Site 7 WWTP**	**Site 8 EBR**
Cryptomonas sp. *(*Cry*)*	x	x	x	x	x	x	
Total genera of the class Cryptophyceae	**1**	**1**	**1**	**1**	**1**	**1**	**0**
CHRYSOPHYCEAE							
Genus	**Site 2 KKD**	**Site 3 BWFS**	**Site 4 AWFS**	**Site 5 BKD**	**Site 6 PD**	**Site 7 WWTP**	**Site 8 EBR**
Dinobryon sp. (Din)	x	x	x	x	x		x
Total genera of the class Chrysophyceae	**1**	**1**	**1**	**1**	**1**	**0**	**1**
DINOPHYCEAE							
Genus	**Site 2 KKD**	**Site 3 BWFS**	**Site 4 AWFS**	**Site 5 BKD**	**Site 6 PD**	**Site 7 WWTP**	**Site 8 EBR**
Ceratium sp. (Cer)		x	x	x	x	x	x
Peridiniopsis sp. (Per)	x			x			
Peridinium (Peri)	x	x	x	x	x	x	x
Total genera of the class Dinophyceae	**2**	**2**	**2**	**3**	**2**	**2**	**2**
EUGLENOPHYCEAE							
Genus	**Site 2 KKD**	**Site 3 BWFS**	**Site 4 AWFS**	**Site 5 BKD**	**Site 6 PD**	**Site 7 WWTP**	**Site 8 EBR**
Euglena sp. *(*Eug)	x	x	x	x	x	x	x
Phacus sp. *(*Phac)		x			x	x	x
Strombomonas sp *(*Str)						x	x
Trachelomonas sp. *(*Tra)	x	x	x		x	x	x
Total genera of the class Euglenophyceae	**2**	**3**	**2**	**1**	**3**	**4**	**4**

The abundance of each of these algal classes per site and for the Mooi River can be seen in Figure 4.2 (a) and Figure 4.2 (b).

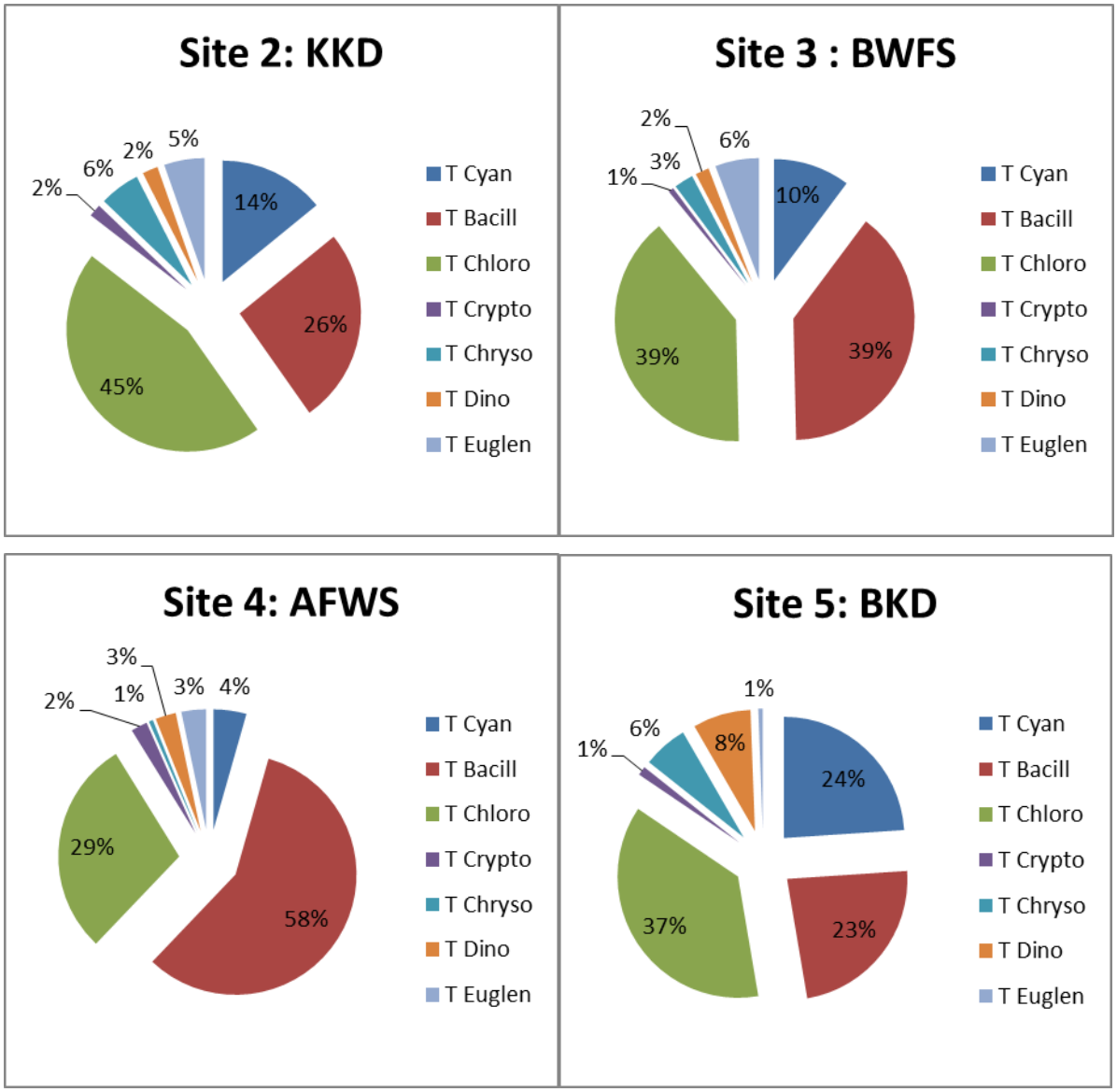


Figure 4-2 (a): Relative abundance of the phytoplankton classes present for the study period January 2014 – October 2015 at Site 2: KKD, Site 3: BWFS, Site 4: AFWS and Site 5: BKD.
T Cyan: Cyanophyceae, T Bacill: Bacillariophyceae, T Chloro: Chlorophyceae, T Crypto: Cryptophyceae, T Chryso: Chrysophyceae, T Dino: Dinophyceae

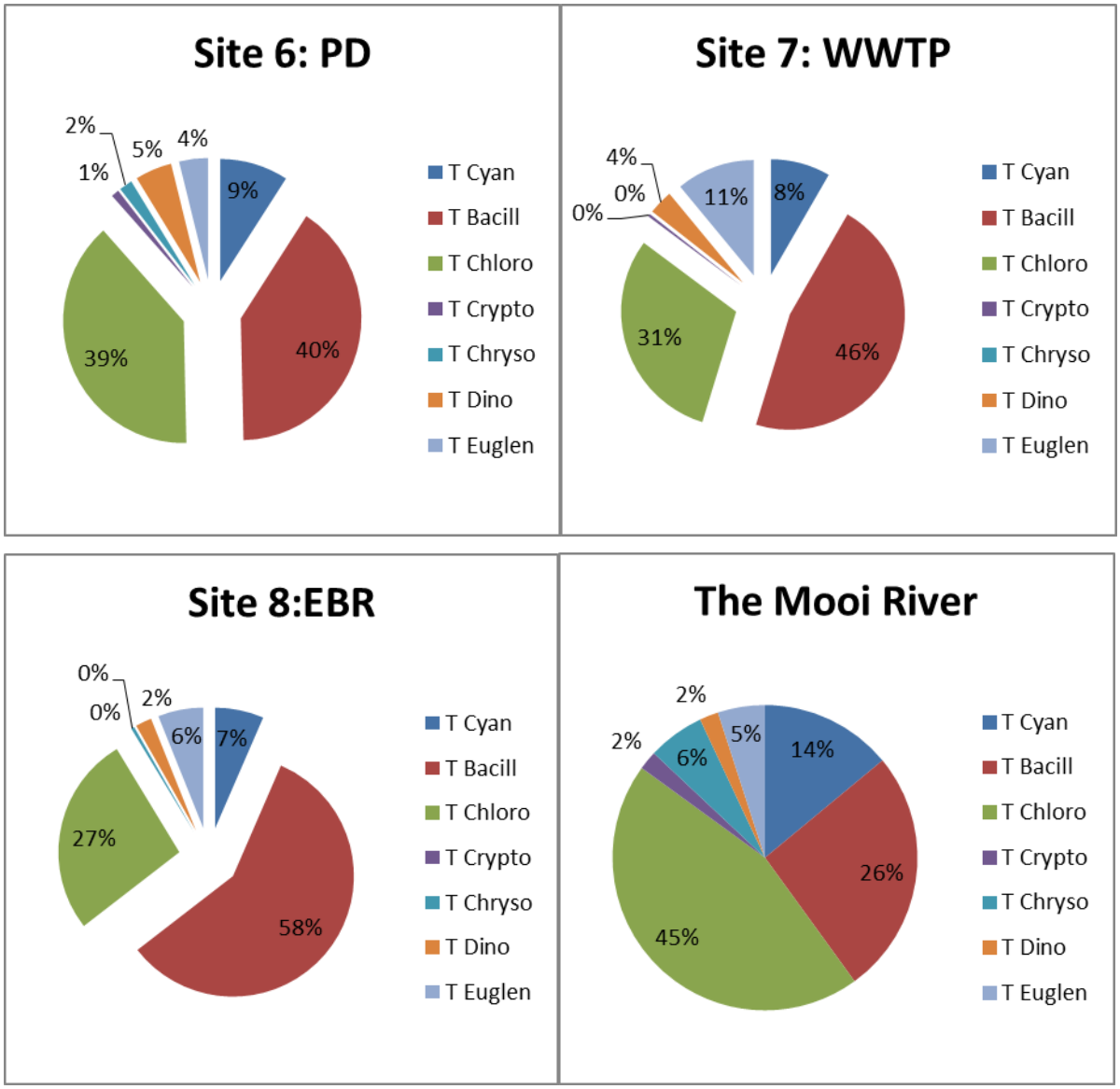


Figure 4-2 (b): **Relative abundance of the phytoplankton classes present for the study period January 2014 – October 2015 at Sites 6: PD, Site 7: WWTP, Site 8: EBR and The Mooi River.**
T Cyan: Cyanophyceae, T Bacill: Bacillariophyceae, T Chloro: Chlorophyceae, T Crypto: Cryptophyceae, T Chryso: Chrysophyceae, T Dino: Dinophyceae

The most abundant algal classes identified during this study for the Mooi River system were Chlorophyceae with 45% occurrence, Bacillariophyceae with 26% occurrence, and Cyanophyceae with 14% occurrence (Figure 4.2 (b)).

The algal classes were further compared between the three reservoirs (Site2: KKD, Site 5: BKD and Site 6: PD) and the in-stream sites (Figure 4.3). The most abundant algal group present in the reservoirs was Chlorophyceae, 45%. In-stream the Bacillariophyceae and Chlorophyceae were in even abundance of 39%.

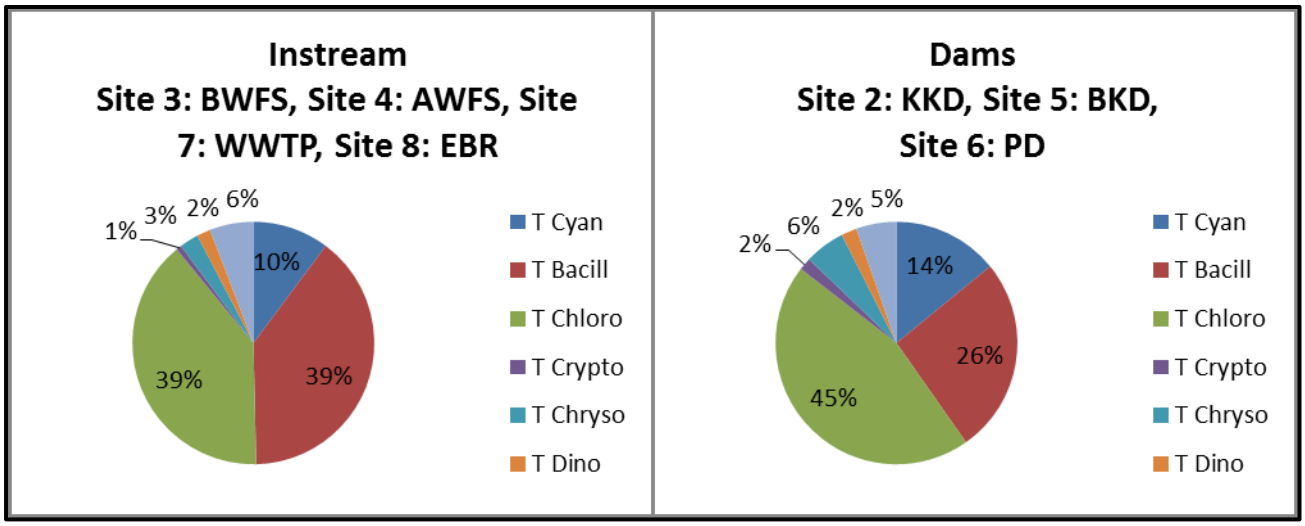


Figure 4-3: **The abundance of the phytoplankton classes comparing in-stream and the reservoirs for the study period January 2014 – October 2015.**
T Cyan: Cyanophyceae, T Bacill: Bacillariophyceae,
T Chloro: Chlorophyceae, T Crypto: Cryptophyceae,
T Chryso: Chrysophyceae, T Dino: Dinophyceae

The three reservoirs, namely Site 2: KKD, Site 5: BKD and Site 6: PD were also compared (Figure 4.4) to determine the occurrence of algal genera.

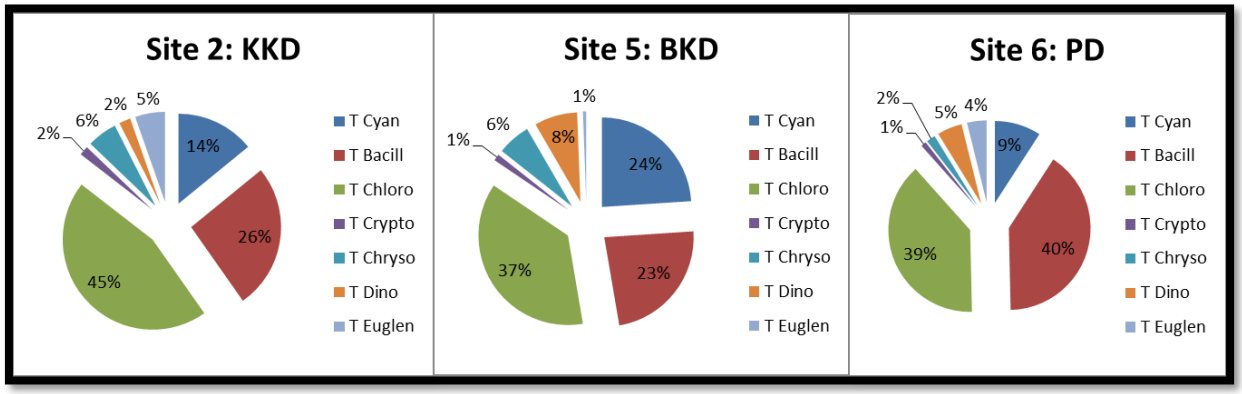


Figure 4-4: **The abundance of the phytoplankton classes compared between the three reservoirs for the study period January 2014 – October 2015.**
T Cyan: Cyanophyceae, T Bacill: Bacillariophyceae,
T Chloro: Chlorophyceae, T Crypto: Cryptophyceae,
T Chryso: Chrysophyceae, T Dino: Dinophyceae

Chlorophyceae was the most abundant algal class present at Site 2: KKD (45%) and Site 5: (37%) and a close second at Site 6: PD. Bacillariophyceae was the most abundant algal class present at Site 6: PD. Cyanophyceae was the second most abundant at Site 5: BKD at 24% occurrence.

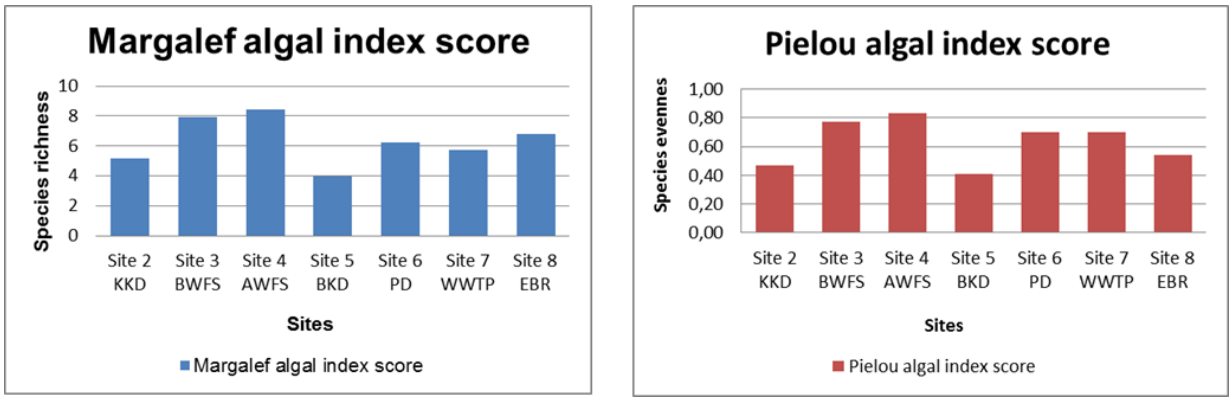
The enumeration and identification data of the algal genera were used during the compilation of the following indices (Table 4.2): the Shannon Wiener Diversity Index, Pielou Species Evenness Index, Margalef Species Richness Index, and Palmer's Algal Genus Pollution Index. These indices' scores reveal the evenness, richness and pollution level of the Mooi River and will assist in the water quality determination with regards to organic pollution. The calculation for these indices can be seen in Appendix C.

The determined biotic indices scores are presented in Table 4.2. Colour keys are allocated to the Palmer- and Shannon-Wiener Diversity Index scores, depicting the level of pollution in accordance with Tables 3.9 and 3.7 respectively.

Table 4-2: **The scores of the four biotic indices calculated for the sites for the study period January 2014 – October 2015.**
Site1: BVO, Site 2: KKD, Site 3: BWFS, Site 4: AWFS, Site 5: BKD, Site 6: PD, Site 7: WWTP, Site 8: EBR

SITE	Palmer algal index score	Shannon Wiener Diversity index score	Margalef Species Richness index score	Pielou Species Evenness index score
Site 2 KKD	29	1.89	5,17	0,47
Site 3 BWFS	37	3.18	7,91	0,77
Site 4 AWFS	28	3.2	8,42	0,83
Site 5 BKD	28	1.58	3,95	0,41
Site 6 PD	38	2.91	6,26	0,70
Site 7 WWTP	37	2.79	5,74	0,70
Site 8 EBR	30	2.11	6,78	0,54

The Margalef and Pielou indices are indicative of the species richness and evenness respectively. These indices are further depicted in Figure 4.5.



(A) (B)

Figure 4-5: **Graphs depicting the species richness (A) and species evenness (B) of the sites along the Mooi River.**
Site1: BVO, Site 2: KKD, Site 3: BWFS, Site 4: AWFS, Site 5: BKD, Site 6: PD, Site 7: WWTP, Site 8: EBR

The Palmer Index indicated high levels of organic pollution at all the sites, with scores higher than 20 (see Table 4.2 and Table 3.9). Organic pollution sources include agricultural runoff, abattoirs, animal feeding lots and cattle grazing (Dallas and Day, 2004). All these activities are present next to the Mooi River thus contributing to the high Palmer Index score. The Palmer identified genera present at each site used for the calculation of the Palmer Index is listed in Table 4.3. These genera are most tolerant to organic pollution.

Low levels of pollution were present at Site 6: PD, Site 7: WWTP and Site 8: EBR with a Shannon Wiener scores between 2.0 and 3.0 (Table 4.2). Between these three sites, Site 8: EBR had the highest species richness, but the lowest species evenness, with 58% of the species identified at this site falling within the class Bacillariophyceae.

The Shannon-Wiener scores for Site 2: KKD and Site 5: BKD indicate moderate pollution levels, with a score between 1.0 and 2.0 (Table 4.2). The lowest species richness and evenness are present at Site 5: BKD (Figure 4.4).

Table 4-3: The Palmer genera present at each site used for the Palmer Index calculation. Site1: BVO, Site 2: KKD, Site 3: BWFS, Site 4: AWFS, Site 5: BKD, Site 6: PD, Site 7: WWTP, Site 8: EBR

Bacillariophyceae Chlorophyceae Cyanophyceae Euglenophyceae

Genus	Pollution Index	Site 2 KKD	Site 3 BWFS	Site 4 AWFS	Site 5 BKD	Site 6 PD	Site 7 WWTP	Site 8 EBR
Ankistrodesmus spp	2	x	x		x	x	x	
Chlamydomonas spp	4	x	x	x	x	x	x	x
Chlorella spp	3	x	x	x	x	x	x	x
Cyclotella spp	1		x	x		x	x	x
Fragilaria spp	2	x	x	x	x	x	x	x
Euglena spp	5	x	x	x	x	x	x	x
Gomphonema spp	1	x	x	x	x	x	x	x
Melosira spp	1	x	x	x			x	x
Navicula spp	3	x	x	x	x	x	x	x
Nitzschia spp	3	x	x	x	x	x	x	x
Oscillatoria spp	5		x			x	x	
Phacus spp	2		x			x	x	x
Phormidium spp	1	x	x	x	x	x	x	x
Scenedesmus spp	4	x	x	x	x	x	x	x
Total Palmer genera present: (14)		11	14	11	10	13	14	12

The Palmer genera that contributed most to the high scores are *Euglena* spp, *Scenedesmus* spp*, Chlamydomonas* spp, present at all sites and *Oscillatoria* spp*,* present at Site 3: BWFS, Site 6: PD and Site 7: WWTP. The Palmer genera present forms part of the most abundant algae classes, Chlorophyceae (45%) Bacillariophyceae (26%) and Cyanophyceae (14%) (Figure 4.1).

4.2 PHYSICO-CHEMICAL AND MICROBIOLOGICAL WATER QUALITY OF THE MOOI RIVER

The descriptive statistics (valid n, mean, minimum, maximum and standard deviation) for all the physico-chemical and biological variables at each respective site as well as the overall average for the river during the duration of the study period are summarised in Table 4.4. This data will serve as an overview of the current water quality status of the Mooi River and how it relates to the set RQO for the Mooi River in the Upper Vaal catchment.

The Mooi River, as mentioned in Chapter 2, is mainly impacted on by mining and agricultural activities. The physico-chemical and microbiological data is therefore expected to exhibit results indicative of aforementioned activities. The variables for this study were chosen with these activities in mind in order to achieve the objectives set out for this study.

High calcium and magnesium concentrations are expected due to dolomitic lithology of the study sites (Figure 2.4). Calcium and magnesium concentrations are expected to increase after the confluence of the Mooi River and WFS due to liming activities occurring in the area upstream from the WFS (Durandt, 2012). An increase in the sulphate concentration and activity of sulphate reducing bacteria is also expected after this confluence.

The abundance of the Bacillariophyceae and Chlorophyceae algal classes (Figure 4.2) are indicative of high phosphate and nitrate and nitrite concentrations expected.

The RQO numerical limits for the Upper Vaal Catchment were gazetted on 22 April 2016. Variables without a set limit that were measured during this study include:

calcium, cyanide, iron, temperature, total dissolved solids, turbidity, alkalinity, total coliforms and sulphate reducing bacteria. Variables listed but which did not form part of the study are; fluoride, aluminium, arsenic, cadmium, chromium, copper, mercury, lead, selenium, zinc, chorine, endosulfan and atrazine.

Table 4-4: Summary of the descriptive statistics for all the physico-chemical and biological variables determined for the whole study period January 2014 - 0ctober 2016.

Site1: BVO, Site 2: KKD, Site 3: BWFS, Site 4: AWFS, Site 5: BKD, Site 6: PD, Site 7: WWTP, Site 8: EBR

Variable	Descriptive	River	Site 1 BVO	Site 2 KKD	Site 3 BWFS	Site 4 AWFS	Site 5 BKD	Site 6 PD	Site 7 WWTP	Site 8 EBR
Calcium Mg/l	Valid N	174	21	22	22	22	22	22	21	22
	Mean	48.362	42.19	37.909	41.773	63.5	47.409	47.045	53.14	53.864
	Minimum	21	34	26	29	47	29	21	34	34
	Maximum	97	49	64	58	97	61	64	76	66
	Standard deviation	12.3	4.75	9.3346	8.2689	13.269	9.7573	11.445	10.341	8.357
Magnesium Mg/l	Valid N	174	21	22	22	22	22	22	21	22
	Mean	35.828	25.286	29.091	30.273	37.545	39.318	41	41.52	42.364
	Minimum	16	16	21	21	27	30	29	27	27
	Maximum	52	32	50	46	45	47	50	52	52
	Standard deviation	8.459	4.014	6.3614	6.1037	5.3339	4.9124	6.0317	7.4808	6.485
Nitrate and Nitrite Mg/l	Valid N	167	20	21	21	21	21	21	21	21
	Mean	0.877	1.528	0.25	0.25	1.137	0.367	0.36	1.684	1.47
	Minimum	0.25	0.25	0.25	0.25	0.8	0.25	0.25	0.78	0.25
	Maximum	4.3	2.9	0.25	0.25	1.3	1.3	1.9	4.3	3.9
	Standard deviation	0.813	0.444	0	0	0.1333	0.2576	0.3803	0.92	1.138
Orthophosphate Mg/l	Valid N	174	21	22	22	22	22	22	21	22
	Mean	0.163	0.028	0.035	0.049	0.035	0.035	0.113	0.567	0.464
	Minimum	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025
	Maximum	2.0	0.07	0.25	0.32	0.25	0.25	1.1	2.0	1.1
	Standard deviation	0.305	0.011	0.048	0.0773	0.048	0.048	0.2416	0.5339	0.284

Table 4-4: **Summary of the descriptive statistics for all the physico-chemical and biological variables determined for the whole study period January 2014 - 0ctober 2016.**

Site1: BVO, Site 2: KKD, Site 3: BWFS, Site 4: AWFS, Site 5: BKD, Site 6: PD, Site 7: WWTP, Site 8: EBR

Variable	Descriptive	River	Site 1 BVO	Site 2 KKD	Site 3 BWFS	Site 4 AWFS	Site 5 BKD	Site 6 PD	Site 7 WWTP	Site 8 EBR
Sulphate mg/l	Valid N	174	21	22	22	22	22	22	21	22
	Mean	73.554	4.411	4.669	9.501	108.4	110.81	100.56	120.24	128.82
	Minimum	0.025	0.025	0.025	0.025	77	91	83	80	93
	Maximum	288	20	15	112	129	126	117	146	288
	Standard deviation	55.779	4.517	3.1238	23.049	12.676	10.777	10.205	16.251	38.97
Cyanide mg/l	Valid N	174	21	22	22	22	22	22	21	22
	Mean	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	Minimum	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	Maximum	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	Standard deviation	0	0	0	0	0	0	0	0	0
Iron mg/l	Valid N	174	21	22	22	22	22	22	21	22
	Mean	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
	Minimum	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
	Maximum	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
	Standard deviation	**0**	0	0	0	0	0	0	0	
Manganese mg/l	Valid N	174	21	22	22	22	22	22	21	22
	Mean	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
	Minimum	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
	Maximum	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
	Standard deviation	0	0	0	0	0	0	0	0	0

Table 4-4: **Summary of the descriptive statistics for all the physico-chemical and biological variables determined for the whole study period January 2014 - 0ctober 2016.**

Site1: BVO, Site 2: KKD, Site 3: BWFS, Site 4: AWFS, Site 5: BKD, Site 6: PD, Site 7: WWTP, Site 8: EBR

Variable	Descriptive	River	Site 1 BVO	Site 2 KKD	Site 3 BWFS	Site 4 AWFS	Site 5 BKD	Site 6 PD	Site 7 WWTP	Site 8 EBR
Uranium mg/l	Valid N	174	21	22	22	22	22	22	21	22
	Mean	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.007	0.008
	Minimum	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
	Maximum	0.05	0.018	0.015	0.015	0.014	0.019	0.018	0.05	0.05
	Standard deviation	0.005	0.003	0.0025	0.0031	0.0025	0.0034	0.0033	0.0099	0.01
Temp °C	Valid N	174	21	22	22	22	22	22	21	22
	Mean	18.8	20.105	19.205	18.125	18.235	18.709	19.431	18.42	18.814
	Minimum	6.11	9.6	6.11	9.4	9.28	10.7	10.98	9.33	10.6
	Maximum	26.4	22.9	23.5	23.2	25.1	25.2	26.4	25.7	26.3
	Standard deviation	4.365	2.659	4.6744	4.5342	4.282	4.4714	4.6849	4.4321	4.911
pH	Valid N	174	21	22	22	22	22	22	21	22
	Mean	7.953	7.235	7.848	7.949	7.885	8.299	8.224	7.982	8.167
	Minimum	6.6	6.6	7.01	7.54	7.2	7.8	7.6	7.3	7.5
	Maximum	10	8.5	8.8	8.5	9.6	9.7	10	9.8	9.8
	Standard deviation	0.579	0.405	0.4306	0.2508	0.5786	0.524	0.5446	0.642	0.526
EC	Valid N	174	21	22	22	22	22	22	21	22
	Mean	70.59	48.614	53.318	46.636	73.773	99.59	74.955	82.714	86.736
	Minimum	33	41	38	33	59	57	51	51	63
	Maximum	211.20	120	99	118	175	169	188	209	211.2
	Standard deviation	27.14	17.101	17.761	16.678	23.415	23.42	26.691	30.118	28.407

Variable	Descriptive	River	Site 1 BVO	Site 2 KKD	Site 3 BWFS	Site 4 AWFS	Site 5 BKD	Site 6 PD	Site 7 WWTP	Site 8 EBR
TDS mg/l	**Valid N**	174	21	22	22	22	22	22	21	22
	Mean	469.42	338.05	340.96	337.14	521.5	508.63	517.36	576.14	614.46
	Minimum	191	236	245	255	325	408	191	360	451
	Maximum	1370	781	642	767	1142	1100	1222	1359	1370
	Standard deviation	178.35	107.25	88.283	100.98	148.48	138.55	180.76	189.2	178.7
Turbidity NTU	**Valid N**	174	21	22	22	22	22	22	21	22
	Mean	1.693	0.259	1.436	1.519	0.78	0.582	1.805	3.15	4.014
	Minimum	0.1	0.1	0.82	0.68	0.54	0.44	0.94	1.14	1.85
	Maximum	17.2	0.8	1.92	3.02	1.71	1.01	4.29	12.6	17.2
	Standard deviation	1.885	0.15	0.2283	0.5769	0.2898	0.1426	0.6724	2.4465	3.24
Alkalinity mg/l	**Valid N**	163	20	21	21	21	21	20	19	20
	Mean	242.767	234.05	241.05	258.05	246.48	232.81	231.2	239.9	258.1
	Minimum	115	223	188	222	235	166	137	115	206
	Maximum	285	241	268	273	253	285	266	272	284
	Standard deviation	27.201	3.98	16.076	15.506	4.9155	35.733	43.081	39.131	15.134
***E.coli* cfu/100ml**	**Valid N**	174	21	22	22	22	22	22	21	22
	Mean	828	65	81	497	1124	145	209	11	4425
	Minimum	3	31	36	219	420	89	121	3	1414
	Maximum	4840	111	114	722	4840	235	308	26	4840
	Standard deviation	1504	22	21	175	966	36	45	5	1082

Table 4-4: Summary of the descriptive statistics for all the physico-chemical and biological variables determined for the whole study period January 2014 - 0ctober 2016.

Site1: BVO, Site 2: KKD, Site 3: BWFS, Site 4: AWFS, Site 5: BKD, Site 6: PD, Site 7: WWTP, Site 8: EBR

Variable	Descriptive	River	Site 1 BVO	Site 2 KKD	Site 3 BWFS	Site 4 AWFS	Site 5 BKD	Site 6 PD	Site 7 WWTP	Site 8 EBR
Sulphate Reducing Bacteria (SRB) cfu/100ml	**Valid N**	174	21	22	22	22	22	22	21	22
	Mean	15	3	4	9	24	15	18	20	25
	Minimum	0	0	0	0	9	4	6	10	9
	Maximum	63	9	11	32	36	26	33	34	63
	Standard deviation	11	3	3	8	8	6	8	7	13
Chlorophyll *a* ug/l	**Valid N**	174	21	22	22	22	22	22	21	22
	Mean	7.8	2.4	16.2	10.6	2.9	7.6	12.31	5.2	4.6
	Minimum	0.1	0.1	0.4	0.5	0.4	0.5	1.2	1.5	0.91
	Maximum	202	7.5	202	124	10	67	95	14	12
	Standard deviation	20.8	2.1	44.8	26.9	2.8	13.9	21.2	3.5	3.1

The numerical limits set for the RQO of the measured variables during this study are presented in Table 4.5. The required percentile was calculated for each result as per the Government Gazette 39943 for the Mooi River, and listed in Table 4.5. The percentile results exceeding the set limit is highlighted in red. Variables that exceeds the RQO numerical limit are; orthophosphate, Chlorophyll *a*, magnesium, pH and *E.coli*

Table 4-5: The numerical limits for the RQO variables as listed in the government gazette 39943 for the Upper Vaal and the calculated percentile for the Mooi River.

Indicates that the measurement exceeds the set numerical limit

Variable	Units	Limit (GG No. 39943)	Percentile	
Nitrate and Nitrite	mg/L	≤ 4	95th	2.8
Orthophosphate	mg/L	≤ 0.125	95th	0.7925
Electrical Conductivity	mS/m	≤111	95th	103.75
Sulphate	mg/L	≤ 500	95th	131.75
Magnesium Dissolved	mg/L	≤ 33	95th	49
pH at 25°C	pH units	>5.8	5th	7.1
pH at 25°C	pH units	≤ 8.8	95th	8.94
Dissolved manganese	mg/L	≤ 1.3	95th	0.05
Dissolved uranium	mg/L	≤0.015	95th	0.01425
E.coli	MPN/100 ml	≤ 130	95th	4840

The numerical limit for the uranium RQO is set at <0.0015mg/l. The average uranium concentration measured for the Mooi River was 0.006mg/l (Table 4.4). The 95th percentile uranium measured for the Mooi River is below the RQO numerical limit (Table 4.5). Uranium naturally occurs within the study area and can leach from the lithology and is also contributed by the mining activities along the WFS.

The measurements for manganese, iron and cyanide were below the detection limit of the methods used and had no significant contribution to the water quality study.

The average temperature measured for the Mooi River was 18.8°C, with a maximum of 26.4°C during the summer months and a minimum of 6.11°C during the winter months. Temperature was considered during this study as it can have an effect on the chemical toxicity of substances as well as the overall water quality. According to Dallas

and Day (2004), the water temperature variation should not exceed 2°C or 10% from the average water background temperature. To be able to establish if effluent or runoff has an effect on the water temperature an hourly measurement would have to be taken over a set time.

The measurements of the parameters depicted in Figure 4.5 are the most likely to be influenced directly by the mining activities of the West Rand. Most of the changes occur after the confluence with the WFS.

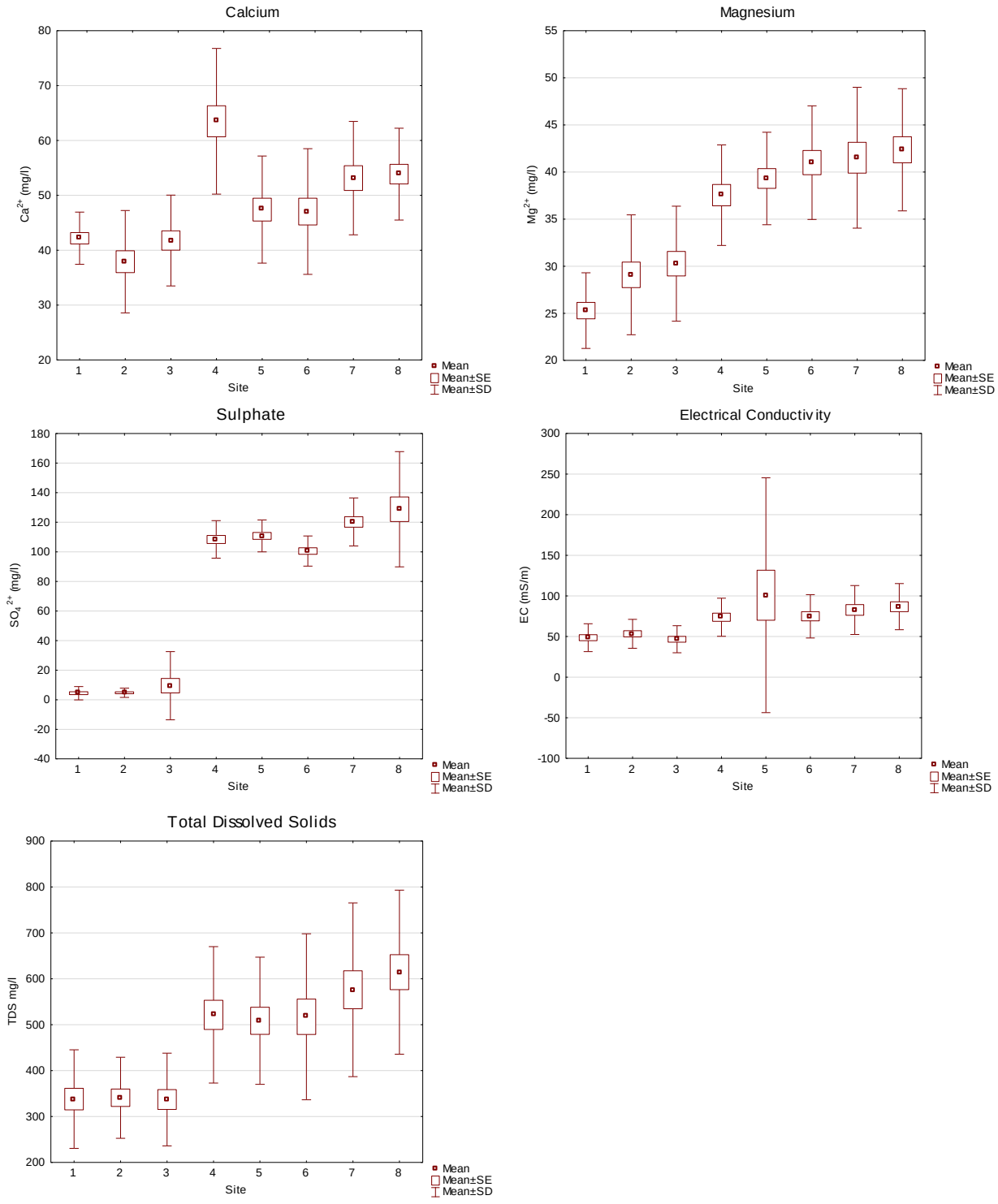


Figure 4-5: **Box and whiskers plots illustrating the electrical conductivity, total dissolved solids, calcium, magnesium and sulphate concentration measured along the Mooi River gradient during the study period January 2014 - October 2016.**

±SE (Standard Error); ±SD (Standard Deviation); Site 1: BVO, Site 2:

KKD, Site 3: BWFS, Site 4: AWFS, Site 5: BKD, Site 6: PD, Site 7: WWTP, Site 8: EBR

The highest average calcium concentration was observed at Site 4: AFWS below the confluence of the WFS and the Mooi River (Figure 4.2). This site had a maximum calcium concentration of 97mg/l with an average concentration of 63.5mg/l (Table 4.4). The calcium concentration did decrease at the next site, Site 5: BKD to an average of 47.409mg/l probably due to the dilution effect of the dam and then gradually increased downstream at the next site to an average of 53.864mg/l. The lowest average calcium concentration of 37.909mg/l (Table 4.4) was measured at Site 2: KKD (Figure 4.4). Site 4: AWFS significantly differs from Site 1: BVO, Site 2: KKD, Site 3: BWFS, Site 5: BKD and Site 6: PD ($p<0.05$).

The magnesium concentration gradually increased downstream of the Mooi River towards the confluence with the Vaal River. The lowest and highest average concentrations being Site 1: BVO with average concentration of 25.286mg/l and Site 8: EBR with an average of 42.364mg/l respectively (Table 4.4). Site 1: BVO shows a significant difference, $p<0.05$, from Site 4: AWFS, Site 5: BKD, Site 6: PD, Site 7: WWTP and Site 8: EBR. The average measurement for Site 1: BVO, Site 2: KKD and Site 3: BWFS is below the set RQO. The average measurement for the sites after the confluence of the WFS exceeds the set RQO for the Upper Vaal. The average magnesium concentration measured for the Mooi River is 35.828mg/l (Table 4.4). The 95th percentile of the magnesium levels calculated for the Mooi River exceeds the set numerical limit for the RQO with 16mg/l (Table 4.4).

Both the magnesium and calcium contribute to the elevated hardness of the water in the Potchefstroom area.

An eleven fold increase in the average sulphate concentration was visible at Site 4: AWFS. Increasing from an average of 9.501mg/l (Site 3: BWFS) to an average of 108.4mg/l (Site 4: AWFS). Site 1: BVO, Site 2: KKD and Site 3: BWFS shows a significant difference from Site 4: AWFS, Site 5: BKD, Site 6: PD, Site 7: WWTP and Site 8: EBR. This increase is most probably due to the confluence of the WFS.

The study sites were originally chosen based on the increase in the average value of EC along the Mooi River. The average measured EC for the Mooi River is 70.59mS/m. The highest average EC was measured at Site 5: BKD.

A significant ($p<0.05$) increase in the average TDS concentration measured was observed at Site 4: AWFS (Figure.4.2). This phenomenon can once again be attributed to the confluence of the WFS. An average TDS of 469.42mg/l was measure during the study time for the Mooi River (Table 4.2). No set limit is present for the TDS concentration for the Upper Vaal study area.

pH is determined by the concentration of hydrogen ions present and alkalinity the concentration hydroxyl, bicarbonate and carbonate ions in the water (Dallas and Day, 2004). Alkalinity refers to the buffering effect of water. The results show that there is a correlation between alkalinity and calcium and magnesium, $p<0.05$ (Appendix B). As previously mentioned calcium and magnesium are characteristic of the dolomitic lithology of the Mooi River area. It is the dissociation of the dolomitic lithology that has a buffering effect and explains this correlation with alkalinity.The pH and alkalinity measured during this study is depicted in Figure 4.6.

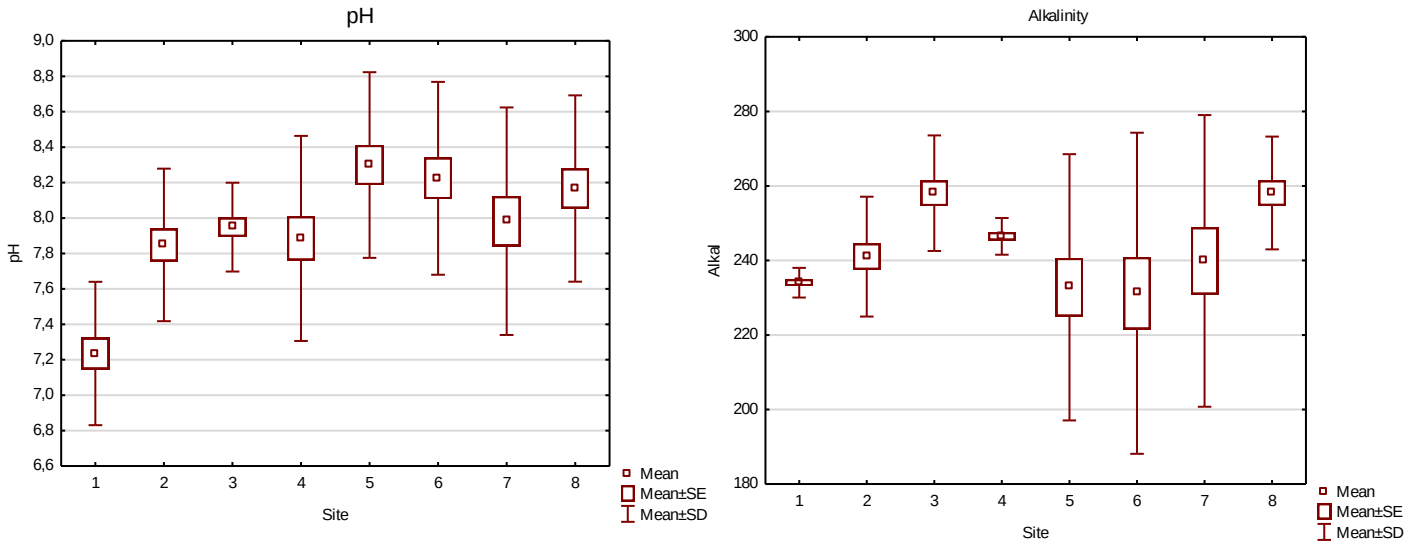


Figure 4-6: Box and whiskers plots illustrating the pH and alkalinity measured along the Mooi River gradient during the study period January 2014 - October 2016.
±SE (Standard Error); ±SD (Standard Deviation); Site 1: BVO, Site 2: KKD, Site 3: BWFS, Site 4: AWFS, Site 5: BKD, Site 6: PD, Site 7: WWTP, Site 8: EBR

The average pH of the Mooi River was 7.953, falling well within the set criteria. A minimum pH of 6.6 was measured at Site 1: BVO with a maximum pH 10 at Site 6: PD (Table 4.3). The 95th percentile for the Mooi River for the study falls outside of the set RQO criteria of 8.8 (Table 4.4). The overall high pH of the Mooi River can be attributed to the dolomitic lithology as well as liming taking place at various mines of which the effluent ends up in the Mooi River.

The average alkalinity measured during the study for the river was 242.767mg/l (Table 4.3). No set limits are present for alkalinity.

Nutrients are introduced into the Mooi River mostly due to agricultural surface runoff. The nutrients contribute to the trophic status of the river. These activities can also be the reason for the high Palmer Index scores (values above 20) (Table 4.2). The nitrate and nitrite and orthophosphate concentrations measured during the study are depicted in Figure 4.7.

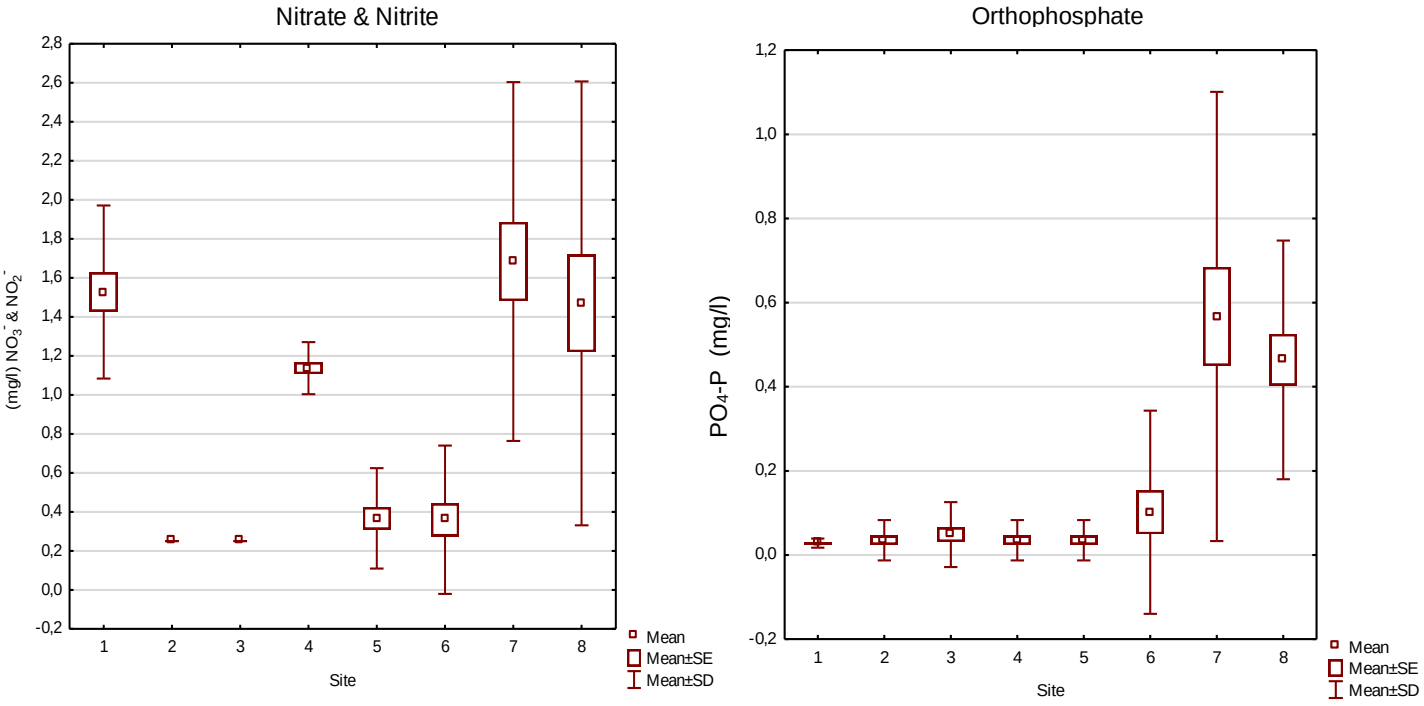


Figure 4-7: **Box and whiskers plots illustrating the orthophosphate, nitrate and nitrite concentration measured along the Mooi River gradient during the study period January 2014 - October 2016.**
±SE (Standard Error); ±SD (Standard Deviation); Site 1: BVO, Site 2: KKD, Site 3: BWFS, Site 4: AWFS, Site 5: BKD, Site 6: PD, Site 7: WWTP, Site 8: EBR

The highest average nitrate and nitrite concentration were measured at Site 7: WWTP, 1.684mg/l, and differs significantly ($p<0.05$) from the lowest average concentration at Site 2: KKD and Site 3: BWFS both with an average of 0.25mg/l. The average nitrate and nitrite measured for the Mooi River was well below the limit of <4mg/l.

The average orthophosphate concentration for the Mooi River was 0.163mg/l (Table 4.4). The highest average orthophosphate concentration, 0.567mg/l, which was measured at Site 7: WWTP. This is characteristic of waste water effluent. The 95th percentile of the Mooi River exceeds the set numerical limit for the RQO (Table 4.4). The orthophosphate concentration at Site 8: EBR also contributes to the exceedance of the RQO numerical limit (Figure 4.4). The high average orthophosphate concentration at Site 8: EBR is most probably due to the agricultural runoff as well as the piggery situated in area before Site 8: EBR. The highest Nitrate and Nitrite- as well as orthophosphate concentrations were measured at Site 7: WWTP.

4.3 BACTERIOLOGICAL RESULTS

4.3.1 *E.COLI* AND TOTAL COLIFORMS

Bacteria occur naturally in all rivers and streams; however pollution from livestock discharge, agricultural activities and human waste discharge can increase the microbial load of a water body.

E.coli forms part of the larger faecal coliform group, which forms part of the larger total coliform group. *E.coli* is a non-pathogenic coliform bacterium, present in the intestinal tracts of all warm blooded mammals. *E.coli* is commonly used to detect faecal pollution and is referred to as an indicator organism. Coliform bacteria are a group of bacteria that occur naturally in most environments and are used during the evaluation of the quality of drinking water. The *E.coli* and total coliform concentrations determined during the study is depicted in Figure 4.8.

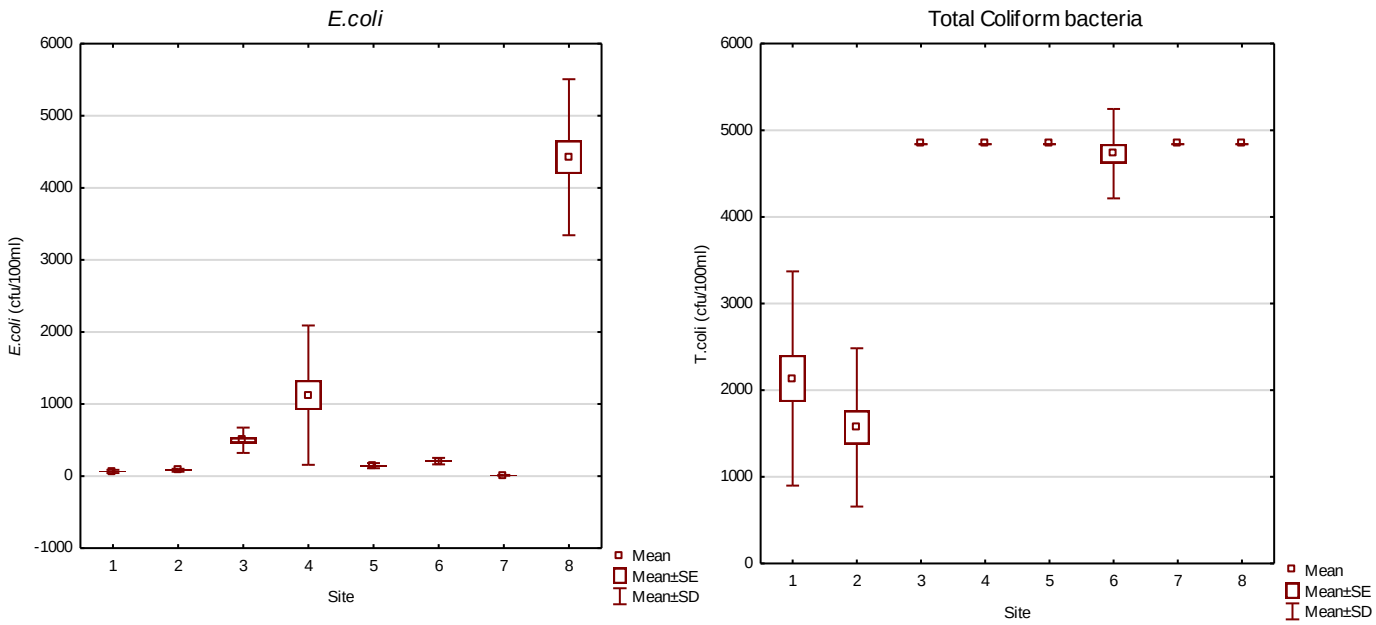


Figure 4-8: **Box and whiskers plots illustrating the *E.coli* and total coliform counts measured along the Mooi River gradient during the study period January 2014 - October 2016.**
±SE (Standard Error); ±SD (Standard Deviation); Site 1: BVO, Site 2: KKD, Site 3: BWFS, Site 4: AWFS, Site 5: BKD, Site 6: PD, Site 7: WWTP, Site 8: EBR

The average *E.coli* count for the Mooi River was 828.46cfu/100ml (Table 4.4). There is no set *E.coli* numerical limit for the Mooi River but, should the 95th percentile be considered for the Vaal River, the Mooi River's 95th percentile exceeds the RQO numerical limit by almost 18 fold. It can be accepted that the Mooi River has a high load of faecal pollution. The sites contributing to this high count were mainly Site 4: AWFS, where cattle grazing were evident, and Site 8: EBR, influenced by agricultural activities and the runoff from a piggery (Figure 4.5). This was expected as the Palmer Index was also indicative of high levels organic pollution.

The sites with the lower *E.coli* counts were Site 1: BVO, Site 2: KKD and Site 7: WWTP. It can therefore be reasoned that the effluent from the waste water treatment plant is adequately treated.

Total coliforms occur naturally in soil and are an indicator of pollution. "Total coliforms" is a collective name for all the coliforms present. The average total coliform count for the study was 4086cfu/100ml (Table 4.4). No set limit is presently set regarding the coliform count for the Upper Vaal. The Total coliforms increased significantly from Site 3: BWFS onward (Figure 4.8). This can be an indication that Site 1: BVO and Site 2: KKD are mainly untouched by human impacts and pollution.

4.3.2 SULPHATE REDUCING BACTERIA

Sulphate Reducing Bacteria (SRB's) are a group of strictly anaerobe prokaryotes found in a variety of anaerobic environments such as soil, mud and sediments of freshwaters, mining waters, industrial waste waters and gastrointestinal tract of man and animals (Luptakova, 2007).

The SRB counts determined during this study is depicted in Figure 4.9.

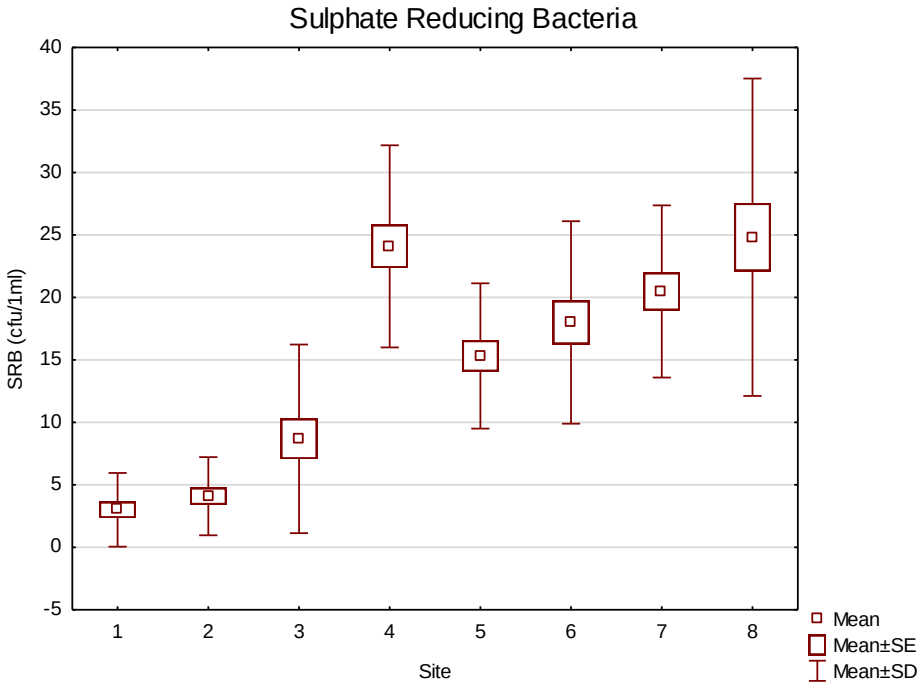


Figure 4-9: **Box and whisker plot illustrating the SRB counts measured along the Mooi River gradient during the study period January 2014 - October 2016.**
±SE (Standard Error); ±SD (Standard Deviation); Site 1: BVO, Site 2: KKD, Site 3: BWFS, Site 4: AWFS, Site 5: BKD, Site 6: PD, Site 7: WWTP, Site 8: EBR

The average SRB count measured was 14.845cfu/1ml (Table 4.4). A peak in the SRB count can be seen ate Site 4: AWFS (Figure. 4.6). A significant correlation exists between the SRB counts and the Sulphate concentration ($p<0.05$).

CHAPTER 5: DISCUSSION

A Bio-indicator is described by Wiley *et al* (2010) as species that provides information on the physical of chemical characteristics of a specific environment or site. Due to their high reproductive rates, algae respond rapidly to natural and/or anthropogenic changes in their environmental conditions (Wiley *et al*, 2010). As such they can make a valuable contribution as bio-indicators of a water body's health. Dominant genera in algal groupings change not only spatially but also seasonally, as physical, chemical and biological conditions in a water body change (Wetzel, 2001). The surface water quality of the Mooi River is influenced by both naturally and anthropogenic activities (Barnard *et al*, 2013). It is therefore important to monitor algal assemblages in a water system that serve agricultural, recreational and potable uses not only as indicators of water health but also to identify problematic algae that can harm human health.

The algal classes identified during this study in the Mooi River are indicative of a mesotrophic to eutrophic river system. The chlorophyll *a* measured during the study for the Mooi River ranged between a minimum of 0.1mg/l measured at Site 1:BVO to a maximum of 202mg/l measured at Site 2; KKD. No large blooms were observed of any harmful algae. Currently no RQO numerical limit is set for chlorophyll *a*, however the 95% percentile set for sites in the rest of the Upper Vaal is 20mg/l. The 95% percentile chlorophyll *a* concentration for the Mooi River currently exceeds this with 22.3mg/l. A need thus exist to monitor the chlorophyll *a* concentration.

In their paper on the algal groups of the reservoirs in the Mooi River system, Venter *et al* (2013) compared the algal compositions present in 1999-2000 to that of 2010-2011. The chlorophyll *a* concentration observed during this study have almost doubled for the reservoirs when compared with their study. Our study showed that the Cyanophyceae showed the biggest increase in abundance for both Site 2: KKD and Site 5: BKD but showed little change at Site 6: PD. The Cyanophyceae abundance doubled at Site 2: KKD increasing from a 7% abundance in 1999-2000 and 2010-2011 to 14% abundance during the current study. The Cyanophyceae's abundance increased almost five fold at Site 5: BKD from an occurrence of 5% in 1999 - 2000 to 24% during the current study. It is this increase in abundance of this algal class that is of concern as the water is abstracted for drinking water from Site 5: BKD. This algal

class is usually considered as nuisance algae as they contribute to taste and odours (Bishop, 2015) (Midvaal Water Company, 2008). It is in particular phosphorus enrichment that favours the growth of Cyanophyceae (Venter *et al*, 2013). Problematic Cyanophyceae genera identified at Site 2: KKD and Site 5: BKD are *Microcystis* sp. and *Oscillatoria* sp. *Microcystis* is known for producing cyanotoxins (Table 4.1) which pose a health risk for both humans and animals (Bishop, 2015). *Oscillatoria* is known to be a taste and odour causing culprit, and was also identified at Site 3: BWFS, Site 6: PD and Site 7: WWTP (Midvaal Water Company: 2008).

The abundance of the class Chrysophyceae decreased especially at Site 5: BKD from a 10% abundance to but 1% abundance. The class Chrysophyceae is usually considered to be an indicator of oligotrophic water (Venter *et al,* 2013) and the decrease thereof thus also confirms a decrease in the water quality. The class Bacillariophyceae had the biggest increase in abundance at Site 6: PD where the group increased from 15% in 1999 - 2000 to 40% abundance for the current study.

When considering the change in algal abundance from 1999-2000 to 2010-2011 to 2015, the current study, it can be assumed the water quality deteriorated in terms of nutrient concentration. Venter *et al,* 2013, classified the three major reservoirs in their study as oligotrophic to mesotrophic, where the algal abundance of the current study is more suggestive of a mesotrophic to eutrophic state, thus suggesting an increase in the nutrient concentration. According to the Water Quality Guidelines volume 7 (1996) nitrate and nitrite levels between 0.5 - 2.5mg/l as well as the phosphate levels of 0.005 - 0.025mg/l is indicative of a mesotrophic state. The trophic state is thus further confirmed as mesotrophic by the mean nitrate and nitrite concentration of 0.877mg/l and the mean orthophosphate concentration of 0.163mg/l determined for the whole of the Mooi River. This is most probably contributed to by the agricultural activity. The orthophosphate level also exceeds the set ROQ numerical limit for the Upper Vaal by six times and therefore needs to be monitored and managed.

The Palmer Index for this study concur the above statement as it is indicative of high organic pollution. It is however suggested by Bellinger and Sigee (2010) that care must be taken when making use of the Palmer Index as sites with high organic pollution also sometimes have high inorganic nutrient and algae typically tolerant to both.

According to Bellinger *et al* (2010) algal flora are influenced to a much greater extent by organic pollution than any other aquatic environmental factor.

During this study the Palmer Index scores ranged from 28 at Site 5: BKD and Site 4: AWFS with a total of 10 and 11 Palmer genera present respectively to a score of 38 at Site 5: PD with a total 13 Palmer genera present. It is not only the amount of Palmer genera present that influences the score but also the concentration thereof.

A study of Krhirsagar (2013) on the Mula River in India indicated that the Palmer Index scores ranging from 19 to 37 to 42, very similar to the scores obtained in this study. During the study by Krhirsagar (2013) the genera that repeatedly occurred were; *Oscillatoria, Euglena, Chlorella, Scenedesmus, Gomphonema, Melosira, Pediastrum, Navicula, Nitzschia, Stigeoclonium* and *Synedra*. Except for *Stigeoclonium* and *Synedra* these genera were also identified in the Mooi River system (Table 4.4) and can thus also be seen as adequate indicators of the organic pollution in light of the Palmer index scores. The Palmer Index score determined by *Booyens* (2015) for the Koekemoerspruit, a water body known to be influenced by illegal mining activities, reported scores ranging from 22 to 27. Possible sources of organic pollution include livestock, sewage effluent from informal settlements, agricultural runoff and abattoirs.

Shannon-Wiener Diversity Index is used to indicate the effect of stress and disturbances on community distribution (Fedor and Spellerberg, 2013) The Shannon-Wiener Diversity Index scores determined for the Mooi River ranged from 1.58 to 3.2. This is very similar to the scores found by *Booyens* (2015) for the Koekemoerspruit who concluded that based on these scores the river ranged from being moderately polluted to heavily polluted.

The Shannon-Wiener Diversity Index is indicative of the species diversity, and takes into account both species richness and species evenness. Species richness refers to the total number of species present and species evenness how the individuals are distributed among these species. The species richness and evenness can vary independently and needs to be looked at separately. The Margalef species richness and the Pielou species evenness indices were determined for this purpose. The Jyothi *et al* (2016) states that a higher diversity index is indicative of a healthier, usually oligotrophic, ecosystem and that it would be expected that the Margalef species

richness index and the Pielou evenness index will be lower where pollution is higher. Thus it would be expected that the higher the Palmer Index, indicating higher organic pollution that a lower species evenness and richness is to be expected.

This is however not the norm for the Mooi River. The Margalef species richness ranged from 3.95 at Site 5: BKD to 8.42 at Site 4: AWFS (Table 4.2), thus indicating high levels of algal richness present. Considering the algal composition (Figure 4.2) it can be concluded that the algal classes that contributed the most to this richness are; Chlorophyceae, Bacillariophyceae and Cyanophyceae. The species were not only abundant but were also seen to be evenly distributed when considering the Pielou Index scores ranging from 0.41 to 0.83 and seem to relate to the scores obtained by *Booyens* (2013) ranging from 0.47 to 0.72 as well as a study by Ganai & Parveen (2013), which ranged between 0.64 and 0.89.

The high species richness and evenness can be attributed to the presence of multiple Palmer species being resistant to organic pollution (Table 4.3) and that favourable conditions for these genera are present. *Booyens* (2013) also concluded that sites with lower diversity scores and high Palmer scores would be more indicative of organic pollution expressed through tolerance, rather than conditions favouring more genera. Site 3: BWFS has a total of 15 Palmer genera present (Table 4.3) and has the second highest species richness and species evenness scores (Figure 4.4). This was also visible at Site 4: AWFS which had the highest species richness and evenness (Figure 4.4) with a total of 11 Palmer genera. Site 8: EBR has the third highest species richness, 6.78, but the third lowest species evenness, 0.54. It can thus be concluded that the algal richness at this site is dominated by individual genera, most probably the 12 Palmer genera. Once again Site 5: BKD is highlighted in this study as it is the site with both the lowest species richness and species evenness scores (Table 4.2 and Figure 4.4). Due to these low scores, together with the low diversity score for Site 5: BKD coupled with the high Palmer score of 27, it can thus be concluded that the Boskop Dam is experiencing high levels of organic pollution.

The results obtained during the evaluation of the algal community corresponds to the class III classification of the Mooi River, stating that the river is heavily impacted by human activity but is still ecologically sustainable. The current ecological state of the

Mooi River is a D category (largely modified) and the recommended ecological category is also D. This means that the RQO that were implemented are to sustain the Mooi River as is only and not improve.

The Mooi River has further been under constant scrutiny due to the mining pollution activities of the far West Rand and the confluence of the Mooi River with the WFS. It is especially the uranium concentration levels that were of concern. During a study by Winde (2010) he compared the average uranium measured in 1997 to that measured in 2004. When compared to this study no noteworthy change in concentrations were observed. The average uranium concentration for Site 2: KKD showed a slight increase from 0.0054mg/l measured in 2004 to an average of 0.006mg/l, the same is seen for Site 6: PD with an increase from an average of 0.0042mg/l in 2004 to an average of 0.006mg/l. The uranium concentration for Site 5: BKD, where water is abstracted for drinking water, increased from 1997, with an average of 0.0024mg/l to an average of 0.011mg/l in 2004. A decrease in average uranium concentration was seen for Site 5: BKD when compared with the current study with an average current measurement of 0.006mg/l. The 95% percentile for uranium is also below the RQO set numerical limit for the Upper Vaal. It can thus be assumed that the mining activities are better monitored and managed to pose a smaller threat to the Mooi River.

Another variable synonymous with mining pollution is sulphate. Mine pollution originating from the decanting of closed down mine shafts, is acidic mainly due to the presence of sulphuric acid (Durandt, 2012). The dolomite reacts with this acidic inflow, buffering the pH, increasing the amount of dissolved ions, and increasing both the EC and TDS (Usher and Scott, 2000). This reaction can also explain the increase in these variables after the confluence of the WFS at Site 4: AWFS. This also releases sulphate in the underground water resources, eventually resulting in an increased risk of sinkholes (Usher *et al*, 2000).

The reaction for dolomite with sulphuric acid is as follows:

$$2H_2SO_4 + CaCO_3.MgCO_3 \longrightarrow Ca^{2+} + Mg^{2+} + 2H_2O + 2CO_2 + 2SO_4$$

A study performed by Barnard et al (2013) showed a decline in the sulphate concentration from 1995 until 2010. This decline in sulphate concentration is a further indication of the mining pollution being managed. The average sulphate concentration

for this study, 131.75mg/l, is five times less that the 95% percentile RQO set numerical limit for the Upper Vaal of 500mg/l. The Mooi River is an up-stream tributary of the Vaal River which has a much lower RQO numerical limit of 120mg/l. Water is abstracted by Midvaal Water Company for drinking water purposes. It is thus accepted that the Vaal River has a diluting effect on the sulphate concentration.

Even though the sulphate levels are currently not of threat when considering the RQO, the activity of sulphate reducing bacteria may pose a threat to due to the formation of H_2S. Anaerobic respiration of the sulphates occurs in two ways, depending on the energy source (Luptakova, 2007).

(i) Organic energy source: Heterotrophic reduction of sulphates: An organic substance is used as energy source, producing large quantities of gaseous hydrogen sulphide (H_2S). The hydrogen sulphide reacts easily with any heavy metal cations present in the water, forming insoluble sulphides of the given metals.

(ii) Inorganic energy source: Autotrophic reduction of sulphates: SRB's utilises inorganic hydrogen as the energy source, producing sulphide (Luptakova, 2007).

A significant correlation, $p<0.05$, exists between the sulphate concentration seen in Figure 4.5 and the SRB count illustrated in Figure 4.6 (Appendix B). Favourable conditions for SRB and thus the presence of H_2S are present along the Mooi River.

SRB's are classified as a nuisance bacterium because of the rotten egg smell caused by the production of H_2S gas. SRB's can be a problem in the Mooi River as they initiate corrosion processes in metal fittings and react with dissolved metals. SRB's also generate deposits that increase turbidity (Luptakova, 2007). Lith *et al* (2000) found that SRBs also plays a fundamental role in the deposition of dolomite at earth surface temperatures.

The average pH measured for Mooi River when compared with the study of Bezuidenhout (2013) decreased slightly from an average of 8.12 to an average of 7.95 measured during the current study. High photosynthetic activity, as mentioned previously, is usually associated with a higher pH. As the algae takes up the CO_2 during photosynthesis the OH^- ions increases thus increasing the pH (Glasgow &

Hannon, 2004). In hard water the pH increase may lead to $CaCO_3$ precipitation. The mine pollution is expected to contribute to a lower pH. It is suspected that the dolomitic lithology of the Mooi River area has a buffering effect and ensures a pH between 6 and 9. The algal activity may also contribute to the increased pH. The 95% percentile pH for the Mooi River, 8.94, exceeds the RQO numerical limit for the Upper Vaal of 8.8. This pH is acceptable for swimming according to the target water quality guidelines for recreational use (1996), but some eye, skin, ear and mucus membrane irritation is expected.

The magnesium and calcium are found to be abundant in the Mooi River. This is most probably due to the dolomitic lithology of the Mooi River area as well as the contribution of the liming activities occurring in the area upstream from the WFS. In water dolomite dissociates as follows:

$CaCO_3.MgCO_3 + 2H_2CO_3^{+2} \longrightarrow Ca^{2+} + Mg^{2+} + 4HCO_3$ (Usher *et al*: 2000)

It is thus clear that a source of calcium and magnesium can be the dolomitic lithology. Usher and Scott (2000) during is study regarding post mining impacts, found that the average calcium and magnesium concentration for mixed surface water are 47mg/l and 42mg/l respectively. The average calcium concentration 48.36mg/l and the average magnesium concentration 35.83mg/l measured for this study is thus suggestive of mixed surface water of both mine water and dolomitic water. This confirms that it is not only the lithology contributing to these concentrations but also the mine waters. Dolomite has a buffer pH of 5.3 – 6.8 and thus assists with keeping the pH in a desired range of 6 – 8.8 (Usher and Scott, 2000). Factors that would have an influence on lowering the pH are the photosynthetic activity and the acid mine drainage contribution that occurs when water decants from mines.

According to Hubert and Wolkersdorfer (2015) the conversion factor for the estimation of total dissolved solids from the electrical conductivity measurements varies greatly for mine water related samples. This statement is confirmed during this study as no linear regression for electrical conductivity or total dissolved solids exists.

E.coli, a faecal indicator is present in high concentrations in the Mooi River. An average of 828cfu/100ml was detected. These concentrations are less than the *E.coli* concentrations determined for the Schoonspruit and Jagspruit, National Microbial

Monitoring Programme (NMMP) points, that are heavily impacted by sewage pollution with reported *E.coli* concentrations of >242000cfu/100ml. The average concentrations are however higher than those measured for Koekemoerspruit and Vaal River at the Midvaal intake where concentrations less than a100cfu/100ml has been reported. It is assumed that the major sources of faecal pollution for the Mooi River are livestock such as cattle and pigs and sewage effluents from informal settlements. The impact of the waste water treatment effluent on the Mooi River can be seen at Site 7: WWTP. However the maximum *E.coli* concentrations measured for this site were only 26cfu/100ml and it can thus be accepted that that the effluent is sufficiently treated. A study by Soller et al (2010) on the estimated human health risk from the exposure to recreational waters impacted by human and non-human sources of faecal contamination, found that the impact of cattle manure does not substantially differ from those impacted by a human source. It was found that cattle manure were associated with pathogens such as *C. jejuni*, *Cryptosporidium, Giardia* spp, and Shiga-toxin producing *E.coli* strains. Soller et al (2010) did however find that a lower risk for water borne disease exist for water impacted on by chicken ad pig's faeces and even suggested an alternative water quality standard for these faeces effluents.

CHAPTER 6: CONCLUSION

Considering the physico-chemical, phytoplankton and biological analyses it can be concluded that the Mooi River system has high levels of organic pollution with a high faecal pollution load. The nutrient pollution needs intervention as it is rapidly contributing to an eutrophic system.

The phytoplankton classes identified and enumerated suggests that the Mooi River is in a mesotrophic to eutrophic state. The trophic state is further confirmed as Mesotrophic by the mean nitrate and nitrite concentration of 0.877mg/l and the mean orthophosphate concentration of 0.163mg/l determined for the whole Mooi (Water Quality Guidelines Volume 7 (DWS, 1996D)).

Therefore it is suggested that the Mooi River is a productive system with high levels of species diversity. Blooms of nuisance algae can however be expected. Nuisance algae such as *Microcystis* sp. and *Oscillatoria* sp. have already been identified and can contribute to possible taste and odour problems in future as well as the production of cyanotoxins.

The South African Water Quality Guidelines Volume 5 (DWA, 1996c) states that calcium, magnesium, sulphate and nitrate and nitrite can be potentially hazardous when consumed by livestock. These chemical variables are below that of the target water quality objectives and can thus be seen as safe for livestock consumption. However the average *E.coli* counts (828cfu/100ml) determined for the Mooi River system pose a significant risk of infection to young livestock, swine and poultry. The *E.coli* enumerated in the Mooi River system exceeds the Target Water Quality Guidelines for Recreational Use Volume 2 (DWA, 1996a). *E.coli* counts greater than 400cfu/100ml is unfit for full contact recreation and is associated with an elevated health risks.

The Mooi River is mostly abstracted for irrigation purposes. The average pH measured for the Mooi River system is 7.8. This falls within the target water quality guidelines set out in the South African Water Quality Guidelines Volume 4 (DWA,1996b) and does not present major problems. Other variables measured during this study that might have an effect on crop irrigation is; electrical conductivity and uranium. The average

uranium concentrations measured for the Mooi River system falls within the range where the crop yields are unaffected.

Ironically it is the same land use activities that require the management of the Mooi River water quality that contribute to the deterioration thereof.

The implementation of resource quality objectives are thus of need and must be continuously reconsidered and monitored as the quality of the Mooi River changes.

CHAPTER 7: REFERENCES

ANNENDALE, E. & NEALER, E. 2011. Exploring aspects of the water history of the Potchefstroom region and the local management of it. *New Centree*, 62: 111-124.

ANONYMOUS. 2012. Radioactivity Monitoring Programme: selection of catchment. http://www.dwaf.gov.za/iwqs/radioact/mooi/summary_2.htm
Date of access: 20 October 2013

ASLAM, M. 2009. Diversity, species richness and evenness of moth fauna of Peshawar. *Pakistan Journal of Entomology*, 31(2): 99-102.

BARNARD, S., VENTER, A. & VAN GINKEL, CE. 2013. Overview of the confluences of mining-related pollution on the water quality of the Mooi River system's reservoirs, using basic statistical analyses and self-organised mapping. *WaterSA* 39(5): 655-662

BELLINGER, E.G.& SIGEE, D.C. 2010. Fresh Water Algae: Identification and Use as Bioindicators. John Wiley and Sons. p99-134.

BEZUIDENHOUT, C. 2013. A large scale study of microbial and physico-chemical quality of selected groundwater in the North West province, South Africa. *Water Research commission*. (Report No. 1966/1/13) p25-27

BISHOP, M. 2015. Nuisance algae characterization and management. *SePRO: Journal of Aquatic Plant Management* p76-79

BOOYENS, S. 2015. Water Quality Assessment of the Koekemoerspruit: Integrating water physico-chemistry and phytoplankton assemblages. Potchefstroom: NWU. Thesis submitted in fulfilment of the degree Magister Scientiae in Environmental Sciences at Potchefstroom Campus od the North-West University. Potchefstroom, South Africa. p47-49, p 74-96

BUTLIN, R., ADAMS, M.E. & THOMAS, M. 1948. The Isolation and Cultivation of Sulphate Reducing Bacteria. *Chemical Research Laboratory, Department of Scientific and Industrial Research:* p46-59.

COETZEE, H., WINDE, F. & WADE, P. 2006. An assessment of sources, pathways, mechanisms and risks of current and potential future pollution of water and sediments in gold mining areas of the Wonderfonteinspruit catchment. *Water Research Commission.* (Report No. 1214/1/06) p 1-5, p13-16

CROASDALE, H. & FLINT, E.A. 1986. Flora of New Zealand: Desmids. Vol I. Wellington, New Zealand: V.R. Ward, Government Printer

CROASDALE, H. & FLINT, E.A. 1988. Flora of New Zealand: Desmids. Vol II. Christchurch, New Zealand: DSIR, Botany Division.

CROASDALE, H., FLINT, E.A. & RACINE, M.M. 1994. Flora of New Zealand: Desmids. Vol III. Canterbury, New Zealand: Manaaki Whenua Press, Land care Research.

DALLAS, H.F. & DAY, J.A. 2004. The effect of water quality variables on aquatic ecosystems: A review. *Water Research Commission.* (Report No. TT 224/04) p29-94

DE MEYER, A. & NORTJE, J.P. 2013. Land cover classification and hydrological modelling of the Mooirivier and Koekemoerspruit catchment areas using geographic information system (GIS) techniques to determine the influence of mining activities. Potchefstroom: NWU. (Report – Hons.BSc).

DICKENS, C., PRINGLE, C. & MACFARLANE, D. 2011. Procedures to Develop and Implement Resource Quality Objectives. Department of Water Affairs, Pretoria, South Africa. p12 -42

DURANDT, J.F. 2012. The impact of gold mining on the Witwatersrand on the rivers and karst system of Gauteng and North West Province, South Africa. *Journal of African Science* 68: 24 - 43

DWA (Department of Water Affairs). 1996a. South African Water Quality Guidelines: Recreational use. 2nd ed. (Report Volume 2) p37, p75

DWA (Department of Water Affairs). 1996b. South African Water Quality Guidelines: Agricultural use - irrigation. 2nd ed. (Report Volume 4) p95, p105, p127, p165

DWA (Department of Water Affairs). 1996c. South African Water Quality Guidelines: Agricultural use – Livestock watering. 2nd ed. (Report Volume 5) p109, p115

DWA (Department of Water Affairs). 1996d. South African Water Quality Guidelines: Aquatic Ecosystems. 2nd ed. (Report Volume 7) p79, p123

DWA (Department of Water Affairs). 2016. Classes and Resource Quality Objectives for the Upper Vaal Catchment. Government Gazette No. 39943.

ENTWISLE, T.J., SONNEMAN, J.A. & LEWIS, S.H. 1996. Freshwater Algae in Australia. Potts Point: Sainty & Associates.

FEDOR, P.J. & SPELLERBERG, I.J. 2013. Shannon-Wiener Index. *Earth Systems and Environmental Sciences*: 1- 4.

GANAI, A.H. & PARVEEN, S. 2013. Effect of physico-chemical conditions on the structure and composition of the phytoplankton community in Wular Lake at Lankrishipora, Kashmir. *International Journal of Biodiversity and Conservation* 6(1): 71-84

GASSERT, F., REIG, P., LUO, T., MADDOCKS, A. 2013. Aqueduct Country and River Basin Rankings - A Weighted Aggregation of Spatially Distinct Hydrological Indicators. http://www.wri.org/publication/aqueduct-country-river-basin-rankings Date of access: 19 November 2015

GELL, P.A., SONNEMAN, J.A., REID, M.A., ILLMAN, M.A. & SINCOCK, A.J. 1999. An illustrated key to common diatom genera from southern Australia. Identification Guide no. 26. Albury: Cooperative Research Centre for Freshwater Ecology

GLASGOW, H. & HANNON, E. 2004. Removal strategies for drinking water treatment plants. AWWA Research Foundation United States of America. P26

GRUNDLING, G., RETIEF, R.F. 2005. Peatlands and Mires of South Africa. *Neue Serie* 35: 379-396

GUIRY, M.D., RINDI, J.F. & McCARTHY, T.K. 2007. New survey of Clare Island: The freshwater and terrestrial algae. Vol 6. Dublin: Royal Irish Academy.

HINDÁK, F. 2008. Colour atlas of cyanophytes. Bratislava: VEDA, Publishing House of the Slovak Academy of Science.

HUBERT, E., WOLKERSDORFER, C. 2015. Establishing a conversion factor between electrical conductivity and total dissolved solids in South African mine waters. *Water SA,* 41(4): 490 - 500.

JANSE VAN VUUREN, S., TAYLOR, J., GERBER, A. & VAN GINKEL, C. 2006. Easy identification of the most common freshwater algae. North-West University and Department of Water Affairs and Forestry, Pretoria, South Africa.

JOHN, D.M., WHITTON, B.A. & BROOK, A.J. 2002. The Freshwater Algal Flora of the British Isles: An Identification Guide to Freshwater and Terrestrial Algae. Cambridge: Cambridge University Press.

JOSKA, M.A. & BOLTON, J.J . 1993. Guide to common filamentous freshwater macroalgae in South Africa. *Water Research Commission* (WRC Report TT66/94).

JYOTI, V., PRAKASH, N., PRATEEK, S. 2016. Diversity of Diatoms in the Rivers Of Bundelkhand Plateau: A Multivariate Approach For Floral Patterns. *International Journal of Geology, Earth & Environmental Sciences* 6 (1): 66-77

KRHIRSAGAR, A.D. 2013. Diversity of aquatic macrophytes from River Mula Pune City, MS, India. *Science Research Reporter* 3(1):09-14

LAD, D. 2015. Analysis of Pollution Status of Wetland Areas using Various Species Diversity Index. *Scholars Academic and Scientific Publisher* 3(7):616-617

LITH, Y., VASCONCELOS, C., WARTHMANN, R. & MCKENZIE, J. 2000. Role of Sulfate Reducing Bacteria During Microbial Dolomite Precipitation as Deduced from Culture Experiments. *Journal of Conference Abstracts* 5(2): 1038.

LUND, J.W.G., KIPLING, C. & LE CREN, E.D. 1958. The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. *Hydrobiologia,* 11**:** 143-170.

LUPTAKOVA, A. 2007. Importance of Sulphate Reducing Bacteria in the environment. Nova Biotechnologies VII - I: 17 – 22

MCDONALD, M. 2014. Business Case for the Establishment of the Vaal River CMA. Department of Water and Sanitation. p57 - 69

MIDVAAL WATER COMPANY. 2008. Phew! The water stinks! Klerksdorp Record: 14, 1 February. p40

PALMER, C.M. 1969. A composite rating of algae tolerating organic pollution. *Journal of Phycology*, (5): 78-82.

PARUCH, A. 2010. Mikrobiologiske analyser (E.coli). http://www.bioforsk.no/ikbViewer/page/fagomrader/fagomrade/omrade/tema/artikkel?p_dimension_id=15097&p_document_id=65347
Date of access: 15 October 2015

PRESCOTT, G.W. 1983. How to know the freshwater algae. 3rd ed. Dubuque: The Picture Key Nature Series. Prescott, Harley, and Klein's microbiology. 7th ed. New York: McGraw-Hill Higher Education.

RIJSBERMAN, F. 2004. Water Scarcity: Fact or Fiction. *Water Science and Technology* 47(6): 53-62

SERAGELDIN, I. 1995. World Bank Vice President for Environmental Affairs. https://www.canneslions.com/resources/downloads/Young%20Lions%20Media/2011/Austria.pdf Date of access: 15 January 2016

SOLLER, J.A., SCHOEN, M.E, BARTRAND, T., RAVENSCROFT J.E., ASHBOLT, & N.J. 2010. Estimated human health risks from exposure to recreational waters impacted by human and non-human sources of faecal contamination. *Water Research*: 1- 18.

TAYLOR, J.C., HARDING, W.R. & ARCHIBALD, C.G.M. 2007. An illustrated guide to some common diatom species from South Africa. *Water Research Commission* (WRC Report TT282/07).

THELWELL, E. 2014. South Africa's looming water crisis - News 24. http://www.news24.com/SouthAfrica/News/South-Africas-looming-water-disaster-20141103 Date of access: 19 November 2015

USHER, B. & SCHOTT, R. 2000. Post-mining Impacts of Gold Mining on the West Rand and West Wits Line: *Hydro chemistry and observations*. 5: 33- 55

UTERMÖHL, H. 1931. Über das umgekehrte Mikroskop. *Archiv für Hydrobiologie und Plankton,* 22: 643-645.

VAN DER WALT, IJ., WINDE F. & NELL B. 2002. Integrated catchment management: the Mooi River (Northwest Province, South Africa). A case study. *Cuadernos de Investigacion Geografica* 28:109-126

VENTER, A., BARNARD, S., DICKINSON, M.A., JANSE VAN VUURES, S., LEVANETS, A. & TAYLOR, J.C. 2013. Planktonic algae and cyanoprokaryotes as indicators of ecosystem quality in the Mooi River system in the North-West Province, South Africa. *Water SA*, 39(5): 707-719.

WEHR, J.D. & SHEATH, R.G. 2002. Freshwater Algae of North America: Ecology and Classification. 1st ed. Armonk, NY: Academic Press.

WETZEL, R.G. 2001. Limnology Lake and River Ecosystems 3rd Edition. Academic Press, San Francisco. p332 -390

WILLEY, J. M., SHERWOOD, L., WOOLVERTON, C. J., & PRESCOTT, L. M. 2008. Prescott, Harley, and Klein's microbiology. 7th ed. New York: McGraw-Hill Higher Education.

WINDE, F. 2010. Uranium pollution of the Wonderfonteinspruit: 1997-2008. Part 1: Uranium in water – concentrations, loads and associated risks. *WaterSA* 36 (3): 239 – 256.

WINDE, F. 2011. Peatlands as filters for polluted mine water? – A case study from uranium- contaminated karst system in South Africa Part VI: Quantifying the chemical filter component. Water 3: 391-423

WINDE, F. & VAN DER WALT, I.J. 2004. The significance of groundwater–stream interactions and fluctuating stream chemistry on waterborne uranium contamination of streams—a case study from a gold mining site in South Africa. *Journal of Hydrology*, 287:178–196.

WORLD WILDLIFE FUND. 2016. Freshwater- threats. https://www.worldwildlife.org/habitats/freshwaters Date of access: 15 January 2016

RIJSBERMAN, F. 2004. Water Scarcity: Fact or Fiction. *Water Science and Technology* 47(6): 53-62

APPENDIX A

Sequencing of the 16SrDNA gene via PCR amplification was attempted in order to identify the sulphate reducing bacteria present in the surface water of the Mooi River.

Sulphate reducing bacteria (SRB) are a group of chemo-organotrophic and strictly anaerobic bacteria that may be divided into four groups based on rRNA sequence analyses: Gram-negative mesophilic SRB, Gram-positive spore forming SRB, thermophilic bacterial SRB and thermophilic archaeal SRB (Luptakova, 2007). Common SRB genera found in aquatic environments include Desulfovibrio, Desulfomicrobium, Desulfobacter, Desulfosarcin*a, Desulfotomacullum, Thermodesulfobacterium, Archaeoglobus, Thiobacillus, Sulfolobus, Thiospira,* and *Thiobacterium* (Luptakova, 2007).

As mentioned in Chapter 5, SRBs can utilise either organic or inorganic food sources. Thus by identifying the SRB one can gain knowledge on the state of the water body.

2. MATERIALS AND METHODS

2.1 SAMPLING

Refer to chapter 3.1.2: MICROBIOLOGICAL SAMPLING

2.2 CULTURING OF SINGLE COLONIES

A pour plate method using Merck Sulphite Iron Agar with 20ml 7% Iron sulphate per one litre (1Litre) of agar was used. A dilution range of zero to a 10^{-4} dilution of each water sample was made and one millilitre aseptically pipetted into a sterile petri dish. Cooled agar was then poured into each petri dish, gently swirling the plate and leaving it to solidify. Plates were inverted and placed in an anaerobic jar with an AnaeroPack-Anaero sachet. The AnaeroPack-Anaero sachet absorbs the oxygen and generates carbon dioxide, creating an anaerobic environment. Plates were then incubated at 35°C for 2-4 days.

Difco plate count broth (18.5g) was suspended in 1 litre distilled water and autoclaved. The pH was adjusted to 7±0.2. Single black colonies were then transferred to a test

tube containing 10 ml Difco plate count broth with 2ml 7% iron sulphate solution. Latex gloves were used when handling plates after incubation. Test tubes were then incubated in an anaerobic container with AnaeroPack-Anaero sachets at 35°C for another 48 hours.

After 48 hours the test tubes with growth was centrifuged at 3000rpm (Sigma 4-16S) for 5 minutes, forming a pellet. This pellet was then washed twice with 1/4 strength Ringers solution (Merck) at 2500rpm for 3 minutes. The wash step is to ensure that the broth and iron solution is removed. Ringers solution was used to in order to avoid that the bacterial cells go into osmotic shock.

2.3 DNA ISOLATION

During this study several DNA isolation methods were attempted.

2.3.1 MICROWAVE METHOD (Butlin *et al*, 1948)

The microwave method was first used to isolate the DNA from the cells. A washed pellet (see section 2.2) was suspended in 20µl PCR grade water in a 1.5ml micro-centrifuge tube and briefly centrifuged in a desktop centrifuge. The micro-centrifuge tube was subsequently placed in the microwave oven for two minutes, at 700W. After microwaving the sample was centrifuged again for two minutes at 13400rpm. The supernatant was kept and the pellet with impurities was discarded. The DNA was present in the supernatant and used directly for PCR.

2.3.2 DNA ISOLATION KITS

Both the Macherey Nagel Nucleo spin kit and the Qiagen DNeasy blood and tissue kit were also used for DNA isolation according to the manufacturers' instructions. During the use of these kits the bacterial pellet was taken as is. The supernatant obtained from the microwave method was also used to try and optimised the method to insure cell breakage.

2.3.3 CTAB METHOD

The cell pellet was suspended in a 200µl solution of 50mM Tris-HCl, (pH 8.0) and 50mM EDTA. After suspension, the cells were treated with proteinase K at a final concentration of 100µg/ml at 55°C for 10 minutes before addition of the extraction buffer. Then 600µl of pre-warmed (55°C) extraction buffer (3% (w/v) cetyltrimethyl ammonium bromide (CTAB), 1% (W/v) sarkosyl, 20mM EDTA, 1.4M NaCl, 0.1M Tris HCl , pH8, 0 1% (v/v) 2-mercaptoethanol, freshly prepared) were added and incubated at 55°C in a water bath for a further 30 minutes with gentle inversion every 5-10 minutes. The mixture was mixed by gentle inversion (30 times) until an emulsion formed. After centrifugation (12000 x g for 5 minutes at 25°C), the supernatant was transferred to a sterile microcentrifuge, 2 volumes of 4 M NaCl were added and the solution mixed by gentle inversion. Equal volumes of isopropanol was then added to the mixture and incubated for 1 hour at room temperature to precipitate the DNA. The mixture was centrifuged for 20 minutes at 12000 *g* and the supernatant discarded. One millilitre 70% (v/v) ethanol was added and the DNA was centrifuged for a further 10 minutes at 12000 *g*. The supernatant was again removed and the ethanol wash step was repeated with absolute (96% v/v) ethanol. The pellet was then air-dried in a desiccator with silica gel crystals. The DNA-pellet was resuspended in 500µl of TE buffer (10mMTRIS-HCl, pH 8.0, 1mM EDTA). A final concentration of 750mM ammonium acetate was added to the DNA containing solution followed by a chloroform extraction using equal volumes of chloroform. After centrifugation (5 minutes at 12 000x g) the DNA in the supernatant was precipitated overnight with 1ml of absolute ethanol and then washed twice with 70% (v/v) ethanol. The DNA was subsequently dried and dissolved in TE buffer (10mMTris-HCl, pH8.0, 1mM EDTA).

After each isolation method the quality and concentration of the DNA sample was determined using the Nanodrop-1000 spectophotometer.

2.4 16S rRNA PCR AMPLIFICATION

Primers used during this study are: the forward primer, 16SUF: 5'-AGA GTT TGA TCM TGG CTC AG-3' Tm: 53.2°C and the reverse primer, 16SUR: 5' – TAC GGY TAC CTT GTT AGG AC -3' Tm 52.9°C.

PCR amplification was performed in a 25µl (total volume) reaction mixture containing 12.5µl KAPPA Taq ready mix with Mg^{2+}, 0.8µl forward primer (1µM), 0.8µl reverse primer (1µM), DNA template as required (100ng/µl) and water to fill up to 25µl.

Amplification was carried out with a Thermal cycler and the following cycling parameters were used: Initial denaturation for 5 minutes at 95°C, followed by 30 cycles of 30 seconds at 95°C, 30 seconds at annealing temperature gradient ranging from 50°C to 53°C and 1 minute at 72°C, followed by a final elongation step of 5 minutes at 72°C.

After amplification the sample was loaded onto a 1.5% agarose gel stained with Gel Red and Orange DNA loading dye. A 1kb DNA ladder was used to determine the size of the DNA fragments.

OPTIMISATION

During this study several possibilities were considered for optimisation. To enhance the PCR reaction 5% DMSO was added as well as 1,5 mM $MgCl_2$. The annealing temperature was also adjusted over a range of 48°C to 55°C also.

3. Results:

3.1 SRB colonies

Single black colonies were visible after the dilution range (see Fig. 3.3).

Bacterial growth was visible in the test tubes. A distinct cream colour pellet was present after the cells were centrifuged and washed.

3.2 DNA isolation

The absorbance ratio at 260nm/280nm of pure double stranded DNA is between 1.8 and 1.9 and for RNA it is 2.0. The spectrophotometric results showed that the quality of the DNA isolated (A_{260}/A_{280}) was low and that contamination was present.

The results for the microwave method can be seen in Figure A.1. From all the methods used, the highest concentration of DNA was isolated with the microwave method.

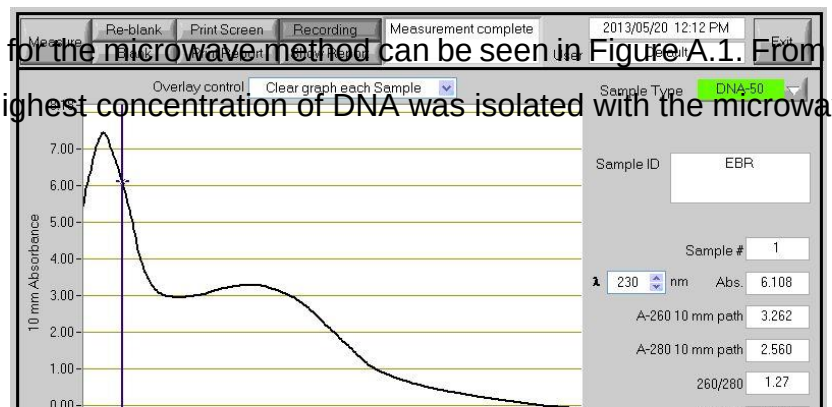


Figure A.1: Absorption spectrum of the DNA sample obtained from the microwave isolation method. High concentration of DNA was obtained but the A_{260}/A_{280} ratio was low.

The results obtained for the Macherey Nagel Nucleo spin kit is depicted in Figure A.2. A concentration of 2.5ng/µl was isolated. The graph shows that it is of low quality.

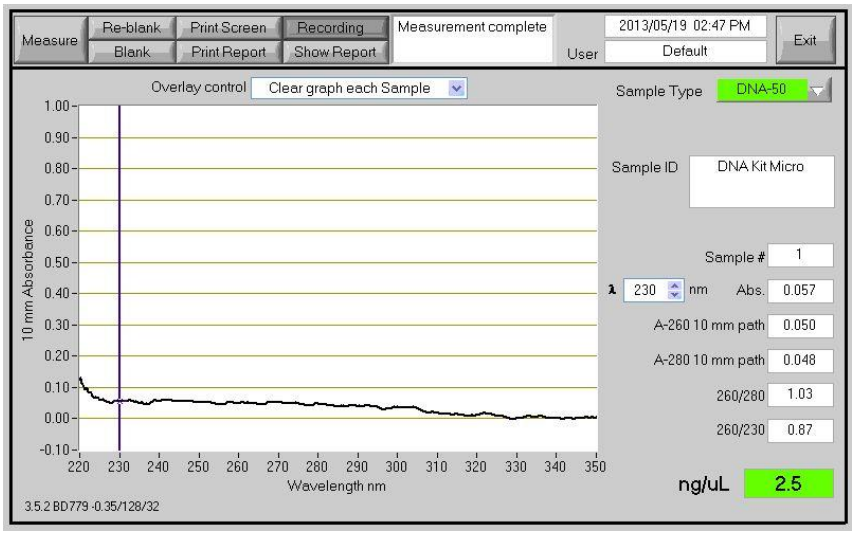


Figure A.2: Absorption spectrum of the DNA sample obtained from the the Macherey Nagel Nucleo spin kit isolation method. Low concentration of DNA was obtained and the A_{260}/A_{280} ratio was low.

The results obtained for the Qiagen DNeasy blood and tissue kit was similar to that obtained by the Macherey Nagel Nucleo spin kit with a yield of 3.7ng/l DNA. (Figure A.3)

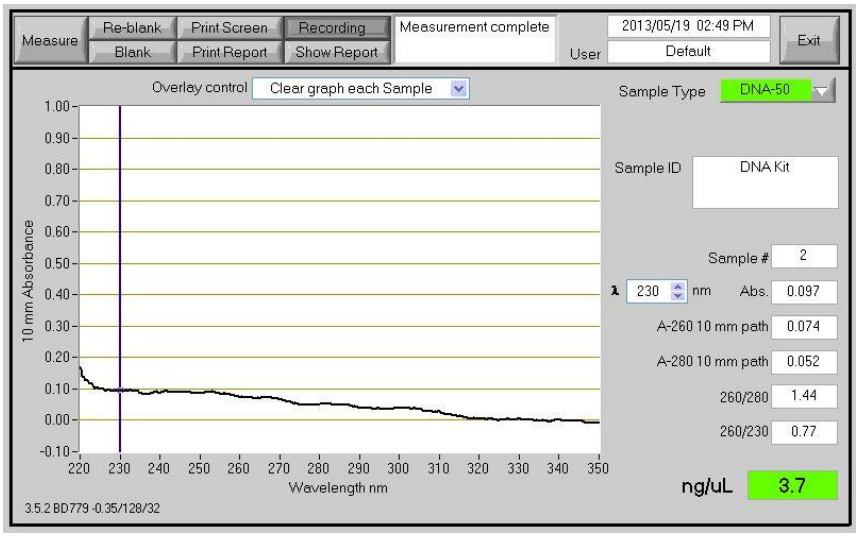


Figure A.3: Absorption spectrum of the DNA sample obtained from the Qiagen DNeasy blood and tissue kit isolation method. Low concentration of DNA was obtained and the A_{260}/A_{280} ratio was low.

3.3 PCR results

Unfortunately no PCR results were obtained for the SRB samples. Primer Dimers were however visible with each run. The set out objective to classify the SRB growth present was thus not reached.

APPENDIX B

Kruskal-Wallis multiple comparisons of p-values (2 tailed) that indicate significant variable differences ($p<0.05$) between all sites from 2014 to 2015. ***Note that the Rank values coloured in red indicate significant differences***

±SE (Standard Error); ±SD (Standard Deviation); Site1: BVO, Site 2: KKD, Site 3: BWFS, Site 4: AWFS, Site 5: BKD, Site 6: PD, Site 7: WWTP, Site 8: EBR

VARIABLE	SITE							
Calcium	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1		1,000000	1,000000	0,000001	1,000000	1,000000	0,021440	0,003651
2	1,000000		1,000000	0,000000	0,073702	0,082092	0,000269	0,000026
3	1,000000	1,000000		0,000001	1,000000	1,000000	0,021624	0,003599
4	0,000001	0,000000	0,000001		0,011059	0,009756	1,000000	1,000000
5	1,000000	0,073702	1,000000	0,011059		1,000000	1,000000	1,000000
6	1,000000	0,082092	1,000000	0,009756	1,000000		1,000000	1,000000
7	0,021440	0,000269	0,021624	1,000000	1,000000	1,000000		1,000000
8	0,003651	0,000026	0,003599	1,000000	1,000000	1,000000	1,000000	
Magnesium	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1		1,000000	1,000000	0,000076	0,000002	0,000000	0,000000	0,000000
2	1,000000		1,000000	0,018475	0,000939	0,000044	0,000038	0,000004
3	1,000000	1,000000		0,095857	0,006815	0,000428	0,000365	0,000050
4	0,000076	0,018475	0,095857		1,000000	1,000000	1,000000	1,000000
5	0,000002	0,000939	0,006815	1,000000		1,000000	1,000000	1,000000
6	0,000000	0,000044	0,000428	1,000000	1,000000		1,000000	1,000000
7	0,000000	0,000038	0,000365	1,000000	1,000000	1,000000		1,000000
8	0,000000	0,000004	0,000050	1,000000	1,000000	1,000000	1,000000	
Nitrate & Nitrite	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1		0,000000	0,000000	1,000000	0,000002	0,000000	1,000000	1,000000
2	0,000000		1,000000	0,000042	1,000000	1,000000	0,000000	0,000071
3	0,000000	1,000000		0,000042	1,000000	1,000000	0,000000	0,000071
4	1,000000	0,000042	0,000042		0,002082	0,000525	1,000000	1,000000
5	0,000002	1,000000	1,000000	0,002082		1,000000	0,000008	0,003220
6	0,000000	1,000000	1,000000	0,000525	1,000000		0,000001	0,000838
7	1,000000	0,000000	0,000000	1,000000	0,000008	0,000001		1,000000
8	1,000000	0,000071	0,000071	1,000000	0,003220	0,000838	1,000000	

APPENDIX B: Continued

Phosphate	1	2	3	4	5	6	7	8
1		1,000000	1,000000	1,000000	1,000000	1,000000	0,000012	0,000006
2	1,000000		1,000000	1,000000	1,000000	1,000000	0,000004	0,000002
3	1,000000	1,000000		1,000000	1,000000	1,000000	0,000014	0,000007
4	1,000000	1,000000	1,000000		1,000000	1,000000	0,000004	0,000002
5	1,000000	1,000000	1,000000	1,000000		1,000000	0,000004	0,000002
6	1,000000	1,000000	1,000000	1,000000	1,000000		0,000078	0,000041
7	0,000012	0,000004	0,000014	0,000004	0,000004	0,000078		1,000000
8	0,000006	0,000002	0,000007	0,000002	0,000002	0,000041	1,000000	
Sulphate	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1		1,000000	1,000000	0,000001	0,000000	0,000577	0,000000	0,000000
2	1,000000		1,000000	0,000012	0,000002	0,003556	0,000000	0,000000
3	1,000000	1,000000		0,000037	0,000006	0,008549	0,000000	0,000000
4	0,000001	0,000012	0,000037		1,000000	1,000000	1,000000	1,000000
5	0,000000	0,000002	0,000006	1,000000		1,000000	1,000000	1,000000
6	0,000577	0,003556	0,008549	1,000000	1,000000		0,112122	0,083708
7	0,000000	0,000000	0,000000	1,000000	1,000000	0,112122		1,000000
8	0,000000	0,000000	0,000000	1,000000	1,000000	0,083708	1,000000	
Chl a	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1		1,000000	1,000000	1,000000	0,505514	0,036354	0,135254	0,462784
2	1,000000		1,000000	1,000000	1,000000	1,000000	1,000000	1,000000
3	1,000000	1,000000		1,000000	1,000000	0,923420	1,000000	1,000000
4	1,000000	1,000000	1,000000		1,000000	0,114372	0,375865	1,000000
5	0,505514	1,000000	1,000000	1,000000		1,000000	1,000000	1,000000
6	0,036354	1,000000	0,923420	0,114372	1,000000		1,000000	1,000000
7	0,135254	1,000000	1,000000	0,375865	1,000000	1,000000		1,000000
8	0,462784	1,000000	1,000000	1,000000	1,000000	1,000000	1,000000	
pH	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1		0,005321	0,000326	0,038348	0,000000	0,000000	0,007616	0,000001
2	0,005321		1,000000	1,000000	0,252648	1,000000	1,000000	1,000000
3	0,000326	1,000000		1,000000	1,000000	1,000000	1,000000	1,000000
4	0,038348	1,000000	1,000000		0,045971	0,236543	1,000000	0,429665
5	0,000000	0,252648	1,000000	0,045971		1,000000	0,239176	1,000000
6	0,000000	1,000000	1,000000	0,236543	1,000000		0,949511	1,000000
7	0,007616	1,000000	1,000000	1,000000	0,239176	0,949511		1,000000
8	0,000001	1,000000	1,000000	0,429665	1,000000	1,000000	1,000000	

APPENDIX B: Continued

Electrical conductivity	1	2	3	4	5	6	7	8
1		1,000000	1,000000	0,000348	0,003991	0,000109	0,000000	0,000000
2	1,000000		1,000000	0,034219	0,218433	0,013854	0,000024	0,000000
3	1,000000	1,000000		0,000159	0,002052	0,000047	0,000000	0,000000
4	0,000348	0,034219	0,000159		1,000000	1,000000	1,000000	0,341481
5	0,003991	0,218433	0,002052	1,000000		1,000000	0,612152	0,058050
6	0,000109	0,013854	0,000047	1,000000	1,000000		1,000000	0,673041
7	0,000000	0,000024	0,000000	1,000000	0,612152	1,000000		1,000000
8	0,000000	0,000000	0,000000	0,341481	0,058050	0,673041	1,000000	
TDS	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1		1,000000	1,000000	0,000241	0,002346	0,000421	0,000000	0,000000
2	1,000000		1,000000	0,000564	0,005133	0,000970	0,000000	0,000000
3	1,000000	1,000000		0,000514	0,004722	0,000886	0,000000	0,000000
4	0,000241	0,000564	0,000514		1,000000	1,000000	1,000000	0,390603
5	0,002346	0,005133	0,004722	1,000000		1,000000	1,000000	0,080504
6	0,000421	0,000970	0,000886	1,000000	1,000000		1,000000	0,275643
7	0,000000	0,000000	0,000000	1,000000	1,000000	1,000000		1,000000
8	0,000000	0,000000	0,000000	0,390603	0,080504	0,275643	1,000000	
NTU	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1		0,000008	0,000001	0,178781	1,000000	0,000000	0,000000	0,000000
2	0,000008		1,000000	0,424382	0,006060	1,000000	0,034364	0,000717
3	0,000001	1,000000		0,145216	0,001341	1,000000	0,114881	0,003407
4	0,178781	0,424382	0,145216		1,000000	0,008210	0,000000	0,000000
5	1,000000	0,006060	0,001341	1,000000		0,000028	0,000000	0,000000
6	0,000000	1,000000	1,000000	0,008210	0,000028		1,000000	0,071553
7	0,000000	0,034364	0,114881	0,000000	0,000000	1,000000		1,000000
8	0,000000	0,000717	0,003407	0,000000	0,000000	0,071553	1,000000	
Alkalinity	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1		1,000000	0,000006	0,156935	1,000000	0,145814	0,022554	0,000004
2	1,000000		0,026883	1,000000	1,000000	1,000000	1,000000	0,018380
3	0,000006	0,026883		0,418160	0,018866	0,530787	1,000000	1,000000
4	0,156935	1,000000	0,418160		1,000000	1,000000	1,000000	0,301782
5	1,000000	1,000000	0,018866	1,000000		1,000000	1,000000	0,012832
6	0,145814	1,000000	0,530787	1,000000	1,000000		1,000000	0,386591
7	0,022554	1,000000	1,000000	1,000000	1,000000	1,000000		1,000000
8	0,000004	0,018380	1,000000	0,301782	0,012832	0,386591	1,000000	

APPENDIX B: Continued

E.coli	1	2	3	4	5	6	7	8
1		1,000000	0,000001	0,000000	0,319246	0,006901	1,000000	0,000000
2	1,000000		0,000028	0,000000	1,000000	0,062266	0,414460	0,000000
3	0,000001	0,000028		1,000000	0,080112	1,000000	0,000000	0,241806
4	0,000000	0,000000	1,000000		0,001624	0,114372	0,000000	1,000000
5	0,319246	1,000000	0,080112	0,001624		1,000000	0,000429	0,000001
6	0,006901	0,062266	1,000000	0,114372	1,000000		0,000001	0,000230
7	1,000000	0,414460	0,000000	0,000000	0,000429	0,000001		0,000000
8	0,000000	0,000000	0,241806	1,000000	0,000001	0,000230	0,000000	
T.coli	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1		1,000000	0,000073	0,000073	0,000073	0,000200	0,000095	0,000073
2	1,000000		0,000000	0,000000	0,000000	0,000001	0,000001	0,000000
3	0,000073	0,000000		1,000000	1,000000	1,000000	1,000000	1,000000
4	0,000073	0,000000	1,000000		1,000000	1,000000	1,000000	1,000000
5	0,000073	0,000000	1,000000	1,000000		1,000000	1,000000	1,000000
6	0,000200	0,000001	1,000000	1,000000	1,000000		1,000000	1,000000
7	0,000095	0,000001	1,000000	1,000000	1,000000	1,000000		1,000000
8	0,000073	0,000000	1,000000	1,000000	1,000000	1,000000	1,000000	
SRB	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1		1,000000	0,857217	0,000000	0,000125	0,000004	0,000000	0,000000
2	1,000000		1,000000	0,000000	0,001003	0,000042	0,000001	0,000000
3	0,857217	1,000000		0,000036	0,393871	0,048134	0,002427	0,000179
4	0,000000	0,000000	0,000036		0,475985	1,000000	1,000000	1,000000
5	0,000125	0,001003	0,393871	0,475985		1,000000	1,000000	1,000000
6	0,000004	0,000042	0,048134	1,000000	1,000000		1,000000	1,000000
7	0,000000	0,000001	0,002427	1,000000	1,000000	1,000000		1,000000
8	0,000000	0,000000	0,000179	1,000000	1,000000	1,000000	1,000000	

APPENDIX C

Site 2 KKD	N	pi	ln(pi)	pi* ln(pi)
Ana	196,63	0,00569	-5,169	-0,0294
Aph	1402,3	0,040581	-3,204	-0,13
Apha	365,09	0,010565	-4,55	-0,0481
Gei	286,23	0,008283	-4,794	-0,0397
Lep	44,15	0,001278	-6,663	-0,0085
Mer	229,08	0,006629	-5,016	-0,0333
Mic	2262	0,065459	-2,726	-0,1785
Pho	1080,1	0,031257	-3,466	-0,1083
Pse	598,13	0,017309	-4,057	-0,0702
Syne	86,569	0,002505	-5,989	-0,015
Aul	794,45	0,02299	-3,773	-0,0867
Coc	13,493	0,00039	-7,848	-0,0031
Cra	6,7465	0,000195	-8,541	-0,0017
Cymb	66,668	0,001929	-6,251	-0,0121
Diad	1,6	4,63E-05	-9,98	-0,0005
Eun	53,184	0,001539	-6,477	-0,01
Frag	35,75	0,001035	-6,874	-0,0071
Fru	11,917	0,000345	-7,972	-0,0027
Gom	33,263	0,000963	-6,946	-0,0067
Mel	65,958	0,001909	-6,261	-0,012
Nav	51,194	0,001481	-6,515	-0,0097
Nit	191,59	0,005544	-5,195	-0,0288
Pen	42,35	0,001226	-6,704	-0,0082
Pin	32,556	0,000942	-6,967	-0,0066
Rhop	11,917	0,000345	-7,972	-0,0027
Sen	170	0,00492	-5,315	-0,0261
Sta	1826	0,052843	-2,94	-0,1554
Anki	51,241	0,001483	-6,514	-0,0097
Car	20,33	0,000588	-7,438	-0,0044
Chla	84,417	0,002443	-6,015	-0,0147
Chlo	177,75	0,005144	-5,27	-0,0271
Chloro	236,65	0,006848	-4,984	-0,0341
Clo	14,3	0,000414	-7,79	-0,0032
Coe	95,333	0,002759	-5,893	-0,0163
Cos	107,25	0,003104	-5,775	-0,0179
Cruc	192,67	0,005576	-5,189	-0,0289
Cruci	39,466	0,001142	-6,775	-0,0077
Des	84,391	0,002442	-6,015	-0,0147
Dic	548,17	0,015863	-4,144	-0,0657
Ela	133,26	0,003856	-5,558	-0,0214
Gon	29,792	0,000862	-7,056	-0,0061
Mon	47,608	0,001378	-6,587	-0,0091
Mou	226,42	0,006552	-5,028	-0,0329
Nep	49,267	0,001426	-6,553	-0,0093
Ooc	501,21	0,014504	-4,233	-0,0614
Ped	138,23	0,004	-5,521	-0,0221
Sce	287,2	0,008311	-4,79	-0,0398
Stau	144,03	0,004168	-5,48	-0,0228
Tet	25,033	0,000724	-7,23	-0,0052
Cry	189,95	0,005497	-5,204	-0,0286
Din	20889	0,604501	-0,503	-0,3043
Per	0,3333	9,65E-06	-11,55	-0,0001
Peri	2,6667	7,72E-05	-9,47	-0,0007
Eug	113,08	0,003272	-5,722	-0,0187
Tra	167,76	0,004855	-5,328	-0,0259
	34556	1		-1,894

Site 3 BWFS	N	pi	ln(pi)	pi* ln(pi)
Ana	5,3333	0,0021	-6,168	-0,0129
Aph	23,833	0,0094	-4,671	-0,0437
Lep	9,3333	0,0037	-5,608	-0,0206
Mer	309,44	0,1216	-2,107	-0,2562
Osc	48	0,0189	-3,971	-0,0749
Pho	15,833	0,0062	-5,08	-0,0316
Pse	21,667	0,0085	-4,766	-0,0406
Syn	24,5	0,0096	-4,643	-0,0447
Syne	25,5	0,01	-4,603	-0,0461
Ach	79,625	0,0313	-3,465	-0,1084
Ancn	5,9583	0,0023	-6,057	-0,0142
Amp	5	0,002	-6,233	-0,0122
Aul	12,667	0,005	-5,303	-0,0264
Coc	41,609	0,0163	-4,114	-0,0672
Cra	0,3333	0,0001	-8,941	-0,0012
Cyc	2,9167	0,0011	-6,772	-0,0078
Cyma	0,3334	0,0001	-8,941	-0,0012
Cymb	27,142	0,0107	-4,541	-0,0484
Diat	0,1667	7E-05	-9,634	-0,0006
Frag	32,167	0,0126	-4,371	-0,0552
Fru	0,5	0,0002	-8,535	-0,0017
Gom	3,1667	0,0012	-6,689	-0,0083
Gyr	15,217	0,006	-5,12	-0,0306
Mel	20,167	0,0079	-4,838	-0,0383
Nav	77,309	0,0304	-3,494	-0,1061
Nit	112,3	0,0441	-3,121	-0,1377
Pen	118,81	0,0467	-3,065	-0,143
Pin	46,208	0,0182	-4,009	-0,0728
Rhop	0,3333	0,0001	-8,941	-0,0012
Sel	0,6666	0,0003	-8,248	-0,0022
Sen	60,56	0,0238	-3,738	-0,0889
Sta	27,333	0,0107	-4,534	-0,0487
Act	5,3333	0,0021	-6,168	-0,0129
Anki	2,3834	0,0009	-6,974	-0,0065
Car	0,8333	0,0003	-8,024	-0,0026
Chla	10,383	0,0041	-5,502	-0,0224
Chlo	63,101	0,0248	-3,697	-0,0917
Chloro	39,133	0,0154	-4,175	-0,0642
Coe	35,601	0,014	-4,27	-0,0597
Cos	3,2167	0,0013	-6,674	-0,0084
Cruc	96,125	0,0378	-3,276	-0,1237
Cruci	18,25	0,0072	-4,938	-0,0354
Des	166,1	0,0653	-2,729	-0,1781
Dic	375,38	0,1475	-1,914	-0,2823
Ela	1,6667	0,0007	-7,331	-0,0048
Gon	2,3333	0,0009	-6,995	-0,0064
Mon	39,775	0,0156	-4,159	-0,065
Mou	2,6667	0,001	-6,861	-0,0072
Nep	2	0,0008	-7,149	-0,0056
Ooc	72,067	0,0283	-3,564	-0,1009
Ped	26,217	0,0103	-4,576	-0,0471
Sce	285,35	0,1121	-2,188	-0,2453
Stau	2,3834	0,0009	-6,974	-0,0065
Tet	9,2584	0,0036	-5,617	-0,0204
Tetr	2	0,0008	-7,149	-0,0056
Tre	0,5	0,0002	-8,535	-0,0017
Cry	1,25	0,0005	-7,619	-0,0037
Din	68,167	0,0268	-3,62	-0,0969
Cer	0,5	0,0002	-8,535	-0,0017
Peri	2,0833	0,0008	-7,108	-0,0058
Eug	10,784	0,0042	-5,464	-0,0231
Phac	1,5	0,0006	-7,437	-0,0044
Tra	25,183	0,0099	-4,616	-0,0457
	2545,5	1		-3,1796

APPENDIX C: Continued

Site 4 AWFS	N	pi	ln(pi)	pi* ln(pi)
Lep	34,5	0,1464	-1,921	-0,281
Pho	6	0,0255	-3,671	-0,093
Ach	1,1667	0,005	-5,308	-0,026
Ancn	0,3333	0,0014	-6,561	-0,009
Amp	1,5	0,0064	-5,057	-0,032
Aul	0,6667	0,0028	-5,868	-0,017
Coc	5	0,0212	-3,853	-0,082
Cra	2	0,0085	-4,769	-0,04
Cyc	6	0,0255	-3,671	-0,093
Cyma	0,1667	0,0007	-7,254	-0,005
Cymb	0,1667	0,0007	-7,254	-0,005
Diat	6,3332	0,0269	-3,617	-0,097
Eun	0,6667	0,0028	-5,868	-0,017
Frag	10,333	0,0438	-3,127	-0,137
Fru	0,6667	0,0028	-5,868	-0,017
Gom	10,333	0,0438	-3,127	-0,137
Gyr	2,3334	0,0099	-4,615	-0,046
Mel	8,5001	0,0361	-3,322	-0,12
Nav	27,167	0,1153	-2,16	-0,249
Nit	22,333	0,0948	-2,356	-0,223
Pen	5,0001	0,0212	-3,853	-0,082
Pin	5,0001	0,0212	-3,853	-0,082
Sel	2,1668	0,0092	-4,689	-0,043
Sen	2,5	0,0106	-4,546	-0,048
Sta	3,8333	0,0163	-4,119	-0,067
Act	1,1667	0,005	-5,308	-0,026
Chla	8,3333	0,0354	-3,342	-0,118
Chlo	7,0001	0,0297	-3,516	-0,104
Chloro	6,3333	0,0269	-3,617	-0,097
Clo	0,6667	0,0028	-5,868	-0,017
Cruc	0,6667	0,0028	-5,868	-0,017
Cruci	4,6666	0,0198	-3,922	-0,078
Des	2,6668	0,0113	-4,482	-0,051
Gon	3,1667	0,0134	-4,31	-0,058
Mon	4,1666	0,0177	-4,035	-0,071
Mou	9,1667	0,0389	-3,247	-0,126
Nep	1	0,0042	-5,462	-0,023
Ooc	0,5	0,0021	-6,156	-0,013
Ped	5	0,0212	-3,853	-0,082
Sce	5,3334	0,0226	-3,788	-0,086
Sph	1,3333	0,0057	-5,175	-0,029
Cry	4,4999	0,0191	-3,958	-0,076
Din	0,3334	0,0014	-6,561	-0,009
Cer	0,1667	0,0007	-7,254	-0,005
Peri	1,5001	0,0064	-5,057	-0,032
Eug	1,1666	0,005	-5,308	-0,026
Tra	2,1666	0,0092	-4,689	-0,043
	235,67	1		-3,237

Site 5 BKD	N	pi	ln(pi)	pi* ln(pi)
Aph	1854,49	0,02103	-3,8617	-0,081218994
Gei	159,29	0,00181	-6,3164	-0,011410564
Lep	4534,01	0,05142	-2,9677	-0,152601143
Mer	1418,31	0,01609	-4,1299	-0,066429256
Mic	53742,4	0,60949	-0,4951	-0,301776648
Pho	1800,54	0,02042	-3,8912	-0,079458973
Pse	2073,31	0,02351	-3,7502	-0,088179656
Syn	0,6667	7,6E-06	-11,793	-8,91637E-05
Syne	92,95	0,00105	-6,855	-0,007226203
Ancn	68,2032	0,00077	-7,1646	-0,005541765
Aul	21,5373	0,00024	-8,3173	-0,00203154
Coc	27,805	0,00032	-8,0619	-0,002542204
Cymb	2,2755	2,6E-05	-10,565	-0,000272642
Diad	148,96	0,00169	-6,3834	-0,010783855
Frag	1276,4	0,01448	-4,2353	-0,061308437
Gom	27,9717	0,00032	-8,0559	-0,002555549
Nav	245,251	0,00278	-5,8848	-0,016367969
Nit	392,188	0,00445	-5,4153	-0,024086398
Pen	202,58	0,0023	-6,076	-0,013959266
Sen	27,305	0,00031	-8,08	-0,002502109
Sta	126,538	0,00144	-6,5465	-0,009394721
Anki	109,221	0,00124	-6,6937	-0,008291344
Car	51,197	0,00058	-7,4514	-0,004326477
Chla	84,7336	0,00096	-6,9476	-0,006676373
Chlo	134,275	0,00152	-6,4872	-0,009878813
Chloro	230,194	0,00261	-5,9482	-0,015528441
Coe	1,3333	1,5E-05	-11,099	-0,000167834
Cos	1	1,1E-05	-11,387	-0,000129141
Cruc	35,9333	0,00041	-7,8054	-0,003180865
Des	126,686	0,00144	-6,5454	-0,00940403
Dic	491,48	0,00557	-5,1897	-0,028926566
Ela	27,305	0,00031	-8,08	-0,002502109
Gem	6840,2	0,07757	-2,5565	-0,198320941
Mon	109,885	0,00125	-6,6877	-0,008334178
Mou	3	3,4E-05	-10,288	-0,000350045
Ooc	843,693	0,00957	-4,6493	-0,044486004
Sce	45,498	0,00052	-7,5694	-0,003905769
Sph	95,566	0,00108	-6,8273	-0,007399496
Stau	0,1667	1,9E-06	-13,179	-2,49148E-05
Tet	52,0802	0,00059	-7,4343	-0,004391011
Cry	114,31	0,0013	-6,6482	-0,008618629
Din	10186,7	0,11553	-2,1583	-0,249336341
Cer	1	1,1E-05	-11,387	-0,000129141
Per	47,725	0,00054	-7,5216	-0,00407108
Peri	287,475	0,00326	-5,7259	-0,018668079
Eug	11,917	0,00014	-8,9091	-0,001204075
	88175,6	1		-1,57799

APPENDIX C: continued

Site 7 WWTP	N	pi	ln(pi)	pi* ln(pi)
Aph	672,1	0,0556	-2,889	-0,1607
Lep	38,052	0,0031	-5,761	-0,0181
Mer	2,6667	0,0002	-8,419	-0,0019
Osc	2207,3	0,1827	-1,7	-0,3105
Pho	2906,1	0,2405	-1,425	-0,3427
Pse	117,73	0,0097	-4,631	-0,0451
Syne	1,5	0,0001	-8,994	-0,0011
Amp	7,15	0,0006	-7,433	-0,0044
Aul	1123	0,0929	-2,376	-0,2208
Coc	21,42	0,0018	-6,335	-0,0112
Cra	8,8166	0,0007	-7,223	-0,0053
Cyc	736,53	0,061	-2,798	-0,1705
Cyma	5,0958	0,0004	-7,771	-0,0033
Cymb	0,1667	1E-05	-11,19	-0,0002
Diat	31,587	0,0026	-5,947	-0,0155
Frag	92,994	0,0077	-4,867	-0,0375
Fru	0,5	4E-05	-10,09	-0,0004
Gom	21,783	0,0018	-6,318	-0,0114
Gyr	27,57	0,0023	-6,083	-0,0139
Mel	146,88	0,0122	-4,41	-0,0536
Nav	187,26	0,0155	-4,167	-0,0646
Nit	513,36	0,0425	-3,159	-0,1342
Pen	138,48	0,0115	-4,469	-0,0512
Pin	39,083	0,0032	-5,734	-0,0185
Sel	43,149	0,0036	-5,635	-0,0201
Sen	54,55	0,0045	-5,401	-0,0244
Sta	129,75	0,0107	-4,534	-0,0487
Sur	1,5	0,0001	-8,994	-0,0011
Try	48,245	0,004	-5,523	-0,0221
Act	71,673	0,0059	-5,128	-0,0304
Anki	282,16	0,0233	-3,757	-0,0877
Car	377,29	0,0312	-3,467	-0,1082
Chla	97,365	0,0081	-4,821	-0,0388
Chlo	224,15	0,0185	-3,987	-0,074
Chloro	254,39	0,0211	-3,861	-0,0813
Clo	7,55	0,0006	-7,378	-0,0046
Coe	143	0,0118	-4,437	-0,0525
Cruc	1,3333	0,0001	-9,112	-0,001
Cruci	19,066	0,0016	-6,452	-0,0102
Des	96,111	0,008	-4,834	-0,0384
Gon	2,3333	0,0002	-8,552	-0,0017
Mon	157,46	0,013	-4,34	-0,0566
Mou	7,3333	0,0006	-7,407	-0,0045
Ooc	89,744	0,0074	-4,903	-0,0364
Ped	38,133	0,0032	-5,759	-0,0182
Sce	186,04	0,0154	-4,174	-0,0643
Tet	39,825	0,0033	-5,715	-0,0188
Tre	10,239	0,0008	-7,073	-0,006
Cry	2,8333	0,0002	-8,358	-0,002
Cer	39,913	0,0033	-5,713	-0,0189
Peri	42,928	0,0036	-5,64	-0,02
Eug	300,91	0,0249	-3,693	-0,092
Phac	116,45	0,0096	-4,642	-0,0447
Str	55,245	0,0046	-5,388	-0,0246
Tra	96,152	0,008	-4,834	-0,0385
	12084	1		-2,7873

Site 6 PD	N	pi	ln(pi)	pi* ln(pi)
Ana	596	0,025201	-3,681	-0,0928
Aph	4428,4	0,187253	-1,675	-0,3137
Apha	3,6667	0,000155	-8,772	-0,0014
Gei	95,333	0,004031	-5,514	-0,0222
Lep	538,88	0,022786	-3,782	-0,0862
Mer	382,27	0,016164	-4,125	-0,0667
Osc	178,75	0,007558	-4,885	-0,0369
Pho	4418,6	0,186836	-1,678	-0,3134
Pse	421,92	0,017841	-4,026	-0,0718
Syne	59,061	0,002497	-5,993	-0,015
Ach	21,41	0,000905	-7,007	-0,0063
Amp	88,74	0,003752	-5,585	-0,021
Aul	2233,9	0,094457	-2,36	-0,2229
Coc	46,764	0,001977	-6,226	-0,0123
Cra	3,9722	0,000168	-8,692	-0,0015
Cyc	82,747	0,003499	-5,655	-0,0198
Cymb	263,1	0,011125	-4,499	-0,05
Diad	227,75	0,00963	-4,643	-0,0447
Diat	2,6667	0,000113	-9,09	-0,001
Frag	534,07	0,022582	-3,791	-0,0856
Fru	0,3333	1,41E-05	-11,17	-0,0002
Gom	97,961	0,004142	-5,487	-0,0227
Gyr	7,4833	0,000316	-8,058	-0,0025
Lut	0,3333	1,41E-05	-11,17	-0,0002
Nav	179,24	0,007579	-4,882	-0,037
Nit	1009,3	0,042676	-3,154	-0,1346
Pen	74,972	0,00317	-5,754	-0,0182
Pin	7,9929	0,000338	-7,993	-0,0027
Rhop	12,417	0,000525	-7,552	-0,004
Sel	42,91	0,001814	-6,312	-0,0115
Sen	1712,8	0,072423	-2,625	-0,1901
Sta	1492,5	0,063109	-2,763	-0,1744
Try	0,5	2,11E-05	-10,76	-0,0002
Anki	537,44	0,022725	-3,784	-0,086
Car	0,3333	1,41E-05	-11,17	-0,0002
Chla	28,805	0,001218	-6,711	-0,0082
Chlo	143,23	0,006057	-5,107	-0,0309
Chloro	194,75	0,008235	-4,799	-0,0395
Clo	39,555	0,001673	-6,393	-0,0107
Coe	532,28	0,022507	-3,794	-0,0854
Cos	7,1595	0,000303	-8,103	-0,0025
Cruc	188,15	0,007956	-4,834	-0,0385
Cruci	42,556	0,001799	-6,32	-0,0114
Des	408,9	0,01729	-4,058	-0,0702
Dic	631,8	0,026715	-3,623	-0,0968
Ela	2	8,46E-05	-9,378	-0,0008
Gem	47,667	0,002016	-6,207	-0,0125
Gon	1	4,23E-05	-10,07	-0,0004
Mon	94,976	0,004016	-5,517	-0,0222
Mou	214,26	0,00906	-4,704	-0,0426
Ooc	226,21	0,009565	-4,65	-0,0445
Ped	23	0,000973	-6,936	-0,0067
Sce	215,76	0,009123	-4,697	-0,0429
Sph	81,914	0,003464	-5,665	-0,0196
Stau	10,798	0,000457	-7,692	-0,0035
Tet	37,563	0,001588	-6,445	-0,0102
Tetr	27,972	0,001183	-6,74	-0,008
Cry	30,443	0,001287	-6,655	-0,0086
Din	42,305	0,001789	-6,326	-0,0113
Cer	369,03	0,015604	-4,16	-0,0649
Peri	169,82	0,007181	-4,936	-0,0354
Eug	15,25	0,000645	-7,346	-0,0047
Phac	0,5	2,11E-05	-10,76	-0,0002
Tra	17,417	0,000736	-7,214	-0,0053
	23650	1		-2,908

APPENDIX C: continued

Site 8 EBR	N	pi	ln(pi)	pi* ln(pi)
Apha	841,94	0,5269	-0,641	-0,3376
Lep	56,833	0,0356	-3,336	-0,1187
Pho	96,367	0,0603	-2,808	-0,1694
Pse	7,4	0,0046	-5,375	-0,0249
Aul	6,2	0,0039	-5,552	-0,0215
Coc	37,573	0,0235	-3,75	-0,0882
Cra	1,1667	0,0007	-7,222	-0,0053
Cyc	29,6	0,0185	-3,989	-0,0739
Cyma	2,6667	0,0017	-6,396	-0,0107
Cymb	1,1667	0,0007	-7,222	-0,0053
Diat	42,535	0,0266	-3,626	-0,0965
Frag	1,8	0,0011	-6,789	-0,0076
Fru	1,3333	0,0008	-7,089	-0,0059
Gom	1,6667	0,001	-6,866	-0,0072
Gyr	9,7333	0,0061	-5,101	-0,0311
Lut	2,0667	0,0013	-6,651	-0,0086
Mel	145,08	0,0908	-2,399	-0,2178
Nav	37,035	0,0232	-3,765	-0,0873
Nit	47,518	0,0297	-3,515	-0,1045
Pin	7,5	0,0047	-5,362	-0,0252
Rhoi	3,2	0,002	-6,213	-0,0124
Rhop	0,3333	0,0002	-8,475	-0,0018
Sel	11,333	0,0071	-4,949	-0,0351
Sen	1,5	0,0009	-6,971	-0,0065
Sta	1,2	0,0008	-7,194	-0,0054
Sur	15,533	0,0097	-4,633	-0,045
Try	24,237	0,0152	-4,189	-0,0635
Act	5,2	0,0033	-5,728	-0,0186
Car	3,8667	0,0024	-6,024	-0,0146
Chla	27,667	0,0173	-4,056	-0,0702
Chlo	31,871	0,0199	-3,915	-0,0781
Chloro	9,7843	0,0061	-5,096	-0,0312
Clo	1,3333	0,0008	-7,089	-0,0059
Coe	5,1333	0,0032	-5,741	-0,0184
Cos	0,1667	0,0001	-9,168	-0,001
Des	4,2667	0,0027	-5,926	-0,0158
Dic	1,3333	0,0008	-7,089	-0,0059
Gem	10,667	0,0067	-5,009	-0,0334
Gon	3,8333	0,0024	-6,033	-0,0145
Mon	14	0,0088	-4,737	-0,0415
Ooc	1,8333	0,0011	-6,77	-0,0078
Sce	6	0,0038	-5,585	-0,021
Tet	1,4	0,0009	-7,04	-0,0062
Tre	1	0,0006	-7,376	-0,0046
Din	1,3333	0,0008	-7,089	-0,0059
Cer	2,8333	0,0018	-6,335	-0,0112
Peri	1,9	0,0012	-6,735	-0,008
Eug	21,8	0,0136	-4,295	-0,0586
Phac	0,5	0,0003	-8,07	-0,0025
Str	1,6667	0,001	-6,866	-0,0072
Tra	4	0,0025	-5,99	-0,015
	1597,9	1		-2,114