

Changes in the algal abundance and composition along the Mooi River in the Potchefstroom area

LN Koekemoer



orcid.org 0000-0003-2771-7135

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Supervisor: Prof MS Janse van Vuuren

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ABSTRACT

Rivers and dams are the main sources of fresh water in South Africa, and the quality of drinking water is rapidly deteriorating. More than 80% of the country's rivers and dams experience pressure as a result of utilisation and pollution. The inhabitants of Potchefstroom are dependent on the Mooi River (specifically Boskop Dam) as its only source of drinking water. The Mooi River originates in the Boons area and flows southwards through Klerkskraal, Boskop, and Potchefstroom dams until it reaches the city of Potchefstroom. From here, the Mooi River flows for 25 km to where it converges with the Vaal River.

The Mooi River experiences surface water pollution, as a result of various anthropogenic activities including agricultural activities and effluents from urban, industrial and informal settlement areas. These anthropogenic activities in the catchment contribute to nutrient pollution that stimulates the growth of phytoplankton (algae and cyanobacteria). Excessive amounts of nutrients, particularly orthophosphates, stimulate the growth of harmful cyanobacterial and algal species, and reduce the water quality.

Apart from anthropogenic activities, the river is influenced by several tributaries feeding it. The Wonderfontein Spruit enters the Mooi River approximately 3 km downstream from its source and it is influenced by large scale mining, resulting in acid mine drainage and heavy metal (especially uranium) pollution. The second tributary entering the Mooi River is the Gerhard Minnebron, situated in an area where peat mining and mining effluents (via Wonderfontein Spruit) are problematic. The Wasgoed Spruit enters the Mooi River in the city of Potchefstroom and it feeds the Mooi River with urban effluent, sewage effluents, and wastewater from industries.

Water samples were collected once a month at eight different sites in the Mooi River, as well as one site in each tributary. Phytoplankton samples were enumerated and phytoplankton was identified to genus level. The main aims of the study were to investigate spatial changes in physico-chemical variables and phytoplankton concentration and composition in the Mooi River, and to relate it to the effect of the inflowing tributaries. Furthermore, the physico-chemical variables were compared to known limits, namely the resource quality objectives and recommended water quality objectives, goals set to strive to a certain desired water quality. The orthophosphate and nitrogen concentrations were used to determine the current trophic status of the Mooi River. This represents the first study on the influence of tributaries on the water quality and phytoplankton dynamics of the Mooi River.

Results of the study indicated that the tributaries contributed to elevated nutrient levels in the Mooi River. High nutrient concentrations were the result of agricultural activities and sewage effluents. The mean ammonia and nitrate concentrations for the Mooi River and its tributaries are indicative of mesotrophic conditions, while mean orthophosphate concentrations indicated hypertrophic conditions. Nutrients were positively correlated with green algae, diatoms, cyanobacteria, and euglenophytes. Orthophosphates, in particular, showed a strong positive correlation with the concentration of cyanobacteria. High nutrient concentrations stimulated the growth of phytoplankton, amongst others harmful genera such as *Microcystis* and *Anabaena*. These genera are known to produce toxins and also cause taste and odour problems. High concentrations of these genera were accompanied by high turbidity levels and relatively high pH values. Relatively low abundance and diversity of cryptophytes and chrysophytes, groups generally associated with low nutrient conditions, indicate that the Mooi River and its tributaries can be regarded as polluted systems.

High TDS concentrations and EC levels in the tributaries, primarily due to irrigation and sewage effluent, had a huge and observable effect on the Mooi River downstream from the points of inflow. Elevated calcium, magnesium, and sulfate concentrations, as a result of mining in the catchment, can be dangerous if consumed by livestock. In general, calcium and magnesium concentrations were high, and can be attributed to the catchment underlined by dolomite - an anhydrous carbonate composed mainly of calcium and magnesium. The Wonderfontein Spruit contributed to elevated magnesium levels in the Mooi River, while Gerhard Minnebron mainly contributed to elevated sulfate concentrations. Mining and sewage effluents could have been responsible for high chloride concentrations in the catchment. Both Wonderfontein Spruit and Gerhard Minnebron contributed to elevated chloride levels in the Mooi River system. Manufacturing industries surrounding the Wasgoed Spruit area could have elevated fluoride concentrations in downstream reaches of the Mooi River. Wonderfontein Spruit also contributed significantly to elevated concentrations of heavy metals such as manganese, hexavalent chromium, and uranium. The Gerhard Minnebron was responsible for higher iron, and manganese concentrations in the Mooi River.

Most of the mean values for physico-chemical variables measured in the Mooi River and tributaries exceeded the limits of the resource quality objectives and recommended resource water quality objectives.

Phytoplankton concentration in the tributaries did not have a huge effect on phytoplankton in the Mooi River main stream. Similarities between genera observed in the tributaries and the

Mooi River can be the result of importation from the tributaries, their preference for high nutrients concentrations or seasonal aspects favouring their growth.

It is important that the Mooi River and its tributaries must be monitored regularly, to ensure proper management of the river. Nutrients, especially orthophosphate concentrations, should be reduced. Constant monitoring of phytoplankton dynamics in relation to physico-environmental variables is recommended for future effective management of the Mooi River system.

Keywords: anthropogenic activities, Mooi River, nutrients, phytoplankton, physico-chemical variables, tributary, trophic status, resource quality objectives, water quality

DEDICATION

IN LOVING MEMORY OF MY
GRANDMOTHER, LAZIA MARIA LOOTS
AND
GODMOTHER, NANCY-LAZIA KOEKEMOER

You are dearly loved and greatly missed. Thank you for all the years of unconditional love and support. You were such strong figures to look up to. The following song written by John Rutter always reminds me of you:

“look at the world: Everything all around us
look at the world: and marvel everyday
look at the world: So many joys and wonders
So many miracles along our way

Praise to Thee o Lord for all creation
give us thankful hearts that we may see
all the gifts we share and every blessing all things come of Thee

Look at the earth: Bringing forth fruit and flower
look at the sky: The sunshine and the rain
Look at the hills, look at the trees and mountains,
Valley and flowing river field and plain.

Think of the spring, Think of the warmth of summer
Bringing the harvest before the winters cold
Everything grows, everything has a season
‘Til it is gathered to the fathers fold

Every good gift, all that we need and cherish
Comes from the Lord in token of His love
We are His hands, stewards of all His bounty
His is the earth and His the Heavens above”

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CHAPTER 1: INTRODUCTION

“Water is the basis of life and the blue arteries of the earth! Everything in the non-marine environment depends on fresh water to survive.” Sandra Postel

About 71% of the earth’s surface is covered with water (Miller & Spoolman, 2012), of which about 99% is either located in the ocean or Arctic areas (Wetzel, 1992). Water is of crucial importance for the foundation and maintenance of all life on earth (Hickman, *et al.*, 2008). It is an irreplaceable element with distinctive properties (Miller & Spoolman, 2012), and all organisms, including humans, need a continuous flux of water or at least a water-rich environment for survival (Strahler, 2005). Only about 1% of the planet’s water is available as freshwater in accessible groundwater deposits, lakes, rivers, and streams (Miller & Spoolman, 2012; Wetzel, 1992). Accessibility of fresh water can be difficult in some areas, due to water being unevenly distributed across the surface of the earth (Huntley *et al.*, 1987; Mukheibir & Sparks, 2003). In other words, even though the planet mainly consists of water, there is only a small percentage that is useable.

The small percentage of freshwater available is used for many different purposes, such as domestic, irrigation, agriculture, industrial, and mining (DWAF, 2002; Mackay, 2000). Water users in the above mentioned sectors put pressure on freshwater resources, often over utilising it (Ashton *et al.*, 2005), and, at the same time, contributing to water pollution (Nkwonta & Ochieng, 2009). Pollution reduces water quality, restricting the use of water as a commodity (Sen *et al.*, 2013).

Freshwater has become limited worldwide, especially in dry countries such as South Africa, which currently experiences high water pressure (Keating, 2013; Mukheibir & Sparks, 2003). The country is regarded as semi-arid, with a mean annual rainfall of approximately 497 mm (Mantel *et al.*, 2010), which is low compared to the world average of 860 mm per year (DWAF, 1986; Germs *et al.*, 2004). South Africa also has relatively high rates of evaporation, contributing to a water scarce country (Mukheibir & Sparks, 2003). Cape Town, one of the largest cities in South Africa, faced the worst drought recorded in history in 2017 (PWC, 2017). The country further faces severe problems, such as an increase in large scale mining, increases in large industries, poorly managed sanitation systems, informal settlements established around rivers, developing communities, and extensive agriculture practices, all of which can have an effect on the limited water resources (DWAF, 1986). Agriculture uses the most water in South Africa (62%; Figure 1 in Askham & Van der Poll, 2017). Although a small percentage of water is used by mining (3%), it can have significant impacts on water quality through contamination, which reduces the water

quality for human consumption and large amounts of money must be spend to recycle the contaminated water (Askham & Van der Poll, 2017).

The increasing population of South Africa depends on the country's rivers and aquifers as a freshwater resource (Dallas & Day, 2004), placing it under constant exploitation. About 60% of the country's rivers are currently under threat, while 23% are critically endangered, in terms of utilisation and pollution (WWF, 2013).

In the dry North West Province of South Africa, with extreme climate conditions, rivers and dams are the main sources of surface water and therefore extremely important (Davies & Day, 1986; DWAF, 1986; 2011; SOER, 2002). The Mooi River system, located in the North West Province of South Africa, has been chosen for monitoring, because the river is a significant water resource forming part of the Upper Vaal catchment area (McDonald, 2014). Not only is the Mooi River a tributary of the Vaal River, one of the largest rivers in South Africa, but it is a significant fresh water resource for the city of Potchefstroom. Potchefstroom, a city in the North West Province, is dependent on the Mooi River and its dams (especially Boskop Dam) for drinking water (Annandale & Nealer, 2011). The last syllable in the word "Potchefstroom", namely, "stroom", means "stream" and refers to the Mooi River. This is an indication of the importance of the river for the inhabitants of Potchefstroom.

Although disputed, it is generally accepted that the Mooi River (which means "beautiful" river) obtained its name from its once beautiful, clear stream of water. However, currently the Mooi River experiences different impacts in its catchment, affecting the water quality in terms of pollution and utilisation. Climatic factors, including high summer temperatures, low average rainfall, unevenly distributed rainfall patterns, and high evaporation rates, contribute to insufficient water availability of the Mooi River (Van der Walt *et al.*, 2002; Winde & Van der Walt, 2004). Surface water pollution, as a result of various anthropogenic activities, is common in the area surrounding the Mooi River, and includes effluents from agricultural, urban, industrial, and informal settlement areas, diamond diggings, and recreational activities (NWDACE, 2008), all putting pressure on the river. Aside from this, the river is further impacted by several tributaries, of which the water quality is influenced by anthropogenic activities in their vicinity (discussed in detail in Chapter 2, section 2.2). In turn these tributaries affect the water quality of the Mooi River when entering the system. The Wonderfontein Spruit (WFS), the Loop Spruit (LS), the Gerhard Minnebron (GM), and the Wasgoed Spruit (WS) are the major tributaries feeding the Mooi River (De la Rey *et al.*, 2004).

The WFS is of major concern, notorious for mining activities in its catchment (Bomman *et al.*, 2013). Acid mine drainage (AMD) and heavy metal (especially uranium) pollution are two main concerns regarding water quality in the WFS (Coetzee *et al.*, 2006). This is especially important, because Potchefstroom receives its drinking water from Boskop Dam, which is located just downstream from the inflow of the WFS into the Mooi River. The whole catchment area is underlined by dolomite, and therefore water from the WFS can reach the Mooi River, not only as surface water inflow, but also directly through dolomitic outcrops (Barnard *et al.*, 2013). According to Bomman *et al.* (2013) it is assumed that in future, the ground and surface water from the WFS may deteriorate to such an extent, that it will become almost unusable. They further stated that, if drastic steps are not taken to improve the water quality, the area will face a serious shortage in useable water resources.

The deterioration of the water quality of the tributaries, such as the WFS, is responsible for a deterioration of the water quality in the Mooi River. All these negative impacts are equally problematic for humans and aquatic organisms inhabiting the waters. It is therefore extremely important to monitor the water quality in the Mooi River regularly to see if the water quality improves or deteriorates. This will raise awareness so that proper management can take place in order to protect the water quality of the Mooi River.

Many studies have been done on the Mooi River and its tributaries. Most were, however, focussed on the WFS tributary with regards to mining pollution (Coetzee *et al.*, 2006; De Waard, 2012; Liefferink, 2015; Opperman, 2008; Schrader & Winde, 2014; Swart *et al.*, 2002), heavy metal pollution (Aucamp, 2000), and especially uranium pollution (Winde, 2006, 2009, 2010, 2013). A few studies were done on the water quality of the GM tributary (Bekker, 2010), especially on peat mining (DWAf, 2010; Winde, 2008; 2011a,b,c; Winde & Erasmus, 2011). In the LS, a study was conducted using macroinvertebrates as indicators of water quality (Erasmus & De Kock, 2015). Studies on the water quality of the WS mainly focused on diatoms (Kriel, 2008) and birds of the riparian corridors (Wyma, 2012).

Other studies were limited to only a small fraction of the Mooi River, such as the study conducted at Kromdraai on the impact of the gold mining industry on water quality (Malan, 2002), a water catchment management plan for Kromdraai (Riedel, 2003), wetlands in the area (Cilliers *et al.*, 1998; Coetzee *et al.*, 2002), and on dams located in the river (Barnard *et al.*, 2013; Bomman *et al.*, 2013; Van Aardt & Erdmann, 2004; Venter *et al.*, 2013).

Several studies aimed to investigate the effects of tributaries on the Mooi River, but sampling sites were only located in the main stream of the river and dams. These studies focused

mainly on fish (Van Heerden *et al.*, 2006), bacteria (Bezuidenhout, 2013; Jordaan, 2015; Jordaan & Bezuidenhout, 2015), yeast (Van Wyk, 2012), contaminants in sediments (Fosso-Kankeu *et al.*, 2015), distribution of inorganic contaminants (Manyatshe *et al.*, 2016, 2017), diatoms and macroinvertebrates (Pelser; 2015), and a comparison of water quality with TWQR and RQO's of the Middle Vaal, in order to determine RQO's for the Upper Vaal, as well as to contribute to algal data (Labuschagne, 2017).

Only a few studies included physical sampling on the Mooi River and one or more tributaries. These studies concentrated mainly on water chemistry (Van der Walt *et al.*, 2002), gold and uranium mining (Hamman, 2012), yeast (Monapathi, 2014), Mollusca diversity (Wolmarans *et al.*, 2015), diatoms (De la Rey, 2007; De la Rey *et al.*, 2004; Harding *et al.*, 2004), and birds combined with algal dynamics (Luyt, 2018). Luyt (2018) sampled the Mooi River's main stream and WFS tributary. No literature is available about the influence of the tributaries on the Mooi River regarding algal (including cyanobacteria) abundance and composition.

Janse van Vuuren & Taylor (2015) stated the importance of awareness of freshwater algae in dams and rivers, since they can effect, determine, and give an overall indication of water quality. In general, algae production has positive outcomes, because these organisms not only play an important role in the "self-purification of water bodies", but are also primary producers in the food chain (Sen *et al.*, 2013). Algal productivity is largely determined by the availability of nutrients in the water (Paerl *et al.*, 2001).

Anthropogenic activities in the catchment contribute to pollution with nutrients such as inorganic nitrogen (nitrites, nitrates, and ammonium) and phosphorus (orthophosphates; Hasler, 1947; Yang *et al.*, 2008), that constantly enrich the Mooi River and its tributaries. Excessive amounts of these nutrients lead to eutrophication that stimulates the growth of algal species, some of which can reduce water quality and affects its use (Sen *et al.*, 2013). Janse van Vuuren & Taylor (2015) stated that the presence of some cyanobacteria species can cause serious problems, because they are able to secrete toxins. Under favourable environmental conditions (sufficient amounts of nutrients available and suitable temperatures), cyanobacteria are able to reproduce at a high rate forming blooms. These blooms can result in scums covering the water's surface, causing taste and odour problems. Decomposition of the blooms result in anoxia in the water, causing fish kills (Janse van Vuuren & Taylor, 2015).

The trophic status of water bodies can be classified in four major groups, namely oligotrophic, mesotrophic, eutrophic, and hypertrophic (Matthews & Bernard, 2014; Rast & Thornton, 1996). An oligotrophic water body has low nutrient levels, low algal productivity, and

usually very few water quality problems. Eutrophic water bodies have high nutrient levels, high algal productivity (often associated with cyanobacterial blooms), and increasing water quality problems. Nutrient levels for mesotrophic water bodies fall between oligotrophic and eutrophic status. Hypertrophic water bodies have extremely high nutrient levels and serious water quality problems are experienced (Rast & Thornton, 1996).

Two previous studies were conducted by Venter *et al.* (2013) between 1999–2000, and 2010-2011 on the Klerkskraal, Boskop, and Potchefstroom dams in the Mooi River system. The most recent study (2010-2011) showed a decline in algal and cyanobacterial blooms, and indicates a general improvement in water quality since the period of the first study (1999-2000). However, increases in the number of diatom species characteristic of eutrophic waters, were observed. The second study showed that the Mooi River system was still in an acceptable condition in terms of its water quality, but with a further addition of nutrients, the river has the potential to produce problem species. A study by Labuschagne (2017), between the years 2014-2015, showed that the Mooi River falls in a mesotrophic to eutrophic state and phytoplankton (algae and cyanobacteria) species present were indicative of mesotrophic to eutrophic water. A recent study (2016-2017) by Luyt (2018) showed that the orthophosphate concentrations (mean of 17 mg/l) in the Mooi River are indicative of hypertrophic conditions. However, nitrogen concentrations (mean of 0.051 mg/l) were low during the same period, hence preventing hypertrophic symptoms.

Bio-indicators can be defined as organisms that reflect signs that they are affected through anthropogenic activities (Kshirsagar, 2013). Bio-monitoring (using bio-indicators) will be used in this study in an attempt to understand the effects and changes in water quality of the Mooi River. Phytoplankton is chosen as bio-indicators for this particular study. According to Sen *et al.* (2013) the absence or presence of phytoplankton can reflect the overall condition in a river. For example, cyanobacteria are mostly known to occur in nutrient-rich waters (Janse van Vuuren & Taylor, 2015; Sen *et al.*, 2013), while desmids are generally known to appear in oligotrophic waters (Brook, 1965). Other aspects making phytoplankton good indicators include: many species are present all year; they are diverse organisms found in large quantities and they are easy to sample and identify (Sen *et al.*, 2013). Phytoplankton is also able to respond quickly to physical and chemical environmental changes (Kshirsagar, 2013), which make them an excellent choice to use as indicators. Their presence can provide information regarding physical and/or chemical environments at a particular site (Bellinger & Sigeo, 2010). Bio-indicators (phytoplankton in this case) seldom provide insight into what is causing the issues (Dallas & Day, 2004). So it is important to also look at the physical and chemical data when performing bio-monitoring, in order

to identify possible factors that may cause damage to ecosystems so that proper and helpful actions can be implemented (Dallas & Day, 2004). Physical and chemical variables (together known as the physico-chemical variables) will therefore be included in the study, as they may provide insight on all factors contributing to the current pollution and deterioration of water quality of the Mooi River. The physico-chemical variables used during this study will be listed in Chapter 2, section 2.4.3.

The National Water Act (NWA), Act No 36 of 1998, uses different sets of scientific criteria, one of which focuses on the physico-chemical variables (DWA, 2016). These criteria are known as the resource quality objectives (RQO's) and consist of qualitative and quantitative information. The criteria make use of limits and can be seen as goals set to strive to a certain desired water quality for an area. The notion is to compare these set limits (goals) to current findings to see if the limits are reached (Dickens *et al.*, 2011; DWA, 2016). RQO's limits are, however not a replacement for other monitoring programmes with their own goals (Labuschagne, 2017). Yet, RQO's plays an important role in water resource management, because not only is it easier to have clear sets of criteria to work towards, but the protection of water becomes a reality in this way (Dickens *et al.*, 2011). The criteria differ for each of the nine water management areas (WMA's) of South Africa. The Vaal catchment is divided into the Upper Vaal, Middle Vaal, and Lower Vaal (Labuschagne, 2017). The Mooi River forms part of the Upper Vaal WMA (McDonald, 2014). DWA (2009) also recommended water quality objectives (RWQO's) which specifically target the Mooi River catchment. RWQO's work according to the RQO framework. Although the RQO's for the Vaal WMA are currently under review (Labuschagne, 2017), Dickens *et al.* (2011) stated that current available information should be used where possible.

The aims of this study were therefore to:

- Investigate the physico-chemical variables at eight different sites along the length of the Mooi River (from its source to before the confluence with the Vaal River), and at one site located in each tributary (WFS, GM and WS). Changes in physico-chemical water quality at the eight sites located in the Mooi River, will be related to the effect of inflowing streams polluted by various sources of pollution, such as the WFS (mining), the GM (peat mining) and WS (industrial and urban pollution);
- Investigate changes in phytoplankton abundance and composition at the same sites than the physico-chemical variables, to note if there are any changes in the total algal concentration and composition downstream from the inflow of the tributaries;

- Compare physico-chemical variables to the current RWQO and RQO limits;
- Determine the current trophic status of the Mooi River in terms of the orthophosphate and nitrogen concentrations.

CHAPTER 2: STUDY AREA, MATERIAL AND METHODS

“While it may seem small, the ripple effects of small things is extraordinary.” Matt Bevin

2.1 Study area

The Mooi River catchment is located across two provinces – the North West and the western part of Gauteng (Le Roux, 2005; Van der Walt *et al.*, 2002). The study area (Figure 2-1) stretches from the Mooi River’s northern origin in the Boons area, in the North West province, until it joins the Vaal River further southwards (Curie, 2001), and it forms part of the Upper Vaal water management area (McDonald, 2014). Klerkskraal, Boskop, and Potchefstroom Dams, are major dams located in the Mooi River (Van der Walt *et al.*, 2002).

Some of the tributaries entering the Mooi River have water quality impacts on the study area (De la Rey *et al.*, 2004), and will therefore also be discussed in section 2.2. The Mooi River has two main tributaries, namely the Wonderfontein Spruit (WFS; north-eastern reach), and the Loop Spruit (LS; eastern reach). Tudor Lancaster, and Donaldson Dams are the major dams in the WFS, and Klipdrift Dam is the major dam in the LS (Curie, 2001; Nel, 2011). Gerhard Minnebron (GM) and the Wasgoed Spruit (WS; Figure 2-2) are two smaller tributaries. GM is located upstream from Boskop Dam (Winde, 2008), and the WS is located in the city of Potchefstroom (De la Rey *et al.*, 2004). The three tributaries included in this study are the WFS, the GM and the WS. Although the GM and WS are not considered main tributaries, they may have a huge impact on the Mooi River system. WS is well-known for extremely poor water quality over long periods of time (personal communication: Prof. S Janse van Vuuren). GM can obtain groundwater directly from the upper part of the WFS, or from the Boskop-Turffontein compartment that gains underground water from the WFS Eye (well-known for mining pollution; Winde, 2008). GM feeds the Boskop Dam, where Potchefstroom’s potable water is stored. Although no samples were taken in the LS, a brief discussion of its possible influences on the Mooi River will be given in section 2.2.5.

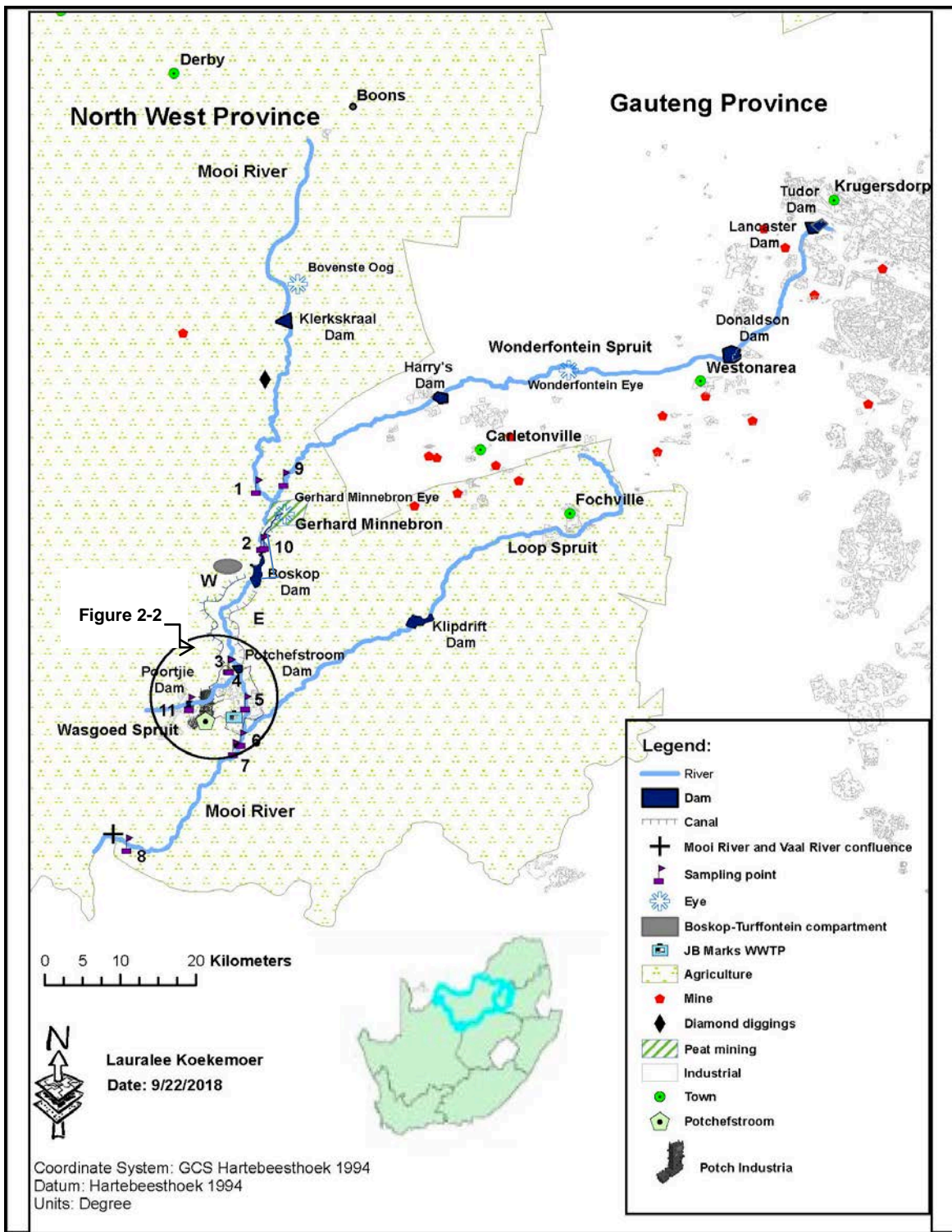


Figure 2-1 Catchment of the Mooi River from its source to the confluence with the Vaal River. Site 1: UBD, Site 2: BDI, Site 3: BDC, Site 4: PD, Site 5: RB, Site 6: SB, Site 7: DFE, Site 8: Krom, Site 9: WFS, Site 10: GM, Site 11: WS. See section 2.3 for descriptions of abbreviations.

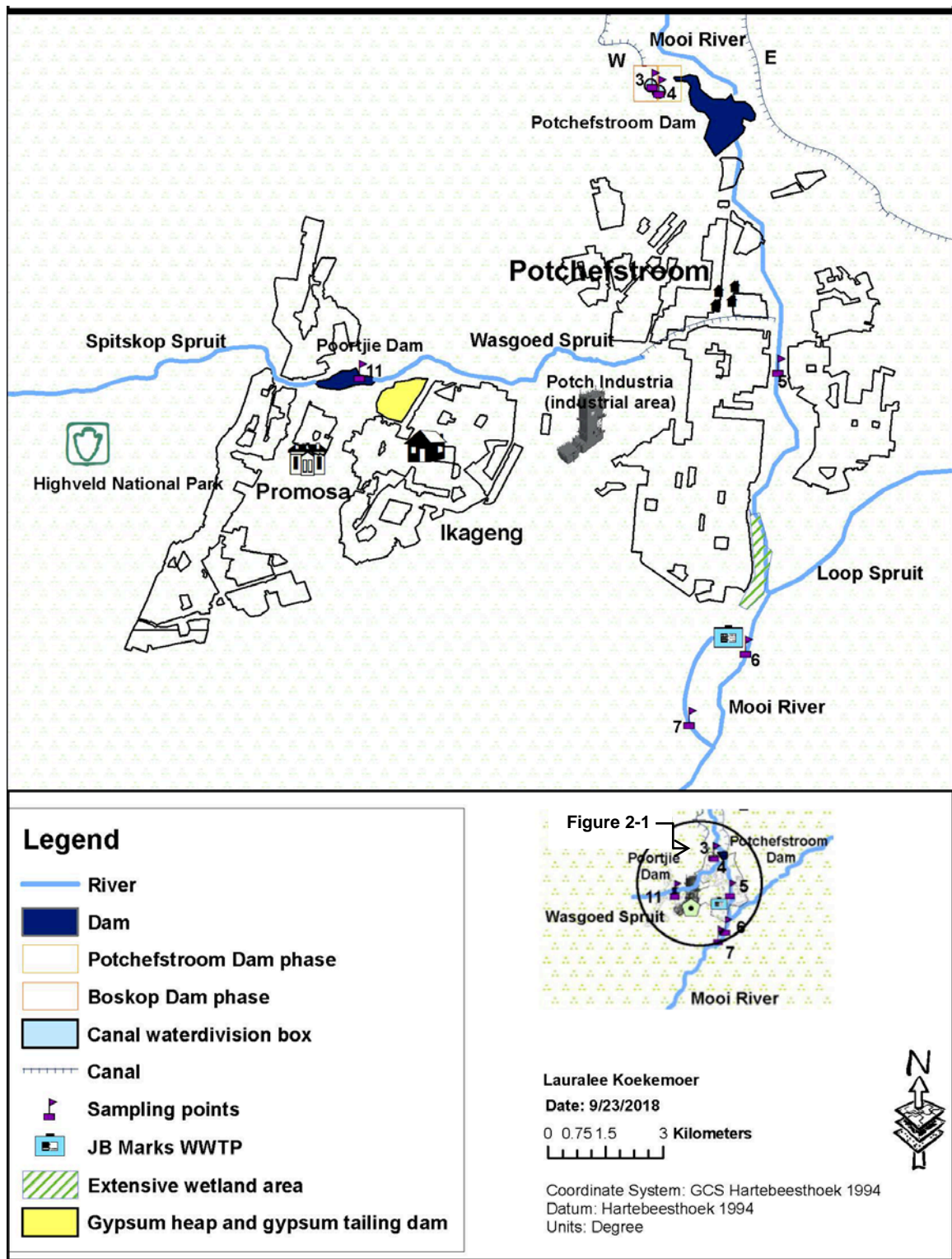


Figure 2-2 The location of the Mooi River and the inflow of the Wasgoed Spruit system (WS) in Potchefstroom.

The study area is situated in a summer rainfall region, and although rainfall is unevenly distributed throughout the area (Pelser, 2015), it usually ranges around an average of 507 mm per annum (Winde & Van der Walt, 2004). Appendix A, Figure A-1 contains the rainfall data for the study period (January to December 2015). The karst landscape area is underlain by dolomite (Le Roux, 2005), and due to extensive dolomitic outcrops, only 44.2% of the catchment yields significant runoff (Winde & Van der Walt, 2004). Rainfall in the rest of the catchment may end up as groundwater, feeding the Mooi River and its tributaries through dolomitic outcrops (Van der Walt *et al.*, 2002). The meandering Mooi River is relatively flat, ranging from 1520 m above sea level in the north to about 1300 m above sea level in the area where it converges with the Vaal River. The flat topography in the lower Mooi River catchment explains the extensive wetland areas downstream of Potchefstroom (Le Roux, 2005; Figure 2-2).

Appendix A, Figure A-2 illustrates the monthly maximum, minimum, and average temperatures for the study period. On average, the maximum summer temperatures in the area range between 27.2°C and 29.2°C, while the average winter minimum temperatures range between 0.5°C to 0.7°C (Aucamp, 2000).

The Mooi River originates near the town of Derby, in the Boons area, and flows southwards into the Klerkskraal Dam (Curie, 2001; Venter *et al.*, 2013; Figure 2-1). The Bovenste Oog (a natural spring), north of Klerkskraal Dam, also contributes significantly to the flow of the upper Mooi River region (Riedel, 2003). The Bovenste Oog has extremely clear and shallow waters, with a deep pool (Pelser, 2015). The original purpose of the Klerkskraal Dam, including its cement canals, was to manage irrigation and flow of the Mooi River (Annandale & Nealer, 2011; Barnard *et al.*, 2013). In general, water quality in the upper section of the Mooi River, from its origin to below the Klerkskraal Dam, is excellent, because it's not directly influenced by the WFS tributary or any other land use impacts (Booyens, 2016). The water quality south of Klerkskraal Dam is influenced by different impacts, amongst others the WFS, which is highly impacted by mining (Le Roux, 2005).

From the Klerkskraal Dam, the Mooi River flows further south to where the WFS joins it (Nealer & Raga, 2008). The WFS, surrounded by a couple of active/old and abandoned mines (Winde & Van der Walt, 2004), originates in the southern part of Krugersdorp (Nel, 2011) in the far West-Rand (Gauteng) at the Tudor Dam (Riedel, 2003) and flows for about 80 km from its origin to its confluence with the Mooi River (Swart *et al.*, 2002; Figure 2-1). It is believed that before gold mining commenced, the original stream of WFS was probably in a pristine condition, fed by perennial springs. The Tudor Dam was used as a storage dam for the Luipaardsvlei Gold plant (Opperman, 2008). From the Tudor Dam, water flows into the Lancaster Dam (Nel, 2011),

also used as a storage dam for the plant. Both dams became entirely silted up as a result of spillages from the plant, causing a lifetime buildup of gold containing sediment. Water from old mineshafts (treated with lime) fed the Cooke Attenuation Dam downstream, and then flows through a constructed wetland into the lower WFS (Opperman, 2008). From here, water flows towards the town of Westonaria into the Donaldson Dam (Nel, 2011; Figure 2-1). The Donaldson Dam obtains water from various sources, such as mining, sewage facilities and informal settlements (Barnard *et al.*, 2013). The WFS joins the Mooi River about 31 km downstream from Klerkskraal Dam near the GM Eye (Nealer & Raga, 2008; Figure 2-1). The GM Eye (active spring) is directly fed by dolomitic spring water that emerges from the Boskop-Turffontein compartment (Winde & Van der Walt, 2004). GM also forms part of a huge underground karst network that extends well into the upstream catchment of the WFS (impacted by heavy mining activity), while at the same time contributes significantly to the inflow into Boskop Dam (Winde, 2011b; section 2.3.3). Boskop Dam is fed with underground and canalised water from the GM Eye, located on the GM farm (Nealer & Raga, 2008). From the GM Eye, water flows in a cement canal for about 8 km to the Boskop Dam.

Boskop Dam is located about 7 km downstream from the confluence of the Mooi River and WFS, and about 41 km downstream from Klerkskraal Dam (Figure 2-1). The JB Marks Municipality in Potchefstroom is dependent on the Mooi River as its only source of raw water for approximately 400 000 inhabitants (Bomman *et al.*, 2013). Potchefstroom with its growing population, university, and large industries highly depends on the Mooi River for potable water (Pelser, 2015; Van der Walt *et al.*, 2002). The water is collected from surface and groundwater, and is stored in Boskop Dam (Annandale & Nealer, 2011; Van der Walt *et al.*, 2002). Various amounts of water from the Mooi River are canalised into two main open cement canals (East Bank canal and the West Bank canal) used primarily for agriculture. Most of the city's raw water is obtained from the West Bank canal (Figure 2-1; Figure 2-3) that passes through Potchefstroom, and is linked with the water purification plant. Both canals finally end up in the Mooi River, just north of the confluence with the Vaal River (TCEPM, 2013). The city of Potchefstroom and a few downstream farmers are the final users of water from the Mooi River, before it joins the Vaal River (Van der Walt *et al.*, 2002).

From Boskop Dam the Mooi River continues its flow to Potchefstroom Dam, located about 12 km downstream. Potchefstroom Dam's main purpose was originally for irrigation, but it has become popular for recreational activities (Annandale & Nealer, 2011).

One of the sampling sites used in this study is the Poortjie Dam that obtains water from the Spitskop Spruit arising from the western side of Potchefstroom, near the Highveld National Park

(Nel, 2011; Figure 2-2). From Poortjie Dam, water flows through the industrial area of Potchefstroom. Industrial effluents have a huge effect on water entering the Mooi River system (discussed in section 2.2.4.3). From the industrial area water is converted into a concrete lined canal, the WS. The WS flows eastwards through the city of Potchefstroom, thereby splitting the city into a northern and southern area (Nealer & Raga, 2008). The WS joins the Mooi River, in the eastern part of Potchefstroom, about 3 km downstream from Potchefstroom Dam (Nealer & Raga, 2008; Nel, 2011; Wyma, 2012). The Mooi River flows southwards through Potchefstroom, to its confluence with the LS, just downstream of the city.

The LS originates about 8 km north-east of Fochville. The source of the LS arises from various springs. The LS then flows through Fochville and informal settlements. The LS provides water to various impoundments (Erasmus & De Kock, 2015), and flows through Klipdrift Dam before it converges with the Mooi River on the southern side of Potchefstroom (Figure 2-1). Unfortunately no data on the LS was available for use in this study.

Downstream from the convergence of the LS and Mooi River, the water flows past the waste water treatment plant (WWTP) of Potchefstroom, located at the southern entrance of the Viljoenskroon road. Treated effluents from the WWTP are converted and recycled back into the Mooi River system. From here, the Mooi River flows about 25 km in a south-westerly direction, until it joins the Vaal River between Kromdraai farm (northern bank), and Hoogtekraal farm (southern bank; Nel, 2011).

2.2 Land Uses in Study Area

The following paragraphs contain a summary of land uses in the Mooi River, as well as in the catchment of each tributary in the study area.

2.2.1 Mooi River

2.2.1.1 Agriculture and irrigation

Agriculture plays a very important part in South Africa's economy and environment, and farming activities must be managed well. If not, it can have severe negative impacts on the natural environment (WWF, 2011). The North West province's climate and soil conditions make it suitable for a variety of farming practices (DARD, 2013). During the planting months of crops (March, April, August, and October), pesticides are used. Pesticides, herbicides and fertilisers (combined with extensive irrigation), can be fed into the aquatic environment (Pelser, 2015). According to the WWF (2011), an overuse of synthetic fertilisers, pesticides and herbicides is able to reduce long-

term soil richness (result in soil erosion), pollutes water supplies, poisons delicate ecosystems, exposes farmers and farm workers to toxins, and it can also contribute to climate change through greenhouse gas releases. Agriculture is prominent in the whole Mooi River system, from its origin to the confluence with the Vaal River (Henderson-Sellers, 1991; Pelsler, 2015). Crop farming and grazing are most evident in the northern area of the catchment (Van der Walt *et al.*, 2002). Wheat, maize and lucerne are pre-dominantly produced under irrigation (DARD, 2013). According to Earle *et al.* (2005) irrigation may cause a large amount of water loss due to evapotranspiration, which may result in negative impacts on the river's ecosystem, as well as for crop production further downstream.

Water allocations in the Mooi River area are controlled by the Mooi River Government Water Scheme. Most of the allocations can be managed by releases from Klerkskraal, Boskop, and Potchefstroom Dams. Other diffuse irrigation users gain water from farm dams, the Mooi River itself and boreholes, making it difficult to control the precise demand for water in the catchment (Le Roux, 2005).

2.2.1.2 Diamond diggings

Diamond diggings occur near the Klerkskraal Dam (Van der Walt *et al.*, 2002), as well as in the area where the Mooi River flows towards the Vaal River (Curie, 2001). According to Van der Walt *et al.* (2002) these diggings destroy the floodplain, and cause the removal of riparian vegetation, thus reducing habitat integrity (Curie, 2001; Van der Walt *et al.*, 2002).

2.2.1.3 Waste water

Upstream from Potchefstroom effluents are being discharged in the Mooi River through agricultural activities, diamond diggings, and peat mining. Two pump stations, namely the Botha and Eland street pump stations, located downstream from Potchefstroom, have the potential of overflowing into the Mooi River. The waste water treatment plant of Potchefstroom is located at the southern edge of the town. One of their core objectives is to treat sewage and discharge it back in the Mooi River, and to ensure that the effluent does not pose threats to human health and the ecosystem (Nel, 2011). However, the plant may overflow during high rainfall conditions and the effluents (untreated/semi-treated) may end up in the Mooi River.

2.2.1.4 Recreation

The Mooi River, its tributaries, and dams are also used for recreational activities, such as fishing, motor and sail boating, kayaking, and swimming.

2.2.2 Wonderfontein Spruit

In the WFS, mining and waste water treatment plants are the main contributors to pollution.

2.2.2.1 Mining (heavy metals)

From the 1930's large scale mining ruled the upper and middle catchment of the WFS. The mines are mostly concentrated in the Krugersdorp and Carletonville areas (Van der Walt *et al.*, 2002). Underground flooding of abandoned mines, together with current large scale mines that discharge their effluent and storm water into the WFS (Riedel, 2003; Van der Walt *et al.*, 2002), resulted in pollution of both surface and underlying dolomitic water resources (Coetzee *et al.*, 2006; Le Roux, 2005). Waste effluent, due to mining activity, can cause a profound and irreversible destruction of ecosystems. Gold mining is the biggest, single source of waste, and contributes to dust, soil, surface and groundwater pollution (Lieverink, 2015). Underground dolomite is divided into numerous compartments (Van der Walt *et al.*, 2002), and groundwater flow forms continuous connections between mining areas (Barnard *et al.*, 2013). Some of these compartments have dewatered (Van der Walt *et al.*, 2002), resulting in Acid Mine Drainage (AMD), and dams in the WFS catchment are contaminated with the effluent (Pelser, 2015).

In the middle section of the WFS, the stream is diverted into a 1 m wide, 32 km long pipeline, in order to prevent water flowing back into dewatered compartments (Pelser, 2015; Van der Walt *et al.*, 2002). If storm water exceeds the capacity of the pipeline, water is discharged across a side-spill weir into the original WFS streambed (Barnard *et al.*, 2013).

The lower WFS is also dominated by large scale mining (Van der Walt *et al.*, 2002), that can result in a lower water table and the formation of sink holes (Pelser, 2015). Winde (2010) stated that, although the WFS often dries up during dry months before converging with the Mooi River, it is able to reach Potchefstroom indirectly through the Boskop-Turffontein compartments.

Concentrations of heavy metals and radioactive compounds are concerns regarding mine related pollution in the WFS (Le Roux, 2005). Sediments of the WFS and dams located in the tributary are constantly enriched with heavy metals, such as uranium and thorium (Winde, 2009). If not controlled, radionuclides, and their daughter products, could be released into downstream water, and it can be hazardous to human health. The water quality may exceed drinking water limits for radioactivity (Le Roux, 2005). However, Winde (2009), cited by Annandale (2014), claimed that the WFS contains toxic uranium waste as a result of AMD, but pollution is minimised through dilution, and the drinking water purification works of Potchefstroom purifies the water to such an extent that traces of uranium in Potchefstroom's drinking water are below limits set by

the World Health Organization (Annandale, 2014). The mines in the WFS area use lime in order to treat their effluents and this, together with the dolomitic geology, help neutralising the effects of AMD (Pelser, 2015). However, if tailings dams have pH levels as low as 1.7, concerns can be raised regarding AMD (Wittmann & Förstner, 1977).

2.2.2.2 Informal settlements

Informal settlements surrounding the Donaldson Dam sometimes have poor sewage systems and the waste water effluents contribute to elevated loads of nutrients (Opperman, 2008).

2.2.2.3 Waste water

Communities live near the WFS (Pelser, 2015), and numerous WWTP's discharge their effluent back into the WFS (Nel, 2011). In the upper WFS, Flip Human (treated sewage for Roodepoort, Kagiso and Krugersdorp) and Randfontein Estates Gold Mining Company Limited (REGM) Cooke 2 (treated sewage for REGM mine) are two major WWTP's. There are also six additional WWTP's in the lower WFS (Le Roux, 2005).

2.2.3 Gerhard Minnebron

2.2.3.1 Peat Mining

The GM Eye is one of the lowest outflow points of the interconnected dolomitic compartments (Winde, 2008), and it's well-known for peat that is mined on the farm in the wetland below the eye (Le Roux, 2005). Le Roux (2005) argued that, although peat mines do not contribute to point sources of pollution, there might be a possibility of sedimentation and trace metal pollution. According to DWA (2009), peat mining is able to decrease habitat integrity in this area. However, Winde (2008) stated that although former studies discussed the possibility of peat mine related pollution of GM, there is still a lot of unknown factors regarding this topic. This study of Winde (2008) was conducted to see whether there is a possibility that peat has the ability to remove uranium and other heavy metals from polluted waters. A previous study by Coetzee *et al.* (2006) was done to see if wetlands are able to aid with mining pollution due to their ability to concentrate heavy metals. The outcome of the Coetzee *et al.* (2006) and Winde (2008) studies was that the wetlands act as pollution sinks, but metals may be remobilised, which can pose a threat to downstream water users. DWAF (1999) did a study on the Mooi River between 1997 and 1998, and found higher radionuclide levels in streams near mining areas, but that the quality improved downstream for Potchefstroom's water users. In their study, other factors that help to reduce radionuclides (like rainfall, and the underlain dolomite resulting in the neutralisation of acid

mine waters) were also taken into consideration (DWAF, 1999). According to Walmsley (1988) wetlands do not only play an important role as water reservoirs, but they can act as stream flow regulators, flood attenuators, natural water filters, and soil erosion regulators.

2.2.4 Wasgoed Spruit, including Poortjie Dam

The WS contains effluents from Poortjie Dam, industrial effluents from Potchefstroom, along with urban and storm water runoff (Figure 2-2). All these effluents flow into the Mooi River without prior treatment (Pelser, 2015). The water quality in the WS can thus have a major impact on water quality in the Mooi River.

2.2.4.1 Waste water

Spitskop Spruit, as well as Poortjie Dam (Figure 2-2), directly impacts the water of the Mooi River, because they contribute to the flow in the WS. The water of Spitskop Spruit, drains the north-western part of Ikageng (forms a small wetland area; Wyma, 2012). Ikageng, as well as Promosa, are informal and semi-formal communities (Van Aardt & Erdman, 2004) and besides utilising the water, these areas also deposit effluents back. The Poortjie Dam, downstream from the Spitskop Spruit (Wyma, 2012) is often polluted by sewage, due to blocked pump stations (Nel, 2011), and as a result the Poortjie Dam often experiences severe blooms of potentially toxic cyanobacteria.

2.2.4.2 Phospho-gypsum heap

A phospho-gypsum heap is located east of the Poortjie Dam, and can pollute the water, especially with sulfates and phosphates (Nel, 2011). Phosphorus rich gypsum “clouds” often hang over the dam (personal observation), as a result of eastern winds picking up and carrying gypsum particles. Gypsum particles settling down into the water, contribute to high phosphorus and sulfate concentrations in the dam.

2.2.4.3 Industrial effluents

Effluents from the industrial area of Potchefstroom flow into the WS opposite the Nestlé factory. Sources of these effluents are unknown as they arise from a variety of underground pipe systems, but according to Nel (2011) it is possible that illegal discharges may find their way into the WS. Annual studies, conducted with third year students from the North-West University and UNISA, show consistently and extremely high levels of total dissolved salts (TDS) and electrical conductivity (EC) in the WS in the industrial area. High TDS levels are mainly the result of high

sulfate concentrations, arising from effluents in this area (personal communication: Prof. S. Janse van Vuuren and Prof. J. Taylor).

2.2.5 Loop Spruit

No sampling was done in the LS, but several land-use activities in the LS may have an effect on water quality of the Mooi River and therefore it is briefly mentioned below.

2.2.5.1 Mining

The LS is also affected by mining activities, but to a much lesser extent than the WFS. Some of the goldmines located between the WFS and the LS also discharge water into the LS (Van der Walt *et al.*, 2002).

2.2.5.2 Agriculture and irrigation

In the LS, as well as the Mooi River downstream from the confluence, farming activities, including irrigation, take place. In the LS farmers extract water mostly from Klipdrift Dam for irrigation of their crops (Nel, 2011) and animals (Erasmus & De Kock, 2015).

2.2.5.3 Waste water

The LS obtains waste water from Khokosi at Fochville and other smaller WWTP's (Nel, 2011).

2.3 Sampling sites

The same sampling sites monitored on a regular basis by the JB Marks Municipality were selected for this study. Data on environmental variables at these sites will be used for comparison with phytoplankton results obtained during this study.

In the following paragraphs a short description of each sampling site monitored during this study is given, as well as information on why these locations were selected. The position of all sampling sites is illustrated in Figure 2-1 and Figure 2-2.

2.3.1 Mooi River sites

2.3.1.1 • Upstream from Boskop Dam (UBD: 26°26'42.25"S; 27°7'6.02"E)

This site is located about 48 km downstream from the Mooi River's origin and about 3 km upstream from the confluence of the Mooi River and WFS tributary. At this site, clear water flows

slowly over the rocky streambed. The substrate is sandy, and aquatic vegetation is prominent. The stream is shaded by overhanging riparian vegetation. This site is affected by agricultural activities, specifically feedlots, fertiliser pollution and the impacts of diamond diggings upstream. UBD is located about 10 km upstream from the Boskop Dam Inlet (BDI) site.

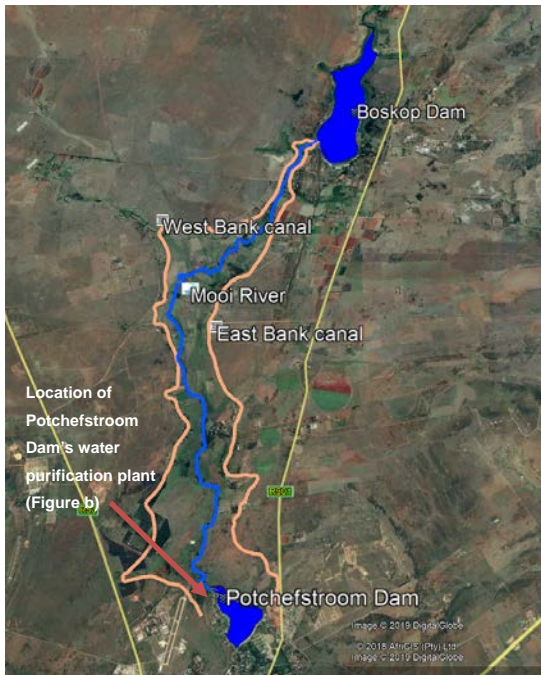
2.3.1.2 • Boskop Dam Inlet (BDI: 26°30'52.52"S; 27°7'28.34"E)

This site has a small, relatively slow flowing ripple stream and a substrate that consists of mud. Reeds and big trees overshadow this area. This site is affected by both the water of the main stream of the Mooi River and the WFS tributary that is situated upstream, as well as agricultural activities that take place in between (from the confluence to the Boskop Dam inlet). GM may also have an influence on this site. BDI is located about 15 km upstream from the Boskop Dam Canal (BDC) site.

2.3.1.3 • Boskop Dam Canal (BDC: 26°39'44.32"S; 27°5'7.69"E)

The Potchefstroom Dam water treatment works (WTW's) can be divided into two phases. One of the phases receives water from the West Bank canal (Figure 2-3b; TCEMP, 2013). Before purification, samples were taken from a division box at the plant. Activities between BDI and BDC include all possible upstream pollution mentioned above, together with agricultural activities and pollution by an ammunition manufacturing company situated near Boskop Dam. Dust containing pollutants from the ammunition manufacturing company are blown in the area, resulting in the contamination of both the surrounding soil and surface water (Hamilton-Atwell, 1999). BDC is located about 0.16 km from the Potchefstroom Dam (PD) site.

a)



b)

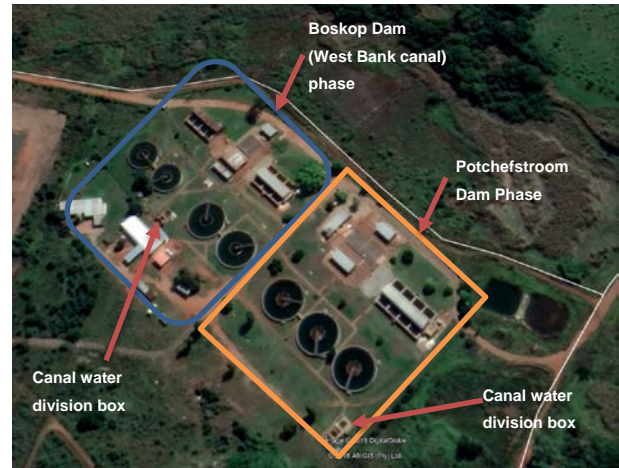


Figure 2-3 a) Location of the West Bank and East Bank canals; b) Layout of the Potchefstroom Dam's water treatment works located next to the Potchefstroom Dam (Google Earth Pro, 2018).

2.3.1.4 • Potchefstroom Dam (PD: 26°39'48.20"S; 27°5'11.54"E)

The other phase at the Potchefstroom Dam's WTW's, receives raw water from the Mooi River at the Potchefstroom Dam (TCEMP, 2013). Samples were taken at the division box (before purification) from the phase of Potchefstroom Dam (Figure 2-3b). Agricultural activities are the main sources of pollution between Boskop and Potchefstroom Dams. PD is located about 4 km upstream from the confluence of the Mooi River with the WS tributary, and about 5 km's from the Retief Street Bridge (RB) site.

2.3.1.5 • Retief Street Bridge (RB: 26°42'28.40"S; 27°6' 19.87"E)

This site has a clay covered substrate, with water moving at a relatively slow pace (ripple stream). Large trees cover this area. This location may be impacted by the WS flowing into the Mooi River about 1 km upstream from this site. The WS carries high loads of TDS and often cyanobacteria. Urban and storm water runoff from the city of Potchefstroom can also influence this site. RB is located about 4.5 km upstream from the South Bridge (SB) site.

2.3.1.6 • South Bridge (SB: 26°45'9.40"S; 27°6'1.19"E)

This site is located just outside Potchefstroom on the road to Viljoenskroon. It is located just upstream from the JB Marks local WWTP. The Mooi River passes an extensive area of wetlands and reed beds before reaching the SB. The water at this site is covered by willow trees. The streambed consists of clay, and water has a ripple effect. The cumulative influence of all activities in the city, as well as the contributions of the WFS, GM, WS and LS, may affect this area. The WWTP is located about 500 m downstream from SB, and 1.5 km before the downstream final effluent (DFE) site.

2.3.1.7 • Downstream final effluent (DFE: 26°45'50.44"S; 27°5'29.00"E)

This site is located 1 km downstream from the JB Marks WWTP that releases purified water back into the Mooi River. This site is shallow, consisting of very rapidly flowing water. The substrate at this site is mostly covered with rocks and a cement bridge. No trees or aquatic plants cover the area where the samples were taken. A company selling instant lawn, is located near this site and fertilisers used on lawns may have an influence on water quality at this site.

2.3.1.8 • Kromdraai (Krom: 26°52'49.48"S; 26°57'51.55"E)

This site is located approximately 25 km downstream from the previous site, and it is located about 1.5 km before the confluence of the Mooi and Vaal Rivers. The area has a variety of aquatic and land vegetation. Samples were taken just downstream from a weir built in the river (Figure 2-4). As a result of overflow at the weir, water is rapidly flowing. The influence of agricultural activities, including, farming, grazing, and irrigational activities, may have an impact on the water quality in this area.



Figure 2-4 Location of Kromdraai, about 25 km downstream from the WWTP and upstream from the confluence with the Vaal River (Nel, 2011).

2.3.2 WFS site

2.3.2.1 • Wonderfontein Spruit at Muiskraal (WFS: 26°26'11.18"S; 27°9'4.03"E)

The water flows from a round cement canal, just before the site where samples were taken. The water at this site is very shallow, aquatic vegetation (especially reeds), as well as shrubs and overhanging trees cover the area. This site is situated in the WFS tributary and will illustrate the effect of mining activities on the Mooi River. Other anthropogenic activities include untreated sewage and effluents from small communities. This site is located about 3 km upstream from the confluence of the WFS tributary and the Mooi River, and 7 km from BDI.

2.3.3 GM site

2.3.3.1 • Gerhard Minnebron (GM: 26°30'48.13"S; 27°7'41.59"E)

Supplying 60-80 Megalitres (ML) of water per day, the GM Eye is known as the largest natural fountain in the southern hemisphere (TCEMP, 2013). The fountain is fed by underground dolomitic water, which forms part of the larger Boskop-Turffontein dolomite compartment (Winde, 2006; discussed in section 2.1). Although GM has a surface catchment area originating at the Eye (Figure 2-5), it is categorised as a groundwater resource. Due to constant interaction between surface and groundwater in this area, GM is regarded as a very complex catchment, which makes

the exact contribution of the GM supply to Boskop Dam uncertain (Le Roux, 2005). Because GM is also located at a very low outflow point (Winde, 2008), it makes this area extremely vulnerable with regard to exploitation and pollution from upstream water users (TCEMP, 2013). The GM sampling site is located about 5 km downstream from the GM Eye.

Water is transported from the GM Eye to Boskop Dam in a concrete lined cement canal. The slow moving water transported in the cement canal is extremely clear and non-turbid, because it originates from a natural fountain, but unfortunately it is directly and indirectly affected by die polluted WFS tributary. A substantial amount of peat is also mined around this area. The GM site is located about 3 km upstream from the Boskop Dam, and its water reaches the dam through the canal described above (Figure 2-1).

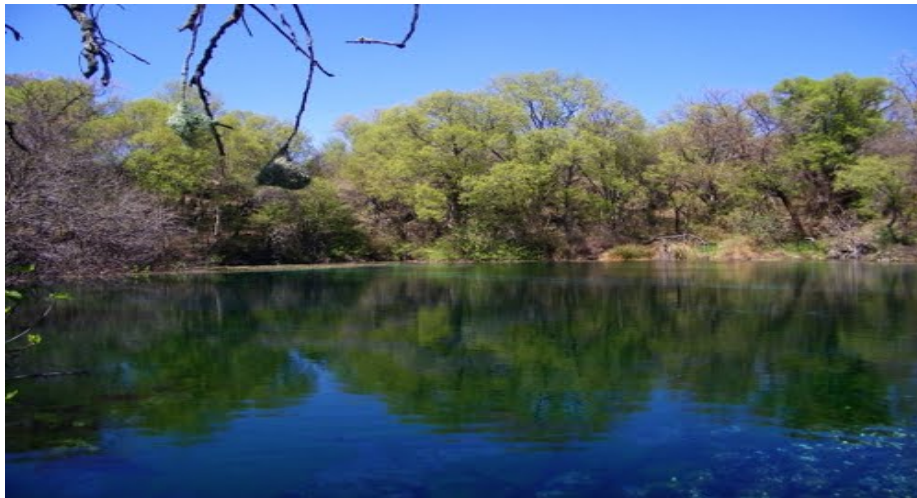


Figure 2-5 The Eye of Gerhard Minnebron showing clear fountain water (Muller, 2011).

2.3.4 WS site

2.3.4.1 • Poortjie Dam (WS: 26°42'31.44"S; 27°2'19.80"E)

Poortjie Dam is not situated on the Mooi River system, but its water flows via the WS into the Mooi River in the city of Potchefstroom and it might, therefore, have a huge influence on the water quality of the Mooi River. This site is well-known for extensive blooms of cyanobacteria, coupled with high levels of turbidity as was observed during the study period (Figure 2-6). Cyanobacteria blooms often form large green scums on the water surface, and colonies (particularly those of *Microcystis wesenbergii*) were visible with the naked eye. This site may be affected by anthropogenic impacts, such as untreated and illegal effluent discharges, through informal settlements, and blocked pump stations. Pollution by the phospho-gypsum heap may also find its way into the water resource. Water from this site flows into the WS for about 7.5 km

until it joins the Mooi River. From the confluence of the WS and the Mooi River it is about 800 meters downstream to the RB site.

a)



b)



Figure 2-6 a) Location of sample site at the Poortjie Dam b) Surface scum of cyanobacteria floating on the surface of Poortjie Dam (photographs: Marianke Saayman & Evashi Jansen, Potchefstroom Herald, 08/07/2016).

2.4 Material and methods

2.4.1 Water sampling for phytoplankton analysis

Eleven sites, subjected to different sources of pollution, were selected in the Potchefstroom area, eight of which were located along the Mooi River (described in section 2.3). In addition, one sample was taken from each tributary. A sampling site was situated in the WFS to show whether mining effluents have an impact on the water quality of the Mooi River. Another site was situated in the GM tributary, because this tributary obtains water directly from the WFS through dolomitic compartments that feed the Boskop Dam (source of drinking water for Potchefstroom). A third sampling site was located in the WS, contributing to high inflows of TDS and cyanobacteria in the Mooi River.

Surface water grab samples were collected on a monthly basis from January to December 2015. Phytoplankton sampling was done at the exact same locations and dates that water physico-chemistry analyses were done by the JB Marks Municipality in Potchefstroom. Sampling was done in the mornings, starting upstream from the Mooi River at Muiskraal and ending downstream at Kromdraai (Figure 2-1), just before the confluence of the Mooi and Vaal Rivers.

Water for phytoplankton analyses was sampled by lowering a bucket about 30 cm into the water. At each site a sub-sample of water was transferred to a 100 ml plastic bottle. Two millilitres of 37% formaldehyde solution (formalin) were added to each 100 ml sub-sample in order to preserve the phytoplankton. According to John *et al.* (2002) formalin is the most common liquid to use as preservative, although it is hazardous and can cause changes in cell dimensions and slight distortion of chloroplasts. The lower the concentration the less damage it causes, but the concentration must still be sufficient for successful preservation. Cell contents must be clearly visible for accurate identification of phytoplankton, and Janse van Vuuren & Taylor (2015) prefer using formalin over Lugol's solution, because the latter often discolours cell contents thereby obscuring cell structures.

2.4.2 Phytoplankton enumeration

Each 100 ml sub-sample was shaken vigorously to ensure even distribution of phytoplankton cells. Water from the sub-sample was transferred to a metal container, after which a mechanical hammer was used to pressure-deflate the gas vacuoles of cyanobacteria. The hammer applied a pressure of 49.5 kPa, required to collapse the gas vacuoles of cyanobacteria (Walsby, 1971).

Depending on the concentration and density of the phytoplankton and suspended material, a known volume of water (50 or 100 ml) was filtered through a cellulose nitrate filter with a pore size of 0.45 μm . If the concentration of the phytoplankton or suspended material was relatively high, 50 ml of sample was filtered, while 100 ml of sample was filtered when phytoplankton concentrations were low or when the water was relatively free of suspended material. The filtering process aids in concentrating the phytoplankton cells for counting. The phytoplankton gathered on the filter paper was transferred to 10 ml tubes filled with distilled water. A vortex mixer was used to remove the phytoplankton from the filter paper and to re-suspend it into 10 ml of distilled water. WS was always rich in phytoplankton and therefore this sample was not filtered.

Depending on the phytoplankton concentration, 1 to 6 ml of the sample, containing suspended phytoplankton, was extracted with a Finn pipette and transferred to sedimentation chambers. The remaining volume of the sedimentation chamber was filled with distilled water and a circular glass cover slip was placed on top of the chamber in order to avoid evaporation. Brierley *et al.* (2007) recommended the use of sedimentation chambers, applying the Utermöhl technique (Utermöhl, 1931, 1958), for studies of the abundance, composition and biovolume of phytoplankton in rivers and lakes.

The sedimentation chambers were placed in a desiccator for 48 hours (24 hours settling time per cm length of the sedimentation tube) to allow the phytoplankton to settle to the bottom. The bottom of the desiccator was filled with water to ensure humid conditions, preventing evaporation from the samples. The above mentioned procedures were repeated for each sample. This method was described by Utermöhl (1931, 1958) and modified by Lund *et al.* (1958). After a minimum of two days the phytoplankton was identified to genus level using an inverted Zeiss light microscope and a variety of literature and text books such as Taylor *et al.* (2007), John *et al.* (2002), Wehr & Sheath (2003), and Janse van Vuuren *et al.* (2006).

It proved to be useful to scan the samples at a variety of magnifications before quantitative analysis was undertaken. Low magnification revealed large taxa, while high magnification revealed some of the smaller taxa. Phytoplankton counting was done using a whipple eyepiece graticule inserted into one of the eyepieces of the microscope (Figure 2-7). The transect counting technique, described by Lund *et al.* (1958), was used for phytoplankton counting and it is illustrated in Figure 2-7. The glass bottoms of the sedimentation tubes were examined in transects and all phytoplankton cells inside the graticule were counted. According to Janse van Vuuren & Taylor (2015) a minimum of 200 cells need to be counted in each sample. This helps to ensure and strengthen statistical calculation of the data. The counting procedure involves recording the taxa observed and the number of phytoplankton cells for each taxon in a known area (transect) of the counting chamber. For densely packed colonies, like *Microcystis*, an estimation of cells were made within each small square of the Whipple graticule and then multiplied by the number of squares in the grid that the colony occupied. According to Lund *et al.* (1958) a single count is enough to give an estimate of phytoplankton abundance for phytoplankton cells that have settled randomly in the sedimentation chamber. The sample site name, date of sampling, sub-sample volume, number of transects counted, and the number of cells counted for each phytoplankton genus were noted.

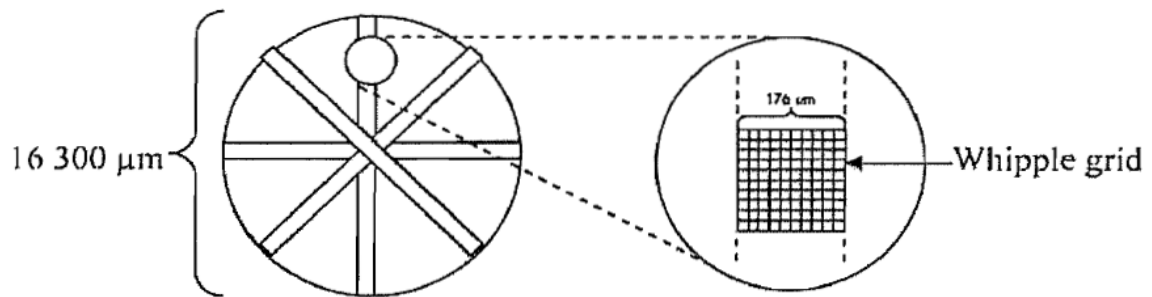


Figure 2-7 A line diagram showing the orientation of transects and the Whipple grid used for algal enumeration (Swanepoel *et al.*, 2008).

The quantification of the phytoplankton genera present at each site was done using Microsoft Excel spreadsheet calculations. The sub-sample volume transferred to the sedimentation chambers, as well as the number of cells and transects counted, were considered when the calculations were made. The results were used to determine the number of genera, total biomass in terms of cells/ml, successional patterns, as well as the percentage composition of phytoplankton taxa at a given time.

2.4.3 Physico-chemical analysis

Water quality data, for each of the eleven sampling sites mentioned above, was obtained from the water testing laboratory of the JB Marks Municipality in Potchefstroom. Although the municipality has a non-accredited water testing laboratory, they verify their results by participating in the South African Bureau of Standards' proficiency testing scheme. Physico-chemical variables, along with their method numbers, are presented in Table 2-1. Physico-chemical data for the WS are limited, and the crosses in the last column ("WS") of Table 2-1 indicate the available variables for the WS.

Table 2-1 Physico-chemical variables (including their method numbers) obtained from the JB Marks Municipality for all sampling sites from January to December 2015.

Physico-chemical environmental variables	Abbreviation	Unit of measurement	WS
Electrical Conductivity (method number: SANS 7888)	EC	mS/m	X
Total Dissolved Solids (method number: SANS 5213)	TDS	mg/l	X
Calcium (method number: SANS 6265)	Ca ²⁺	mg/l	
Magnesium (method number: SANS 6265)	Mg ²⁺	mg/l	
Chloride (method number: SANS 374)	Cl ⁻	mg/l	
Fluoride (method number: SANS 10359-1)	F ⁻	mg/l	
Sulfate (method number: SANS 6310)	SO ₄ ²⁻	mg/l	X
Ammonia (method number: SANS 5217)	NH ₃ ⁺	mg/l	X
Nitrate (method number: SANS 5210)	NO ₃ ⁻	mg/l	X
Orthophosphate (method number: SANS 6055)	PO ₄ -P	mg/l	X
pH (method number: SANS 10523)		pH units	X
Turbidity (method number: SANS 375)	Turb	NTU	X

Heavy metals analyses were done by the Council of Geoscience, but obtained from JB Marks Municipality. Data on heavy metal variables are presented in Table 2-2. Only one method (TM-CHE017) was used for the analyses of all heavy metals listed. Heavy metal concentrations were only measured at the first 4 sites located in the Mooi River (UBD, BDI, BDC, and PD) and at the sites located in the WFS and GM tributaries.

Table 2-2 Heavy metal variables obtained from JB Marks Municipality (analysed by the Council for Geoscience) for UBD, BDI, BDC, and PD located in the Mooi River and the two sites located in the WFS and GM tributaries (January to December 2015).

Heavy metals TM-CHE017	Abbreviation	Unit of measurement
Copper	Total Cu	µg/l
Hexavalent Chromium	Total Cr(VI)	µg/l
Iron	Total Fe	µg/l
Lead	Total Pb	µg/l
Manganese	Total Mn	µg/l
Nickel	Total Ni	µg/l
Uranium	Total U	µg/l
Zinc	Total Zn	mg/l

2.4.4 Statistical analysis of data

Statistical analysis for phytoplankton and physico-chemical environmental variables was done using STATISTICA 13.3 (StatSoft Inc ©, 2017) software. In order to determine if the data was normally distributed, the Kolmogorov-Smirnov and Lilliefors tests for normality were used. It was decided to apply non-parametric statistics, because most of the data did not meet the assumption of normality. This result led to the following tests being performed: 1) The Spearman Rank Order Correlation test was used to determine if significant correlations existed between variables and the different sites. The heavy metal variables were excluded when correlations were done, because heavy metal data was not available for all sites. 2) In order to compare multiple independent variables, Kruskal-Wallis ANOVA was used, to determine whether significant differences existed between the data sets and between different sites. Descriptive statistics determined the valid N, mean, minimum, average, maximum and standard deviation.

Canonical Correspondence Analysis (CCA) was performed using CANOCO 4.5 (Ter Braak & Prentice, 1988). CCA's were used to determine the relationships between distribution of phyla and related environmental factors (Kent & Coker, 1992). Phytoplankton data analyses was performed using $\log(y + 1)$. The limited amount of heavy metal data can skew the data and was therefore excluded in CCA analyses.

Line graphs, area- and pie charts were composed using STATISTICA 13.3 (StatSoft Inc ©, 2017) software.

CHAPTER 3: RESULTS

“There is no such thing as “away”. When we throw anything away it must go somewhere.” Annie Leonard

3.1 Physio-chemical Environmental Variables

Anthropogenic activities taking place in the Mooi River catchment (discussed in Chapter 2, sections 2.2 and 2.3) can lead to eutrophication of this once pristine river system (Luyt, 2018). One of the main aims of the study was to see if three tributaries, namely the Wonderfontein Spruit (WFS), Gerhard Minnebron (GM) and Wasgoed Spruit (WS), have impacts on the Mooi River's water quality. The confluence of the Mooi River and WFS is located near the origin of the Mooi River and any activities taking place in the upstream section of a river can have an effect down the entire length of the river system (Dallas & Day, 2004). GM, another tributary, obtains its water from the GM Eye (Chapter 2, section 2.1), and feeds the Boskop Dam supplying potable water to Potchefstroom. Poortjie Dam flows into the WS tributary that converges with the Mooi River in Potchefstroom. Aside from these tributaries, there are many other activities (discussed in Chapter 2, section 2.2) in the catchment of the Mooi River that may contribute to water pollution, which emphasise the importance for monitoring the water quality regularly.

Phytoplankton (algae and cyanobacteria) require certain conditions for their highly selective habitats, and therefore react to physical and chemical changes in water (Prescott, 1969). The importance of these variables on algal dynamics has been discussed in several studies for the managing of surface waters and protection of aquatic ecosystems (Boney, 1989; Damásio *et al.*, 2007; De Huszar & Caraco, 1998; Hassan *et al.*, 2004; Kagalou *et al.*, 1999).

Physical variables are important because they directly influence aquatic organisms (Wetzel, 1983). Despite the importance of physical variables, only turbidity data was available for use in this study. Janse van Vuuren (2001) stated that: “...chemical variables (especially nutrients) play an extremely important role in the presence and succession of phytoplankton associations...” These two environmental variables are collectively known as the physico-chemical environmental variables (Oosthuizen, 2012).

Physico-chemical (including heavy metal) data of variables described in the paragraphs, will illustrate the influence of anthropogenic activities (Chapter 2, sections 2.2 and 2.3) on the water quality of the Mooi River. The influence of major tributaries (WFS, GM and WS) and their constituents on the water quality of the river will also become apparent. A comprehensive set of

physico-chemical data, that contains, amongst others, all data used for correlations (statistical significant differences; Appendix B and Appendix D), as well as descriptive statistics (Appendix C and Appendix E), will be presented in Appendices.

3.1.1 Nutrients

Nutrients contribute to the trophic status of the river, and nutrient concentrations in the Mooi River system and its surroundings are illustrated in Figure 3-1 to Figure 3-3.

The availability of nutrients, particularly inorganic nitrogen and phosphorus, results in algal growth and can cause eutrophication in rivers and lakes. The trophic status of rivers and lakes is therefore influenced by the amount of algae present (Carlson, 1977; Vollenweider, 1976). Three forms of nitrogen are generally measured in water bodies, namely ammonia, nitrates and nitrites (EPA, 2013), while phosphorus is measured in its dissolved form, namely inorganic orthophosphate ions (Dallas & Day, 2004). The only nutrient data available during this study was ammonia, nitrates, and orthophosphates. The JB Marks Municipality only measures nitrites for drinking water, not raw water. The sum of nitrates, nitrites and ammonia concentrations is regarded as dissolved inorganic nitrogen (DIN) – the components available for uptake by algae and other photosynthesising aquatic organisms (Dallas & Day, 2004). According to Dallas & Day (2004), the nitrate concentration contributes the largest fraction of DIN. The concentration of nitrite is usually so small, that it does not add much to DIN. Nitrite concentration sometimes barely exceeds 5%, while the ammonia and nitrate concentrations contribute to the rest of the sum (Dallas & Day, 2004). According to Borja & Collins (2004), the concentration that each variable contributes to DIN varies in different water bodies, and sometimes the nitrate concentration represents about 55% to 80% of the total DIN. Depending on the conditions in the water, it is possible that ammonia concentrations can exceed nitrate concentrations (Borja & Collins, 2004). Borja & Collins (2004) also found that, when nutrients are almost exhausted and phytoplankton populations decay, the ammonia concentration increases. The orthophosphate concentration is regarded as being equivalent to the dissolved inorganic phosphorus (DIP) concentration, which is the phosphorus source available for uptake by algae and aquatic organisms (Dallas & Day, 2004).

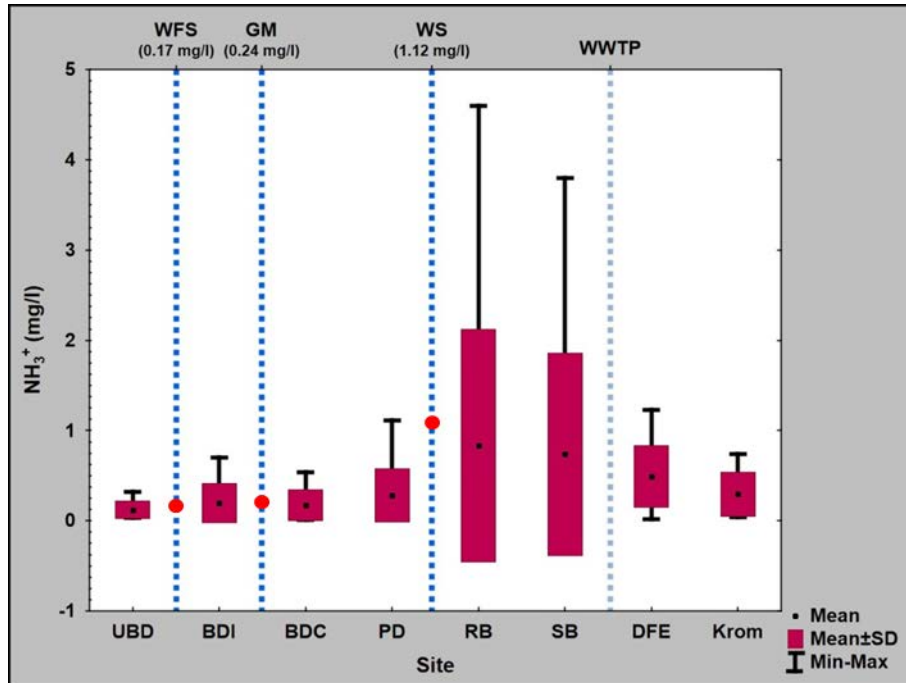


Figure 3-1 Box plot illustrating the ammonia (NH_3^+) concentration at the sites located in the Mooi River for the study period January to December 2015. The dark blue dotted lines indicate the position of the inflows of the WFS, GM and the WS, while the grey dotted line indicates the location of the WWTP. Red dots represent the mean ammonia concentrations for the WFS, GM and WS, while exact values are given in brackets.

The mean ammonia concentration was recorded 0.12 mg/l (Appendix C, Table C-1) at the origin of the Mooi River system (site UBD). After this site the WFS (mean ammonia concentration of 0.17 mg/l) joins the Mooi River. At BDI the mean ammonia concentration (0.20 mg/l) increased with 40%, compared to that at the origin. The GM site is situated about 20 km upstream from BDC, and at GM the mean ammonia concentration was measured as 0.24 mg/l. However, the inflow of GM does not seem to affect the Mooi River downstream, because the mean ammonia concentration at BDC (0.17 mg/l) decreased with 15%, from the BDI site located upstream. The mean ammonia concentration from the BDC site downstream to PD (mean of 0.28 mg/l) increased with 39% until the inflow of the WS in the city of Potchefstroom. The mean ammonia concentration for the WS was very high (1.12 mg/l), and showed a statistical significant difference with UBD, BDI, and BDC located upstream (Appendix B, Table B-1). It also had a huge influence on elevating ammonia levels further downstream in the Mooi River. A mean concentration of 0.83 mg/l was recorded at RB, located just downstream from the inflow of the WS. The maximum value for ammonia in the Mooi River was also recorded at RB (4.60 mg/l). From here the mean

concentrations gradually decreased downstream to the Mooi River's confluence with the Vaal River.

The ammonia concentration in the Mooi River ranged from <0.01 at BDI to 4.60 mg/l at RB (Appendix C, Table C-1). The overall mean ammonia concentration for the entire Mooi River system (excluding tributaries), over the whole study period, was 0.39 mg/l which is 56% higher than that of the WFS (0.17 mg/l) and 39% higher than the GM (0.24 mg/l) site, but 65% lower compared to that in the WS (1.12 mg/l).

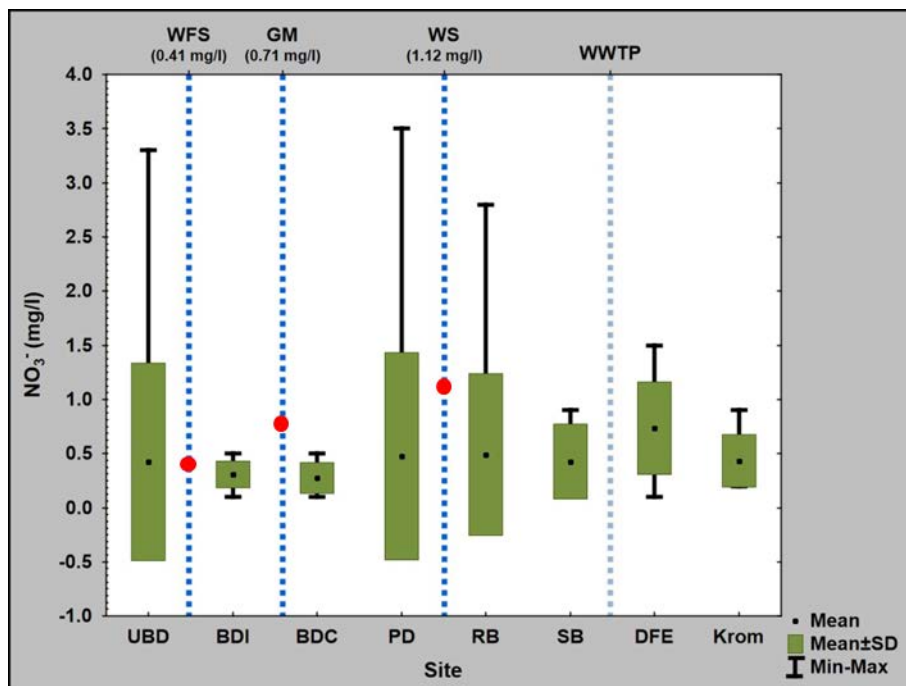


Figure 3-2 Box plot illustrating the nitrate (NO_3^-) concentration at the sites located in the Mooi River for the study period January to December 2015. The dark blue dotted lines indicate the position of the inflows of the WFS, GM and the WS, while the grey dotted line indicates the location of the WWTP. Mean nitrate concentration values for the WFS, GM and WS are given in brackets. Red dots represent the nitrate concentrations for the WFS, GM and WS, while exact values are given in brackets.

The mean nitrate concentration at the source of the Mooi River (UBD) was relatively high (0.43 mg/l, Appendix C, Table C-1). The WFS, flowing into the Mooi River after UBD, had a mean nitrate concentration of 0.41 mg/l, which is comparable to that at the source of the river. The mean nitrate concentration declined with 28% at BDI (0.31 mg/l) when compared to UBD. At the GM site a high mean concentration of 0.71 mg/l nitrate was recorded. However, the mean concentration in the Mooi River remained more or less constant after the inflow of GM (0.28 mg/l at BDC) when compared to BDI. Yet, PD showed an increase in mean nitrate concentration (0.48

mg/l), and the mean nitrate concentration at this site was 10% higher than the value recorded at the source of the river. The maximum nitrate concentration recorded for the Moor River, was at PD (3.50 mg/l). After PD, the WS flows into the Mooi River. In the WS, a high mean nitrate concentration of 1.12 mg/l was measured over the study period. However, high nitrate levels in the WS did not seem to have a huge effect on the concentrations at the sites downstream, namely RB (mean of 0.49 mg/l) or SB (mean of 0.43 mg/l), as the mean concentrations remained similar to the mean value of PD. At site DFE, situated 1 km downstream from the Waste Water Treatment Plant (WWTP) of the city of Potchefstroom, a mean nitrate concentration of 0.73 mg/l was recorded. Unfortunately no nitrate measurements were made at the WWTP to determine whether outflows from this plant could have contributed to maximum nitrate averages at site DFE. From this site the concentration decreased to the confluence with the Vaal River.

The nitrate concentration in the Mooi River ranged from <0.01 at UBD to 3.50 mg/l at PD (Appendix C, Table C-1). The overall mean nitrate concentration for the Mooi River was 0.45 mg/l, which was 9% higher than that of the WFS (0.41 mg/l), but 37% lower than that of GM (0.71 mg/l), and 60% lower than WS (1.12 mg/l).

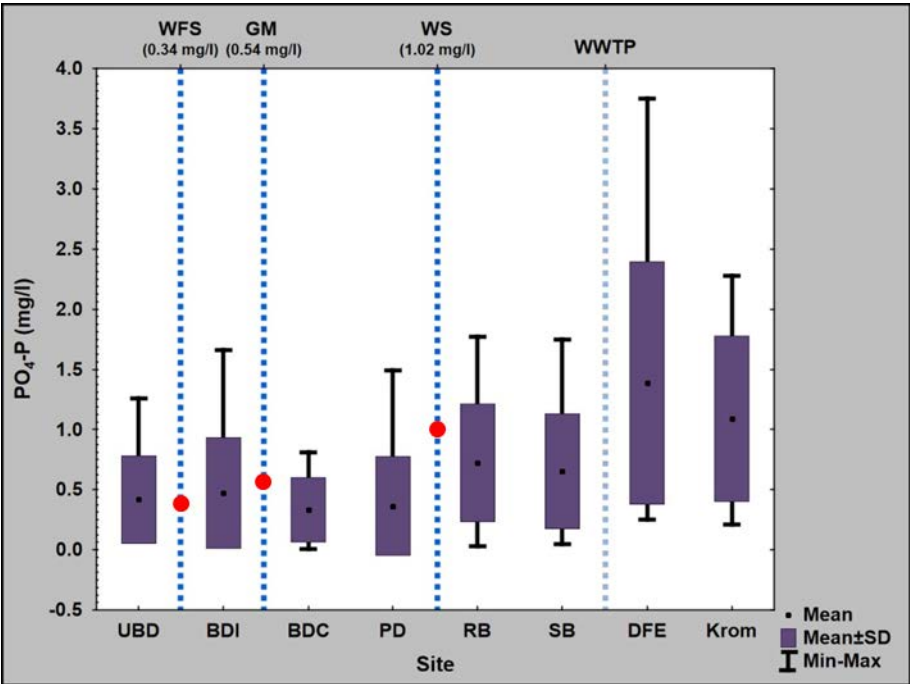


Figure 3-3 Box plot illustrating the orthophosphate ($PO_4\text{-P}$) concentration at the sites located in the Mooi River for the study period January to December 2015. The dark blue dotted lines indicate the position of the inflows of the WFS, GM and the WS, while the grey dotted line indicates the location of the WWTP. Red dots represent the mean orthophosphate concentrations for the WFS, GM and WS, while exact values are given in brackets.

At the origin of the Mooi River (UBD), orthophosphate concentrations were relatively high, displaying a mean of 0.42 mg/l (Appendix C, Table C-1). After UBD, the WFS converges with the Mooi River, importing a 19% lower mean orthophosphate concentration (0.34 mg/l) to the Mooi River. In spite of this, the mean orthophosphate concentration remained more or less constant downstream to BDI (mean of 0.47 mg/l). GM flows into the Mooi River with a 22% higher mean orthophosphate concentration (0.54 mg/l) compared to UBD. In spite of the fact that GM was rich in orthophosphates, it did not elevate the mean concentration at BDC, as the mean recorded at this site (0.33 mg/l) was 30% lower than at BDI (mean of 0.47 mg/l), located upstream from the point of inflow. From BDC to PD (mean of 0.36 mg/l), the mean concentration remained more or less constant. In the WS, a high mean orthophosphate concentration of 1.02 mg/l was recorded, and high concentrations in this tributary had a huge visible influence on concentrations at the RB (mean of 0.72 mg/l) and SB (mean of 0.65 mg/l) sites located downstream from the point of inflow. DFE, located downstream from the WWTP, showed the highest mean (1.39 mg/l) and maximum (3.75 mg/l) orthophosphate concentrations for the Mooi River system, and showed a statistical significant difference with BDC and PD upstream (Appendix B, Table B-1). From DFE, the mean concentration declined to the confluence with the Vaal River.

The orthophosphate concentration in the Mooi River ranged from 0.01 at BDC to 3.75 mg/l at DFE (Appendix C, Table C-1). The overall mean orthophosphate concentration for the Mooi River was 0.68 mg/l, which was 50% higher than that of the WFS (0.34 mg/l), 21% higher than GM (0.54 mg/l), but it decreased three-fold compared to WS (1.02 mg/l).

3.1.2 Total Dissolved Solids (TDS), Electrical Conductivity (EC) and Major ions

Although total dissolved solids (TDS) are naturally present in water, its concentration may increase as a result of mining, industrial effluents, treatment of industrial water and other anthropogenic inputs, such as agriculture (Srinivas, 2008; Weber-Scannel & Duffy, 2007). TDS is the sum of the total cation concentrations, such as sodium, potassium, calcium, magnesium, and the total anion concentrations, such as bicarbonate, carbonate, chloride, sulfate, and fluoride. Together these ions are known as the “major ions”. Because these ions carry a positive or negative charge, it gives water a conductive ability. TDS concentration therefore usually has a direct relationship with the electrical conductivity (EC) of the water (Marandi *et al.*, 2013). Increasing TDS concentrations may result in the proliferation of potential problematic algae, such as the dinoflagellates that have high salt tolerances and prefer higher salt concentrations (Janse van Vuuren, 2001). Data on major ions received from the JB Marks Municipality, include: calcium, magnesium, chloride, fluoride, and sulphate.

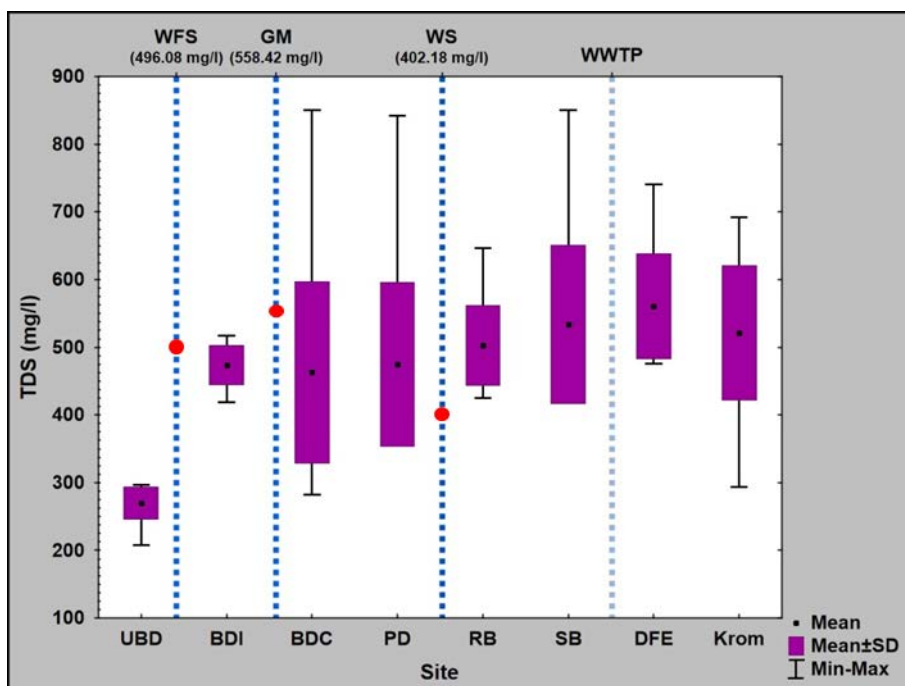


Figure 3-4 Box plot illustrating the TDS concentration at the sites located in the Mooi River for the study period January to December 2015. The dark blue dotted lines indicate the position of the inflows of the WFS, GM and the WS, while the grey dotted line indicates the location of the WWTP. Red dots represent the mean TDS concentrations for the WFS, GM and WS, while exact values are given in brackets.

The lowest mean TDS concentration (270 mg/l) was recorded at the source (UBD) of the Mooi River (Appendix C, Table C-1). UBD also showed a statistical significant difference with WFS, GM, and the last four sites downstream in the Mooi River (RB, SB, DFE, Krom; Appendix B, Table B-1). The WFS had a high mean TDS concentration of 496 mg/l which clearly influenced the TDS concentration in the Mooi River downstream from the convergence, as a mean TDS concentration of 474 mg/l was recorded at site BDI. After this site, GM (displaying a high mean of 558 mg/l) converges with the Mooi River. At BDC TDS concentrations (mean of 463 mg/l) were still high. This site, as well as SB, further downstream, showed the maximum TDS concentration (850 mg/l) recorded in the Mooi River over the entire study period. As the river continued its path downstream to PD, TDS levels remained high (mean of 475 mg/l). Downstream from PD, the WS (mean TDS of 402 mg/l) flows into the Mooi River. After the inflow of the WS, the mean TDS concentration kept on increasing downstream in the river. The highest mean TDS concentration recorded for the Mooi River, was just downstream from the WWTP, at DFE (mean 561 mg/l). From this site, TDS concentrations decreased towards Krom, located approximately 1.5 km upstream from the Mooi River's confluence with the Vaal River.

The TDS concentration in the Mooi River ranged from 208 at UBD to 850 mg/l at BDC (Appendix C, Table C-1). The overall mean TDS concentration for the Mooi River was 475 mg/l, which was 4% and 15% lower than that of the WFS (496 mg/l) and GM (558 mg/l) respectively, but 15% higher than that of WS (402 mg/l).

In general, similar patterns were observed between TDS concentration and EC in the Mooi River (compare Figure 3-4 and Figure 3-5).

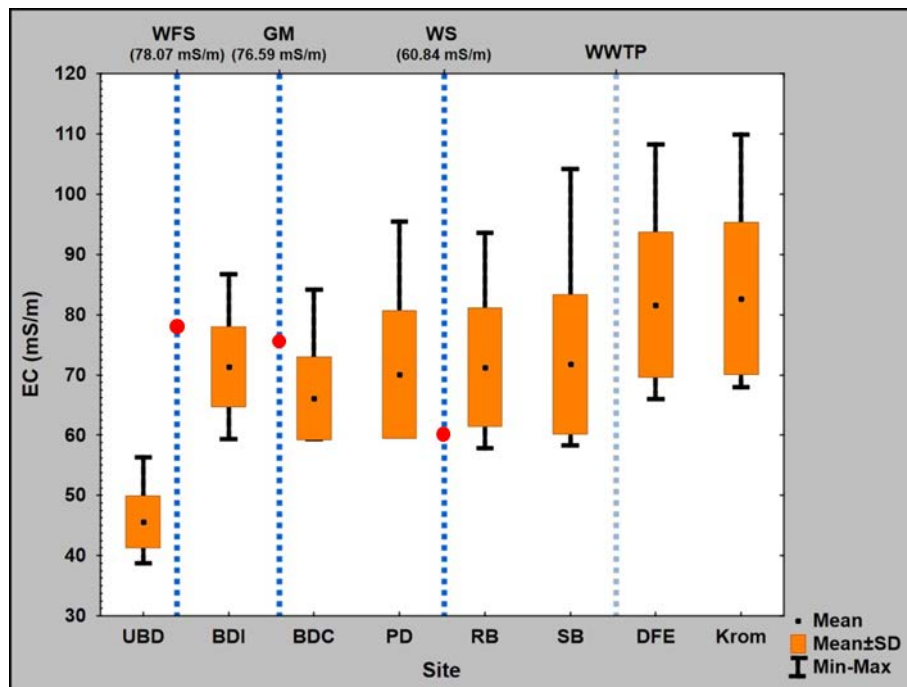


Figure 3-5 Box plot illustrating EC levels at the sites located in the Mooi River for the study period January to December 2015. The dark blue dotted lines indicate the position of the inflows of the WFS, GM and the WS, while the grey dotted line indicates the location of the WWTP. Red dots represent the mean EC levels for the WFS, GM and WS, while exact values are given in brackets.

In general, the lowest EC levels for the Mooi River were recorded at the origin of the river at site UBD (46 mS/m; Appendix C, Table C-1). EC at UBD also showed a statistical significant difference with all other sites during the study period, except the site in the WS tributary, and BDC in the Mooi River (Appendix B, Table B-1). The WFS flowed into the Mooi River with a high mean EC level of 78 mS/m, and contributed to a two-fold elevation in the mean EC level at BDI (71 mS/m). After this, the GM joins the Mooi River with a mean EC level of 777 mS/m. Apart from the high EC levels upstream from BDC, this site showed a 7% decrease in mean EC (66 mS/m) compared to BDI. The WS, with lower EC levels (mean of 61 mS/m), joins the Mooi River and it does not seem to have much of an impact on levels further downstream in the river. The high EC

levels remained more or less constant downstream to SB. The WWTP may have been responsible for higher mean EC levels found at DFE (82 mS/m) and Krom (83 mS/m). Krom also showed the maximum EC (110 mS/m) recorded for the whole study period in the Mooi River system.

The mean EC levels in the Mooi River ranged from 39 at the most upstream site (UBD) to 110 mS/m at the most downstream site (Krom; Appendix C, Table C-1). The overall mean EC for the Mooi River (70 mS/m) was 10% and 9% lower than that of the WFS (78 mS/m) and GM (77 mS/m) respectively, but 13% higher than that of the WS (61 mS/m).

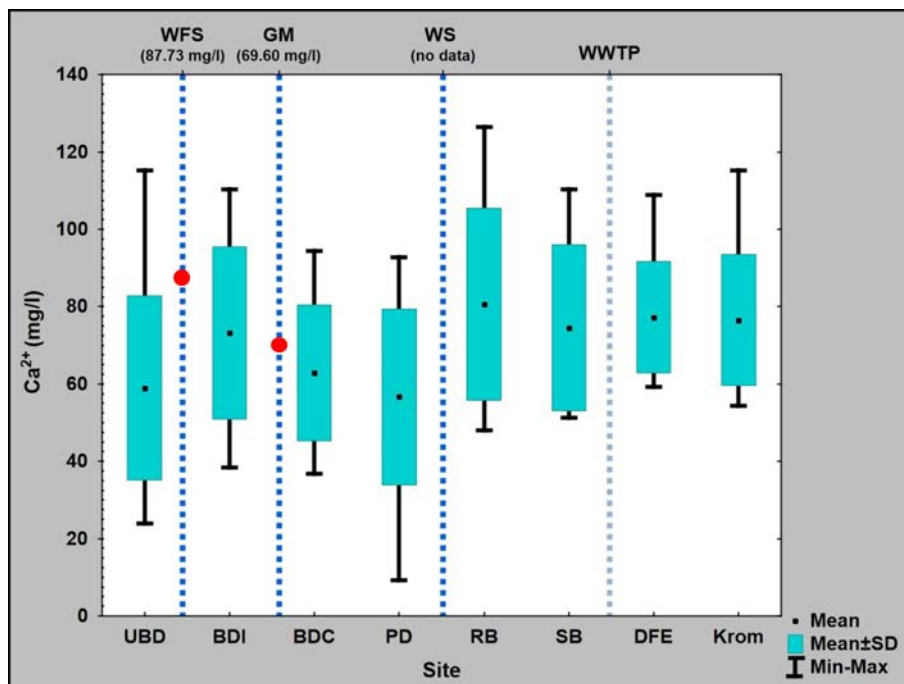


Figure 3-6 Box plot illustrating the calcium (Ca^{2+}) concentration at the sites located in the Mooi River for the study period January to December 2015. The dark blue dotted lines indicate the position of the inflows of the WFS and GM, while the grey dotted line indicates the location of the WWTP. Red dots represent the mean calcium concentrations for the WFS and GM, while exact values are given in brackets.

A mean calcium concentration of 58.93 mg/l was recorded at UBD (Appendix C, Table C-1). Inputs of calcium from the WFS (mean of 87.73 mg/l) explain the high mean concentration (73.20 mg/l) found at BDI. The GM, which flows into the Mooi River downstream from BDI, had the highest overall calcium concentration (maximum of 134.40 mg/l; Appendix C, Table C-1) recorded for the study period, although the mean value was much lower at 69.60 mg/l. As a result, the mean calcium concentration decreased at BDC, and further downstream to PD. Due to a lack of data, it is difficult to determine the influence of the WS on downstream sites where increases

in mean values were observed. The maximum calcium concentration in the Mooi River was measured after the WS inflow at RB (maximum of 126.40 mg/l; mean of 80.67 mg/l). From RB downstream to the confluence with the Vaal River, the mean calcium concentrations remained high and relatively constant.

The calcium concentration in the Mooi River ranged from 9.20 at PD to 126.40 mg/l at RB (Appendix C, Table C-1). The overall mean calcium concentration for the Mooi River was 70.06 mg/l, 20% lower than that of the WFS (87.73 mg/l), but comparable to that of GM (69.60 mg/l).

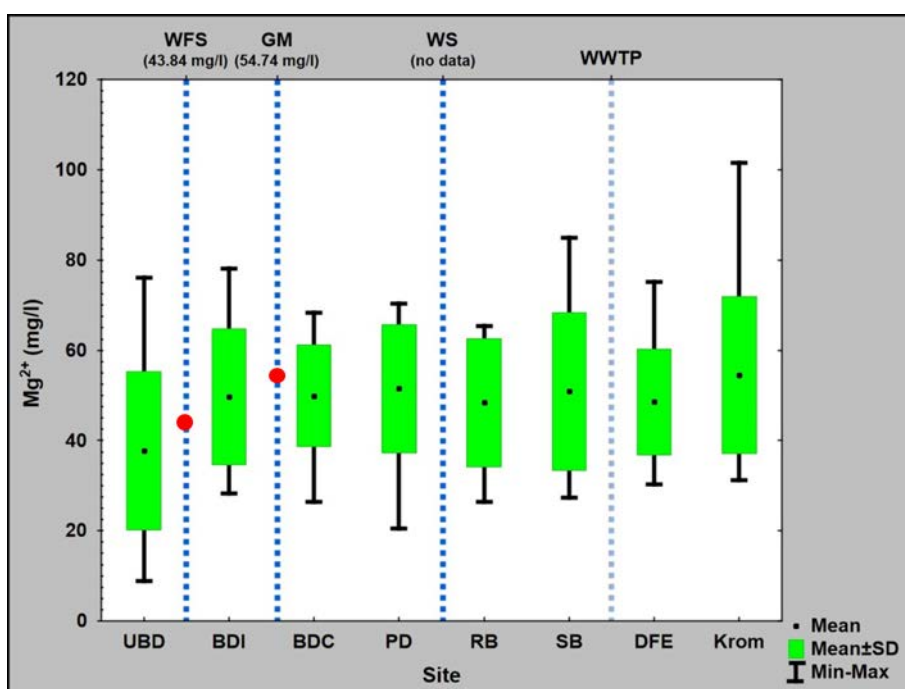


Figure 3-7 Box plot illustrating the magnesium (Mg^{2+}) concentration at the sites located in the Mooi River for the study period January to December 2015. The dark blue dotted lines indicate the position of the inflows of the WFS and GM, while the grey dotted line indicates the location of the WWTP. Red dots represent the mean magnesium concentrations for the WFS and GM, while exact values are given in brackets.

The minimum magnesium concentration (8.80 mg/l) was measured at UBD (mean of 37.74 mg/l Appendix C, Table C-1). The WFS showed higher magnesium concentrations (mean of 43.84 mg/l), that can explain the increase in the mean value of 49.70 mg/l at BDI. After BDI the GM (mean of 54.74 mg/l) converges with the Mooi River. The means of BDI and GM were comparable and therefore the inflow of GM had no apparent effect on magnesium concentrations downstream from site BDI (BDC and PD) where means of 49.93 and 51.49 mg/l, respectively were recorded. While, no magnesium data was available for the WS, it does not seem as if the concentrations at downstream sites were affected by the inflow of the WS, because mean concentrations at these

sites were similar to those at upstream sites (BDI, BDC and PD) of the river. The most downstream site in the Mooi River (Krom), showed the maximum magnesium concentration (101.50 mg/l).

The magnesium concentration in the Mooi River ranged from 8.80 at UBD to 101.50 mg/l at Krom (Appendix C, Table C-1). The overall mean magnesium concentration for the Mooi River (48.90 mg/l) was 10% higher than that of the WFS (43.84 mg/l), and 11% lower than that of GM (54.74 mg/l).

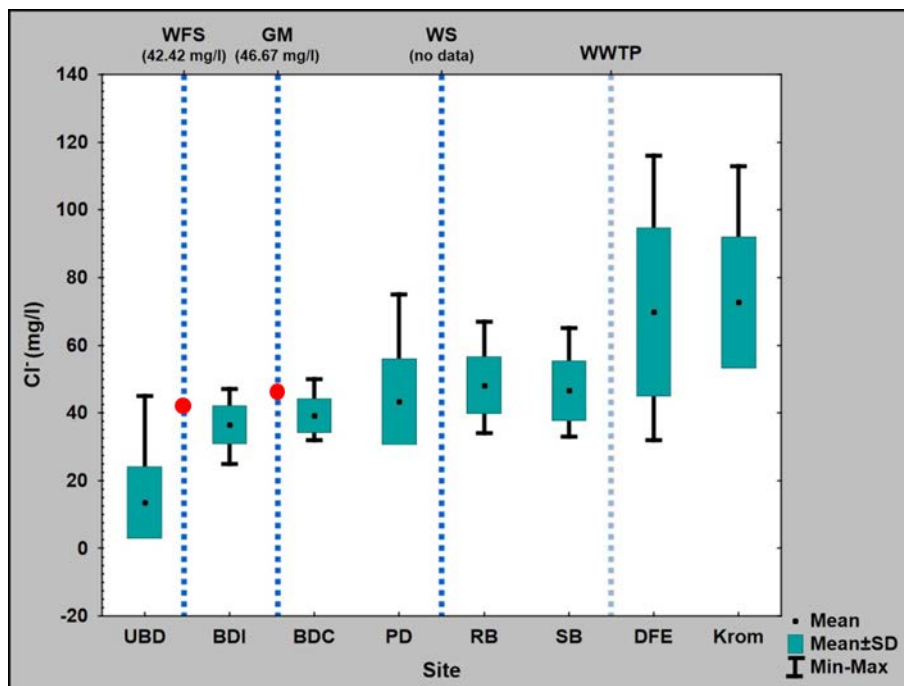


Figure 3-8 Box plot illustrating the chloride (Cl⁻) concentration at the sites located in the Mooi River for the study period January to December 2015. The dark blue dotted lines indicate the position of the inflows of the WFS and, GM, while the grey dotted line indicates the location of the WWTP. Red dots represent the mean chloride concentrations for the WFS and GM, while exact values are given in brackets.

The minimum chloride concentration (5.00 mg/l) was recorded at UBD (Appendix C, Table C-1). This concentration is extremely low compared to that in the WFS (minimum of 35.00 mg/l and mean of 42.42 mg/l). It is clear that the inflow of WFS into the Mooi River had an effect on chloride concentrations downstream of UBD, because a huge increase in chloride concentration was observed at BDI just downstream from the confluence (mean of 36.50 mg/l). Downstream from BDI, the GM also joins the Mooi River with high chloride concentrations (mean of 46.67 mg/l), contributing to elevated concentrations downstream from this site. Even though there was no chloride data for the WS, no extraordinary increase in mean values were noted at RB and SB.

However, after the water has been treated by the WWTP, and re-introduced into the river, the chloride concentrations increased significantly, clearly visible at DFE (mean of 69.83 mg/l) and Krom (mean of 72.67 mg/l). DFE also had the maximum chloride concentration (116.00 mg/l) recorded for the study period.

The chloride concentration in the entire Mooi River ranged from 5.00 at UBD to 116.00 mg/l at DFE (Appendix C, Table C-1). The overall mean chloride concentration (46.25 mg/l) for the Mooi River was only 8% higher than that of the WFS (mean of 42.42 mg/l), and very similar to GM's mean concentration of 46.67 mg/l.

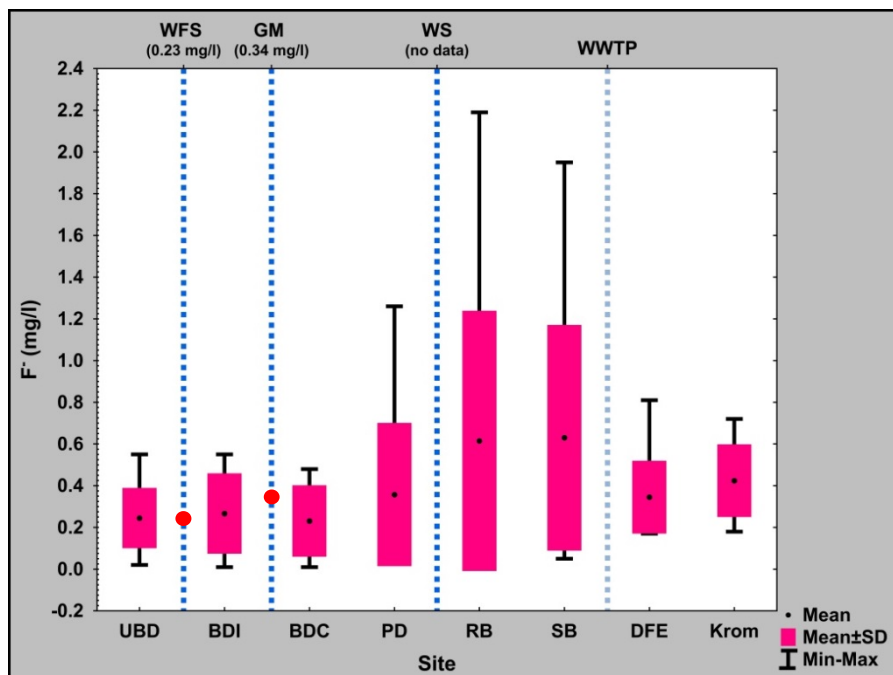


Figure 3-9 Box plot illustrating the fluoride (F^-) concentration at the sites located in the Mooi River for the study period January to December 2015. The dark blue dotted lines indicate the position of the inflows of the WFS and GM, while the grey dotted line indicates the location of the WWTP. Red dots represent the mean fluoride concentrations for the WFS and GM, while exact values are given in brackets.

A mean fluoride concentration of 0.25 mg/l was recorded at the source (UBD; Appendix C, Table C-1). After this, the WFS joins the Mooi River, with similar fluoride concentrations (mean of 0.23 mg/l), explaining why the mean fluoride concentration at BDI remained more or less constant. After this, the GM (mean of 0.34 mg/l) flows into the river, but it does not seem to have an effect downstream to BDC. Between BDC and PD a noticeable increase (36%) in mean fluoride concentration (from 0.23 to 0.36 mg/l) occurred. Due to the lack of fluoride data in the WS, it is difficult to judge whether it could have affected downstream sites, although it appears to be the

case, since RB (downstream from the inflow of the WS) showed a much higher mean value (0.62 mg/l), as well as the maximum fluoride concentration (2.19 mg/l) recorded in the Mooi River. The mean chloride concentration remained high to SB, and declined significantly downstream to DFE (mean of 0.35 mg/l) and Krom (mean of 0.42 mg/l).

The fluoride concentration in the Mooi River ranged from 0.01 at BDI and BDC to 2.19 mg/l at RB (Appendix C, Table C-1). The overall mean fluoride concentration recorded for the Mooi River system (0.39 mg/l) was much higher (41%) than the WFS (mean of 0.23 mg/l), and only and 13% higher than that of GM (mean of 0.34 mg/l).

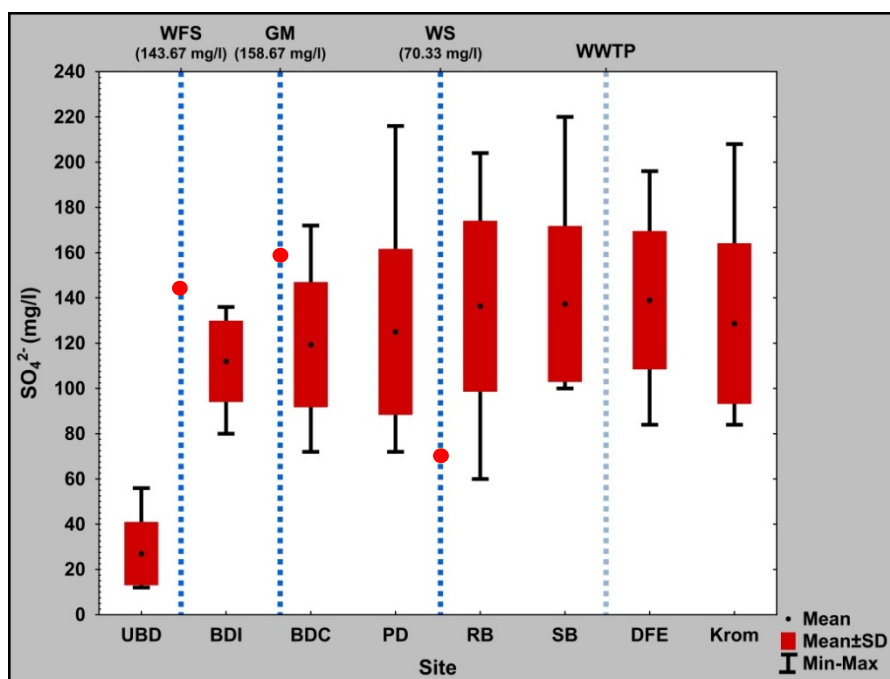


Figure 3-10 Box plot illustrating the sulfate (SO_4^{2-}) concentration at the sites located in the Mooi River for the study period January to December 2015. The dark blue dotted lines indicate the position of the inflows of the WFS, GM and the WS, while the grey dotted line indicates the location of the WWTP. Red dots represent the mean sulfate concentrations for the WFS, GM and WS, while exact values are given in brackets.

The sulfate concentration of 12.00 mg/l at the origin of the Mooi River (UBD) was the minimum recorded for the study period (Appendix C, Table C-1). UBD also showed a statistical significant difference with all the sites measured during the study period, except the sites in the WS tributary, and BDI in the Mooi River (Appendix B, Table B-1). Downstream from this site the WFS, with a high mean sulfate concentration of 143.67 mg/l, converges with the Mooi River. The WFS clearly had a huge impact on sulfate concentrations further downstream in the river, because

the BDI site showed a nine-folded increase in sulfate concentration (mean of 112 mg/l). After BDI the GM flows in the Mooi River with even higher sulfate concentrations (maximum of 344.00 mg/l; mean of 158.67 mg/l). These two tributaries definitely had a major effect downstream, as the mean concentrations further increased downstream to PD. Downstream from PD, the WS joins the river, with considerably lower sulfate levels (mean of 70.33 mg/l). In spite of the fact that relatively low sulfate concentrations were recorded at this site (located in the Poortjie Dam upstream from the actual Wasgoed Spruit), the industrial area located further downstream in the WS is most probably responsible for high sulfate inflows (personal observations) into the Mooi River. This could have contributed to higher mean sulfate concentrations further downstream at RB and SB. From here the sulfate concentrations remained relatively constant downstream to DFE, whereafter it decreased towards the confluence with the Vaal River at Krom.

The sulfate concentration in the Mooi River ranged from 12.00 at UBD to 220.00 mg/l at SB (Appendix C, Table C-1). The overall mean for the Mooi River (115.58 mg/l) was 20% and 27% lower than the mean values measured for the WFS (143.67 mg/l) and GM (158.67 mg/l) respectively, but also 39% higher than the mean of the WS (70.33 mg/l). However the low mean for the WS may be misleading as high sulfate concentrations enter the WS in the industrial area, located just downstream from the sampling site.

A comparison of the major ion concentrations in the Mooi River, WFS and GM (WS excluded because of the lack of data) can be summarised as follows:

Mooi River: $\text{SO}_4^{2-} \gg \text{Ca}^{2+} > \text{Cl}^- > \text{Mg}^{2+} \gg \text{F}^-$;

WFS: $\text{SO}_4^{2-} \gg \text{Ca}^{2+} \gg \text{Mg}^{2+} > \text{Cl}^- \gg \text{F}^-$; and

GM: $\text{SO}_4^{2-} \gg \text{Ca}^{2+} > \text{Mg}^{2+} \gg \text{Cl}^- \gg \text{F}^-$

The overall (Mooi River, WFS, and GM combined) comparison of the ions can be summarised as follows:

$\text{SO}_4^{2-} \gg \text{Ca}^{2+} > \text{Cl}^- > \text{Mg}^{2+} \gg \text{F}^-$

3.1.3 pH

Several factors, of which the most natural are interactions with surrounding rocks, particularly carbonate rocks (WHO, 2003), affect the pH of water. Increases in carbon dioxide concentrations lower pH and thus pH can be affected by precipitation, particularly acid rain (Lal, 2016), as well as point sources such as agricultural runoff, waste water and discharges from

mines (Morrison *et al.*, 2001), all common phenomena in the Mooi River area. According to Morrison *et al.* (2001), point source pollution from mines can increase or decrease the pH, depending on the chemical constituents of the point source. In the WFS catchment area mines often produce acid runoff that can result in acidic groundwater seepage if the surrounding soil is poorly buffered. According to Benamer (2014) pH can affect the solubility and toxicity of chemicals, such as heavy metals, in water. Even a minor change in pH can increase the solubility of inorganic nitrogen and phosphorus (Benamer, 2014), thereby increasing the quantity available for uptake by algae, resulting in the system being more susceptible to eutrophication. Increases in algal growth will result in elevated pH levels (Farrel-Poe, 2000) due to the uptake of carbon dioxide during photosynthesis (Chen & Durbin, 1994).

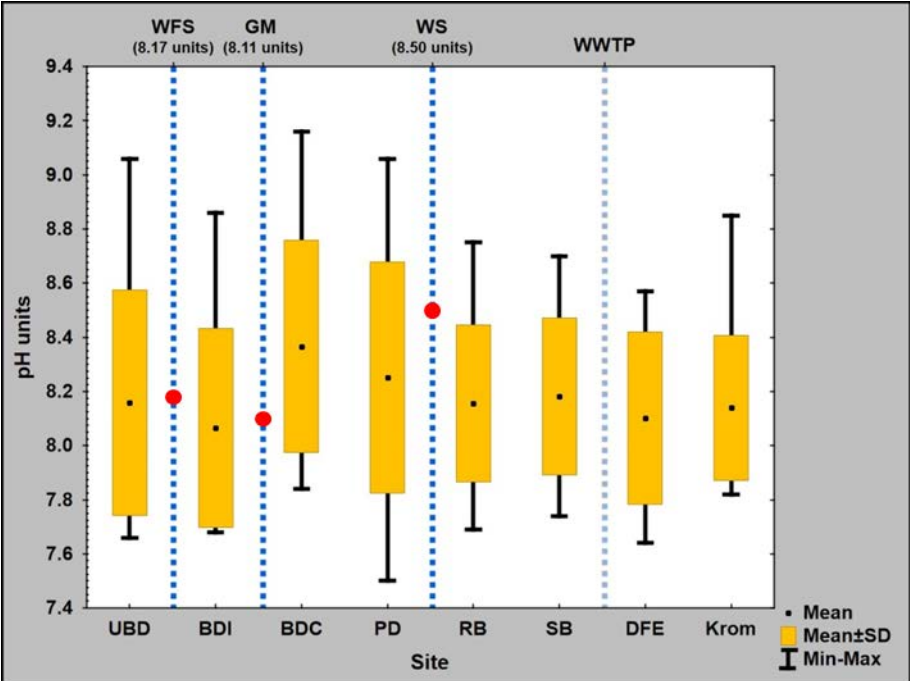


Figure 3-11 Box plot illustrating the pH at the sites located in the Mooi River for the study period January to December 2015. The dark blue dotted lines indicate the position of the inflows of the WFS, GM and the WS, while the grey dotted line indicates the location of the WWTP. Red dots represent the mean pH for the WFS, GM and WS, while exact values are given in brackets.

The pH values measured at the origin (UBD) were relatively high (mean of 8.2 maximum of 9.1; Appendix C, Table C-1). The WFS showed comparable pH values (mean 8.2 maximum of 9.0). Downstream the pH decreased to a mean value of 8.1 (BDI). The mean pH value for GM (8.1) were similar to BDI. At BDC higher pH values were recorded (mean of 8.4) compared to upstream sites. BDC also had the maximum pH value recorded for the Mooi River (9.2 mg/l). Although the second highest value (9.1) was recorded at PD, the mean pH value decreased from

BDC to PD. Downstream from this site, the WS joins the Mooi River. In this tributary (WS) pH values as high as 9.4 (maximum), with a mean of 8.5, were recorded, which represent the highest pH values recorded for the whole study period in any of the water bodies studied. In spite of these high values, the WS did not seem to impact the Mooi River downstream, because the mean values at RB and SB (8.2) were lower than at PD. After the WWTP inflow, the mean pH value decreased to 8.1 at DFE and Krom.

The pH in the Mooi River ranged from 7.5 at PD to 9.2 at BDC (Appendix C, Table C-1). The overall mean pH value measured for the Mooi River (8.2) was comparable to that of the WFS (8.2), higher than that of the GM (8.1), but lower than that of the WS (8.5).

3.1.4 Turbidity

Turbidity is the result of inorganic and organic suspended particles in the water, and measures the depth to which light passes through water (Chapman & Kimstach, 1996). Increasing levels of these particles reduce the clarity of the water, making it more difficult for light to pass through (Dallas & Day, 2004; Ziegler, 2002). Because algae need light to photosynthesise, turbidity affects the rate and regulates the amount of primary productivity (Farrel-Poe, 2000; Mitchell & Stapps, 1986). On the other hand, if algal concentrations in the water are high, it can be responsible for high turbidity levels, not only as a result of high levels of suspended particles, but also the capability of algae to absorb light, thereby reducing the underwater light availability.

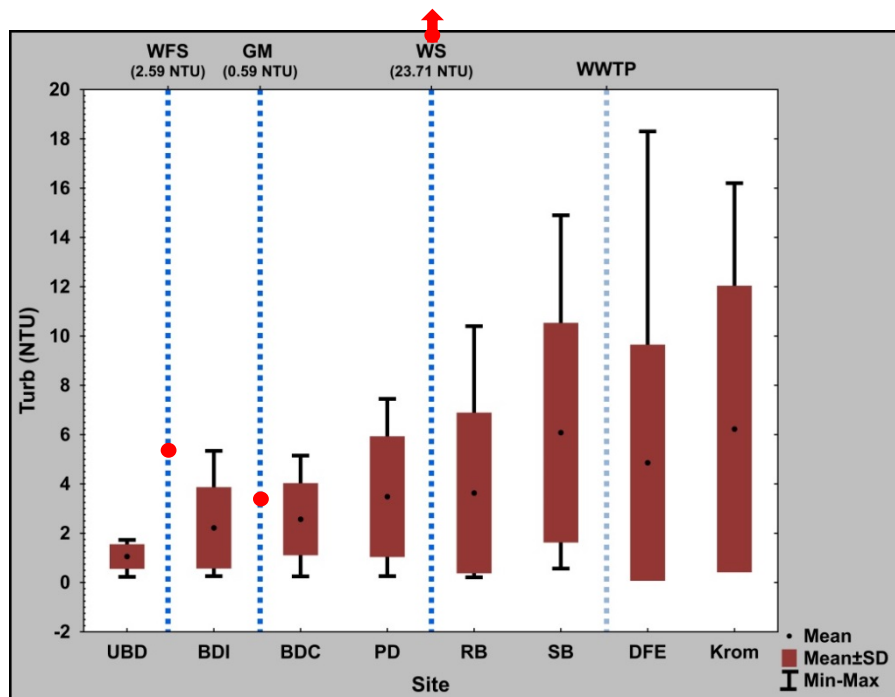


Figure 3-12 Box plot illustrating the turbidity at the sites located in the Mooi River for the study period January to December 2015. The dark blue dotted lines indicate the position of the inflows of the WFS, GM and the WS, while the grey dotted line indicates the location of the WWTP. Red dots represent the mean turbidity for the WFS, GM and WS, while exact values are given in brackets.

It is illustrated in Figure 3-12 that the turbidity at the origin of the river (UBD; 0.23 NTU; Appendix C, Table C-1) was the minimum recorded for the entire Mooi River system. The WFS (mean turbidity of 2.59 NTU), flows into the Mooi River just downstream of this site. After the inflow of the WFS the turbidity increased to reach a mean of 2.22 NTU at BDI. The GM flow into the Mooi River with lower turbidity values (mean of 0.59 NTU), probably due to the fact that water is transported in a concrete canal. Downstream from the GM inflow the mean turbidity increased gradually downstream to SB. The WS displayed the maximum turbidity value (118.00 NTU) for the entire study period (mean of 23.71 NTU). WS also showed a statistical significant difference with all the sites measured during the study period, except PD, DB, DFE and Krom in the Mooi River (Appendix B, Table B-1). Despite higher turbidity levels in the WS, its inflow does not seem to have a huge effect on the site downstream of the inflow (RB; mean of 3.63 NTU). However, further downstream at SB, turbidity increased with 40% to a mean of 6.08 NTU. After the water went through the WWTP'S processes, the mean turbidity decreased, but the maximum turbidity for the Mooi River system was recorded at this site (18.3 NTU). From here the mean turbidity increased downstream to the confluence with the Vaal River at Krom. In general, turbidity levels were quite low and the Mooi River system is characterised by relatively clear, transparent water.

The turbidity in the Mooi River ranged from 0.21 at RB to 18.30 NTU at DFE (Appendix C, Table C-1). The overall mean turbidity recorded in the Mooi River was 3.77 NTU, which was just above that of the WFS (2.59 NTU), higher than the mean of the GM (0.59 NTU) and lower than the WS (mean of 23.71 NTU).

3.1.5 Heavy metals

Heavy metal data was only monitored at the 4 most upstream sites in the Mooi River (UBD, BDI, BDC and PD), and for one site located in each of the WFS and GM tributaries. UBD is upstream from the confluence of the Mooi River and the WFS, and can serve as a control site not subjected to heavy metal pollution. The idea was to investigate to what extent the WFS, known for its mining activities, contributes to heavy metal pollution in the Mooi River system. The concentration of heavy metals found at GM, and its influence on the Mooi River, was also examined. According to Gautam *et al.* (2014) and Jaishankar *et al.* (2014), metals are constituents with a relatively high density that naturally form part of the earth's geology, but the concentration can vary among different locations. Metals are found commonly (in trace amounts) in water as well, and some are usually harmless, depending on the concentration (Booyens, 2016; Salem *et al.*, 2000). However, certain heavy metals are generally toxic or poisonous even at low concentrations (Gautam *et al.*, 2014). A small amount of them such as copper, iron, manganese, and zinc, are necessary in very low concentrations to serve as compounds for enzyme activities. However, higher concentrations of these metals and the presence of other toxic heavy metals, such hexavalent chromium, lead, nickel and uranium can be harmful (Gautam *et al.*, 2014; Salem *et al.*, 2000). Elevated concentrations of heavy metals in water bodies can be due to natural occurrences such as soil erosion, or through various anthropogenic activities including mining, industrial effluents, urban runoff, sewage discharge, insect or disease control agents applied to crops, and many others. In this way they bio-accumulate in water, and can be hazardous (Gautam *et al.*, 2014; Jaishankar *et al.*, 2014). They are also able to cause elevated EC levels (Jaishankar *et al.*, 2014). Heavy metals included in this study were: copper, hexavalent chromium, iron, lead, manganese, nickel, uranium, and zinc.

At very low concentrations, copper is required to activate some enzymes during photosynthesis (Gautam *et al.*, 2014) and it serves as a micro-nutrient supporting certain physiological activities of algae (Mamboya *et al.*, 2002). However, at these low concentrations it can still be toxic to some algae (Dallas & Day, 2004). The effect of toxicity depends mainly on the type of algae, the concentration of the copper, and the amount of time the algae are exposed to the copper. In other words, copper can be toxic to some algae at high concentrations, or at low concentrations over long periods of time. In this way it can limit photosynthesis and algal growth

(Mamboya *et al.*, 2002). At higher concentrations, copper can also be harmful to humans and toxic to a variety of aquatic organisms (Gautam *et al.*, 2014). However, according to Dallas & Day (2004) the toxicity can be reduced in the presence of molybdenum, sulfate, and zinc, and because copper is bound to organic molecules, it does not usually act as toxins even at relatively high concentrations. Mines and industries are the main contributors to copper released into the environment (Gautam *et al.*, 2014), because copper is found in a variety of sources, including electrical wiring, building materials, coins, and many more. Pipes, especially in a corrosive condition, may also contribute to copper pollution in water bodies. Agriculture can also contribute to copper pollution, since insecticides contain copper compounds. Copper is also sometimes added to fertilisers and animal feeds as a growth nutrient. Algicides, fungicides, food flavours and wood preservatives also contain copper compounds (WHO, 2004).

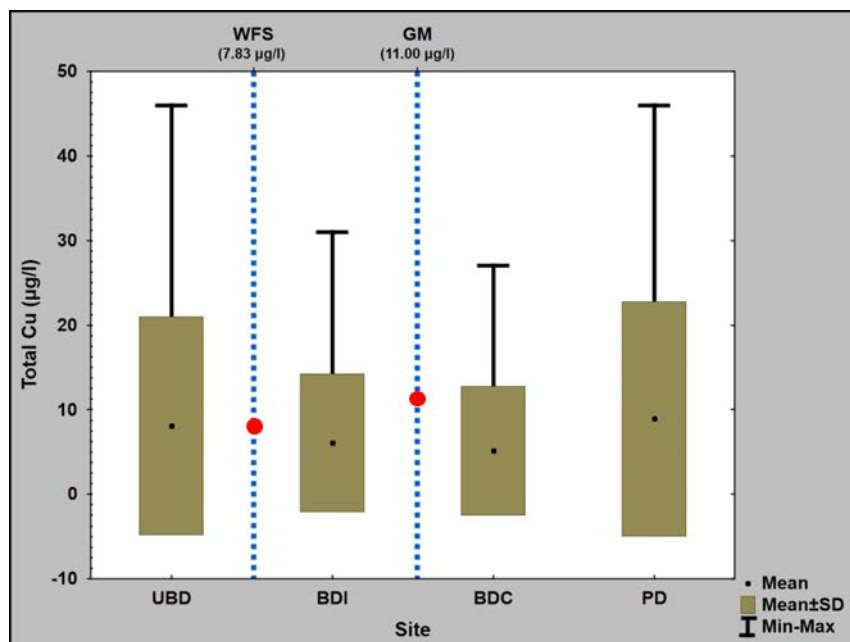


Figure 3-13 Box plot illustrating the total copper (Cu) concentration at the upstream sites located in the Mooi River (UBD, BDI, BDC and PD) for the study period January to December 2015. The dark blue dotted lines indicate the position of the inflows of the WFS and GM. Red dots represent the mean copper concentrations for the WFS and GM, while exact values are given in brackets.

The maximum copper concentrations for the Mooi River were recorded upstream at UBD, before the confluence with the WFS (Appendix E, Table E-1), as well as in PD (46.00 µg/l at each site). Mean copper concentrations at UBD was 8.08 µg/l. The WFS contained a mean copper concentration of 7.83 µg/l and a maximum of 37.00 µg/l. Lower copper concentrations were however found at BDI (mean of 6.08 µg/l). GM flows into the Mooi River with a 45% higher mean

(11.00 µg/l) copper concentration compared to BDI. GM does not seem to have an effect on concentrations further downstream at BDC, because the concentrations at this site declined to a mean of 5.17 µg/l, which is 15% lower compared to the mean of BDI. From BDC, the mean copper concentration increased 42% to PD (mean of 8.92 µg/l).

Copper concentrations in the Mooi River ranged from <1.00 at UBD, BDC, and PD to 46.00 µg/l at UBD and PD (Appendix E, Table E-1). A combination of all Mooi River sites displayed a mean concentration of 7.06 µg/l, which is comparable to that of the WFS (7.83 µg/l), but 36% lower than that of GM (11.00 µg/l).

Chromium is one of the least toxic metals at low concentrations (Dallas & Day, 2004). It is known to bio-accumulate in phytoplankton, aquatic plants, fish and invertebrates (MWC, 2012). According to Dallas & Day (2004) chromium can be accumulated up to 4000 times by some algae, and the reason for this remains unknown. The uptake, accumulation and effects of chromium are influenced by several factors, such as temperature, pH, the size and development stages of algae, and many more - however research on this topic is still very limited (MWC, 2012). Chromium occurs in different oxidation forms (Dallas & Day, 2004; Saha *et al.*, 2011). The hexavalent chromium form, is considered hazardous, and it can pose health risks, because it is known to be mutagenic and carcinogenic (Dallas & Day, 2004; Esmaeili *et al.*, 2010). Hexavalent chromium can be toxic to aquatic organisms in relatively low concentrations, and is able to reduce algal growth and photosynthesis (MWC, 2012). The major causes of hexavalent chromium released into water bodies include pigments for paints, inks, and plastics, anti-corrosion coatings, stainless steel manufacturing, textile dyes, wood preservatives, and leather tanning (Saha *et al.*, 2011). None of the above mentioned activities is prominent in the Mooi River catchment.

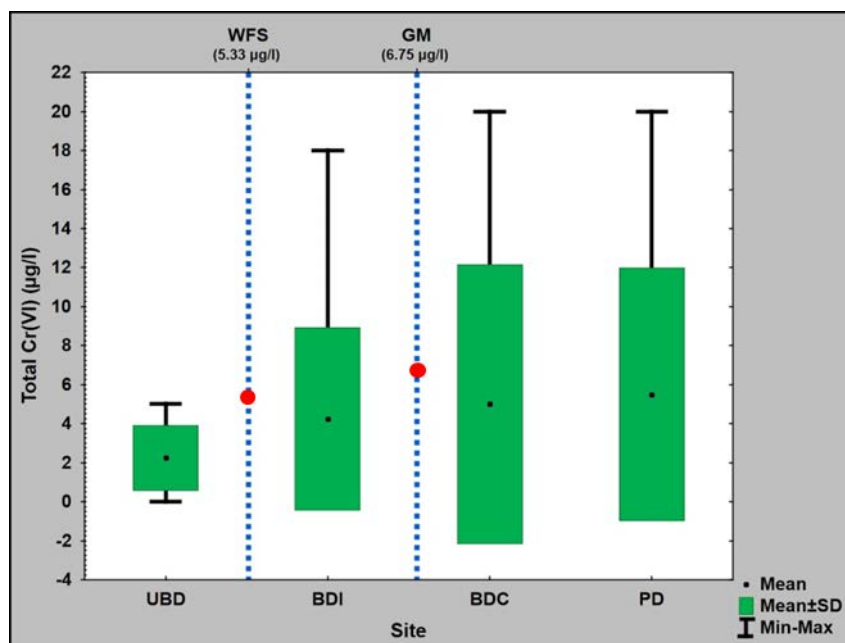


Figure 3-14 Box plot illustrating the total hexavalent chromium (Cr (VI)) concentration at the upstream sites located in the Mooi River (UBD, BDI, BDC and PD) for the study period January to December 2015. The dark blue dotted lines indicate the position of the inflows of the WFS and GM. Red dots represent the mean hexavalent chromium concentrations for the WFS and GM, while exact values are given in brackets.

Hexavalent chromium concentrations were relatively low in the Mooi River system before its confluence with the WFS (mean of 2.25 µg/l at UBD; Appendix E, Table E-1). The WFS carried higher concentrations of hexavalent chromium (mean of 5.33 µg/l), and its inflow can explain the two-fold elevation in concentration (mean of 4.25 µg/l) found further downstream at BDI. Hexavalent chromium concentrations in the GM exceeded the concentrations measured in the WFS (mean of 6.75 µg/l; maximum of 25.00 µg/l). The inflow of the GM tributary may have had an effect on the Mooi River, because the mean values increased with 15% downstream to BDC (mean of 5.00 µg/l) and again with 9% to PD (mean of 5.50 µg/l). These 2 sites also had the maximum recorded values (20.00 µg/l) for hexavalent chromium concentration in the Mooi River over the study period.

Hexavalent chromium concentrations in the Mooi River ranged between <0.01 at UBD, BDC and PD and 20.00 µg/l at BDC and PD (Appendix E, Table E-1). The overall mean hexavalent chromium concentration for the entire Mooi River system was 4.25 µg/l, which is 20% and 37% lower than WFS (5.33 µg/l) and GM (6.75 µg/l) respectively.

Iron and manganese are related as both metals are commonly found naturally in soils and groundwater (McFarland & Dozier, 1996; Zinati, 2005). These two metals normally occur together,

although iron is the more common of the two (McFarland & Dozier, 1996). Both iron and manganese serve as important micro-nutrients found in organisms (Dallas & Day, 2004); they help with the normal growth in algae, and play an important role in the functioning of respiration and photosynthesis (Akbarnezhad *et al.*, 2016). Iron compounds are able to oxidise easily, which results in high concentrations of reduced forms. In this way it can cause oxygen depletion (Dallas & Day, 2004), most probably because it can cause a reduction in photosynthesis at high concentrations (GWA, 2013). Although high manganese concentrations can disturb metabolic systems (Dallas & Day, 2004), it is not known to be hazardous to human health (McFarland & Dozier, 1996). During rainfall these metals can become apparent, and contains bacteria giving iron and manganese a brownish-red and brownish-black colour, respectively (GWA, 2013; McFarland & Dozier, 1996; Zinati, 2005). This can result in unpleasant taste and odour problems in water (McFarland & Dozier, 1996). Iron and manganese, as well as their containing bacteria, can accumulate and block irrigation systems (GWA, 2013; Zinati, 2005), and are therefore able to reach water bodies through irrigation. Both these metals occur widely - they are commonly found at higher concentrations in waters that drain coal and metal mines (Hallberg & Johnson, 2005).

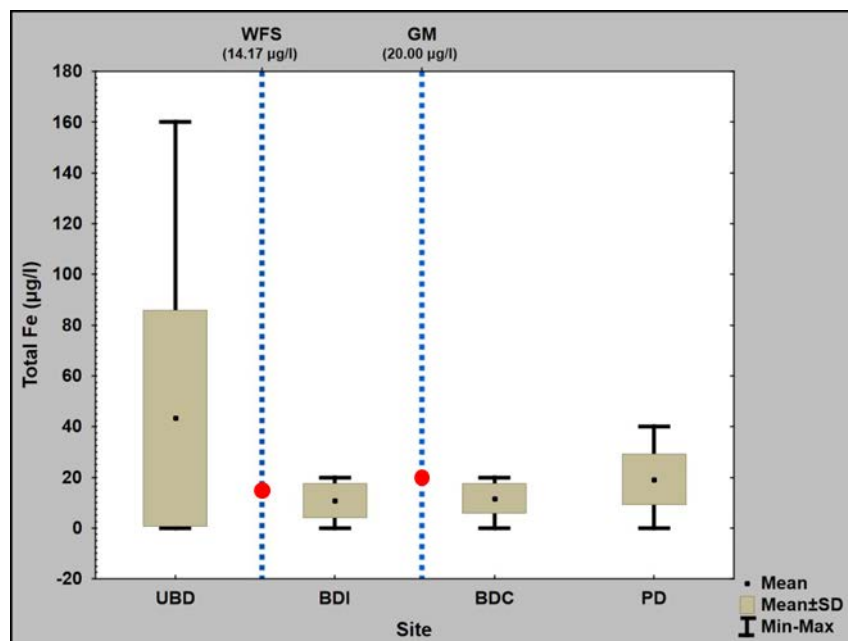


Figure 3-15 Box plot illustrating the total iron (Fe) concentration at the upstream sites located in the Mooi River (UBD, BDI, BDC and PD) for the study period January to December 2015. The dark blue dotted lines indicate the position of the inflows of the WFS and GM. Red dots represent the mean iron concentrations for the WFS and GM, while exact values are given in brackets.

The maximum iron concentration recorded during the entire study period was at UBD (160.00 µg/l; mean of 43.33 µg/l). The WFS was poor in iron (mean concentration of 14.17 µg/l). The mean iron concentration declined significantly (Appendix D; Table D-1) downstream from UBD to BDI (10.83 µg/l; Figure 3-15), probably as a result of dilution with WFS water. GM then joins the Mooi River with a mean iron concentration of 20.00 µg/l. This could have resulted in the 7% increase at BDC (mean of 11.67 µg/l), and 39% increase again at PD (mean of 19.17 µg/l; Appendix E, Table E-1).

Iron concentration ranged between <0.01 (for all the sites) and 160.00 µg/l (UBD) in the Mooi River. An overall mean of 21.25 µg/l was recorded for the river, which is 33% higher than that of the WFS (mean of 14.17 µg/l), but comparable to the mean of GM (20.00 µg/l; Appendix E, Table E-1).

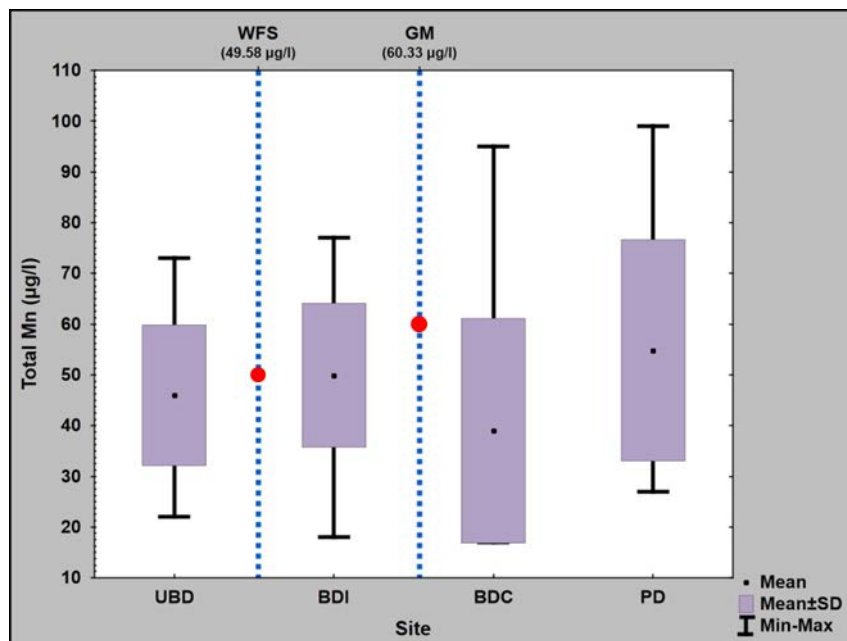


Figure 3-16 Box plot illustrating the total manganese (Mn) concentration at the upstream sites located in the Mooi River (UBD, BDI, BDC and PD) for the study period January to December 2015. The dark blue dotted lines indicate the position of the inflows of the WFS and GM. Red dots represent the mean manganese concentrations for the WFS and GM, while exact values are given in brackets.

Figure 3-16 illustrates that a mean manganese concentration of 46.00 µg/l was recorded at the source (UBD) of the Mooi River (Appendix E, Table E-1). The mean manganese concentration in the WFS was 7% higher (49.58 µg/l) compared to that of the source. After the inflow of the WFS, the mean manganese concentration increased to 49.92 µg/l at BDI. GM had the maximum manganese concentration (115.00 µg/l; mean of 60.33 µg/l) recorded for the study period.

However, BDC situated downstream from the GM inflow, showed a 22% decrease (compared to BDI) in the mean concentration (39.00 µg/l), in spite of a high maximum of 95.00 µg/l. The mean manganese concentration increased with 29% downstream to PD (54.83 µg/l). At this site the maximum manganese concentration (99.00 µg/l) in the main stream of the Mooi River system was recorded.

Manganese concentrations in the Mooi River ranged between 17.00 at BDC and 99.00 µg/l at PD (Appendix E, Table E-1). The overall mean manganese concentration (47.44 µg/l) in the Mooi River was comparable to that in the WFS (49.58 µg/l), but 21% lower than that of the GM (60.33 µg/l).

Lead is an extremely toxic heavy metal (Jaishankar *et al.*, 2014), and is harmful even in low concentrations (Salem *et al.*, 2000). Unlike some of the above discussed metals, such as copper, iron, and manganese, lead does not take part in any biological processes, but rather disturbs physiological processes, and it is also able to damage chlorophyll pigments and photosynthetic processes, suppressing overall growth of aquatic plants and phytoplankton (Jaishankar *et al.*, 2014). Lead can also cause a variety of serious problems in humans, and when exposed to it over long periods of time, it can cause damage to the central nervous system, the brain and kidneys, slackened growth and many more (Salem *et al.*, 2000). Industrial processes are the major causes of lead leaking into aquatic ecosystems through several sources including, food, smoking, gasoline, house paints, and water pipes (Jaishankar *et al.*, 2014; UEEG, 2011). Lead can also be released into the environment through the emission gasses of vehicle exhausts - in the United States, about 100 - 200 000 tons of lead is being released yearly in this manner (Jaishankar *et al.*, 2014).

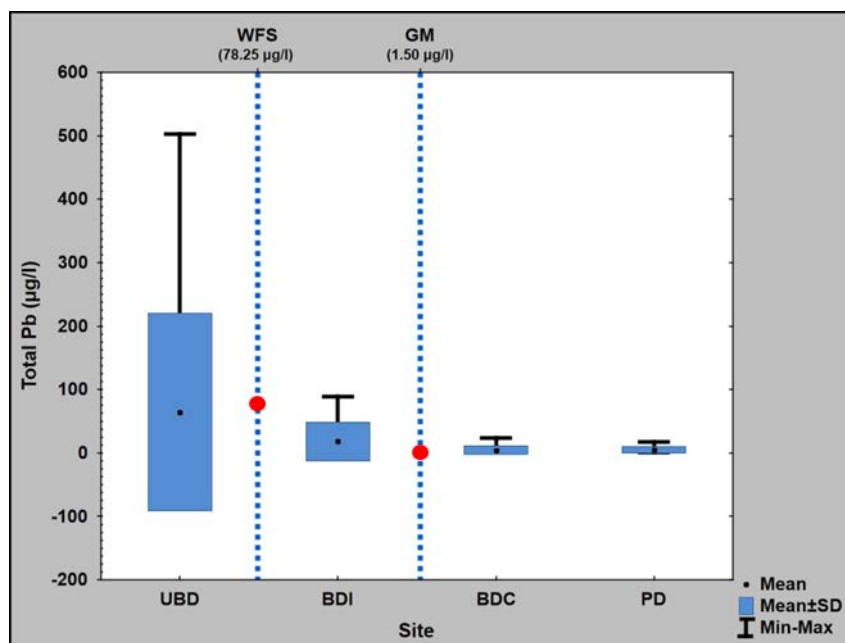


Figure 3-17 Box plot illustrating the total lead (Pb) concentration at the upstream sites located in the Mooi River (UBD, BDI, BDC and PD) for the study period January to December 2015. The dark blue dotted lines indicate the position of the inflows of the WFS and GM. Red dots represent the mean lead concentrations for the WFS and GM, while exact values are given in brackets.

The maximum lead concentration (503.00 µg/l) in the main stream of the Mooi River was recorded at the origin (UBD; mean of 64.42 µg/l). The WFS showed a 4% higher maximum concentration of 524.00 µg/l (mean of 78.25 µg/l). Despite the high lead concentrations found upstream at BDI and WFS, BDI showed a decrease in lead concentrations (mean of 18.25 µg/l; maximum of 89.00 µg/l). GM showed the lowest overall lead concentrations (mean of 1.50 µg/l), and the maximum concentration recorded in this tributary was only 3.00 µg/l. Relatively low mean concentrations were detected in the Mooi River at BDC and PD (4.50 and 4.83 µg/l, respectively), compared to UBD upstream (Appendix E, Table E-1).

Lead concentrations in the Mooi River ranged between 1.00 (for all the sites) and 503.00 µg/l (UBD). The overall mean lead concentration (23.00 µg/l) calculated in the river was 71% lower than that of the WFS (78.25 µg/l), but remarkably higher (94%) than that of GM (1.50 µg/l; Appendix E, Table E-1).

Nickel is commonly found in nature, mainly in basic rocks (EIFAC, 1984), and is considered to be toxic, even at low concentrations (Dallas & Day, 2004). However, nickel at very low concentrations, takes part in microbial enzymatic activities of microorganisms, and several physiological processes of plants. Nickel is known to be accumulated by phytoplankton, while at

the same time can be harmful to these organisms (Shukla *et al.*, 2009). The toxicity of nickel can vary for different phytoplankton species at different concentrations (Wang & Wood, 1984). During the study of Wang & Wood (1984), they found that cyanobacteria are usually more sensitive to high nickel concentrations than green algae. Nickel is released into the environment through various anthropogenic activities, including mining (particularly coal mines), manufacturing, the burning of fossil fuels and residue oils, and sewage sludge (EIFAC, 1984; Shukla *et al.*, 2009).

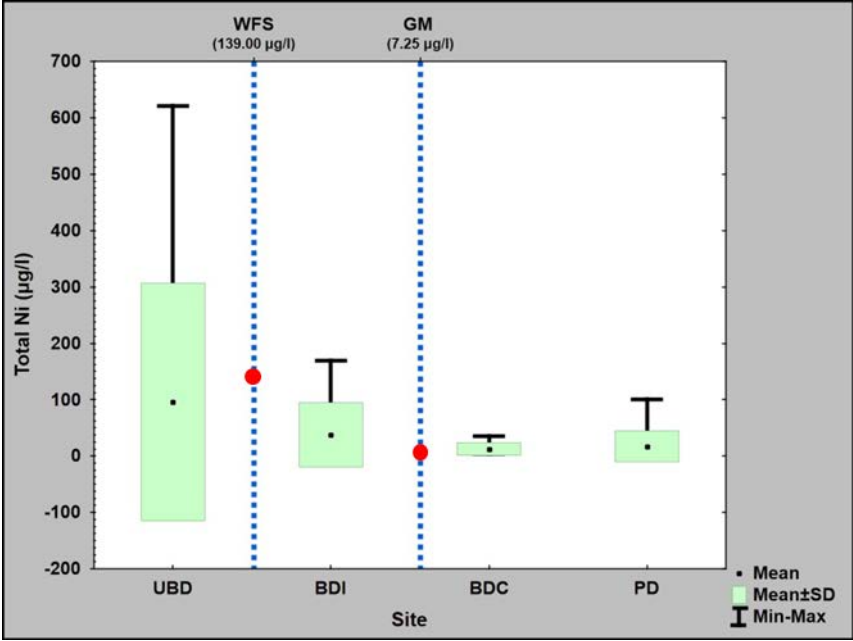


Figure 3-18 Box plot illustrating the total nickel (Ni) concentration at the upstream sites located in the Mooi River (UBD, BDI, BDC and PD) for the study period January to December 2015. The dark blue dotted lines indicate the position of the inflows of the WFS and GM. Red dots represent the mean nickel concentrations for the WFS and GM, while exact values are given in brackets.

Figure 3-18 shows that maximum nickel concentrations in the main stream of the Mooi River were recorded at the origin (site UBD; maximum of 621.00 µg/l; mean of 95.58 µg/l). The WFS site displayed the maximum recorded concentration (656.00 µg/l) amongst all tributaries. The mean concentration in the WFS was 139.00 µg/l. At site BDI, located downstream from the WFS’s inflow, a 60% lower mean nickel concentration (37.83 µg/l) was recorded compared to UBD. The GM joins the Mooi River downstream from this site containing very low nickel concentrations (mean of 7.25 µg/l). The mean nickel concentration decreased with 65% downstream to BDC (12.67 µg/l), probably due to dilution and the low concentrations that GM contributed to the river. However nickel concentrations showed a 25% increase to PD (mean of 17.17 µg/l; Appendix E, Table E-1).

In the Mooi River, nickel concentrations ranged between 4.00 (for all the sites) and 621.00 µg/l (UBD). A mean nickel concentration of 40.81 µg/l was recorded for the entire Mooi River main stream. The WFS exhibited a three-fold increase in the mean concentration (139.00 µg/l) compared to the Mooi River system. In contrast with the WFS, the GM showed a six times lower mean concentration (7.25 µg/l) when compared to the Mooi River (Appendix E, Table E-1).

Although uranium is considered a major hazard due to its radioactivity (Dallas & Day, 2004), it is by no means a rare metal. It occurs naturally as part of the lithology, and dissolved uranium found in natural waters is normally present in very low concentrations (Booyens, 2016). Mining activities can however elevate uranium concentrations (Kalin *et al.*, 2005). Coal mining in particular, releases uranium. Uranium can also be released into the environment by agricultural activities such as phosphate fertilisers because natural phosphates are rich in uranium (Booyens, 2016; Garnier–Laplace *et al.*, 2001). According to Dallas & Day (2004), uranium is normally not toxic as a chemical, except at high concentrations. There is limited knowledge on the toxicity of uranium on algae, however several studies showed that pH plays an important role in the toxicity of uranium on phytoplankton, as is usually the case with heavy metals (Fortin *et al.*, 2007; Kalin *et al.*, 2005; Markich *et al.*, 1996). Fortin *et al.* (2007) also found that the accumulation of uranium declines with higher concentrations of calcium, magnesium, and phosphates (Fortin *et al.*, 2007).

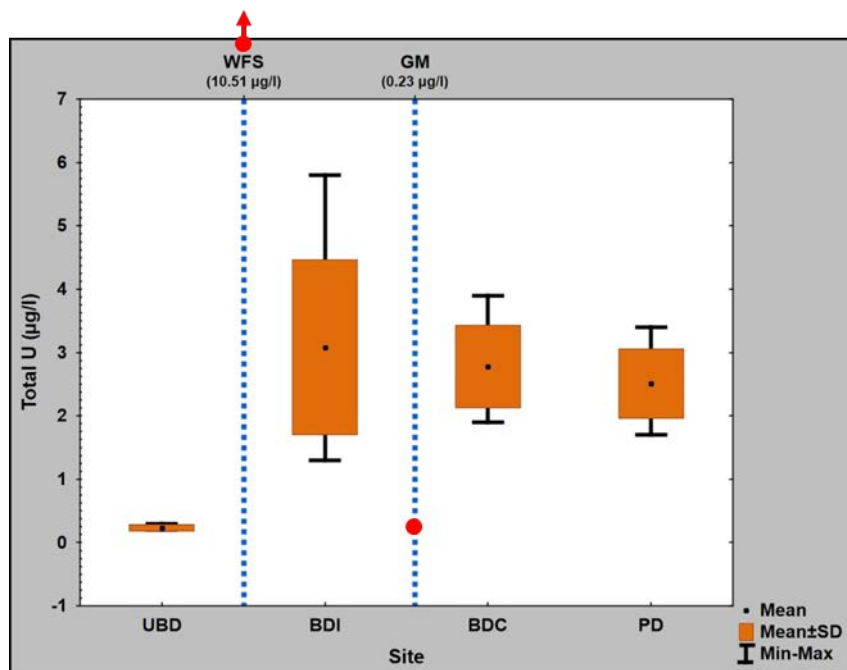


Figure 3-19 Box plot illustrating the total uranium (U) concentration at the upstream sites located in the Mooi River (UBD, BDI, BDC and PD) for the study period January to December 2015. The dark blue dotted lines indicate the position of the inflows of the WFS and GM. Red dots represent the mean uranium concentrations for the WFS and GM, while exact values are given in brackets.

At the headwaters of the river (UBD) the uranium concentration was the minimum (0.20 µg/l; mean of 0.23 µg/l; Appendix E, Table E-1) measured for the Mooi River system. After this site the WFS (mean uranium concentration of 10.51 µg/l) flows into the Mooi River. This tributary also displayed the maximum uranium concentration (22.30 µg/l) recorded at all sites, and showed a statistical significant difference with UBD and GM (Appendix D, Table D-1). The high uranium concentrations found in the WFS influenced BDI, the site located downstream from the confluence of the Mooi River and the WFS. BDI exhibited the highest mean (3.08 µg/l) and maximum (5.80 µg/l) concentrations recorded for the Mooi River system. After this site the GM, with a very low mean uranium concentration of 0.23 µg/l, flows into the river. A dilution effect probably resulted in lower mean concentrations at BDC (2.78 µg/l) and PD (2.51 µg/l).

The uranium concentrations in the Mooi River ranged between 0.20 at UBD and 5.80 µg/l at BDI. The overall mean uranium concentration recorded for the river (2.15 µg/l), was 80% lower than that of the WFS (10.51 µg/l), but also considerably (89%) higher than that of GM (0.23 µg/l; Appendix E, Table E-1).

Zinc is commonly found and is an important micro-nutrient for many organisms (Dallas & Day, 2004), required for the normal functioning of enzyme systems of algae (Johnson *et al.*, 2007). Zinc is non-toxic at low concentrations, but at high concentrations it becomes toxic (Dinesh *et al.*, 2013; Lundqvist, 2016). When zinc exceeds the amount necessary for optimal growth of algae, it interrupts photosynthesis, respiration, production and pigment synthesis, and suppresses cell division (Johnson *et al.*, 2007). The major contributors to zinc in soils and water bodies include mining (particularly coal mines), industries producing zinc materials and products, and metallurgical processing of zinc ores (Gautam *et al.*, 2014).

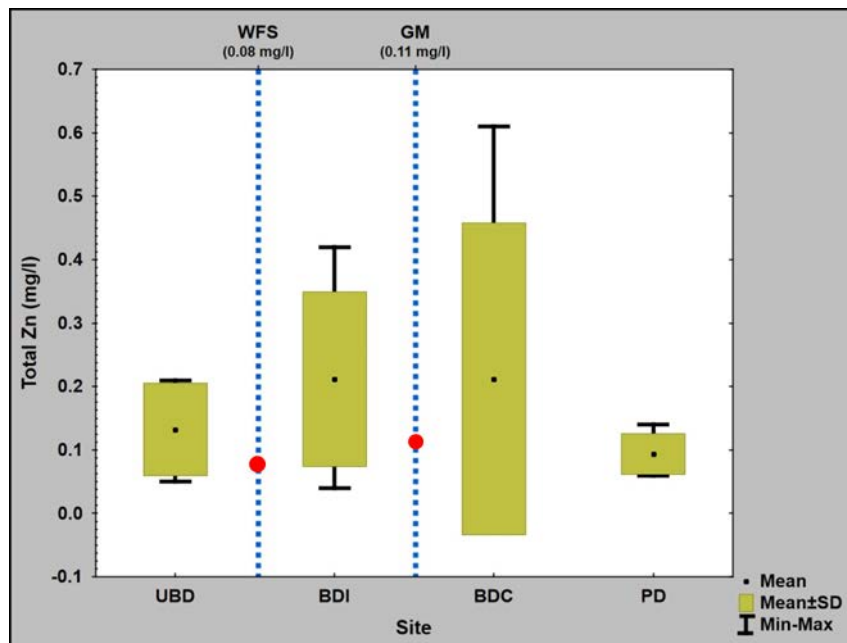


Figure 3-20 Box plot illustrating the total zinc (Zn) concentration at the upstream sites located in the Mooi River (UBD, BDI, BDC and PD) for the study period January to December 2015. The dark blue dotted lines indicate the position of the inflows of the WFS and GM. Red dots represent the mean zinc concentrations for the WFS and GM, while exact values are given in brackets.

A mean zinc concentration of 0.13 mg/l was recorded at the most upstream site located in the Mooi River (UBD; Appendix E, Table E-1). In the WFS the mean zinc concentration was 39% lower (0.08 mg/l) than at UBD. However, downstream at BDI, the mean concentration elevated to a mean of 0.21 mg/l. GM then joins the Mooi River with a 48% lower mean zinc concentration of 0.11 mg/l, but the mean concentration remained constant downstream to BDC (0.21 mg/l). BDC showed the maximum zinc concentration (0.61 mg/l) for the Mooi River. From here, the mean zinc concentration showed a seven-fold decrease downstream to PD (0.09 mg/l).

Zinc concentrations in the Mooi River ranged between 0.03 and 0.61 mg/l at BDC. The overall mean of the Mooi River was 0.16 mg/l. The WFS showed a 50% lower mean (0.08 mg/l), while the mean concentration in the GM (0.11 mg/l) was 31% lower than that in the Mooi River's main stream (Appendix E, Table E-1).

3.2 Resource Quality Objectives and Resource Water Quality Objective

The National Water Act (Act No 36 of 1998) states scientific criteria, known as the resource quality objectives (RQO's; DWA, 2016; Chapter 1), for certain environmental variables. The Mooi River's water resources form part of the Upper Vaal catchment region, and the RQO's are based on this region (Labuschagne, 2017). RQO's for the Upper Vaal catchment appear in Labuschagne (2017) and in the Government Gazette 39943 (22 April 2016; DWA, 2016). DWA (2009) also prescribed recommended water quality objectives (RWQO's), specifically for the Mooi River. Both the RQO's and RWQO's numerical limits are listed in Table 3-1 next to the measured variables for the study period.

Table 3-1 List of the physico-chemical variables, their set numerical limits (RQO's and RWQO's), in comparison to the mean values of the control point (UBD) in the Mooi River, the entire Mooi River, and the tributaries feeding the Mooi River. If a variable is absent from the list, it is because there is no limit set for the specific variable. The means of the control point/upstream site (UBD) located in the Mooi River, the entire Mooi River (including UBD), as well as the WFS, GM and WS tributaries are listed next to the set limits to indicate how their mean values compare with the RQO and RWQO limits. The mean values that exceed one or both the limits are highlighted in red. The mean values exceeding one limit, but are lower than another limit are highlighted in yellow. If the mean values are less than one or both the limits, it is highlighted in green.

Variable and unit:	RQO limit:	RWQO limit:	Mean of control point (UBD) at the source of the Mooi River	Mean of entire Mooi River:	Mean of WFS:	Mean of GM:	Mean of WS:
Ammonia (NH ₃ ⁺) mg/l	No set limit	0.03	0.12	0.39	0.17	0.24	1.12
Nitrate (NO ₃ ⁻) mg/l	≤ 4	0.3	0.43	0.45	0.41	0.71	1.12
Orthophosphate (PO ₄ -P) mg/l	≤ 0.125	0.4	0.42	0.68	0.34	0.54	1.02
Total Dissolved Solids (TDS) mg/l	No set limit	370.5	270.25	475.05	496.08	558.42	402.18
Electrical Conductivity (EC) mS/m	≤ 111	57	45.64	70.07	78.07	76.59	60.84
Sulfate (SO ₄ ²⁻) mg/l	≤ 500	75	27.00	115.58	143.67	158.67	70.33
Calcium (Ca ²⁺) mg/l	No set limit	47	58.93	70.06	87.73	69.60	No data
Chloride (Cl ⁻) mg/l	No set limit	36	13.58	46.25	42.42	46.67	No data
Magnesium (Mg ²⁺) mg/l	≤ 33	30	37.74	48.90	43.84	54.74	No data
Fluoride (F ⁻) mg/l	≤ 3	0.25	0.25	0.39	0.23	0.34	No data
pH units	≤ 8.8	8	8.16	8.18	8.17	8.11	8.50
Copper (Total Cu) µg/l	≤ 8	No set limit	8.08	7.06	7.83	11.00	No data

Variable and unit:	RQO limit:	RWQO limit:	Mean of control point (UBD) at the source of the Mooi River	Mean of entire Mooi River:	Mean of WFS:	Mean of GM:	Mean of WS:
Hexavalent Chromium (Total Cr (VI)) µg/l	≤ 200	No set limit	2.25	4.25	5.33	6.75	No data
Iron (Total Fe) µg/l	No set limit	350	43.33	21.25	14.17	20.00	No data
Lead (Total Pb) µg/l	≤ 13	No set limit	64.42	23.00	78.25	1.50	No data
Manganese (Total Mn) µg/l	≤ 1300	30	46.00	47.44	49.58	60.33	No data
Uranium (Total U) µg/l	≤ 15	No set limit	0.23	2.15	10.51	0.23	No data
Zinc (Total Zn) mg/l	≤ 0.036	No set limit	0.13	0.16	0.08	0.11	No data

3.3 Phytoplankton

Cyanobacteria and algae are collectively referred to as phytoplankton. Cyanobacteria are photosynthetic prokaryotic organisms, regarded as ancient bacteria and not algae (Van Ginkel, 2012). They belong to the Phylum Cyanophyta, and representatives of this phylum can be in the form of single cells (such as *Synechocystis*), colonies (such as *Microcystis*), or filaments (such as *Anabaena*; Bellinger & Sigee, 2010; Janse van Vuuren *et al.*, 2006). They usually appear blue-green in water bodies due to a combination of their green pigments (chlorophyll-*a*) and blue pigments (phycocyanin) that dominate. They also have a red pigment, phycoerythrin, and when all three pigments are equally present, the cells may appear a purplish colour (Janse van Vuuren *et al.*, 2006). Cyanobacteria's DNA are not enclosed by a nuclear membrane, and they do not have membrane bound organelles (such as Golgi bodies, plastids, mitochondria, and microsomes) such as eukaryotic organisms (Bellinger & Sigee, 2010). Cyanobacteria have very thick layered cell walls often surrounded by mucous that is responsible for gliding movements that some perform. They only reproduce asexually, and have no flagella (Van den Hoek *et al.*, 1995).

These organisms have many special adaptations that give them competitive advantages over algae. Some cyanobacteria species can move up and down in the water column by means of gas vacuoles or aerotopes. The gas vacuoles help these species to control their vertical position in the water column, allowing them to also obtain nutrients from deeper layers in the water column, when nutrients in upper layers are depleted (Shapiro, 1990). Due to the presence of phycobilisomes (containing phycocyanin, allophycocyanin and phycoerythrin) they can, unlike algae, utilise green light for photosynthesis that also gives them a competitive advantage. Green light penetrates deeper than blue and red light (Dodds, 2002) and therefore cyanobacteria can live and photosynthesise in deeper areas of the water column - therefore the statement is often made that they are able to tolerate low light intensities and survive in lower layers of the water column (Bellinger & Sigee, 2010). Cyanobacteria are adapted to survive during periods of nutrient deficiencies, because some can convert atmospheric nitrogen, through biological nitrogen fixation, to ammonium. They can also assimilate phosphorus in excessive amounts and store it to use when the water becomes phosphorus-limited (Paerl *et al.*, 2001). Cyanobacteria are very diverse and thrive in different environments that include marine, fresh water, brackish, terrestrial and even aerial or sub-aerial habitats (Janse van Vuuren *et al.*, 2006).

Algae are eukaryotic, meaning the cells contain nuclei and membrane bounded organelles. Algae can be microscopic (such as *Chlorella*), or macroscopic (such as *Chara*). Most algae are autotrophic (photosynthetic), though some can live heterotrophic (Bellinger & Sigee, 2010; Van den Hoek *et al.*, 1995). Algae have a simple vegetative structure without any vascular

tissues, and their sexual organs are not enclosed within protective coverings (Bellinger & Sigee, 2010; Wehr & Sheath, 2003). They are able to reproduce asexually or sexually, and can be in the form of single cells, aggregates, colonies, unbranched or branched filaments or even thallus-like (Bellinger & Sigee, 2010; McCarthy & Orchard, 2007). Algae can be classified into various phyla (such as those listed in Table 3-2). Characteristics used to classify algae in different phyla are based, amongst others, on their photosynthetic pigments that give them unique and different colours. For example, “chryso” in the phylum Chrysophyta means “golden”, and therefore they are commonly referred to as golden algae, because of the accessory pigment, fucoxanthin. “Chloro” in Chlorophyta, refers to “green”, and they are commonly referred to as green algae, because these algae have chlorophyll *a* and *b* pigments that give them a green colour (Van den Hoek *et al.*, 1995). Algae have a variety of mechanisms for movement. Some species are immotile, while others can swim by means of flagella, perform gliding movements, euglenoid movements or amoeboid movements. They can be planktonic (free floating or swimming), or benthic where they live attached to surfaces (Bellinger & Sigee, 2010). Algae are diverse and flourish in different environments, including fresh, marine, and brackish waters. Although algae are primarily found in aquatic environments, they do also occur in terrestrial habitats (Bellinger & Sigee, 2010, McCarthy & Orchard, 2007). Algae are important primary producers in freshwater and marine environments, and form the base of the food chain, where aquatic organisms (such as fish and mussels) feed on them (Paerl *et al.*, 2001).

One of the major causes of algal and cyanobacteria blooms in aquatic ecosystems can be attributed to pollution by anthropogenic activities (Bennett, 2017). Mining, industries, waste water effluents and agricultural run-off are the main components leading to pollution in aquatic ecosystems (Naidoo, 2013). All of these components contribute to pollution in the Mooi River and its tributaries. Non-point source pollution, such as vehicle emissions released into the environment, can also reach aquatic ecosystems by means of rainfall or dry fall out. These pollutants can contain pesticides, heavy metals, oil, and numerous organic and inorganic compounds which are able to affect the water quality (Naidoo, 2013; Singh *et al.*, 2013). Physico-chemical and biological variables are affected by these pollutants and can reflect overall changes in water bodies (Chellappa *et al.*, 2009; Singh *et al.*, 2013). Knowledge regarding these variables in the Mooi River and its tributaries is therefore extremely important. Phytoplankton is used as bio-indicators in this study, because pollutants are able to affect the abundance and composition of phytoplankton (Harris & Vinobaba, 2012). By studying the presence, absence, abundance, and composition, phytoplankton can serve as a helpful tool to provide information regarding the physical and/or chemical environment at a specific site (Singh *et al.*, 2013). The knowledge of phytoplankton dynamics in the Mooi River and its tributaries is important, because it can give

information on the types of algae and cyanobacteria present in the river and its tributaries. Phytoplankton assemblages in the Mooi River can be compared to those of the tributaries to see if and how they affect the river.

Different types of phytoplankton have different tolerance ranges for certain physical and chemical environmental variables; and therefore they can grow, form blooms, and out-compete one another under different water quality conditions (Singh *et al.*, 2013). Some algae (such as *Cymbella*) are generally associated with good water quality and low nutrient levels, while others (such as *Microcystis*) prefer eutrophic water conditions (Janse Van Vuuren *et al.*, 2006). The phytoplankton found in the Mooi River and its tributaries can help to draw conclusions on the general water quality of the river, and to predict potential problems associated with particular phytoplankton genera found at the different sites. Because different phytoplankton genera prefer different water conditions, it can serve to indicate the trophic status in the Mooi River and tributaries.

However, when investigating the phytoplankton composition at a given moment, it is important to note that conditions at the time of the sampling may differ from those triggering their development (Janse van Vuuren, 2001; Oosthuizen, 2012). Oosthuizen (2012) stated that “It takes time for water to move through the system and that is why conditions at a site located later in the sequence are the reflection of conditions that had occurred a few days before that specific date at a site earlier in the sequence”.

Several studies, using algae as bio-indicators of water quality, were conducted on the Mooi River (Barnard *et al.*, 2013; De la Rey *et al.*, 2004; Labuschagne, 2017; Pelsler, 2015; Venter *et al.*, 2013). Some of these studies were limited to diatoms, and focused on the impacts of the WFS and GM tributaries. However, no physical sampling was done to study phytoplankton dynamics and environmental variables in the tributaries to determine the effect of the inflowing tributaries on the Mooi River. The following paragraphs contain results on the phytoplankton in the Mooi River, and its tributaries (WFS, GM, and WS), as well as their effect on the Mooi River system.

Seven phytoplankton phyla were found at nine sites in the Mooi River, and at three sites, each located in a different tributary (Table 3-2). The Bacillariophyta (diatoms) and Chlorophyta (green algae) were most abundant in terms of concentration (total cells per ml) and diversity (number of genera present). The Cyanophyta (cyanobacteria) was less diverse, but one of the most abundant phyla in terms of concentration. These three phyla succeeded one another repeatedly as the dominants. Other, less abundant but still significant, phyla were Cryptophyta

(cryptophytes), Chrysophyta (golden algae), Dinophyta (dinoflagellates) and Euglenophyta (euglenophytes).

In the main stream of the Mooi River, the Chlorophyta and Bacillariophyta were most diverse, each phylum with 30 genera identified. They were followed by Cyanophyta (11 genera), Euglenophyta (four genera), Dinophyta (three genera), Chrysophyta (two genera), and Cryptophyta (one genus). A comparison of the different sites (Table 3-2) revealed that maximum diversity (most genera present) was in PD (64) and BDC (60), while minimum diversity was found in BDI (48). Most Cyanophyta genera were found in PD (ten) and BDC (nine), while UBD, SB and Krom showed the least (five genera at each site). The least amount of Bacillariophyta genera (14) was present at BDI. SB had the highest diatom diversity (23 genera). The Chlorophyta were most diverse at BDC and PD with a total of 25 and 24 genera respectively, and the least abundant at Krom (17 genera). The other phyla were only represented by a few genera at each site. At WFS, the Bacillariophyta was most diverse (20 genera), followed by the Chlorophyta (15 genera), Cyanophyta (nine genera), Euglenophyta (three genera), Dinophyta (two genera). Cryptophyta and Chrysophyta were represented by only one genus each. At GM, the Bacillariophyta was the most diverse (21 genera), followed by the Chlorophyta (17 genera), Cyanophyta and Euglenophyta (four genera each), Dinophyta (two genera), and the Cryptophyta and Chrysophyta (one genus each). At WS, the Chlorophyta was the most diverse (21 genera), followed by the Bacillariophyta (16 genera), Cyanophyta (ten genera), Euglenophyta (four genera), Dinophyta (two genera), and Cryptophyta and Chrysophyta (one genus each).

Interesting observations were made regarding the presence of genera at different site locations in the Mooi River and tributaries. *Nostoc* was observed at the origin of the Mooi River (UBD), but was absent in downstream sections and in the tributaries. *Aphanothece* was only present at BDC and PD in the Mooi River (Table 3-2; phylum Cyanophyta). *Placoneis* was only present at the origin of the Mooi River (UBD), while *Staurosira* was found in all the sites studied in the river and tributaries, except the site at the origin. *Tryblionella* was only found at a few sites in the Mooi River, but was absent in all three tributaries. *Epithemia* was only present at one site in the river (PD) and in GM. *Hippodonta* was only found in WS (Table 3-2; phylum Bacillariophyta). *Carteria* and *Treubaria* were only present in the Mooi River, but not in any of the tributaries. *Chlorolobion* and *Microspora* were only present at the origin of the river (UBD). *Staurostrum* was absent in the downstream section of the Mooi River, and WFS. *Gonatozygon* was only found in GM. *Pediastrum* was also absent at the origin (UBD). *Pandorina* was only present in WS. *Kirchneriella* and *Eudorina* were only present in WFS and in DFE (in the Mooi River; Table 3-2; phylum Chlorophyta).

Table 3-2 List of phytoplankton phyla and genera identified at each site. Respective authors are also indicated. Mooi River sites are arranged in white shaded columns, and arranged from the upstream to the downstream sites. Tributaries (WFS, GM, and WS) are shaded in grey and are arranged according to their position of convergence with the Mooi River. Black crosses indicate the presence of genera at each site, while red crosses indicate dominant genera or genera found in high numbers. * = Genera previously classified under the phylum Chlorophyta (John *et al.*, 2002), but currently classified under the phylum Charophyta according to Algaebase (Guiry & Guiry, 2019).

PHYLUM CYANOPHYTA											
GENUS AND AUTHOR	UBD	WFS	BDI	GM	BDC	PD	WS	RB	SB	DFE	KROM
<i>Anabaena</i> Bory ex Bornet et Flahault	X	X			X	X	X	X	X		X
<i>Aphanocapsa</i> Nägeli	X	X	X		X	X	X	X		X	X
<i>Aphanothece</i> Nägeli					X	X					
<i>Arthrospira</i> Stizenberger ex Gomont							X				
<i>Chroococcus</i> Nägeli							X				
<i>Merismopedia</i> Meyen	X	X	X		X	X	X		X	X	X
<i>Microcystis</i> Kützing ex Lemmermann		X	X	X	X	X	X	X			
<i>Nostoc</i> Vaucher ex Bornet & Flahault	X										
<i>Oscillatoria</i> Vaucher ex Gomont		X			X	X				X	X
<i>Phormidium</i> Kützing ex Gomont			X	X	X	X	X	X	X	X	
<i>Pseudanabaena</i> Lauterborn	X	X	X	X	X	X	X	X	X	X	X
<i>Spirulina</i> Turpin ex Gomont		X	X			X	X				
<i>Synechocystis</i> Sauvageau		X	X		X	X	X	X	X	X	
Unknown round colonies		X		X							
Total Cyanophyta genera	5	9	7	4	9	10	10	6	5	6	5

PHYLUM BACILLARIOPHYTA

GENUS AND AUTHOR	UBD	WFS	BDI	GM	BDC	PD	WS	RB	SB	DFE	Krom
<i>Achnanthidium</i> Kützing	X	X	X	X	X	X	X	X	X	X	X
<i>Amphora</i> Ehrenberg ex Kützing					X				X	X	
<i>Aulacoseira</i> Thwaites	X	X	X	X	X	X	X	X	X	X	X
<i>Cocconeis</i> Ehrenberg	X	X	X	X	X	X	X	X	X	X	X
<i>Craticula</i> Grunow		X	X	X		X		X	X		
<i>Cyclotella</i> Kützing ex Brébisson	X	X	X	X	X	X	X	X	X	X	X
<i>Cymatopleura</i> Smith		X					X	X	X		X
<i>Cymbella</i> Agardh	X	X	X	X	X	X		X	X		X
<i>Diademsis</i> Kützing	X		X	X	X	X		X	X	X	X
<i>Diatoma</i> Bory	X	X		X	X	X	X	X	X		X
<i>Encyonema</i> Kützing		X	X		X	X					
<i>Epithemia</i> Kützing				X		X					
<i>Fragilaria</i> Lyngbye	X	X	X	X	X	X	X	X	X	X	X
<i>Frustulia</i> Agardh										X	
<i>Gomphonema</i> Ehrenberg	X	X	X	X	X	X	X	X	X	X	X
<i>Gyrosigma</i> Hassall							X	X		X	X
<i>Hantzschia</i> Grunow				X	X	X		X	X	X	X
<i>Hippodonta</i> Lange-Bertalot, Witkowski & Metzeltin							X				
<i>Melosira</i> Agardh		X		X	X	X		X	X		X
<i>Navicula</i> Bory	X	X	X	X	X	X	X	X	X	X	X
<i>Nitzschia</i> Hassall	X	X	X	X	X	X	X	X	X	X	X
<i>Pinnularia</i> Ehrenberg	X	X		X	X	X	X	X		X	X

<i>Placoneis</i> Mereschkowsky	X										
<i>Pleurosigma</i> Smith									X		
<i>Rhoicosphenia</i> Grunow	X	X	X	X	X	X	X	X	X	X	X
<i>Sellaphora</i> Mereschowsky		X		X	X	X		X	X		
<i>Staurosira</i> Ehrenberg		X	X	X	X	X	X	X	X	X	X
<i>Surirella</i> Turpin	X	X		X	X	X	X		X		X
<i>Tabellaria</i> Ehrenberg ex Kützing		X		X					X	X	X
<i>Tryblionella</i> Smith	X					X		X	X		X
Unidentified pennate diatom	X	X	X	X	X	X	X	X	X	X	X
Total Bacillariophyta genera	16	20	14	21	20	22	16	21	23	17	21

PHYLUM CHLOROPHYTA

GENUS AND AUTHOR	UBD	WFS	BDI	GM	BDC	PD	WS	RB	SB	DFE	Krom
<i>Ankistrodesmus</i> Corda	X	X	X		X	X	X			X	X
<i>Carteria</i> Diesing	X		X		X	X		X		X	X
<i>Chlamydomonas</i> Ehrenberg	X	X	X	X	X	X	X	X	X	X	X
<i>Chlorella</i> Beijerinck	X	X	X	X	X	X	X	X	X	X	X
<i>Chlorococcum</i> Meneghini	X	X	X	X	X	X	X	X	X	X	
<i>Chlorolobion</i> Korshikov	X										
* <i>Closterium</i> Nitzsch ex Ralfs	X	X	X	X	X	X	X	X	X	X	X
<i>Coelastrum</i> Nägeli	X		X	X	X	X	X	X	X	X	
* <i>Cosmarium</i> Corda ex Ralfs		X	X	X	X	X	X		X	X	
<i>Crucigenia</i> Morren	X	X	X		X	X		X	X	X	X
<i>Crucigeniella</i> Lemmerman	X		X		X	X	X	X	X	X	X
<i>Desmodesmus</i> An, Friedl & Hegewald	X	X	X	X	X	X	X	X	X	X	X

<i>Dictyosphaerium</i> Nägel	X		X		X	X	X		X		X
<i>Eudorina</i> Ehrenberg ex Ralfs							X			X	
<i>Geminella</i> Turpin			X		X	X		X			
* <i>Gonatozygon</i> De Bary				X							
<i>Kirchneriella</i> Scmidle							X			X	
<i>Microspora</i> Thuret	X										
<i>Monoraphidium</i> Komárková-Legnerová	X	X	X	X	X	X	X	X	X	X	X
* <i>Mougeotia</i> Agardh	X	X	X	X				X	X	X	
<i>Oocystis</i> Braun	X	X	X	X	X	X	X	X	X	X	X
<i>Pandorina</i> Bory de Saint-Vincent							X				
<i>Pediastrum</i> Meyen		X	X		X	X	X	X	X	X	X
<i>Scenedesmus</i> Meyen	X	X	X	X	X	X	X	X	X	X	X
<i>Schroederia</i> Lemmermann	X			X	X	X	X	X	X	X	
<i>Sphaerocystis</i> Chodat		X	X	X	X	X					
* <i>Staurastrum</i> Meyen ex Ralfs	X		X	X	X	X	X				
<i>Tetraedron</i> Kützing	X	X	X	X	X	X	X	X	X	X	X
<i>Tetrastrum</i> Chodat	X			X	X	X	X	X			X
<i>Treubaria</i> Bernard emend. Reymond	X				X	X			X	X	X
<i>Ulothrix</i> Kützing					X	X		X	X	X	X
Total Chlorophyta genera	22	15	21	17	25	24	21	19	19	22	17
PHYLUM CRYPTOPHYTA											
GENUS AND AUTHOR	UBD	WFS	BDI	GM	BDC	PD	WS	RB	SB	DFE	Krom
<i>Cryptomonas</i> Ehrenberg	X	X	X	X	X	X	X	X	X	X	X
Total Cryptophyta genera	1	1	1	1	1	1	1	1	1	1	1

PHYLUM CHRYSOPHYTA											
GENUS AND AUTHOR	UBD	WFS	BDI	GM	BDC	PD	WS	RB	SB	DFE	Krom
<i>Dinobryon</i> Ehrenberg	X	X	X	X	X	X	X	X	X	X	X
<i>Synura</i> sp. Ehrenberg								X		X	X
Total Chrysophyta genera	1	1	1	1	1	1	1	2	1	2	2
PHYLUM DINOPHYTA											
GENUS AND AUTHOR	UBD	WFS	BDI	GM	BDC	PD	WS	RB	SB	DFE	Krom
<i>Ceratium</i> Schrank	X	X		X	X	X	X	X		X	
<i>Peridinium</i> Ehrenberg	X		X		X	X	X	X	X	X	X
<i>Sphaerodinium</i> Woloszynska		X	X	X		X		X			
Total Dinophyta genera	2	2	2	2	2	3	2	3	1	2	1
PHYLUM EUGLENOPHYTA											
GENUS AND AUTHOR	UBD	WFS	BDI	GM	BDC	PD	WS	RB	SB	DFE	Krom
<i>Euglena</i> Ehrenberg	X	X	X	X	X	X	X	X	X	X	X
<i>Lepocinclis</i> Perty				X	X	X			X	X	X
<i>Phacus</i> Dujardin							X	X		X	X
<i>Strombomonas</i> Deflandre		X		X			X				
<i>Trachelomonas</i> Ehrenberg	X	X	X	X	X	X	X	X	X	X	X
Total Euglenophyta genera	2	3	2	4	3	3	4	3	3	4	4
TOTAL NUMBER OF GENERA PER SITE	49	51	48	50	60	64	55	55	53	54	51

Figure 3-21 to Figure 3-24 represent seasonal area charts of the phytoplankton concentration (total cells/ml) and composition at eight different sites in the Mooi River. Pie charts illustrate phytoplankton composition in the three tributaries.

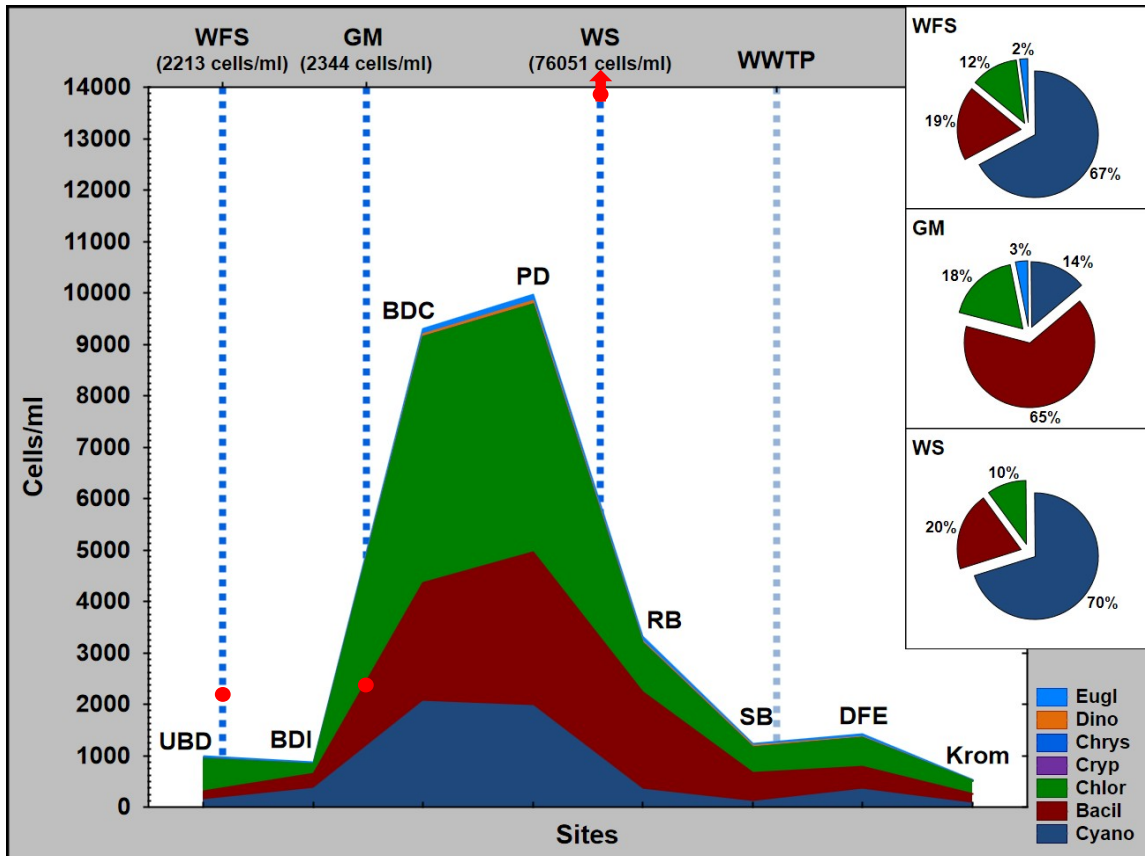


Figure 3-21 Area graph illustrating phytoplankton concentration (cells/ml) and composition during summer months in the Mooi River from January to December 2015. The dark blue dotted lines indicate inflow positions of the WFS, GM and WS, while the light blue dotted line indicates the location of the WWTP. The phytoplankton composition in each tributary is illustrated with a pie chart. Red dots represent the mean phytoplankton concentration (cells/ml) for the WFS, GM, and WS, while the total phytoplankton concentrations are given in brackets. Cyano = Cyanophyta, Bacil = Bacillariophyta, Chlor = Chlorophyta, Cryp = Cryptophyta, Chrys = Chrysophyta, Dino = Dinophyta, and Eugl = Euglenophyta.

At the origin of the Mooi River (UBD) the total phytoplankton concentration was relatively low compared to the rest of the river (Appendix F, Table F-1; Figure 3-21). A mixture of green algae, of which *Chlamydomonas* was the most conspicuous, dominated at this site. WFS showed a much higher phytoplankton concentration than UBD, and was dominated by cyanobacteria (mainly *Anabaena* filaments and *Microcystis* colonies). Cyanobacteria contributed 67% to the total

phytoplankton abundance. BDI showed a slightly lower phytoplankton concentration than UBD, but high numbers of cyanobacteria, particularly *Microcystis*, were present. GM had a higher phytoplankton concentration than WFS, of which 65% comprised of diatoms (especially *Cyclotella*). The phytoplankton concentration increased downstream to BDC and PD due to high concentrations of green algae. *Geminella* filaments were particularly important amongst the green algae. High concentrations of diatoms (especially the filamentous *Aulacoseira* during late December 2015), and cyanobacteria (*Anabaena*) were also found at these two sites. WS showed a very high phytoplankton concentration (Appendix F, Table F-1). Cyanobacteria dominated at this site (70% - see pie chart). Abundant cyanobacteria at WS included *Anabaena*, *Merismopedia* and *Microcystis*. High phytoplankton concentrations at PD and in the WS did not seem to have an effect on RB and SB downstream, where lower phytoplankton concentrations were recorded. Diatoms (specifically *Aulacoseira*) dominated the waters at these two sites. Slightly higher phytoplankton abundance was found 1 km downstream of the WWTP at DFE, where a mixture of green algae was present (mostly *Coelastrum* colonies). From here, the phytoplankton abundance decreased downstream towards the confluence with the Vaal River near Krom. Green algae, especially *Crucigeniella*, were the most apparent at Krom.

To summarise: During the summer months, the Mooi River and its tributaries were dominated by green algae, diatoms, and cyanobacteria. The total number of phytoplankton cells per ml of water in sites sampled in the Mooi River was mostly higher than in the WFS and GM, but much lower compared to concentrations in the WS (Appendix F, Table F-1).

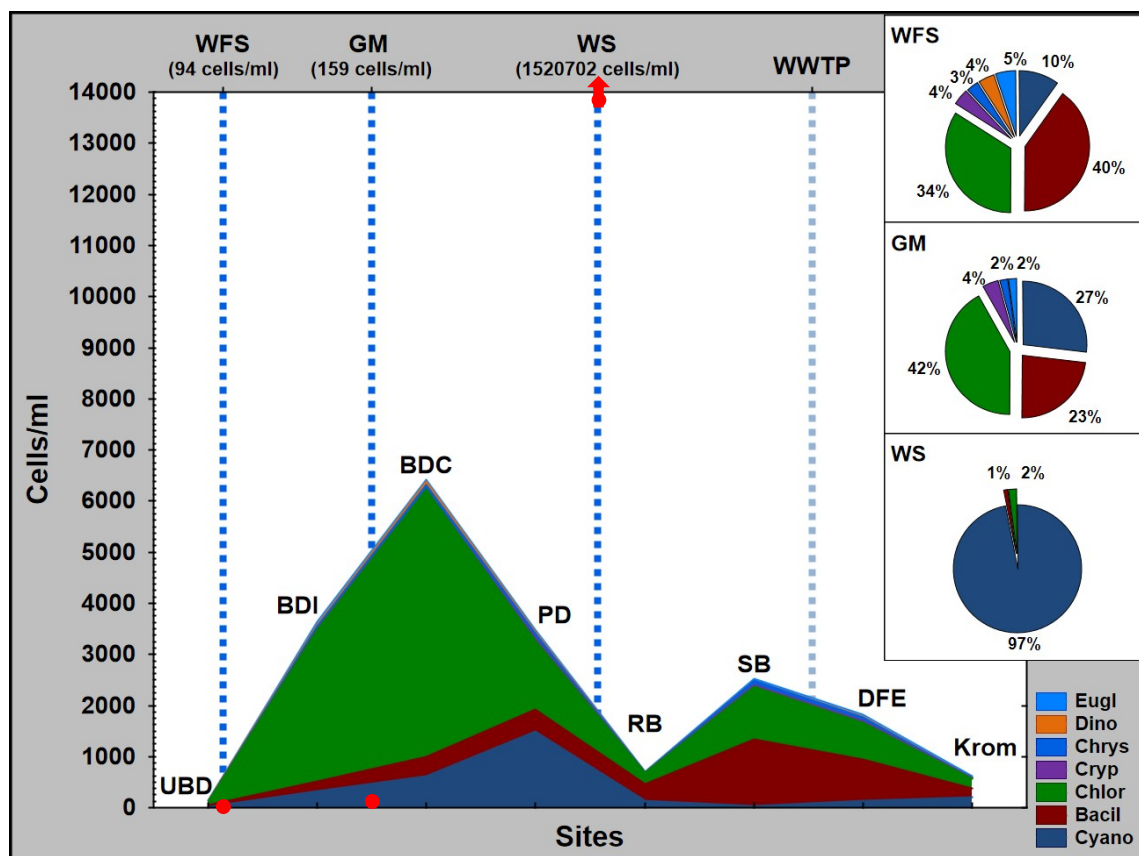


Figure 3-22 Area graph illustrating the phytoplankton concentration (cells/ml) and composition during autumn months in the Mooi River from January to December 2015. The dark blue dotted lines indicate inflow positions of the WFS, GM and the WS, while the light blue dotted line indicates the location of the WWTP. The phytoplankton composition in each tributary is illustrated with a pie chart. Red dots represent the mean phytoplankton concentration (cells/ml) for the WFS, GM, and WS, while the total phytoplankton concentrations are given in brackets. Cyano = Cyanophyta, Bacil = Bacillariophyta, Chlor = Chlorophyta, Cryp = Cryptophyta, Chrys = Chrysophyta, Dino = Dinophyta, and Eugl = Euglenophyta.

During autumn the lowest phytoplankton concentration in the Mooi River was recorded at the headwaters (UBD). Green algae, such as *Chlamydomonas* and *Crucigeniella*, dominated at this site. The lowest phytoplankton concentration in the entire river system was found in the WFS (Appendix F, Table F-2). Phytoplankton in the WFS was, however, diverse and included green algae (representing 34% of the algae; particularly *Chlamydomonas*) and diatoms (40%; mainly *Aulacoseira*). Other phyla present at this site included Cryptophyta (4%), Chrysophyta (3%), Dinophyta (4%) and Cyanophyta (10% - see pie chart of WFS in Figure 3-22). Phytoplankton concentration increased downstream to BDI. The green algae (particularly *Geminella*) dominated, while representatives of the cyanobacteria (mostly *Microcystis*) were also present. Although low

phytoplankton concentrations were recorded in GM, the diversity of algae was high and green algae (particularly *Chlamydomonas*), diatoms (*Fragilaria*) and cyanobacteria (*Phormidium*) were found. Downstream to BDC an increase in phytoplankton concentration was observed. BDC was dominated by green algae (*Geminella*). Cyanobacteria (mostly *Phormidium*) were also present at this site. Located about 0.16 km from this site, PD had higher numbers of cyanobacteria (*Phormidium*), but lower numbers of green algae (mainly *Geminella*). The WS showed high phytoplankton concentrations and was almost entirely dominated by cyanobacteria (97% abundance; particularly *Microcystis*). In spite of the high *Microcystis* numbers transferred into the Mooi River by the WS, phytoplankton abundance at RB was low and consisted mainly of diatoms (mostly *Cyclotella*), green algae and relatively low cyanobacteria numbers. Phytoplankton concentration increased towards SB and consisted of a mixture of green algae, and diatoms (*Cyclotella*). After water was treated by the WWTP and released back into the river, lower phytoplankton concentrations, consisting of diatoms (mostly *Cyclotella*) and green algae (mostly *Pediastrum*), were found at DFE. At Krom, the total phytoplankton concentration decreased further. Three phyla were present, namely Cyanophyta (especially *Merismopedia*), Bacillariophyta (mostly *Cyclotella*), and Chlorophyta (*Dictyosphaerium* which dominated at this site).

Less abundant phyla, such as Euglenophyta, Cryptophyta, Chrysophyta and Dinophyta increased during autumn, although they showed low concentrations compared to that of diatoms, green algae, and cyanobacteria. The total cell concentration recorded for the Mooi River was much higher than the total concentrations recorded in the WFS and GM, but much lower than concentrations in the WS (Appendix F, Table F-2).

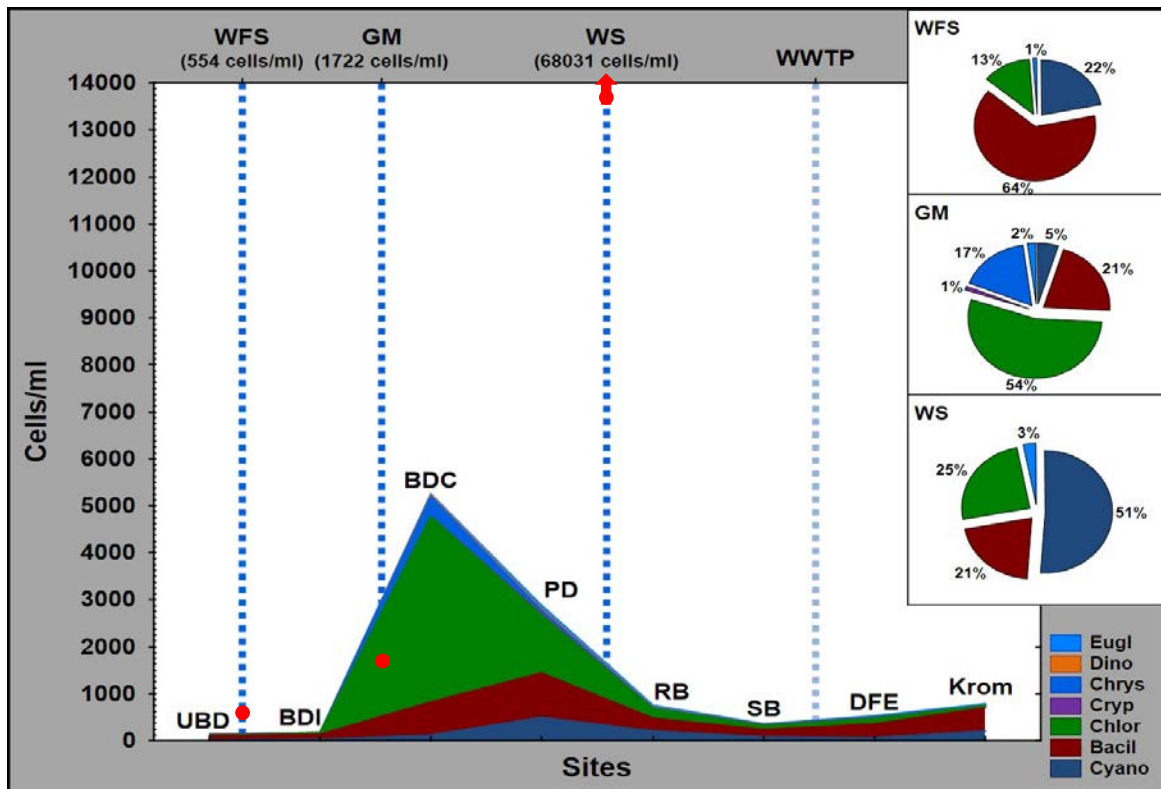


Figure 3-23 Area graph illustrating the phytoplankton concentration (cells/ml) and composition during winter months in the Mooi River from January to December 2015. The dark blue dotted lines indicate inflow positions of the WFS, GM and the WS, while the light blue dotted line indicates the location of the WWTP. The phytoplankton composition in each tributary is illustrated with a pie chart. Red dots represent the mean phytoplankton concentration (cells/ml) for the WFS, GM, and WS, while the total phytoplankton concentrations are given in brackets. Cyano = Cyanophyta, Bacil = Bacillariophyta, Chlor = Chlorophyta, Cryp = Cryptophyta, Chrys = Chrysophyta, Dino = Dinophyta, and Eugl = Euglenophyta.

UBD showed the lowest phytoplankton concentration during the entire winter period. Diatoms, particularly *Cyclotella*, dominated at this site. The WFS joins the Mooi River with higher phytoplankton concentrations (Appendix F, Table F-3). Diatoms, especially *Cyclotella*, were dominated in the WFS. Other diatom genera such as *Navicula* and *Nitzschia* were also present. Cyanobacteria (22% of the phytoplankton composition; such as *Spirulina*), were also present. At BDI, the total phytoplankton concentration remained low, and was dominated by diatoms, particularly *Cyclotella*. GM showed higher phytoplankton concentrations. *Dinobryon*, a colonial chrysophyte, contributed 17% of the total phytoplankton composition in this tributary. Green algae (*Gonatozygon*) and different diatom genera (particularly *Fragilaria*) were also found at this site. Downstream from the inflow of GM, the phytoplankton concentration increased to BDC,

whereafter it decreased again to PD. These two sites were dominated by *Geminella* for an extensive period of time. High numbers of diatoms (particularly *Cyclotella*) were also present. *Dinobryon* was also present at BDC in relatively high concentrations. PD showed high numbers of *Diatoma* (diatom) and *Microcystis* (cyanobacteria). The WS joins the Mooi River with a very high phytoplankton concentration (68 000 cells/ml), of which 51% can be ascribed to cyanobacteria (mostly *Anabaena*, *Microcystis*, and *Merismopedia*). Diatoms (such as *Cymatopleura*, *Diatoma* and *Gomphonema*) and green algae (such as *Coelastrum*, *Pandorina*, *Pediastrum*, and *Scenedesmus*) were also present in the WS in large numbers. Despite high phytoplankton concentrations found in the WS, RB located just downstream from the inflow of the WS, showed relatively low phytoplankton concentrations. Cyanobacteria (particularly *Microcystis*), diatoms (particularly *Diatoma*), and a combination of green algae (such as *Coelastrum* and *Scenedesmus*) were present at this site. The total phytoplankton concentration decreased downstream to SB where diatoms (especially *Diatoma*) dominated. A mixture of green algae and cyanobacteria were also found in relatively low concentrations at SB. The phytoplankton concentration increased to DFE as the result of diatoms (particularly *Nitzschia*) that dominated. DFE also showed relatively high concentrations of green algae (mostly *Pediastrum*). The total phytoplankton concentration increased to Krom with diatoms (such as *Navicula*, *Nitzschia* and *Cocconeis*) being the dominants.

Some of the less conspicuous phyla, such as Euglenophyta, Cryptophyta and Chrysophyta, have been more apparent during winter months, but still showed low concentrations compared to diatoms, green algae and cyanobacteria. Again the total phytoplankton concentration for the entire Mooi River was much higher than the total phytoplankton concentrations recorded in the WFS and GM, but much lower compared to the concentrations found in the WS (Appendix F, Table F-3).

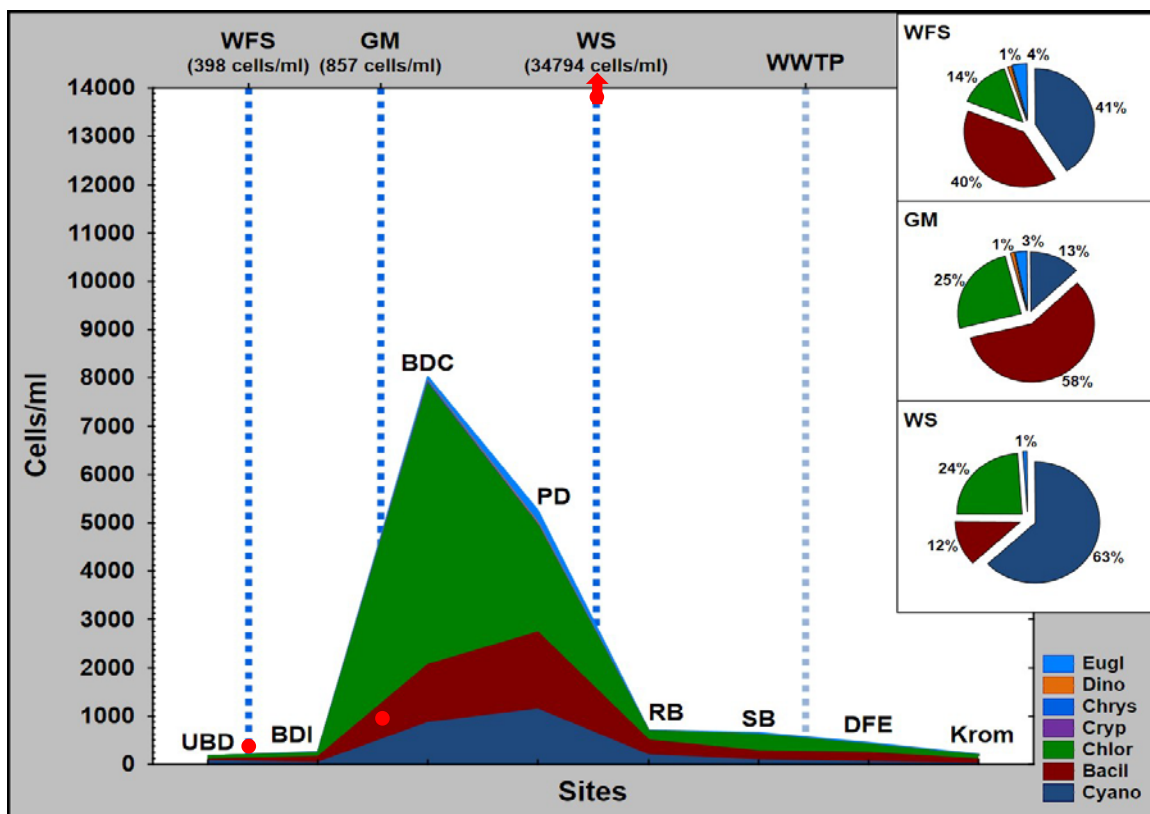


Figure 3-24 Area graph illustrating the phytoplankton concentration (cells/ml) and composition during spring months in the Mooi River from January to December 2015. The dark blue dotted lines indicate inflow positions of the WFS, GM and the WS, while the light blue dotted line indicates the location of the WWTP. The phytoplankton composition in each tributary is illustrated with a pie chart. Red dots represent the mean phytoplankton concentration (cells/ml) for the WFS, GM, and WS, while the total phytoplankton concentrations are given in brackets. Cyano = Cyanophyta, Bacil = Bacillariophyta, Chlor = Chlorophyta, Cryp = Cryptophyta, Chrys = Chrysophyta, Dino = Dinophyta, and Eugl = Euglenophyta.

The phytoplankton concentration at the origin of the Mooi River (UBD) was low during spring. Cyanobacteria (particularly *Nostoc*) and green algae (mostly *Chlamydomonas*) dominated at this site. Higher phytoplankton concentrations were measured in the WFS (Appendix F, Table F-4). The cyanobacteria (especially *Oscillatoria*) and diatoms (*Nitzschia*) dominated this site. Phytoplankton concentrations at BDI were still low. BDI was dominated by diatoms (mostly *Aulacoseira*). GM showed higher phytoplankton concentrations and 58% of the phytoplankton comprised of diatoms (particularly *Fragilaria* and *Navicula*). Green algae (especially *Gonatozygon*), and cyanobacteria (especially *Microcystis*) were also present at GM (25 and 13% respectively – see pie chart). A huge peak in phytoplankton concentration was observed at BDC, mostly due to high concentrations of green algae, in particular *Ulothrix* (filamentous green algae).

Diatoms (especially *Aulacoseira*) and cyanobacteria (mainly *Microcystis*) were also found at this site. PD also had high phytoplankton concentrations, but concentrations were lower than at BDC. Green algae, such as *Ulothrix*, dominated in PD. PD also showed high numbers of cyanobacteria (*Microcystis*) and diatoms (such as *Aulacoseira* and *Diatoma*). Similar to the other seasons described above, the WS was characterised by exceptionally high phytoplankton concentrations (Appendix F, Table F-4) compared to sites in the Mooi River or in the other tributaries. Cyanobacteria (particularly *Microcystis*) comprised 63% of the total abundance. High concentrations of green algae, such as *Coelastrum*, *Eudorina*, *Pandorina* and *Pediastrum*, were also present in the WS. Downstream from the inflow of the WS, the phytoplankton concentration decreased. At this site (RB) there was a mixed assemblage of diatoms (particularly *Aulacoseira*), cyanobacteria (such as *Microcystis*) and green algae (such as *Ulothrix*). The phytoplankton concentration remained constant downstream to SB, with a combination of different green algae (*Coelastrum* dominant). A small decrease in phytoplankton concentration was observed at DFE. Diatoms, particularly *Nitzschia*, dominated. A diversity of green algae (dominated by *Pediastrum*) was also present at DFE. The total phytoplankton concentration further decreased to Krom with diatoms, particularly *Cocconeis* and *Nitzschia*, present at highest concentrations.

The most abundant groups during autumn were again diatoms, green algae and cyanobacteria. The total phytoplankton concentration recorded for the entire Mooi River during the spring months, was much higher than the total phytoplankton concentrations recorded in the WFS and GM, but lower compared to the concentration found in the WS (Appendix F, Table F-4).

The tendencies observed in phytoplankton concentration across all seasons can be summarised as follows: In general the phytoplankton concentration was low at the source of the Mooi River. Maximum concentrations were recorded at BDC and PD. Downstream from BDC the concentration decreased until relatively low concentrations were recorded at Krom, just before the confluence of the Mooi and Vaal Rivers. High phytoplankton concentrations were found in the WS. Concentrations recorded in the WS exceeded that of all other sampling sites. At all sites and during all seasons' green algae, diatoms and cyanobacteria were the dominant groups encountered. Other algal phyla, such as the Cryptophyta, Chrysophyta, Dinophyta and Euglenophyta, were present in low proportions compared to these three phyla. Cyanobacteria reached maximum concentrations at BDC and PD sites. No obvious differences were observed during the different seasons of the year. In general highest phytoplankton concentrations were recorded during the summer period, while lowest concentrations were recorded during the winter months.

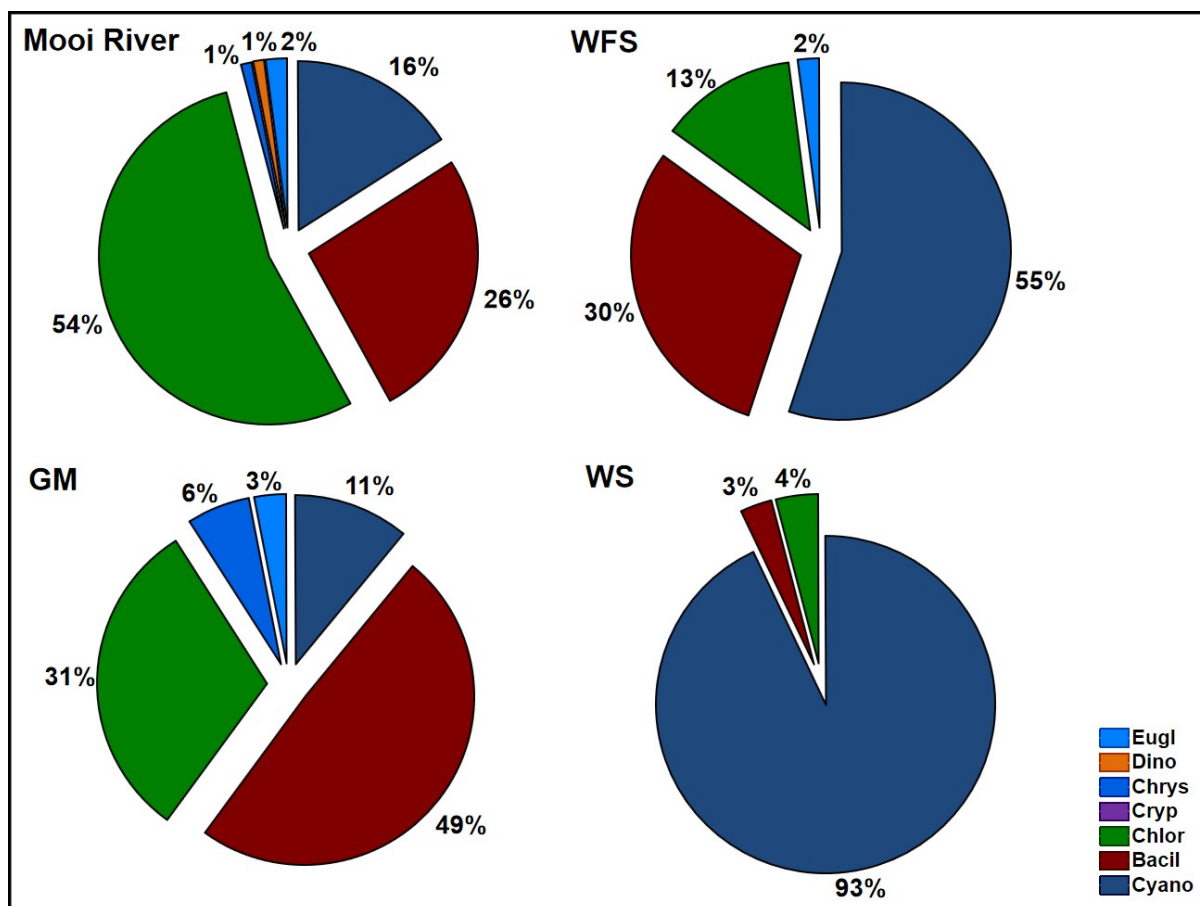


Figure 3-25 Relative abundance of phytoplankton phyla present during the study period January to December 2015 in the Mooi River, WFS, GM and WS.

Figure 3-25 shows that the most abundant phytoplankton phyla in the Mooi River’s main stream (in decreasing order) were Chlorophyta (54%), Bacillariophyta (26%), Cyanophyta (16%), Euglenophyta (2%) and Chrysophyta and Dinophyta (1% each). The concentration of Cryptophyta was so low, relative to the other phyla, that it is not visually illustrated on the graph.

In the WFS, the Cyanophyta showed the highest relative abundance (55%), followed by Bacillariophyta (30%), Chlorophyta (13%), and Euglenophyta (2%). GM was dominated by Bacillariophyta (49%), followed by Chlorophyta (31%), Cyanophyta (11%), Chrysophyta (6%), and Euglenophyta (3%). Other phyla were present in very low concentrations. Cyanophyta almost entirely dominated in the WS (relative abundance of 93%). Chlorophyta (4%) and Bacillariophyta (3%) were present in low concentrations, compared to Cyanophyta.

3.4 Relationships between variables

During the study period (January to December 2015) positive relationships were observed between certain variables in the Mooi River (tributaries excluded). The variables showing the strongest positive relationships were TDS and EC; TDS, chloride, and sulphate; and cyanobacteria and pH (Figure 3-26 to Figure 3-28). TDS and EC both showed a positive relationship with chloride and sulfate, but only one variable (TDS) was chosen for illustration. Chloride and sulfate are major ions contributing to the TDS concentration, which explain why TDS and EC correlate strongly with them.

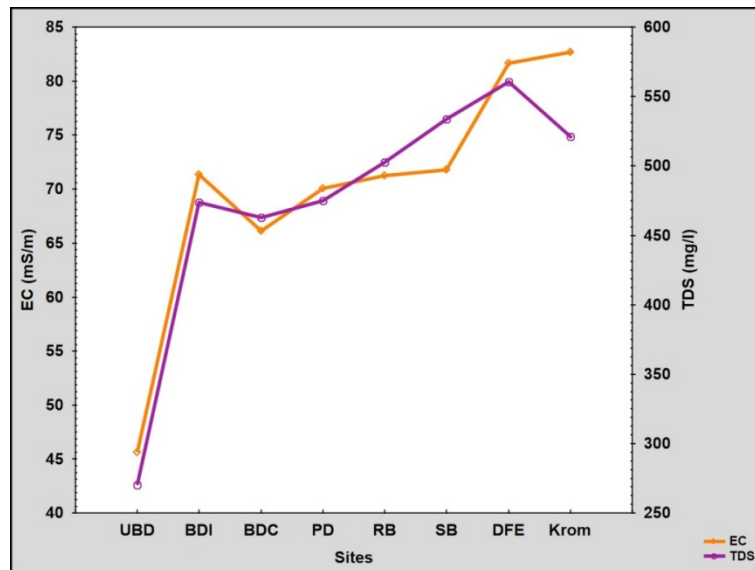


Figure 3-26 Line graph illustrating the relationship between TDS and EC at the different sites located in the Mooi River (January to December 2015).

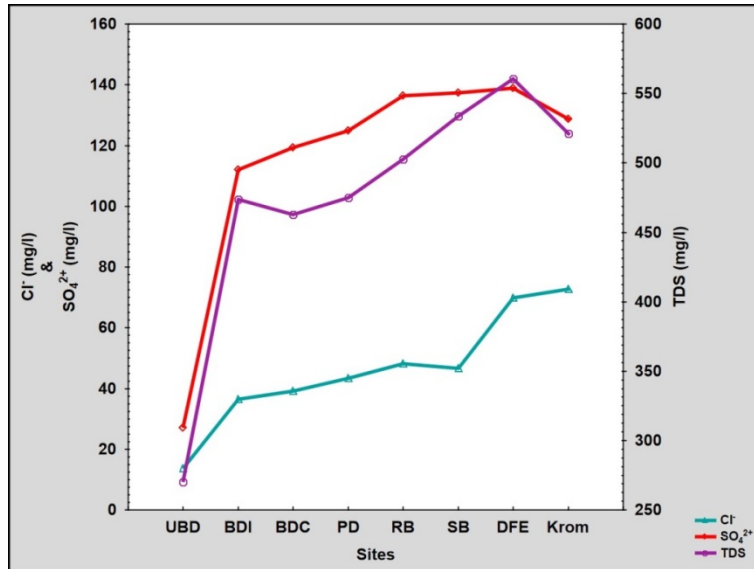


Figure 3-27 Line graph illustrating the relationship between TDS, chloride, and sulfate at the different sites located in the Mooi River (January to December 2015).

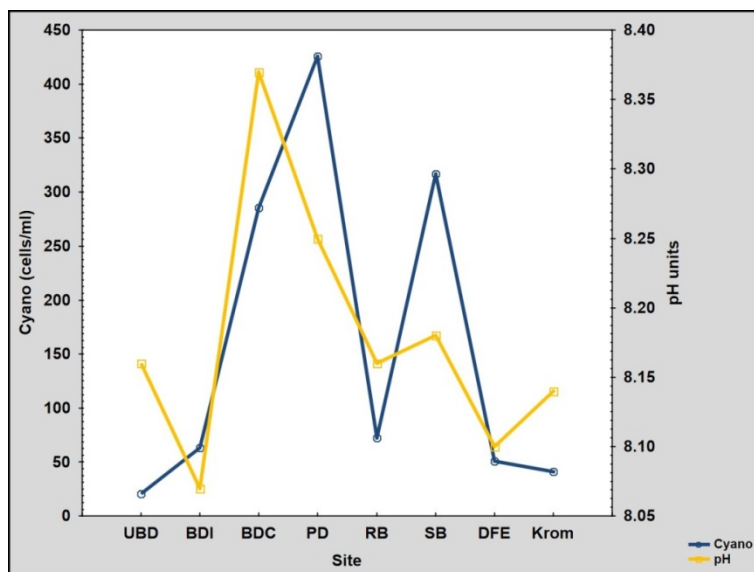


Figure 3-28 Line graph illustrating the relationship between Cyanobacteria (Cyano) and pH at the different sites located in the Mooi River (January to December 2015).

3.5 Multivariate Analyses

Multivariate analyses are statistical tools, which have the ability to allow simultaneous analyses for more than one dataset (Harper *et al.*, 2000). Two data-sets, one on physico-chemical, and the other one on phytoplankton phyla, were used for multivariate analyses in this study. A canonical correspondence analysis (CCA; Figure 3-29) was performed which contained all the variables of the two data-sets to see how they relate to one another and to the axes. The

CCA only includes data of the Mooi River and not the tributaries, as the purpose of the CCA was to draw an overall conclusion for conditions in the river itself, and to see how different water quality variables relates to one another in the main stream. Heavy metal data was also excluded, because it was not available for all sites located in the Mooi River.

Eigenvalues for the first four axes of the CCA is displayed in Table 3-3. The first axis explained 57% of the variance in the species-environmental data, and the second axis explained 25% of the variance in the species-environment relationship.

Table 3-3 Eigen values of the CCA on the phytoplankton and physico-chemical variable data of the Mooi River from January to December 2015.

Axes	1	2	3	4	Total
Eigenvalues	0.033	0.015	0.007	0.002	0.291
Species-environment correlations	0.492	0.454	0.417	0.310	
Cumulative percentage variance of species data	11.4	16.4	18.9	19.7	
Cumulative percentage variance of species-environment relation	57.0	82.0	94.4	98.2	
Sum of Eigenvalues					0.291
Sum of all canonical eigenvalues					0.058

The Monte Carlo Permutation test on all canonical axes (number of permutations = 499) showed a P-value of 0.004 and an F-ratio of 1.731, indicating that the environmental (physico-chemical) variables significantly correlated with the species (phytoplankton) data. The physico-chemical variables for the CCA were interpreted based on a few factors which include:

- if the vectors (arrows) of the variables form a small angle between each other, the variables correlate positively to one other. The smaller the angle, the stronger the positive correlation;
- if the vectors of the variables form a 90° angle, there is no relationship between the variables;
- if the vectors of the variables form a 180° angle (or are directed oppositely), the variables relate negatively to one another; and

- the variable which has the longest vector within an axis, carries the strongest relation on the axis.

The above factors were also applied for the interpretation for the clustering of the sampling sites and phytoplankton phyla, and to the physico-chemical variables (Bhat *et al.*, 2014; Booyens, 2016).

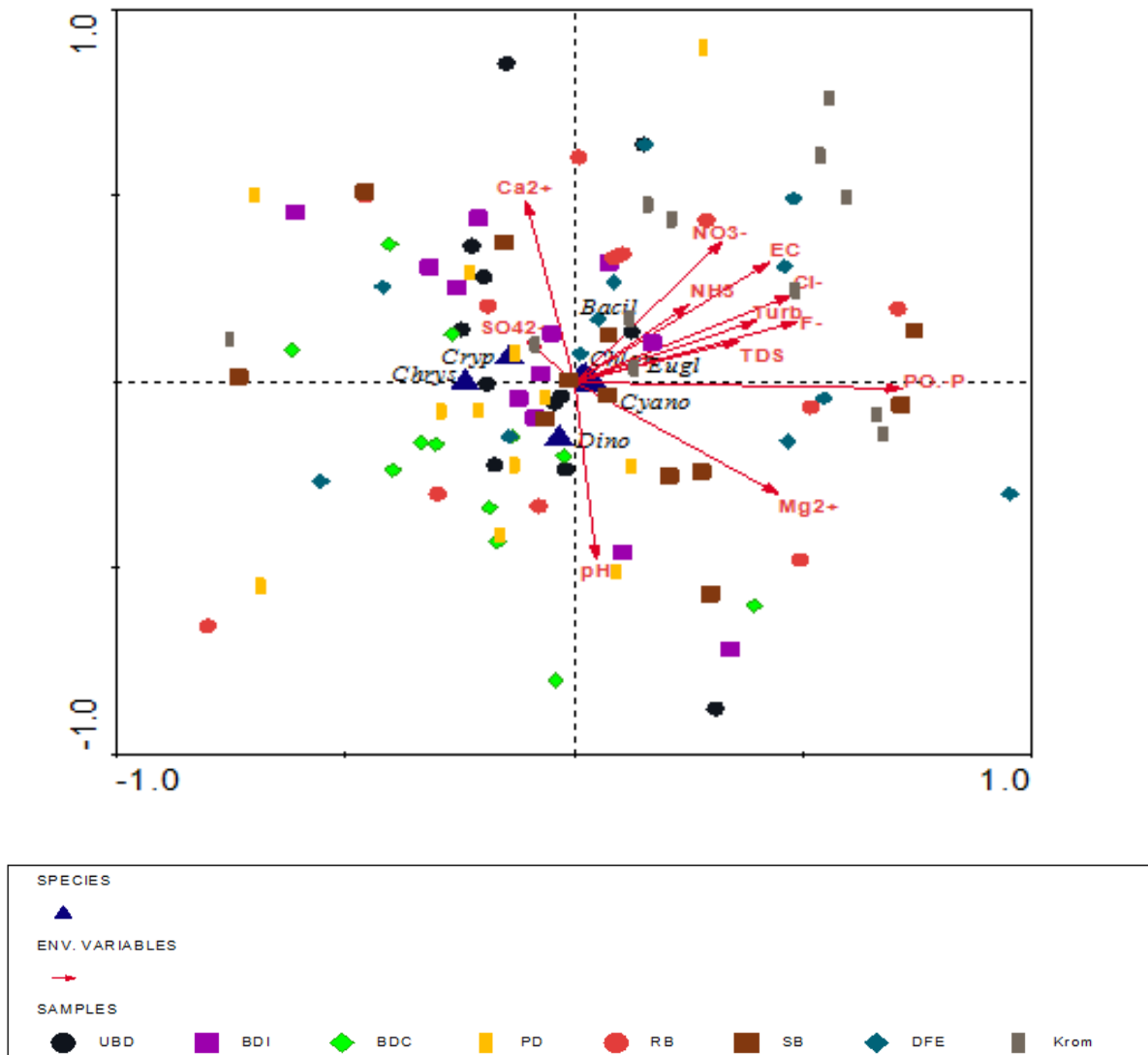


Figure 3-29 Canonical correspondence analyses (CCA) between the physico-chemical environmental variables, natural log of the phytoplankton data, and the different sites located in the Mooi River (January to December 2015).

The CCA (Figure 3-29) confirms significant relationships between different variables illustrated in Figure 3-26 to Figure 3-28. EC, TDS, and turbidity relate well to each other (strong positive correlation) as well as to the anions chloride (Cl⁻), and fluoride (F⁻) – this is also supported by correlations values in Table 3-3. Calcium (Ca²⁺) and Magnesium (Mg²⁺), in contrast, show a negative relation with one another, because their vectors are situated opposite to each other. Cyanobacteria (Cyano) were positively correlated, with orthophosphate (PO₄-P), TDS, pH, magnesium (Mg²⁺), and turbidity. Orthophosphates also seem to be the most important variable with the greatest influence on the first axis (refer to the length of the vector). The positive correlation between cyanobacteria and orthophosphates, coupled with orthophosphates having the most important influence, emphasise the importance of this variable on cyanobacteria dynamics. Besides orthophosphates, cyanobacteria also showed a positive correlation with other nutrients, namely ammonia (NH₃⁺), and nitrates (NO₃⁻). Chlorophytes (Chlor), diatoms (Bacil), and euglenophytes (Eugl) also correlated positively with nutrients. The cryptophytes (Cryp), chrysophytes (Chrys), and dinoflagellates (Dino), did not occur frequently or in high abundance in the Mooi River. These groups are generally associated with low phosphate concentrations, as well as low TDS and turbidity values. pH showed a negative correlation with most physico-chemical variables, except magnesium. BDC and PD, displayed the highest phytoplankton concentration recorded for the Mooi River, and these sites were strongly negatively correlated with nutrients. The negative correlation is probably due to high phytoplankton concentrations that deplete the nutrients in the water (Figure 3-21 to Figure 3-24).

CHAPTER 4: DISCUSSION

“If there is magic on this planet, it is contained in water.” Loran Eisely

4.1 Physico-Chemical Environmental Variables (Including Resource Quality Objectives and Resource Water Quality Objectives)

4.1.1 Nutrients

According to De Villiers (2007) the levels of major nutrients (which include orthophosphates, nitrates, nitrites, and ammonia) increased globally over the last few decades, making water bodies vulnerable to eutrophication. In South Africa, numerous water catchment areas deteriorated to such an extent that it became entirely eutrophic (Van Ginkel, 2011). An example is the Hartbeespoort Dam, once described as an oligotrophic dam, rich in oxygen (Hutchinson *et al.*, 1932), but currently classified as a hypereutrophic impoundment (Botha, 2015; Paerl *et al.*, 2001).

Eutrophication can result in the excessive growth of algae, some of which are harmful, and causing problems such as bad tastes and odours, the reduction of oxygen levels, all interfering with the desirable uses of water (Meyer & Rossouw, 1992). Although eutrophication can be a result of natural causes, it is often driven by inputs of nutrients through anthropogenic activities. Point sources (such as WWTP's), diffuse sources (such as discharges from fertilised agricultural areas), and urban runoff are three major anthropogenic causes responsible for nutrient inputs in water bodies (WHO, 2002). According to De Villiers & Thiar (2007) agricultural activities and sewage effluent are two of the main contributors to enriched nutrient levels in South African rivers.

Between 1985 and 1988, the Department of Water Affairs (DWA) and the Council for Scientific and Industrial Research (CSIR) completed an investigation and set specific water quality guidelines to control nutrient enrichment, in order to prevent water bodies to become eutrophic. They found that the best way to manage eutrophication in rivers and streams is to set target water quality requirements for chlorophyll concentration and total phosphorus (DWA 1988; Van Ginkel 2011). The target water quality requirements set for chlorophyll *a* concentration ($\mu\text{g/l}$) by DWA (1988) and Van Ginkel (2011) are as follows: oligotrophic ($0 < x \leq 10$), mesotrophic ($10 < x \leq 20$), eutrophic ($20 < x \leq 30$), and hypertrophic (> 30), where x represents the variable, whereas the requirements for total phosphorus (mg/l) are set for negligible ($x \leq 0.015$), moderate ($0.015 < x \leq 0.047$), significant ($0.047 < x \leq 0.130$), and serious (> 0.130). Total phosphorus (TP) consists of dissolved inorganic phosphorus (DIP), together with the insoluble forms. Total nitrogen (TN) consists of dissolved inorganic nitrogen (DIN) and organic nitrogen. However, the abbreviation

TP, used in Dallas & Day (2004), does not refer to total phosphorus, but is used for total inorganic phosphorus, which excludes insoluble forms, making TP equivalent to DIP. The same applies to the abbreviation for TN in Dallas & Day (2004) where it is used for total inorganic nitrogen (Dallas & Day, 2004), instead of total nitrogen (organic and inorganic). According to Dallas & Day (2004) chlorophyll is often used as a non-specific indicator when managing eutrophication, and therefore the best way is to manage the total inorganic phosphorus (TP in Dallas & Day or DIP in most other sources) and total inorganic nitrogen (TN in Dallas & Day, or DIN in most other sources) concentrations. The terms DIN and DIP will be used during this study to avoid any confusion. Dallas & Day (2004) further stated that if DIP and DIN concentrations are maintained below certain target limits, it should ensure that the chlorophyll concentration is also below the set limits.

Phosphorus (orthophosphate) usually occurs in dissolved inorganic form (DIP), while nitrogen mostly occurs as nitrate, nitrite and ammonium ions (DIN; Dallas & Day, 2004; Chapter 3, section 3.1.1). Only ammonia and nitrate data was available during the study period, and the sum of their concentrations is compared to the ranges set for DIN. The lack of nitrite data may affect the ranges of the criteria, but unlike nitrate, nitrite concentrations are usually low so that it only has a minor contribution to the DIN (Dallas & Day, 2004).

4.1.1.1 Ammonia and nitrate

Ammonia and nitrate concentrations normally occur in water bodies due to natural phenomena, such as climate change and hydrogeology (Dallas & Day, 2004; Fu *et al.*, 2012). However, anthropogenic activities can result in elevated concentrations of these nutrients. Urban, agricultural (especially pig and poultry farming), and industrial runoffs are the main causes of elevated ammonia levels (Fu *et al.*, 2012), while irrigation, agriculture and sewage effluents are mostly known to elevate nitrate levels in water bodies (Tredoux *et al.*, 2009).

Both ammonia and nitrate concentrations were relatively high at the origin of the Mooi River (before any inflowing tributaries), as it exceeded the RQO and RWQO limits. The mean ammonia concentration at UBD (0.12 mg/l) was four times higher than the RWQO limit (of 0.03 mg/l). The mean nitrate concentration at UBD was 0.43 mg/l, exceeding both the RQO (≤ 4 mg/l) and RWQO (0.3 mg/l) limits (Table 3-1). The origin of the Mooi River is surrounded by agricultural activities (including pig farming), that can explain high ammonia and nitrate concentrations.

Natural waters typically contain ammonia compounds in concentrations below 0.1 mg/l (Dallas & Day, 2004). In general, it seems as if progressively higher mean ammonia concentrations, arising from inflowing tributaries (WFS, GM and WS), had a huge effect on the

ammonia concentrations at sampling sites downstream from the points of inflow (Figure 3-1). High ammonia concentrations in the WFS may be the cause of the various WWTP's (such as Flip Human, REGM, and Cooke 2; Chapter 2, section 2.2.2.3) located in the area, discharging their effluent back into the WFS. The high ammonia concentrations in GM may be the result of peat mining in the area. According to Bourbonniere (2009) drainage by peat mining can result in elevated ammonia concentrations in water bodies through the decomposition of soil. The WS tributary had a major impact on the Mooi River in terms of ammonia concentrations. WS showed a statistical significant difference with most upstream sites (UBD to BDC), before it converges with the Mooi River (Appendix B, Table B-1). High values (mean of 1.12 mg/l) measured in the WS were reflected in the Mooi River at RB and SB, located downstream from the point of inflow, and a clear increase in mean ammonia concentration was observed between PD and RB as a direct effect of the inflow. High ammonia concentrations in the WS tributary can be attributed to various sources, including urban and storm water runoff, and sewage from informal and semi-formal settlements, such as Ikageng and especially Promosa (Chapter 2, section 2.2.4.1).

Similar tendencies were observed for nitrate concentrations, namely that successive tributaries flowing into the Mooi River were responsible for increasing concentrations in the Mooi River (Figure 2-2). It does seem as if the nitrate concentrations in the Mooi River system were influenced to a lesser extent by the inflowing tributaries than the ammonia concentrations. High nitrate concentrations were again recorded in the WS (mean of 1.12 mg/l), which showed a statistical significant difference with the site at the origin of the Mooi River (UBD; Appendix B, Table B-1). The WWTP may have contributed significantly to elevated nitrate levels downstream from its inflow, but unfortunately no measurement was made at the WWTP.

Mean values calculated for the Mooi River and all three tributaries exceeded both the RQO and RWQO limits set for ammonia and nitrates (Table 3-1). The sum of ammonia and nitrate for the entire Mooi was calculated 0.84 mg/l, which is lower than that of all three tributaries, namely the WFS (1.12 mg/l), GM (0.95 mg/l) and WS (2.24 mg/l). When the sum of ammonia and nitrate is compared to the DIN ranges which appears in DWAF (1996) and Dallas & Day (2004), the Mooi River and all three the tributaries range between 0.5 to 2.5 mg/l, and is indicative of mesotrophic state (DWAF, 1996). DIN (consisting of ammonia and nitrate) plays a secondary role in eutrophication, while DIP (orthophosphate) is recognised as the primary cause of eutrophication in fresh water bodies (Paerl *et al.*, 2001; Walmsley, 2000).

4.1.1.2 Orthophosphate

Orthophosphates are mainly influenced by agricultural, industrial and sewage effluents (Muscutt & Withers, 1996; Pelley, 2016). The mean orthophosphate concentration at the origin of the Mooi River (0.34 mg/l) exceeded the RQO limit (<0.125 mg/l), and can be the result of agricultural activities. However, it was still within the RWQO limit (0.4 mg/l; Table 3-1). Again, an increasing trend in the mean orthophosphate concentrations was observed from the WFS to GM to the WS tributaries (Figure 3-3). High concentrations, particularly in the WS tributary (mean of 1.02 mg/l), resulted in increasing concentrations in the Mooi River downstream from the point of inflow. Although orthophosphate data is not available at the WWTP, it seems as if it may have contributed significantly to elevated orthophosphate levels downstream from the inflow.

The mean orthophosphate concentrations for the entire Mooi River (0.68 mg/l), the GM (0.54 mg/l) and WS (1.02 mg/l) exceeded the RQO (<0.125 mg/l) and RWQO (0.4 mg/l) limits. Orthophosphate concentrations in the WFS (0.34 mg/l) only exceeded the RQO limit (Table 3-1). According to DWAF (1996) orthophosphate concentrations above 0.25 mg/l are indicative of hypertrophy. The entire Mooi River, and all three the tributaries, are therefore indicative of hypertrophic conditions.

Venter *et al.* (2013) found that the Mooi River was changing from a mesotrophic to a eutrophic system in terms of nutrient concentrations. Labuschagne (2017), who conducted a study on the Mooi River during the same period (2014 to 2015) than the current study (2015), found a very similar mean orthophosphate value of 0.163 mg/l, indicative of a eutrophic status according to DWAF (1996) guidelines. Phosphate is considered to be the main nutrient regulating the degree of eutrophication in aquatic ecosystems (DWA, 2009). JB Marks municipality (personal communication: Me. Bothma), who supplied the data, confirmed that high orthophosphate concentrations (around 0.68 mg/l) are often recorded in the Mooi River. For further certainty, the orthophosphate data of 2014 and 2017 was obtained from the municipality to see if any other factors, e.g. rainfall (2015 was a very dry year), could have been responsible for the high values during 2015 (Appendix H, Table H-1). From Table H-1 it can be seen that mean orthophosphate values increased slightly from 2014 to 2015, and decreased again from 2015 to 2017. However, all these concentrations were high and fell within the ranges specified for a hypertrophic system.

Luyt (2018) found high mean total phosphorus values for the Mooi River (17 mg/l) and WFS (8.2 mg/l) during 2016 and 2017. During the same period nitrogen concentrations were, however, low and indicative of oligotrophic conditions. Luyt (2018) explained that, in spite of high prevailing phosphorus concentrations, low nitrogen concentrations can prevent hypertrophic symptoms by

limiting algal growth. Yet, it is of utmost importance that attention should be drawn to the Mooi River and tributaries in terms of nutrient, especially phosphorus enrichment.

4.1.2 Total Dissolved Solids (TDS), Electrical Conductivity (EC) and Major ions

Dissolved material in water can be measured in three ways, namely TDS, salinity, and electrical conductivity (EC). These three parameters usually correlate well with one another in most waters, and are seen as some of the most important descriptors in water quality (Dallas & Day, 2004; Dodds, 2002). Only TDS and EC were used in this study and these two variables showed a positive correlation with each other (Figure 3-26; Appendix G, Table G-1).

4.1.2.1 Total Dissolved Solids (TDS)

TDS concentrations in natural waters range from 7 mg/l (freshwater) to 35 000 mg/l (seawater; Dallas & Day 2004), and water is classified as non-saline if concentrations are less than 1000 mg/l (Thirupathi & Muniyan, 2018). The mean salinity of the world's rivers is about 120 mg/l (Weber-Scannell & Duffy, 2007), and most of these rivers' TDS concentrations are less than 100 mg/l (Hutchinson, 1975). In South Africa, the lowest TDS concentration ranges were recorded in the Waterkloof stream (10-27 mg/l; Dallas & Day, 2004), while the highest TDS concentration was recorded in Burgerspan (65 000 mg/l; Silberbauer & King, 1991). TDS is determined by natural factors including atmospheric rainfall, geology of the drainage area, and the water balance (rainfall versus evaporation; Van der Laan *et al.*, 2012; Weber-Scannell & Duffy, 2007). However anthropogenic impacts, including irrigation and sewage effluent, elevate TDS concentrations (Janse van Vuuren & Taylor, 2015).

The mean TDS concentration at the source of the Mooi River (270 mg/l) was the lowest recorded for all sites in the entire Mooi River system and was 27% below the RWQO limit of 370.5 mg/l (Table 3-1). However, the value is high compared to the world average for rivers (100 mg/l; Hutchinson, 1975). Elevated TDS concentrations at the source can be the result of the dolomitic geology underlying the area, and irrigation taking place along the length of the river. High TDS concentrations of WFS, and WS affected the TDS concentration in the Mooi River, because increases were observed at sites downstream from the inflows of these tributaries (Figure 3-4). High TDS concentrations in WFS (mean of 496 mg/l) could be the result of dolomitic geology, leaching and erosion from older slime dams (Booyens, 2016; Van der Walt *et al.*, 2002), and various waste water effluents from dewatering mines, WWTP's, as well as poorly treated sewage effluents from informal settlements. High TDS concentrations in GM may be ascribed to the fact that GM gains water directly from the WFS tributary through the Boskop-Turffontein underground

compartment and the Wonderfontein Eye. Though, Winde (2008) stated that the source contributing to highly polluted groundwater between two peat mining operations, responsible for high EC values, remains unclear. Because EC and TDS showed a positive relation (trend) in the current study (Figure 3-26), it is assumed that it may also be a reason for the high TDS concentrations at GM. High TDS concentrations in the WS (402 mg/l) can be the result of sewage from semi-formal and informal settlements, as well as blocked pump stations, and the phosphogypsum heap (Chapter 2, section 2.2.4.2). No TDS data was available for the WWTP but it seems as if its outflows could have contributed to elevated TDS levels downstream in the Mooi River system.

The TDS concentration in the Mooi River ranged from 208 to 850 mg/l. The mean TDS concentrations for the entire Mooi River (475 mg/l), the WFS (496 mg/l), the GM (558 mg/l), and the WS (402 mg/l) all exceeded the suggested RWQO limit of 370.5 mg/l (Table 3-1).

During the study period a positive relation was observed between TDS, chloride and sulfate (Figure 3-27). TDS is the sum of the major ions (Marandi *et al.*, 2013), and therefore TDS positively relates with the cations (calcium, magnesium, sodium, and potassium) and anions (bicarbonate, carbonate, chloride, sulfate, and fluoride).

4.1.2.2 Electrical Conductivity (EC)

EC and TDS positively correlated (Appendix G, Table G-1). In general, it seems as if especially the WFS and WWTP could have had the most significant effects in elevating EC levels in the Mooi River system (Figure 3-5). The overall mean EC for the Mooi River (70 mS/m), the WFS (78 mS/m), the GM (77 mS/m), and the WS (61 mS/m) all exceeded the RWQO limit of 57 mg/l (Table 3-1), but are below the suggested RQO limit (≤ 111 mS/m). Pelsier (2015) found that the EC levels in the Mooi River ranged between 38 and 211.2 mS/m along the length of the Mooi River during 2015. Although measurements made during this study (39 - 110 mS/m) correspond well to those made by Pelsier (2015), slightly lower maximum values were recorded.

Usually an increase in TDS was responsible for an increase in EC (Figure 3-26). This is because EC is a surrogate measure of TDS (Janse van Vuuren, 2001; Thirupathi & Muniyan, 2018).

4.1.2.3 Calcium and Magnesium

Calcium was the second most abundant ion in the Mooi River, while magnesium was the fourth most abundant (Chapter 3, section 3.1.2, page 43). Mean calcium concentrations at all

sites along the Mooi River (70.06 mg/l), including the source (58.93 mg/l), as well as the tributaries WFS (87.73 mg/l), and GM (46.67 mg/l) exceeded the RWQO limit of 47 mg/l. No RQO limits were set for calcium. The mean magnesium concentration for the entire Mooi River (48.90 mg/l), the source (37.74 mg/l), WFS (43.84 mg/l), and GM (54.74) exceeded both the RQO and RWQO limits (≤ 33 and 30 mg/l respectively; Table 3-1). According to Potasznik & Szymczyk (2015) underlying geology is one of the leading natural causes of calcium and magnesium in water bodies. The geology of the study area is underlined by dolomite, an anhydrous carbonate composed mainly of calcium and magnesium carbonate, explaining the high mean calcium and magnesium concentrations measured during the study. High calcium and magnesium concentrations in the WFS, are probably the result of mining activities in the catchment (Labushagne, 2017). Inflows from the WFS influenced magnesium concentrations downstream at site BDI (Figure 3-7). Although no data was available for calcium and magnesium in the WS, its inflow may have caused the steep increase in mean calcium concentration found downstream of the inflow (Figure 3-6). The WS flows through a wetland and wetlands are known to accumulate calcium during low flow rates (Potasznik & Szymczyk, 2015). With increasing water supply, accompanied by higher flow rate conditions, desorption, which increases calcium levels, may occur. However, dry climate conditions prevailed during the study period, and it is assumed that the gypsum heap, situated in the area of the WS (Chapter 2, section 2.2.4.2), could have been responsible for the higher calcium concentrations, since it mainly consists of calcium and sulfate. Calcium was present in even higher concentrations than chloride - one of the three ions (sulfate, chloride and bicarbonate) usually dominant in water bodies (Dallas & Day, 2004). Bicarbonate – the other abundant major ion – was not measured during this study.

4.1.2.4 Chloride

Chloride was the third most abundant major ion in the Mooi River and its tributaries (combined; Chapter 3, section 3.1.2, page 43). The mean chloride concentration at the origin was low (13.58 mg/l) and well below (62%) the recommended RWQO limit of 36 mg/l (Table 3-1). The mean chloride concentrations gradually increased downstream in the Mooi River. In general, it seems as if inflows of the WFS and GM tributaries, with chloride concentrations of 42.42 and 46.67 mg/l respectively, were responsible for elevated concentrations in the Mooi River (Figure 3-8). The mean chloride concentration for WFS exceeded the RWQO limit (36 mg/l) with 15%, while the mean of GM exceeded the limit with 23%. The mean chloride concentration calculated for the Mooi River was also high (46.25 mg/l) and exceeded the RWQO limit with 22%. According to Hunt *et al.* (2012) sewage effluents and mine salts elevate chloride concentrations, and impacts from these sources can explain the high values in the WFS. GM obtains water from WFS through

the Boskop-Turffontein compartment. Sampling sites in cement canals (WFS, GM, BDC, and PD) displayed higher chloride concentrations. Although no sampling was done at the WWTP, the site downstream from the plant (DFE) showed the maximum (116.00 mg/l) recorded for the study period. According to Hunt *et al.* (2012) sewage effluent raises chloride levels and is not removed by septic tanks during treatment. In this manner it is able to enter the groundwater through septic systems and find its way into water bodies. However, no statistical significant differences were observed between any of the sites examined during the study period in terms of chloride concentration (Appendix B, Table B-1). The Mooi River and tributaries are also surrounded by roads, and road salts contribute to elevated chloride levels in aquatic ecosystems, especially during rainfall (Hunt *et al.*, 2012; Müller & Gächter, 2012). High chloride concentrations in the Mooi River can also be the result of fertilisers.

4.1.2.5 Fluoride

Fluoride was the least abundant major ion found in the Mooi River and tributaries (combined; Chapter 3, section 3.1.2, page 43). High mean fluoride concentrations were found at sites downstream from the inflow of the WS tributary (Figure 3-9). Unfortunately, no fluoride data was available for the WS, but it seems as if water from the WS could have increased fluoride concentrations. Fluoride is used in many manufacturing products, such as steel manufacturing and wood preservatives (DWA, 2009). The WS, flowing through an industrial area, is surrounded by manufacturing companies, and fluoride can enter the water through the effluents of these companies. Fluoride is also used in agricultural activities (such as insecticides) and in water treatment (DWA, 1996), but it is unlikely that any of these two factors contributed to elevated fluoride levels in the Mooi River. The mean fluoride concentration for the entire Mooi River (0.39 mg/l) exceeded both the recommended RQO and RWQO limits (≤ 3 and 0.25 mg/l respectively; Table 3-1).

According to DWA (2009) fluoride responds rapidly with phosphates and calcium ions. During this study fluoride showed a strong positive correlation with orthophosphates, but a negative correlation with calcium (Appendix G, Table G-1). Fluoride is also able to respond freely with magnesium at alkaline pH levels to form complexes that are difficult for aquatic organisms to absorb (DWA, 2009). Fluoride showed a positive correlation with magnesium (Table G-1). Given the overall high magnesium concentrations and pH levels recorded for the study period, it is therefore possible that fluoride can pose a problem to the aquatic life in the Mooi River and tributaries. According to DWA (2009) high water temperature can increase the toxicity of fluoride in water bodies. Data on water temperature was, however, not available for the study period.

4.1.2.6 Sulfate

Sulfate was the most abundant (highest concentration) major ion in the Mooi River and its tributaries (combined; Chapter 3, section 3.1.2, page 43). Very low sulfate concentrations were recorded at the origin of the river, compared to the other sites (Figure 3-10). The sulfate concentrations at the origin (mean of 27.00 mg/l) and in the WS (mean of 70.33 mg/l) were lower than both the RQO (≤ 500 mg/l) and RWQO (75 mg/l) limits (Table 3-1). Sulfate pollution is known to be caused by mining activity (Durandt, 2012), explaining high sulfate concentrations in the WFS tributary, that is surrounded by mines. The dolomite in the area reacts to the sulphuric acid, derived from abandoned mine shafts, at the same time buffering the pH and resulting in elevated sulfate concentrations (Usher & Vermeulen, 2006). The GM tributary also showed relatively high sulfate concentrations (mean of 158.67 mg/l), exceeding the limits set by RWQO, but not those by RQO. GM obtains water directly from the WFS explaining elevated concentrations. Sulfate concentrations were 10% higher in GM than in the WFS (mean of 143.67 mg/l). According to Coleman Wasik *et al.* (2015) peat lands in general, are considered to be sinks for atmospherically deposited sulfate, and coupled with droughts may further elevate sulfate concentrations in aquatic environments. It is clear from Figure 3-10 that the WFS played a huge role elevating sulfate concentrations and, together with GM, it contributed to high sulfate concentrations further downstream in the Mooi River.

The mean sulfate concentrations for the entire Mooi River (mean of 115.58 mg/l), the WFS (mean of 143.67), and GM (mean of 158.67) are within the RQO limit, but higher than the recommended RWQO limit (Table 3-1). The mean sulfate concentration for the Mooi River was lower compared than that found in a previous study by De la Rey *et al.* (2004) where a mean of 134.09 mg/l was found. The decrease in sulfate concentration can be the result of the reduction of mining activity in the WFS catchment (Labuschagne, 2017).

4.1.3 pH

The mean pH values for the entire Mooi River, the source of the river, WFS (8.2 each), GM (8.1), and WS (8.5) exceeded the RWQO limit of 8, but not the RQO limit (≤ 8.8 ; Table 3-1). Generally, pH ranges between 6 and 8.9 in fresh waters (Dallas & Day, 2004). The pH ranges for the entire Mooi River (7.5 – 9.2), WFS (7.6 – 9), GM (7.6 – 8.9); and WS (7.5 – 9.4) can thus be regarded as relatively high, especially that of the WS tributary. If pH values are above 8, ammonium may be converted to ammonia that can be toxic to aquatic organisms (Booyens, 2016; Dallas & Day, 2004). High pH levels in the WS may explain high ammonia concentrations at this site (compare Figure 3-1 and Figure 3-11). During photosynthesis, phytoplankton utilise carbon

dioxide, leading to an increase of hydroxide ions, which simultaneously increases the pH. In this manner high pH values are usually associated with high photosynthetic activity (Chen & Durbin, 1994).

4.1.4 Turbidity

Turbidity levels in the tributaries did not influence turbidity in the Mooi River system to a huge extent. The WFS could have contributed to slightly higher turbidity levels observed at BDI (Figure 3-12). Turbidity can be the result of inorganic and organic suspended solids or dissolved substances (Booyens, 2016; Dallas & Day, 2004; Janse van Vuuren & Taylor, 2015). Janse van Vuuren & Taylor (2015) found that very high turbidity levels at some of the sites located in the Sundays River were caused by high flow rates. Unfortunately flow rate was not measured during the study period to determine its effect on turbidity. High concentrations of living organisms, such as phytoplankton, can also result in high turbidity levels (Janse van Vuuren & Taylor, 2015). Schilling *et al.* (2017) evaluated 43 rivers in Iowa, and found a positive correlation coefficient (r) between orthophosphates and turbidity. Phosphates are the major nutrients used by aquatic organisms, and an increase in phosphates can therefore result in an increase in the concentration of aquatic organisms, which simultaneously results in higher turbidity levels. Another example was found in the WS, which had the maximum orthophosphate concentrations and turbidity levels, as well as the maximum phytoplankton concentration recorded for the study period. Another reason for the positive relationship is that orthophosphate are able to absorb turbidity particles (Meozzi, 2011). In general, a positive relationship between turbidity and orthophosphates was also found for the current study (Appendix G, Table G-1).

4.1.5 Heavy metals

The Mooi River is under constant investigation as a result of mining activities and associated pollution (especially uranium pollution) in its catchment (Labuschagne, 2017). Numerous papers (Aucamp, 2000; Coetzee *et al.*, 2006; De Waard, 2012; Liefferink, 2015; Opperman, 2008; Schrader & Winde, 2014; Swart *et al.*, 2002 Winde, 2006, 2009, 2010, 2013) have been published about pollution, including heavy metal pollution, in the WFS and its surroundings. Pollution in the WFS will affect a large section of the Mooi River as the point of inflow is located just below the source. The GM tributary, which can also obtain water directly from the WFS tributary, was also investigated in the current study to see how it affects the Mooi River in terms of heavy metal pollution.

Although eight different types of heavy metals were investigated during the current study, it seems as if the WFS tributary only impacted the Mooi River in terms of manganese, uranium, and hexavalent chromium, while the GM tributary was responsible for elevated iron and hexavalent chromium concentrations in the river (Chapter 3, Section 3.1.5).

Mining and agriculture are two main contributors to copper released into aquatic ecosystems (Gautam *et al.*, 2014; WHO, 2004; Chapter 3, section 3.1.5), all of which are evident in the upper part of the catchment area. Even though the tributaries had no effect on the Mooi River in terms of copper concentrations (Figure 3-13), the mean copper concentration (8.08 µg/l) at the source of the Mooi River (UBD) exceeded the recommended RQO limit of ≤ 8. The UBD site is surrounded by agricultural activities. The mean concentration in GM (11 µg/l) also exceeded the limit (Table 3-1), and can be the cause of mining, coupled with agriculture in the surrounding area. Copper concentrations can be toxic to some algae, even in low concentrations (Dallas & Day, 2004), and at higher concentrations it can be toxic to a variety of aquatic organisms and humans (Gautam *et al.*, 2014). Because copper binds to organic molecules, the presence of sulfate and zinc can reduce the toxicity of copper (Dallas & Day, 2004). Sulfate concentrations were low at UBD, however the mean zinc concentration (0.13 mg/l) were above the RQO limits (≤ 0.036 mg/l; Table 3-1). Sulfate and zinc concentrations were relatively high at GM, and may reduce the toxicity of copper.

Hexavalent chromium (Figure 3-14) is a product of manufacturing products, such as stainless steel and wood preservatives (Saha *et al.*, 2011). These companies are, however, not prominent in the upstream section of the Mooi River, or around the WFS and GM tributaries. The area is mostly impacted by mining, agricultural and peat mining activities. Although the mean hexavalent chromium concentration for the entire Mooi River (4.25 µg/l), the WFS (5.33 µg/l) and the GM (6.75 µg/l) were generally low and far below the recommended RQO limit of 200 µg/l (Table 3-1), the reason for its presence, though in low concentrations, in this part of the catchment is unknown.

Iron and manganese usually co-occur in aquatic ecosystems (McFarland & Dozier, 1996). Irrigation is known to contribute to iron and manganese (Hallberg & Johnson, 2005). Iron and manganese can accumulate and block irrigation systems and reach water bodies through irrigation (GWA, 2013; Zinati, 2005). Irrigation takes place along the length of the Mooi River, especially at the origin. This can explain the high manganese and iron concentrations found at the origin of the river (Figure 3-15). Iron and manganese in the WFS and GM tributaries can be the result of mining pollution and effluent. The mean iron concentrations for the origin (43.33 µg/l), the entire Mooi River (21.25 µg/l), the WFS (14.17 µg/l) and the GM (20.00 µg/l) were relatively

low and all concentrations were below the RWQO limit of 350 µg/l (Table 3-1). No RQO limit is set for iron concentration. The mean manganese concentrations in the Mooi River (47.44 µg/l), WFS (49.58 µg/l) and GM (60.33 µg/l) were also below the RQO limit for manganese (\leq 1300 µg/l), but exceeded the recommended RWQO limit (30 µg/l).

Industrial processes are the main cause of elevated lead concentrations in water bodies (Jaishankar *et al.*, 2014; UEEG, 2011; Chapter 3, section 3.1.5). Lead can be toxic even at low concentrations (Dallas & Day, 2004), and can suppress the overall growth of phytoplankton (Jaishankar *et al.*, 2014). Although the tributaries seem to have no direct effect on the Mooi River in terms of elevated lead concentrations (Figure 3-17), the mean lead concentrations at the origin (64.42 µg/l), the entire river (23.00 µg/l), and WFS (78.25 µg/l) exceeded the recommended RQO lead limit of \leq 13 µg/l (Table 3-1).

Similar to lead, nickel can be toxic at low concentrations (Dallas & Day, 2004), but unlike lead, it takes part in several physiological processes (Shukla *et al.*, 2009). Nickel can accumulate in phytoplankton and at the same time be harmful to these organisms, depending on the sensitivity of these organisms to nickel concentrations (Shukla *et al.*, 2009; Wang & Wood, 1984; Chapter 3, section 3.1.5). Elevated nickel concentrations in aquatic environments are mainly caused by mining, manufacturing, and sewage sludge (EIFAC, 1984; Shukla *et al.*, 2009). Mining and sewage works are evident in the WFS, however the tributaries did not seem to have an effect on the Mooi River in terms of elevated nickel concentrations (Figure 3-18). There are no recommended RQO or RWQO limit set for nickel for comparative purposes.

Uranium in the Mooi River and its tributaries (especially the WFS) is a serious point of concern due to it being radioactive. Mining activity is the main cause for uranium pollution in aquatic ecosystems, and mining explains why the WFS had the highest mean (10.51 µg/l) calculated at all sites during the study period. The mean uranium concentration of 10.51 µg/l in the WFS is still 30% below the recommended RQO limit of 15 µg/l (Table 3-1). The maximum uranium recorded in the WFS (22.30 µg/l) did, however, exceed the limit with 33%. The WFS had a huge effect on the Mooi River in terms of uranium concentrations (Appendix E, E-1; Figure 3-19) as its inflow increased the mean concentration with 93% from UBD (0.23 µg/l) to BDI (3.08 µg/l). Winde (2010) found that the mean uranium concentration in the Mooi River (calculated at three sites) increased from 0.004 mg/l in 1997 to 0.007 mg/l in 2004. When compared to the current study (mean uranium concentration of 2.15 mg/l at four sites in the Mooi River), a steep increase is observed. However, the mean, and even the maximum, uranium concentrations measured for the origin (mean of 0.23 µg/l; maximum of 0.30 µg/l), the entire Mooi River (mean of 2.15 µg/l;

maximum of 5.80 µg/l), and GM (mean of 0.23 µg/l; maximum of 0.60 µg/l) were still below the recommended RQO limit of 15 µg/l.

The mean zinc concentrations (Figure 3-20) for UBD (0.13 mg/l), the entire Mooi River (including UBD; 0.16 mg/l), the WFS (0.08 mg/l) and GM (0.11 mg/l) exceeded the RQO limit of ≤0.036 mg/l (Table 3-1). Mining and industries, producing zinc materials and products, are the main contributors to elevated zinc in aquatic environments (Gautam *et al.*, 2014). However, mining operations located in the WFS area did not seem to affect the Mooi River downstream in terms of elevated zinc concentrations. The mean zinc concentration in WFS was lower than the mean measured at UBD, and WFS showed the lowest mean zinc concentration compared to the Mooi River and GM. Zinc is often used as a supplement in agriculture (Nielsen, 2012), and could have been responsible for the high zinc concentrations at UBD, because this site is affected by agricultural activities.

Even though the WFS and GM tributaries seemed to have had no direct effect on increasing copper, lead, nickel and zinc concentrations in the Mooi River, the concentration of these metals should be monitored regularly.

4.2 Phytoplankton

As any other water body, the Mooi River and its tributaries constantly experience physical, chemical and biological environmental changes due to natural as well as anthropogenic activities (Barnard *et al.*, 2013; Labuschagne, 2017). Due to these changes, dominant phytoplankton genera (Table 3-2) change spatially and seasonally (Wetzel, 2001).

4.2.1 Phytoplankton concentration and composition

The most upstream site in the Mooi River (UBD) served as a control, because this site is not affected by any inflowing tributaries. Nutrient concentrations, especially nitrates and orthophosphates (Figure 3-2 and Figure 3-3) were generally high at UBD, coupled with relatively high pH levels. Nutrients determine the rate of primary production and phytoplankton biomass (Paerl *et al.*, 2001). Elevated nutrient levels can be the result of changes in pH. The pH at UBD experienced fluctuations (Figure 3-11). High pH can be the result of phytoplankton growth (Benamer, 2014). It is therefore expected that UBD should be rich in phytoplankton but, on the contrary, UBD showed very low phytoplankton concentrations during all seasons (total of 1211 cells/ml; Figure 3-21 to Figure 3-24; Appendix F; Table F-1 to F-4). Generally low turbidity levels found at UBD (Figure 3-12) allow for sufficient under water light conditions, favouring photosynthesis, provided an area is not shaded by vegetation. However, overhanging riparian

vegetation at UBD is able to block light, which can reduce photosynthesis. Other factors such as flow rate, light and water temperature can also affect phytoplankton growth (Edwards *et al.*, 2016; Falkowski *et al.*, 1992), but were not measured during the study period.

Genera most abundant at UBD included: *Chlamydomonas*, *Crucigeniella*, *Cyclotella*, and *Nostoc* (Table 3-2). *Chlamydomonas* was often found in high concentrations at UBD. *Chlamydomonas* can form blooms in nutrient-rich waterbodies (Janse van Vuuren *et al.*, 2006), and is indicative of organic pollution (Bellinger & Sigee, 2010). High nutrient concentrations measured in UBD are probably the result of organic pollution through agricultural activities surrounding the area. *Crucigeniella* is commonly found in a wide range of freshwater habitats, and is not responsible for any known problems (Janse Van Vuuren *et al.*, 2006). *Cyclotella* is present in waters ranging from oligo to mesotrophic conditions (Janse van Vuuren *et al.*, 2006), and it has previously been reported to produce taste and odour problems in drinking water (Bellinger & Sigee, 2010; Palmer, 1969). *Nostoc* is commonly associated with low nutrient levels (Douterelo *et al.*, 2004). They are able to fixate atmospheric nitrogen, and can therefore live in nitrogen-deficient habitats (Janse van Vuuren & Taylor, 2015; Liengen, 1997; Wehr & Sheath, 2015). Several studies conducted on river systems (Douterelo *et al.*, 2004, Fernández-Piñas *et al.*, 1991; Janse van Vuuren & Taylor, 2015) found *Nostoc* to be abundant at upstream sites while absent downstream, and ascribed it to lower nutrient concentrations. However, this was not the case for UBD, because nutrient concentrations were generally high and comparable to most sites located further downstream in the Mooi River. According to John *et al.* (2002) many *Nostoc* species are found in nutrient poor waters, but there are at least two species that are consistent indicators of eutrophic freshwater bodies. Some *Nostoc* species are also able to produce toxins in fresh water systems (Bellinger & Sigee, 2010). Genera such as *Chlorolobion*, *Placoneis*, and *Microspora* were not abundantly at UBD, but were exclusively present at this site, and absent at all other sites investigated in the Mooi River and tributaries (Table 3-2). According to John *et al.* (2002), *Chlorolobion* can be present in many aquatic ecosystems where they rarely becomes planktonic. This genus is, however, defined vague, and resembles *Keratococcus* (John *et al.*, 2002). *Placoneis* species can be present in different types of water chemistry that includes unpolluted water, slightly polluted water, brackish water, and sediments (Taylor *et al.*, 2007). The presence of *Microspora* can probably be attributed to high concentrations of heavy metals measured at UBD (Chapter 3, section 3.1.5, Figure 3-13 - Figure 3-19). *Microspora* often dominates in stream sites contaminated by high concentrations of heavy metals (John *et al.*, 2002).

The site located in the WFS tributary almost showed a three-fold increase in phytoplankton concentrations (3259 cells/ml; Figure 3-21 to Figure 3-24; Appendix F; Table F-1 to F-4) when compared to concentrations at the source (UBD). Genera most evident in the WFS included: *Anabaena*, *Aulacoseira*, *Chlamydomonas*, *Cyclotella*, *Microcystis*, *Navicula*, *Nitzschia*, *Oscillatoria* and *Spirulina* (Table 3-2). From this list, it is clear that cyanobacteria were more prominent in the WFS than at UBD. Nutrient concentrations in the WFS were high (Figure 3-1 - Figure 3-3), and probably stimulated the growth of cyanobacteria, a phylum known to prefer high nutrient conditions (Fogg *et al.*, 1973; Goldman & Horne, 1983; Paerl *et al.*, 2001; Smith, 2003). Common phytoplankton genera, associated with eutrophic conditions, include *Chlamydomonas*, *Anabaena*, *Microcystis*, and *Nitzschia*, while *Navicula* can be found in water ranging from meso to eutrophic conditions (Janse van Vuuren *et al.*, 2006). *Aulacoseira* is often associated with slightly eutrophic conditions (Janse van Vuuren *et al.*, 2006), and prefers high EC levels (Luyt, 2018). Luyt (2018) found *Anabaena*, *Oscillatoria*, and *Spirulina* to be abundant in the WFS during 2016 and 2017, consistent with the effects of increased phosphorus loadings. High conductivity and TDS concentrations in the WFS may explain high concentrations of *Aulacoseira*.

In general, it does not seem as if higher phytoplankton concentrations in the WFS influenced the total phytoplankton concentration downstream from the point of inflow. The total phytoplankton concentration in WFS, is still low compared to total phytoplankton concentrations recorded in the middle section of the Mooi River (BDI, and especially BDC, and PD; Figure 3-21 - Figure 3-24; Appendix F; Table F-1 – F-4). Increases in phytoplankton concentration from UBD to BDI were observed during the winter and spring months. Sections of the WFS, prior to the convergence with the Mooi River, are often dry. It is only during high rainfall periods that the WFS is able to feed the Mooi River. The Mooi River can obtain underground water from WFS (Winde 2010), but it is uncertain whether it influences the inlet of Boskop Dam (where BDI is located). Low rainfall during winter and high rainfall during spring did not seem to have an effect (Appendix A, Table A-1). The distance between sites may also play a role - the distance between UBD to BDI and WFS to BDI are 10 km and 7 km respectively. Other factors that may affect the water quality could occur between sites. Only two genera (*Microcystis* in summer and *Cyclotella* in winter) were present in higher numbers at BDI and relates to higher numbers found upstream (particularly at WFS). However it is not known if importation of WFS water was responsible for the presence of these two genera at BDI, because *Microcystis* is known to usually occur in summer months when high water temperatures prevail (Janse van Vuuren *et al.*, 2006).

BDI (total of 4650 cell/ml) showed 74% and 30% higher phytoplankton concentration compared to UBD and WFS respectively (Figure 3-21 to Figure 3-24; Appendix F; Table F-1 to

F-4). Nutrient concentrations were generally high, especially the mean orthophosphate concentration that was higher at BDI, than at UBD and WFS (Figure 3-3). Genera most apparent in BDI included: *Aulacoseira*, *Cyclotella*, *Geminella*, and *Microcystis* (Table 3-1). High orthophosphate concentrations, coupled with high summer temperatures, could have stimulated the growth of *Microcystis*, while high EC levels (Figure 3-5), could have favoured the growth of *Aulacoseira*. *Geminella* is widely distributed in a variety of freshwater habitats, including shallow waters of lakes and ponds, swamps, and even in terrestrial habitats (Cambra & Aboal, 1992), and usually prefers slightly shallow and acidic water (Bellinger and Sigeo, 2010; John *et al.*, 2002). The water at BDI is shallow, but not acidic. Labuschagne (2017) found *Geminella* in Boskop and Potchefstroom dams between 2014 and 2015, coupled with non-acidic water conditions. During the current study period, *Geminella* was also found in BDC and PD, also reflecting non-acidic water conditions.

The site located in the GM tributary showed a four-fold increase in phytoplankton concentration (total of 5082 cells/ml) compared to UBD, if the average over all seasons is taken into account (Figure 3-21 to Figure 3-24; Appendix F; Table F-1 to F-4). High nutrient concentrations (especially nitrate and orthophosphate; Figure 3-1 to Figure 3-3), coupled with low turbidity (Figure 3-12) could have favoured phytoplankton growth. Genera most conspicuous in GM included: *Cyclotella*, *Dinobryon*, *Fragilaria*, *Gonatozygon*, *Microcystis*, *Navicula*, *Nitzschia*, and *Phormidium* (Table 3-2). According to the Palmer index calculations of Labuschagne (2017) on the Mooi River *Cyclotella*, *Fragilaria*, *Navicula*, and *Nitzschia* are tolerant to high levels of organic pollution. GM is surrounded by peat lands that cause an increase in organic sediment loads (Glooschenko, 1990). The presence of *Gonatozygon* can also be the result of peat mining, because this genus is often present in *Sphagnum* bogs (peat moss; Guiry & Guiry, 2019). *Phormidium* is very common, and can even be found in extreme habitats (Guiry & Guiry, 2019). Luyt (2018) found *Phormidium* and *Dinobryon*, to be abundant in the Mooi River catchment during 2016 and 2017, consistent with the effects of increased phosphorus loadings. According to Bellinger & Sigeo (2010) *Dinobryon* is widely distributed in waters ranging from nutrient-enriched to nutrient poor conditions, and it can cause odour problems in drinking water.

In general, it does not seem as if GM affected the Mooi River in terms of phytoplankton concentration. The overall phytoplankton concentration (average for all seasons) for GM (total of 5082 cells/ml) was 83% lower than at BDC (28 991 cell/ml), and 77% lower than at PD (21 597 cells/ml), which are located downstream from the GM inflow (Figure 3-21 to Figure 3-24; Appendix F; Table F-1 to F-4). Distance could have played a role, as water from GM flows for about 25 km before it reaches BDC and PD, with different unknown factors occurring between the sites. Only

two genera (*Dinobryon* during autumn and winter, and *Phormidium* during autumn) present in higher numbers at BDC and PD relates to high numbers found upstream (particularly at GM). However, again, it cannot be sure that importation of GM water was responsible for the presence of these two genera at BDC and PD, because *Phormidium* is very common (Guiry & Guiry, 2019; Bellinger & Sigee, 2010), and chrysophytes (such as *Dinobryon*) were generally present in higher concentrations during autumn and winter months.

The maximum phytoplankton concentrations recorded for the Mooi River during all seasons were at BDC and PD (Figure 3-21 - Figure 3-24; Appendix F; Table F-1 – F-4). Located 0.16 km apart, BDC and PD reflect very similar water quality conditions (Chapter 3; section 3.1; Figure 3-1 - Figure 3-20). Both these sites have high nutrients levels, coupled with relatively low turbidity levels, which make them suitable for phytoplankton growth. BDC and PD had high mean pH values, and the maximum pH recorded for the Mooi River was at BDC (Figure 3-11). Photosynthesis and the uptake of carbon dioxide can elevate pH (Chen & Durbin, 1994; Farrel-Poe, 2000). Genera most evident in BDC and PD included: *Anabaena*, *Aulacoseira*, *Cyclotella*, *Diatoma*, *Geminella*, and *Ulothrix* (Table 3-2). *Diatoma* is indicative of hard water, coupled with high nutrient levels (Janse van Vuuren *et al.*, 2006; Taylor *et al.*, 2007). Magnesium and calcium are the two major ions responsible for hard water (Sengupta, 2013). High concentrations of *Diatoma* at PD can be the result of high nutrient, coupled with high calcium and magnesium concentrations (Figure 3-1 - Figure 3-3; Figure 3-6 and Figure 3-7). Luyt (2018) found *Anabaena* to be consistent with the effects of increased phosphorus loadings in the Mooi River. High concentrations of *Anabaena* can result in problems including, taste and odour, toxicity, and dermatitis (Janse van Vuuren *et al.*, 2006). High numbers of *Aulacoseira* found in BDC and PD can most probably be attributed to high EC levels, corresponding with the findings of Luyt (2018). *Aulacoseira* can cause taste and odour problems (Bellinger & Sigee, 2010; Janse van Vuuren *et al.*, 2006), while *Cyclotella* can known to cause severe filter clogging problems in water treatment plants (Palmer, 1980). *Ulothrix* also prefers eutrophic conditions (Guiry & Guiry, 2019). The genus *Aphanothece* was not abundant in BDC and PD, but was only present at these sites, and absent from all other sites in the Mooi River and tributaries (Table 3-2). According to John *et al.* (2002), there is a limited knowledge about the habitat of *Aphanothece* although it has been reported as widespread on humid terrestrial surfaces, including brackish habitats.

The site located in the WS tributary (Poortjie Dam) showed the highest phytoplankton concentration (total of 1 699 578 cells/ml for all seasons) during the entire study period (Figure 3-21 to Figure 3-24; Appendix F; Table F-1 to F-4). High phytoplankton concentrations were expected, because this site had the highest nutrient concentrations (Figure 3-1 - Figure 3-3), pH

(Figure 3-11), and turbidity (Figure 3-12). High turbidity levels are probably the result of high phytoplankton concentrations (Chapter 3, section 3.1.4), rather than inorganic suspended particles.

WS is often polluted by sewage, due to blocked pump stations (Nel, 2011), resulting in blooms of cyanobacteria, particularly *Microcystis*. During the present study blooms were present throughout the year and *Microcystis* formed a thick blue green layer on the water's surface, coupled with scums (personal observation). *Microcystis* has gas vacuoles (aerotopes), helping them to control their vertical position in the water column to obtain sufficient nutrients and light for photosynthesis (Chapter 3, section 3.3, page 62). In this manner they can float on the water surface, sometimes in such high concentrations, that it becomes dense enough to prevent sunlight from reaching other phytoplankton. This gives *Microcystis* an advantage to dominate over other phytoplankton. Dense concentrations result in taste and odour problems, depletion of oxygen in the water, and fish kills (Janse van Vuuren *et al.*, 2006; Janse van Vuuren & Taylor, 2015; Chapter 1). Genera present in high concentrations at WS include: *Anabaena*, *Coelastrum*, *Cymatopleura*, *Diatoma*, *Eudorina*, *Gomphonema*, *Merismopedia*, *Pandorina*, *Pediastrum*, and *Scenedesmus* (Table 2-2). All these genera are known to prefer nutrient rich water (Bellinger & Sigee, 2010; Janse van Vuuren *et al.*, 2006). *Gomphonema* also often occur in water enriched with sewage effluent (Janse van Vuuren *et al.*, 2006). The genus *Hipodonta* was not abundant in WS. It was, however, only present in the WS but absent from all other sites (Table 3-2). According to Taylor *et al.* (2007), *Hipodonta* is typically found in eutrophic conditions and can tolerate critical pollution levels.

Very high phytoplankton concentrations were recorded in WS for all seasons (total of 1 699 578 cells/ml). However, WS did not affect downstream sites including RB (5482 cells/ml), SB (4794 cells/ml), DFE (4214 cells/ml), and Krom (2150 cells/ml), because these sites reflect very low phytoplankton concentrations (Figure 3-21 to Figure 3-24; Appendix F; Table F-1 to F-4). The distance (9.5 km) between the WS and RB can have an influence on the phytoplankton concentration downstream, as other factors may have an effect on the water quality between the sites. Water from WS flows through different sources, including a wetland and cement canal, before it reaches RB. The cement canal serves as an illegal dumping site for garden refuse and plant material. The plants often establish and grow in the WS and can absorb bio-available nutrients, thereby indirectly causing a reduction in phytoplankton numbers.

Downstream sites in the Mooi River (RB, SB, DFE, Krom) showed low phytoplankton concentrations - almost comparable to concentrations found at the origin of the Mooi River (1 211 cells/ml; UBD; Figure 3-21 to Figure 3-24; Appendix F; Table F-1 to F-4). It cannot be explained

why low phytoplankton concentrations were found at these sites, because the nutrient concentrations were high (Figure 3-1 to Figure 3-3). However, these sites experienced the highest turbidity (Figure 3-12) recorded for the Mooi River - high turbidity levels resulting from organic and/or inorganic suspended particles could influence the rate of primary productivity, by limiting under water light availability (Chapter 3, Section 3.1.4). Other factors affecting phytoplankton concentrations include:

- Large trees covering RB and SB may block light, reducing photosynthesis.
- DFE is situated downstream from the WWTP (treats the water, and re-introduces it back into the river), and the plant may affect water quality at DFE.
- Light, temperature, and flow rate (not measured). For example, at Krom there is a weir with rapidly flowing water. The flow rate may suppress the development of phytoplankton, resulting in low concentrations.

Seven genera (*Aulacoseira*, *Coelastrum*, *Cyclotella*, *Diatoma*, *Microcystis*, *Scenedesmus* and *Ulothrix*) that were present in higher numbers downstream (e.g. at RB) can be related to high numbers found upstream (particularly at PD and WS). The presence of these genera downstream could be the result of imports from the tributaries, or high nutrient concentrations measured at RB (Figure 3-1 - Figure 3-3) - most of these genera are also indicative of high nutrient conditions.

Genera which were the most abundant (Table 3-2) at RB included *Aulacoseira*, *Coelastrum*, *Cyclotella*, *Diatoma*, *Microcystis*, *Scenedesmus*, and *Ulothrix*, SB, included *Aulacoseira*, *Cyclotella*, *Diatoma*, and *Coelastrum*, DFE, included *Coelastrum*, *Cyclotella*, *Pediastrum*, and *Nitzschia*, Krom, included *Cocconeis*, *Crucigeniella*, *Cyclotella*, *Dictyosphaerium*, *Merismopedia*, *Navicula*, and *Nitzschia*.

Genera preferring nutrient enriched conditions include *Aulacoseira*, *Coelastrum*, *Diatoma*, *Dictyosphaerium*, *Merismopedia*, *Microcystis*, *Pediastrum*, *Scenedesmus*, and *Ulothrix* (Bellinger & Sigee, 2010; Guiry & Guiry, 2019; Janse van Vuuren *et al.*, 2006; Taylor *et al.*, 2007). *Navicula* and *Cyclotella* can be found in oligo to highly eutrophic conditions, depending on the species (Taylor *et al.*, 2007). High nutrient concentrations (Figure 3-1 to Figure 3-3) at RB, SB, DFE, and Krom could have favoured the growth of these genera. *Aulacoseira* also prefers high EC levels (Luyt, 2018), that can explain their presence at RB and SB, where very high TDS and EC levels were recorded (Figure 3-4 and Figure 3-5). *Diatoma* prefers hard water conditions (Taylor *et al.*, 2007). Very high calcium and magnesium concentrations at RB and SB (Figure 3-6 and Figure 3-7) could, therefore have stimulated the growth of *Diatoma*. *Cocconeis* and *Crucigeniella* are

common in freshwater systems, and they are not known for causing any water quality problems (Janse Van Vuuren *et al.*, 2006).

Carteria, *Treubaria* and *Tryblionella* were present in the Mooi River, but absent from the tributaries. *Tryblionella* occurs in freshwaters with high EC levels (Louvrou & Economou-Amilli, 2015; Round *et al.*, 1990). Although the mean EC for the Mooi River was lower than that of WFS and GM (Figure 3-5), EC levels recorded for the Mooi River were relatively high (exceeded the recommended RWQO limit), and could have favoured the growth of *Tryblionella*. *Carteria* prefers similar nutrient-rich water conditions than *Chlamydomonas*, and they are usually found in still waters or slow flowing rivers (Bellinger & Sigee, 2010; Janse van Vuuren *et al.*, 2006). In contrast to *Carteria*, *Treubaria* is rare in standing water, slow flowing rivers, or very nutrients enriched conditions (Janse van Vuuren *et al.*, 2006) such as those experienced in the Mooi River. This genus was, however, only present in low concentrations in the Mooi River.

To summarise: In general the phytoplankton concentration across all seasons was low at the source of the Mooi River, and downstream from RB to Krom (Figure 3-21 to Figure 3-24; Appendix F; Table F-1 to F-4). Maximum concentrations were recorded in the middle reaches of the Mooi River at BDC and PD. Cyanobacteria reached maximum concentrations at these sites and their presence was usually associated with water quality problems. High nutrient concentrations (Figure 3-1 to Figure 3-3), coupled with high pH values (Figure 3-11), were recorded at BDC and PD and excess nutrients may have resulted in the high phytoplankton concentrations at these sites. The maximum pH was recorded in the Mooi River at BDC and PD. Phytoplankton growth can elevate pH as a result of the uptake of CO₂ during photosynthesis (Chen & Durbin, 1994). Therefore high nutrient levels may have been responsible for blooms of phytoplankton, that may, in turn, caused elevated pH levels. Agricultural activities and an ammunition manufacturing company (Chapter 2, section 2.3.1.3 and 2.1.3.4) are the only known factors that may have contributed to water pollution at BDC and PD.

In general it does not seem as if the inflow of tributaries has had a huge effect on the Mooi River in terms of phytoplankton concentration. Research carried out in Brazil (Nogueira *et al.*, 2010; Rodrigues *et al.*, 2009; Soares *et al.*, 2007), showed that medium and large tributaries are able to influence main rivers in terms of phytoplankton dynamics. Outcomes of the research for smaller tributaries differ, because of lower flow rates and smaller volumes of water (Perbiche-Neves *et al.*, 2011) resulting in limited depth and velocity, that play an important role in phytoplankton dynamics (Bahnwart *et al.*, 1999). None of these variables were investigated during the study period. The Mooi River and WFS are classified as small rivers (Luyt, 2018), while the GM and WS are classified as perennial streams (Kriel, 2008; Winde, 2008). However, similar

genera were often observed between the tributaries and the sites downstream from their inflows. For example GM was dominated by *Dinobryon* during winter, and at the same period high concentrations of *Dinobryon* were also present at BDC located just downstream from the GM inflow. It is uncertain whether the tributaries are responsible for these similarities, or if it could be the result of other factors, such as high nutrients concentrations in general or seasonally bound genera.

WS was clearly different from the other tributaries (WFS, GM), as well as the Mooi River, not only in terms of algal concentration that was high, but also in terms of algal composition. Concentrations recorded in the WS exceeded that of all other sampling sites. The availability of nutrients, particularly inorganic nitrogen (ammonia, nitrate, nitrite) and phosphorus (orthophosphates), results in phytoplankton growth (Carlson, 1977; Vollenweider, 1976). The WS (Poortjie Dam) often experiences pollution by sewage, as a result of blocked pump stations (Nel, 2011). Sewage effluent is one of the main contributors to enriched nutrient levels in South African rivers (De Villiers & Thiar, 2007). High mean ammonia, nitrate and orthophosphate concentrations were measured in the WS (Figure 3-1 to Figure 3-3). This could have resulted in the dominance of cyanobacteria, particularly *Microcystis* - a genus known to thrive in nutrient enriched conditions. The maximum turbidity for the study period was recorded at WS (Figure 3-12), and can be attributed to high phytoplankton concentrations. The presence of high phytoplankton numbers, resulting in high photosynthetic rates and the co-current absorption of carbon dioxide, could have elevated the pH. Maximum pH levels were recorded in the WS (Figure 3-11).

4.2.2 Diversity of phytoplankton phyla in the Mooi River

Of the seven phytoplankton phyla encountered in the Mooi River, the green algae and diatoms were the most diverse (20 genera each; Table 3-2, section 3.3, page 65), followed by the cyanobacteria (11 genera), euglenophytes (four genera), dinoflagellates (three genera), chrysophytes (two genera) and cryptophytes (one genus). More or less the same diversity structure was observed in the Krishna River in Maharashtra, and the Sabarmati River in India (Kumar *et al.*, 2012; Sarwade & Kamble, 2013), also rivers that are influenced by various anthropogenic activities. Although mixed assemblages of diatoms and green algae are usually the most common algae found in river systems (Round, 1985), green algae can outcompete diatoms when nutrient concentrations become high (Akin-Oriola, 2003). The Great KWA River in India is, to a lesser extent, also influenced by anthropogenic activities, and it is dominated by diatoms that also had the highest diversity of all taxa (12 genera), followed by green algae (six genera), chrysophytes and cyanobacteria (four genera each; Eyo & Paul, 2015). According to

Palmer (1980), the presence of the cryptophytes and chrysophytes indicates that the water is clean and unpolluted. The low abundance of these two genera in the Mooi River can be an indication of pollution in the river.

4.3 Multivariate Analyses on the Mooi River (Excluding Tributaries)

Multivariate analyses are statistical tools, which have the ability to allow simultaneous analyses for more than one dataset (Harper *et al.*, 2000). Two data-sets, one on physico-chemical, and the other one on phytoplankton, were used for the multivariate analyses in this study. A canonical correspondence analysis (CCA; Figure 3-29) was performed which contained all the variables of the two data-sets contributing to the variance within the data, their relation to the axes, along with the individual samples at each site. Note that the CCA only includes data of the Mooi River, excluding the tributaries, as the CCA is used to draw an overall conclusion for the river, and to see how different water quality variables relates to one another. The heavy metal data was also excluded in the CCA, because metal data was not available for all sites located in the Mooi River.

Nutrients (including ammonia, nitrates and orthophosphates) determine the rate of primary production and phytoplankton biomass (Paerl *et al.*, 2001). Nutrients showed a positive correlation with diatoms, green algae, cyanobacteria and euglenophytes (Figure 3-29). Of all the nutrients, orthophosphates had the greatest influence (length of the vector), and can therefore be regarded as the main nutrient responsible for the occurrence of these phyla. Orthophosphates were strongly positively correlated with Cyanophyta (small angle between vectors; Figure 3-28 and Figure 3-29) and results of this study therefore support those of other studies, such as Akin-Oriola (2003), Elser *et al.* (1990), and Okogwu & Ugwumba (2013) who illustrated that phosphates stimulate the growth of cyanobacteria.

Green algae and diatoms were the most abundant and diverse phyla in the Mooi River. The green algae showed the highest relative abundance (54%), followed by the diatoms (26%), and the cyanobacteria (16%; Figure 3-25). Felisberto *et al.* (2011) found green algae to benefit from increased phosphorus in the Corvo River (Rosana reservoir). Although the cyanobacteria were less diverse, they were highly abundant in terms of concentration, especially at BDC and PD. BDC and PD, in general, displayed the highest phytoplankton concentration recorded for the Mooi River. However these two sites correlated negatively with nutrients, probably as a result of high phytoplankton concentrations that depleted the nutrients in the water. BDC also had the maximum pH recorded for the Mooi River, followed by PD with the second highest values (Figure 3-11).

Positive relationship between cyanobacteria and turbidity, and cyanobacteria and pH, can be the result of cyanobacteria responsible for elevated turbidity levels and increases in pH. According to Chorus & Bartram (1999) cyanobacteria have a competitive advantage in turbid conditions, because they are specially adapted to absorb green light, and can therefore live in environments with lower light intensities. In some cases cyanobacteria blooms may be the result of high turbidity levels, while in other cases cyanobacterial blooms may cause high turbidity levels. This can explain the positive correlations found between cyanobacteria and turbidity (Figure 3-29). Some cyanobacteria, like *Microcystis* and *Anabaena* have gas vacuoles which give them the ability to float, and they can form large green scums on the water surface, which can reduce or prevent light passing through the water column (Šejnohová, 2008). Increases in phytoplankton growth may increase the pH, as a result of the uptake of carbon dioxide during photosynthesis (Chen & Durbin, 1994; Farrel-Poe, 2000).

The euglenophytes was one of the less conspicuous phyla encountered during the study period (2% relative abundance). Although present in relatively low concentrations, euglenophytes were more abundant than cryptophytes, chrysophytes and dinoflagellates. Figure 3-29 indicates that Euglenophyta was positively correlated with nutrient concentrations, TDS/EC and turbidity. This is in accordance to descriptions in Janse van Vuuren *et al.* (2006), stating that most species within this phylum are known to occur in nutrient-rich habitats. On the contrary, the cryptophytes and chrysophytes showed a negative correlation with orthophosphates and turbidity (Figure 3-29), that supports Palmer (1980), who found that these phyla are generally associated with clean and unpolluted water.

CHAPTER 5: CONCLUSION AND FUTURE RECOMMENDATIONS

“When we save a river, we save a significant part of an ecosystem, and we save ourselves as well because of our dependence – physical, economic, spiritual, - on the water and its community of life.” Anonymous

5.1 Conclusions

It can be concluded that the WFS, GM, and WS tributaries affected physico-chemical variables in the Mooi River downstream from their points of inflow. The Loop Spruit (LS) and WWTP, not included in the current study, also seem to have influenced the Mooi River downstream from its inflow. Most mean values for physico-chemical variables measured in the Mooi River and its tributaries exceeded the recommended RQO and RWQO limits.

In terms of nutrient concentration it can be concluded that the all three inflowing tributaries affected the Mooi River downstream from their points of inflow, especially with regards to ammonia concentrations. WS and the WWTP seem to have had a leading effect in terms of elevated nitrate and orthophosphate levels. However, nitrates and orthophosphates were not measured in the WWTP, and tendencies observed were not scientifically proven. The mean ammonia, nitrate, and orthophosphate concentrations for the Mooi River and all three the tributaries exceeded the RQO and RWQO limits. The mean orthophosphate concentration in the WFS was, however, below the RWQO limit. The mean ammonia and nitrate concentrations for the Mooi River main stream (0.84 mg/l), WFS (1.12 mg/l), GM (0.95 mg/l) and WS (2.24 mg/l) are indicative of mesotrophic conditions (0.5-2.5 mg/l; DWA, 1996; Dallas & Day, 2004), while the mean orthophosphate concentration for the Mooi River (0.68 mg/l), WFS (0.34 mg/l), GM (0.54 mg/l) and WS (1.02 mg/l) is indicative of hypertrophic conditions (0.25 mg/l; DWA, 1996; Dallas & Day, 2004).

High TDS concentrations and EC levels in the tributaries also had an effect on the Mooi River downstream from the points of inflow. Although no TDS and EC data was available for the WWTP, it seems as if it had a significant effect at downstream sites in the Mooi River. The mean TDS concentration for the Mooi River, and that of all the tributaries, exceeded the RWQO limit, while the mean EC levels exceeded both the RQO and RWQO limits.

According to DWA (1996), elevated calcium, magnesium, and sulfate concentrations can be dangerous if consumed by livestock. The overall (Mooi River, WFS, and GM combined) abundance of major ions in the study area can be summarised as follows: $\text{SO}_4^{2-} \gg \text{Ca}^{2+} > \text{Cl}^- > \text{Mg}^{2+} \gg \text{F}^-$. It can be concluded that the WFS contributed to elevated magnesium levels in the Mooi River, while GM elevated sulfate concentrations. Although no calcium data was available for WS, it is suspected that it affected the Mooi River significantly downstream in terms of calcium concentrations. Mean magnesium concentrations in the Mooi River, and WFS exceeded the RWQO limit, while the mean sulfate concentration of the GM exceeded the RWQO limits. The WFS and GM (and probably also the WS and WWTP) contributed to elevated chloride levels in the Mooi River. Mean chloride concentrations in the Mooi River, WFS, and GM exceeded the RWQO limit. Although fluoride was not measured in the WS, it seems as if it was responsible for an elevation in fluoride concentration downstream from its point of inflow.

It can be concluded that the WFS could have contributed to slightly higher turbidity levels in the Mooi River.

In general the mean pH values for the entire Mooi River, WFS, GM, and especially WS, were relatively high and exceeded the RWQO limit, but not the RQO limit.

With regards to heavy metal pollution, WFS contributed to higher manganese, hexavalent chromium, and uranium concentrations, while the GM was responsible for higher iron, and manganese concentrations in the Mooi River. However, the mean hexavalent chromium, and uranium concentrations for the Mooi River, WFS, and GM were all below the recommended RQO limits, and the mean iron concentrations were below the recommended RWQO limits. Although the mean hexavalent chromium concentration was below the RQO limit, it exceeded the RWQO limit. Attention should be placed on the copper concentrations in GM, as the mean exceeded the RQO limit. According to Dallas & Day (2004) copper and zinc can become toxic at high concentrations, and lead is toxic even in low concentrations. The mean lead, and zinc concentrations for the Mooi River and WFS exceeded the RQO limits.

The whole catchment area was subjected to pollution as a result of various anthropogenic activities that contributed to elevated nutrient concentrations in the Mooi River and tributaries. High nutrient concentrations stimulate the growth of phytoplankton, some of which are harmful. Despite the fact that high nutrient concentrations were measured spatially along the Mooi River, some sites showed lower phytoplankton concentrations than others. Factors such as flowrate, temperature, light and overhanging riparian vegetation could have had an influence on phytoplankton concentrations.

In general, phytoplankton concentrations were relatively low in the upper and lower reaches of the Mooi River, compared to the middle reaches (BDC and PD) where maximum phytoplankton concentrations were recorded. Cyanobacteria are often associated with water quality problems and highest concentrations of this problematic phylum were also found in the middle reaches of the river (BDC and PD). Most of the genera found at BDC and PD were indicative of high nutrients concentrations.

It does not seem as if the phytoplankton concentration and composition in the tributaries have had an effect on the Mooi River's phytoplankton further downstream. A possible reason for this is that the WFS is often dry and only feeds the Mooi River during high rainfall periods. Water from the sites in the tributaries often flows a long distance before reaching the Mooi River sites, and unknown factors between sites could have affected phytoplankton concentration and composition. Beside this, water flows through different areas (such as canals, wetlands, dams) from one site to another. It was, however, often observed that similar genera were abundant in the tributaries and in the Mooi River downstream from their inflows. It is uncertain whether the tributaries are responsible for similarities observed, or whether it was the result of other factors, such as high nutrients concentrations or seasonally bound genera.

Most phytoplankton found in the Mooi River and tributaries can be responsible for nuisance conditions. *Microcystis* and *Anabaena* are known to produce toxins, and cause taste and odour problems (Janse van Vuuren *et al.*, 2006; Janse van Vuuren & Taylor, 2015). The WS was entirely dominated by cyanobacteria and *Microcystis* reached bloom proportions, forming thick scums, at this site. These blooms were accompanied by high nutrient concentrations, high turbidity levels, and relatively high pH values.

Of the seven phytoplankton phyla found, the green algae, diatoms and cyanobacteria were the most abundant and diverse, while euglenophytes, dinoflagellates, chrysophytes, and cryptophytes were found in low concentrations and were less diverse. Nutrients, that determine the rate of primary production and phytoplankton biomass (Paerl *et al.*, 2001), positively correlated with diatoms, green algae, cyanobacteria, and euglenophytes. Orthophosphates and cyanobacteria showed a strong positive correlation, emphasising the important effect of this nutrient on cyanobacterial abundance. High orthophosphate concentrations recorded during the study period, can, therefore, explain the abundance of cyanobacteria during the study. Relatively low abundance and diversity of chrysophytes and cryptophytes, phyla generally associated with clean and unpolluted water (Palmer, 1980), emphasise the fact that the Mooi River and its tributaries can be regarded as polluted systems that show a continuous deterioration in water quality over time.

5.2 Future Recommendations

During the course of the study, it was realised that inclusion of the following aspects will contribute and improve future knowledge of the Mooi River system:

- inclusion of sites in the the Loop Spruit tributary and the WWTP
- monitoring of more than one site in each tributary to allow comparison between different sites in the same tributary, as well as comparisons between the tributaries and the Mooi River
- review RQO's and RWQO's regularly with reference to further studies, to see if some of the ranges can be adjusted according to changes in water chemistry
- identification of phytoplankton to species level (where possible) will be valuable for the calculation of indices and use of indicator species as a measure of pollution
- include measurements on temperature, flow rate, nitrite, and chemical oxygen demand (COD)
- choose sites in such a way that they are comparable to one another, e.g. standing vs flowing water, concrete bottoms (canals) vs natural sediments
- manage nutrient especially orthophosphate concentrations in the Mooi River and its tributaries
- continuous monitoring of cyanobacteria, algae and their relationship with physico-environmental variables over a longer period of time will also contribute to more knowledge that can lead to recommendations and management of the Mooi River system in future.

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ANNEXURES

“A drop of water is worth more than a sack of gold to a thirsty man.” Unknown

APPENDIX A: RAINFALL AND TEMPERATURE DURING THE STUDY PERIOD

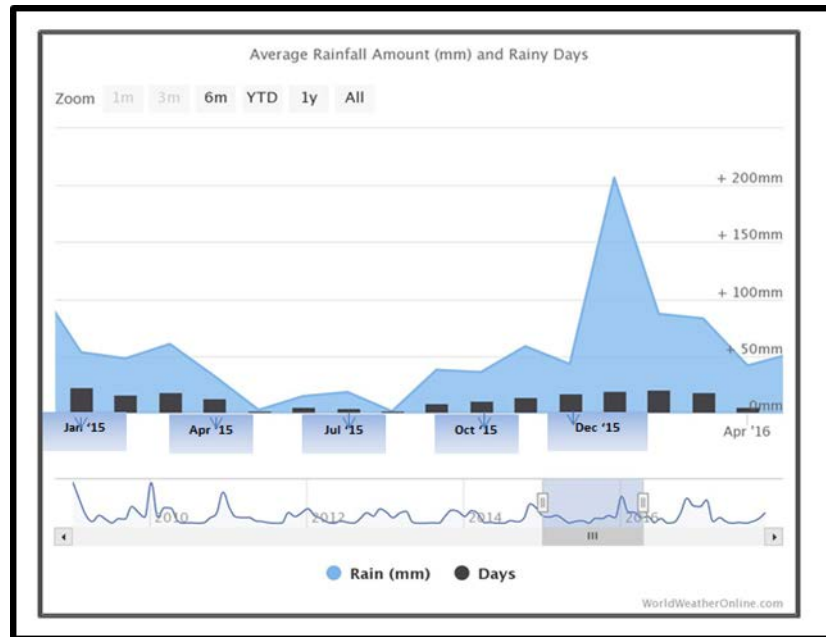


Figure A-1: Rainfall data for 2015 (WWO, 2018).

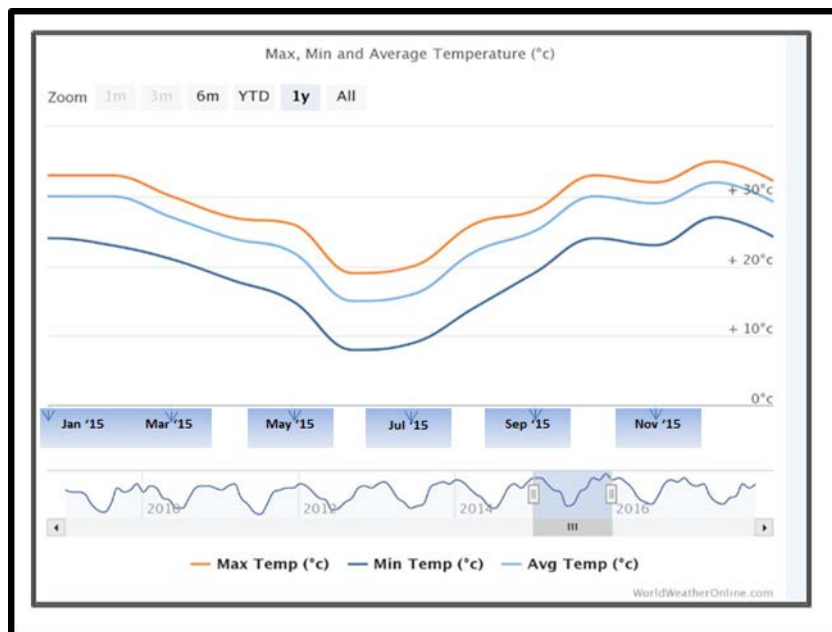


Figure A-2: Temperature data for 2015 (WWO, 2018).

APPENDIX B: KRUSKAL-WALLIS TESTS INDICATING THE STATISTICAL SIGNIFICANT DIFFERENCES FOR PHYSICO-CHEMICAL VARIABLES

BETWEEN SITES (EXCLUDING HEAVY METALS)

Table B-1: The Kruskal-Wallis multiple comparisons of p-values (2 tailed) showing the significant variable differences (p<0.05) for the physico-chemical variables at all sites from January to December 2015. The columns illustrating the tributaries (WFS, GM, and WS) are shaded in grey. Statistical significant p-values <0.05 are indicated in red.

Ammonia (NH ₃ ⁺)	SITE										
	UBD	WFS	BDI	GM	BDC	PD	WS	RB	SB	DFE	Krom
UBD		1.000000	1.000000	1.000000	1.000000	1.000000	0.006628	1.000000	1.000000	0.371878	1.000000
WFS	1.000000		1.000000	1.000000	1.000000	1.000000	0.012318	1.000000	1.000000	0.586373	1.000000
BDI	1.000000	1.000000		1.000000	1.000000	1.000000	0.032691	1.000000	1.000000	1.000000	1.000000
GM	1.000000	1.000000	1.000000		1.000000	1.000000	0.124782	1.000000	1.000000	1.000000	1.000000
BDC	1.000000	1.000000	1.000000	1.000000		1.000000	0.027627	1.000000	1.000000	1.000000	1.000000
PD	1.000000	1.000000	1.000000	1.000000	1.000000		0.604593	1.000000	1.000000	1.000000	1.000000
WS	0.006628	0.012318	0.032691	0.124782	0.027627	0.604593		1.000000	1.000000	1.000000	1.000000
RB	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000		1.000000	1.000000	1.000000
SB	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000		1.000000	1.000000
DFE	0.371878	0.586373	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000		1.000000
Krom	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	

Table B-1: Continues

Nitrate (NO ₃ ⁻)	SITE											
	SITE	UBD	WFS	BDI	GM	BDC	PD	WS	RB	SB	DFE	Krom
UBD		1.000000	1.000000	1.000000	0.001690	1.000000	1.000000	0.016651	1.000000	1.000000	0.017710	1.000000
WFS	1.000000		1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000
BDI	1.000000	1.000000		0.217466	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000
GM	0.001690	1.000000	0.217466		0.045075	0.007882	1.000000	0.146282	0.457338	1.000000	1.000000	1.000000
BDC	1.000000	1.000000	1.000000	0.045075		1.000000	0.288919	1.000000	1.000000	0.303534	1.000000	1.000000
PD	1.000000	1.000000	1.000000	0.007882	1.000000		0.064061	1.000000	1.000000	0.067760	1.000000	1.000000
WS	0.016651	1.000000	1.000000	1.000000	0.288919	0.064061		0.786709	1.000000	1.000000	1.000000	1.000000
RB	1.000000	1.000000	1.000000	0.146282	1.000000	1.000000	0.786709		1.000000	0.822377	1.000000	1.000000
SB	1.000000	1.000000	1.000000	0.457338	1.000000	1.000000	1.000000	1.000000		1.000000	1.000000	1.000000
DFE	0.017710	1.000000	1.000000	1.000000	0.303534	0.067760	1.000000	0.822377	1.000000		1.000000	1.000000
Krom	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000		1.000000

Table B-1: Continues

Orthophosphate (PO ₄ -P)	SITE											
	SITE	UBD	WFS	BDI	GM	BDC	PD	WS	RB	SB	DFE	Krom
UBD		1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	0.177135	0.396504
WFS	1.000000		1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	0.046394	0.114129
BDI	1.000000	1.000000		1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	0.279532	0.604593
GM	1.000000	1.000000	1.000000		1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000
BDC	1.000000	1.000000	1.000000	1.000000		1.000000	1.000000	1.000000	1.000000	1.000000	0.032054	0.080814
PD	1.000000	1.000000	1.000000	1.000000	1.000000		1.000000	1.000000	1.000000	1.000000	0.027353	0.069682
WS	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000		1.000000	1.000000	1.000000	1.000000	1.000000
RB	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000		1.000000	1.000000	1.000000	1.000000
SB	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000		1.000000	1.000000	1.000000
DFE	0.177135	0.046394	0.279532	1.000000	0.032054	0.027353	1.000000	1.000000	1.000000			1.000000
Krom	0.396504	0.114129	0.604593	1.000000	0.080814	0.069682	1.000000	1.000000	1.000000	1.000000	1.000000	

Table B-1: Continues

Total Dissolved Solids (TDS)	SITE										
	UBD	WFS	BDI	GM	BDC	PD	WS	RB	SB	DFE	Krom
UBD		0.000238	0.070262	0.000001	0.816999	0.525042	1.000000	0.001797	0.000485	0.000001	0.000104
WFS	0.000238		1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000
BDI	0.070262	1.000000		0.682227	1.000000	1.000000	1.000000	1.000000	1.000000	0.899381	1.000000
GM	0.000001	1.000000	0.682227		0.055988	0.096283	0.031388	1.000000	1.000000	1.000000	1.000000
BDC	0.816999	1.000000	1.000000	0.055988		1.000000	1.000000	1.000000	1.000000	0.079330	1.000000
PD	0.525042	1.000000	1.000000	0.096283	1.000000		1.000000	1.000000	1.000000	0.134462	1.000000
WS	1.000000	1.000000	1.000000	0.031388	1.000000	1.000000		1.000000	1.000000	0.044787	0.665792
RB	0.001797	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000		1.000000	1.000000	1.000000
SB	0.000485	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000		1.000000	1.000000
DFE	0.000001	1.000000	0.899381	1.000000	0.079330	0.134462	0.044787	1.000000	1.000000		1.000000
Krom	0.000104	1.000000	1.000000	1.000000	1.000000	1.000000	0.665792	1.000000	1.000000	1.000000	

Table B-1: Continues

Electrical Conductivity (EC)	SITE										
	UBD	WFS	BDI	GM	BDC	PD	WS	RB	SB	DFE	Krom
UBD		0.000001	0.003258	0.000007	0.682599	0.042132	1.000000	0.006208	0.011205	0.000000	0.000000
WFS	0.000001		1.000000	1.000000	0.119347	1.000000	0.010856	1.000000	1.000000	1.000000	1.000000
BDI	0.003258	1.000000		1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000
GM	0.000007	1.000000	1.000000		0.291310	1.000000	0.031427	1.000000	1.000000	1.000000	1.000000
BDC	0.682599	0.119347	1.000000	0.291310	1.000000	1.000000	1.000000	1.000000	1.000000	0.048672	0.026283
PD	0.042132	1.000000	1.000000	1.000000		1.000000	1.000000	1.000000	1.000000	0.763695	0.471941
WS	1.000000	0.010856	1.000000	0.031427	1.000000		1.000000	1.000000	1.000000	0.003772	0.001834
RB	0.006208	1.000000	1.000000	1.000000	1.000000	1.000000			1.000000	1.000000	1.000000
SB	0.011205	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000		1.000000	1.000000
DFE	0.000000	1.000000	1.000000	1.000000	1.000000	1.000000	0.003772	1.000000	1.000000		1.000000
Krom	0.000000	1.000000	1.000000	1.000000	0.048672	0.763695	0.001834	1.000000	1.000000	1.000000	

Table B-1: Continues

Calcium (Ca ²⁺)	SITE:											
	SITE	UBD	WFS	BDI	GM	BDC	PD	WS	RB	SB	DFE	Krom
UBD		0.042489	1.000000	1.000000	1.000000	1.000000	1.000000	no data	1.000000	1.000000	1.000000	1.000000
WFS	0.042489		1.000000	1.000000	1.000000	0.226096	0.061966	no data	1.000000	1.000000	1.000000	1.000000
BDI	1.000000	1.000000		1.000000	1.000000	1.000000	1.000000	no data	1.000000	1.000000	1.000000	1.000000
GM	1.000000	1.000000	1.000000		1.000000	1.000000	1.000000	no data	1.000000	1.000000	1.000000	1.000000
BDC	1.000000	0.226096	1.000000	1.000000		1.000000	1.000000	no data	1.000000	1.000000	1.000000	1.000000
PD	1.000000	0.061966	1.000000	1.000000	1.000000		1.000000	no data	1.000000	1.000000	1.000000	1.000000
WS	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data
RB	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	no data		1.000000	1.000000	1.000000
SB	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	no data	1.000000		1.000000	1.000000
DFE	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	no data	1.000000	1.000000		1.000000
Krom	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	no data	1.000000	1.000000	1.000000	

Table B-1: Continues

Magnesium (Mg ²⁺)	SITE										
	UBD	WFS	BDI	GM	BDC	PD	WS	RB	SB	DFE	Krom
SITE											
UBD		1.000000	1.000000	0.592962	1.000000	0.618383	no data	1.000000	1.000000	1.000000	0.689062
WFS	1.000000		1.000000	1.000000	1.000000	1.000000	no data	1.000000	1.000000	1.000000	1.000000
BDI	1.000000	1.000000		1.000000	1.000000	1.000000	no data	1.000000	1.000000	1.000000	1.000000
GM	0.592962	1.000000	1.000000		1.000000	1.000000	no data	1.000000	1.000000	1.000000	1.000000
BDC	1.000000	1.000000	1.000000	1.000000		1.000000	no data	1.000000	1.000000	1.000000	1.000000
PD	0.618383	1.000000	1.000000	1.000000	1.000000		no data	1.000000	1.000000	1.000000	1.000000
WS	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data
RB	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	no data		1.000000	1.000000	1.000000
SB	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	no data	1.000000		1.000000	1.000000
DFE	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	no data	1.000000	1.000000		1.000000
Krom	0.689062	1.000000	1.000000	1.000000	1.000000	1.000000	no data	1.000000	1.000000	1.000000	

Table B-1: Continues

Chloride (Cl ⁻)	SITE										
SITE	UBD	WFS	BDI	GM	BDC	PD	WS	RB	SB	DFE	Krom
UBD		0.127730	1.000000	0.000577	1.000000	0.428233	no data	0.000765	0.004055	0.000000	0.000000
WFS	0.127730		1.000000	1.000000	1.000000	1.000000	no data	1.000000	1.000000	0.191075	0.013161
BDI	1.000000	1.000000		0.205981	1.000000	1.000000	no data	0.250269	0.773092	0.000451	0.000010
GM	0.000577	1.000000	0.205981		1.000000	1.000000	no data	1.000000	1.000000	1.000000	1.000000
BDC	1.000000	1.000000	1.000000	1.000000		1.000000	no data	1.000000	1.000000	0.005842	0.000204
PD	0.428233	1.000000	1.000000	1.000000	1.000000		no data	1.000000	1.000000	0.051920	0.002724
WS	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data
RB	0.000765	1.000000	0.250269	1.000000	1.000000	1.000000	no data		1.000000	1.000000	0.968677
SB	0.004055	1.000000	0.773092	1.000000	1.000000	1.000000	no data	1.000000		1.000000	0.322709
DFE	0.000000	0.191075	0.000451	1.000000	0.005842	0.051920	no data	1.000000	1.000000		1.000000
Krom	0.000000	0.013161	0.000010	1.000000	0.000204	0.002724	no data	0.968677	0.322709	1.000000	

Table B-1: Continues

Fluoride (F ⁻)	SITE											
	SITE	UBD	WFS	BDI	GM	BDC	PD	WS	RB	SB	DFE	Krom
UBD		1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	no data	1.000000	1.000000	1.000000	1.000000
WFS	1.000000		1.000000	1.000000	1.000000	1.000000	1.000000	no data	1.000000	0.845333	1.000000	1.000000
BDI	1.000000	1.000000		1.000000	1.000000	1.000000	1.000000	no data	1.000000	1.000000	1.000000	1.000000
GM	1.000000	1.000000	1.000000		1.000000	1.000000	1.000000	no data	1.000000	1.000000	1.000000	1.000000
BDC	1.000000	1.000000	1.000000	1.000000		1.000000	1.000000	no data	1.000000	0.792233	1.000000	1.000000
PD	1.000000	1.000000	1.000000	1.000000	1.000000		1.000000	no data	1.000000	1.000000	1.000000	1.000000
WS	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data
RB	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	no data		1.000000	1.000000	1.000000
SB	1.000000	0.845333	1.000000	1.000000	1.000000	0.792233	1.000000	no data	1.000000		1.000000	1.000000
DFE	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	no data	1.000000	1.000000		1.000000
Krom	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	no data	1.000000	1.000000	1.000000	

Table B-1: Continues

Sulphate (SO ₄ ²⁻)	SITE										
	SITE	UBD	WFS	BDI	GM	BDC	PD	WS	RB	SB	DFE
UBD		0.000020	0.117234	0.000001	0.012981	0.008498	1.000000	0.000062	0.000244	0.000029	0.001771
WFS	0.000020		1.000000	1.000000	1.000000	1.000000	0.033341	1.000000	1.000000	1.000000	1.000000
BDI	0.117234	1.000000		0.687758	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000
GM	0.000001	1.000000	0.687758		1.000000	1.000000	0.005039	1.000000	1.000000	1.000000	1.000000
BDC	0.012981	1.000000	1.000000	1.000000		1.000000	1.000000	1.000000	1.000000	1.000000	1.000000
PD	0.008498	1.000000	1.000000	1.000000	1.000000		1.000000	1.000000	1.000000	1.000000	1.000000
WS	1.000000	0.033341	1.000000	0.005039	1.000000	1.000000		0.072999	0.184927	0.042954	0.682599
RB	0.000062	1.000000	1.000000	1.000000	1.000000	1.000000	0.072999		1.000000	1.000000	1.000000
SB	0.000244	1.000000	1.000000	1.000000	1.000000	1.000000	0.184927	1.000000		1.000000	1.000000
DFE	0.000029	1.000000	1.000000	1.000000	1.000000	1.000000	0.042954	1.000000	1.000000		1.000000
Krom	0.001771	1.000000	1.000000	1.000000	1.000000	1.000000	0.682599	1.000000	1.000000	1.000000	

Table B-1: Continues

pH	SITE										
SITE	UBD	WFS	BDI	GM	BDC	PD	WS	RB	SB	DFE	Krom
UBD		1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000
WFS	1.000000		1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000
BDI	1.000000	1.000000		1.000000	0.692952	1.000000	0.270422	1.000000	1.000000	1.000000	1.000000
GM	1.000000	1.000000	1.000000		1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000
BDC	1.000000	1.000000	0.692952	1.000000		1.000000	1.000000	1.000000	1.000000	1.000000	1.000000
PD	1.000000	1.000000	1.000000	1.000000	1.000000		1.000000	1.000000	1.000000	1.000000	1.000000
WS	1.000000	1.000000	0.270422	1.000000	1.000000	1.000000		1.000000	1.000000	1.000000	1.000000
RB	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000		1.000000	1.000000	1.000000
SB	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000		1.000000	1.000000
DFE	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000		1.000000
Krom	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	

Table B-1: Continues

Turbidity	SITE										
SITE	UBD	WFS	BDI	GM	BDC	PD	WS	RB	SB	DFE	Krom
UBD		1.000000	1.000000	1.000000	1.000000	1.000000	0.000002	1.000000	0.028802	0.296460	0.079330
WFS	1.000000		1.000000	0.397782	1.000000	1.000000	0.007269	1.000000	1.000000	1.000000	1.000000
BDI	1.000000	1.000000		1.000000	1.000000	1.000000	0.001363	1.000000	1.000000	1.000000	1.000000
GM	1.000000	0.397782	1.000000		0.314113	0.063949	0.000000	0.126318	0.000510	0.009805	0.001819
BDC	1.000000	1.000000	1.000000	0.314113		1.000000	0.009877	1.000000	1.000000	1.000000	1.000000
PD	1.000000	1.000000	1.000000	0.063949	1.000000		0.058564	1.000000	1.000000	1.000000	1.000000
WS	0.000002	0.007269	0.001363	0.000000	0.009877	0.058564		0.028903	1.000000	0.295560	0.928439
RB	1.000000	1.000000	1.000000	0.126318	1.000000	1.000000	0.028903		1.000000	1.000000	1.000000
SB	0.028802	1.000000	1.000000	0.000510	1.000000	1.000000	1.000000	1.000000		1.000000	1.000000
DFE	0.296460	1.000000	1.000000	0.009805	1.000000	1.000000	0.295560	1.000000	1.000000		1.000000
Krom	0.079330	1.000000	1.000000	0.001819	1.000000	1.000000	0.928439	1.000000	1.000000	1.000000	

APPENDIX C: DESCRIPTIVE STATISTICS OF PHYSICO-CHEMICAL VARIABLES (EXCLUDING HEAVY METALS)

Table C-1: Summary of the descriptive statistics for the physico-chemical environmental variables determined at all sites during the study period January to December 2015. The column shaded in light blue contains the overall descriptive statistics for the Mooi River (values of tributaries excluded in calculation for Mooi River). The columns containing the descriptive statistics of the tributaries (WFS, GM, and WS) are shaded in grey. SD = Standard Deviation.

Variables, abbreviations and unit	Descriptive	Mooi River	UBD	WFS	BDI	GM	BDC	PD	WS	RB	SB	DFE	Krom
Ammonia (NH ₃ ⁺) mg/l	Valid N	96	12	12	12	12	12	12	12	12	12	12	12
	Mean	0.39	0.12	0.17	0.20	0.24	0.17	0.28	1.12	0.83	0.74	0.49	0.29
	Minimum	< 0.01	0.03	0.01	< 0.01	< 0.01	0.01	0.02	0.22	0.03	0.03	0.02	0.04
	Maximum	4.60	0.32	0.56	0.70	0.82	0.54	1.11	5.16	4.60	3.80	1.23	0.74
	SD	0.67	0.09	0.18	0.21	0.24	0.17	0.30	1.36	1.29	1.12	0.34	0.24
Nitrate (NO ₃) mg/l	Valid N	96	12	12	12	12	12	12	12	12	12	12	12
	Mean	0.45	0.43	0.41	0.31	0.71	0.28	0.48	1.12	0.49	0.43	0.73	0.43
	minimum	< 0.01	< 0.01	0.10	0.10	0.30	0.10	0.10	0.20	0.10	0.10	0.10	0.20
	Maximum	3.50	3.30	0.70	0.50	1.10	0.50	3.50	2.80	2.80	0.90	1.50	0.90
	SD	0.57	0.91	0.20	0.12	0.24	0.14	0.96	1.00	0.75	0.35	0.43	0.24
Orthophosphate (PO ₄ -P) mg/l	Valid N	96	12	12	12	12	12	12	12	12	12	12	12
	Mean	0.68	0.42	0.34	0.47	0.54	0.33	0.36	1.02	0.72	0.65	1.39	1.09
	Minimum	0.01	0.09	0.09	0.05	0.05	0.01	0.03	0.02	0.03	0.05	0.25	0.21
	Maximum	3.75	1.26	0.69	1.66	1.06	0.81	1.49	5.23	1.77	1.75	3.75	2.28
	SD	0.65	0.36	0.22	0.46	0.32	0.27	0.41	1.52	0.49	0.48	1.01	0.69
Total Dissolved Solids (TDS) mg/l	Valid N	96	12	12	12	12	12	12	11	12	12	12	12
	Mean	475.05	270.25	496.08	473.92	558.42	462.92	475.00	402.18	502.50	533.92	560.75	521.17
	Minimum	208.00	208.00	162.00	419.00	472.00	282.00	391.00	226.00	425.00	440.00	476.00	294.00
	Maximum	850.00	297.00	660.00	517.00	707.00	850.00	842.00	662.00	647.00	850.00	741.00	692.00
	SD	121.68	23.75	150.88	28.99	62.31	133.64	120.78	125.57	59.21	117.26	77.52	99.48

Variables, abbreviations and unit	Descriptive	Mooi River	UBD	WFS	BDI	GM	BDC	PD	WS	RB	SB	DFE	Krom
Electrical Conductivity (EC) mS/m	Valid N	96	12	12	12	12	12	12	12	12	12	12	12
	Mean	70.07	45.64	78.07	71.34	76.59	66.11	70.05	60.84	71.27	71.81	81.64	82.69
	Minimum	38.77	38.77	66.71	59.35	66.16	59.32	60.08	48.81	57.84	58.29	66.02	67.95
	Maximum	109.90	56.30	96.96	86.69	93.80	84.18	95.47	79.61	93.63	104.20	108.20	109.90
	SD	14.24	4.29	7.89	6.65	6.96	6.88	10.58	9.42	9.84	11.59	12.00	12.65
Calcium (Ca ²⁺) mg/l	Valid N	96	12	12	12	12	12	12	-	12	12	12	12
	Mean	70.06	58.93	87.73	73.20	69.60	62.80	56.63	-	80.67	74.53	77.20	76.53
	Minimum	9.20	24.00	52.80	38.40	19.20	36.80	9.20	-	48.00	51.20	59.20	54.40
	Maximum	126.40	115.20	116.80	110.40	134.40	94.40	92.80	-	126.40	110.40	108.80	115.20
	SD	21.81	23.82	19.53	22.23	28.82	17.58	22.77	-	24.86	21.51	14.38	16.94
Magnesium (Mg ²⁺) mg/l	Valid N	96	12	12	12	12	12	12	-	12	12	12	12
	Mean	48.90	37.74	43.84	49.70	54.74	49.93	51.49	-	48.39	50.90	48.58	54.49
	Minimum	8.80	8.80	28.30	28.30	17.60	26.40	20.50	-	26.40	27.30	30.30	31.20
	Maximum	101.50	76.10	63.40	78.10	105.40	68.30	70.30	-	65.40	84.90	75.20	101.50
	SD	15.18	17.49	10.73	15.00	20.33	11.21	14.26	-	14.23	17.44	11.75	17.35
Chloride (Cl ⁻) mg/l	Valid N	96	12	12	12	12	12	12	-	12	12	12	12
	Mean	46.25	13.58	42.42	36.50	46.67	39.25	43.42	-	48.17	46.58	69.83	72.67
	Minimum	5.00	5.00	35.00	25.00	41.00	32.00	32.00	-	34.00	33.00	32.00	55.00
	Maximum	116.00	45.00	50.00	47.00	52.00	50.00	75.00	-	67.00	65.00	116.00	113.00
	SD	21.98	10.63	4.21	5.57	2.96	4.99	12.63	-	8.39	8.77	24.90	19.36
Fluoride (F ⁻) mg/l	Valid N	96	12	12	12	12	12	12	-	12	12	12	12
	Mean	0.39	0.25	0.23	0.27	0.34	0.23	0.36	-	0.62	0.63	0.35	0.42
	minimum	0.01	0.02	< 0.01	0.01	0.06	0.01	0.03	-	0.06	0.05	0.17	0.18
	Maximum	2.19	0.55	0.41	0.55	0.59	0.48	1.26	-	2.19	1.95	0.81	0.72
	SD	0.36	0.14	0.14	0.19	0.16	0.17	0.34	-	0.62	0.54	0.17	0.17
Sulphate (SO ₄ ²⁻) mg/l	Valid N	96	12	12	12	12	12	12	12	12	12	12	12
	Mean	115.58	27.00	143.67	112.00	158.67	119.33	125.00	70.33	136.33	137.33	139.00	128.67
	Minimum	12.00	12.00	84.00	80.00	96.00	72.00	72.00	36.00	60.00	100.00	84.00	84.00

Variables, abbreviations and unit	Descriptive	Mooi River	UBD	WFS	BDI	GM	BDC	PD	WS	RB	SB	DFE	Krom
	Maximum	220.00	56.00	252.00	136.00	344.00	172.00	216.00	156.00	204.00	220.00	196.00	208.00
	SD	45.51	13.97	43.00	17.97	63.30	27.65	36.63	43.30	37.77	34.47	30.56	35.48
pH	Valid N	96	12	12	12	12	12	12	12	12	12	12	12
	Mean	8.18	8.16	8.17	8.07	8.11	8.37	8.25	8.50	8.16	8.18	8.10	8.14
	Minimum	7.50	7.66	7.55	7.68	7.60	7.84	7.50	7.48	7.69	7.74	7.64	7.82
	Maximum	9.16	9.06	9.02	8.86	8.87	9.16	9.06	9.38	8.75	8.70	8.57	8.85
	SD	0.35	0.42	0.42	0.37	0.37	0.39	0.43	0.58	0.29	0.29	0.32	0.27
Turbidity NTU	Valid N	96	12	12	12	12	12	12	11	12	12	12	12
	Mean	3.77	1.06	2.59	2.22	0.59	2.57	3.48	23.71	3.63	6.08	4.86	6.23
	Minimum	0.21	0.23	0.44	0.26	0.14	0.25	0.26	6.97	0.21	0.57	0.55	0.96
	Maximum	18.30	1.73	6.57	5.34	0.89	5.15	7.45	118.00	10.40	14.90	18.30	16.20
	SD	3.79	0.49	1.81	1.65	0.26	1.46	2.44	31.87	3.26	4.45	4.79	5.81

APPENDIX D: KRUSKAL-WALLIS TESTS OF HEAVY METALS

Table D-1: The Kruskal-Wallis multiple comparisons of p-values (2 tailed) showing differences in significant variables for the heavy metals at UBD, BDI, BDC, and PD in the Mooi River, as well as at two sites located in the WFS and GM tributaries (January to December 2015). The columns illustrating the tributaries (WFS and GM) are shaded in light grey. Statistical significant p-values (<0.05) are indicated in red.

Copper (Total Cu)	SITE					
SITE	UBD	WFS	BDI	GM	BDC	PD
UBD		1.000000	1.000000	1.000000	1.000000	1.000000
WFS	1.000000		1.000000	1.000000	1.000000	1.000000
BDI	1.000000	1.000000		1.000000	1.000000	1.000000
GM	1.000000	1.000000	1.000000		1.000000	1.000000
BDC	1.000000	1.000000	1.000000	1.000000		1.000000
PD	1.000000	1.000000	1.000000	1.000000	1.000000	
Hexavalent Chromium (Total Cr(VI))	SITE					
SITE:	UBD	WFS	BDI	GM	BDC	PD
UBD		1.000000	1.000000	1.000000	1.000000	1.000000
WFS	1.000000		1.000000	1.000000	1.000000	1.000000
BDI	1.000000	1.000000		1.000000	1.000000	1.000000
GM	1.000000	1.000000	1.000000		1.000000	1.000000
BDC	1.000000	1.000000	1.000000	1.000000		1.000000
PD	1.000000	1.000000	1.000000	1.000000	1.000000	
Iron (Total Fe)	SITE					
SITE	UBD	WFS	BDI	GM	BDC	PD
UBD		0.396451	0.046801	1.000000	0.086740	1.000000
WFS	0.396451		1.000000	1.000000	1.000000	1.000000
BDI	0.046801	1.000000		1.000000	1.000000	1.000000
GM	1.000000	1.000000	1.000000		1.000000	1.000000
BDC	0.086740	1.000000	1.000000	1.000000		1.000000
PD	1.000000	1.000000	1.000000	1.000000	1.000000	

Table D-1: Continues

Lead (Total Pb)	SITE					
SITE	UBD	WFS	BDI	GM	BDC	PD
UBD		1.000000	1.000000	1.000000	1.000000	1.000000
WFS	1.000000		1.000000	1.000000	1.000000	1.000000
BDI	1.000000	1.000000		1.000000	1.000000	1.000000
GM	1.000000	1.000000	1.000000		1.000000	1.000000
BDC	1.000000	1.000000	1.000000	1.000000		1.000000
PD	1.000000	1.000000	1.000000	1.000000	1.000000	
Manganese (Total Mn)	SITE					
SITE	UBD	WFS	BDI	GM	BDC	PD
UBD		1.000000	1.000000	1.000000	1.000000	1.000000
WFS	1.000000		1.000000	1.000000	1.000000	1.000000
BDI	1.000000	1.000000		1.000000	1.000000	1.000000
GM	1.000000	1.000000	1.000000		1.000000	1.000000
BDC	1.000000	1.000000	1.000000	1.000000		0.965297
PD	1.000000	1.000000	1.000000	1.000000	0.965297	
Nickel (Total Ni)	SITE					
SITE	UBD	WFS	BDI	GM	BDC	PD
UBD		0.201009	1.000000	1.000000	1.000000	1.000000
WFS	0.201009		1.000000	0.166453	1.000000	0.273337
BDI	1.000000	1.000000		1.000000	1.000000	1.000000
GM	1.000000	0.166453	1.000000		1.000000	1.000000
BDC	1.000000	1.000000	1.000000	1.000000		1.000000
PD	1.000000	0.273337	1.000000	1.000000	1.000000	
Uranium (Total U)	SITE					
SITE	UBD	WFS	BDI	GM	BDC	PD
UBD		0.000001	0.012732	1.000000	0.016296	0.077108
WFS	0.000001		1.000000	0.000000	1.000000	0.608351
BDI	0.012732	1.000000		0.003573	1.000000	1.000000
GM	1.000000	0.000000	0.003573		0.004660	0.024986
BDC	0.016296	1.000000	1.000000	0.004660		1.000000
PD	0.077108	0.608351	1.000000	0.024986	1.000000	

Table D-1: Continues

Zinc (Total Zn)	SITE					
SITE	UBD	WFS	BDI	GM	BDC	PD
UBD		1.000000	1.000000	1.000000	1.000000	1.000000
WFS	1.000000		1.000000	1.000000	1.000000	1.000000
BDI	1.000000	1.000000		1.000000	1.000000	1.000000
GM	1.000000	1.000000	1.000000		1.000000	1.000000
BDC	1.000000	1.000000	1.000000	1.000000		1.000000
PD	1.000000	1.000000	1.000000	1.000000	1.000000	

APPENDIX E: DESCRIPTIVE STATISTICS OF HEAVY METALS

Table E-1: Summary of the descriptive statistics for heavy metals determined at UBD, BDI, BDC, and PD in the Mooi River, as well as at two sites located in the WFS and GM tributaries during the study period January to December 2015. The column shaded in light blue contains the overall descriptive statistics for the Mooi River (values of tributaries excluded in calculation for Mooi River). The columns containing the descriptive statistics of the tributaries (WFS and GM) are shaded in grey. (SD = Standard Deviation).

Variable, abbreviation and unit	Descriptive	Mooi River	UBD	WFS	BDI	GM	BDC	PD
Copper (Total Cu) µg/l	Valid N	48	12	12	12	12	12	12
	Mean	7.06	8.08	7.83	6.08	11.00	5.17	8.92
	Minimum	< 1.00	< 1.00	< 1.00	1.00	1.00	< 1.00	< 1.00
	Maximum	46.00	46.00	37.00	31.00	32.00	27.00	46.00
	SD	10.74	12.89	13.03	8.17	11.02	7.59	13.87
Hexavalent Chromium (Total Cr (VI)) µg/l	Valid N	48	12	12	12	12	12	12
	Mean	4.25	2.25	5.33	4.25	6.75	5.00	5.50
	minimum	< 0.01	< 0.01	1.00	1.00	1.00	< 0.01	< 0.01
	Maximum	20.00	5.00	13.00	18.00	25.00	20.00	20.00
	SD	5.40	1.66	3.63	4.67	8.72	7.16	6.47
Iron (Total Fe) µg/l	Valid N	48	12	12	12	12	12	12
	Mean	21.25	43.33	14.17	10.83	20.00	11.67	19.17
	Minimum	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	Maximum	160.00	160.00	30.00	20.00	60.00	20.00	40.00
	SD	25.32	42.50	10.84	6.69	15.95	5.77	9.96
Lead (Total Pb) µg/l	Valid N	48	12	12	12	12	12	12
	Mean	23.00	64.42	78.25	18.25	1.50	4.50	4.83
	Minimum	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	Maximum	503.00	503.00	524.00	89.00	3.00	24.00	17.00
	SD	80.81	155.73	163.81	30.64	0.80	7.17	5.36
Manganese (Total Mn) µg/l	Valid N	48	12	12	12	12	12	12
	Mean	47.44	46.00	49.58	49.92	60.33	39.00	54.83
	Minimum	17.00	22.00	29.00	18.00	31.00	17.00	27.00
	Maximum	99.00	73.00	93.00	77.00	115.00	95.00	99.00
	SD	18.75	13.80	20.10	14.16	27.09	22.12	21.80
Nickel (Total Ni) µg/l	Valid N	48	12	12	12	12	12	12
	Mean	40.81	95.58	139.00	37.83	7.25	12.67	17.17
	Minimum	4.00	4.00	7.00	4.00	4.00	4.00	4.00
	Maximum	621.00	621.00	656.00	169.00	11.00	35.00	101.00
	SD	111.83	211.04	224.73	57.12	2.30	10.94	27.55
Uranium (Total U) µg/l	Valid N	48	12	12	12	12	12	12
	Mean	2.15	0.23	10.51	3.08	0.23	2.78	2.51
	Minimum	0.20	0.20	1.00	1.30	0.20	1.90	1.70

Variable, abbreviation and unit	Descriptive	Mooi River	UBD	WFS	BDI	GM	BDC	PD
	Maximum	5.80	0.30	22.30	5.80	0.60	3.90	3.40
	SD	1.38	0.05	5.96	1.38	0.12	0.65	0.55
Zinc (Total Zn) mg/l	Valid N	20	5	5	5	5	5	5
	Mean	0.16	0.13	0.08	0.21	0.11	0.21	0.09
	Minimum	0.03	0.05	0.06	0.04	0.01	0.03	0.06
	Maximum	0.61	0.21	0.11	0.42	0.25	0.61	0.14
	SD	0.14	0.07	0.02	0.14	0.11	0.25	0.03

APPENDIX F: DESCRIPTIVE STATISTICS FOR PHYTOPLANKTON DATA

Table F-1 - F-4: Seasonal descriptive statistics for phytoplankton variables determined at all sites during the study period January to December 2015. The column shaded in light blue contains the overall descriptive statistics for the Mooi River (tributaries excluded). The columns containing the descriptive statistics of the tributaries (WFS, GM, and WS) are shaded in grey.

Table F-1: Summer (SD = Standard Deviation, Cyano = Cyanophyta, Bacil = Bacillariophyta, Chloro = Chlorophyta, Crypto = Cryptophyta, Chryso = Chrysophyta, Dino = Dinophyta and Eugl = Euglenophyta).

Algal phyla	Descriptive	Mooi River	UBD	WFS	BDI	GM	BDC	PD	WS	RB	SB	DFE	KROM
Cyano (cells/ml)	Valid N	24	3	3	3	3	3	3	3	3	3	3	3
	Mean	224	45	496	118	67	687	658	17813	116	33	115	22
	Minimum	0	5	78	60	0	538	310	7772	36	15	65	0
	Maximum	1329	111	1229	229	146	888	1329	32627	175	44	192	47
	SD	325	57	637	96	73	180	581	13097	72	16	67	23
	Sum	5382	115	1488	354	348	2061	1974	53439	349	99	344	66
Bacil (cells/ml)	Valid N	24	3	3	3	3	3	3	3	3	3	3	3
	Mean	369	59	136	99	317	765	996	5084	629	192	146	66
	Minimum	26	54	53	45	84	73	138	303	170	39	75	26
	Maximum	1445	64	204	168	466	1140	1445	9966	902	388	222	94
	SD	471	5	76	63	204	601	743	4832	400	179	74	36
	Sum	8854	118	409	296	1502	2296	2987	15253	1887	575	438	199
Chloro (cells/ml)	Valid N	24	3	3	3	3	3	3	3	3	3	3	3
	Mean	529	208	88	61	92	1595	1609	2380	319	166	192	82
	Minimum	21	82	50	42	29	1168	655	1031	124	108	45	21
	Maximum	2900	393	153	71	124	2119	2900	4001	567	196	295	113
	SD	743	163	56	16	54	483	1160	1504	227	50	130	52

Algal phyla	Descriptive	Mooi River	UBD	WFS	BDI	GM	BDC	PD	WS	RB	SB	DFE	KROM
	Sum	12698	474	264	183	428	4786	4828	7141	958	499	575	245
Crypto (cells/ml)	Valid N	24	3	3	3	3	3	3	3	3	3	3	3
	Mean	0	0	0	1	0	0	0	24	0	0	0	0
	Minimum	0	0	0	0	0	0	0	0	0	0	0	0
	Maximum	2	0	0	2	0	0	0	73	0	0	0	0
	SD	0	0	0	1	0	0	0	42	0	0	0	0
	Sum	2	0	0	2	0	0	0	73	0	0	0	0
Chryso (cells/ml)	Valid N	24	3	3	3	3	3	3	3	3	3	3	3
	Mean	1	6	0	0	0	0	0	0	0	0	0	0
	Minimum	0	0	0	0	0	0	0	0	0	0	0	0
	Maximum	17	17	0	0	0	0	0	0	0	0	0	0
	SD	4	10	0	0	0	0	0	0	0	0	0	0
	Sum	17	17	0	0	0	0	0	0	0	0	0	0
Dino (cells/ml)	Valid N	24	3	3	3	3	3	3	3	3	3	3	3
	Mean	9	0	0	3	1	21	18	0	11	8	6	2
	Minimum	0	0	0	0	0	0	0	0	0	0	0	0
	Maximum	39	1	0	8	2	35	39	0	24	12	12	5
	SD	12	1	0	5	1	19	20	0	12	7	6	2
	Sum	209	1	0	8	2	64	53	0	32	24	19	7
Eugl (cells/ml)	Valid N	24	3	3	3	3	3	3	3	3	3	3	3
	Mean	21	14	17	11	15	34	43	49	30	15	15	7
	Minimum	1	10	7	5	3	1	7	0	10	6	12	2
	Maximum	102	20	29	19	24	98	102	109	51	19	18	14
	SD	27	6	11	8	11	55	51	56	21	7	3	6
	Sum	505	41	52	32	64	103	129	145	90	45	46	20
Total cells/ml		27666	756	2213	575	2344	9310	9971	76051	3315	1242	1422	537

Table F-2: Autumn (SD = Standard Deviation, Cyano = Cyanophyta, Bacil = Bacillariophyta, Chloro = Chlorophyta, Crypto = Cryptophyta, Chryso = Chrysophyta, Dino = Dinophyta and Eugl = Euglenophyta).

Algal phyla	Descriptive	Mooi River	UBD	WFS	BDI	GM	BDC	PD	WS	RB	SB	DFE	KROM
Cyano (cells/ml)	Valid N	24	3	3	3	3	3	3	3	3	3	3	3
	Mean	124	8	3	109	7	206	499	491406	45	45	45	68
	Minimum	0	0	0	0	1	35	85	20515	0	0	0	23
	Maximum	1251	13	9	315	11	480	1251	1231097	93	93	120	133
	SD	265	7	5	179	5	240	652	648512	47	47	66	58
	Sum	2970	25	9	327	44	617	1497	1474218	134	33	134	203
Bacil (cells/ml)	Valid N	24	3	3	3	3	3	3	3	3	3	3	3
	Mean	152	11	13	65	6	125	142	3650	109	109	273	58
	Minimum	6	8	6	6	5	15	18	1309	27	27	39	7
	Maximum	1115	15	23	170	8	335	284	6984	268	268	432	129
	SD	243	4	9	91	2	182	134	2965	138	138	207	63
	Sum	3655	32	38	196	37	374	427	10948	326	1305	820	175
Chloro (cells/ml)	Valid N	24	3	3	3	3	3	3	3	3	3	3	3
	Mean	492	21	11	990	11	1749	454	10645	75	75	236	65
	Minimum	6	14	2	13	7	33	27	2619	11	11	17	6
	Maximum	5085	26	16	2934	14	5085	1251	21897	189	189	441	179
	SD	1170	6	8	1684	3	2889	691	10036	99	99	213	99
	Sum	11804	63	32	2970	67	5247	1362	31936	225	1034	709	194
Crypto (cells/ml)	Valid N	24	3	3	3	3	3	3	3	3	3	3	3
	Mean	6	1	1	1	1	0	13	133	1	1	11	5
	Minimum	0	1	0	0	0	0	0	0	0	0	1	0
	Maximum	36	2	2	1	2	0	36	218	2	2	19	15
	SD	10	0	1	1	1	0	20	117	1	1	9	8
	Sum	132	4	3	2	6	0	40	400	4	34	32	15

Algal phyla	Descriptive	Mooi River	UBD	WFS	BDI	GM	BDC	PD	WS	RB	SB	DFE	KROM
Chryso (cells/ml)	Valid N	24	3	3	3	3	3	3	3	3	3	3	3
	Mean	16	1	1	17	0	28	31	0	1	1	24	0
	Minimum	0	0	1	0	0	0	0	0	0	0	0	0
	Maximum	78	1	1	49	1	73	58	0	3	3	73	0
	SD	27	1	0	27	1	39	29	0	2	2	42	0
	Sum	393	2	3	51	2	85	92	0	3	87	73	0
Dino (cells/ml)	Valid N	24	3	3	3	3	3	3	3	3	3	3	3
	Mean	6	2	1	11	0	18	9	49	0	0	8	0
	Minimum	0	0	0	0	0	0	3	0	0	0	0	0
	Maximum	51	3	2	34	0	51	15	145	0	0	25	0
	SD	13	2	1	20	0	28	6	84	0	0	15	0
	Sum	147	5	4	34	0	55	27	145	0	0	25	0
Eugl (cells/ml)	Valid N	24	3	3	3	3	3	3	3	3	3	3	3
	Mean	11	2	2	20	1	11	15	1019	7	7	9	11
	Minimum	0	1	1	0	0	2	3	436	4	4	5	0
	Maximum	44	3	4	44	1	22	22	1891	9	9	15	29
	SD	11	1	2	22	0	10	11	770	3	3	5	16
	Sum	258	5	5	59	3	33	45	3055	20	36	26	32
Total cells/ml		19359	137	94	3639	159	6412	3491	1520702	713	2528	1818	621

Table F-3: Winter (SD = Standard Deviation, Cyano = Cyanophyta, Bacil = Bacillariophyta, Chloro = Chlorophyta, Crypto = Cryptophyta, Chryso = Chrysophyta, Dino = Dinophyta and Eugl = Euglenophyta).

Algal phyla	Descriptive	Mooi River	UBD	WFS	BDI	GM	BDC	PD	WS	RB	SB	DFE	KROM
Cyano (cells/ml)	Valid N	24	3	3	3	3	3	3	3	3	3	3	3
	Mean	53	7	40	15	13	44	169	11664	67	32	26	69
	Minimum	0	0	0	0	0	0	12	3492	0	23	14	30
	Maximum	436	19	87	41	38	131	436	22442	184	44	38	140
	SD	95	11	44	22	21	76	233	9741	102	11	12	62
	Sum	1283	20	121	45	78	131	507	34991	202	95	77	206
Bacil (cells/ml)	Valid N	24	3	3	3	3	3	3	3	3	3	3	3
	Mean	126	31	119	30	60	231	314	4668	95	47	97	166
	Minimum	3	3	2	4	3	3	21	1528	16	6	26	52
	Maximum	862	82	333	75	175	666	862	6802	206	107	224	333
	SD	214	44	185	39	99	377	475	2777	99	53	111	148
	Sum	3034	92	357	91	362	693	942	14004	285	142	290	498
Chloro (cells/ml)	Valid N	24	3	3	3	3	3	3	3	3	3	3	3
	Mean	237	6	22	12	157	1316	410	5529	68	32	39	17
	Minimum	2	2	1	5	2	41	39	2983	33	16	13	2
	Maximum	3601	9	55	23	462	3601	1058	8657	92	47	75	44
	SD	748	4	29	10	264	1984	563	2882	31	16	32	24
	Sum	5698	18	66	36	939	3947	1231	16586	204	97	116	50
Crypto (cells/ml)	Valid N	24	3	3	3	3	3	3	3	3	3	3	3
	Mean	3	1	1	0	2	4	12	73	2	1	5	2
	Minimum	0	0	0	0	0	0	1	0	0	1	2	1
	Maximum	33	1	1	1	4	11	33	182	2	2	10	4
	SD	7	1	1	1	2	6	18	96	1	1	4	2
	Sum	81	3	2	1	10	12	35	218	5	4	14	6

Algal phyla	Descriptive	Mooi River	UBD	WFS	BDI	GM	BDC	PD	WS	RB	SB	DFE	KROM
Chryso (cells/ml)	Valid N	24	3	3	3	3	3	3	3	3	3	3	3
	Mean	22	0	0	0	50	142	23	8	4	2	3	0
	Minimum	0	0	0	0	0	1	1	0	0	0	0	0
	Maximum	393	0	0	0	149	393	65	24	12	7	8	0
	SD	80	0	0	0	86	218	36	14	7	4	5	0
	Sum	525	0	0	0	300	426	70	24	12	7	9	0
Dino (cells/ml)	Valid N	24	3	3	3	3	3	3	3	3	3	3	3
	Mean	4	0	1	1	0	8	12	28	6	1	1	0
	Minimum	0	0	0	0	0	0	1	0	0	0	0	0
	Maximum	25	0	2	2	1	18	25	49	15	4	2	0
	SD	7	0	1	1	1	9	13	25	8	2	1	0
	Sum	85	0	2	2	2	24	35	85	18	4	2	0
Eugl (cells/ml)	Valid N	24	3	3	3	3	3	3	3	3	3	3	3
	Mean	8	4	2	1	5	6	21	707	8	6	8	8
	Minimum	0	1	1	0	1	1	2	546	1	1	4	3
	Maximum	58	10	4	2	11	16	58	873	19	13	16	18
	SD	12	5	1	1	5	9	32	164	10	7	6	8
	Sum	190	12	7	4	30	19	62	2122	25	17	25	25
Total cells/ml		10895	145	554	179	1722	5253	2884	68031	751	367	533	784

Table F-4: Spring (SD = Standard Deviation, Cyano = Cyanophyta, Bacil = Bacillariophyta, Chloro = Chlorophyta, Crypto = Cryptophyta, Chryso = Chrysophyta, Dino = Dinophyta and Eugl = Euglenophyta).

Algal phyla	Descriptive	Mooi River	UBD	WFS	BDI	GM	BDC	PD	WS	RB	SB	DFE	KROM
Cyano (cells/ml)	Valid N	24	3	3	3	3	3	3	3	3	3	3	3
	Mean	102	23	54	10	18	290	377	7273	61	30	18	6
	Minimum	0	0	3	0	12	0	255	4183	20	2	2	5
	Maximum	542	53	153	27	27	542	458	10312	138	70	47	8
	SD	166	27	86	14	8	273	108	3065	67	36	25	2
	Sum	2446	70	162	30	109	870	1131	21818	183	91	54	17
Bacil (cells/ml)	Valid N	24	3	3	3	3	3	3	3	3	3	3	3
	Mean	156	10	54	44	83	397	537	1419	105	61	65	28
	Minimum	4	4	6	32	66	276	244	691	23	4	23	19
	Maximum	973	19	143	66	103	594	973	1819	224	97	102	33
	SD	229	8	78	20	19	172	385	631	106	50	40	8
	Sum	3736	30	161	131	497	1191	1610	4256	314	184	194	83
Chloro (cells/ml)	Valid N	24	3	3	3	3	3	3	3	3	3	3	3
	Mean	1	22	19	28	36	1935	737	2744	62	115	56	32
	Minimum	0	4	3	18	22	822	495	1722	1	3	10	5
	Maximum	18	57	41	41	43	4128	866	4219	141	269	127	64
	SD	4	30	20	12	12	1899	210	1309	72	138	62	29
	Sum	8967	67	56	84	214	5806	2212	8233	186	346	168	97
Crypto (cells/ml)	Valid N	24	3	3	3	3	3	3	3	3	3	3	3
	Mean	374	0	0	0	0	6	2	0	0	0	0	0
	Minimum	1	0	0	0	0	0	0	0	0	0	0	0
	Maximum	4128	0	0	0	0	18	5	0	0	0	0	0
	SD	858	0	0	0	0	11	3	0	0	0	0	0
	Sum	24	0	0	0	0	18	5	0	0	0	0	0

Algal phyla	Descriptive	Mooi River	UBD	WFS	BDI	GM	BDC	PD	WS	RB	SB	DFE	KROM
Chryso (cells/ml)	Valid N	24	3	3	3	3	3	3	3	3	3	3	3
	Mean	3	0	0	0	0	10	2	0	1	0	0	0
	Minimum	0	0	0	0	0	0	0	0	0	0	0	0
	Maximum	22	0	0	0	1	29	7	0	2	0	0	0
	SD	6	0	0	0	0	17	4	0	1	0	0	0
	Sum	38	0	0	0	1	29	7	0	2	0	0	0
Dino (cells/ml)	Valid N	24	3	3	3	3	3	3	3	3	3	3	3
	Mean	2	0	1	1	2	9	13	0	0	1	0	0
	Minimum	0	0	0	0	1	7	7	0	0	0	0	0
	Maximum	29	1	2	4	4	12	22	0	0	3	0	0
	SD	6	1	1	2	2	3	8	0	0	2	0	0
	Sum	75	1	4	4	11	27	40	0	0	3	0	0
Eugl (cells/ml)	Valid N	24	3	3	3	3	3	3	3	3	3	3	3
	Mean	17	2	5	2	4	25	82	163	6	11	9	4
	Minimum	0	0	1	0	4	0	5	100	0	1	1	1
	Maximum	124	3	14	5	5	51	124	206	13	27	20	7
	SD	34	1	8	3	1	26	66	56	7	14	10	3
	Sum	419	5	16	7	25	74	246	488	18	33	26	11
Total cells/ml		15705	173	398	257	857	8016	5251	34794	703	657	441	208

APPENDIX G: KRUSKAL-WALLIS TESTS INDICATING THE STATISTICAL SIGNIFICANT DIFFERENCES BETWEEN THE DIFFERENT PHYSICAL-CHEMICAL AND PHYTOPLANKTON VARIABLES (EXCLUDING HEAVY METALS)

Table G-1: The Kruskal-Wallis multiple comparisons of p-values (2 tailed) indicating the overall statistical significant differences (p<0.05) between the physico-chemical and phytoplankton variables (heavy metals excluded) from January to December 2015. Statistical significant p-values <0.05 are indicated in red.

Variables	Ammonia (NH ₃ ⁺)	Nitrate (NO ₃ ⁻)	Orthophosphate (PO ₄ ⁻ P)	Total Dissolved Solids (TDS)	Electrical Conductivity (EC)	Calcium (Ca ²⁺)	Magnesium (Mg ²⁺)	Chloride (Cl ⁻)	Fluoride (F ⁻)	Sulphate (SO ₄ ²⁻)	pH	Turbidity	Cyanophyta	Bacillariophyta	Chlorophyta	Cryptophyta	Chrysoophyta	Dinophyta	Euglenophyta	
Ammonia (NH ₃ ⁺)	1.0000	0.3021	0.1818	0.0293	0.0790	0.1890	-	0.3705	-	0.0578	-	0.3672	0.0935	0.2054	0.2518	-	-	-	-	0.2538
Nitrate (NO ₃ ⁻)	0.3021	1.0000	0.4050	0.4162	0.4074	0.1678	-	0.3792	0.2115	0.1912	-	0.2840	0.0334	0.1283	0.0746	-	-	-	-	0.0121
Orthophosphate (PO ₄ ⁻ P)	0.1818	0.4050	1.0000	0.3576	0.3150	-	0.1080	0.1941	0.3972	0.5162	0.0902	-	0.0711	0.2054	0.1609	-	-	-	-	0.1090
Total Dissolved Solids (TDS)	0.0293	0.4162	0.3576	1.0000	0.7312	0.3889	0.0992	0.5539	0.2971	0.6026	-	-	-	-	-	-	-	-	-	-
Electrical Conductivity (EC)	0.0790	0.4074	0.3150	0.7312	1.0000	0.3580	0.1835	0.6193	0.2076	0.5827	-	-	0.1175	0.0549	0.1196	0.0564	0.1691	0.0663	0.1374	0.1479
Calcium (Ca ²⁺)	0.1890	0.1678	-	0.3889	0.3580	1.0000	-	0.2830	-	0.4978	-	-	0.2465	0.0126	0.2701	0.1630	0.2577	0.1334	0.1000	0.1788
Magnesium (Mg ²⁺)	-	-	0.1080	0.0992	0.1835	-	1.0000	0.2296	0.1118	-	0.1351	0.1343	0.2636	0.2863	0.2402	-	-	-	-	0.3606
Chloride (Cl ⁻)	0.3705	0.3792	0.3972	0.5539	0.6193	0.2830	0.2296	1.0000	0.3259	0.4991	-	0.2436	-	0.1362	-	-	-	-	-	0.0764
Fluoride (F ⁻)	-	0.2115	0.5162	0.2971	0.2076	-	0.1118	0.3259	1.0000	0.2887	-	0.2894	0.0009	0.2621	0.1405	-	-	-	-	0.1110
Sulphate (SO ₄ ²⁻)	0.0578	0.1912	0.0902	0.6026	0.5827	0.4978	-	0.4991	0.2887	1.0000	-	-	0.1753	0.2621	0.1405	-	-	-	-	-
pH	-	-	-	-	-	-	0.1351	-	-	-	1.0000	0.1339	0.3494	0.3237	0.3071	0.0843	-	-	-	0.3291
Turbidity	0.3672	0.2840	0.3486	-	0.0126	0.1373	-	0.0113	0.1249	0.2223	-	0.1339	1.0000	0.4305	0.4799	0.4967	-	-	-	0.4839
Cyanophyta	0.0935	0.0334	0.0711	-	-	-	0.1343	0.2436	0.2894	0.2325	0.1753	0.1339	1.0000	0.4305	0.4799	0.4967	-	-	-	0.4839
Bacillariophyta	0.2054	0.1283	0.2054	0.0564	0.1630	0.1333	-	0.1362	0.2621	0.1795	0.3237	0.4799	0.7524	1.0000	0.8529	0.0607	0.0076	0.3119	0.7820	

Chlorophyta	0.2518	0.0746	0.1609	-	-	-	0.2402	-	0.1405	-	0.3071	0.4967	0.7137	0.8529	1.0000	0.0270	0.1048	0.3920	0.7480	
Cryptophyta	-	-	-	0.1691	0.2577	0.1975	-	0.0170	-	0.2045	-	0.0843	-	-	0.0607	0.0270	1.0000	0.3917	0.1037	0.0123
Chrysophyta	0.1165	0.2075	0.3504	0.0663	0.1334	0.0720	0.1592	0.0560	0.2437	0.0959	0.0843	0.1685	0.0609	0.0076	0.1048	0.3917	1.0000	0.2695	-	0.0540
Dinophyta	0.0922	0.2037	0.2491	0.1374	0.1000	0.0786	0.1263	0.1399	0.1049	-	0.1239	-	-	0.0076	0.1048	0.3917	1.0000	0.2695	-	0.0540
Euglenophyta	0.1145	0.2596	0.0766	0.1855	0.1788	0.1697	0.0615	0.1367	0.0199	0.0903	0.1060	0.0455	0.2421	0.3119	0.3920	0.1037	0.2695	1.0000	0.2123	-
	0.2538	-	0.1090	-	-	-	0.3606	0.0764	0.1110	-	0.3291	0.4839	0.7308	0.7820	0.7480	0.0123	-	0.2123	1.0000	0.0540
		0.0121		0.1479	0.2505	0.1274				0.2473										

APPENDIX H:

Table H-1: Summary of the mean orthophosphate concentrations (mg/l) in the Mooi River and tributaries during 2014, 2015 and 2017.

	2014	2015 (current study)	2017
Mooi River	0.59	0.68	0.49
WFS tributary	0.25	0.34	0.30
GM tributary	0.42	0.54	0.46
WS tributary	0.63	1.02	0.38