Water Quality Assessment of the Koekemoerspruit: Integrating water physico-chemistry and phytoplankton assemblages

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ABSTRACT

Midvaal Water Company, situated on the banks of the Vaal River in the North West Province, supplies potable water to the Greater Municipality of Matlosana, as well as mining and industrial undertakings in the area. Water is abstracted downstream from the confluence of the Koekemoerspruit (KMS) and Vaal River. The KMS represents an affected (mining- and urbanisation associated pollution) water resource and the middle-Vaal River system acts as the receiving water body. This emphasised the need to assess the water quality of the KMS and its influence on the Vaal River. The main aim of this study was to integrate the use of phytoplankton assemblages and water physico-chemistry, hypothesising that it would provide a more accurate and comprehensive means to determine and assess water quality.

The descriptive statistics revealed that nutrient enrichment and salinity, as the result of urbanisation and gold mining, contributed most to the deterioration of water quality in the KMS. Nutrient enrichment at Site 1, reflecting water quality impacts from the informal settlement of Khuma, was indicated by the high mean values for TOC (9.82 mg/ℓ), Faecal coliforms (3444.46 cfu/100mℓ), NH_4 (22.80 mg/ℓ) and PO_4 (3.19 mg/ℓ). Salinisation at Site 3 reflected mining impacts and was indicated by high mean values for turbidity (40.54 NTU), EC (238 mS/m), Na (258.05 mg/ℓ), Cl (182.76 mg/ℓ) and SO_4 (932.95 mg/ℓ).

In the Vaal River Site 6 was chosen to reflect water quality downstream of the confluence with the KMS and to show any impact on the Vaal River. Descriptive statistics revealed that trace metals contributed most to deteriorating water quality at this site. The impact of trace metals at Site 6 was indicated by the high mean values for Fe (3.39 mg/ℓ), Mn (7.29 mg/ℓ) and As (2.94 mg/ℓ) and was the result of heavy rain experienced during March 2014 that caused a sudden influx of polluted runoff from nearby tailings dumps.

The phytoplankton data confirmed that Cyanophyceae dominated in the KMS, except at Site 3 (canal) that was dominated by Chlorophyceae. This confirmed that Chlorophyceae is more tolerant to saline conditions and that Cyanophyceae is influenced more by the availability of nutrients. In addition, the application of the Shannon-Wiener Diversity Index, Pielou's Species Evenness Index, Margalef's Species Richness Index, and Palmer's Algal Genus Pollution Index to the phytoplankton data, concluded that the KMS is more organically polluted than the Vaal River. Chlorophyceae dominated in the Vaal River, and Cryptophyceae and Dinophyceae were only present in the Vaal River.

Amongst other techniques, the data were subjected to multivariate statistical analysis. The principal component analysis (PCA) did not succeed in reducing the amount of variables, but could be used to explain variability within the data more effectively. Projections of the variables on a component-plane (that plotted the variables captured by the 1st and 3rd principal components), as well as the PCA ordination, were however successful in separating the KMS from the Vaal River, combining both phytoplankton and physico-chemical data. It was also possible to assign individual sites to specific sets of variables that correlated with the descriptive statistics, clearly illustrating that the KMS was mostly impacted by nutrient enrichment (Site 1) and salinity (Site 3), and that Site 6 (after KMS) in the Vaal River was separated from the other sites by grouping it along with the trace metals. Most important, the fact that a clear distinction can be made between the KMS and Vaal River concluded that the KMS does not have a significant impact on the water quality of the Vaal River.

Keywords: water quality, phytoplankton assemblages, physico-chemical variables, multivariate statistical analysis, indices.

OPSOMMING

Midvaalwater Maatskappy, geleë op die oewer van die Vaalrivier in die Noord-Wes Provinsie, voorsien drinkwater aan die Matlosana Munisipaliteit, asook aan myn- en industriële aktiwiteite in die gebied. Water word stroomaf van die samevloeiing van die Koekemoerspruit (KMS) en die Vaalrivier onttrek. Die Koekemoerspruit word beïnvloed deur besoedeling geassosieer met myne en verstedeliking, en die middel-Vaalriviersisteem word moontlik beïnvloed deur hierdie besoedelde water. Dit beklemtoon die behoefte wat ontstaan om die waterkwaliteit van die KMS, asook die invloed van die KMS op die Vaalrivier, te assesseer. Die hoofdoel van hierdie studie was om data oor fitoplanktonbevolkings en fisies-chemiese veranderlikes te integreer, met die doel om sodoende 'n meer akkurate en volledige metode daar te stel om waterkwaliteit te assesseer.

Die beskrywende statistiestiese metodes wat tydens hierdie studie gebruik is, het aangetoon dat voedingstofverryking en versouting, as die direkte gevolge van verstedeliking en goudmyn aktiwiteite, die meeste bygedra het tot die afname in waterkwaliteit van die KMS. Voedingstofverryking by Versamelpunt 1 dui die waterkwaliteitsimpak, afkomstig vanaf die informele nedersetting van Khuma, aan. Dit word weerspieël deur hoë Totale Organiese Koolstof konsentrasies (TOC; 9.82 mg/ℓ), Fekale coliforme (F.coli; 3444.46 cfu/100mℓ), NH_4 (22.80 mg/ℓ) en PO_4 (3.19 mg/ℓ). Versouting by Versamelpunt 3 weerspieël die impak van myne en word gedemonstreer deur hoë waardes vir troebelheid (40.54 NTU), elektriese geleiding (238 mS/m), Na (258.05 mg/ℓ), Cl (182.76 mg/ℓ) en SO_4 (932.95 mg/ℓ).

In die Vaalrivier weerspieël Versamelpunt 6 die waterkwaliteit stroom-af van die samevloeiing van die KMS en dit sal enige impak wat die KMS op die Vaalrivier mag hê, aantoon. Beskrywende statistiek het aangetoon dat spoormetale die hoof bydra gelewer het tot 'n verswakking van die waterkwaliteit by hierdie punt. Die invloed van spoormetale by hierdie versamelpunt word deur hoë Fe (3.39 mg/ℓ), Mn (7.29 mg/ℓ) en As (2.94 mg/ℓ) konsentrasies aangetoon en is waarskynlik die gevolg van swaar reënval gedurende Maart 2014 wat verantwoordelik was vir die invloei van besoedelstowwe vanaf nabygeleë slikdamme.

Die fitoplanktondata het aangedui dat Cyanophyceae dominant was in die KMS, met die uitsondering van Versamelpunt 3 (kanaal), waar Chlorophyceae gedomineer het. Dit bevestig dat Chlorophyceae meer verdraagsaam is teenoor hoë soutgehalte, terwyl die Cyanophyceae hoofsaaklik deur die beskikbaarheid van voedingstowwe beïnvloed word. Verdermeer het die toepassing van die Shannon-Wiener diversiteitsindeks, Pielou se spesie-ewekansigheidsindeks, Margalef se spesierykheidsindeks, asook Palmer se alggenus besoedelingsindeks tot die gevolgtrekking gelei dat die KMS meer organies besoedel is as die Vaalrivier. Rakende die fitoplanktondata van die Vaalrivier, is aangedui dat Chlorophyceae dominant was, terwyl die Cryptophyceae en Dinophyceae slegs in die Vaalrivier teenwoordig was.

Meervoudige statistiese analises is op die data gedoen. Hoofkomponentanalises ("principal component analysis", PCA) het nie daarin geslaag om die aantal veranderlikes te verminder nie, maar dit kon gebruik word om variasie in die data aan te toon. Die voorstelling van veranderlikes op die komponentvlak ('n eendimensionele rangskikking van veranderlikes wat vasgevang is deur die eerste en derde hoofkomponente), sowel as die PCA-ordinasie, was suksesvol om die KMS van die Vaalrivier te onderskei deur die fitoplankton en fisies-chemiese data te kombineer. Dit was ook moontlik om individuele versamelpunte te koppel aan spesifieke groeperings van veranderlikes. Dit het duidelik geïllustreer dat voedingsstofverryking (Versamelpunt 1) en soutgehalte (Versamelpunt 3) die grootste invloed gehad het op die KMS, terwyl Versamelpunt 6 (na die KMS) in die Vaalrivier geskei was van die ander versamelpute, deurdat dit saam met die spoormetale gegroepeer was. Baie belangrik is dat daar 'n duidelike onderskeid getref kon word tussen die KMS en die Vaalrivier, waaruit die gevolgtrekking kon gemaak word dat die KMS nie 'n noemenswaardige invloed gehad het op die waterkwaliteit van die Vaalrivier nie.

Sleutelwoorde: waterkwaliteit, fitoplanktonbevolkings, fisies-chemiese veranderlikes, meervoudige statistiese analises, indekse.

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"*2 When you pass through the waters, I will be with you;*
and through the rivers, they shall not overwhelm you;
when you walk through fire you shall not be burned,
and the flame shall not consume you.
3 For I am the LORD your God,
the Holy One of Israel, your Saviour.
I give Egypt as your ransom,
Cush and Seba in exchange for you.
4 Because you are precious in my eyes,
and honoured, and I love you,
I give men in return for you,
peoples in exchange for your life."

Isaiah 43: 2-4.

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ACRONYMS AND SHORT FORMS

AMD	Acid Mine Drainage
APDI	Artois-Picardie Diatom Index
BDI	Biological Diatom Index
COM	City of Matlosana
DWA	Department of Water Affairs
EC	Electrical Conductivity
FRAI	Fish Response Assessment Index
GDI	Generic Diatom Index
HBF	Hartebeesfontein
IDWG-LUP	Interdepartmental Working Group on Land Use Planning
IHI	Index of Habitat Integrity
ISO	Internation Organization for Standardization
KMS	Koekemoerspruit
KOSH	Klerksdorp, Orkney, Stilfontein, Hartebeesfontein
MIRAI	Macro-invertebrate Response Assessment Index
MWC	Midvaal Water Company
NTU	Nephelometric Turbidity Units
NWU	North-West University
PCA	Principal Component Analysis
RHP	River Health Programme
SANAS	South African National Accreditation System
SANS	South African National Standards
SASS	South African Scoring System

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

SD	Standard Deviation
SE	Standard Error
SMD	Saline Mine Drainage
T.Chl	Total Chlorophyll
TDI	Trophic Diatom Index
TDS	Total Dissolved Salts
TOC	Total Organic Carbon
USEPA	United States Environmental Protection Agency
VEGRAI	Riparian Vegetation Response Assessment Index
VMR	Village Main Reef
WHO	World Health Organisation
WMA	Water Management Area
WWTP	Waste Water Treatment Plant
WWTW	Wastewater Treatment Works

CHAPTER 1: INTRODUCTION

Water is an essential resource required to sustain life and critical to humans for potable purposes. Industries such as agriculture, manufacturing and mining also depend on freshwater resources to remain operational and economically viable, placing water resources under enormous pressure (Ragush, 2011). Ironically, it is industries like these that often pollute freshwater resources (Nkwonta & Ochieng, 2009). South Africa, a semi-arid developing country (Janse van Vuuren & Pieterse, 2004) is no exception, with freshwater resources also being our most limiting natural commodity (Oosthuizen, 2012). South Africa is currently experiencing water shortages and headings such as "*Water restrictions in Pretoria as heatwave causes water shortage*" (Shange, 2015), "*Cape Town facing summer water restrictions*" (Petersen, 2015) and "*Water restrictions imposed on parts of Kwa-Zulu Natal*" (Hartleb, 2015) frequents the local news. Hence, the monitoring and protection of finite freshwater resources currently enjoy increasing interest on both a local and global scale (Ragush, 2011).

Midvaal Water Company is a water service provider that supplies potable water to the Greater Municipality of Matlosana (Klerksdorp), as well as the mining and industrial undertakings in the area. It is situated 15 km from Stilfontein in the North West Province on the banks of the Vaal River and abstracts water downstream from the confluence of the KMS and the Vaal River (Anon, 2011). This emphasised the need to assess the water quality of the KMS, as well as the receiving middle-Vaal River system.

According to Spangenberg (2000) there are four major gold mines operating in the Klerksdorp area, namely Stilfontein, Hartebeesfontein, Buffelsfontein and Vaal Reefs. The towns of Orkney, Stilfontein, Potchefstroom and the rural settlement of Khuma are situated in the vicinity. Within this region, the Koekemoerspruit (KMS) represents an affected (mining- and urbanisation associated pollution) water resource and the middle-Vaal River system the receiving water body (Spangenberg, 2000). The KMS is a non-perennial stream with a total catchment area of approximately 860 km^2 (Winde & Van der Walt, 2004). The area is not only affected by mining and municipal development, but also agriculture (Spangenberg, 2000).

According to Chapman (1996), water quality assessment may only be achieved through the appropriate monitoring of three critical components, namely hydrology, physico-chemistry, and biology. Olguin *et al.* (2004) stated the following: “Physical and chemical analyses, bioassays, and bio-assessments may detect, each one, essential effects which the other may fail to reveal”. Yet, due to a lack of funding and skilled taxonomists (phycologists), the majority of data analyses for water quality assessment fails to properly integrate both the physico-chemical and biological data (Olguin *et al*., 2004).

The main aim of this study is to integrate results from two key assessment approaches in the KMS and Vaal River, hypothesising that the integrated use of phytoplankton assemblages and water physico-chemistry is a more accurate and comprehensive means of assessing water quality. The following are objectives for this study:

- To describe the different land-use practices at each study site.
- To determine changes in phytoplankton assemblages and water physico-chemistry over a 24 month study period.
- To compare differences between phytoplankton assemblages and water physico-chemistry at each site and correlate each to the various land-use practices.
- To properly integrate and interpret the two sets of data, using multivariate statistical analysis.
- To confirm that the integrated use of source water management practices could determine the influence of the KMS on the water quality of the Vaal River.

CHAPTER 2: LITERATURE REVIEW

The Vaal River is one of South Africa's main sources of freshwater and plays a vital role in the country's economic growth as it sustains a population of about 12 million people (DWA, 2009). The Vaal River system is recognised as South Africa's primary drainage system and consists of four water management areas (WMA's): Upper Vaal, Middle Vaal, Lower Vaal and partially the Upper Orange. Increased development within these WMA's has led to deteriorating source water quality. These four WMA's form part of an inter-correlated network of draining systems, emphasising the need to develop and implement a source water management plan. The aim of this management plan would be to meet the specific water requirements within each WMA without compromising water transfer requirements between WMA's, and the quality of water being distributed for various uses (DWA, 2009).

There is, however, a series of challenges associated with the development of such a management plan. The most important challenge is to obtain a comprehensive understanding of the existing water quality status for each of the WMA's within the primary drainage system, as well as the processes responsible for altering source water quality (DWA, 2009).

The latter represents the second of three sequential building blocks or processes in assembling a sustainable source water management plan. The three processes (in this order) are monitoring, assessment and management. Water quality monitoring can be defined as "*the actual collection of information at set locations and at regular intervals in order to provide the data which may be used to define current conditions and establish trends*" (Chapman, 1996), whereas water quality assessment can be defined as "*the overall process of evaluation of the physical, chemical and biological nature of water in relation to natural quality, human effects and intended uses, particularly uses which may affect human health and the health of the aquatic system itself*" (Chapman, 1996). In other words, certain attributes of the water of a specified area are first monitored over a given period of time to determine the water quality status. The observations made through monitoring are then assessed to identify tendencies for determining "cause and effect" interactions. Part of the assessment process is to interpret the results obtained from monitoring and finally to propose a proper source water management plan (Chapman, 1996).

As mentioned in CHAPTER 1, the aim of this study was to assess the surface water quality of the KMS catchment, specifically because it was identified as one of the principal contributing components in terms of deteriorating water quality of the Middle Vaal MWA, and ultimately then also the primary drainage system of South Africa, the Vaal River (DWA, 2006a; Winde & Van der Walt, 2004). Thus, through the processes of monitoring and assessment, this study attempts to obtain a comprehensive understanding of the existing water quality status for one of the catchments within the primary drainage system, as well as the processes responsible for altering the catchments' water quality. The aim of this chapter is therefore to review some of the most widely used means for monitoring and assessing surface water quality, and the processes influencing it.

2.1 SOURCE WATER QUALITY ASSESSMENT PRACTICES

Chapman (1996) states that a simplified definition for water quality proves difficult, due to the vast amount of contributing factors and variables to consider when the status of water quality is determined. In addition, one should also be aware that defining the term water quality in terms of status (good or bad water quality) will vary according to the water user. For example, what one may consider as "decent" water quality from a human perspective (water suitable for drinking and industrial uses), will not necessarily be considered "decent" water quality for aquatic inhabitants (Dallas & Day, 2004).

For the purpose of this study the following definition of water quality seemed to be most suitable: "*The chemical, physical and biological characteristics of water, usually in respect to its suitability for a designated use*" (Daniels *et al*., 2007). Although this definition for water quality appeared to be the most comprehensive it seems to lack a very important aspect of water quality, namely its adherence to approved water quality guidelines. According to Carr and Neary (2008), water quality can only be determined through comparisons between the true physico-chemical and biological attributes of water, and the limits set for these characteristics by water quality guidelines or standards for specific uses. An example of these standards is the South African National Standards (SANS). Thus, for possible future reference the definition will be rephrased as follows: *The physico-chemical and biological characteristics of water, with respect to its suitability for a specific use, as clearly stipulated by either national or international water quality guidelines or standards.*

According to Chapman (1996), there are multiple ways for assessing water quality of a specific aquatic environment, which can be classified as either quantitative or qualitative. Quantitative measurements would typically be represented by the physico-chemical characteristics of the water, whereas the use of biological indices or species composition would represent a qualitative approach. Since the primary components for characterising any water body include the hydrology, physico-chemical properties as well as biotic properties, the accurate assessment of water quality could not be executed without the consideration of all three of these critical components. Carr and Neary (2008) also state that the constant monitoring and assessment of an aquatic ecosystems' physico-chemical and biological state is the only means to allow for the early detection of often irreversible ecosystem deterioration.

Literature indicates that the physico-chemical components used for water quality status assessment usually consist of the same set of key variables. Examples include Li *et al*. (2011), Mihulka (2011) and Ragush (2011), all of which monitored pH, electrical conductivity (EC) and turbidity (NTU). Depending on the aim of each study, as well as the land-use practices impacting on the study area, some nutrients, trace metals and bacteriological indicators may be monitored as well. In contrast, a wide variety of biological components can be used to assess water quality status. The use of these biological components to assess the status of environmental quality is known as bio-monitoring (DWA, 2008). Markert *et al.* (1999) define the process of bio-monitoring as follows: "*Bio-monitoring is a method of observing the impact of external factors on ecosystems and their development over a long period of ascertaining differences between one location and another*".

The South African River Health Programme (RHP) is a monitoring program that was developed for the purpose of assessing the ecological state of the nations' rivers. This initiative mainly makes use of bio-monitoring techniques to achieve this goal, arguing that the condition of the organisms inhabiting a river, represents a direct and comprehensive suggestion of the health of the entire stretch of river. The RHP usually employs one of the following biological indices: The Diatom Index, Macro-invertebrates or the South African Scoring System (SASS) and Macro-invertebrate Response Assessment Index (MIRAI), Fish Response Assessment Index (FRAI), Riparian Vegetation Response Assessment Index (VEGRAI) and the Index of Habitat Integrity (IHI) (DWA, 2008).

2.1.1 PHYSICO-CHEMICAL ANALYSIS & KEY VARIABLES

Physico-chemical variables are also commonly expressed as environmental variables (Oosthuizen, 2012). Though these variables are often categorised into two groups, they are almost always interpreted and discussed concurrently (Junshum *et al*., 2008; Ramakrishnan, 2003). The first group contains physical attributes which usually include temperature, turbidity, electrical conductivity and colour. The second group contains the chemical components such as the total dissolved solids, pH and a variety of different trace metals and ions (Dallas & Day, 2004). Some of these chemical constituents, including a number of trace metals, may be toxic, whilst others are not particularly detrimental at low concentrations (Dallas & Day, 2004; Van Loon & Duffy, 2005). The key physico-chemical variables that formed an integral part of the source water quality assessment for this study are listed in CHAPTER 4, Table 4.1, and will be briefly discussed in this section.

Turbidity & Colour

Ziegler (2002) defines turbidity as "*the decreased clarity of a solution due to the presence of both suspended and, to a lesser extent, dissolved substances, causing the light entering the solution to either be dispersed or absorbed*". He further states that increased light dispersal is coupled with increased turbidity values, expressed as nephelometric turbidity units (NTU). Colour and turbidity are closely related, because both are influenced by the same constituents and can visually be observed, making them two of the most apparent physical water quality variables (Dallas & Day, 2004).

According to DWA (1996), the main contributing components of turbidity and colour include the following: **dissolved organic substances** of which some of the organic acids may result in discolouration of the water, **dissolved inorganic substances** such as ions and minerals (though their contribution to turbidity and colour is minimal), **suspended organic substances** such as pollen or phytoplankton that, depending on the fluorescence and refractive index of the dominant component, may contribute to both turbidity and colour, and **suspended inorganic substances** resulting from the underlying and surrounding geomorphology that also, depending on the fluorescence and refractive index, may contribute to both turbidity and colour.

Though the natural and seasonal alternations in environmental conditions may cause fluctuations in turbidity, a rapid increase in turbidity, often due to anthropogenic inputs, may negatively impact aquatic ecology (Dallas & Day, 2004). The severity of the impact will vary according to the kind and duration of source contribution (Wood & Armitage, 1997). For example, when the contribution is chronic and significant in volume (see discussion under section 2.1.2), chances are that the aquatic ecosystem will undergo rapid and sometimes irreversible changes (Wood & Armitage, 1997). Phytoplankton communities, as a major part of the primary producers, are usually the first biological indicators of elevated turbidity due to decreased photosynthesis. Successive biological communities will ultimately be affected as well (Davies-Colley & Close, 1990).

Anthropogenic contributors of turbidity can be divided into either point- or diffuse sources. Point sources include treated or untreated sewage effluent, as well as mine– and industrial effluents. Diffuse sources include municipal and industrial solid waste disposal sites, as well as agricultural and urban runoff (Chapman, 1996).

pH

According to Dallas and Day (2004), the hydrogen (H^+), hydroxyl (OH^-), bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions determine the pH of water. Naturally occurring freshwater usually has a neutral pH of 6 to 8, and if the pH drastically deviate from this value, it could not only indicate a possible source of pollution, but will also change the availability and toxicity of a variety of other chemical water quality constituents, especially some of the trace metals (Van Loon & Duffy, 2005). Huizenga (2011) determined that the pH of surface water in South Africa typically ranges between 8 and 8.5 due to the weathering of certain bedrock that releases bicarbonate. Metals such as aluminium (Al), copper (Cu), manganese (Mn) and zinc (Zn), as well as non-metallic ions such as cyanide (CN^-), seems to be most affected by lowered pH levels. For instance, both CN^- and Al are harmless under relatively alkaline conditions while gradual acidification of water will result in them becoming toxic. To the contrary, other ions like ammonium (NH_4^+) are adversely altered by elevated pH levels, rendering them toxic as well (Campbell & Tessier, 1987). Thus, introducing pollutants to the water which may alter the pH, could proliferate the toxicity of otherwise harmless chemical elements (Dallas & Day, 2004). Furthermore, the adsorption of nutrients such as phosphates (PO_4^{-3}), trace metals (Al, Cu, Mn and Zn) and other constituents of biocides to components of turbidity, are impaired by fluctuation in pH levels, since the pH levels determine the electrical charge of the molecules (Dallas & Day, 2004).

According to Peng *et al.* (2009), pH is fundamentally responsible for trace metal transferences between surface water and sediments and different metals become mobile at different pH levels, called the limit pH. For example, the limit pH for some metals used as chemical variables in this study is: Zn = pH 6–6.5, arsenic (As) = pH 5.5–6, Cu = pH 4.5, Al = pH 2.5, and iron (Fe) = pH 2.5 (Peng *et al.,* 2009).

Anthropogenic causes for pH fluctuations in freshwater sources include industrial effluent inputs, mine drainage effluent inputs and air pollution, that result in acid precipitation. The latter results in acidification, with alkaline inputs being a less common occurrence. Pollution sources causing alkalinisation of freshwater include specific industrial effluent inputs and is mostly associated with urban and agricultural runoff with a high salt or organic content (Dallas & Day, 2004).

Electrical Conductivity (EC)

Chapman (1996) defines electrical conductivity as *a means to measure the ability of water to conduct an electrical current in units of mili-siemens per metre (mS/m), of which the capacity is determined by the concentrations of dissolved organic and inorganic substances present in the water*. By this definition, it is logical that pH and EC would be closely related.

Of the dissolved substances, inorganic ions and minerals are the most significant contributing constituents. These include cations such as sodium (Na^+) and calcium (Ca^{2+}), anions such as chloride (Cl^-) and sulphate (SO_4^{2-}), nutrients such as nitrate (NO_3^-) and PO_4^{3-} and some trace metals (Zn, Cu, Al and Fe). The main reason why dissolved organic ions are less abundant than dissolved inorganic ions, is because the pH levels often determine whether organic constituents, like humic and fulvic acids (the by-products of decaying plant material), are ionised or not (Van Loon & Duffy, 2005). The reason for mentioning this is because EC is only representative of the ionised constituents present in water, which is then ultimately determined by pH (Dallas & Day, 2004). In addition, the term salinity refers to the saltiness or concentration of ions (particularly that of Cl^-) present in water and is measured as EC. In other words, EC is also a direct reflection of the salinity of water (Dallas & Day, 2004). Most freshwater sources reflects EC values between 10 to 1,000 mS/m, therefore EC values exceeding this maximum usually indicate some type of polluted input, such as decanted mine water, or urban, agricultural or industrial runoff (Chapman, 1996).

Major Ions, Nutrients, Trace metals and Biological and Bacteriological indicators

Dallas & Day (2004) state that although the major chemical constituents that ultimately determine turbidity, pH and EC are measured as such, the results derived from these variables could at most speculate which of the individual chemical constituents makes the biggest contribution. Thus, by also monitoring and assessing the concentrations of some of the individual chemical constituents, a more specified and accurate assumption can be made concerning the source water quality and sources of pollution.

Major Ions

Almost all freshwater sources contain **Na+** and **Cl^-** as they are essential ions required by aquatic biota (Chapman, 1996; Dallas & Day, 2004). The occurrence of Cl^- is especially common in South African freshwater sources (Dallas & Day, 2004). Elevated levels of Na^+ and Cl^- may indicate anthropogenic inputs, derived from sewage or industrial effluent, and are usually monitored in water allocated for potable purposes, livestock watering and irrigation. The association between elevated Cl^-, Na^+ and sewage effluent also makes these ions suitable indicators for possible faecal pollution that can indicate the total affected area of contamination (Chapman, 1996).

SO_4^{2-} is an ionic form of sulphur and an essential element required for aquatic biota. In moderate concentrations, SO_4^{2-} is completely harmless, though sulphuric acids may form when high concentrations of SO_4^{2-} prevail. This would result in declining pH levels as found in mine water seepage or mine shaft decanting, as well as inputs of industrial effluent (Chapman, 1996; Dallas & Day, 2004).

CN^- can occur in a variety of forms in freshwater. It can either be in ionic form (as a hydrocyanic acid), or as part of intricate complexes with metals. The ionic and acidic forms are the most toxic, however CN^- can also form unstable complexes with metals like Zn, lead (Pb) and cadmium (Cd), which are extremely toxic. Complexes formed between CN^- and Cu^{2+} is less toxic. The main source of CN^- in freshwater seems to be industrial effluent, such as effluent from electroplating industries. Source water used for potable purposes is meticulously monitored for traces of CN^-, due to its highly toxic nature (Chapman, 1996).

Nutrients

According to Ragush (2011), carbon, nitrogen and phosphorus represent the three major nutrients usually monitored when studying environmental systems. He further states that the stability and speciation of these particular nutrients within a given environmental system, usually serves as a good indication of ecosystem health.

Similar to Na^+ and SO_4^{2-}, **nitrogen** and **phosphorus** are also essential elements required by all aquatic biota. Phytoplankton assimilates inorganic forms of nitrogen and phosphorus, and converts it to organic, unavailable nitrogen and phosphorus (Chapman, 1996). According to Camargo and Alonso (2006) and Rabalais (2002), the dissolved ionic, inorganic forms of nitrogen most readily found in freshwater include ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-). On the other hand, phosphorus is most readily found in freshwater as soluble inorganic phosphorus or orthophosphates (PO_4^{3-}), polyphosphates, and organic phosphates (Chapman, 1996).

NH_4^+ concentrations, as well as the availability of inorganic ammonia (NH_3) in freshwater, strongly depend on pH levels. Elevated pH levels (alkalinisation) cause NH_3 to become mobilised and, at certain high concentrations, it can be toxic to aquatic biota. NH_4^+ shows a negative correlation to pH. In other words, the acidification of freshwater would favour the availability of NH_4^+, whereas the opposite is true for NH_3. Typical concentrations of NH_4 NH_4^+ measured in pristine freshwater sources range between 0.2 - 3 mg/ℓ (Chapman, 1996).

NO_3^- is usually the dominant form of nitrogen found in freshwater, aerobic aquatic systems (Chapman, 1996), due to its highly mobile, soluble and stable nature (Ragush, 2011). In pristine freshwater, NO_3^- concentrations rarely exceeds 0.1 mg/ℓ (Chapman, 1996). Values exceeding this concentration are usually indicative of organic pollution (Chapman, 1996). Just like NH_4^+, **PO_4^{-3}** concentrations are determined by pH, and correlates positively to an increase in pH. In surface water, unaffected by organic pollution, PO_4^{-3} concentrations rarely surpass 0.02 mg/ℓ (Chapman, 1996). In freshwater PO_4^{-3}, and to a lesser extent NO_3^-, are considered to be the two principal limiting nutrients for phytoplankton growth, and as such, are extremely important indicators for eutrophication (Chapman, 1996).

Anthropogenic sources of inorganic nitrogen and phosphorus in freshwater systems include wastewater from livestock, sewage and industrial effluent, agricultural runoff (loaded with inorganic nitrogen present in various fertilisers), urban runoff, as well as runoff and seepage from closed mines and mine shafts (modified from Camargo & Alonso, 2006; Chapman, 1996; Dallas & Day, 2004; Rabalais, 2002).

Total organic carbon (TOC) is described by Chapman (1996) as a measure of the content of organic matter in water. TOC consists of both dissolved and particulate carbon, and in pristine surface waters, TOC seldom exceeds a total concentration of 10 mg/ℓ (Chapman, 1996) and it generally reflects the biological processes associated with the stream biota. Elevated concentrations of TOC in surface water usually indicate polluted inputs, such as sewage and industrial effluent or urban runoff, where concentrations sometimes exceed 100 mg/ℓ (Chapman, 1996). Ragush (2011) states that the presence of excessive amounts of organic carbon in source water bodies is problematic, as chlorine dosing (a common disinfectant in the water purification process) may result in the formation of trihalomethanes, which are carcinogenic.

Biological and Bacteriological indicators

Total chlorophyll (T.Chl), measured in µg/ℓ or mg/ℓ, is an indirect estimate of the phytoplankton biomass present in water, since chlorophyll pigments are present in all photosynthetic aquatic flora. Indeed, T.Chl is capable of reflecting the nutrient load of freshwater, as well as the trophic status of the water body. T.Chl is always monitored in water allocated towards potable purposes, since excessive problematic phytoplankton (otherwise known as harmful algal blooms) could require adaptations in the water purification process, to rid the water of aesthetically displeasing tastes, smells and odours, as well as toxins associated with these harmful algal blooms (Chapman, 1996). (See discussion on harmful algal blooms under section 2.1.2)

Faecal coliforms (F.coli) represent bacterial species (including *Escherichia coli*) that are part of a bacterial family known as the Enterobacteriaceae. These bacteria species form a major part of human and other warm-blooded animals' intestinal microbial communities, and as such, have gained immense status as indicator species of faecal contamination (Willey *et al*., 2008). According to Dallas and Day (2004), the presence of Faecal coliforms may be indicative of organic pollution in the form of untreated sewage or animal manure.

Davies and Day (1998) state that the likelihood for South African rivers to be polluted by untreated sewage is a matter of concern, especially considering the amount of informal settlements lacking basic potable water facilities. Most Faecal coliform bacterial species are not pathogenic, although they do indicate the possible presence of pathogens, which can be transferred to humans through the consumption of contaminated water (Larsen *et al*., 1994).

Trace metals

Environmental literature commonly refers to the term 'heavy metals', that are often discussed in context of their toxic effects. Although the term was formerly used to refer to metals, such as lead (Pb) and mercury (Hg), with comparatively high atomic mass and specific gravities, it seemed to be increasingly applied scientifically incorrectly to metals such as Al (with a low atomic mass and specific gravity), and even semi-metals like As. Therefore, since metals in freshwater are usually present in moderate concentrations, they will collectively be referred to as 'trace metals' (Van Loon & Duffy, 2005).

Trace metals are frequent environmental pollutant constituents, as they are cosmopolitan and freely soluble in water. Most trace metals, in their dissolved form, are easily assimilated by aquatic biota as essential components necessary for metabolic processes. Unfortunately, in high concentrations, a variety of trace metals can become toxic as they ultimately alter protein structures and disrupt functional enzyme activity (Chakraborty *et al.,* 2010). The speciation of trace metals in freshwater is principally determined by ionic strength, pH (as mentioned earlier), as well as the redox (E_h) status (Coetzee *et al*., 2006; Van Loon & Duffy, 2005). In this section, the focus will mainly be on pH and its role in the distribution of trace metals in freshwater, because pH was included as one of the physico-chemical variables measured during this study.

Fe is an essential trace metal required by all aquatic biota, especially as a component of the respiratory pigments in phytoplankton (Dallas & Day, 2004). Fe concentrations in pristine freshwater rarely surpasses 0.1 mg/ℓ (Xing & Liu, 2011), however elevated Fe levels may indicate inputs of effluents characterised by low pH levels. Such effluent is commonly derived from mining activities in the form of acid mine drainage (AMD) (Huizenga, 2011), waste water treatment plants (WWTP's) and agricultural runoff (present in many pesticides and fertilisers) (Phippen *et al.,* 2008).

Mn is a common and essential trace metal required by aquatic biota as an important component of enzymes (Dallas & Day, 2004; Peters *et al.,* 2010). Mn concentrations in pristine freshwater rarely surpasses 1 mg/ℓ and often occur in concentrations less than 0.2 mg/ℓ (Reimer, 1999). The solubility of Mn is promoted by the acidification of freshwater, as well as the presence of Cl, SO_4 and NO_3, and it most commonly occur as Mn^{2+} or Mn^{4+} ions. In contrast, elevated pH levels promote the formation of organic compounds with Mn, which are more readily assimilated by aquatic flora (Reimer, 1999). According to Peters *et al.* (2010), literature indicates that high concentrations of Mn^{2+} ions are the most likely cause of toxic effects in freshwater biota and that increased concentrations of H^+ and Ca^{2+} may counteract this effect. Anthropogenic sources of Mn include effluent from WWTP's, sewage effluent or sludge, as well as industrial effluent from steel productions and coal mining activities (Reimer, 1999).

As with Mn, both **Zn** and **Cu** are vital trace metals, present in numerous enzymes of all biota (Dallas & Day, 2004). Zn concentrations in freshwater, unaffected by pollution, seldom exceed 0.01 mg/ℓ (WHO, 2003a), and is most commonly derived from the surrounding geomorphology. Cu concentrations in freshwater, unaffected by pollution, seldom exceed 0.03 mg/ℓ, though in surface water affected by anthropogenic inputs, Cu concentrations may range from 0.1 mg/ℓ to 200 mg/ℓ, especially in mining areas (USEPA, 2007). The most common anthropogenic sources of dissolved Zn and Cu include mining, WWTP's and steel manufacturing effluent, as well as urban runoff (Pistelok & Galas, 1999; USEPA, 2007). Elevated concentrations of Zn and Cu present in freshwater can be toxic to aquatic biota and, according to Chapman (1996), the level of toxicity is mainly determined by the hardness (Ca and magnesium (Mg) content) of the water.

Al is not an essential element for biological processes and is one of the trace metals with potential for exerting toxic effects. As mentioned earlier, the toxic nature of Al is mainly determined by pH (Dallas & Day, 2004). Al in pristine freshwater sources (within the pH range 5.5–6.0) is less soluble and concentrations usually range between 0.001 to 0.05 mg/ℓ (Dallas & Day, 2004). Freshwater sources affected by the decanting of mine water are typically more acidic, resulting in the mobilisation of dissolved Al (Huizenga, 2011) and it has been found that Al concentrations in such situations may reach values of up to 90 mg/ℓ (WHO, 2003b). The occurrence of Al in freshwater sources can be expected since it is one of the most abundant trace metals present in geomorphological components.

In addition, anthropogenic activities may contribute significantly to elevated Al concentrations in source water. $Al_2(SO_4)_3$ is, for example, commonly used as a flocculent in the water purification process and may increase the Al loading in freshwater systems through WWTP effluent (Butcher, 1988; WHO, 2003b).

Similar to Al, some species of arsenic (**As**) can be exceptionally toxic, in particular the two forms most abundant in freshwater, namely the inorganic As(III) and As(V) species (Dallas & Day, 2004; Rahman & Hasegawa, 2012). Arsenic is naturally present in freshwater, as it is a common trace element found in geological components (Rahman & Hasegawa, 2012).

Numerous studies have indicated that anthropogenic activities also contribute to elevated arsenic concentrations in freshwater systems. These include mine and sewage effluents, as well as agricultural runoff where many pesticides and herbicides contain arsenic (Dallas & Day, 2004; Morin & Calas, 2006). Arsenic concentrations in lentic freshwater bodies normally don't exceed 0.01 mg/ℓ (Rahman & Hasegawa, 2012) and concentrations exceeding this value are particularly alarming when the water is used for potable purposes, seeing that arsenic is a well known carcinogen (Dallas & Day, 2004).

Uranium (**U**) is one of the trace metals causing an enormous threat to the health of aquatic ecosystems, as well as humans, due to its radioactive nature (Dallas & Day, 2004). It is fairly common for low concentrations of U to be present in natural freshwater, as it is a constituent of various geomorphological surroundings. Anthropogenic activities may cause surface water to be contaminated, thus elevating the overall U concentration (Small *et al*., 2008; Wade *et al*., 2004). According to Van Eeden *et al*. (2009), U is the most notorious contaminant emanated through gold-mining activities, and besides being toxic and radioactive, it also has an exceptionally long half-life, which implicates long-term ecological effects. Though U in surface water usually occur as dissolved UO_2^{2+}, the speciation of U in freshwater is fairly complex and strongly depends on the pH levels (Wade *et al*., 2004). SANS (2015) sets the operational limit for U concentrations at ≤ 30 µg/ℓ, and also indicate that long-term intake of potable water exceeding this value would result in chronic health implications.

NOTE: Van Loon and Duffy (2005) contain a table on the principal environmentally important aqueous trace metal species under various pH and redox conditions. Also, in the above discussion on arsenic (As), the component's name was written out in full, even after the acronym was provided, to facilitate reading that particular section.

2.1.2 QUANTITATIVE PHYTOPLANKTON ANALYSIS AND CONCEPTS OF BIOMONITORING

Naturally, all biological entities within an ecosystem are impacted upon by stress factors, ranging from fluctuations within the physical environment (climate change), stress induced between biological units (e.g. predation) or even within a single biological unit (competition for food and suitable mating partners). Although a negative connotation is made to the term “stress”, from an ecological point of view, natural stress factors are essential driving forces behind the advancement of individual species and ecosystems, reflected through their ability to react and adapt to such stressors. However, these natural evolutionary adaptations usually occur naturally in response to prolonged and constant exposure to stress factors (Markert *et al*., 2003). Unfortunately, the concept of ecological stress has regained its negative connotation over the late centuries.

Increasing anthropogenic development introduces entirely different stress factors, with regards to both the nature and quantitative input of these factors (Markert *et al*., 2003). Many of these anthropogenic stressors were discussed under section 2.1.1 of this chapter and include a variety of ions, nutrients and trace metals. More than often, the sudden exposure of the environment to great quantities of these substances is met by the inability to react and adapt to such stressors within a short period of time. This phenomenon also brought about the new ecological concept of tolerance, whereby the biological species which survive such stress, is considered to possess over a tolerance range, accommodating the particular set of substances and certain quantities thereof (Markert *et al*., 2003).

Sharov (2008), states that pristine freshwater ecosystems, unaltered by anthropogenic inputs, are nowadays challenging to find. In addition, Li *et al*. (2010) state that globally, lotic freshwater bodies, in particular, are increasingly gaining status as endangered ecosystems. With this realisation came the growing need to develop practical, yet comprehensive ways to assess not only the given state of these freshwater systems, but also the rate of ecosystem degradation.

At first, the most common means for monitoring freshwater systems were based on the analysis of chemical constituents (Friberg, *et al*., 2011). Later, the additional use of bacteriological components gained preference, due to the health risks contaminated freshwater posed for humans. However, in lotic freshwater bodies, the use of these variables alone proved to be inadequate (Friberg, *et al*., 2011; Li *et al*., 2010).

The physico-chemical analysis of freshwater, sampled from a river or stream, would only reflect a given moment in time and in principle, won't be indicative of long-term impacts on the system or the integrated effect of several stress factors. Because water is essential for all living organisms, the quality thereof can only be determined by assessing the organisms that inhabit it (Sharov, 2008). Furthermore, because these organisms integrate their responses over time and space, using them could result in lowered sampling frequencies as well as costs, compared to the often high sampling frequencies required when depending on chemical properties alone to identify assured impacts (Friberg, *et al*., 2011). Therefore, bio-monitoring proved to be a crucial addition to the "old-fashioned" freshwater monitoring methodologies (Li *et al*., 2010).

Li *et al*. (2010) define bio-monitoring as "*the systematic use of living organisms or their responses, to determine the current condition or changes of the environment*". These living organisms are called bio-indicators. Markert *et al*. (2003) distinguish between two types of bio-indicators. The first type are organisms (or a community of organisms) that reflect the existing quality of the environment in which they occur, and is simply called bio-indicators. The second type, known as bio-monitors, are defined by Markert *et al*. (2003) as "*organisms (or a part of an organism or a community of organisms) that contains information on the quantitative aspects of the quality of the environment*" and are naturally always bio-indicators as well.

Markert *et al*. (2003) explain that one should also distinguish between two types of bio-monitoring. Active bio-monitoring makes use of laboratory cultured organisms which are exposed to known concentrations of a certain toxin for a pre-determined period, after which their reactions are recorded. During passive bio-monitoring, the reactions of organisms are studied within their natural environment. Passive bio-monitoring is considered to be much more comprehensive compared to active bio-monitoring, because it is extremely challenging to mimic the natural environment without neglecting any of a number of constantly fluctuating stress factors, characteristic of especially lotic freshwater systems (Friberg, *et al*., 2011; Markert *et al*., 2003). Therefore, the definition for bio-monitoring, given by Markert *et al*. (1999; 2003) and used in section 2.1 of this study, seems to relate more to what they describe as passive bio-monitoring. That definition also represents the methodological procedures followed during this study: "*a method of observing the impact of external factors on ecosystems and their development over a period, or of ascertaining differences between one location and another*."

According to Li *et al*. (2010), bio-monitors can be selected from all biological units of an ecosystem, for example individual species, populations or communities. Even though initial bio-monitoring trends seemed to favour the use of higher levels of biological organisation (Li *et al*., 2010), the twentieth century brought about a new trend of bio-monitoring with the use of microscopic organisms (such as phytoplankton, fungi and protozoa) (Bonada *et al*., 2006), as well as benthic macro-invertebrates and fish (Friberg, *et al*., 2011; Li *et al*., 2010). Friberg, *et al*. (2011) consider the use of these distinct taxonomic groups as "*the single most significant leap forward in bio-monitoring*", because it ultimately initiated the development of biotic indices and major advancements in ecologically applied statistical analysis.

Although numerous methods using fish, phytoplankton and macro-invertebrates have been available to bio-assess the integrity of aquatic environments, bio-monitoring was only implemented to manage South African freshwater systems during 1996 (De la Rey *et al*., 2004). During this time, benthic macro-invertebrates were regarded as valuable bio-monitors, because they are macroscopic (and thus easy to identify), they have rapid life cycles, as well as the relative ease of sampling (due to their sedentary nature) and identification. This set of characteristics lead to the development of SASS (South African Scoring System) in 1998, a macro-invertebrate index, which formed an integral component of the South African River Health Programme (RHP) until recently (see section 2.1) (De la Rey *et al*., 2004).

With increased development and use of indices, based on macro-invertebrates and aquatic animals in general, also came the realisation that these organisms may not be as suitable for use in indices as first thought. Round (1991) provides a list of these shortcomings, amongst others that most aquatic animals have season-bound life cycles (although rapid), they are motile at least to some extent (which means that they are able to leave hostile environments), they may undergo metamorphosis (that complicates the identification process), they are habitat-specific and their distribution is dependent on stream-flow conditions (which also makes it difficult to sample in deep and fast flowing stretches of the river).

Research done by Li *et al*. (2010) suggest that as a result, the use of phytoplankton, especially the diatoms or Bacillariophyceae (Prygiel *et al*., 1999) then became preferential as bio-monitors in rivers. Freshwater phytoplankton is well known for their susceptibility to pollution, that is usually analysed in conjunction with the physico-chemical properties of the water (Sharov, 2008).

Their high rate of reproduction and relatively short life cycles, allow them to respond to and also reflect abrupt habitat alterations caused by anthropogenic activities (Li *et al*., 2010; Sharov, 2008). Wu *et al*. (2014) also state that phytoplankton communities (unlike fish or macro-invertebrates) are usually present before, during and after habitat alterations. This also contributed to the increased use of these organisms to assess the quality of freshwater. In addition, every aspect of phytoplankton, including phytoplankton assemblages (Janse van Vuuren & Pieterse, 2010), phytoplankton biomass (Takamura & Nojiri, 1994), chlorophyll *a* (Felip & Catalan, 2000) and species diversity (Ptacnik *et al*., 2008) have been utilised to indicate environmental stress.

The reasons why the Bacillariophyceae is preferred as bio-monitors in rivers, is because they have a cosmopolitan distribution that is not determined by stream flow, they allow for quick and easy sampling, they have rapid life cycles, they are an extremely diverse group, many species are able to attach to various substrates and their silica cell walls allow for infinitive preservation and archiving of samples (Round, 1993; Taylor *et al*., 2005a). A list of additional advantages for the use of diatoms in bio-monitoring is contained in Harding *et al*. (2005). Some diatom-based indices include the Generic Diatom Index (GDI), the Biological Diatom Index (BDI) the Artois-Picardie Diatom Index (APDI) and the Trophic Diatom Index (TDI), to name but a few (Taylor *et al*., 2005b). Although diatoms are excellent bio-monitors because they are able to attach to various substrates (and as such have the unique ability to reflect water quality impacts occurring over extended periods of time), it is this attribute which also restricts their use as bio-monitors, with specific reference to sampling.

Taylor *et al*. (2005a) listed various considering factors that should be taken into account when diatoms are sampled for water quality analysis. Amongst these considerations were that trivial differences in diatom distribution may occur between substrata that are submerged at varying depths, and given the complex hydrology of lotic freshwater bodies (Li *et al*., 2010), this could result in a common sampling restriction, especially because it is required that sampling (for the purpose of water quality monitoring) should occur at pre-determined sites. Also, boulder collection for diatom sampling should be avoided when covered in filamentous phytoplankton, or even a thin layer of sediment, as both represent modified substrata, which could house different diatom communities. Furthermore, the collection of boulders proves to be difficult during episodes of flooding.

Phytoplankton genera are used less frequently as bio-monitors compared to benthic diatoms (Li *et al*., 2010) and even though they share many of the advantages with diatoms for use as bio-monitors (Sharov, 2008), they do not have any of the above-mentioned disadvantages. One of the main reasons why the monitoring of free-floating phytoplankton is extremely important, is the fact that the notorious bloom forming Cyanophyceae is included amongst them.

It is well known that phytoplankton productivity is predominantly determined by the availability of nutrients, especially PO_4^{3-}, and to a lesser extent NO_3^- (Chapman, 1996). The enrichment of freshwater bodies with such nutrients (that are commonly present in many by-products of anthropogenic activities), also known as eutrophication (Harding, 2006), often results in phytoplankton bloom formation. Although a number of phytoplankton genera are capable of forming blooms, it is the cyanobacteria which are most notorious in this regard (Paerl *et al*., 2001). Scientific literature, documenting the occurrence of these harmful algal blooms, dates back to over 130 years (O'Neil *et al*., 2012), but the frequency as well as severity are increasing over time (O'Neil *et al*., 2012; Paerl *et al*., 2001; Trainer & Hardy, 2015).

Cyanobacterial blooms are called "harmful" algal blooms because they are often associated with water quality deterioration, in terms of recreation (tastes and odour problems), toxicity (the release of collectively called cyano-toxins) as well as ecosystem or food-chain alterations (hypoxia and anoxia) (Paerl *et al*., 2001). The toxins, that some of these genera produce, are particularly problematic because of the health risks it poses to humans when consumed (Harding, 2006). Cyano-toxins that are fatal to animals after exposure include microcystins and cylindrospermopsins that affect the liver, as well as anatoxin-a and saxitoxins, that affect the nervous system (Trainer & Hardy, 2015). A table summarising the major cyano-toxins, their effect on humans, as well as the genera that produce each toxin is contained in O'Neil *et al*. (2012). Freshwater bodies that experience these toxic blooms are often utilised for recreational purposes and as sources of potable water (Trainer & Hardy, 2015), resulting in elevated costs to eliminate toxins from the source water (Lopez *et al*., 2008). The toxic nature of blooms is also highly unpredictable, because a bloom which may not be toxic at the time can become toxic at a later stage. Phytoplankton monitoring programmes are increasingly being implemented, involving local, state and federal scientists to perform routine analysis as a measure of precaution (Trainer & Hardy, 2015). They also represent the bio-monitor organisms selected for use in this study.

To the contrary, only two phytoplankton-based indices, known as Palmer's Algal Genus Pollution Index and Palmer's Algal Species Pollution Index, are commonly used to indicate the extent of organic pollution and they are almost always used in conjunction with indices of diversity (Junshum *et al*., 2008; Ramakrishnan, 2003), richness and evenness (Motwani *et al*., 2014), partly because their applicability are not restricted to specific organisms. Junshum *et al*. (2008) state that phytoplankton species composition and diversity are determined as a means of indicating water quality. For instance, decreased diversity, caused by a decline in the number of species accompanied with an increasing number of individuals of each species, would be indicative of polluted water.

When considering the above statement by Junshum *et al*. (2008), two very important aspects regarding diversity, that is not pertinently mentioned either in the definition of diversity, or the reasoning behind the use of diversity indices, come to light such as richness and evenness. Aslam (2009) defines diversity as "*the number of different items and their relative frequency*". In terms of ecology, these 'items' may represent all levels of biological organisation (species, genera, populations etc.). Heip *et al*. (1998) state that the aim of a diversity index would be to "*attain a measurable estimate of biological variability, which can be used to compare biological units, composed of discrete components, in space or time*". The two concepts are known as richness and evenness. Aslam (2009) believes that failing to recognise these two critical components of diversity has, in the past, lead to the misuse of diversity as a principal characteristic of communities (such as phytoplankton). One of the most common misconceptions is that species richness and diversity refer to the same attribute of a population. Heip *et al*. (1998) define species richness as "*a measure of the total number of species in the community*" and species evenness as "*how evenly the individuals in the community are distributed over the different species*". In other words, the definition of diversity given by Aslam (2009) indirectly refers to both richness and evenness in the following way: "*the number of different items* (**richness**) *and their relative frequency* (**evenness**)". For this reason, Heip *et al*. (1998) argue that the use of diversity indices should be coupled by the separate use of both richness and evenness indices, to reflect the contribution made by each in terms of diversity. Indices of diversity, richness and evenness that are well established in ecological literature include the Shannon-Wiener Diversity Index, the Simpsons Diversity Index, Margalef's Richness Index, McIntosh's Richness Index and Pielou's Evenness Index (see section 4.2.3.) (Motwani *et al*., 2014).

2.2 IMPACTS OF GOLD-MINING INDUSTRIES ON SOURCE WATER QUALITY

South Africa's gold resources were first discovered in 1886, resulting in the gold-mining industry becoming one of the country's largest economic, political and social drivers (Adler *et al*., 2007; Naicker *et al*., 2003;). The economic value of this asset alone, allowed the mining industry to be privileged in many ways, which included separating water policies that applied to mines from that of other industries. At the time (and with the definite aim of profit maximisation), sustainability was still a foreign concept, with little thought given to the long-term effects of mining on receiving environment (Adler *et al*., 2007).

The Far West Rand held the largest gold deposit worldwide, that initiated the first mining-based economic development in the country. However, the immense depth at which these gold deposits were situated made the extraction process much more difficult and perilous, especially because it was overlain by enormous dolomitic aquafers (Adler *et al*., 2007).

The current and historic mining activities associated with, and affecting the surface and groundwater quality of the KMS catchment are discussed comprehensively in section 3.3.2 of this dissertation. The aim of this section is however to give a brief overview of the various ways in which gold-mining could impact on freshwater quality, with specific reference to various gold-mining procedures and by-products.

According to Coetzee *et al*. (2006), gold-mining can impact on freshwater sources in two ways, namely the quality and quantity thereof. Concerning the quantitative feature, there are two ways in which gold-mining affects the availability of source water, the first being the bulk water demand required for gold-mining operations to take place (DWA, 2002). In the KOSH area, that includes the KMS catchment, the water demand required for mining operations alone amounts to roughly 41.7 Mℓ/day (Table 3.1) (COM, 2013; DWA, 2006; MWC, 2013; Smith, 2012), putting immense pressure on the KMS and the Vaal River. The second, and main culprit, according to Coetzee *et al*. (2006), involves the decanting of dolomitic groundwater compartments to prevent mine shafts from flooding (DWA, 2006). This is especially the case in areas where the surface lithology is mainly comprised of dolomitic earth, well known for its porous nature, which allows for the rapid infiltration and thus recharge of groundwater (DWA, 2004; Winde & Stoch, 2010).

Although one may assume that this characteristic of dolomitic earth will counteract some of the effects resulting from decanting groundwater, the opposite is often true. In order to prevent the re-infiltration of groundwater that is pumped to the surface, the groundwater is deliberately discharged outside the boundaries of the decanted underground compartments by means of canals and pipelines.

In some instances, such as in the vicinity of the Wonderfonteinspruit catchment, this resulted in drastically lowered groundwater levels and reports of dried up boreholes. In addition, the lowered groundwater levels also resulted in the formation of large sinkholes that, together with a lack of available water, severely altered the land-use practices in the area, especially agriculture (Coetzee *et al*., 2006). Coetzee *et al*. (2006) stated that the water being discharged from operational and closed gold mines, often holds far reaching consequences for the receiving water bodies in terms of an alteration in water quality. In assessing these impacts, it is important to distinguish between point and diffuse mine water discharge sources.

Point discharge water sources typically include process water, entering freshwater bodies from canals or pipelines. The quality of these types of effluent can be regulated so that the water is diverted into settling ponds intended to decrease sediment loads as well as trace metal concentrations (Coetzee *et al*., 2006). Both elevated sediment loads and trace metal concentrations (Coetzee *et al*., 2006), as well as increased salinity and SO_4^{2-} concentrations (Winde, 2010), are typical symptoms of freshwater impacted by mine effluent discharges.

Diffuse discharge water sources are commonly derived from seepages through tailings deposits (slimes dams, metallurgical plants, rock dumps, ore piles and unlined return-water dams), undetected pipeline and canal leakages, as well as accidental spills (see section 3.3.2). These mine effluents are extremely difficult to regulate and pose even greater risks in introducing pollutants to freshwater resources. Tailings dumps are of specific concern in this regard, since their sole purpose is to act as large contaminant reservoirs (Coetzee *et al*., 2006). Naicker *et al*. (2003) describe the two main processes used for extracting gold from ore. In order to fully understand the impact of these tailings deposits on source water quality, a short summary will be given here.

Naicker *et al*. (2003) state that the Hg-amalgam method was originally implemented to extract gold in the Far West Rand. During this process mined ore brought to the surface, was first ground to the consistency of fine sand, where after it was exposed to a thin layer of Hg applied to Cu-plates. After a period of time, the plates were removed and the Hg-gold amalgam was scraped off and distilled to extract the gold. The tailings were disposed in dumps close to the extraction plant (Naicker *et al*., 2003). Although this method seemed to be perfectly adequate at the time, gold extraction from deeper levels of the Witwatersrand conglomerates containing pyrite (FeS_2), the most common sulphide mineral on earth (Johnson & Hallberg, 2005), severely interfered with this method of extraction (Naicker *et al*., 2003).

Instead, the MacArthur-Forrest Cyanide method was implemented to extract gold from the conglomerate ores. During this extraction process, the ore brought to the surface is ground to an even finer consistency, after which a cyanide-based solution is applied to dissolve the gold. In order to regulate the pH of the gold containing product, lime is usually added (De Beer, 2005). This product then undergoes further processing to extract the gold, while the tailings are disposed of in large slimes dumps. However, the cyanide method is not as effective in extracting all the gold from the conglomerate ores and as a result, some of the slimes dumps have and are being reprocessed to recover the remaining traces of gold (Naicker *et al*., 2003).

A large number of these tailings and slimes dumps have not been reprocessed, and they were left undisturbed for nearly a century. During this time, the upper layers of pyrite (a few meters in depth) have been exposed to oxygen and precipitation, both of which oxidises the pyrite and a number of other sulphides (Naicker *et al*., 2003). The oxidation of pyrite and formation of by-products are explained through the following equation, given by Johnson and Hallberg (2005):

$$4FeS_2 + 15O_2 + 14H_2O \longrightarrow 4Fe(OH)_3 + 8SO_4^{2-} + 16H^+$$

The water leaching through these dumps is thus acidic given the H^+ and OH ions (section 2.1.1 under *pH)*, and eventually enters the groundwater (Naicker *et al*., 2003). Also, in cases such as the KMS catchment, where mining deposits are most likely situated on top of porous dolomitic earth (see section 3.3.2 under *Anglo Gold Mines*), the leached acidic and pollutant rich water will rapidly contaminate the groundwater (Coetzee *et al*., 2006).

An example of the combined effects of both point- and diffuse sources of mine water on the receiving freshwater body can be seen in the Blesbokspruit. According to Roychoudhury and Starke (2006), the Grootvlei Gold Mine, located in the East Rand basin (Witwatersrand), discharges between 80 and 100 Mℓ/day (Schoeman & Steyn, 2001) underground mine effluent into the Blesbokspruit to keep the mine operational. This action was necessitated by the closure of neighbouring mines in the area, which were allowed to be flooded (Roychoudhury & Starke, 2006; Schoeman & Steyn, 2001). As a direct result of exposure to mine effluent, the Blesbokspruit is under threat of Fe, Mn, SO_4^{2-}, Ca, Mg, Na and Cl contamination (Schoeman & Steyn, 2001).

As mentioned before, the lithology of the Witwatersrand area (besides dolomitic earth) contains sulphide-bearing pyrite. This means that groundwater seepage, accumulating in underground compartments, typically has elevated concentrations of Fe, SO_4^{2-}, Cu, As and U. Fe and SO_4^{2-} are of main concern as contaminants entering the Blesbokspruit in the form of mine effluent that is pumped to the surface after leaching. Furthermore, the various mining activities in the area also produced surface rock piles and slimes dams, as well as subsurface backfill rock piles (to prevent the formation of sinkholes). Thus, besides the direct introduction of trace metal- and SO_4^{2-}-rich effluents to the Blesbokspruit via canals or pipelines, diffuse sources of contaminants are also entering the spring by means of seepage through vast slimes dams, as well as atmospheric deposition of tailings particles (Orlekowsky *et al*., 2013; Roychoudhury & Starke, 2006).

In addition, and similar to the KMS catchment (see section 3.3.1), another source of trace metals and minerals (especially ions which contribute to salinity) into the Blesbokspruit is due to the fact that the upper reaches of the stream flows through urbanised and industrial areas before it reaches the wetland where mine effluent is being discharged (Roychoudhury & Starke, 2006). Roychoudhury and Starke (2006) stated that after trace metals are released into the aquatic environment (assuming that the prevailing redox status and pH level represent that of neutral water), they are not easily degraded and have a low solubility potential, causing them to accumulate in the sediments. However, these trace metals may be remobilised from the sediments under changing redox and pH conditions, causing the sediments to act as potential long-term sources of surface water pollution.

In section 2.1.1 (under *Trace Metals*), it was stated that elevated Fe, and other trace metal concentrations, may indicate inputs of effluents characterised by low pH levels (Phippen *et al.,* 2008). In the case of gold-mining, where acid mine drainage (AMD) is a common side effect (Huizenga, 2011), the remobilisation of trace metals such as Al, Mn and Fe due to increased solubility as a result of lowered pH (Orlekowsky *et al*., 2013) from sediment depositories , is a very likely possibility. However, Roychoudhury and Starke (2006) found that even though mine effluent containing trace metals is directly discharged into the Blesbokspruit, the pH of the water tends to be more alkaline because it ciphers through dolomitic [$CaMg(CO_3)_2$] (Akande & Agbalajobi, 2013) earth. Also, before the mine effluent gets discharged into the Blesbokspruit, it undergoes meticulous separation procedures and is treated with lime (CaO) (De Beer, 2005). In effect, both these aspects favour trace metal precipitation, which is why the mine residue depositories seem to pose a greater risk of polluting the surface water with trace metals and other chemical constituents, through seepage and atmospheric deposition (Roychoudhury & Starke, 2006).

This is not an isolated case, as Venter *et al*. (2013) stated that even though the Klerkskraal Dam is situated upstream from the highly polluted Wonderfonteinspruit (and thus not directly exposed to waterborne mine effluent) it may well be prone to atmospheric deposition of tailings particles. Orlekowsky *et al*. (2013) state that tailings material, as by-products of gold-mining activities, is frequently characterised by elevated As, Pb, Hg and U concentrations.

Although AMD in gold-mining areas underlain by dolomitic earth seems to be of lesser concern, it often introduces another suit of contaminants to source water in the form of Saline Mine Drainage (SMD) (Labuschagne, 2007). In fact, the recirculation of mine water in the KMS catchment area has been found to intensify salinisation, that is worsened by the fact that leakage of the KMS into the dolomites has been established. Also, because the KMS catchment drains towards the Vaal River, water quality alterations in the form of elevated SO_4^{2-} and total dissolved salts is a common and ongoing consequence (DWA, 2004).

2.3 CONCLUSION

To conclude this chapter, the focus will be placed on a statement made under section 2.1.2 concerning the process of monitoring and recalling that it is the first essential process required to assemble a sustainable source water management plan for the Middle Vaal WMA. Passive monitoring represents the preferred approach followed to conduct this study, since the KMS is a notorious multi-stressed catchment (Spangenberg, 2000), hinting that any other monitoring approach would fail to consider multiple and constantly fluctuating stress factors impacting on the KMS. This is emphasised by all the possible and actual (measurable or quantifiable) implications of the land-use practices that can affect the water quality of the KMS catchment. Therefore the various land-use practices affecting the water quality of the KMS will be investigated comprehensively under section 3.3 of this dissertation and include urbanisation, gold-mining and agriculture. However, gold mining activities are the most common industry in the area close to the confluence of the KMS and the Vaal River. It is important to keep in mind that gold mining activities can impact on these freshwater sources not only in terms of quality but also the quantity thereof.

CHAPTER 3: STUDY AREA

Anderson *et al.* (2001) passed the remark that land-use data are required to first comprehend and then to analyse environmental processes and complications for the purpose of improving or maintaining a certain quality of life. However, literature indicated on various occasions that there exists no mutual agreement on the definition of the term "land-use" (Dickinson & Shaw, 1977). According to European Communities (2001), the interdepartmental working group on land use planning (IDWG-LUP) gave the following definition: "*A delineable area of the earth's terrestrial surface, embracing all attributes of the biosphere* ***immediately above or below this surface****, including those of the near surface climate, the soil and terrain forms, the surface hydrology including shallow lakes,* ***rivers****,* ***marshes*** *and swamps, the near-surface sedimentary layers and associated* ***groundwater*** *and geo-hydrological reserves, the plant and animal populations, the human settlement pattern and physical results of past and present* ***human activity*** *(terracing, water storage or drainage structures, roads, buildings, etc.)*". This definition suits the land-use for the purpose of this study perfectly, since the definition does not exclude land-use practices taking place below ground level, for example gold-mining. The aim of this chapter is to give an in depth discussion of all the land-use practices taking place that may have an effect on the surface water quality of the KMS catchment.

3.1 LOCATION & STREAM ORDER

The Vaal River is the main tributary of the Orange River and has been described as one of South Africa's most established and monitored rivers. The highland area west of the Drakensberg range is the point of origin of the Vaal River, that drains the greater part of South Africa's central highveld. Collectively, the Orange and Vaal River basin includes five water management areas (WMA) (DWA, 2006a), of which the Middle Vaal WMA forms the focus point of this study.

The Middle Vaal WMA is situated downstream with respect to the position where the Vaal River and Rietspruit converge and upstream of the Bloemhof Dam. The area then stretches all the way north to the Schoonspruit and also south to the Vet River. The total catchment area of the Middle Vaal WMA amounts to 52 563km^2 (DWA, 2006a), and comprises parts of the Free State and North-West provinces. Within the Middle Vaal WMA lies the KMS catchment, forming one of the main tributaries of the area (DWA, 2006a), and also the study area.

Both the Schoonspruit and KMS catchments form part of the Middle Vaal WMA and Figure 3.1 illustrates their location with respect to each other and their points of convergence with the Vaal River. From this figure it is evident that the Schoonspruit catchment and its convergence with the Vaal River are situated downstream from that of the KMS catchment. Though most literature discuss the Schoonspruit and KMS catchments collectively in terms of their impacts on the Middle Vaal WMA, the focus of this study is on the KMS catchment area and the Schoonspruit catchment will thus not be included as it is suspected to have no effect on the KMS catchment given their locations.

According to Spangenberg (2000), the KMS originates on the Rooipoort and Lustfontein farms, approximately 28 km north to where it crosses the N12 (a major highway running through the KMS). The Kromdraaispruit is the only significant, naturally occurring tributary of the KMS. Kromdraaispruit originates north of the KMS and drains east until it eventually converges with the KMS a mere 3.5 km north to where it crosses the N12. The KMS and Vaal River confluence is about 16.5 km downstream from this point (see Figure 3.1).

Apart from the Kromdraaispruit, a concrete canal, the so called Enviro Canal, also discharges water into the KMS before it converges with the Vaal River. Apparently the canal was built from a settler plant owned by the Buffelsfontein Gold Mine Operations. This was done as a solution after the discovery of polluted ground water. Shafts were sunk to reach and treat the polluted ground water at this plant, after which the treated water is discharged into the KMS via this canal (De Meyer & Nortje, 2013).

The KMS yields a total catchment area of 760.7 km^2 and is considered to be a principal contributing component of the Middle Vaal WMA, due to its status as a multi-stressed area (DWA, 2006a; Winde & Van der Walt, 2004).

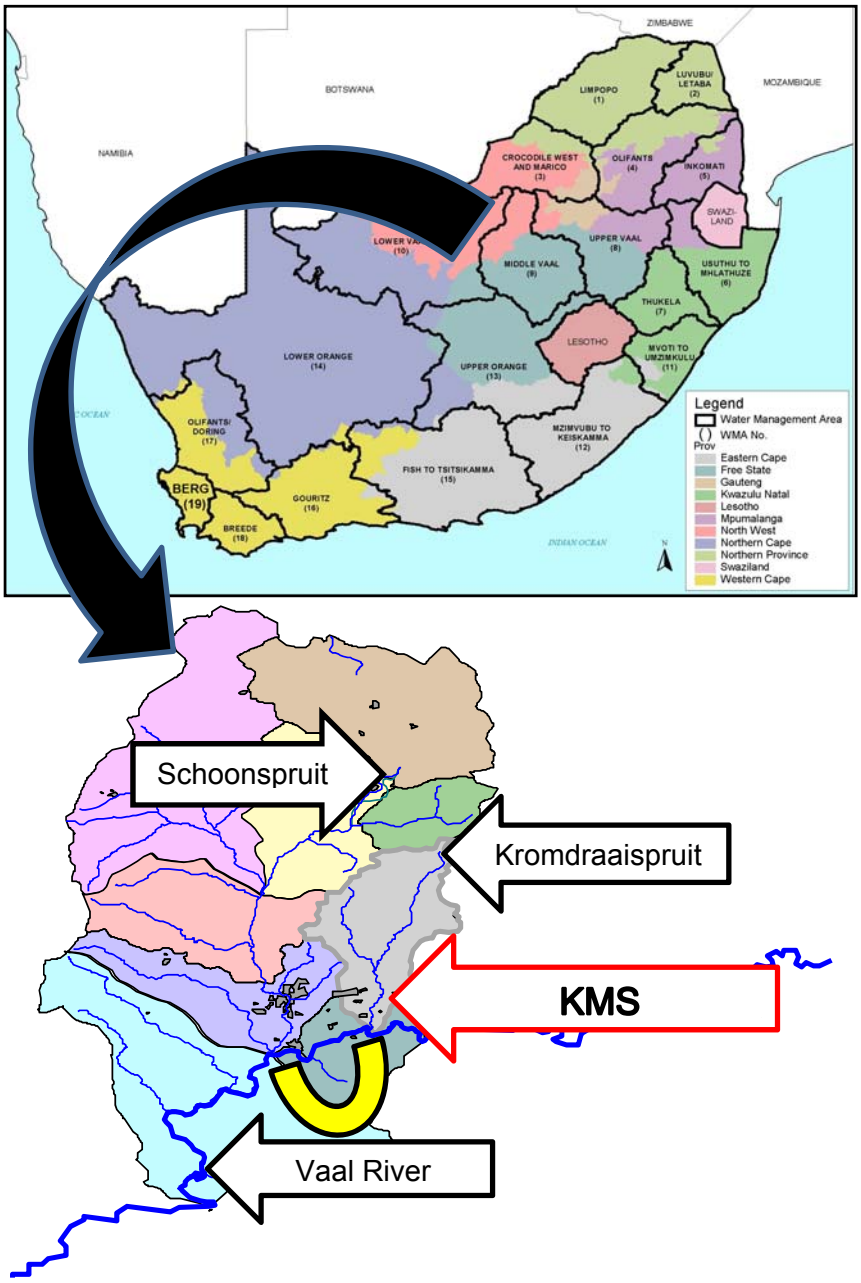

Figure 3.1: Visual orientation of the KMS catchment, its tributaries and confluence with the Vaal River (modified from DWA, 2006a).

According to Dodds (2002), the stream order reflects an estimate of water volume of a specific river or stream and therefore it ultimately reflects the effect of pollutant dilution within a catchment area such as the KMS. Amongst other catchment attributes, tributaries can be used to classify rivers and streams into specific stream orders during which each stream order is assigned a number.

According to the *Strahler classification system*, tributaries which are not subjected to any auxiliary divisions are classified as first order streams. A second order stream is assigned when two first order streams merge. Likewise, a third order stream would be assigned when two second order streams merge and so forth (Dodds, 2002).

The upper stretch of the KMS is thus classified as stream order 1, until it merges with the Kromdraaispruit, thereafter it is classified as stream order 2. The Vaal River is classified as a stream order 5 due to its multiple tributaries (De Meyer & Nortje, 2013), speculating that the KMS will thus have a minimal effect on dilution of the Vaal River.

DWA (2006) states that the KMS had been categorised as a non-perennial stream until surplus groundwater from mining activities in the area was discharged into the KMS during 1959. This caused certain sections of the KMS to be categorised as perennial.

3.2 CLIMATE

The KMS falls within an area characterised by relatively warm, rainy summer months and cold, dry winters during which frost is a common occurrence. The air temperatures range between a minimum of -4˚C during mid-winter to a maximum of 37.2 ˚C during mid-summer (DWA, 2006).

For the purpose of this study, the mean annual precipitation for the KMS will be reflected as the value derived from the Weather Bureau station, Klerksdorp (number 0436294). The data derived from this station are believed to be the most representative of the KMS and is calculated as the average mean annual precipitation from 1973 to 1994. Thus, the estimated mean annual precipitation for the KMS amounts to 553 mm/year (DWA, 2006).

Accordingly, the mean annual evaporation most representative of the KMS will be reflected as the average mean annual evaporation (1958 to 1987) recorded by the Weather Bureau station, Potchefstroom (number 0437104). The mean annual evaporation thus amounts to approximately 178 mm/annum (DWA, 2006).

According to DWA (2002), the mean annual runoff for the Middle Vaal WMA amounts to 887.5 Mℓ/year and humidity reaches its highest relative humidity (62% - 66%) during February and its lowest (52% - 58%) during August.

3.3 LAND-USE

According to DWA (2006b), catchment development has led to severe alterations of the KMS catchment, especially in terms of water quality. Figure 3.2 illustrates that large areas within the KMS catchment are dominated by both urbanisation and mining activities respectively. One can thus make the assumption that these two development entities contribute significantly, if not most, to the deteriorating water quality of the KMS (DWA, 2006b).

To stress the situation even further, Midvaal Water Company abstracts its raw water directly from the Vaal River, just downstream of the KMS/Vaal River confluence (DWA, 2006a; MWC, 2011). Midvaal Water Company is a water service provider which supplies potable water in bulk to the Greater Municipality of Matlosana, Klerksdorp (MWC, 2011). This area of jurisdiction includes the towns of Klerksdorp, Orkney, Stilfontein, Hartebeesfontein (KOSH) as well as each of their associated informal settlements. In addition, the company is also the main supplier of water to the surrounding mines and associated mining activities (DWA, 2006a). Thus, to no exception, the KMS represents a multi-stressed water resource and the Middle Vaal River system the receiving water body (Spangenberg, 2000).

Table 3.1: Summary of Midvaal Water Company water supply for 2013 (COM, 2013; DWA, 2006; MWC, 2013; Smith, 2012).

Clientele	**Town**	**Informal settlement**	**Mining industry**	**Total**
	Klerksdorp	Alabama Jouberton Sakhrol	AngloGold Ashanti & Harmony	
	Orkney	Kanana		
	Stilfontein	Khuma	Stilfontein	
	Hartebeesfontein	Tigane	Hartebeesfontein	
	Buffelsfontein		Buffelsfontein	
Population	428 024 people		N/A	428 024
Bulk water supply demand (Mℓ/day)	84.2		41.7	125.9
Bulk water supply capacity (Mℓ/day)	N/A			250
Estimated water supply demand (Mℓ/day) 2014	133.25			133.25

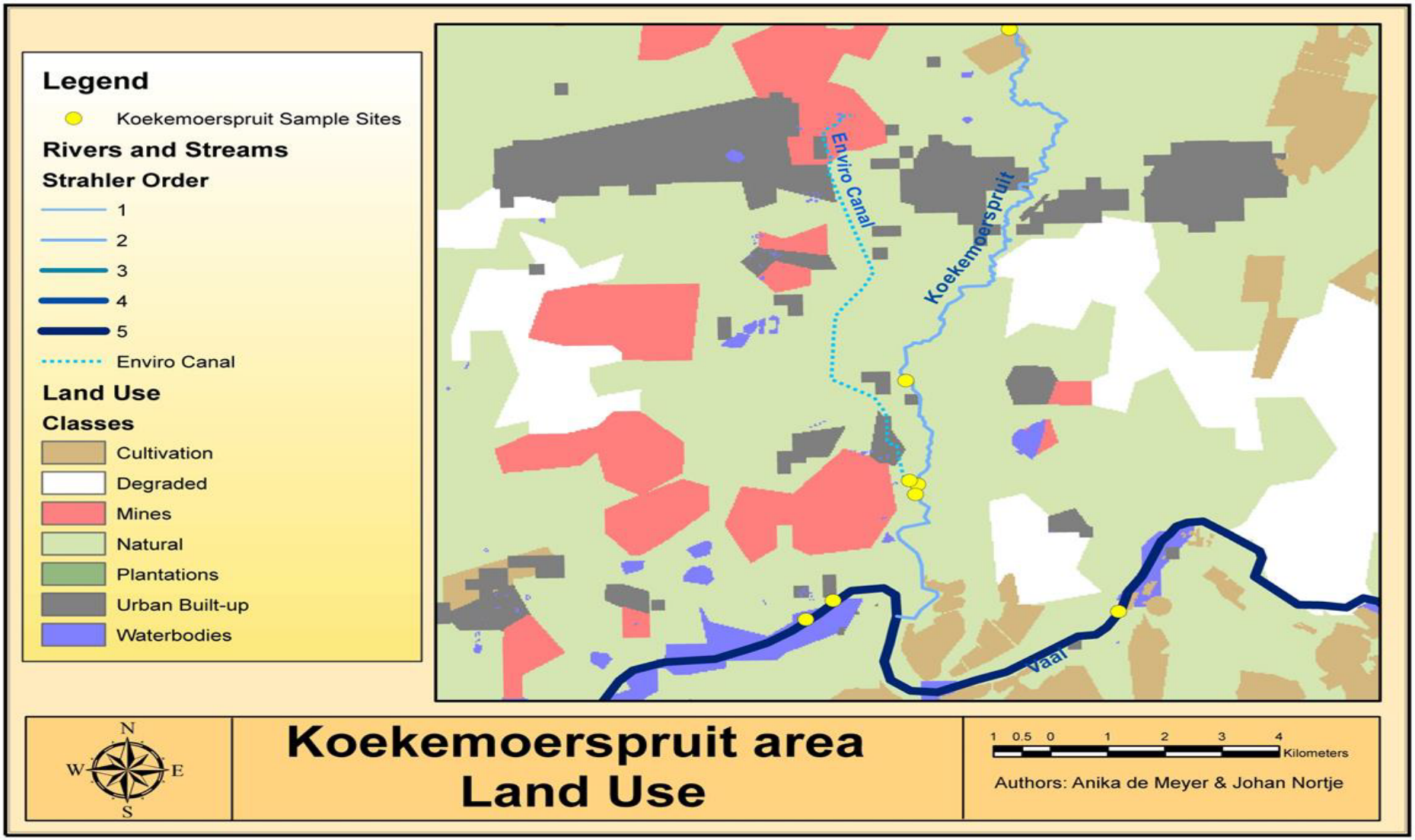

Figure 3.2: Spatial representation of the various land-use practices found in the KMS area (De Meyer & Nortje, 2013).

3.3.1 URBANISATION

The main towns located in the KMS catchment are illustrated in Figure 3.3 and their associated informal settlements are listed in Table 3.1. Water quality problems commonly associated with urbanisation include point-source discharge from numerous trades, effluent from WWTP's as well as urban runoff. These are also the main contributors of deteriorating water quality in the KMS catchment, in terms of increased salinization (DWA, 2006b). The latter statement is especially true for the town of Stilfontein and its informal settlement, Khuma.

As far as effluent from WWTP's are concerned, all of the major towns listed in Table 3.1 have their own WWTP however, only Stilfontein WWTP discharges its effluent into the KMS (DWA, 2006). Furthermore, Figure 3.3 also clearly illustrates that the KMS runs straight through the informal settlement of Khuma, which one would expect to be of great concern if surface water quality is considered.

NOTE: Although the KMS catchment also includes the towns of Klerksdorp, Orkney and their informal settlements, they are not displayed in Figure 3.3 as the focus of this study was on the southern half of the KMS and the crippling effect of the mining industries located within this section.

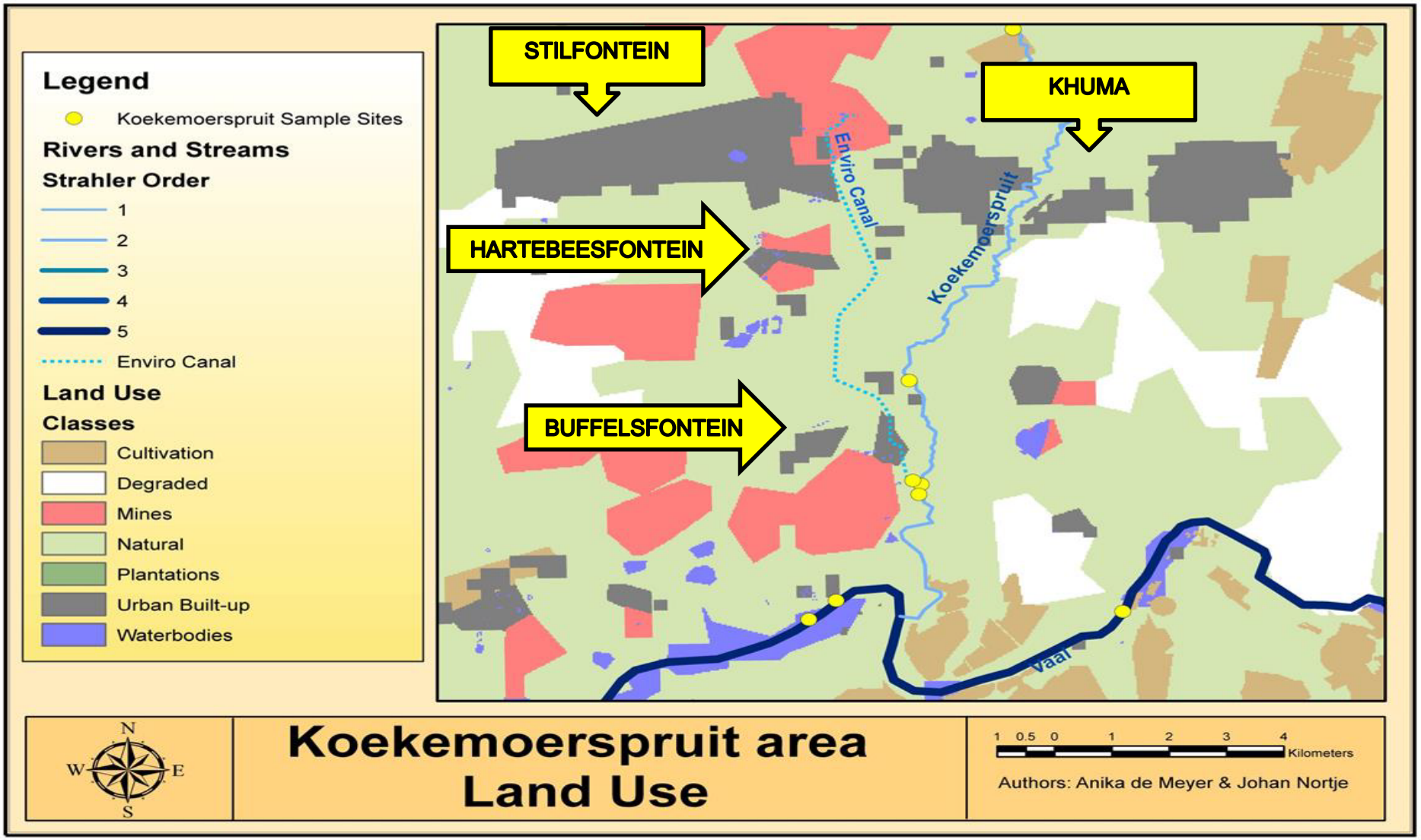

Figure 3.3: Spatial representation of the various urbanised areas found in the KMS area (De Meyer & Nortje, 2013).

3.3.2 MINING ACTIVITIES

According to DWA (2006), some of the world's major gold mines are located within the vicinity of the KMS catchment (see Figure 3.4). **Stilfontein, Buffelsfontein**, Vaal Reefs and **Hartebeesfontein** Gold Mines place immense pressure on the KMS and Vaal River in terms of bulk water demand. Three of these mines are located within the KMS catchment and collectively, these four mines contribute more than 90% of the bulk water requirements in the Middle Vaal WMA (DWA, 2002).

The return flows associated with these mining activities into the KMS can have major conflicting impacts on the catchment. Quantitatively, return flows may have a positive influence on the catchment in terms of stream volume, but at the same time it may also introduce pollutants associated with gold-mining to the ground- and surface water of the catchment (DWA, 2002).

AngloGold Ashanti Vaal River Operations (Anglo Gold Mines)

Anglo Gold Mines all function on the Vaal River banks. Midvaal Water Company provides these mines with potable water and all remaining runoff gets pumped to one of two tailings dams, after which it is mainly used as process water. Anglo Gold Mines consists of four shafts, #8 to #11, and also oversees the monitoring and runoff management of African Rainbow Minerals (Harmony) operating shafts #1 to #7 (Harmony #1, #2 and #5 in Figure 3.4), since it was formerly owned by Anglo Gold Mines (DWA, 2006).

Anglo Gold Mines operates seven Wastewater Treatment Works (WWTW's) located at shafts #1, #2, #3, #4, #8, #10 and #11. The runoff from each WWTW either gets pumped to another Anglo Gold Mines shaft, where it is used as process water, or it is first pumped to Bokkamp or Mispah tailings dams. Leaching from these tailings dams are monitored monthly and none of the process water is being discharged into the Vaal River (DWA, 2006).

Nine waste rock dump areas, situated at shafts #1, #2, #3, #4, #5, #8, #9, #10 and #11, are all underlain by dolomitic earth and are intended for recycling after the mines are no longer operational. Runoff from shaft #10 WWTW is used for irrigational purposes as well as to supress the dust released from the waste rock dumps (DWA, 2006).

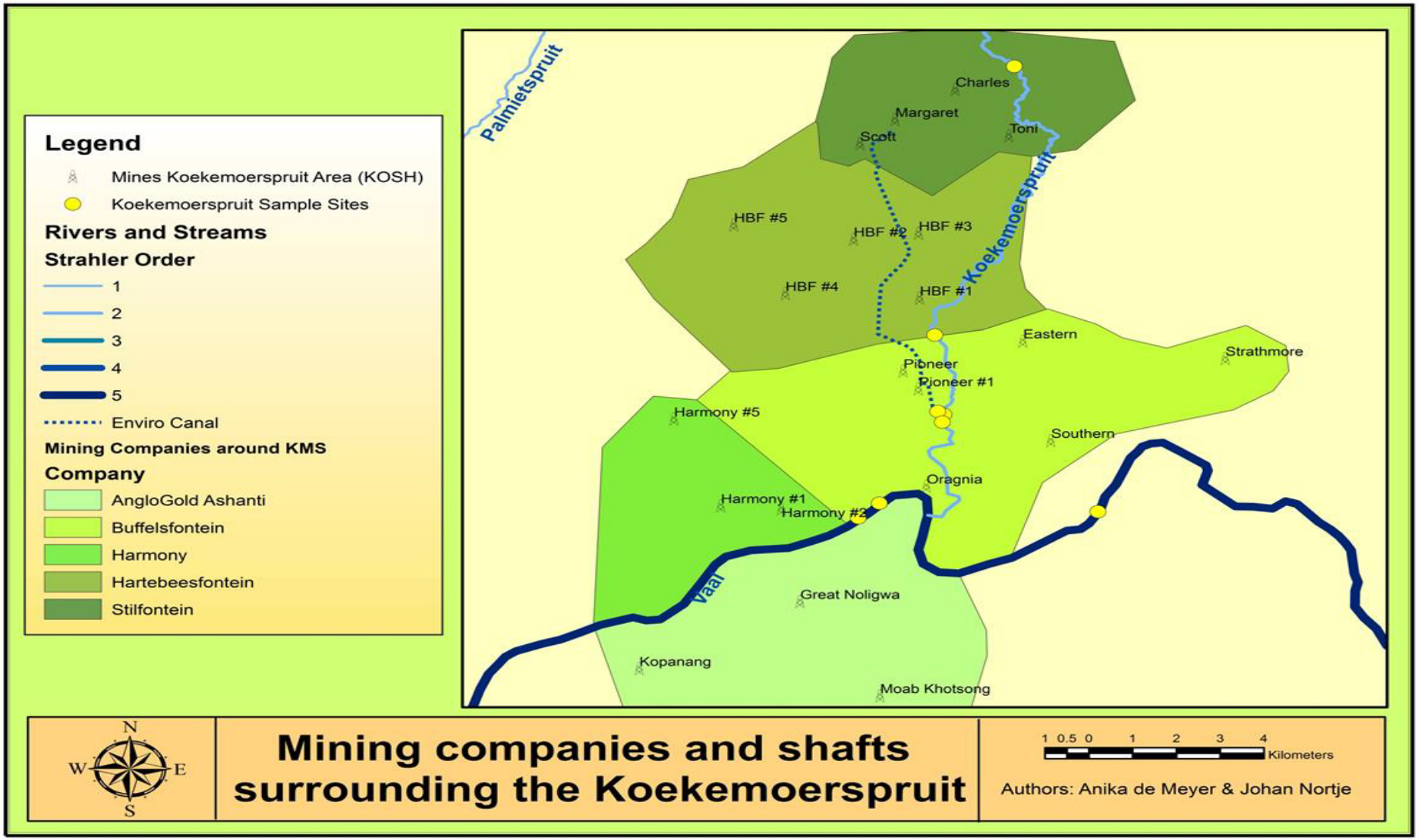

Figure 3.4: Spatial representation of the various gold mines located within the KMS catchment (De Meyer & Nortje, 2013).

It may appear that the KMS catchment is not particularly affected by the Anglo Gold Mines and Harmony gold-mining activities, although DWA (2002) states that seepage from Harmony into the system should not be completely ruled out. In addition, toxic mine waste spills also occur on occasion. On 27 August 2013, toxic waste from Anglo Gold Mines spilled into the KMS near the Vaal River, after which the spokesman of the company stated that, although the spill proved to be harmful to fauna and flora, humans should not be affected (Crowley, 2013). This may have been the case, though one is ultimately compelled to validate whether humans and the environment should be considered to function separate from one another and to then acknowledge that humans are in fact affected as well. The Vaal River is after all the main source of freshwater in the KMS catchment.

Buffelsfontein Gold Mine

The southern subdivision of the Buffelsfontein Gold Mine consists of shafts #9, #10, #11, #12 and #Strathmore, of which shafts #11 and #Strathmore have closed and are no longer operational. As a result, the South Shaft WWTW has also been shut down and water from the remaining shafts is being pumped to the Pioneer WWTW. Both treated effluent from the Pioneer WWTW, as well as raw effluent from the South Shaft WWTW, is discharged into the KMS (DWA, 2006).

The northern subdivision of the Buffelsfontein Gold Mine consists of nine shafts. Midvaal Water Company provides both the southern- and northern subdivision of the Buffelsfontein Gold Mine with potable water, which is supplemented by groundwater from #Margaret shaft, located in the Stilfontein Gold Mines area (DWA, 2006).

Initially, the Buffelsfontein Gold Mine operated five slimes dams and the water from slimes dams 1 and 5 is ultimately recovered by the plant, assuming that it is used as process water. Slime fills the breach between slimes dams 2 and 3, of which the water is transferred to an evaporation dam. The evaporation dam seconds as a flood control measure between the slimes dams (DWA, 2006).

Hartebeesfontein Gold Mine

Figure 3.4 indicates that the Hartebeesfontein Gold Mine consist of five shafts (HBF #1 to #5). According to DWA (2006), the slimes dams located within the Hartebeesfontein Gold Mine area have all been retired as a result of most of the shafts having been closed. Thus, Hartebeesfontein Gold Mine contributes no effluent, except at shaft #2 where the effluent is routed to the Stilfontein WWTP and then ultimately discharged into the KMS.

NOTE: Both Buffelsfontein Gold Mine and Hartebeesfontein Gold Mines' operations are combined and as a result are being managed by the same company, Village, since June 2011 (VMR, 2013).

Stilfontein Gold Mine

Stilfontein Gold Mine consists of five vertical shafts (shafts #Charles, #Margaret, #Scott and #Toni) and two sub-vertical shafts. Midvaal Water Company provides Stilfontein Gold Mine with potable water. During 1992, subsurface mining operations ceased, which lead to shafts #Charles and #Toni having to close down. Both shafts #Margaret and #Scott remained operational for the sole purpose of maintaining pump installations at various levels. The maintenance of these pumps prevents shafts from neighbouring mines to flood (DWA, 2006).

Spangenberg (2000) states that the Stilfontein Gold Mine is located at a higher altitude from the Buffelsfontein Gold mine. This implies that underground water entering Stilfontein Gold Mine will eventually seep through to Hartebeesfontein Gold Mine and then Buffelsfontein Gold Mine, flooding the shafts. There are major concerns regarding the flooding of shafts as they are also a means of underground connections between mines, and serve as escape routes in cases of emergency.

Hartebeesfontein Gold Mine will inevitably be forced to decant the mine as a preventative measure for infiltration into their underground operations from the Stilfontein Gold Mine. An estimated 40 Mℓ/day groundwater is available at the Stilfontein Gold Mine. From this volume, 20 Mℓ/day will be pumped to Buffelsfontein Gold Mine Reservoir 3 to distribute to the south plant for process water. The remaining 20 Mℓ/day will be managed by two underground pump installations at #Margaret shaft which will pump the water to the surface. Some of this surface water will be discarded into the KMS and the rest will be used to rehabilitate the Stilfontein slimes dams (DWA, 2006; Spangenberg, 2000).

Stilfontein Gold Mine only has one operational sewage maturation pond from which the water is used to irrigate the golf course. Furthermore, Stilfontein Gold Mine had previously managed five slimes dams of which slimes dams 1 and 3 are being restored. Slimes dam 5 and some other enclosures serve as runoff management entities (DWA, 2006).

New Machavie Gold Mine

Only a quick mention will be made of the New Machavie Gold Mine since it is not located within the KMS, but its only natural tributary, the Kromdraaispruit (DWA, 2006).

During 1944, New Machavie Gold Mine seized all extensive mining activities and as a result, major pollution of the surrounding area occurred because most of the slimes dams had been left unattended. None of the companies involved could be held accountable for the pollution as it was difficult to determine the exact point source of the pollution, although it could be ascertained that the slimes dams were the probable source. Attempts by Sterling Rand Gold Mine in 1995 to construct and implement an approved environmental management plan failed (DWA, 2006).

DWA, together with the Department of Minerals and Energy, then formed the New Machavie Rehabilitation Working Group. An aerial analysis was performed and the polluted area could then be mapped. The distribution of the slimes could thus be pinned down and it was established that the groundwater pollution was only local, though it proved difficult to determine the impacts on the surface water (DWA, 2006).

3.3.3 AGRICULTURE

According to DWA (2006), the data they obtained from the Department of Agriculture (Agricultural Land Resource Management) indicate that the agricultural land-uses in the KMS catchment are dominated by cultivation and grazing. The most abundant type of crops being cultivated within the Matlosana municipal area are maize, hay, wheat, grasslands, sunflower and potatoes (arranged in descending order of percentage). The total surface area under irrigation, estimated during 2004, amounts to about 1675.85 ha. Return flows from these agricultural land-use activities, containing pesticides, fertilizers and a variety of salts have a definite negative impact on both the surface– and ground water quality of the KMS catchment. It is known however that agricultural land-uses and their effect on the surface water of the KMS catchment in particular is minimal when being compared to the effect of gold-mining activities and inputs resulting from urbanisation (DWA, 2006a).

3.4 GEOLOGY & GEOHYDROLOGY

The interface between ground– and surface water alternates frequently and is determined by a variety of factors, especially climate. For example, the altitude of the groundwater level (relative to the river) will ultimately determine whether the surface water will get recharged by elevated groundwater levels, or whether the surface water will infiltrate the underlying lithology to supplement the groundwater. The location and rate at which the latter takes place are highly dependent on the nature and positioning of the underlying lithology (DWA, 2004).

According to Pulles *et al.* (2005), the surface lithology of the KOSH area, which includes the KMS catchment (see under section 3.3.1) mainly consists of dolomitic earth. This dolomitic earth forms part of the well-known Malmani dolomite stretch and is quite notorious for its ability to store immense volumes of groundwater and thus also for developing rather large shallow aquifers (DWA, 2004). In addition, Winde and Stoch (2010) stated that the extensive gold-mining of the Klerksdorp Goldfields (KOSH area) generated so called underground cavities which, along with the porous nature of the dolomites, allow for the rapid infiltration and thus recharge of groundwater. In fact, it has previously been established that surface water from the KMS does infiltrate the underlying dolomites at certain areas (DWA, 2004). Owing to the above statements, groundwater recharge in the KMS catchment area is comparatively high and progresses along the natural course of streams, with specific reference to the KMS (Veltman & Wilke, 2008).

Beneath this dolomitic layer a much less permeable layer, known as the Ventersdorp lavas, restricts water seepage beyond the dolomitic layer and as such, encourages the development of the above mentioned dolomitic aquifers (Pulles *et al*., 2005). With that being said, it would then be logical to assume that besides naturally occurring faults and dykes, the contours (and depth) of this Ventersdorp lava layer, will in effect determine the altitude of the groundwater levels and also the ground– and surface water interactions in its vicinity accordingly (see Figure 3.5) (Dennis, 2014).

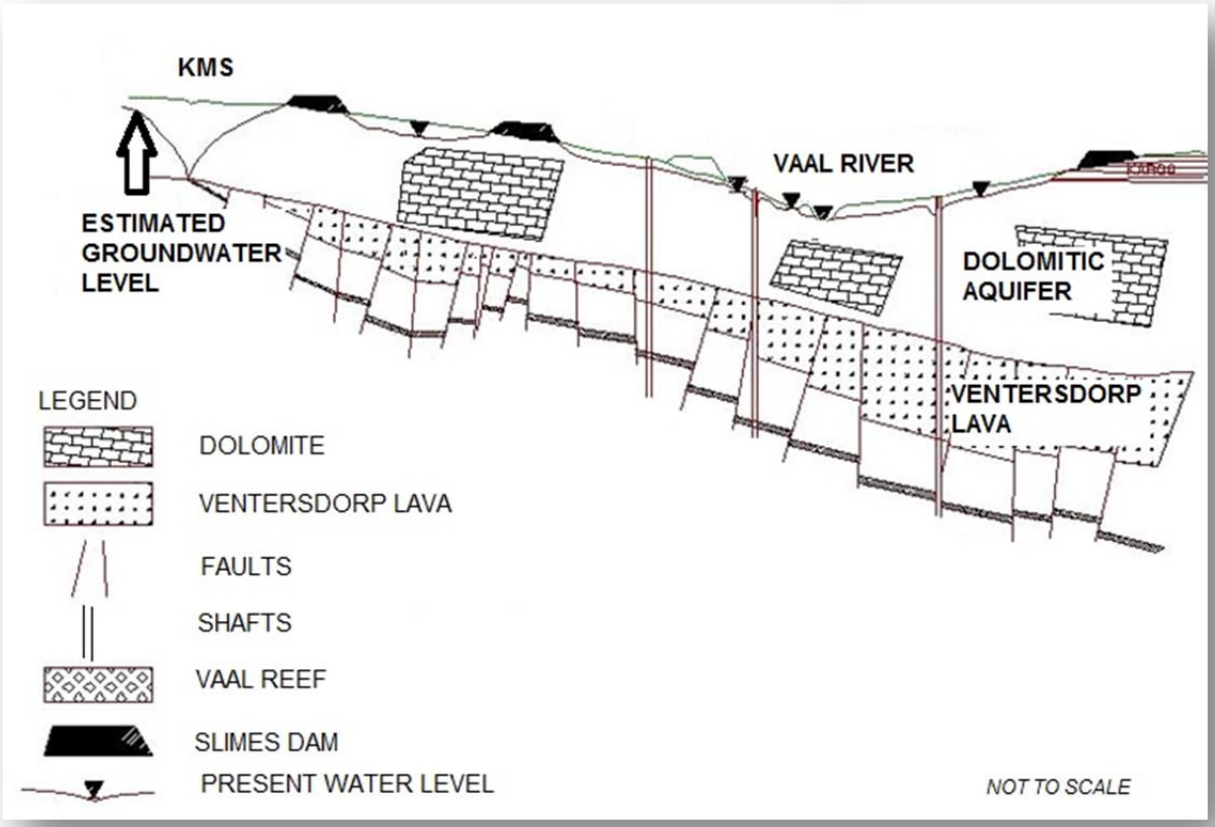

Figure 3.5: Cross-sectional illustration of the geology and geohydrology of the KMS (modified from Veltman & Wilke, 2008).

3.5 SAMPLING SITES

The sampling sites for this study have been carefully and strategically selected. The aim was to have a selection of sites, each of which would deliver data reflecting the various inputs and resulting influences on the KMS. The sampling for and analysis of the water physico-chemistry were carried out by Midvaal Water Company. The sampling for and analysis of the phytoplankton assemblages were performed by the North-West University (NWU). Because this study integrates the use of both the physico-chemical variables, as well as the quantitative phytoplankton data, the selection of sites was also partially co-ordinated with that of Midvaal Water Company in terms of sampling dates and locations. Table 3.2 summarizes the various sampling site locations as well as correlated sites between Midvaal Water Company and the NWU.

Table 3.2: Summary of the sampling sites for this study and each of their location.

Site no.	Site name	Midvaal	NWU	Coordinates		GPS no.
1	After Khuma (KMS)	✓	✓	S 26.91122	E 26.81553	345
2	Vermaasdrift bridge, before KMS (Vaal River)	✓	✓	S 26.93626	E 26.85024	346
3	Enviro Canal (KMS)	✓	✓	S 26.91040	E 26.81418	347
4	Canal and Khuma (KMS)	✓	✓	S 26.91323	E 26.81515	348
5	Khuma (KMS)	X	✓	S 26.89106	E 26.81266	349
6	MidVaal Intake, after KMS (Vaal River)	✓	✓	S 26.93445	E 26.80061	350
7	After Margaret (Vaal River)	✓	✓	S 26.93826	E 26.79579	351
8	N12 Golf Club (KMS)	X	✓	S 26.82011	E 26.83184	352

✓ = Sites at which monthly sampling took place

X = Sites at which no sampling took place for physico-chemical variables

Figure 3.6 illustrates the location for each of the eight sampling sites in the KMS catchment. It is speculated that the various land-use practices in the catchment will have varied effects on the water quality at each sampling site and that they will differ from each other accordingly. Each site and its influences from surrounding land-use practices, will be discussed as part of the results and discussion, CHAPTER 5, though a brief overview is given here.

Five sampling sites (sites 1, 3, 4, 5 and 8) from the total of eight sampling sites were situated within the KMS, whilst the remaining three sites (sites 2, 6 and 7) lie within the Vaal River. Site 8 is situated downstream from the KMS and Kromdraaispruit confluence, but upstream with respect to all the other sampling sites. Following Site 8 is sampling Site 5, which is situated just downstream from the informal settlement of Khuma. Site 1 follows downstream from Site 5 and is but a few meters upstream from where the Enviro Canal (Site 3) enters the KMS at Site 4. Site 2 represents the first of three sampling sites within the Vaal River at the Vermaasdrift bridge and is situated upstream from the point where the KMS enters the Vaal River. Downstream from Site 2 is Site 6 which is situated at Midvaal Water Company's raw water abstraction point in the Vaal River, followed by Site 7. Decanted mine water from #Margaret shaft is discharged into the Vaal River between sites 6 and 7.

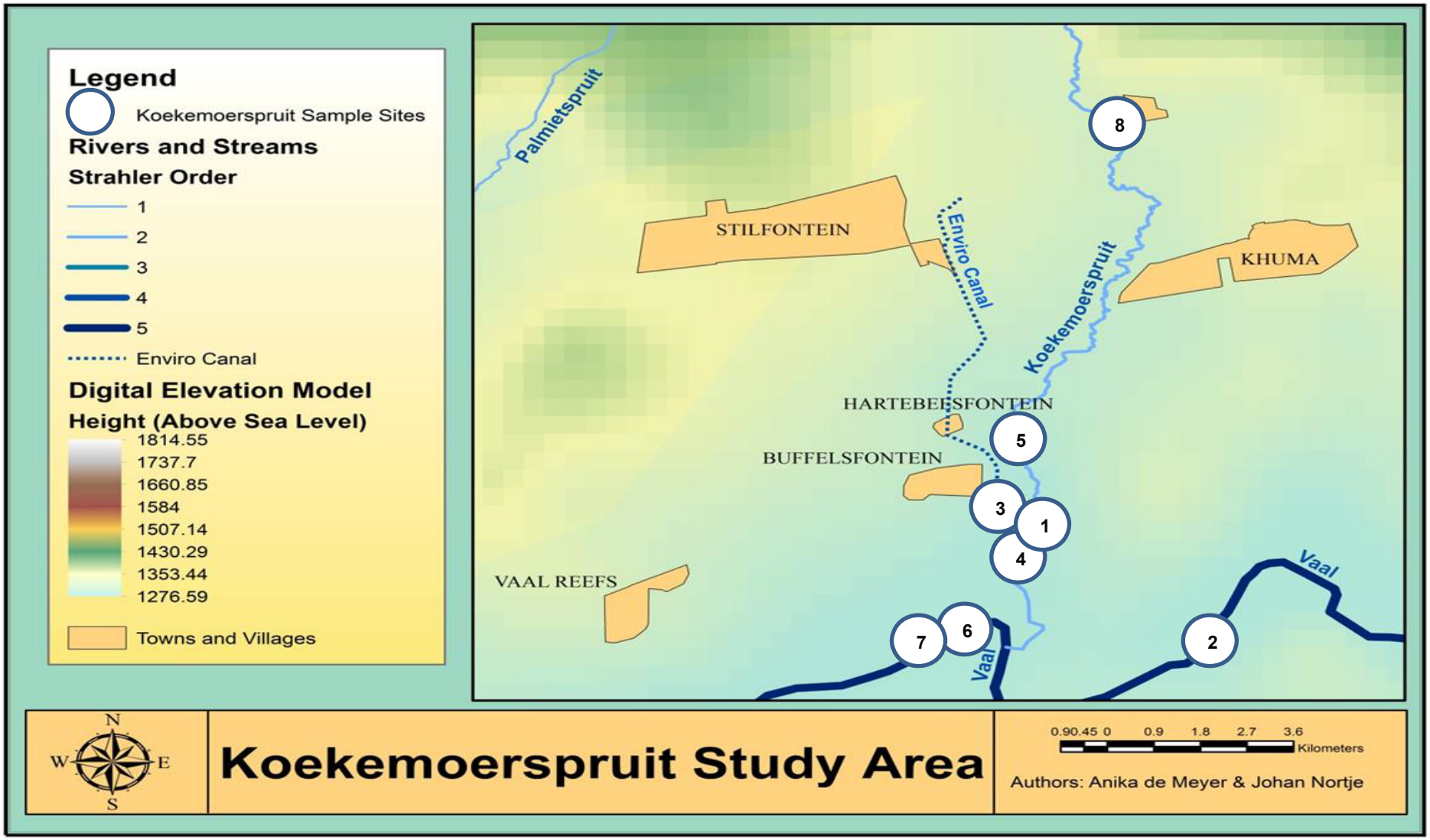

Figure 3.6: Base map of the KMS catchment area indicating the location of the sampling sites (De Meyer & Nortje, 2013).

CHAPTER 4: METHODOLOGY

The aim of this chapter is to provide a brief overview of the methodological procedures followed for sample and data collection, sample preparation, phytoplankton identification and enumeration, as well as the statistical analyses performed on both the physico-chemical and phytoplankton data.

4.1 SAMPLING

Phytoplankton sampling took place on a monthly basis from November 2012 to October 2014 over a 24 month period. Monthly sample collection for physico-chemical analysis was performed by Midvaal Water Company that also provided the physico-chemical data for this project. The dates and sampling sites were coordinated as a quality assurance measure (see Table 3.2). At each site, surface grab samples were taken using a dipstick– and bucket sampler (Hötzel & Croome, 1998). Physico-chemical data for 2001 and 2002 were also provided by Midvaal Water Company. These data were used to conduct a comparison of the water physico-chemistry over time, as an estimate of surface water quality deterioration or improvement within the catchment area.

The surface water grab samples for phytoplankton analysis were immediately transferred to brown, 250 mℓ polyethylene storage bottles, each containing 3 mℓ acidified formaldehyde (2 % final preservative concentration) (Throndsen, 1978). All equipment was thoroughly rinsed between sampling sites (quality assurance) to avoid cross-contamination of phytoplankton genera (ISO, 1998). As stipulated in Swanepoel *et al*. (2008), the samples were kept cool in a small cooler box until all eight sites were sampled. After that the samples were transported to the NWU, Potchefstroom Campus for enumeration.

4.2 PHYTOPLANKTON ANALYSIS

The method used for phytoplankton sample preparation and enumeration is commonly known as “The Inverted Microscope Method of Estimating Algal Numbers”. This method was first described by Utermöhl (1931; 1958), and later adjusted by Lund *et al.* (1958). This section will provide a basic description of the adjusted method from Lund *et al.* (1958) applied in this study.

4.2.1 SAMPLE PREPARATION

The preserved samples needed to be prepared for phytoplankton analysis. Some of these samples contained species of cyanobacteria which have gas vacuoles to maintain their position in the water column. These vacuoles needed to be pressure-deflated for settlement and accurate identification and enumeration. Pressure-deflation was accomplished by transferring the sample (that was shaken well to suspend all phytoplankton) from the storage bottle to a thick-walled metal container (marked accordingly), closing it with a rubber stopper and applying pressure to the stopper with a rubber hammer (see Figure 4.1a) (Swanepoel *et al*., 2008).

The containers were again shaken vigorously to allow the uniform dispersal of phytoplankton present in the sample. A test sample was then transferred with a dispenser pipette to a 6 mℓ sedimentation chamber and left undisturbed for 30 min, allowing the phytoplankton cells to settle to the bottom of the chamber. A new pipette tip was used for each sample to avoid cross-contamination. Once the phytoplankton and suspended particles settled to the bottom, the density of each test sample was studied using an inverted light microscope to determine the actual volume of sub-sample to be enumerated from each site. The sub-sample volumes varied between sites. After the appropriate sub-sample volume from each site was transferred to the sedimentation chambers, it was filled to the top with distilled water and sealed with a glass cover slide. The sedimentation chambers were placed in desiccators for approximately 48 hours to allow for the phytoplankton to settle to the bottom and also to prevent sample evaporation (see Figure 4.1b). The sub-samples were then ready for phytoplankton identification and enumeration (Lund *et al.,* 1958; Swanepoel *et al*., 2008; Utermöhl, 1958).

4.2.2 PHYTOPLANKTON IDENTIFICATION AND ENUMERATION

Each sedimentation chamber was carefully removed from the desiccator and fitted to the round slot of a Zeiss inverted light microscope table (see Figure 4.1c). The 40x objective lens and 10x eyepiece were used to identify and quantify the phytoplankton in each sample. The microscope's eyepiece contained a Whipple-grid of a known size that marked the exact field within which the phytoplankton was counted. One would start by positioning the grid at the diameter of the sedimentation chamber at the far left-hand side, counting every phytoplankton cell which fell within the grid and moving the microscope table one grid at a time, all the way to the far right-hand side. This represented one strip.

A minimum of at least 200 phytoplankton cells were counted and identified for each sample. Thus, in case the first strip did not contain a minimum count of 200 phytoplankton cells, the sedimentation chamber was rotated clockwise to a 90˚ angle and a second strip was completed, this time starting at the far right-hand side of the chamber and moving the grid all the way left. In some instances, up to five strips were counted in this manner before the enumeration process could be halted prior to counting a minimum of 200 phytoplankton cells (Lund *et al.,* 1958; Swanepoel *et al*., 2008; Utermöhl, 1958).

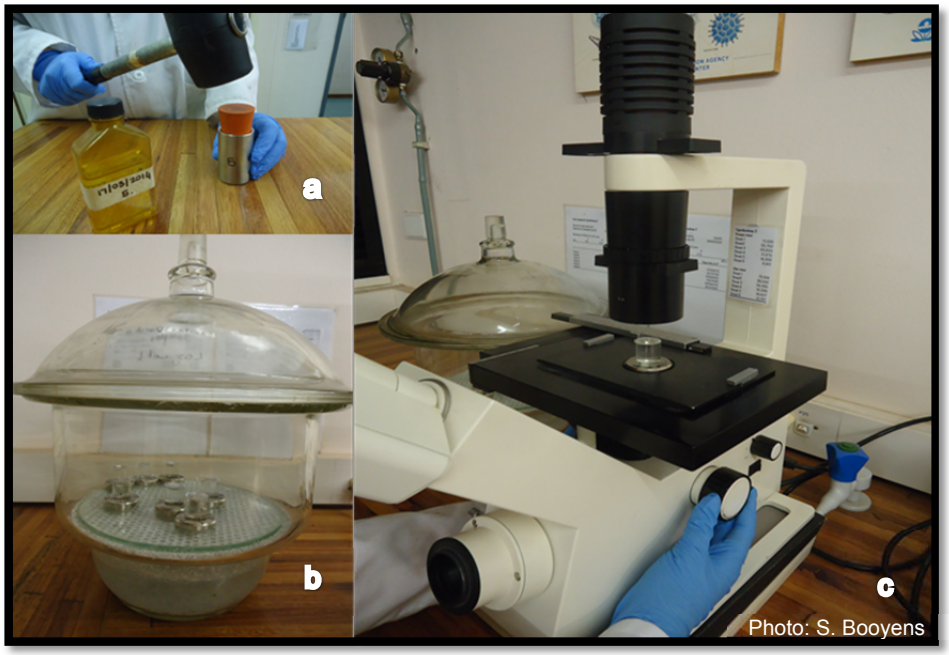

Figure 4.1: Comprehensive illustration of (a) pressure-deflation, (b) phytoplankton settling process and (c) the use of the inverted light microscope.

The phytoplankton was identified to species level as far as possible, with all phytoplankton being identified to genus level. Phytoplankton identification guides such as Croasdale and Flint (1986, 1988), Croasdale *et al.* (1994), Entwisle *et al.* (1996), Gell *et al*. (1999), Guiry *et al*. (2007), Hindák (2008), Janse van Vuuren *et al*. (2006), John *et al*. (2002), Joska and Bolton (1993), Prescott (1983), Taylor *et al*. (2007) and Wehr and Sheath (2002) were used.

Note: All identified species were grouped according to genus for the interpretation of results.

The sampling date, sampling site, volume of each sub-sample, lens objective, number of strips counted, conversion factor as well as the cell count for each phytoplankton genus present in each sample were recorded. These recordings are necessary to determine the cell density (cells/mℓ), percentage composition and successional patterns of the different genera that were present at each site, using an Excel spreadsheet containing the different equations captured in Lund *et al.* (1958), Swanepoel *et al*. (2008) and Utermöhl, (1958).

4.2.3 BIOTIC INDICES

According to Heip *et al*. (1998), the use of diversity indices have gained popularity as part of the methodological proceedings of ecological studies, especially the ones aimed at determining environmental pollution impacts such as this study. Due to lack of a better phrase Heip *et al*. (1998), attributes the latter to "*the never ending quest for indicators of the status of the environment*". It is important however to acknowledge that when diversity indices are being applied to biotic units, for instance phytoplankton genera, two important determinant aspects of diversity should also be considered. Those are species richness as well as species evenness (Aslam, 2009). For this reason, the phytoplankton data recorded during this study were also subjected to four different biotic indices:

1. Shannon-Wiener Diversity Index (*H*) (Shannon & Wiener, 1949 *cited by* Aslam, 2009).

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

$Pi = S/N$

S = Number of individuals of one species

N = Total number of all individuals in the sample

In = Logarithm to base

2. Margalef's Richness Index (Margalef, 1958 *cited by* Aslam, 2009).

$$\boldsymbol{e = (S - 1) / In \, S}$$

S = Total number of species

N = Total number of individuals in the sample

In = Natural logarithm

3. Pielou's Evenness Index ($\boldsymbol{e}$) (Pielou, 1966 *cited by* Aslam, 2009).

$$\boldsymbol{e = H/In\, S}$$

H = Shannon-Wiener Diversity Index

S = Total number of species in the sample

4. Palmer's Algal Genus Pollution Index (Palmer, 1969).

Palmer developed both phytoplankton species and genus pollution indices to be used as tool for rating water samples according to the extent of organic pollution. Table 4.1 contain the 20 phytoplankton genera most tolerant to organic pollution, as was concluded by Palmer. Each phytoplankton genus is assigned a pollution index factor, with 1 being less tolerant than a genus assigned a factor of 2, 3, 4 or 5, and 5 representing the genera most tolerant to organic pollution. The presence of phytoplankton genera (on average > 50 cells/mℓ over 24 months) in a sample of water, if listed under the 20 most tolerant genera, was recorded and assigned their Palmer's pollution index factor. The sum of these factors for a given sample is calculated, and the total would indicate the following:

> 20 = Evidence of high organic pollution

15–19 = Probable evidence of high organic pollution

< 15 = Probable low organic pollution/ Sample is not representative/ Interference of other influential factor

Note: The original Palmer's Algal Genus Pollution Index contained three genera that were later discovered to be taxonomically identified otherwise according to various literatures. These genera are Synedra, that is also identified as the genera Fragilaria (Medlin et al., 2008), Scenedesmus, of that some species are now being identified as Desmodesmus (John et al., 2011), and species of Planktothrix (commonly found in this specific study area) that, based on molecular level analyses, were wrongly identified as Oscillatoria (Conradie et al., 2007). However, to avoid confusion with the use of Palmer's Algal Genus Pollution Index, and because the first edition of John et al. (2002) were used to identify phytoplankton for this study, the original genera will be used. Changes in taxonomy are noted.

Table 4.1: Palmer's Algal Genus Pollution Index in order of decreased tolerance to organic pollution (Palmer, 1969).

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

Note: Please refer to section 2.1.2 of this dissertation for a detailed description of the use of various biotic indices as well as definitions for the different terminology.

4.3 PHYSICO-CHEMICAL VARIABLES

The physico-chemical variables, along with each of their South African National Standards (SANS) operational limit, and South African National Accreditation System's (SANAS) accredited methods for determining present concentrations, are summarised in Table 4.2.

Table 4.2: Summary of the physico-chemical variables and their recommended operational limits (modified from Janse van Rensburg, 2014; SANS, 2015).

Parameter	Abbreviation	Units	SANS 241-1: 2015 Operational Limit	SANAS method number & analytical technique	SANAS accreditation status
Colour	_	mg/ℓ Pt	≤ 15	WL4 - Colorimetric	Not accredited
pH (25 ºC)	_	pH units	≥ 5.0 - ≤ 9.7	WL1 - Electrode	Accredited
Conductivity (25 ºC)	EC	mS/m	≤ 170	WL2 - Electrode	Accredited
Turbidity	NTU	NTU	≤ 1	WL3 – NTU's	Accredited
Chloride	Cl^-	mg/ℓ	≤ 300	GL – 7-5 – Discrete analyser	Accredited
Sulphate	SO_4^{2-}	mg/ℓ	≤ 500	GL – 7-4 – Discrete analyser	Accredited
Nitrates	NO_3^-	mg/ℓ	≤ 0.9	GL – 7-2 – Discrete analyser	Accredited
Ammonium	NH_4^+	mg/ℓ	≤ 1.5	GL – 7-1 – Discrete analyser	Accredited
Soluble orthophosphate	PO_4^{-3}	mg/ℓ	_	CFA – 1B – Cont. flow analyser	Not accredited
Cyanide (recoverable)	CN	mg/ℓ	≤ 0.2	CFA – 1D – Cont. flow analyser	Not accredited
Total chlorophyll	T.Chl	µg/ℓ	_	AI 2 - Extraction	Accredited
Faecal coliform bacteria	F.coli	cfu/100 mℓ	Not detected	BL 3 – Membrane filter	Accredited
Iron	Fe	mg/ℓ	≤ 2.0	ICP 1 – Inductively coupled plasma	Accredited
Manganese	Mn	mg/ℓ	≤ 0.4	ICP 1 – Inductively coupled plasma	Accredited
Zinc	Zn	mg/ℓ	≤ 5.0	ICP 1 – Inductively coupled plasma	Accredited
Copper	Cu	mg/ℓ	≤ 2.0	ICP 1 – Inductively coupled plasma	Accredited
Aluminium	Al	mg/ℓ	≤ 0.3	ICP 1 – Inductively coupled plasma	Accredited
Sodium	Na^+	mg/ℓ	≤ 200	ICP 1 – Inductively coupled plasma	Accredited
Arsenic	As	mg/ℓ	≤ 0.01	ICP 1 – Inductively coupled plasma	Accredited
Uranium	U	mg/ℓ	≤ 0.03	ICP 1 – Inductively coupled plasma	Accredited
Total organic carbon	TOC	mg/ℓ	≤ 10	AAL 5 - Spectrophotometric	Accredited

4.4 STATISTICAL ANALYSIS

According to Gevrey *et al*. (2004), numerous studies have correlated phytoplankton assemblages to physico-chemical (environmental) variables. In most cases these approaches proved to deliver questionable results because the phytoplankton assemblage and physico-chemical data were considered separate from one another. This could mainly be attributed to the fact that both phytoplankton assemblage and physico-chemical data commonly deliver immense data sets that proved difficult to be processed collectively by most statistical programmes (Giske *et al*., 1998).

Multivariate statistical analysis using programmes such as CANOCO 4.5 (Ter Braak & Šmilauer, 2002) and STATISTICA 12 (StatSoft Inc©, 2013) have however enabled researchers to process and correlate intricate sets of environmental data simultaneously (Gevrey *et al*., 2004). According to Simeonov *et al*. (2003), the use of multivariate statistical analysis such as PCA's (principal component analyses), result in more simplified interpretations and better comprehension of water quality assessment studies, similar to this one.

Subjecting these intricate data sets to multivariate statistical analyses could ultimately narrow down the most likely influences on the water quality of catchments, making it a dependable means for managing water resources and most important, the prompt generation of solutions to solve or prevent environmental problems commonly associated with pollution (Simeonov *et al*., 2003).

PCA's are designed for pattern recognition whereby it attempts to explain the variance within the data. Basically, PCA's transform the inter-correlated variables (physico-chemical variables) within the data to a more compact set of uncorrelated variables. It does so by assigning an eigenvalue (weighted average) to each of the variables. The eigenvalues are representative of the significance in terms of variance exhibited by each of the variables. A process of elimination is applied based on these eigenvalues and the remaining set of variables, the principal components, represent the variables within the data set responsible for the most variance (Singh *et al*., 2005; Kazi *et al*., 2009).

Because it was the aim of this study to integrate the use of water physico-chemical variables and phytoplankton assemblages as a means of water quality assessment, the data were subjected to multivariate statistical analysis using CANOCO 4.5 and STATISTICA 12.

The following tests were performed: 1) **Kolmogorov – Smirnov and Lilliefors test** for normality, that clearly indicated that the data did not meet the assumption for normality. This finding led to the following two tests being performed 2) **Kruscal – Wallis multiple comparison between p-values** for non-parametric data, to determine whether significant differences exist between the sets of data and also between the different sites 3) **Spearman Rank Order Correlation test**, to determine whether significant correlations exist between the two sets of data and also the different sites 4) **Descriptive statistics** to determine the valid N, mean, minimum, maximum and standard deviation of the samples and 5) **PCA's** in an attempt to reduce the number of variables and to extract a smaller number of components. Statistical analysis 1 to 4 were performed using STATISTICA 12 (StatSoft Inc©, 2013), whereas the PCAs (number 5) were performed using CANOCO 4.5 (Ter Braak & Šmilauer, 2002).

Further data analyses in the form of line graphs, area graphs and pie graphs were also conducted, using Microsoft Excel 2010. These results were used to illustrate and interpret phytoplankton assemblages for each site over the study period.

CHAPTER 5: RESULTS & DISCUSSION

After the data from the eight sampling sites in the study area were subjected to statistical analysis, it was decided that the results derived from only five of the eight sampling sites will be displayed and discussed here. The selection criteria were based on the importance of the sites' location with respect to their influence on water quality in the study area, as well as the availability of physico-chemical data. The sites which best fitted the selection criteria include sites 1, 2, 3, 4 and 6 (see Table 3.2). It is important however to separate these sites on the basis of their location within the sampling area, in order to get an understanding of the influence of the KMS on the water quality of the Vaal River. For this reason, sites that were located within the KMS were distinguished from Vaal River sites.

Site 1 represents the first site within the KMS that would reflect the possible water quality impacts of the informal settlement of Khuma and is situated upstream from where the Enviro Canal, that represents Site 3, enters the KMS at Site 4. Contrary to Site 1, that would reflect water quality deterioration typically associated with informal settlements in the form of organic pollution, Site 3's outflows would be indicative of mining impacts, indicating how subsurface mine water is decanted into the KMS via this canal (see section 3.1). Site 4 would reflect the combined effects from both sites 1 and 3 and is also the last site sampled before the KMS converges with the Vaal River. Photographs of sites 1, 3 and 4 in the KMS are illustrated in Figure 5.1.

Site 2 represents the first sampling site in the Vaal River at the Vermaasdrift bridge and is situated upstream from the point where the KMS enters the Vaal River. Site 2 would indicate water quality of the Vaal River unaffected by the KMS. Downstream from Site 2 is Site 6 that is situated at Midvaal Water Company's raw water abstraction point and also downstream from the point where the KMS converges with the Vaal River. Site 6 would thus reflect the water quality impacts of the KMS on the Vaal River. Photographs of sites 2 and 6 in the Vaal River are illustrated in Figure 5.2.

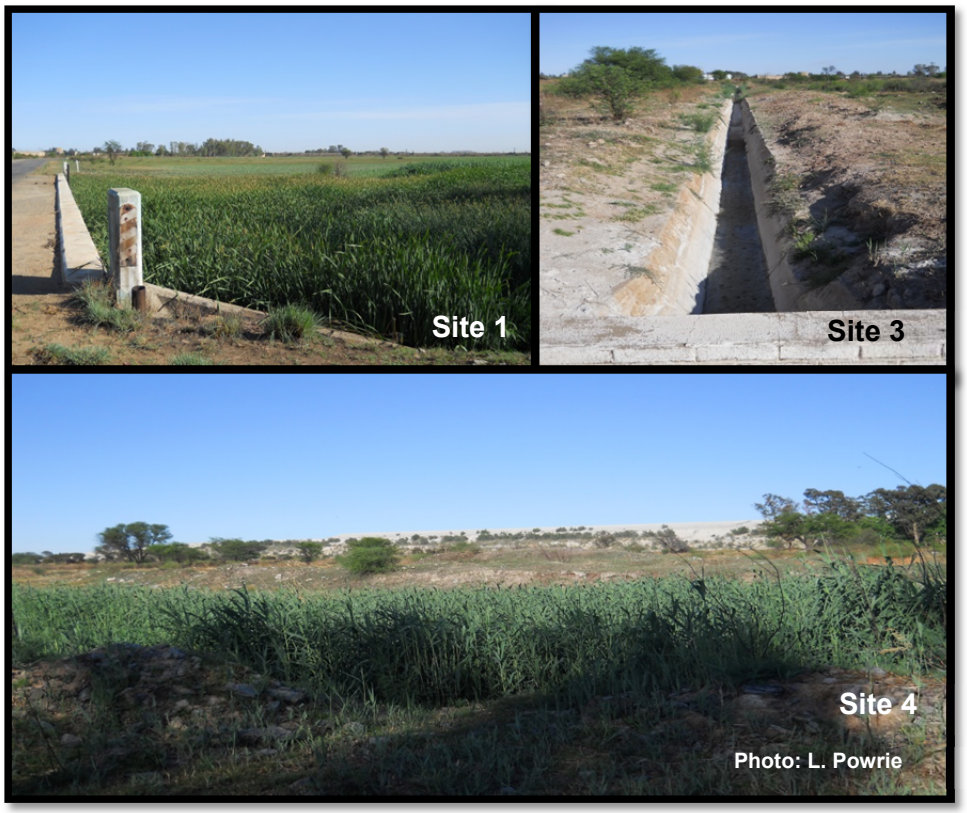

Figure 5.1: Illustration of sites 1, 3 and 4 situated within the KMS.

Figure 5.2: Illustration of sites 2 and 6 situated within the Vaal River.

5.1 DESCRIPTIVE STATISTICS

Table 5.1 summarises the descriptive statistics (valid n, mean, minimum, maximum and standard deviation) for all the physico-chemical and biological variables measured at sites 1, 2, 3, 4 and 6 over the 24 month study period (n = 24). Table 5.2 contains the phytoplankton descriptive statistics for the same five sites. These data serve as an overview of the current water quality status of the sampling area.

Table 5.1: Summary of the descriptive statistics for all the physico-chemical variables determined over a 24 month study period (2012 - 2014).

		KMS			VAAL RIVER	
VARIABLE	**DESCRIPTIVE**	**SITE 1**	**SITE 3**	**SITE 4**	**SITE 2**	**SITE 6**
pH	Valid n	24	21	24	24	24
(pH units)	Mean	7.67	8.65	7.70	8.78	8.75
	Minimum	7.23	7.47	7.11	7.80	7.70
	Maximum	8.14	10.64	8.60	9.55	9.50
	Standard Deviation	0.25	0.92	0.35	0.49	0.49
Colour	Valid n	24	21	24	24	24
(mg/ℓ Pt)	Mean	61.67	6.45	61.33	96.25	92.29
	Minimum	2.50	2.50	2.50	40.00	25.00
	Maximum	250.00	45.00	250.00	300.00	300.00
	Standard Deviation	75.08	10.21	74.97	73.97	69.44
Turbidity	Valid n	24	21	24	24	24
(NTU)	Mean	14.41	40.54	15.26	28.01	24.41
	Minimum	2.90	1.10	1.10	8.40	10.20
	Maximum	46.60	334.00	53.30	120.00	120.00
	Standard Deviation	11.94	77.76	14.55	27.95	25.32
EC	Valid n	24	21	24	24	24
(mS/m)	Mean	114.67	238.91	129.28	55.18	57.10
	Minimum	28.60	96.00	29.00	25.00	24.30
	Maximum	154.00	314.00	279.00	78.60	75.00
	Standard Deviation	26.46	50.17	45.01	14.33	13.93
Fe	Valid n	24	21	24	24	24
(mg/ℓ)	Mean	0.10	0.05	0.10	0.11	3.39
	Minimum	0.01	0.01	0.01	0.01	0.01
	Maximum	0.93	0.40	0.90	0.79	28.10
	Standard Deviation	0.19	0.09	0.18	0.23	7.99
Mn	Valid n	24	21	24	24	24
(mg/ℓ)	Mean	0.29	0.04	0.29	0.03	7.29
	Minimum	0.01	0.01	0.01	0.01	0.01
	Maximum	2.00	0.22	2.00	0.05	59.40
	Standard Deviation	0.41	0.05	0.42	0.02	17.17

Table 5.1 (cont.): Summary of the descriptive statistics for all the physico-chemical variables determined over a 24 month study period (2012-2014).

		KMS			VAAL RIVER	
VARIABLE	**DESCRIPTIVE**	**SITE 1**	**SITE 3**	**SITE 4**	**SITE 2**	**SITE 6**
Cl^-	Valid n	24	21	24	24	24
(mg/ℓ)	Mean	95.59	182.76	121.13	49.71	47.78
	Minimum	9.20	79.00	8.00	18.00	14.07
	Maximum	146.00	327.00	330.00	81.00	64.90
	Standard Deviation	29.87	54.73	63.63	15.45	14.15
SO_4^{2-}	Valid n	24	21	24	24	24
(mg/ℓ)	Mean	191.08	932.95	291.38	111.91	97.47
	Minimum	28.00	231.00	28.00	46.00	29.48
	Maximum	375.00	1435.00	900.00	218.00	149.50
	Standard Deviation	96.94	244.70	232.04	44.20	30.46
Na^+	Valid n	23	20	23	23	24
(mg/ℓ)	Mean	90.00	258.05	109.17	45.51	44.29
	Minimum	9.00	104.00	9.00	12.00	0.12
	Maximum	137.00	380.00	314.00	76.00	70.10
	Standard Deviation	24.21	73.15	69.41	16.51	21.95
As	Valid n	24	21	24	24	24
(mg/ℓ)	Mean	0.01	0.02	0.01	0.01	2.94
	Minimum	0.01	0.01	0.01	0.01	0.01
	Maximum	0.02	0.07	0.10	0.02	28.50
	Standard Deviation	0.00	0.02	0.01	0.00	7.21
CN^-	Valid n	24	21	24	24	24
(mg/ℓ)	Mean	0.03	0.01	0.02	0.01	0.02
	Minimum	0.01	0.01	0.01	0.01	0.01
	Maximum	0.35	0.02	0.10	0.07	0.20
	Standard Deviation	0.07	0.00	0.02	0.01	0.03
Zn	Valid n	24	21	24	24	24
(mg/ℓ)	Mean	0.04	0.02	0.03	0.01	0.01
	Minimum	0.01	0.01	0.01	0.01	0.01
	Maximum	0.19	0.09	0.10	0.06	0.10
	Standard Deviation	0.04	0.02	0.02	0.01	0.01
Cu	Valid n	24	21	24	24	24
(mg/ℓ)	Mean	0.02	0.01	0.01	0.01	0.02
	Minimum	0.01	0.01	0.01	0.01	0.01
	Maximum	0.19	0.09	0.10	0.10	0.10
	Standard Deviation	0.04	0.02	0.01	0.02	0.03
Al	Valid n	24	21	24	24	24
(mg/ℓ)	Mean	0.09	0.04	0.08	0.15	0.02
	Minimum	0.01	0.01	0.01	0.01	0.01
	Maximum	1.20	0.15	1.10	1.30	0.10
	Standard Deviation	0.26	0.05	0.23	0.36	0.02

Table 5.1 (cont.): Summary of the descriptive statistics for all the physico-chemical and biological variables determined over a 24 month study period (2012-2014).

		KMS			VAAL RIVER	
VARIABLE	**DESCRIPTIVE**	**SITE 1**	**SITE 3**	**SITE 4**	**SITE 2**	**SITE 6**
U	Valid n	24	21	24	24	24
(mg/ℓ)	Mean	0.01	0.04	0.02	0.01	0.00
	Minimum	0.01	0.01	0.01	0.01	0.01
	Maximum	0.03	0.14	0.10	0.01	0.00
	Standard Deviation	0.01	0.04	0.03	0.00	0.00
TOC	Valid n	24	21	24	23	22
(mg/ℓ)	Mean	9.82	2.92	9.44	5.79	5.03
	Minimum	5.80	0.30	2.30	4.70	0.01
	Maximum	15.00	16.00	15.00	7.00	8.10
	Standard Deviation	2.64	4.01	3.63	0.71	2.57
Faecal coliforms	Valid n	24	21	24	24	24
(cfu/100 mℓ)	Mean	3444.46	420.00	3342.67	235.00	521.59
	Minimum	20.00	0.00	24.00	9.00	0.05
	Maximum	16000.00	4200.00	16000.00	1800.00	4840.00
	Standard Deviation	5814.37	977.46	5844.05	411.82	1365.84
T. Chl	Valid n	24	21	24	23	24
(µg/ℓ)	Mean	61.83	21.01	52.10	127.18	145.96
	Minimum	4.00	0.25	5.20	8.00	0.14
	Maximum	235.00	176.00	227.00	399.00	412.90
	Standard Deviation	74.64	39.59	65.98	82.52	104.86
NH_4^+	Valid n	24	21	24	24	24
(mg/ℓ)	Mean	22.80	1.36	20.86	0.22	0.38
	Minimum	0.25	0.10	0.10	0.10	0.10
	Maximum	47.00	11.00	47.00	0.25	2.10
	Standard Deviation	14.83	2.46	16.11	0.06	0.45
PO_4^{-3}	Valid n	24	21	24	24	24
(mg/ℓ)	Mean	3.19	0.13	3.52	0.09	0.12
	Minimum	0.22	0.03	0.05	0.03	0.03
	Maximum	19.00	1.50	23.00	0.21	0.60
	Standard Deviation	3.91	0.32	4.70	0.06	0.12
NO_3^-	Valid n	24	21	24	24	24
(mg/ℓ)	Mean	3.45	5.43	3.50	1.70	1.32
	Minimum	0.50	0.60	0.50	0.25	0.25
	Maximum	8.60	12.00	10.00	7.90	4.90
	Standard Deviation	2.01	4.24	2.72	1.75	1.07

NOTE: Please refer to APPENDIX A.1 for the multiple comparisons of p-values (2 tailed) that indicate significant differences ($p<0.05$) between sites.

Rather than providing an in depth discussion of all the different physico-chemical and biological variables which had been monitored, only those that differed most between sites will form the focus point in this section.

Amongst the five sites, colour was the first variable to show noticeable differences, with mean values ranging between 6.45 mg/ℓ Pt at Site 3 (canal), and 96.25 mg/ℓ Pt at Site 2 (before KMS). Though the mean value for colour at Site 3 (canal) is markedly lower than all of the other sites, a clear distinction can be made between the sites in the KMS and the Vaal River, with overall higher values in the Vaal River sites. Both sites 2 (before KMS) and 6 (after KMS) in the Vaal River reached maximum values of 300.00 mg/ℓ Pt that, along with the maximum values for all three sites in the KMS, far exceeds the SANS 241-1:2015 operational limit of ≤ 15 mg/ℓ (Table 4.2). In section 2.1.1 under *Turbidity and Colour*, suspended organic substances such as phytoplankton, represents one of the main contributing components of both turbidity and colour (DWA, 1996). Therefore, the overall higher observed mean values for colour in the Vaal River (sites 2 and 6) are to be expected, since overall higher total phytoplankton cell density were observed at both these sites according to Figure 5.8.

Accordingly, and given the strong correlation between colour and turbidity (Dallas & Day, 2004), the mean turbidity for both sites 2 (28.01 NTU) and 6 (24.41 NTU) in the Vaal River are not only more closely related, but also higher than the mean turbidity of sites 1 (14.41 NTU) and 4 (15.26 NTU) in the KMS, except for Site 3 (canal), which had the highest mean turbidity of 40.54 NTU compared to the other sites. The SANS 241-1:2015 operational limit for turbidity is ≤ 1 NTU, which is far less than the mean turbidity indicated for Site 3 (Table 4.2).

In a study by Matowanyika (2010) to determine the impact of the informal settlement of Alexandra on the water quality of the Jukskei River, turbidity had reached a maximum of 50 NTU. Thus, in comparison, the mean turbidity for Site 3 (canal) in the KMS is becoming comparable to one of the most polluted rivers in South Africa (Matowanyika, 2010). More alarming is that the maximum of 334 NTU observed at Site 3 greatly exceeds the maximum of 50 NTU observed in the Jukskei River between May and December 2009, but also that the 2014 raw data revealed turbidity exceeded 50 NTU's on six separate occasions. It was mentioned under section 2.2 that elevated sediment loads is one of the typical symptoms noticeable in freshwater bodies subjected to mine water discharges (Coetzee *et al*., 2006). Thus, it was anticipated that Site 3 would (in comparison) show increased turbidity.

Also, Winde and Van der Walt (2004) revealed that dolomitic groundwater being discharged from Stilfontein Gold Mine at Site 3 is saturated with Ca and CO_3^{-2}. The carbonates readily precipitate to form thick scales along the canal (Winde & Van der Walt, 2004), which was clearly visible during the sampling sessions. Unfortunately, Ca was not monitored as a physico-chemical variable by Midvaal Water Company.

Contrary to colour and turbidity (with the exception of Site 3), the values measured for EC were much higher in the KMS sites than in the Vaal River sites, with the lowest mean of 55.18 mS/m measured at Site 2 (before KMS) in the Vaal River and the highest mean of 238 mS/m at Site 3 (canal) in the KMS compared to the other sites. This could also be traced back to the high turbidity values measured at Site 3 (canal), since Ca^{2+} (a dissolved inorganic ion) represents one of the most significant contributing constituents to high EC (Van Loon & Duffy, 2005), that may also contribute to elevated turbidity (DWA, 1996).

In the Blesbokspruit that is also subjected to mine water discharges from the Grootvlei Gold Mine (see section 2.2), the EC of the surface water samples ranged between 521 mS/m and 2400 mS/m, showing a steady increase downstream of the liming (CaO) plant before the treated water enters the wetland (Roychoudhury & Starke, 2006). Although the highest mean EC of 238 mS/m observed at Site 3 (canal) is lower than the maximum of 2400 mS/m observed for the Blesbokspruit, it is still above the SANS 241-1:2015 operational limit of ≤ 170 mS/m (Table 4.2).

In the current study, a clear distinction could be drawn between the KMS and Vaal River based on colour, turbidity and EC, requiring a closer look at the individual contributing constituents which were monitored.

Throughout section 2.2 of this study, Fe, Mn and As are constantly mentioned as contaminants of concern in freshwater impacted by gold-mining activities. This was also clearly the case in the Blesbokspruit where tailings deposits posed a definite threat (Roychoudhury & Starke, 2006; Schoeman & Steyn, 2001). Site 6 (after KMS) in the Vaal River displayed the highest mean values for Fe (3.39 mg/ℓ), Mn (7.29 mg/ℓ) and As (2.94 mg/ℓ), with exceptionally high maximum values (Fe = 28.10 mg/ℓ; Mn = 59.40 mg/ℓ; As = 28.50 mg/ℓ). These values are even much higher than that observed for surface water samples in the Blesbokspruit (Roychoudhury & Starke, 2006). The mean values observed are also well above the SANS 241-1:2015 operational limits for Fe (≤ 2.0 mg/ℓ), Mn (≤ 0.4 mg/ℓ) and As (≤ 0.01 mg/ℓ) (Table 4.2).

The large variance observed between the data of Site 6 (after KMS) and the remaining sites (Table 5.1) revealed that the maximum values observed at Site 6 represent single “spikes” in Fe, Mn and As. All three of these variables reached their maximum concentrations during April 2014. Winde and Van der Walt (2004) stated that the high evaporation rates and elevated water tables prevailing in the immediate vicinity of the slimes dams in the KMS, create the perfect conditions for crust formation. This raises concern, especially after extended dry episodes when the initial influx of runoff into the KMS is particularly contaminated, adding to the concentrations of trace metals (Winde & Van der Walt, 2004).

Furthermore, it is also known that the KMS received a great deal of rain during March 2014, since this was the only month in 2014 during which Site 8 (N12 after eye) in the KMS could be sampled for phytoplankton analysis. Site 8 ran completely dry for the remaining months of 2014 (discussion follows under section 5.2, Figure 5.13). This can therefore explain the sudden “spike” in Fe, Mn and As during April 2014 as most probably the result of the initial influx of polluted runoff after the rain experienced in March 2014.

The maximum values for Fe (0.79 mg/ℓ), Mn (0.05 mg/ℓ) and As (0.02 mg/ℓ) indicated for Site 2 (before KMS) in the Vaal River are equivalent to, or only fractionally lower than, the values measured for the same variables at the KMS sites. This could either indicate the influence of the KMS on the Vaal River, or the sudden influx of polluted mine effluent entering the Vaal River between the KMS’s convergence with the Vaal River and Site 6 (after KMS). The mean values for these variables at the KMS sites were higher than that of Site 2 (before KMS), but still much lower compared to that of Site 6 (after KMS), suggesting that the probable cause of elevated mean values at Site 6 (after KMS) could have been an initial influx of polluted runoff entering the Vaal River after it converges with the KMS.

Na^+, Cl- and SO_4^{2-} were present at much higher concentrations in the KMS than in the Vaal River. This occurrence would account for the high EC values measured at the KMS sites, since Na^+, Cl^- and SO_4^{2-} (like the Ca^{2+} at Site 3) represent ions which contribute most significantly to EC (Van Loon & Duffy, 2005) as well as salinity, particularly elevated Cl^- concentrations (Dallas & Day, 2004). The mean values for Na^+ ranged between 44.29 mg/ℓ (Site 6) and 258.05 mg/ℓ (Site 3). Cl^- ranged between 47.78 mg/ℓ (Site 6) and 182.76 mg/ℓ (Site 3), and SO_4^{2-} between 97.47 mg/ℓ (Site 6) and an exceptional high of 932.95 mg/ℓ at Site 3. Without exception, the mean values determined for these variables at each of the KMS sites had followed the same pattern, namely: Site 3 (canal) reached the highest mean values, followed by Site 4 (canal and Khuma combined) and then Site 1 (after Khuma).

This indicates that for the given variables, Site 3 (canal) consistently made the biggest contribution to the values observed at Site 4 (canal and Khuma combined).

Similar to increased sediment loads, elevated salinity and SO_4^{2-} concentrations are also typical symptoms of freshwater impacted by mine effluent discharges (Winde, 2010). It has been mentioned under section 2.2 that SMD in gold-mining areas underlain by dolomitic earth poses a greater risk than AMD (Labuschagne, 2007). In fact, it has been established that the recirculation of mine water in the KMS, repeatedly leaching through the dolomites, intensifies salinity and SO_4^{2-} concentrations (DWA, 2004). Therefore, the high concentrations of Na^+, Cl^- and SO_4^{2-} at Site 3 (canal) were anticipated, since it represents the KMS Site most likely to be indicative of gold-mining impacts.

The mean Na^+ concentration at Site 3 (258.05 mg/ℓ) is above the SANS 241-1:2015 operational limit of ≤ 200 mg/ℓ, whereas the mean Cl^- concentration (182.76 mg/ℓ) is below that of SANS 241-1:2015 at ≤ 300 mg/ℓ. The mean SO_4^{2-} concentration (932.95 mg/ℓ) is well above the SANS 241-1:2015 operational limit of ≤ 500 mg/ℓ (Table 4.2).

Compared to the Blesbokspruit, only the maximum observed concentration of Cl^- (327 mg/ℓ) at Site 3 (canal) was about twice as much as the maximum observed Cl^- concentration (161 mg/ℓ) in the Blesbokspruit, whereas the opposite was true for Na^+ (855 mg/ℓ) and SO_4^{2-} (4194 mg/ℓ) in the Blesbokspruit (Roychoudhury & Starke, 2006) against the much lower Na^+ (258.05 mg/ℓ) and SO_4^{2-} (932.95 mg/ℓ) maximum concentrations at Site 3 (canal) in the KMS. Also, the lowest mean concentrations observed for Na^+, Cl^- and SO_4^{2-} at Site 6 (after KMS) in the Vaal River, gives an indication of how little impact the KMS has on the water quality of the Vaal River, at least for this given set of variables.

TOC and Faecal coliforms displayed different trends amongst the KMS sites, where Site 1 (after Khuma) reached the highest mean values for both TOC (9.82 mg/ℓ) and Faecal coliforms (3444.46 cfu/100 mℓ), followed by Site 4 (canal and Khuma combined) and then Site 3 (canal). From this it can be concluded that Site 1 (after Khuma) consistently made the largest contribution to both the mean TOC and Faecal coliforms values at Site 4 (canal and Khuma combined), as Site 3 (canal) displayed the lowest mean TOC (2.92 mg/ℓ) and Faecal coliforms (420.00 cfu/100 mℓ) values, including values at sites 2 (before KMS) and 6 (after KMS) in the Vaal River.

Given that Site 1 is situated below the informal settlement of Khuma, the extremely high mean and maximum values observed for TOC and Faecal coliforms can be expected, since increased Faecal coliform counts is not only indicative of faecal pollution (Dallas & Day, 2004; Willey *et al*., 2008), but would naturally be related to increased TOC's as the direct result of bacterial decomposition (Chapman, 1996; Matowanyika, 2010).

In the study by Matowanyika (2010) that determined the impact of Alexandra on the water quality of the Jukskei River (see above), higher *Escherichia coli* (included under Faecal coliforms) counts were observed at the site downstream from the informal settlement. Even though the same trends were observed at Site 1 (after Khuma) in the KMS, the mean TOC (9.82 mg/ℓ) still measures below the SANS 241-1:2015 operational limit of ≤ 10 mg/ℓ. According to the SANS 241-1:2015 operational limit, Faecal coliforms shouldn't be present in any of the samples collected. This was however only the case at Site 3 (canal), where the minimum Faecal coliforms counts were 0.0 cfu/100mℓ (see Table 5.1).

Likewise, the observed mean values for both NH_4^+ and PO_4^{-3} reached a maximum at sites 1 (after Khuma) and 4 (canal and Khuma combined) in the KMS. These values are well above the observed mean values for sites 2 (before KMS) and 6 (after KMS) in the Vaal River. Site 1 (after Khuma) had the highest mean level for NH_4^+ (22.80 mg/ℓ), followed by Site 4 (20.86 mg/ℓ), whereas Site 4 (canal and Khuma combined) had the highest mean level for PO_4^{-3} (3.52 mg/ℓ), followed by Site 1 (3.19 mg/ℓ). In the Hartbeespoort Dam, classified as hyper-eutrophic, a study by Ololo (2013) on the limnological factors affecting phytoplankton biodiversity revealed that the maximum observed value for NH_4^+ between February 2011 and March 2012 was 1.58 mg/ℓ, whereas PO_4^{-3} reached a maximum of 3.50 mg/ℓ. Thus, in the KMS, the highest mean PO_4^{-3} concentration (3.52 mg/ℓ) for Site 4 (canal and Khuma combined), nearly matches the maximum observed PO_4^{-3} concentrations in the Hartbeespoort Dam, whereas the highest mean NH_4^+ concentration (22.80 mg/ℓ) at Site 1 (after Khuma) exceeded that measured in the Hartbeespoort Dam by almost 15 fold. This is most likely owed to nutrient inputs derived from the informal settlement of Khuma, that would naturally be more concentrated in smaller streams such as the KMS compared to the Hartbeespoort Dam. Nevertheless, in accordance with the specifications given by Ololo (2013) this section of the KMS could then be classified as hyper-eutrophic. It is acknowledged that river and dam limnology differs significantly however, the deliberate comparison of the KMS to Hartebeespoort Dam is to indicate the extent of nutrient input from Khuma at Site 1 (after Khuma). The highest mean NH_4^+ concentration determined for the KMS exceeds the SANS 241-1:2015 operational limit of ≤ 1.5 mg/ℓ by far. No SANS 241-1:2015 operational limit for PO_4^{-3} is stipulated (see Table 4.2).

The observed mean values for T.Chl were much higher at both sites 2 (before KMS) and 6 (after KMS) in the Vaal River compared to the KMS sites. The uptake of PO_4^{-3} and NH_4^+ by phytoplankton in the Vaal River could account for the observed lower mean PO_4^{-3} and NH_4^+ levels present at the Vaal River sites, and vice versa. Studies performed by Ololo (2013) and Ariyadej *et al*. (2004) led to a similar finding. T.Chl concentration was highest at Site 6 (after KMS) with a mean value of 145.96 µg/ℓ and lowest at Site 3 (canal), with a mean value of 21.01 µg/ℓ. The results obtained from Ololo (2013) revealed a maximum Chl-*a* concentration of 8693 µg/ℓ in the Hartbeespoort Dam. No SANS 241-1:2015 operational limit for T.Chl was stipulated (see Table 4.2).

According to Table 5.2, Vaal River sites (2 and 6) exhibited highest mean phytoplankton cell density (cells/mℓ) for all the phytoplankton taxa over the study period, except Euglenophyceae. The highest Euglenophyceae density was recorded at Site 1 (after Khuma) in the KMS. According to the descriptive statistics (Table 5.1), the high Faecal coliform counts and NH_4^+ concentrations indicate high organic pollution at this site. This, together with the fact that Euglenophyceae is considered highly tolerant to organic pollution (Palmer, 1969), can explain why greater densities of Euglenophyceae occur at Site 1 (after Khuma).

Throughout the study period, the dominant phytoplankton taxa reached highest mean cell density at Site 6 (after KMS) and include (in descending order) Chlorophyceae (31,293 cells/mℓ), Cyanophyceae (20,710 cells/mℓ) and Bacillariophyceae (12,248 cells/mℓ). Euglenophyceae reached the highest mean cell density at Site 2 (1,177 cells/mℓ). The mean Euglenophyceae density were very similar between Site 2 (before KMS) in the Vaal River and Site 1 (1,117 cells/mℓ) in the KMS.

In the KMS, dominant phytoplankton taxa reached highest mean cell densities throughout the study period at Site 4 (Cyanophyceae 11,697 cells/mℓ; Bacillariophyceae 7,440 cells/mℓ; Chlorophyceae 5,449 cells/mℓ), with the exception of Euglenophyceae, that reached highest cell density at Site 1 (after Khuma). Site 3 (canal) constantly exhibited the lowest mean cell densities for all dominant phytoplankton taxa (Chlorophyceae 2,031 cells/mℓ; Cyanophyceae 1,121 cells/mℓ; Bacillariophyceae 291 cells/mℓ; Euglenophyceae 84 cells/mℓ). It is suspected that the high salinity and lack of nutrients (Table 5.1) may cause lower phytoplankton densities at Site 3 (canal).

Table 5.2: Summary of the descriptive statistics for the cell densities of phytoplankton taxa determined over a 24 month study period (2012-2014).

		KMS			VAAL RIVER	
VARIABLE	**DESCRIPTIVE**	**SITE 1**	**SITE 3**	**SITE 4**	**SITE 2**	**SITE 6**
Cyanophyceae	Valid n	24	24	24	24	24
(cells/mℓ)	Mean	3,938	1,121	11,697	13,543	20,710
	Minimum	0	0	0	0	0
	Maximum	27,885	5,586	204,252	67,210	130,202
	Standard Dev.	7,748	1,490	41,264	18,284	35,663
Bacillariophyceae	Valid n	24	24	24	24	24
(cells/mℓ)	Mean	1,679	291	7,440	10,446	12,248
	Minimum	116	0	30	626	1,859
	Maximum	11,154	2,610	90,090	38,253	56,056
	Standard Dev.	2,719	557	19,602	9,913	12,376
Chlorophyceae	Valid n	24	24	24	24	24
(cells/mℓ)	Mean	2,919	2,031	5,449	25,642	31,293
	Minimum	244	124	416	4,862	6,900
	Maximum	11,967	17,446	33,605	99,564	93,427
	Standard Dev.	2,666	3,866	9,519	22,536	26,292
Cryptophyceae	Valid n	24	24	24	24	24
(cells/mℓ)	Mean	0	0	15	282	780
	Minimum	0	0	0	0	0
	Maximum	0	6	358	5,005	7,865
	Standard Dev.	0	1	73	1,023	2,046
Chrysophyceae	Valid n	24	24	24	24	24
(cells/mℓ)	Mean	1	1	4	21	149
	Minimum	0	0	0	0	0
	Maximum	18	14	72	358	3,075
	Standard Dev.	4	4	15	77	623
Dinophyceae	Valid n	24	24	24	24	24
(cells/mℓ)	Mean	3	3	2	90	321
	Minimum	0	0	0	0	0
	Maximum	55	50	21	715	5,434
	Standard Dev.	11	10	5	176	1,101
Euglenophyceae	Valid n	24	24	24	24	24
(cells/mℓ)	Mean	1,117	84	564	1,177	802
	Minimum	72	0	0	0	0
	Maximum	16,445	491	3,933	6,971	5,601
	Standard Dev.	3,299	101	1,016	1,749	1,142

5.2 PHYSICO-CHEMICAL VARIABLES

It was mentioned in section 4.1 that, in addition to the physico-chemical data supplied for 2012 to 2014, Midvaal Water Company also provided data for 2001 to 2002. These data was subjected to the Kruskal-Wallis test for multiple comparisons of p-values between the two sets of data. Group one represents the physico-chemical data obtained from 2001 to 2002, and group 2 from 2012 to 2014. The intention was to determine the change in the water's physico-chemistry over time, as an estimate of surface water quality deterioration or improvement within the catchment area. Figures 5.3 to 5.7 represent the boxplots derived from the Kruskal-Wallis test for multiple comparisons of p-values between the two sets of data. The boxplots only represent the variables deemed important at each site according to the descriptive statistics, their potential impacts on phytoplankton assemblages (that is why T.Chl will be included for all sites) and whether significant differences in concentrations were noticeable over time.

In the KMS, the most important variables according to the descriptive statistics at Site 1 (after Khuma) include TOC, Faecal coliforms, NH_4^+ and to a lesser extent PO_4^{-3}. These variables all reached their highest mean concentrations at Site 1, followed by Site 4 (canal and Khuma combined), except for PO_4^{-3} that was highest at Site 4. This indicates the influence of Site 1 on Site 4 regarding these variables. Most significant differences between data groups 1 and 2 at Site 1 were indicated for TOC, NH_4^+, PO_4^{-3} and T.Chl, excluding Faecal coliforms. Figure 5.3 shows that all these variables increased significantly over time. Since N and P are regarded as principal limiting nutrients for phytoplankton growth (Chapman, 1996), an increase in NH_4^+ and PO_4^{-3} concentrations would naturally lead to increased T.Chl concentrations. Also, in order to establish the possible impact of each site on the water quality of the Vaal River, the variables deemed important at Site 6 (after KMS) will be included as well, which were colour (sharing the highest mean concentration with Site 2), Fe, Mn and As. At Site 1 (after Khuma), only colour, Fe and As showed significant increases over time, excluding Mn.

NOTE: Please refer to APPENDIX A.2 for the comparison of p-values (2 tailed) that indicate significant differences ($p<0.05$) between data groups 1 and 2.

The most important variables at Site 3 (canal) include turbidity, EC, Na^+, Cl^- and SO_4^{2-}. All of these variables reached highest mean concentrations at Site 3, indicating its influence on Site 4 (canal and Khuma combined). Most significant differences between data sets at Site 3 were indicated for Na^+, Cl^-, SO_4^{2-}, and T.Chl, with increases indicated for all of these variables (Figure 5.4). No significant differences were indicated for EC. Regarding important variables at Site 6 (after KMS), Mn decreased by approximately 94 and As increased by approximately 2400.0 % at Site 3 (canal). No significant differences were indicated for colour and Fe.

Changes in water quality at Site 4 (canal and Khuma combined) are similar to that of Site 1 (after Khuma) and 3 (canal) in terms of the selected variables and the trends they display. Variables influenced by Site 1 include TOC, NH_4^+ and PO_4^{-3} and variables influenced by Site 3 include Na^+, Cl^-, SO_4^{2-} and EC. T.Chl was also included. Figure 5.5 show increases for all these variables except EC, which decreased by approximately 24 %. This is somewhat inexplicable given that both Na^+ and Cl^- increased at this site. Regarding important variables at Site 6 (after KMS) colour, Fe and As showed significant increases at Site 4 (canal and Khuma combined). No significant differences were indicated for Mn, which was expected because Mn decreased at Site 3 (canal).

In the Vaal River, important variables at Site 2 (before KMS) according to the descriptive statistics are colour (sharing the highest mean with Site 6) and turbidity (that was only highest in the Vaal River, but compared to all sites it was highest at Site 3 in the KMS). To distinguish between the potential impacts of the KMS on the Vaal River, impacts from sources upstream from the Vaal River and KMS confluence, variables that are important at Site 4 (representing the combined impacts of Khuma and the gold mines) and Site 6 (after KMS) are included for Site 2 (before KMS).

Significant differences between data groups 1 and 2 at Site 2 (before KMS) are indicated for colour, NH_4^+, PO_4^{-3}, Na^+, Cl^-, SO_4^{2-}, EC and As. T.Chl was again included. Figure 5.6 shows that these variables display more fluctuating trends with increased NH_4^+, T.Chl, Na^+, Cl^-, SO_4^{2-} and As, and decreased colour, EC and PO_4^{-3}.

Important variables at Site 6 (after KMS) include colour, Fe, Mn and As. For similar reasons mentioned above to determine the true impact of the KMS on the Vaal River, variables deemed important at Site 4 (Khuma and canal combined) and Site 2 (before KMS) will be included for Site 6 (after KMS). These differences are exhibited by TOC, Faecal coliforms, NH_4^+, PO_4^{-3}, T.Chl, Cl^-, SO_4^{2-}, EC, colour, Mn, and As. Figure 5.7 shows that the variables for Site 6 are evenly divided in displaying increases (T.Chl, NH_4^+, Faecal coliforms, Mn and As) and decreases (colour, EC, PO_4^-3, Cl^-, SO_4^{2-} and TOC).

It is important to notice that Faecal coliforms and Mn showed significant increases at Site 6 (after KMS), even though they did not indicate significant differences at either sites 4 (canal and Khuma combined) or 2 (before KMS). Faecal coliforms were expected to feature at Site 1 (after Khuma) where it reached its highest mean concentration (Table 5.1) and to then reflect its impact on Site 4 (canal & Khuma combined). Neither of these sites showed significant differences in Faecal coliforms over the ten year period. No change in Faecal coliforms concentrations in the KMS do not necessarily imply that the KMS does not impact on the Vaal River regarding Faecal coliforms.

Mn did feature at Site 3 (canal) where it decreased by approximately 94 %, even though no significant differences occurred at Site 4 (canal and Khuma combined). This indicates that Site 3 has no noticeable impact on Site 4 concerning Mn. Furthermore, Mn showed a approximately 100 % increase at Site 6 (after KMS). Since Mn showed a remarkable decrease in the KMS, yet no difference at Site 2 (before KMS) in the Vaal River, the probable cause of increased Mn at Site 6 still favours that of an initial influx of polluted runoff entering the Vaal River after it converges with the KMS (see discussion under section 5.1).

To conclude this section of the results, it is crucial to refer back to section 3.1, specifically the discussion on stream order. It was stated that the KMS is classified as a stream order 2, and the Vaal River as a stream order 5 (De Meyer & Nortje, 2013). Site 4 (canal and Khuma combined) in the KMS is thus a stream order 2, whereas both sites 2 (before KMS) and 6 (after KMS) in the Vaal River are stream orders 5. This led to the conclusion that if any, the KMS would have a minimal effect on the water quality of the Vaal River at Site 6 (after KMS).

To support this, TOC decreased at Site 6 (after KMS), despite the increase indicated at Site 4 (canal and Khuma combined). In addition, no change in TOC was indicated at Site 2 (before KMS). Therefore, and given the stream orders, the decrease in TOC at Site 6 can be ascribed to the dilution effect.

NH_4^+ increases were indicated for both sites 2 (before KMS) and 4 (canal and Khuma combined), of which the combined impact is reflected by the increased NH_4 at Site 6 (after KMS). The same principle applies to T.Chl and As, leading to increases in both variables at Site 6.

Based on the same principle, the opposite is true for EC and colour, where decreases in both variables at sites 2 (before KMS) and 4 (canal and Khuma combined) was reflected by decreased EC and colour at Site 6 (after KMS).

The decreases in Cl^- and SO_4^{2-} at Site 6 (after KMS) over time can currently not be explained, since increases were indicated for both variables at sites 2 (before KMS) and 4 (canal and Khuma combined) over the ten year period.

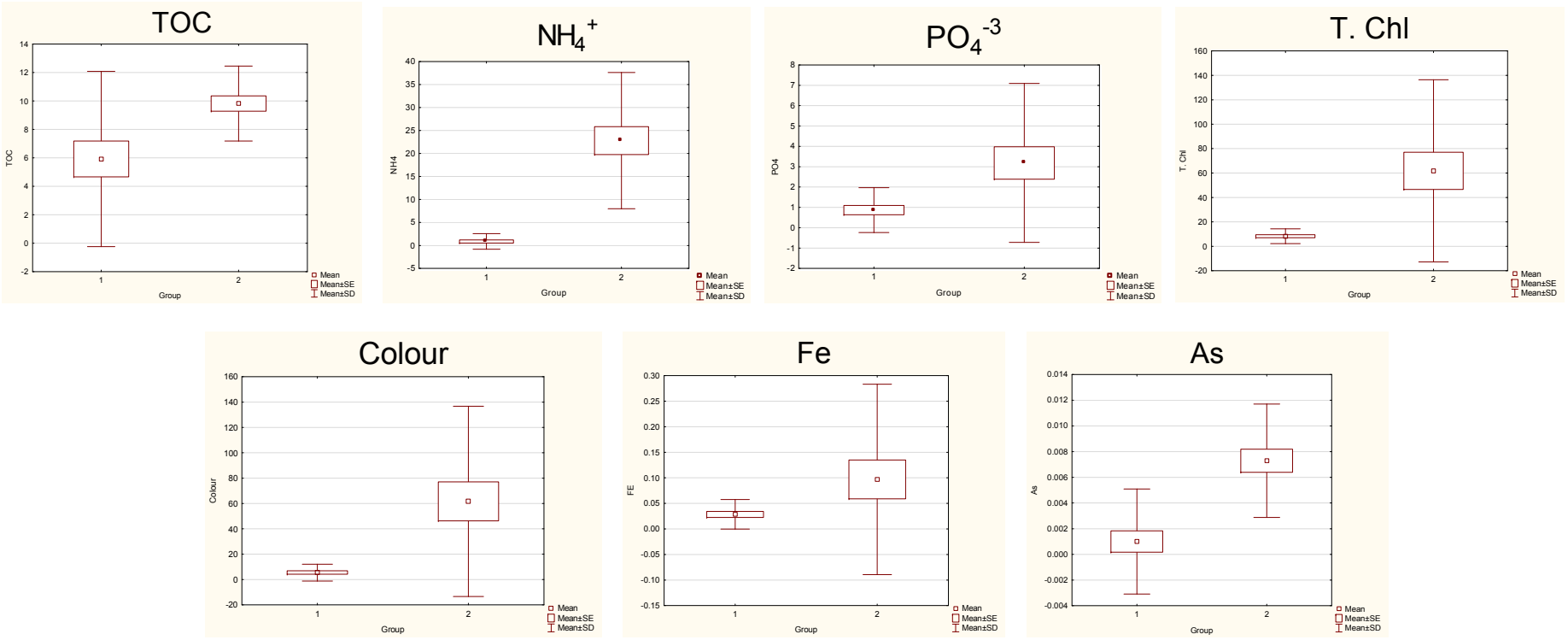

Figure 5.3: Kruskal-Wallis boxplot illustration of significant differences in the concentrations of important water quality variables at Site 1 (after Khuma) in the KMS, over a ten year period. *n = 24; ±SE (Standard Error); ±SD (Standard Deviation)*

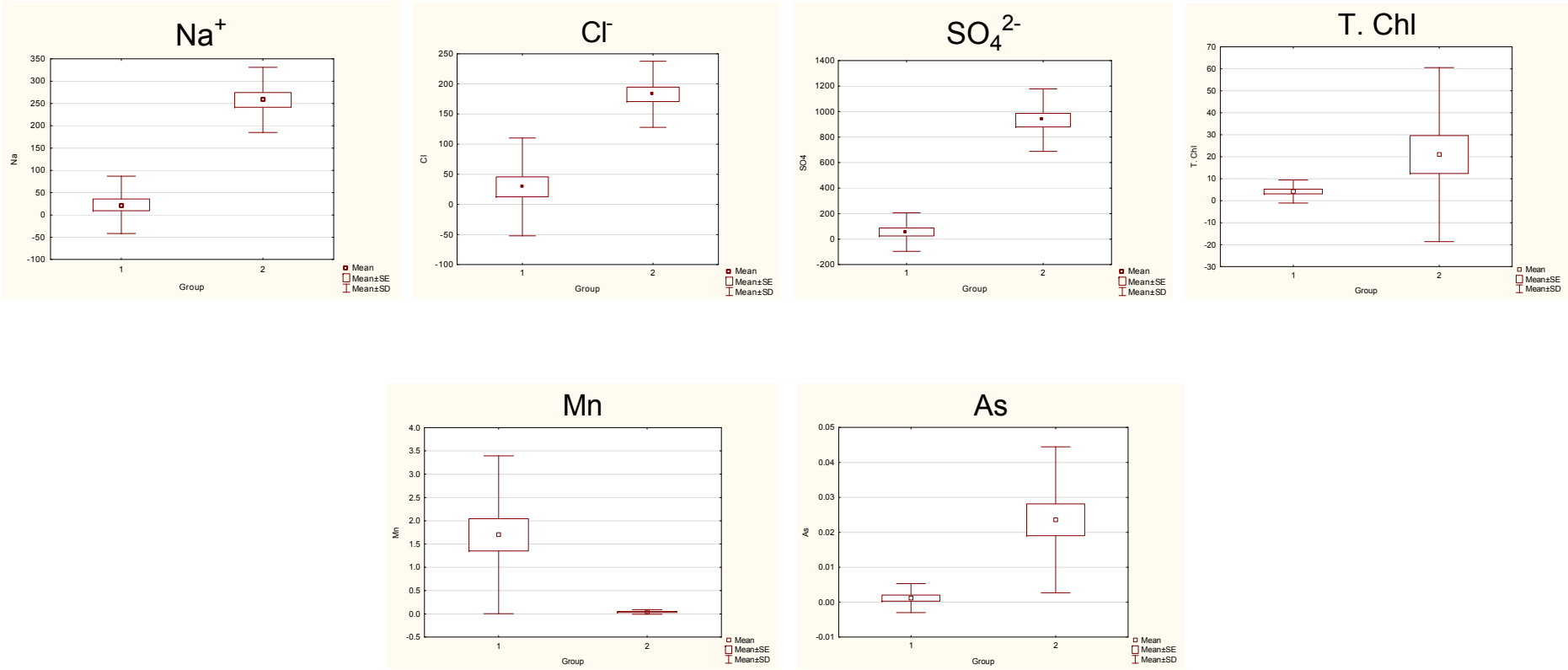

Figure 5.4: Kruskal-Wallis boxplot illustration of significant differences in the concentrations of important water quality variables at Site 3 (canal) in the KMS, over a ten year period. *n = 24; ±SE (Standard Error); ±SD (Standard Deviation)*

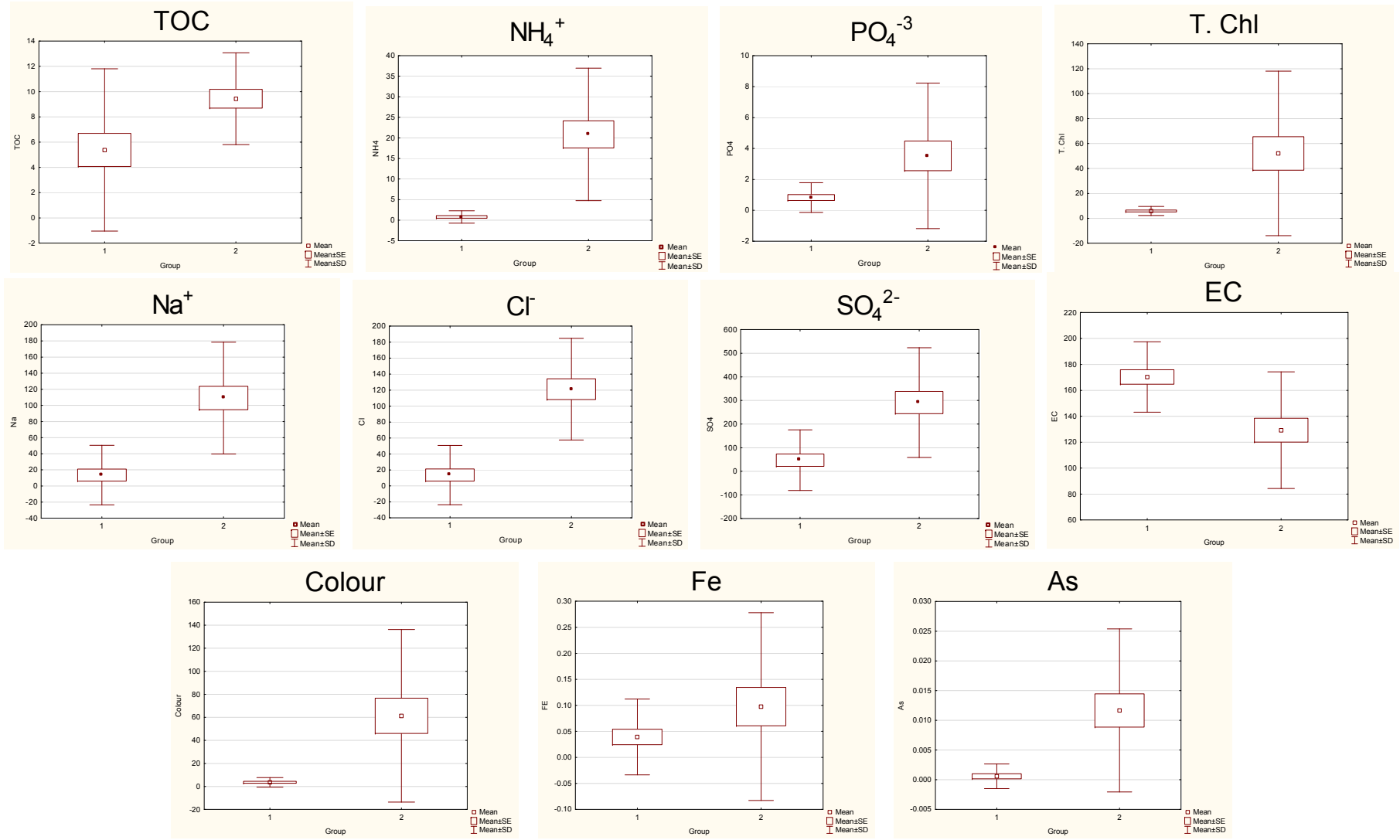

Figure 5.5: Kruskal-Wallis boxplot illustration of significant differences in the concentrations of important water quality variables at Site 4 (canal and Khuma combined) in the KMS, over a ten year period. *n = 24; ±SE (Standard Error); ±SD (Standard Deviation)*

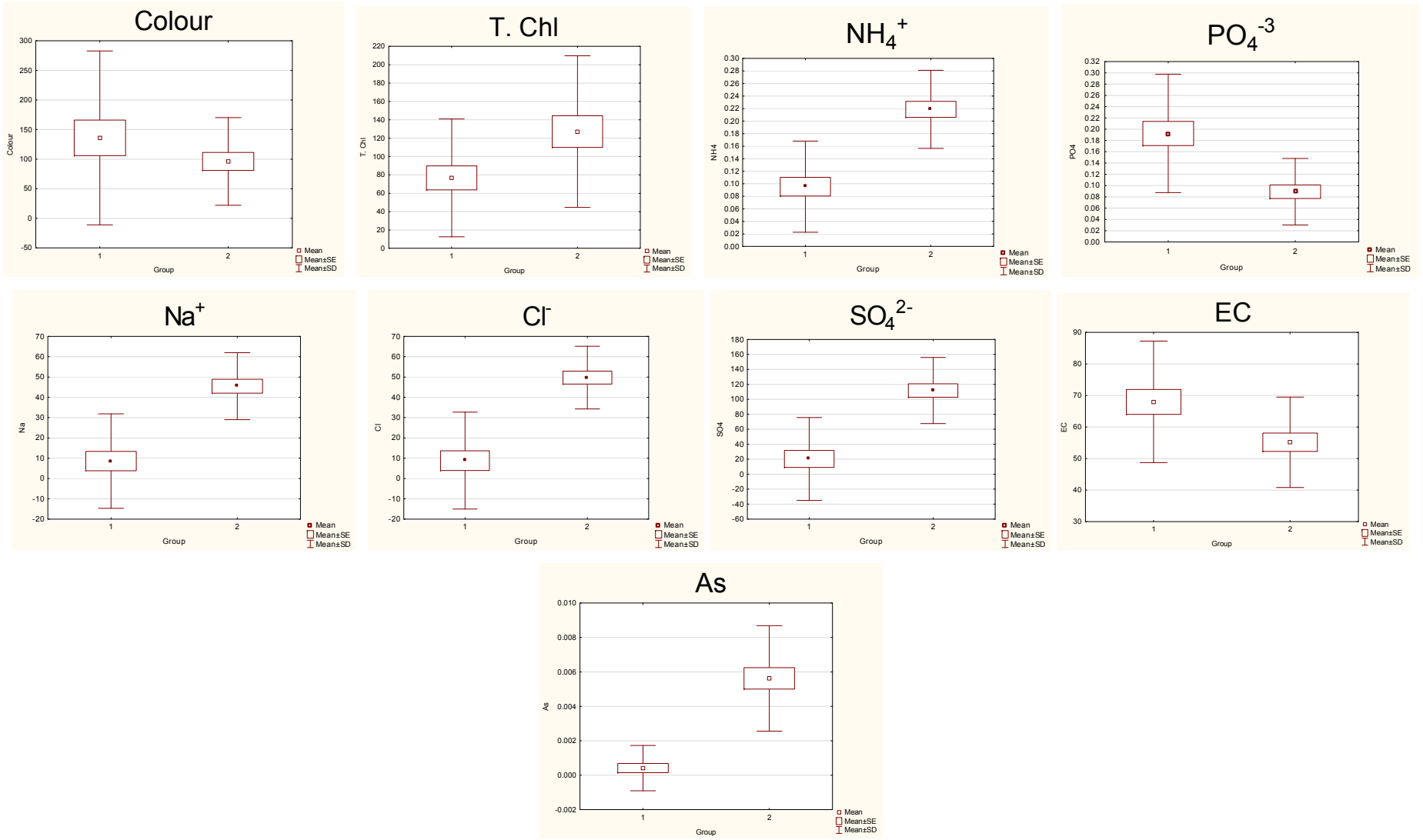

Figure 5.6: Kruskal-Wallis boxplot illustration of significant differences in the concentrations of important water quality variables at Site 2 (before KMS) in the Vaal River, over a ten year period. *n = 24; ±SE (Standard Error); ±SD (Standard Deviation)*

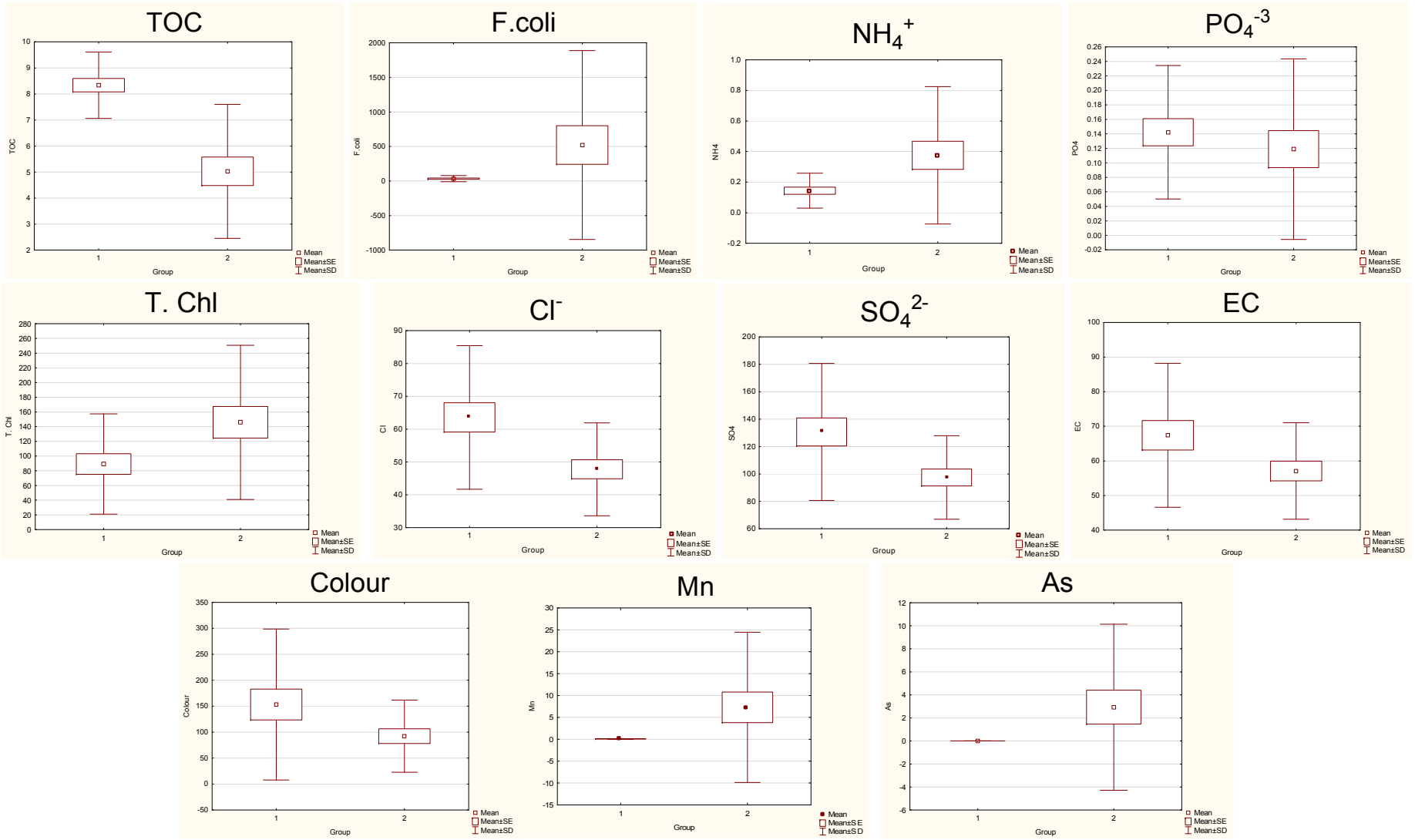

Figure 5.7: Kruskal-Wallis boxplot illustration of significant differences in the concentrations of important water quality variables at Site 6 (after KMS) in the Vaal River, over a ten year period. *n = 24; ±SE (Standard Error); ±SD (Standard Deviation)*

5.3 PHYTOPLANKTON

Phytoplankton results were generated for all eight sampling sites within the study area, though it later became apparent that some of the sites did not meet the selection criteria mentioned under the introduction of section 5. Phytoplankton data for all eight sites will be displayed, followed by a short explanation why these particular results contributed to the selection of only 5 of the eight sampling sites.

Table 5.3 was constructed to display, in a colour coordinated fashion, the frequency of occurrence of each phytoplankton genus which had been identified at each Site over the 24 month study period. The % frequency was determined from the number of times genera had occurred over the 24 months of sampling, divided into four colour coded increments of 25 %:

1-6	=	25%
7-12	=	50%
13-18	=	75%
19-24	=	100%

Note: The following discussion will state the genera in order of decreased frequency of occurrence.

Table 5.3 illustrates that genera most frequently present at the majority of the sites include *Carteria*, *Chlamydomonas*, *Chlorolobion* and *Euglena*. Genera which had occurred as frequent but at fewer sites include *Cyclotella*, *Navicula*, *Nitzschia*, *Monoraphidium*, *Oocystis* and *Scenedesmus*.

NOTE: Please refer to APPENDIX A.3 for a summary of genera that were identified over the 24 month study period, including each of their authors.

Table 5.3: Colour coordinated illustration of percentage occurrence of genera at each site over the 24 month study period.

ABREVIATION	GENUS	SITES							
CYANOPHYCEAE		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Ana	*Anabaena*	3	2			2	2	3	7
Aphan	*Aphanocapsa*	8	12	7	9	14	13	14	7
Arthr	*Arthrospira*		1	1				1	1
Calo	*Calothrix*	2	1				2		
Gloeo	*Gloeocapsa*	2			1				
Meris	*Merismopedia*	3	6		1	2	7	6	1
Micro	*Microcystis*		3		2		2	2	3
Nost	*Nostoc*	1	2		1	1	1		1
Oscil	*Oscillatoria*	15	5	8	12	19	6	7	3
Phor	*Phormidium*	14	5	19	14	18	3	4	6
BACILLARIOPHYCEAE									
Achs	*Achnanthes*	8	1	3	1	8		1	5
Achm	*Achnanthidium*	1	1	4	4	1	1	1	
Amphi	*Amphiprora*				2	1			
Aula	*Aulacoseira*	11	2	9	6	8	19	22	7
Cocco	*Cocconeis*	1	1		2	1		1	2
Cyclo	*Cyclotella*	18	23	9	16	9	24	24	9
Cymb	*Cymbella*	1		5	4	3	1	4	1
Dia	*Diadesmus*	2		1	2	2			
Frag	*Fragilaria*	3	4	6	8	3	5	3	1
Gomph	*Gomphonema*	11	5	5	5	12	1	1	1
Gyro	*Gyrosigma*	2	1	1	1				3
Melos	*Melosira*	4	1		1		2	1	1
Navic	*Navicula*	23	6	18	19	23		4	11
Nitz	*Nitzschia*	24	16	21	24	22	11	13	12
Suri	*Surirella*	2	7		2	1	6	9	
CHLOROPHYCEAE									
Actin	*Actinastrum*	1	7	1			6	5	
Ankis	*Ankistrodesmus*		4			1		2	
Aster	*Asterococcus*	2	2	1	1	1	4	2	1
Cart	*Carteria*	23	22	21	22	22	22	23	12
Chaet	*Chaetophora*			1	3				
Char	*Characium*	11	2	4	5	6	1	1	
Chlam	*Chlamydomonas*	24	23	23	24	23	24	24	11
Chloa	*Chlorella*		2	1		1	1	2	
Chlom	*Chlorococcum*	1	6	1	4		9	7	1
Chlon	*Chlorolobion*	22	16	21	23	23	19	18	1
Closs	*Closteriopsis*	7	6	6	8	5	5	8	8
Closm	*Closterium*	4	1	1	3	3	1	2	2
Coel	*Coelastrum*		13			1	13	14	1
Cosma	*Cosmarium*	1	3	6	4		1	1	
Cruci	*Crucigenia*	2			1		1	2	2
Dict	*Dictyosphaerium*	3	8		1	1	8	6	4
Didy	*Didymogenes*		7	2			7	5	
Elak	*Elakatothrix*	2	3		1		4	3	1
Golen	*Golenkinia*		8			2	7	8	
Gonio	*Goniochloris*		4	1			6	9	2
Kirch	*Kirchneriella*	2	13	1	3	2	7	9	1
Lager	*Lagerheimia*	1	4	1		1	3	6	
Micra	*Micractinium*	1	5			1	1	1	
Monor	*Monoraphidium*	2	21	12	11	18	23	19	12
Oocys	*Oocystis*	13	15	18	13	11	22	21	6
Pand	*Pandorina*	2	4	3	3		6	6	1
Pedia	*Pediastrum*		17		1		14	13	
Ptero	*Pteromonas*	1	7		1	1	8	6	
Scen	*Scenedesmus*	11	23	15	13	7	23	22	11
Spiro	*Spirogyra*		1	2	1				3
Staura	*Staurastrum*		2					1	
Teton	*Tetraedron*	2	6	1	1	4	7	8	4
Tetum	*Tetrastrum*	7	12	2	3	3	16	17	2
Uloth	*Ulothrix*	1		1	2		1		
CHRYSOPHYCEAE									
Dino	*Dinobryon*						1		2
Mallo	*Mallomonas*	3	2	4	2	3	4	6	1
DINOPHYCEAE									
Cerat	*Ceratium*	1	1	1	1	2	3	1	1
Peri	*Peridinium*	4	6	2	3	5	1	8	2
CRYPTOPHYCEAE									
Crypt	*Cryptomonas*		7	1	1		1	1	
EUGLENOPHYCEAE									
Eug	*Euglena*	24	19	21	22	24	19	16	11
Phac	*Phacus*	21	1		2	12	1	2	3
Strom	*Strombomonas*	4	7	5	5	1	4	8	8
Trach	*Trachelomonas*	14	13	11	1	9	12	13	8

Note that the numbers captured under each site represent the times out of 24 months that a specific genus was present

Considering the KMS, genera which had occurred most frequently at Site 1 (after Khuma) include *Nitzschia*, *Chlamydomonas*, *Euglena*, *Navicula*, *Carteria*, *Chlorolobion* and *Phacus*. The most frequently occurring genera at Site 3 (canal) include *Chlamydomonas*, *Nitzschia*, *Carteria*, *Chlorolobion*, *Euglena*, and *Phormidium*, though none of these genera had a 100% occurrence frequency. The genera at Site 4 (canal and Khuma combined) showed more similarity to that of Site 1, in terms of composition and scoring an overall higher frequency of occurrence, and they include *Nitzschia*, *Chlamydomonas*, *Chlorolobion*, *Carteria*, *Euglena*, and *Navicula*. Similar to sites 1 and 4, the most frequently occurring genera at Site 5 (Khuma) include *Euglena*, *Navicula*, *Chlamydomonas*, *Chlorolobion*, *Nitzschia*, *Carteria* with the addition of *Oscillatoria*. Table 5.3 points out that none of the genera which had occurred at Site 8 (N12 after eye) even fell within the 75% or more categories.

In the Vaal River, genera which had occurred most frequently at Site 2 (before KMS) include *Cyclotella*, *Chlamydomonas*, *Scenedesmus*, *Carteria*, *Monoraphidium* and *Euglena*. At Site 6 (after KMS), the genera include *Cyclotella*, *Chlamydomonas*, *Monoraphidium*, *Scenedesmus*, *Carteria*, *Oocystis*, *Aulacoseira*, *Chlorolobion* and *Euglena*. The most frequently occurring genera at Site 7 (after Margaret) include *Cyclotella*, *Chlamydomonas*, *Carteria*, *Aulacoseira*, *Scenedesmus*, *Oocystis* and *Monoraphidium*.

Since the occurrence of phytoplankton contained in Table 5.3 could provide some insight on the qualitative aspects of phytoplankton composition in the study area, Figure 5.8 illustrates and compares the trends followed by total phytoplankton cell densities (cells/mℓ) at all eight sites, to reveal the quantitative aspect required to determine water quality status. It should be noted that the graph contains two y-axes because phytoplankton density for sites 4 (275990 cells/mℓ) and 5 (673173 cells/mℓ) far exceed that of the remaining sites. Figure 5.8 also indicates the seasons (Anon, 2014), each of which repeated twice over the 24 month study period.

In the KMS, the maximum phytoplankton cell density for Site 1 (after Khuma) occurred during early spring, September 2014, and reached 63063 cells/mℓ. Site 3 (canal) reached a maximum of 17643 cells/mℓ during mid-spring, October 2014, which is also the lowest maximum reached in comparison to the other seven sites. Site 4 (canal and Khuma combined) reached the second highest maximum of 275990 cells/mℓ during September 2014, which is markedly higher than all the other sites, except for Site 5 (Khuma) which reached a maximum of 673173 cells/mℓ during mid-summer, January 2014.

Once again, the influence of Site 1 on Site 4 becomes apparent as both these sites reached their maximum cell densities during the same time. It should however be noted that, given the locations of sites 1 (after Khuma), 4 (canal and Khuma combined) and 5 (Khuma) as well as the noticeable quantitative similarity between sites 4 and 5, it is safe to assume that the influence of Site 5 (Khuma) on Site 1 (after Khuma) is what ultimately causes Site 1 to have such a huge influence on Site 4 (canal and Khuma combined). The maximum cell density for Site 8 (N12 after eye) was observed during early summer, December 2012 and reached densities of 22737 cells/mℓ.

In the Vaal River, Site 2 (before KMS), a maximum phytoplankton cell density of 146754 cells/mℓ was reached during late spring, November 2013, whereas Site 6 (after KMS) experienced its maximum cell density of 173459 cells/mℓ during late summer, February 2013, together with Site 7 (after Margaret) where cell densities reached a maximum of 223938 cells/mℓ during the same month.

Some of the trends displayed by total phytoplankton cell densities in Figure 5.8 could be attributed to seasonal variation in temperature and rainfall. Swanepoel (2014) found that chlorophyll-*a* in the Vaal Dam (2000–2012) reached its maximum concentration during summer, followed by autumn, then spring and finally winter. It was speculated that the higher chlorophyll-*a* concentrations during autumn, as supposed to spring, could be attributed to the high summer inoculum, which in itself is the result of increased water temperature during summer (Swanepoel, 2014). Similar trends are displayed in Figure 5.8 with clear peaks during late summer and early autumn 2013, and again during late spring 2013, summer and early autumn 2014. During winter, the lowest cell density was observed for both 2013 and 2014.

Phytoplankton composition and succession during periods of maximum cell density at each site are vital to determine water quality status. This is of special interest regarding operational aspects of water treatment plants. For instance, cyanobacterial blooms are often associated with the production of cyano-toxins (Paerl *et al*., 2001) which is particularly concerning because of the health risks it poses to humans if consumed (Harding, 2006). Also, since not all Cyanophyceae genera produce these toxins (O'Neil *et al*., 2012), knowledge of the composition of the specific genera is extremely important.

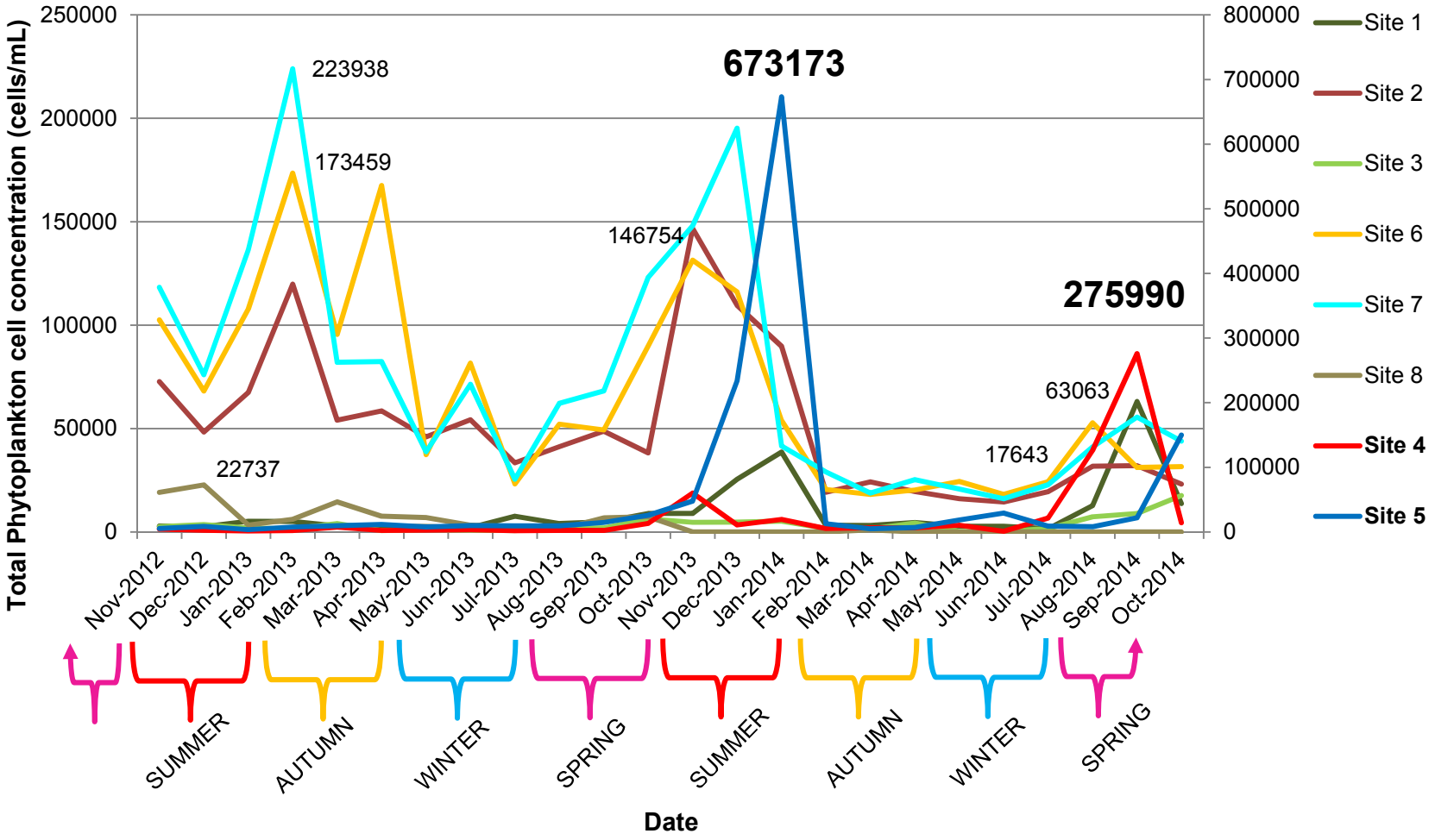

Figure 5.8: Variation in total phytoplankton cell density (cells/mL) over the 24 month study period representing all eight sampling sites. *Note that sites 4 and 5 are plotted on the secondary y-axes due to high values.*

Phytoplankton data were also subjected to further analyses in the form of area graphs, aimed at obtaining a better understanding of the water quality at each site. Area graphs (Figures 5.10 to 5.17) indicate the percentage composition of the various phytoplankton taxa for each site over the 24 month sampling period, rather than phytoplankton density. By using these graphs, it can be established which phytoplankton taxon made the biggest contribution to the maximum phytoplankton cell density for each site at a given time.

Figure 5.8 indicates that the maximum phytoplankton cell density for Site 1 (after Khuma) occurred during September 2014. According to Figure 5.9, at the time, four phytoplankton taxa were present at Site 1 (after Khuma), of which Cyanophyceae accounted for 44.2% of the total cell density, followed by Euglenophyceae (26.1%), Bacillariophyceae (17.7%) and Chlorophyceae (12.0%).

The figure also provides information about varying trends in phytoplankton dominance. When interpreting this figure, it was difficult to decide which phytoplankton taxon was most dominant, though it seemed to be either Cyanophyceae or Chlorophyceae. For this reason, the phytoplankton data were subjected to further graphical analysis in the form of pie graphs to reveal the overall dominant phytoplankton taxon, representative of the entire study period. From this, it was established that the dominant phytoplankton taxon at Site 1 was Cyanophyceae, with 41% dominance (Figure 5.17a).

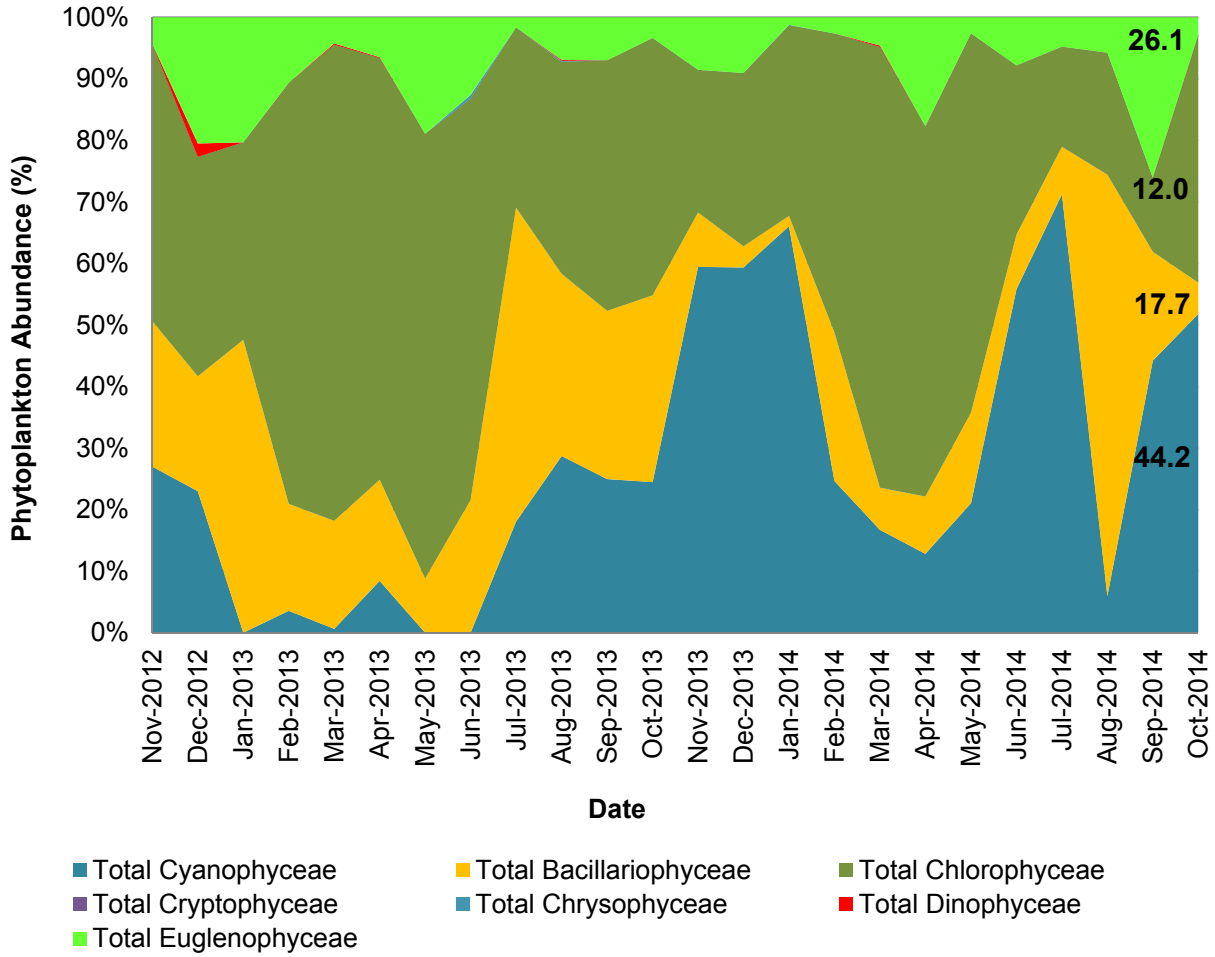

Figure 5.9: Variation in phytoplankton composition over the 24 month study period at Site 1 (after Khuma) in the KMS. *Note that the values to the right side of the graph represent the % composition of different taxa present during maximum phytoplankton cell density.*

The maximum phytoplankton cell density at Site 3 (canal) was found during October 2014 and Figure 5.10 indicates that it mostly comprised of Chlorophyceae (98.9%) representatives. One can also derive from Figure 5.10 that the phytoplankton dominance varied mostly between Chlorophyceae and Cyanophyceae over the study period. The taxon Cyanophyceae was however absent during the time that maximum phytoplankton cell density was reached. Figure 5.17b revealed that Chlorophyceae (58% dominance) was the dominant phytoplankton taxon throughout the study period.

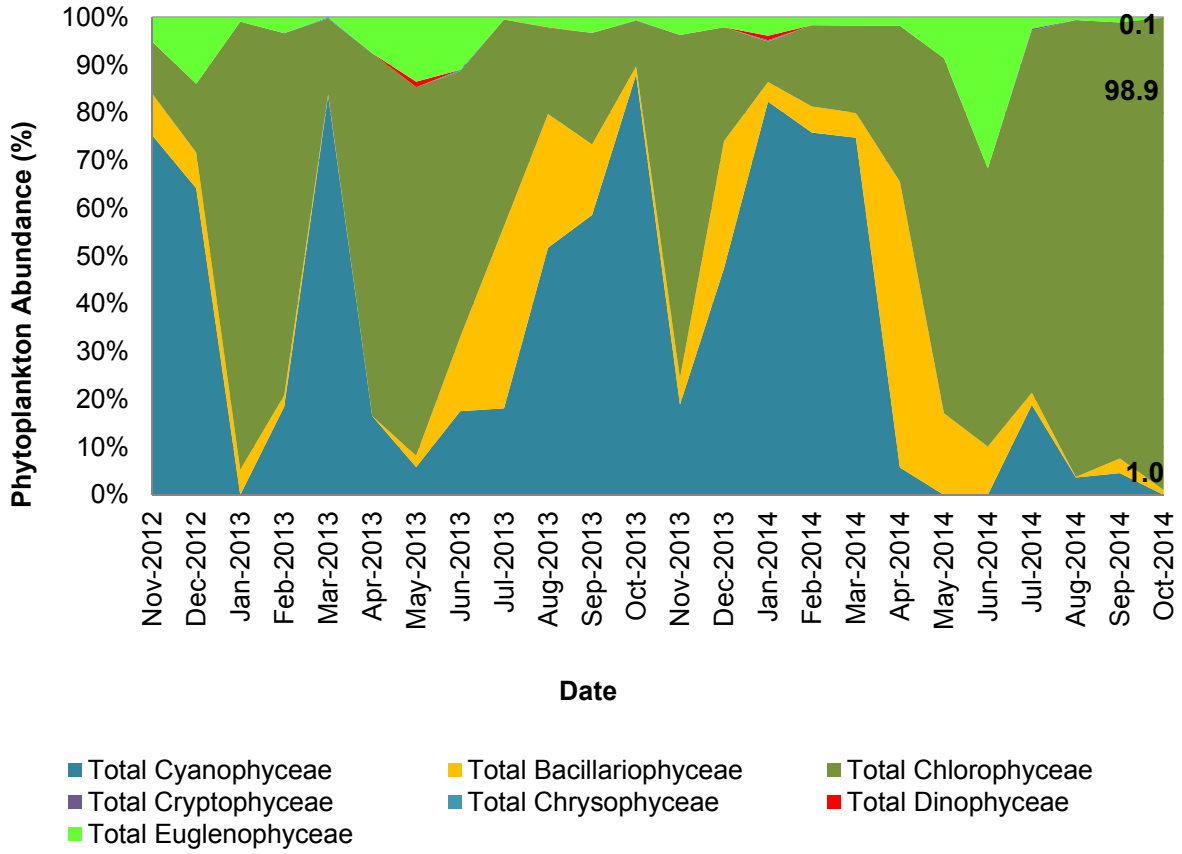

Figure 5.10: Variation in phytoplankton composition over the 24 month study period at Site 3 (canal) in the KMS. *Note that the values to the right side of the graph represent the % composition of different taxa present during maximum phytoplankton cell density.*

Site 4 (canal and Khuma combined) experienced maximum phytoplankton cell density during September 2014 and consisted of Cyanophyceae (74%), Bacillariophyceae (13.6%), Chlorophyceae (12.2%) and Euglenophyceae (0.2%). At this point in the KMS the distinction between dominant phytoplankton taxa at the various sites becomes more apparent. Figure 5.11 indicates that the dominant phytoplankton taxon at Site 4 was Cyanophyceae. This is confirmed in Figure 5.17c, revealed the percentage dominance to be 46%.

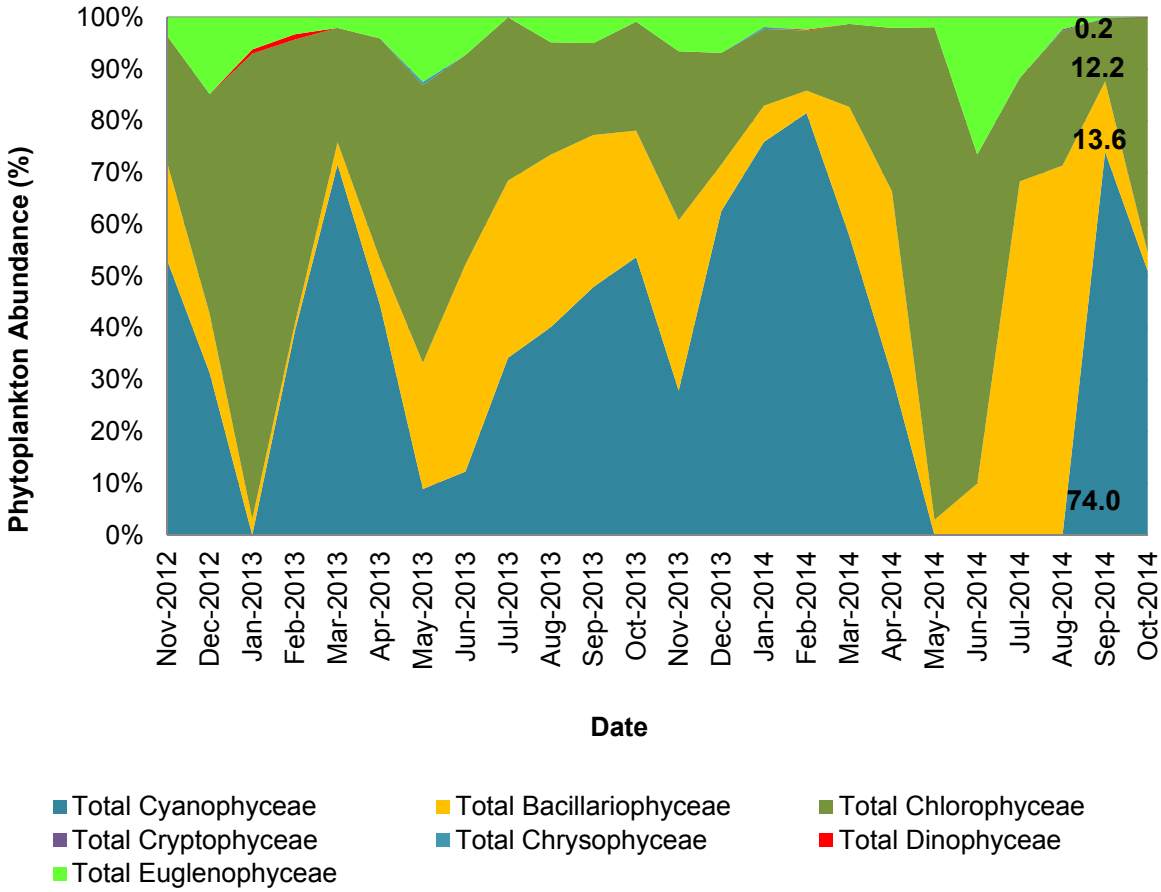

Figure 5.11: Variation in phytoplankton composition over the 24 month study period at Site 4 (canal and Khuma combined) in the KMS. *Note that the values to the right side of the graph represent the % composition of different taxa present during maximum phytoplankton cell density.*

January 2014 showed maximum phytoplankton cell density at Site 5 (Khuma) and consisted mainly of Cyanophycheae (96.8%). Figure 5.12 illustrates that Cyanophyceae was dominant for most of the study period. Figure 5.17d confirmed this with the overall percentage composition of Cyanophyceae (85%) far exceeding that of any other phytoplankton taxon.

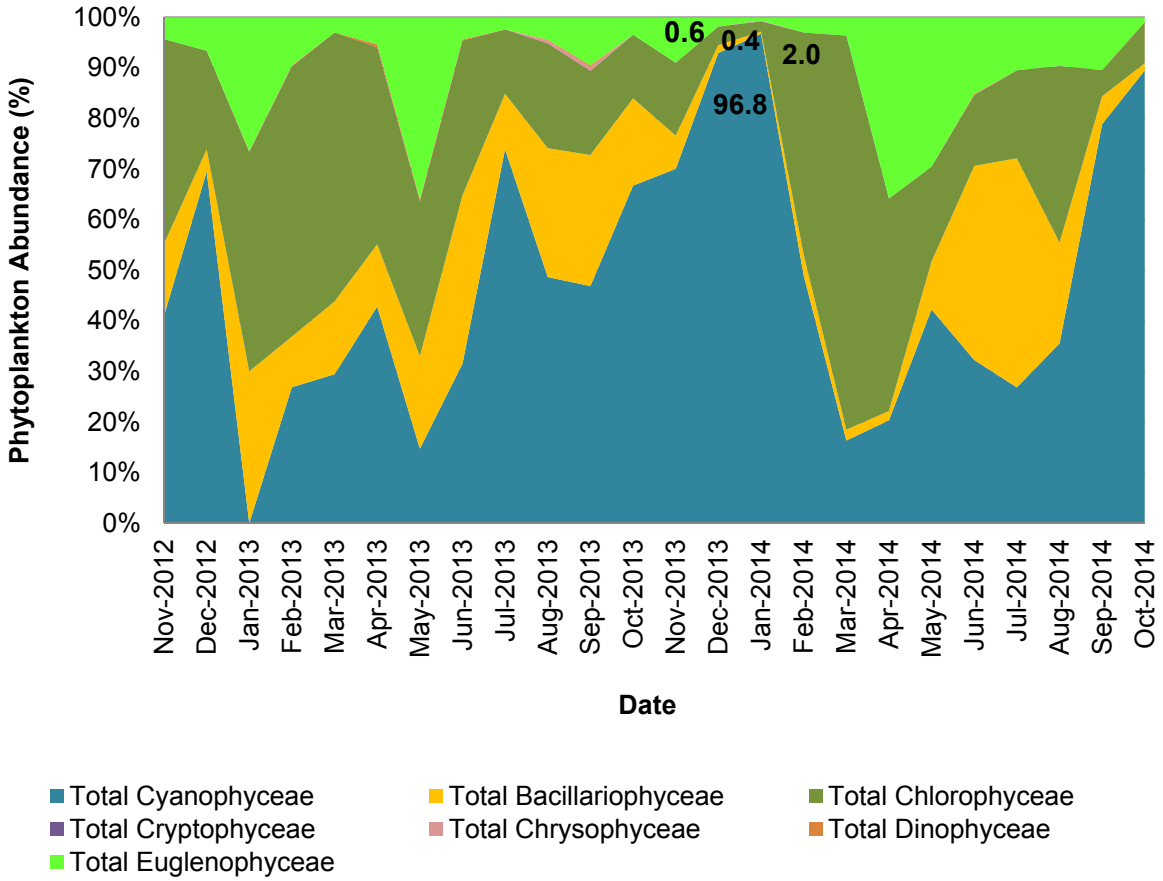

Figure 5.12: Variation in phytoplankton composition over the 24 month study period at Site 5 (Khuma) in the KMS. *Note that the values near the centre of the graph represent the % composition of different taxa present during maximum phytoplankton cell density.*

Figure 5.13 shows that the maximum phytoplankton cell density at Site 8 (N12 after eye) was found during December 2012 and was comprised of Cyanophyceae (74.8%), Chlorophyceae (18.4%), Bacillariophyceae (3.3%), Euglenophyceae (3.3%) and Dinophyceae (0.2%). Figure 5.17e revealed Cyanophyceae (51% dominance) as the dominant phytoplankton taxon during of the study period.

Figure 5.13 also shows that only limited data were available for Site 8, the reason being that this site had in fact been completely dry during most sampling sessions. March 2014 was the only exception. The phytoplankton data derived from Site 8 thus proved to be an unscientific means to support any conclusions made on the use of phytoplankton as a tool for determining surface water quality in this area. As mentioned in section 3.4 of CHAPTER 3, the unique geology and geohydrology of the KMS is the most important contributing factor causing Site 8 to run completely dry for most of 2014.

It is speculated that a combination of the porous dolomitic earth (Winde & Stoch, 2010), a possible underground cavity created by extensive gold-mining (Winde & Stoch, 2010), as well as the less permeable layer of Ventersdorp lavas situated at a greater depth in the area directly underlying Site 8 (Pulles *et al.*, 2005) could have caused this phenomenon.

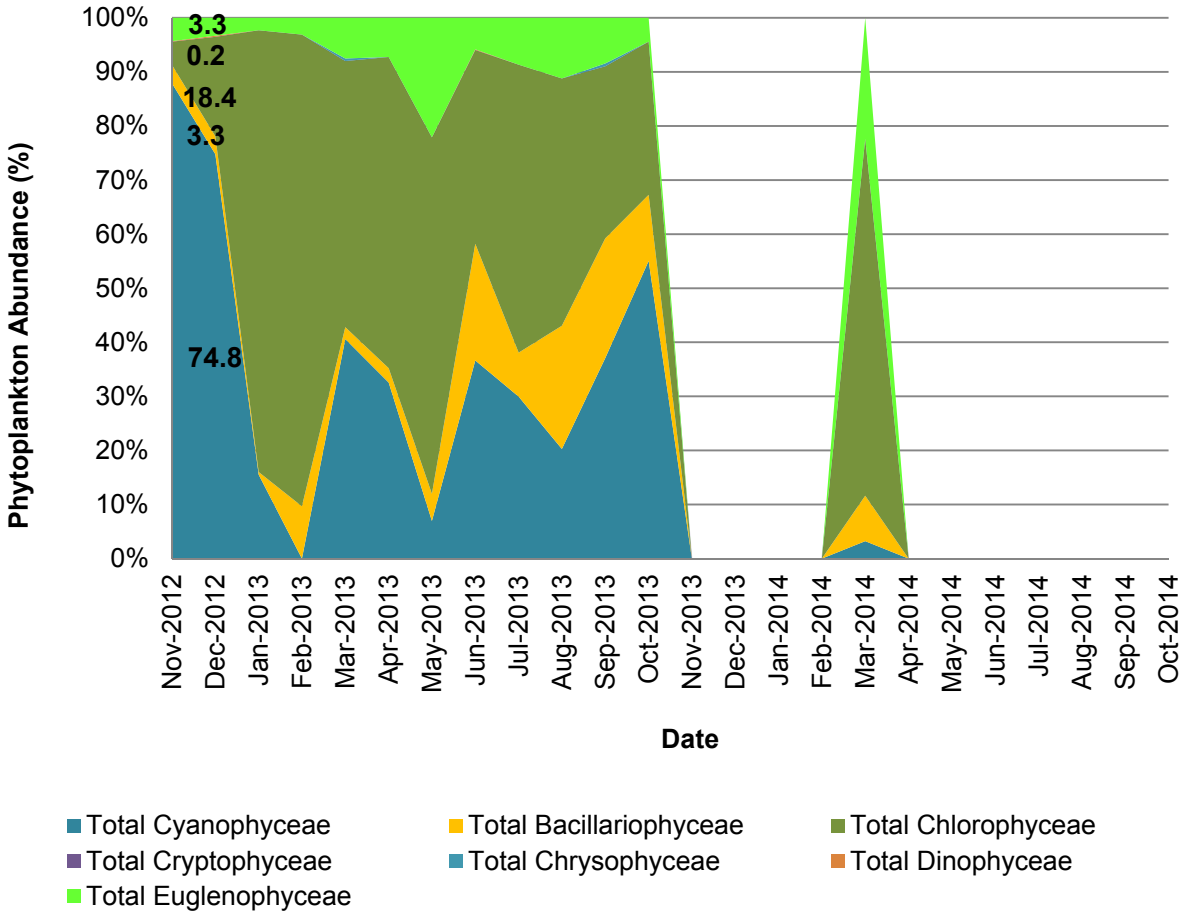

Figure 5.13: Variation in phytoplankton composition over the 24 month study period at Site 8 (N12 after eye) in the KMS. *Note that the values to the left side of the graph represent the % composition of different taxa present during maximum phytoplankton cell density.*

Figure 5.14, illustrating data of Site 2 (before KMS) in the Vaal River, shows that 6 phytoplankton taxa were present during November 2013 when the maximum phytoplankton cell density occurred. These were Chlorophyceae (67.8%), Bacillariophyceae (17.9%), Cyanophyceae (11.2%), Euglenophyceae (2.6%), Chrysophyceae (0.2%) and Dinophyceae (0.2%). The graph also gives the impression that Chlorophyceae was the dominant phytoplankton taxon over the 24 month study period, and it was confirmed by Figure 5.18a showing the 50% composition of Chlorophyceae relatively to other phytoplankton taxa.

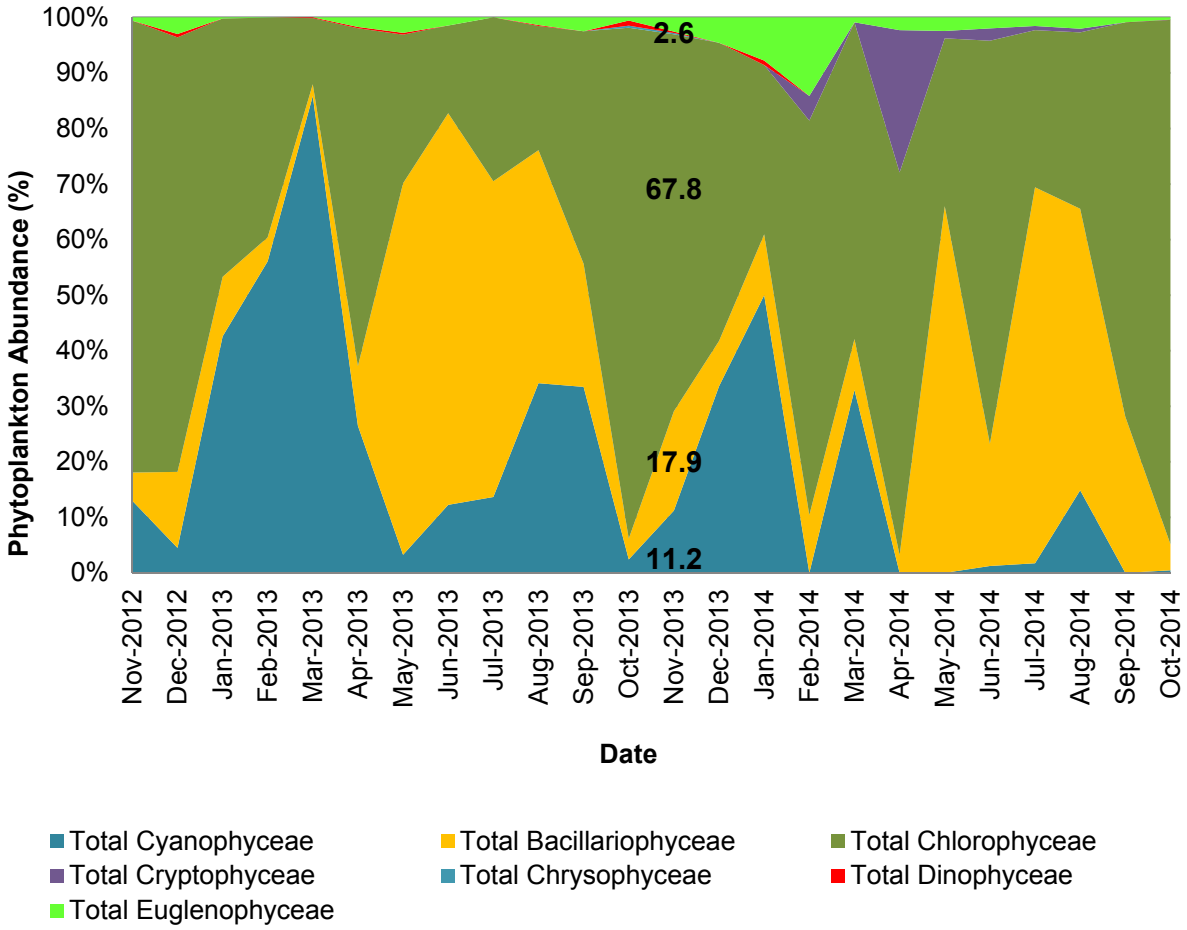

Figure 5.14: Variation in phytoplankton composition over the 24 month study period at Site 2 (before KMS) in the Vaal River. *Note that the values in the centre of the graph represent the % composition of different taxa present during maximum phytoplankton cell density.*

Site 6 (after KMS) in the Vaal River experienced its maximum phytoplankton cell density during February 2013, consisting of Cyanophyceae (75.1%), Chlorophyceae (20%), Bacillariophyceae (4.7%) and Euglenophyceae (0.2%). Figure 5.15 indicates that the dominant phytoplankton taxon for Site 6 was Chlorophyceae. This is confirmed in Figure 5.18b, at a percentage dominance of 47%.

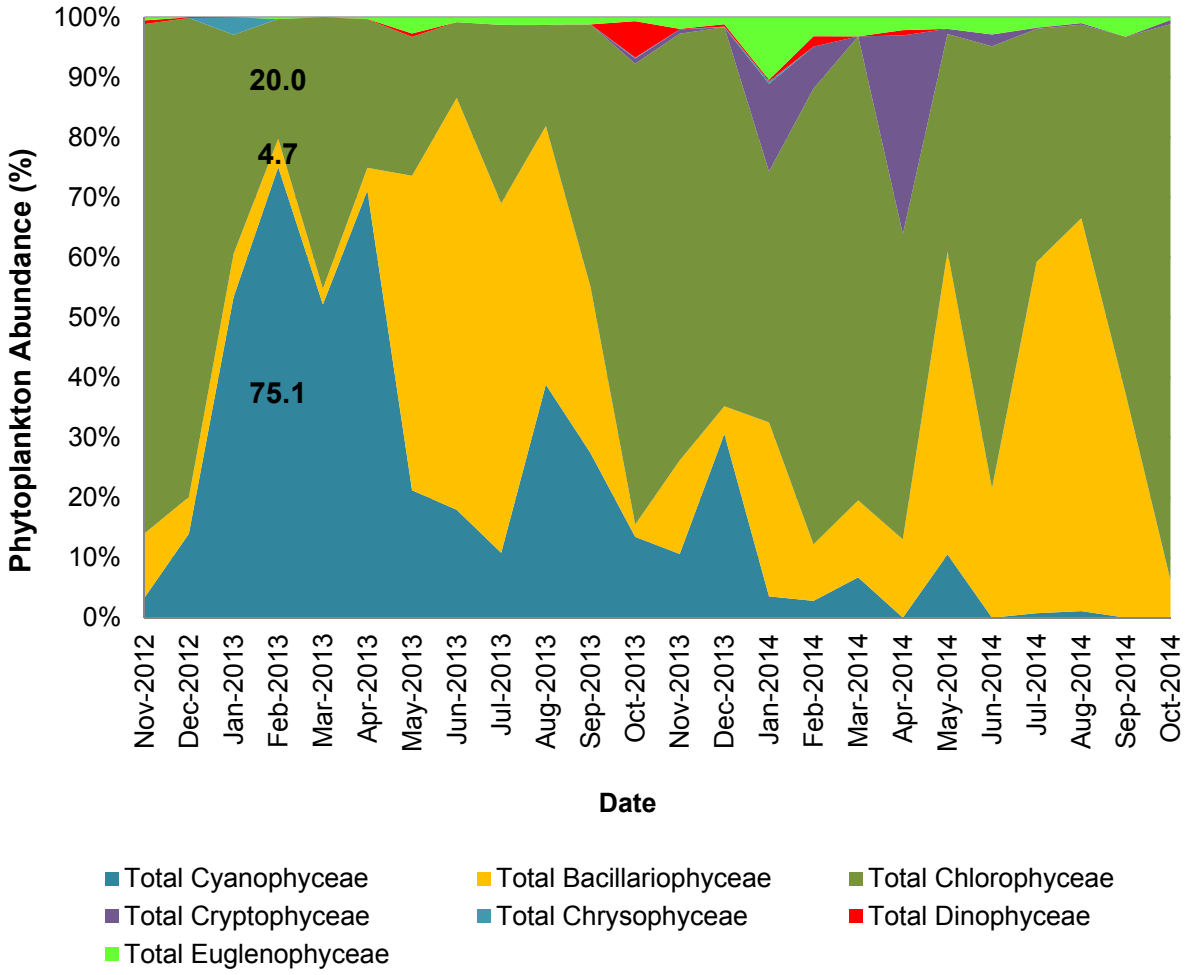

Figure 5.15: Variation in phytoplankton composition over the 24 month study period at Site 6 (after KMS) in the Vaal River. *Note that the values to the left side of the graph represent the % composition of different taxa present during maximum phytoplankton cell density.*

The maximum phytoplankton cell density at Site 7 (after Margaret) also occurred during February 2012 and was comprised of Cyanophyceae (82%), Chlorophyceae (14.9%), Bacillariophyceae (3%) and Euglenophyceae (0.1%) as can be observed in Figure 5.16. Figure 5.18c revealed Chlorophyceae dominance (46%).

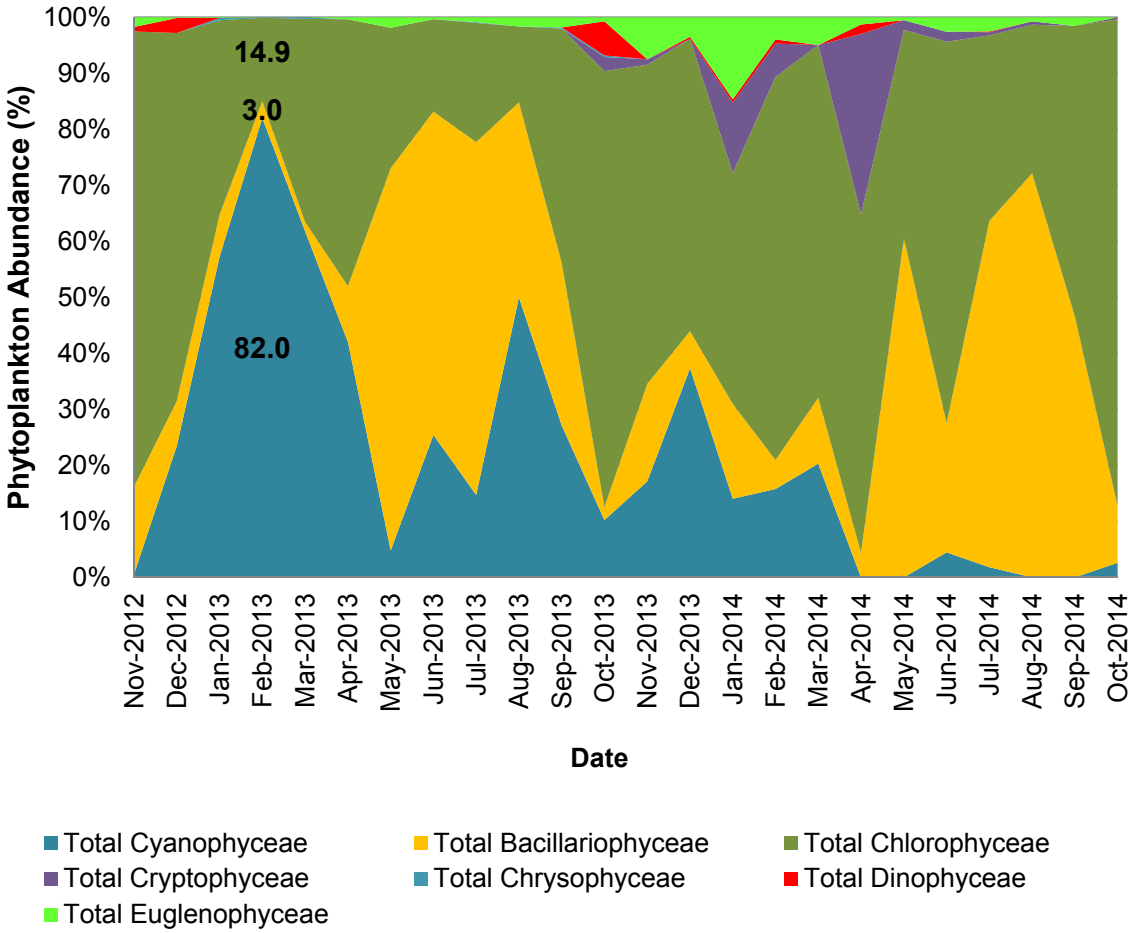

Figure 5.16: Variation in phytoplankton composition over the 24 month study period at Site 7 (after Margaret) in the Vaal River. *Note that the values to the left side of the graph represent the % composition of different taxa present during maximum phytoplankton cell density.*

When comparing Figure 5.15 and Figure 5.16, the trends for phytoplankton composition at sites 6 and 7 are strikingly similar. Both these sites also reached their maximum phytoplankton cell densities during February 2013 (illustrated in Figures 5.9 and 5.19b and c).

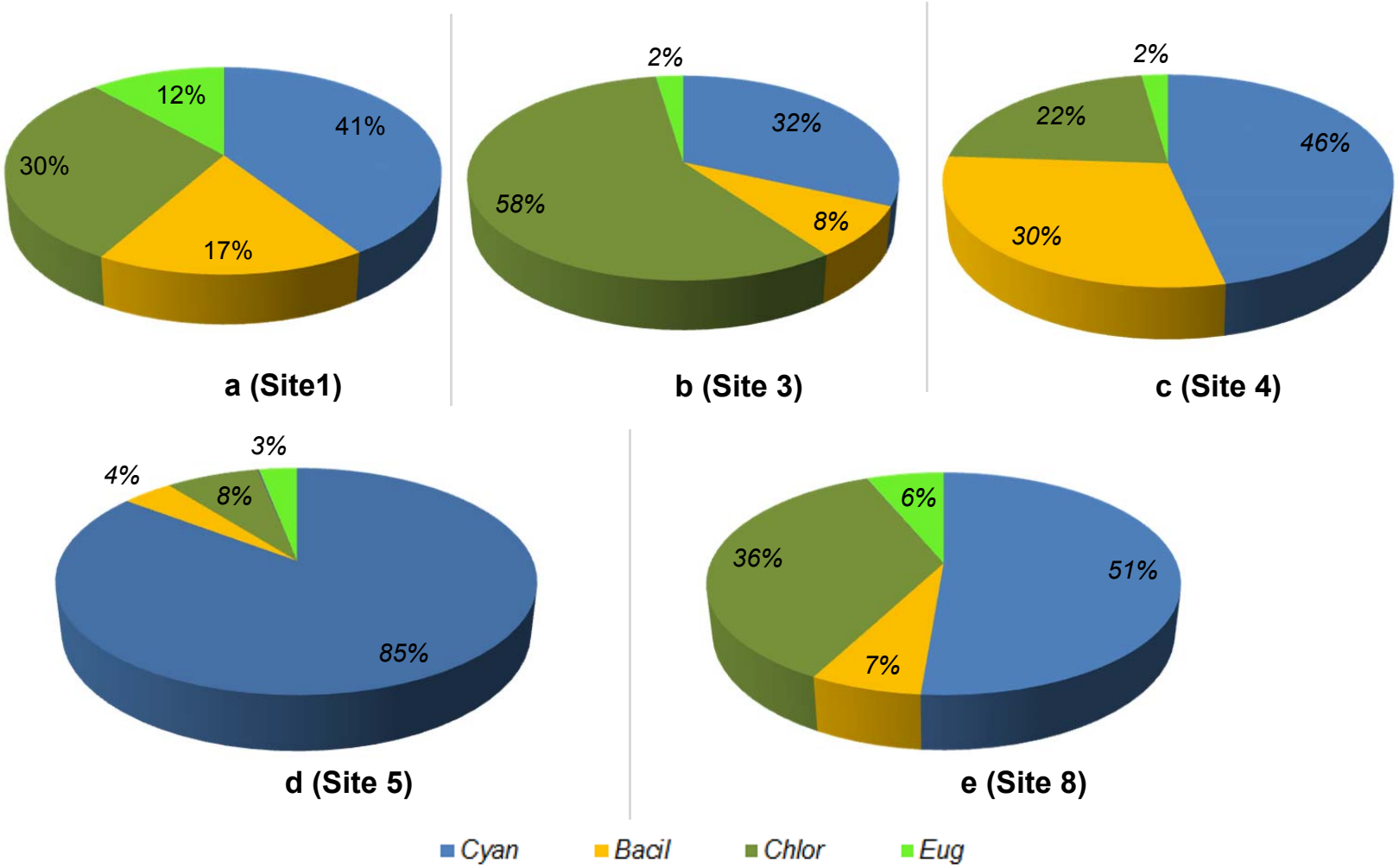

Figure 5.17: Relative abundance and dominance of the phytoplankton taxa present for the duration of the study at different sites in the KMS. *Cyan* = Cyanophyceae, *Bacil* = Bacillariophyceae, *Chlor* = Chlorophyceae, *Eug* = Euglenophyceae.

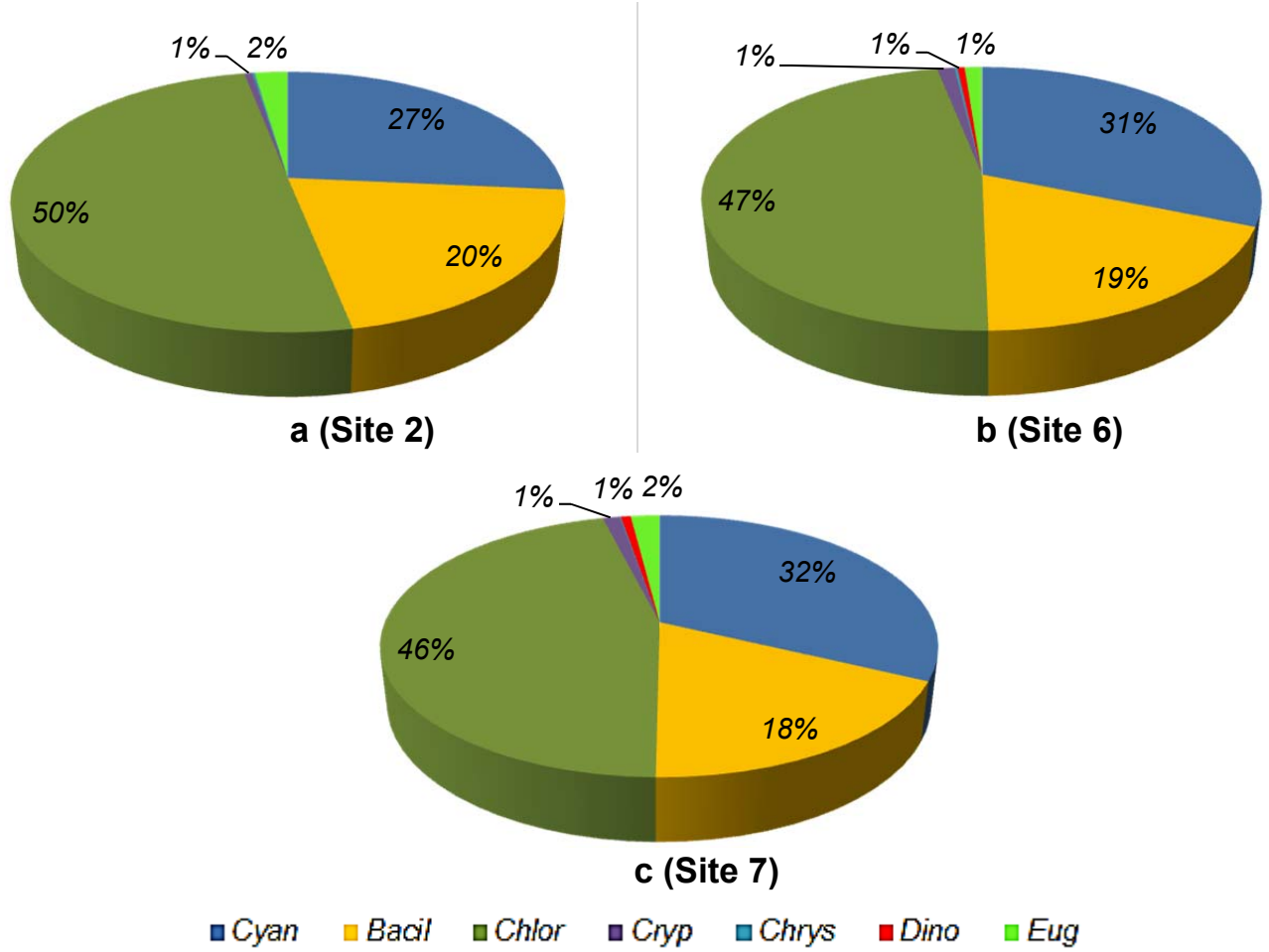

Figure 5.18: Relative abundance and dominance of the phytoplankton taxa present for the duration of the study at different sites in the Vaal River. *Cyan* = Cyanophyceae, *Bacil* = Bacillariophyceae, *Chlor* = Chlorophyceae, *Cryp* = Cryptophyceae, *Chrys* = Chrysophyceae, *Dino* = Dinophyceae, *Eug* = Euglenophyceae.

Figure 5.17 illustrates that, in general, the phytoplankton composition amongst 4 sites in the KMS were dominated by Cyanophyceae at 51% (see calculation of x below), which is especially true for Site 5 (Figure 5.17d), where Cyanophyceae dominated by 85%. At Site 3 however (Figure 5.17b), where Chlorophyceae dominated by 58%, followed by Cyanophyceae at 32%. Once again, the influence of Site 1 (Figure 5.17a) on Site 4 (Figure 5.17c) is visible as Cyanophyceae percentage dominance at both sites differed by only 5% (Site 1 = 41% and Site 4 = 46%).

$$x\ (\%) = \frac{a}{b}(100)$$

a = sum of the actual percentage composition of a specific phytoplankton taxon at all sites

b = sum of the percentage composition of the phytoplankton taxon in question, representing 100% dominance at each site and thus calculated as:

$$b = n\ (100\%)$$

n = number of sites

Figure 5.18 illustrates that the phytoplankton composition at all 3 sites in the Vaal River was dominated by Chlorophyceae at 48% (using the above calculation of x). The similarities seen between Figure 5.15 and Figure 5.16 were once again reflected in the pie graphs for Site 6 (Figure 5.18b) and Site 7 (Figure 5.18c).

When comparing the KMS to the Vaal River, it can be seen that only 4 phytoplankton taxa (Cyanophyceae, Bacillariophyceae, Chlorophyceae and Euglenophyceae) contributed to the phytoplankton composition in the KMS, as opposed to 7 (Cyanophyceae, Bacillariophyceae, Chlorophyceae, Cryptophyceae, Chrysophyceae, Dinophyceae and Euglenophyceae) in the Vaal River (compare Figures 5.18 and 5.19). A study by Janse van Vuuren and Pieterse (2010) from 1992-1997 also found that the phytoplankton in the middle Vaal River mainly consisted of the same 7 taxa. Janse van Vuuren and Pieterse (2010) also found that Bacillariophyceae and Chlorophyceae dominated and succeeded each other, with occasional Cyanophyceae blooms during summer. Similar trends were observed during this study at Site 2 (Figure 5.14), Site 6 (Figure 5.15) and Site 7 (Figure 5.16) in the Vaal River.

Phytoplankton genera present, their cell densities, as well as the relation between different phytoplankton taxa were revealed by this study. However, when considering the KMS sites, the influence of Site 5 (Khuma) on Site 1 (after Khuma) is so pronounced, that when compared to Site 3 (canal), Site 1 had in almost every instance have the greater impact on Site 4 (canal and Khuma combined).

Since the aim of this study was to determine the impact of the KMS on the Vaal River and Site 5 (Khuma), which should be indicative of organic pollution, have now been identified through the surrounding land-use practices and phytoplankton data as one of the main contributing sites in the KMS regarding surface water quality impacts, it was decided to apply the phytoplankton data to four different biotic indices for a better indication whether phytoplankton could be a valuable contribution for determining surface water quality status.

Table 5.4 contains the scores of four biotic indices, each revealing a different aspect of the phytoplankton assemblages present at each site. The indices used included the Shannon-Wiener Diversity Index, the Pielou's Species Evenness Index, Margalef's Species Richness Index, and Palmer's Algal Genus Pollution Index. A combination of these indices scores will reveal the diversity, evenness, richness and genera indicative of organic pollution.

Table 5.4: Index scores for each site over the 24 month study period.

	Site	Shannon's Index Score	Pielou's Index Score	Margalef's Index Score	Palmer's Index Score
KMS	**1**	2.42	0.71	2.35	24
	3	2.03	0.62	2.21	22
	4	2.00	0.59	2.18	27
	5	1.54	0.47	1.85	24
	8	2.21	0.69	2.10	23
Vaal River	**2**	2.67	0.72	2.93	27
	6	2.57	0.70	2.74	24
	7	2.64	0.71	2.79	27

Shannon-Wiener Diversity Index scores ranged between 1.54 at Site 5 (Khuma) in the KMS and 2.67 at Site 2 in the Vaal River. In a study by Ganai and Parveen (2013) about the effect of physico-chemical conditions on the phytoplankton composition of Wular Lake (India), the Shannon-Wiener Diversity Index scores ranged between 1.67 and 2.21, which is very similar to the scores obtained in this study. Ganai and Parveen (2013) concluded that based on these scores, the water ranged from being moderately polluted (index scores between 3 and 4) to heavily polluted (index scores less than 2).

The Pielou's Species Evenness Index scores ranged between 0.47 at Site 5 (Khuma) in the KMS and 0.72 at Site 2 in the Vaal River, and also seem to relate to the evenness scores obtained by Ganai and Parveen (2013), which ranged between 0.64 and 0.89.

The Margalef's Species Richness Index scores ranged between 1.85 at Site 5 (Khuma) in the KMS and 2.93 at Site 2 in the Vaal River, which is fairly similar to the Margalef's Species Richness Index scores (1.25-2.11) obtained by Onyema (2013) in a study on Onijedi Lagoon (Lagos).

Finally, the Palmer's Algal Genus Pollution Index scores ranged between 22 at Site 3 (canal) in the KMS and 27 at Site 4 (canal and Khuma combined) in the KMS, as well as sites 2 (before KMS) and 7 (after Margaret) in the Vaal River. Literature indicates that the Palmer's Algal Genus Pollution Index scores of various studies may differ quite significantly. To give an example, the Palmer scores obtained by Noel and Rajan (2015) on Vaigai River (India) ranged between 15 and 24, whereas the scores obtained by Jafari and Gunale (2006) on the Mutha River (India) ranged between 16 and 41.

In the KMS, Site 1 (after Khuma) had the highest diversity score (2.42), followed by Site 8 (N12 after eye = 2.21), Site 3 (canal = 2.03), Site 4 (canal and Khuma combined = 2.00) and finally Site 5 (Khuma) with the lowest diversity score of 1.54. Ganai and Parveen (2013) stated that for Indian lakes, Shannon-Wiener Diversity Index scores less than 2 is indicative of severely polluted water. Applying this, Site 5 (Khuma) in the KMS could be considered as heavily polluted, since it had a diversity score less than 2. Site 1 also had the highest evenness score (0.71) followed by Site 8 (0.69), Site 3 (0.62), Site 4 (0.59) and finally Site 5 with the lowest evenness score of 1.54. Again, Site 1 had the highest richness score of 2.35, only this time it's followed by Site 3 (2.21), Site 4 (2.18), Site 8 (2.10) and finally Site 5 (1.85). The Palmer index shows that Site 4 had the highest score of 27, sites 1 and 5 collectively the second highest score of 24, followed by Site 8 (23) and Site 3 (22).

In the Vaal River, Site 2 (before KMS) had the highest diversity score of 2.67, followed by Site 7 (after Margaret = 2.64) and Site 6 (after KMS = 2.57). As expected, Site 2 also had the highest evenness score (0.72) followed by Site 7 (0.71) and finally Site 6 (0.70). Contrary to the KMS sites, the same pattern was also observed for richness at the Vaal River sites where Site 2 had the highest richness score of 2.93, followed by Site 7 (2.79) and Site 6 (2.74). Even the Palmer index exhibited similar patterns with sites 2 and 7 each scoring 27, followed by Site 6 (24).

When comparing the Vaal River to the KMS based on these indices, the three Vaal River sites obtained overall higher scores for all four indices. The scores obtained at Site 1 (after Khuma) for diversity, evenness and richness were more closely related to the scores obtained at the Vaal River sites. The exception was Site 4 (canal and Khuma combined) that shared the highest Palmer score (27) with sites 2 (before KMS) and 7 (after Margaret) in the Vaal River.

Contrary to Site 1 (after Khuma), Site 5 (Khuma) had the lowest scores for diversity, evenness and richness, showing the weakest relation to the Vaal River sites, except for the Palmer score (24) which relates to both Site 1 (after Khuma) in the KMS and Site 6 (after KMS) in the Vaal River.

This indicates that Palmer's Algal Genus Pollution Index would be more indicative of the KMS's impact on the Vaal River. Since this index is based on the occurrence of indicator phytoplankton genera, Table 5.3 was used to assemble Table 5.5, which limits the genera that occurred most frequently at each site, to genera most tolerant to organic pollution (in order of decreased tolerance to organic pollution), according to the Palmer's Algal Genus Pollution Index.

Table 5.5: Summary of the Palmer Index phytoplankton genera that occurred most frequently amongst the sampling sites.

Genus	**Index Score assigned by Palmer**	**KMS**					**Vaal River**		
		1	**3**	**4**	**5**	**8**	**2**	**6**	**7**
Euglena	5	✓	✓	✓	✓	✓	✓	✓	✓
Oscillatoria	5	✓	✓	✓	✓	✓	✓	✓	✓
Chlamydomonas	4	✓	✓	✓	✓	✓	✓	✓	✓
Scenedesmus	4		✓	✓		✓	✓	✓	✓
Navicula	3	✓		✓	✓				
Nitzschia	3	✓	✓	✓	✓	✓	✓		✓
Phacus	2	✓			✓				
Cyclotella	1	✓		✓		✓	✓	✓	✓
Phormidium	1	✓	✓	✓	✓	✓	✓	✓	✓
Total Palmer's index genera present: 9		**8**	**6**	**8**	**7**	**7**	**7**	**6**	**7**

It is noted that the presence of other genera (*Ankistrodesmus*, *Gomphonema*, *Pandorina*, *Fragilaria* and *Micractinium*) made minor contributions to the final Palmer Index scores at some sites, though they did not occur as frequently as the genera listed in Table 5.5 and are thus considered to be less indicative of water quality status.

It was stated (section 4.2.3) that a Palmer Index score greater than 20 indicates high organic pollution, which is then applicable to all the sites according to Table 5.4. As mentioned before, Site 5 (Khuma) had the lowest diversity score of all the sites but a Palmer score equivalent to that of sites 1 (after Khuma) in the KMS and 6 (after KMS) in the Vaal River.

Table 5.5 shows that 7 out of the 9 indicator genera, which had occurred most frequently, had occurred at Site 5 (Khuma) and in fact exceeded that of Site 6 (after KMS) in the Vaal River by one genus. This leads to the conclusion that the higher the diversity score, the higher the Palmer score would be due to the presence of more genera at a given site. Therefore, the genera present at sites with lower diversity scores and high Palmer scores would be more indicative of organic pollution expressed through tolerance, rather than conditions favouring more genera. Conditions that favour the occurrence of more genera would naturally also include genera tolerant to organic pollution, as was the case in the Vaal River. The KMS which obtained lower diversity scores, yet high Palmer scores, would then be more indicative of organic pollution compared to the Vaal River. Table 5.5 also confirms this with two of the tolerant genera, *Navicula* and *Phacus* only occurring in the KMS, and this is further emphasised by the fact that the KMS were dominated by Cyanophyceae (Figure 5.17).

To conclude the phytoplankton data's contribution to the second site selection process, the sites that did not meet the final selection criteria (as indicated throughout this chapter) include sites 5 (Khuma) and 8 (N12 after eye) in the KMS, as well as Site 7 (after Margaret) in the Vaal River.

Phytoplankton data derived from Site 5 (Figure 5.19) in the KMS could most definitely be indicative of surface water quality alterations in the KMS, since it differed from the data derived from the sites that did meet the final selection criteria. In summary, Site 5 (Khuma) reached the highest maximum phytoplankton cell density (Figure 5.8), it showed the highest percentage composition of Cyanophyceae (Figure 5.17d) and the lowest diversity, richness and evenness index scores (Table 5.4).

Unfortunately, there were no available physico-chemical data for Site 5 (Table 3.2), defeating the main purpose of this study. For that reason, both sites 1 (after Khuma) and 4 (canal and Khuma combined) in the KMS will serve to reflect the impact of the informal settlement of Khuma on the surface water quality of the KMS at that specific location.

Phytoplankton data of Site 8 (N12 after eye) in the KMS were excluded due to the lack of physico-chemical data, as well as the fact that it could only be sampled during one month for the entire 2014 (Figure 5.20) as a result of being dried up.

Figure 5.19: Photograph of Site 5 (Khuma) located within the KMS.

Figure 5.20: Photograph of Site 8 (N12 after eye) located within the KMS.

Though physico-chemical data for Site 7 (Figure 5.21) were readily available, the trends in phytoplankton data displayed at both sites 6 (after KMS) and 7 (after Margaret) were similar (Figure 5.8). No distinction could for instance be made between Figures 5.16 and 5.17 as well as Figures 5.19b and 5.19c for phytoplankton composition. The fact that these sites are located close to each other, can explain the similarities. Therefore, to avoid repetition it was decided to rather include the data from Site 6 (after KMS) as it is situated at the Midvaal Water Company's raw water abstraction point and therefore considered more important in terms of water quality monitoring.

These similarities between sites 6 (after KMS) and 7 (after Margaret) also indicate that the water being pumped from Margaret shaft had no impact on the water quality of the Vaal River.

Figure 5.21: Photograph of Site 7 (after Margaret) located within the Vaal River.

5.4 MULTIVARIATE STATISTICAL ANALYSIS: PHYSICO-CHEMICAL VARIABLES AND PHYTOPLANKTON DATA

In an attempt to integrate the physico-chemical and phytoplankton data in such a manner that would allow for a simplified interpretation of these immense data sets (without compromising the contribution made by each of the variables under consideration), a Principal Component Analysis (PCA) was performed using Statistica version 12.

Although the PCA was not successful in reducing the number of variables (28), it could be used to generate components that could explain the variability in the data set more effectively. During the interpretation of a PCA, the rule of thumb states that only the principal components that have eigen-values greater than 1 should be considered (Hair *et al*., 2009). Eigen-values greater than 1 were exhibited by 8 of the principal components. Table 5.6 shows that the first four components all displayed eigen-values greater than 1, and collectively accounted for 53% of the total variance within the data set.

According to Hair *et al*. (2009), the selection of the number of principal components to be retained are also supported by the number of principal components occurring before a clear break in the scree plot (Figure 5.22). By combining these two criteria, only the first four components will be retained for this study. These principles have also been applied by Swanepoel (2014) and Bhat *et al*. (2014).

NOTE: Please refer to APPENDIX A.4 for the eigenvalues and related percentages on all 28 contributing principal components.

Table 5.6: Eigenvalues and related percentages on the main contributing principal components.

Principal components	Eigenvalue	% Total variance	Cumulative Eigenvalue	Cumulative % variance
1	5.759234	20.56869	5.75923	20.5687
2	4.060394	14.50141	9.81963	35.0701
3	3.249376	11.60491	13.06900	46.6750
4	1.805225	6.44723	14.87423	53.1222

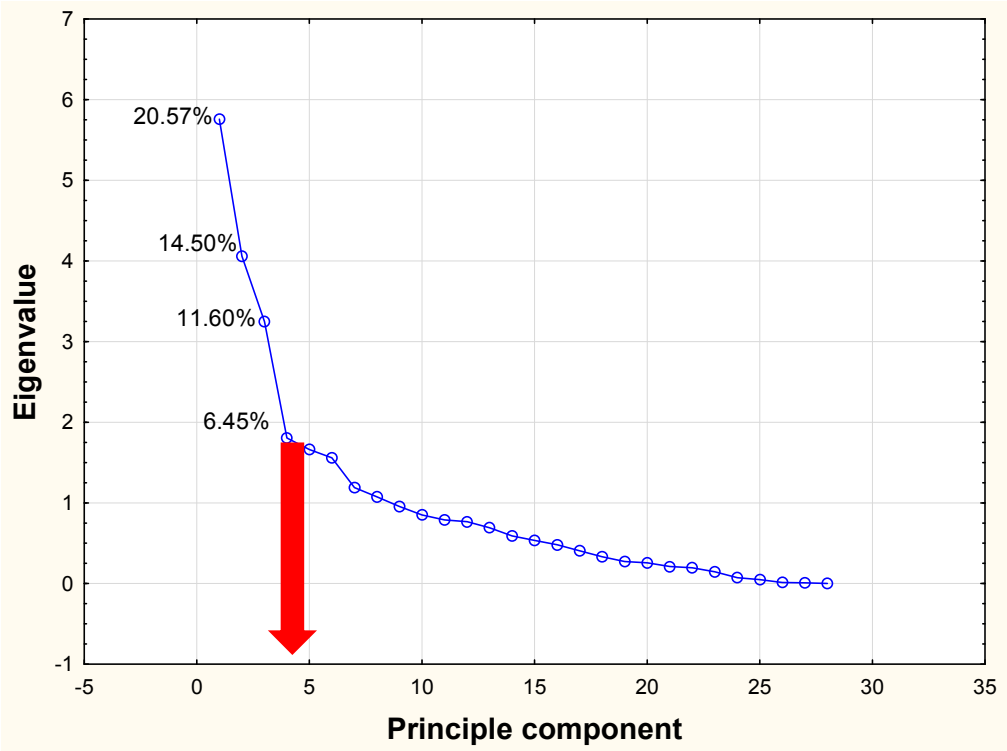

Figure 5.22: Scree plot illustrating eigenvalues against principal components, including the % variance contributed by each component. *Note that the red arrow indicates the clear break in the scree plot.*

Table 5.7: Factor-variable correlations of the first four principal components (PC's) – All variables 2012-2014.

Variable	PC1	PC2	PC3	PC4
Cyan	-0.253691	0.251501	0.219139	0.064853
Bacil	-0.300335	0.164834	0.248701	0.206361
Chlor	-0.454119	0.082046	**0.545445**	0.248287
Cryp	-0.451755	**-0.631159**	-0.266564	0.168052
Chrys	-0.132925	0.003627	0.223937	0.123864
Dino	-0.163319	-0.006288	0.169895	0.448375
Eug	-0.291120	0.095801	-0.128809	-0.003290
Colour	**-0.665269**	-0.016101	-0.228377	-0.292266
pH	0.068441	-0.174652	**0.723400**	0.287996
EC	**0.908597**	-0.183072	-0.147077	0.091052
NTU	0.004153	-0.329748	-0.067642	-0.316327
TOC	-0.136247	**0.759417**	-0.469901	0.034013
T. Chl	-0.383358	0.378317	0.414039	0.234280
F.coli	-0.091372	0.373495	-0.386606	-0.118709
NO_3^-	**0.543208**	-0.104259	-0.188495	0.133991
NH_4^+	0.017830	**0.533026**	**-0.690772**	0.194643
PO_4^{-3}	0.006374	0.430218	**-0.599813**	0.237544
Fe	-0.492702	**-0.750115**	-0.366330	0.141251
Mn	-0.484157	**-0.737518**	-0.373461	0.166280
Cl^-	**0.848744**	-0.070505	-0.209187	0.173081
SO_4^{2-}	**0.847311**	-0.372780	0.065742	-0.020822
CN^-	-0.122916	-0.191949	-0.193024	0.115506
Zn	0.090721	0.277002	-0.402397	0.374487
Cu	0.018575	0.057805	-0.073710	**0.548285**
Al	-0.247809	0.063076	-0.086760	**-0.636632**
Na^+	**0.903032**	-0.234191	-0.015344	0.065211
As	-0.465701	**-0.746060**	-0.353607	0.170795
U	**0.538726**	-0.245644	-0.017957	-0.148734

Factor loadings in red show most significant correlations and variance to the principal component under consideration

From Tables 5.10 and 5.11 it can be derived that the first principal component accounted for 20.6% of the total variance, due to its negative correlation with colour and positive correlations with EC, Cl^-, SO_4^{2-}, Na^+ and U. This component showed very strong positive correlations with EC (0.908597), Cl^- (0.848744), SO_4^{2-} (0.847311) and Na^+ (0.903032). The second component explained 14.5% of the total variance due to its negative correlation with Cryptophyceae, Fe, Mn and As, as well as positive correlations with TOC and NH_4^+. Noticeably strong negative correlations with Fe (-0.750115), Mn (-0.737518) and As (-0.746060) were exhibited by PC2. The third component showed positive correlations with Chlorophyceae and pH, negative correlations with NH_4^+ and PO_4^{-3}, and explained 11.6% of the total variance in the data. The negative correlations to both NH_4^+ (-0.690772) and PO_4^{-3} (-0.599813) was most likely owed to the presence (positive correlation) of Chlorophyceae. Finally, the fourth component was responsible for 6.4% of the variance in the data because of its negative correlation with Al (-0.636632) and positive correlation with Cu (0.548285).

Bhat *et al*. (2014) used the plane (axes) projections as an alternative for interpreting their PCA, mainly because it only considers two principal components at a time. This permitted them to better distinguish between variable groupings that were not apparent in the original PCA. The use of plane projections was also applied in this study (Figure 5.24 and 5.29) as the same problem was encountered regarding the PCA. The plane projections group variables (physico-chemical variables and phytoplankton taxa) together without assigning samples. Since this study requires the consideration of variables from four principal components, we had to make use of more than 1 plane projection. Relating individual samples of specific sites to the variable groupings, required a Kruskal-Wallis test to compare multiple independent samples (groups) from the different sites (Figures 5.26 to 5.28). This will help to explain the correlation of the variables deemed important to each component as indicated in Table 5.7. Figure 5.23 represents the plane projections of PC2 against PC3, illustrating more defined variable cluster formations.

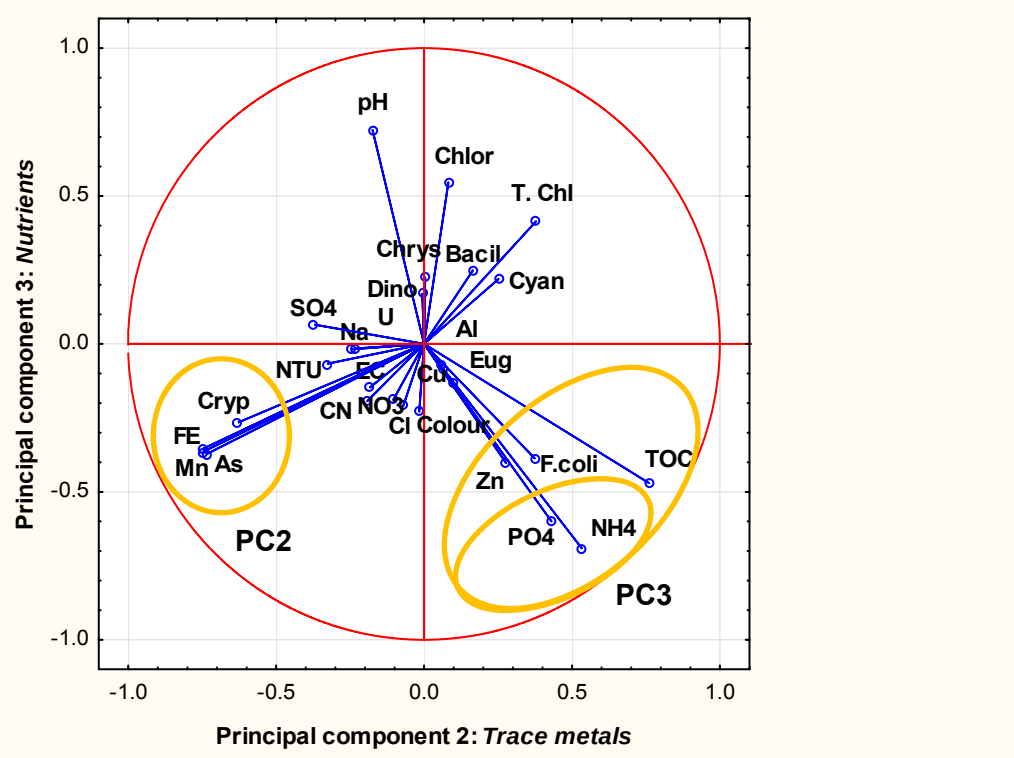

Figure 5.23: Projections of the variables on the component-plane (PC2:PC3).

PC2 show clear correlations between Fe, Mn, As and Cryptophyceae (Figure 5.23), that the PCA was not able to do. The length of the vectors shows that the variance contributed by the trace metals is more significant as opposed to that of Cryptophyceae (slightly shorter vector). To explain this association, Figure 5.24 demonstrates that Site 6 (after KMS) in the Vaal River exhibited significantly higher Fe, Mn and As concentrations, suggesting that the greater percentage of variance explained by this component is owed mostly to the concentrations observed at Site 6. The reason why these correlations are negative may be because Fe, Mn and As occurred at concentrations below the detection limit at the remaining sites. The slightly smaller negative correlation between this component and Cryptophyceae can also be clarified. Figure 5.24 shows that Cryptophyceae occurred in significantly higher cell densities ($p<0.05$) at both Vaal River sites and not only Site 6 (such as the trace metals).

PC2 distinguishes between the KMS and the Vaal River because it also correlates positively with TOC and NH_4^+ (Table 5.7), which had reached overall higher mean values at sites 1 (after Khuma) and 4 (canal and Khuma combined) in the KMS (Table 5.1 and Figure 5.24). These positive correlations are less significant in their contribution to the variance of the second component, due to less variance in the samples between different sites, unlike that of Fe, Mn and As.

PC3 also formed a cluster on the component-plane, revealing the correlation between NH_4^+ and PO_4^{-3}. This cluster is less apparent compared to that of PC2, because it also includes TOC, F.coli and Zn that were not included amongst the most significant contributing variables of this component. Figure 5.25 demonstrates that sites 1 (after Khuma) and 4 (canal and Khuma combined) in the KMS exhibited significantly higher ($p<0.05$) NH_4^+ and PO_4^{-3} concentrations and less Chlorophyceae, especially compared to the Vaal River sites. This implies that the greater percentage of variance exhibited by this component is owed to high nutrient concentrations in the KMS, highlighting the influence of Site 1 (after Khuma) on Site 4 (canal and Khuma conbined). The reason why these correlations are negative (Table 5.7) may be because NH_4^+ and PO_4^{-3} were practically undetected at the majority of sites (Figure 5.25 and Table 5.1), whereas pH and Chlorophyceae showed less variance in the samples between different sites and therefore correlated positively with the component (Figure 5.25). The correlations between PC3 and TOC, Faecal coliforms and Zn (Figure 5.23) were expected, since it was established that this component is representative of the influence that Site 1 (after Khuma) has on the water quality of the KMS.

Figure 5.23 lack the ability to distinguish between variables (Cu and Al) captured by PC4. This was expected because this component was not selected to be projected. It was however established through Figure 5.26 that the component's negative correlation to Al (Table 5.7) is perhaps owed to Site 2 (before KMS) in the Vaal River, since it exhibited the highest mean Al concentration (Table 5.1) over the course of this study. Figure 5.26 also illustrates that besides Site 2 (and given the mean Al concentrations illustrated in Table 5.1), Al would in effect relate more to sites 1 (after Khuma) and 4 (canal and Khuma combined) in the KMS than to Site 6 (after KMS) in the Vaal River. Similar to Al, Cu concentrations also exhibit smaller variance in the data amongst all sites, rather than relating to the Vaal River or KMS in particular. This explains the minor contribution made by Al and Cu to the variance exhibited by this component.

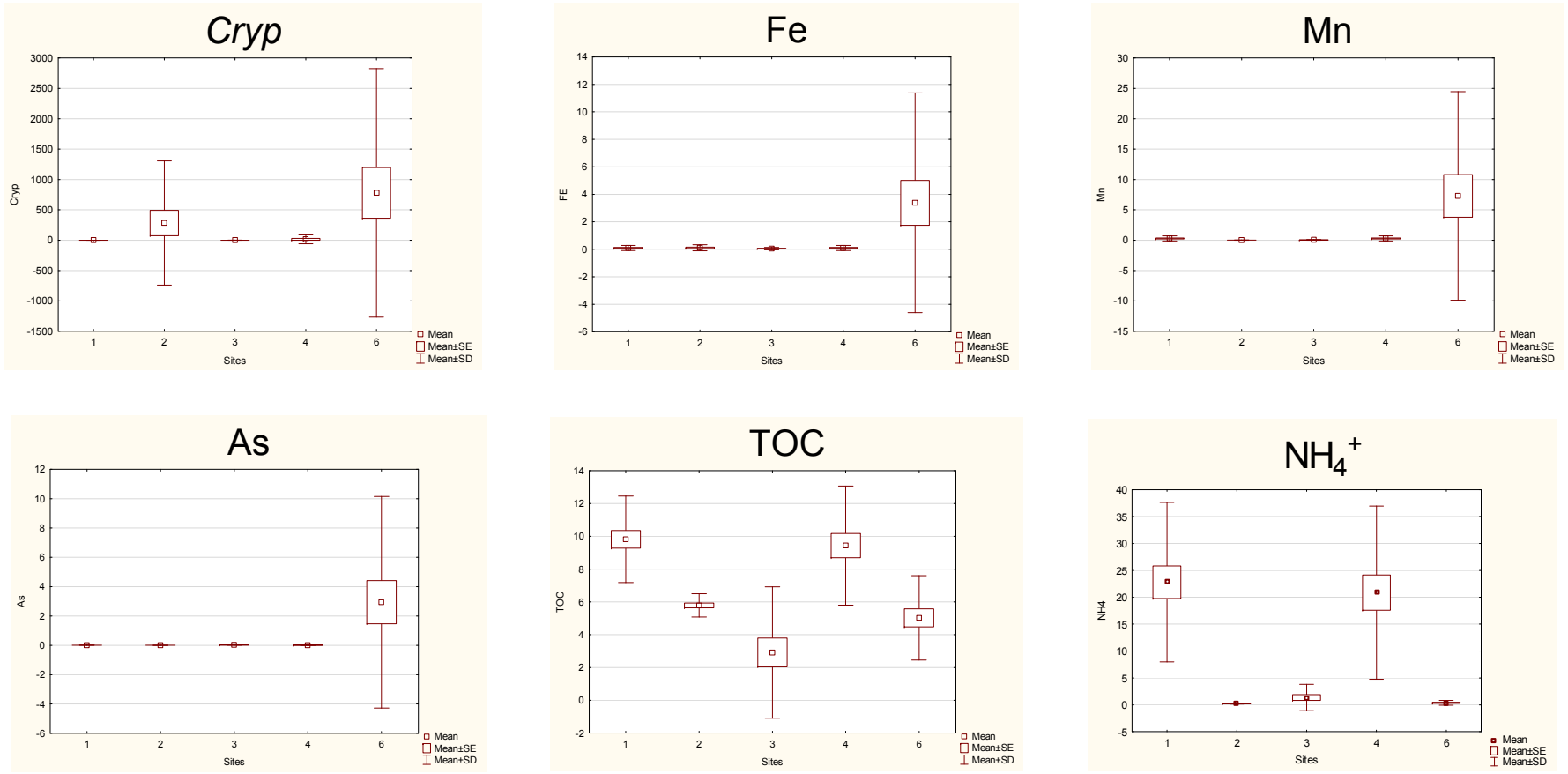

Figure 5.24: Kruskal-Wallis boxplot illustrating the variables that contributed most to the variance of the second principal component (PC2) between different sites.

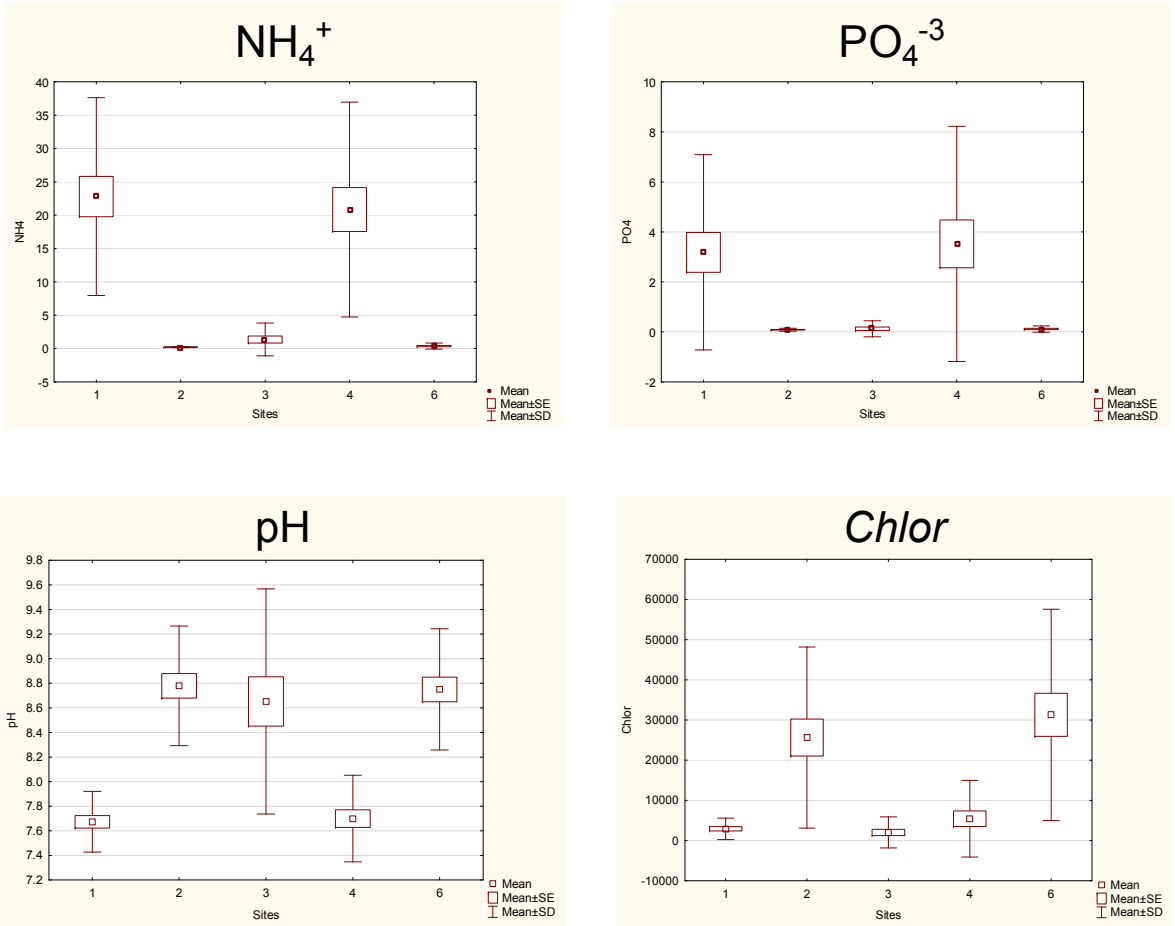

Figure 5.25: Kruskal-Wallis boxplot illustrating the variables that contributed most to the variance of the third principal component (PC3) between different sites.

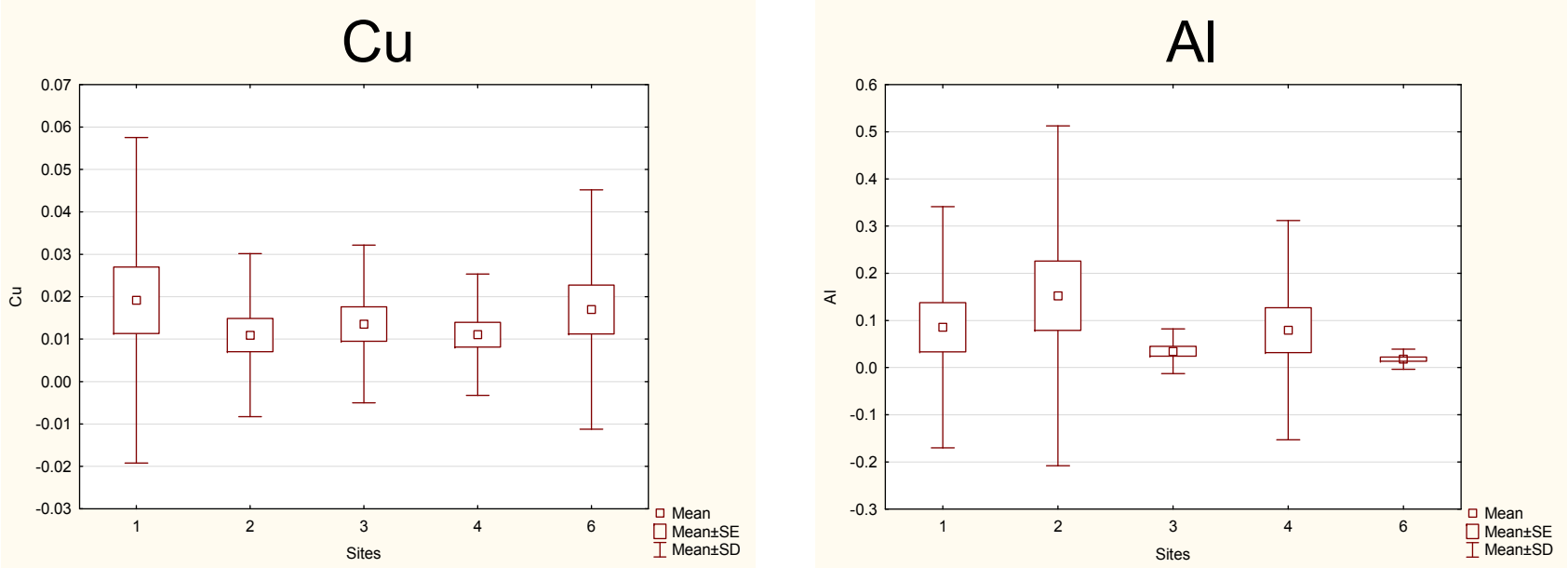

Figure 5.26: Kruskal-Wallis boxplot illustrating the variables that contributed most to the variance of the fourth principal component (PC4) between different sites.

In the process of analysing various combinations of component-plane projections, it was discovered that the projection of PC1 against PC3 could potentially serve as a main representative of the PCA. The reasoning behind it is supported by this particular projection's ability to display separate clusters of the variables captured by each principal component. Figure 5.27 represents the plane projections of PC1 against PC3, illustrating more defined variable cluster formations, relating to all four principal components respectively, as well as the appropriate sites which were assigned to them.

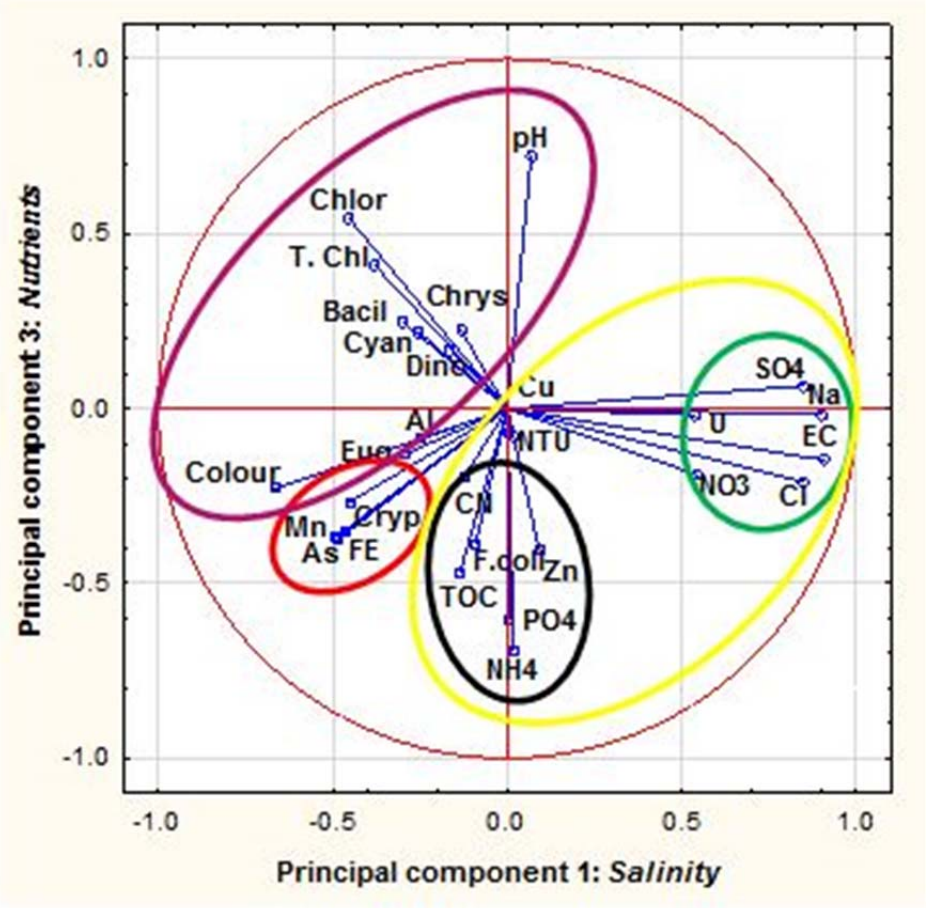

Figure 5.27: Projections of the variables on the component-plane (PC1:PC3) with manual assignment of clusters for each Site. *Site 1■; Site 2■; Site 3■; Site 4■; and Site 6■.*

In summary, Figure 5.27 clearly separates the KMS (yellow, black and green) from the Vaal River (purple and red). Within the KMS, Site 1 (after Khuma) has the biggest influence on the water quality of Site 4 (canal and Khuma combined) regarding PO_4^{-3} and NH_4^{+} (longer vectors). According to Table 5.7, Site 1 then represents PC3. Site 3 (canal) has the biggest influence on Site 4 (canal and Khuma combined) regarding Na^{+}, Cl^{-}, SO_4^{2-} and EC (longer vectors), and represents PC1.

In the Vaal River, Figure 5.27 shows that pH, Chlorophyceae and colour are strongly associated (longer vectors) with Site 2 (before KMS). The descriptive statistics (Table 5.1) indicate that these variables reached highest concentrations at Site 2, even though they are also closely correlated to Site 6 (after KMS). This implies that Site 2 (before KMS) did not contribute as much to the variance in the data compared to other sites, but that Figure 5.27 was rather able to separate the Vaal River sites from the KMS, based on the lack of salinity and nutrients in the Vaal River at both sites. Because Al also reached highest concentrations at Site 2, it represents PC4. Site 6 (after KMS) however has the biggest influence on the Vaal River regarding Fe, Mn and As (longer vectors), and represents PC2.

The software package Canoco 4.5 was used to illustrate the PCA ordination site plot. Figure 5.28 displays the PCA site plot containing all the variables contributing to the variance within the data, their relation to the first and second axes, as well as the individual samples at different sites. All physico-chemical and biological variables were used as concentrations but centred and standardised to compensate for unit differences in the PCA. Ordinations were interpreted using the following rationale: Variables are 1) positively correlated with each other if their vectors subtend a small angle, 2) not correlated if their vectors are arranged greater than 90° apart, 3) negatively correlated if their vectors are directed oppositely (180°) and 4) variables with the longest vector relative to an axis have the greatest influence on that axis. The same principle applies for the clustering of sampling sites, with respect to each other as well as the variables (Bhat *et al*., 2014).

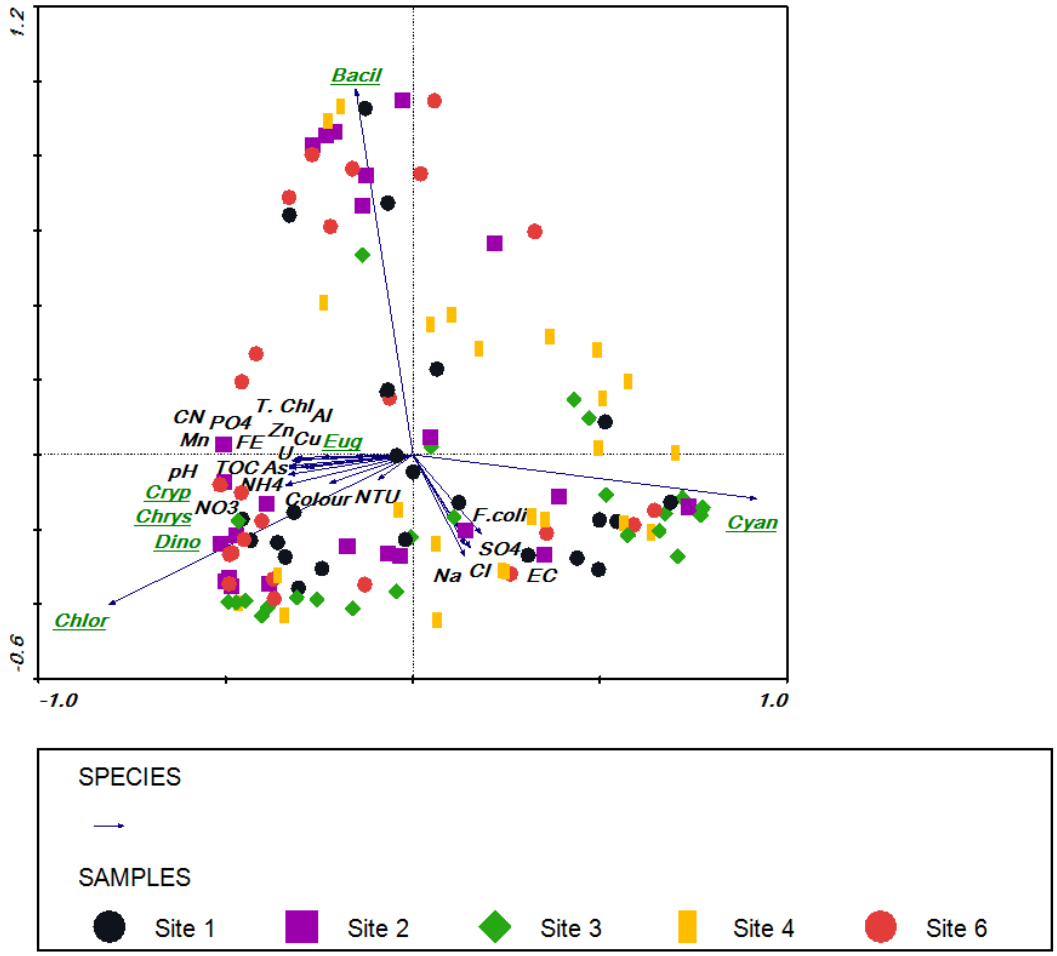

Figure 5.28: A PCA site plot illustrating correlations between the principal physico-chemical water quality variables, phytoplankton taxa and the samples of the different sites in the KMS and Vaal River.

Table 5.8: Eigenvalues and cumulative percentage variance contributed by the four axes on the PCA ordination.

Axes	Eigenvalue	% Total variance	Cumulative Eigenvalue	Cumulative % variance of species data
1	0.392	39.2	0.392	39.2
2	0.261	26.0	0.653	65.2
3	0.165	16.5	0.818	81.7
4	0.127	12.7	0.945	94.4

According to Table 5.8, the first axis explained 39.2% of the variance in the data. Both Chlorophyceae and Cyanophyceae strongly associated with the first axis, and their vectors (situated opposite each other) indicate that they have a strong negative correlation (Figure 5.28). This is likely because they tend to form large assemblages at different times of the year (Janse van Vuuren & Pieterse, 2010).

However, it was established that the KMS (Figure 5.17) was dominated by Cyanophyceae at 51%, whereas the Vaal River (Figure 5.18) was dominated by Chlorophyceae at 48%. More samples from sites 2 (before KMS) and 6 (after KMS) associate closer to Chlorophyceae, suggesting that it did succeed in separating the KMS from the Vaal River. Figure 5.28 indicates that sites 2 and 6 in the Vaal River also correlate stronger with Cryptophyceae, Chrysophyceae, Dinophyceae, Euglenophyceae and Bacillariophyceae. This is supported by Figures 5.19a and 5.19b, where Cryptophyceae only featured at the Vaal River sites, and Dinophyceae solely at Site 6 (after KMS).

Not only did the PCA separate the KMS from the Vaal River regarding phytoplankton composition, but also with regards to selective physico-chemical variables. The second axis explained 26% of the variance in the data (Table 5.8). Figure 5.28 shows positive associations of Na^+, Cl^-, SO_4^{2-} and EC with the second axis, with the addition of Faecal coliforms that wasn't captured previously. It is also illustrated that sites 1 (after Khuma), 3 (canal) and 4 (canal and Khuma combined) in the KMS share mutual correlations with this particular set of variables. The descriptive statistics (see section 5.1) revealed that mean values for Na^+, Cl^-, SO_4^{2-} and EC were much higher in the KMS than in the Vaal River, particularly at Site 3 (canal). It was also mentioned that Site 3 (canal) has the most influence on Site 4 (canal and Khuma combined) regarding these variables, which is why it relates to PC1. Faecal coliforms were highest at Site 1 (after Khuma).

Bacillariophyceae had the biggest influence (longest vector) on the variance of the data and shows a strong association with the first axis. It is also indicated that sites 2 (before KMS) and 6 (after KMS) in the Vaal River associated stronger with Bacillariophyceae as opposed to the KMS sites. This is supported by the abundance of phytoplankton taxa (Figures 5.18 and 5.19), where Bacillariophyceae contributed more to the phytoplankton composition in the Vaal River (Figure 5.18). The exception here is Site 4 (canal and Khuma combined) in the KMS, where Bacillariophyceae contributed up to 30% of the total phytoplankton density. In the PCA, samples from Site 4 (yellow blocks) are associated with Bacillariophyceae.

Given these results, in summary it seems that not only does PC1 suggest the importance of Site 3 (canal) and its impact on the water quality of the KMS, but in conjunction with the PCA ordination, it also distinguishes between the KMS and the Vaal River by correlating negatively with colour, Fe, Mn and As, all of which reached higher values in the Vaal River (Table 5.1).

In the Vaal River, sites 2 (before KMS) and 6 (after KMS) seem to correlate with variables which contributed most to the variance exhibited by the second, third and fourth principal components. The PCA ordination shows that NH_4^+ and PO_4^{-3} correlate with the physico-chemical variables (Fe, Mn, As and colour) and phytoplankton taxa (Chlorophyceae, Cryptophyceae, Chrysophyceae and Dinaphyceae) associated with the Vaal River sites. This is most probably the case because of the large concentrations of phytoplankton that depend on it as nutrients. The descriptive statistics revealed that both NH_4^+ and PO_4^{-3} had reached their maximum mean concentrations at sites 1 (after Khuma) and 4 (canal and Khuma combined) in the KMS respectively, rather than the Vaal River where the T.Chl- and phytoplankton cell densities were much higher, suggesting that it was used for phytoplankton growth.

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

Large areas within the KMS catchment are dominated by both urbanisation and mining activities (Figure 3.2). The assumption that these two factors contribute most to deteriorating water quality in the KMS (DWA, 2006b) was confirmed by the physico-chemical and phytoplankton results obtained during this study.

As anticipated in the KMS, the descriptive physico-chemical results confirmed that Site 1 was influenced by the informal settlement of Khuma and showed high TOC, Faecal coliforms and nutrient concentrations. The concentration of these variables increased since 2002 at Site 1, except for Faecal coliforms. The Enviro Canal (Site 3) was influenced mainly by mining activities and reflected high NTU, EC, and salinity values. Salinity increased from the 2002 period. Site 4 illustrated the combined impacts of urbanisation (Site 1) and mining (Site 3), because TOC, nutrients, as well as salinity increased since 2002. During this study the phytoplankton and physico-chemical results showed that Site 1 (after Khuma) had the largest influence on water quality alterations in the KMS regarding organic pollution from Khuma and not mining, as anticipated. The influence of Site 1 was particularly noticeable at Site 4 (canal and Khuma combined), because Cyanophyceae dominated at both sites 1 and 4, while Site 3 was dominated by Chlorophyceae. It also demonstrated that Chlorophyceae is likely more tolerant to saline conditions, while Cyanophyceae was influenced by the availability of nutrients, as was found by Paerl *et al*. (2001).

Regarding the Vaal River, Site 2 (before KMS) showed no noticeable correlation to a particular set of physico-chemical variables, except turbidity, colour and pH. Site 6 (after KMS), had the highest concentration of trace metals. It was established as single “spikes” in the data due to the initial influx of polluted runoff from tailings dumps after heavy rain during March 2014. The levels of trace metals increased since 2002 at Site 6. The phytoplankton data confirmed that Chlorophyceae dominated in the Vaal River, sometimes succeeded by Bacillariophyceae and with occasional Cyanophyceae blooms. The same tendency was observed by Janse van Vuuren and Pieterse (2010). Also, since Table 5.3 lists all Cyanophyceae genera that occurred at the different sites over the study period, it is recommended that cyano-toxins be measured as part of future research in this area and that the shift in phytoplankton functional groups be investigated as well at all the sites. It is also recommended that line diagrams for Mn, Fe and As should be included for future research to determine the impact of such sudden spikes on water quality.

Although no physico-chemical data were available for Site 5 (Khuma), the phytoplankton data, particularly the indices, were indicative of surface water quality alterations in the KMS. Maximum phytoplankton densities, dominated by Cyanophyceae, were reached at Site 5. Site 5 also showed the lowest diversity-, richness- and evenness index scores, and a Palmer score equivalent to that of Site 6 (after KMS) in the Vaal River. This leads to the following conclusions: Firstly, it must be remembered that conditions that stimulate phytoplankton growth would necessarily also favour genera tolerant to organic pollution, like in the Vaal River. Secondly, the KMS is more prone to organic pollution, particularly Site 5, because it showed lower diversity and high Palmer index scores. And finally, results obtained by the indices could serve as a recommendation for the selection of sites at which physico-chemical variables should ideally be monitored. It is therefore recommended that physico-chemical variables must also be monitored at Site 5, to complement the phytoplankton data in indicating the impact of Khuma on the water quality of the KMS.

Although Site 8 (N12 after eye) in the KMS did not meet the selection criteria for sites to be included in this study, it is recommended that the area's hydrology as well as phytoplankton data obtained for March 2014 should be investigated for its impact on the downstream sites, given that Site 8 would ideally represent the reference site in the KMS.

The multivariate results showed that the plane projection (Figure 5.27) and PCA ordination (Figure 5.28) were able to distinguish the KMS from the Vaal River, by combining both phytoplankton and physico-chemical data. Regarding the KMS, it also indicated the impacts of individual sites on one-another. It can therefore be concluded that through the use of multivariate statistical analysis, it was possible to integrate and interpret the two sets of data and to correlate it successfully to the descriptive statistics. The fact that multivariate results successfully separated the KMS from the Vaal River, led to one of the main conclusions of the study, namely that the KMS did not have an impact on the water quality of the Vaal River. This was anticipated because the physico-chemical results (section 5.2) over a 10 year period indicated that the KMS had a negligible impact on the water quality of the Vaal River at Site 6 (after KMS). This is most probably owed to the differences in stream orders.

Other recommendations are to include the redox (E_h) status as part of the physico-chemical variables to be monitored in the study area. Van Loon and Duffy (2005) stated that the redox (E_h) status plays a key role in determining the speciation of especially trace metals. Site 6 (after KMS) in the Vaal River strongly associated with trace metals and the results also showed that its concentration increased since 2002. It is also recommended that Ca must be monitored in the study area, in addition to the redox (E_h) status.

The high turbidity at Site 3 (canal) in the KMS is probably due to high Ca concentrations in the water flowing from Stilfontein Gold Mine, but this has to be confirmed. Monitoring Ca in the study area would narrow down the cause of high turbidity in the study area.

These recommendations will contribute to the development and implementation of a source water management plan for the Middle Vaal region.

CHAPTER 7: REFERENCES

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CHAPTER 8: APPENDICES

APPENDIX A.1: Kruskal-Wallis multiple comparisons of p-values (2 tailed) that indicate significant variable differences ($p<0.05$) between all sites from 2012 to 2014. ***Note that he Rank values coloured in red indicate significant differences.***

VARIABLE	SITE				
	1	2	3	4	6
Colour	**R:55.646**	**R:81.729**	**R:17.286**	**R:54.313**	**R:80.813**
1		0.077251	0.001538	1.000000	0.101629
2	0.077251		0.000000	0.051099	1.000000
3	0.001538	0.000000		0.002589	0.000000
4	1.000000	0.051099	0.002589		0.068016
6	0.101629	1.000000	0.000000	0.068016	
pH	**R:27.833**	**R:84.625**	**R:73.190**	**R:28.625**	**R:82.500**
1		0.000000	0.000076	1.000000	0.000000
2	0.000000		1.000000	0.000000	1.000000
3	0.000076	1.000000		0.000110	1.000000
4	1.000000	0.000000	0.000110		0.000000
6	0.000000	1.000000	1.000000	0.000000	
EC	**R:68.542**	**R:25.354**	**R:104.29**	**R:75.000**	**R:27.479**
1		0.000103	0.004208	1.000000	0.000274
2	0.000103		0.000000	0.000004	1.000000
3	0.004208	0.000000		0.038586	0.000000
4	1.000000	0.000004	0.038586		0.000012
6	0.000274	1.000000	0.000000	0.000012	
NTU	**R:50.646**	**R:77.250**	**R:45.333**	**R:48.958**	**R:71.104**
1		0.065867	1.000000	1.000000	0.366735
2	0.065867		0.016379	0.038599	1.000000
3	1.000000	0.016379		1.000000	0.110001
4	1.000000	0.038599	1.000000		0.237145
6	0.366735	1.000000	0.110001	0.237145	
TOC	**R:85.667**	**R:46.391**	**R:25.429**	**R:80.604**	**R:43.795**
1		0.000466	0.000000	1.000000	0.000177
2	0.000466		0.356162	0.003892	1.000000
3	0.000000	0.356162		0.000000	0.685421
4	1.000000	0.003892	0.000000		0.001613
6	0.000177	1.000000	0.685421	0.001613	
T. Chl	**R:55.875**	**R:79.217**	**R:27.643**	**R:50.188**	**R:76.583**
1		0.173751	0.049626	1.000000	0.329192
2	0.173751		0.000004	0.030939	1.000000
3	0.049626	0.000004		0.248663	0.000011
4	1.000000	0.030939	0.248663		0.065498
6	0.329192	1.000000	0.000011	0.065498	
Faecal coliforms	**R:81.500**	**R:44.667**	**R:41.286**	**R:82.625**	**R:42.708**
1		0.001687	0.000725	1.000000	0.000744
2	0.001687		1.000000	0.001059	1.000000
3	0.000725	1.000000		0.000453	1.000000
4	1.000000	0.001059	0.000453		0.000457
6	0.000744	1.000000	1.000000	0.000457	
NO_3^-	**R:71.917**	**R:42.792**	**R:78.500**	**R:66.708**	**R:37.521**
1		0.029347	1.000000	1.000000	0.004434
2	0.029347		0.004264	0.145829	1.000000
3	1.000000	0.004264		1.000000	0.000527
4	1.000000	0.145829	1.000000		0.028742
6	0.004434	1.000000	0.000527	0.028742	

APPENDIX A.1: CONTINUE.

VARIABLE	SITE				
	1	2	3	4	6
NH_4^+	R:92.521	R:30.958	R:48.405	R:87.167	R:34.625
1		0.000000	0.000134	1.000000	0.000000
2	0.000000		0.851851	0.000000	1.000000
3	0.000134	0.851851		0.001311	1.000000
4	1.000000	0.000000	0.001311		0.000001
6	0.000000	1.000000	1.000000	0.000001	
PO_4^{-3}	R:92.396	R:39.271	R:29.619	R:89.167	R:40.875
1		0.000001	0.000000	1.000000	0.000001
2	0.000001		1.000000	0.000003	1.000000
3	0.000000	1.000000		0.000000	1.000000
4	1.000000	0.000003	0.000000		0.000008
6	0.000001	1.000000	1.000000	0.000008	
Fe	R:70.208	R:53.458	R:50.833	R:68.250	R:51.229
1		0.871450	0.559216	1.000000	0.525848
2	0.871450		1.000000	1.000000	1.000000
3	0.559216	1.000000		0.857190	1.000000
4	1.000000	1.000000	0.857190		0.821556
6	0.525848	1.000000	1.000000	0.821556	
Mn	R:82.854	R:37.958	R:45.024	R:78.625	R:48.792
1		0.000045	0.001895	1.000000	0.005037
2	0.000045		1.000000	0.000328	1.000000
3	0.001895	1.000000		0.009155	1.000000
4	1.000000	0.000328	0.009155		0.023126
6	0.005037	1.000000	1.000000	0.023126	
Cl^-	R:68.083	R:27.646	R:101.74	R:76.583	R:26.292
1		0.000363	0.008983	1.000000	0.000197
2	0.000363		0.000000	0.000006	1.000000
3	0.008983	0.000000		0.130681	0.000000
4	1.000000	0.000006	0.130681		0.000003
6	0.000197	1.000000	0.000000	0.000003	
SO_4^{2-}	R:59.229	R:36.646	R:105.38	R:67.667	R:31.875
1		0.210881	0.000053	1.000000	0.052118
2	0.210881		0.000000	0.015343	1.000000
3	0.000053	0.000000		0.001984	0.000000
4	1.000000	0.015343	0.001984		0.002568
6	0.052118	1.000000	0.000000	0.002568	
CN^-	R:60.000	R:59.521	R:60.214	R:59.938	R:55.479
1		1.000000	1.000000	1.000000	1.000000
2	1.000000		1.000000	1.000000	1.000000
3	1.000000	1.000000		1.000000	1.000000
4	1.000000	1.000000	1.000000		1.000000
6	1.000000	1.000000	1.000000	1.000000	
Zn	R:83.792	R:42.125	R:54.643	R:75.625	R:38.271
1		0.000209	0.040277	1.000000	0.000033
2	0.000209		1.000000	0.006232	1.000000
3	0.040277	1.000000		0.384322	1.000000
4	1.000000	0.006232	0.384322		0.001362
6	0.000033	1.000000	1.000000	0.001362	

APPENDIX A.1: CONTINUE.

VARIABLE	SITE				
	1	2	3	4	6
Cu	**R:62.792**	**R:50.917**	**R:64.452**	**R:55.521**	**R:62.000**
1		1.000000	1.000000	1.000000	1.000000
2	1.000000		1.000000	1.000000	1.000000
3	1.000000	1.000000		1.000000	1.000000
4	1.000000	1.000000	1.000000		1.000000
6	1.000000	1.000000	1.000000	1.000000	
Al	**R:53.042**	**R:67.521**	**R:60.690**	**R:56.625**	**R:57.333**
1		1.000000	1.000000	1.000000	1.000000
2	1.000000		1.000000	1.000000	1.000000
3	1.000000	1.000000		1.000000	1.000000
4	1.000000	1.000000	1.000000		1.000000
6	1.000000	1.000000	1.000000	1.000000	
Na^+	**R:68.771**	**R:27.521**	**R:104.90**	**R:72.042**	**R:27.500**
1		0.000252	0.003636	1.000000	0.000250
2	0.000252		0.000000	0.000054	1.000000
3	0.003636	0.000000		0.011850	0.000000
4	1.000000	0.000054	0.011850		0.000054
6	0.000250	1.000000	0.000000	0.000054	
As	**R:56.750**	**R:44.833**	**R:82.310**	**R:61.083**	**R:52.938**
1		1.000000	0.116741	1.000000	1.000000
2	1.000000		0.002176	0.969971	1.000000
3	0.116741	0.002176		0.362338	0.037554
4	1.000000	0.969971	0.362338		1.000000
6	1.000000	1.000000	0.037554	1.000000	
U	**R:60.521**	**R:44.833**	**R:77.619**	**R:73.104**	**R:41.250**
1		1.000000	0.916023	1.000000	0.490562
2	1.000000		0.012172	0.038861	1.000000
3	0.916023	0.012172		1.000000	0.003327
4	1.000000	0.038861	1.000000		0.011410
6	0.490562	1.000000	0.003327	0.011410	

APPENDIX A.1: CONTINUE.

VARIABLE	SITE				
	1	2	3	4	6
Cyananophyta	**R:53.792**	**R:72.333**	**R:43.667**	**R:58.854**	**R:73.854**
1		0.648212	1.000000	1.000000	0.457229
2	0.648212		0.043064	1.000000	1.000000
3	1.000000	0.043064		1.000000	0.026449
4	1.000000	1.000000	1.000000		1.000000
6	0.457229	1.000000	0.026449	1.000000	
Bacillariophyta	**R:49.396**	**R:87.333**	**R:19.938**	**R:53.750**	**R:92.083**
1		0.001581	0.033502	1.000000	0.000213
2	0.001581		0.000000	0.008245	1.000000
3	0.033502	0.000000		0.007592	0.000000
4	1.000000	0.008245	0.007592		0.001348
6	0.000213	1.000000	0.000000	0.001348	
Chlorophyta	**R:46.229**	**R:91.333**	**R:25.375**	**R:44.438**	**R:95.125**
1		0.000071	0.378217	1.000000	0.000011
2	0.000071		0.000000	0.000030	1.000000
3	0.378217	0.000000		0.576491	0.000000
4	1.000000	0.000030	0.576491		0.000004
6	0.000011	1.000000	0.000000	0.000004	
Cryptophyta	**R:51.000**	**R:68.188**	**R:53.125**	**R:53.563**	**R:76.625**
1		0.869643	1.000000	1.000000	0.107141
2	0.869643		1.000000	1.000000	1.000000
3	1.000000	1.000000		1.000000	0.192698
4	1.000000	1.000000	1.000000		0.216359
6	0.107141	1.000000	0.192698	0.216359	
Chrysophyta	**R:60.104**	**R:58.396**	**R:62.250**	**R:58.021**	**R:63.729**
1		1.000000	1.000000	1.000000	1.000000
2	1.000000		1.000000	1.000000	1.000000
3	1.000000	1.000000		1.000000	1.000000
4	1.000000	1.000000	1.000000		1.000000
6	1.000000	1.000000	1.000000	1.000000	
Dinophyta	**R:56.750**	**R:66.708**	**R:50.833**	**R:51.042**	**6R:77.167**
1		1.000000	1.000000	1.000000	0.420311
2	1.000000		1.000000	1.000000	1.000000
3	1.000000	1.000000		1.000000	0.087305
4	1.000000	1.000000	1.000000		0.092768
6	0.420311	1.000000	0.087305	0.092768	
Euglenophyta	**R:68.313**	**R:75.354**	**R:25.958**	**R:54.958**	**R:77.917**
1		1.000000	0.000247	1.000000	1.000000
2	1.000000		0.000009	0.422410	1.000000
3	0.000247	0.000009		0.038771	0.000002
4	1.000000	0.422410	0.038771		0.222352
6	1.000000	1.000000	0.000002	0.222352	

Note: Values in red show most significant variable differences ($p < 0.05$) between sites.

APPENDIX A.2: Kruskal-Wallis multiple comparisons of p-values (2 tailed) that indicate significant variable differences ($p<0.05$) between data groups 1 (2001-2002) and 2 (2012-2014) at all the sites (1, 2, 3, 4, and 6).

SITE 1: After Khuma in the KMS.			
VARIABLE	**GROUP 1**	**GROUP 2**	**P-VALUE**
Colour	R:15.313	R:33.688	0.000005
pH	R:30.688	R:18.313	0.002198
EC	R:33.375	R:15.625	0.000011
NTU	R:21.354	R:27.646	0.119522
TOC	R:16.208	R:32.792	0.000041
T. Chl	R:14.896	R:34.104	0.000002
Faecal coliforms	R:24.167	R:24.833	0.868978
NO_3^-	R:18.667	R:30.333	0.003892
NH_4^+	R:13.229	R:35.771	0.000000
PO_4^{-3}	R:17.521	R:31.479	0.000553
Fe	R:17.625	R:31.375	0.000668
Mn	R:23.958	R:25.042	0.788657
Cl^-	R:14.583	R:34.417	0.000001
SO_4^{2-}	R:15.354	R:33.646	0.000006
CN^-	R:12.938	R:36.063	0.000000
Zn	R:13.063	R:35.938	0.000000
Cu	R:13.750	R:35.250	0.000000
Al	R:15.125	R:33.875	0.000003
Na^+	R:14.563	R:33.848	0.000001
As	R:13.458	R:35.542	0.000000

Note: Values in red show most significant variable differences ($p < 0.05$) between data sets.

SITE 3: Canal in the KMS.			
VARIABLE	**GROUP 1**	**GROUP 2**	**P-VALUE**
Colour	R:21.417	R:24.810	0.387297
pH	R:19.125	R:27.429	0.034360
EC	R:25.750	R:19.857	0.133213
NTU	R:25.438	R:20.214	0.183215
TOC	R:28.375	R:16.857	0.003337
T. Chl	R:17.875	R:28.857	0.005136
Faecal coliforms	R:23.000	R:23.000	1.000000
NO_3^-	R:19.396	R:27.119	0.049075
NH_4^+	R:26.438	R:19.071	0.060526
PO_4^{-3}	R:22.000	R:24.143	0.585053
Fe	R:25.938	R:19.643	0.108729
Mn	R:30.104	R:14.881	0.000105
Cl^-	R:14.458	R:32.762	0.000003
SO_4^{2-}	R:12.625	R:34.857	0.000000
CN^-	R:23.875	R:22.000	0.632816
Zn	R:15.125	R:32.000	0.000017
Cu	R:15.125	R:32.000	0.000017
Al	R:14.375	R:32.857	0.000002
Na^+	R:12.958	R:33.950	0.000000
As	R:12.979	R:34.452	0.000000

Note: Values in red show most significant variable differences ($p < 0.05$) between data sets.

SITE 4: Canal and Khuma combined in the KMS.			
VARIABLE	**GROUP 1**	**GROUP 2**	**P-VALUE**
Colour	R:14.979	R:34.021	0.000002
pH	R:27.833	R:21.167	0.099030
EC	R:32.896	R:16.104	0.000033
NTU	R:24.021	R:24.979	0.812559
TOC	R:16.563	R:32.438	0.000086
T. Chl	R:14.667	R:34.333	0.000001
Faecal coliforms	R:24.625	R:24.375	0.950675
NO_3^-	R:21.667	R:27.333	0.160875
NH_4^+	R:14.292	R:34.708	0.000000
PO_4^{-3}	R:18.750	R:30.250	0.004434
Fe	R:18.500	R:30.500	0.002985
Mn	R:27.354	R:21.646	0.157819
Cl^-	R:13.979	R:35.021	0.000000
SO_4^{2-}	R:14.792	R:34.208	0.000002
CN^-	R:17.000	R:32.000	0.000206
Zn	R:13.438	R:35.563	0.000000
Cu	R:14.667	R:34.333	0.000001
Al	R:15.000	R:34.000	0.000003
Na^+	R:14.375	R:34.043	0.000001
As	R:13.229	R:35.771	0.000000

Note: Values in red show most significant variable differences ($p < 0.05$) between data sets.

SITE 2: Before the KMS in the Vaal River			
VARIABLE	**GROUP 1**	**GROUP 2**	**P-VALUE**
Colour	R:29.125	R:19.875	0.022092
pH	R:23.125	R:25.875	0.496221
EC	R:30.625	R:18.375	0.002437
NTU	R:25.042	R:23.958	0.788657
TOC	R:27.729	R:20.109	0.056821
T. Chl	R:19.167	R:29.043	0.013563
Faecal coliforms	R:22.646	R:26.354	0.358842
NO_3^-	R:18.021	R:30.979	0.001344
NH_4^+	R:15.271	R:33.729	0.000005
PO_4^{-3}	R:31.813	R:17.188	0.000296
Fe	R:22.521	R:26.479	0.327366
Mn	R:27.688	R:21.313	0.114703
Cl^-	R:15.250	R:33.750	0.000005
SO_4^{2-}	R:15.125	R:33.875	0.000006
CN^-	R:15.854	R:33.146	0.000019
Zn	R:15.000	R:34.000	0.000003
Cu	R:14.354	R:34.646	0.000001
Al	R:15.000	R:34.000	0.000003
Na^+	R:15.104	R:33.283	0.000006
As	R:13.458	R:35.542	0.000000

Note: Values in red show most significant variable differences ($p < 0.05$) between data sets.

SITE 6: After the KMS in the Vaal River.			
VARAIBLES	**GROUP 1**	**GROUP 2**	**P-VALUE**
Colour	R:31.167	R:17.833	0.000970
pH	R:23.000	R:26.000	0.457901
EC	R:29.292	R:19.708	0.017728
NTU	R:25.750	R:23.250	0.536187
TOC	R:32.917	R:13.227	0.000001
T. Chl	R:20.208	R:28.792	0.033685
Faecal coliforms	R:20.000	R:29.000	0.025952
NO_3^-	R:20.938	R:28.063	0.077904
NH_4^+	R:18.292	R:30.708	0.002124
PO_4^{-3}	R:27.104	R:21.896	0.197493
Fe	126.646	R:22.354	0.288276
Mn	R:28.646	R:20.354	0.040203
Cl^-	R:30.417	R:18.583	0.003412
SO_4^{2-}	R:29.667	R:19.333	0.010563
CN^-	R:13.417	R:35.583	0.000000
Zn	R:30.854	R:18.146	0.001664
Cu	R:20.479	R:28.521	0.046613
Al	R:34.792	R:14.208	0.000000
Na^+	R:25.917	R:23.083	0.483260
As	R:14.167	R:34.833	0.000000

Note: Values in red show most significant variable differences ($p < 0.05$) between data sets.

APPENDIX A.3: Summary of genera that were identified over the 24 month study period, including each of their authors.

GENUS	
CYANOPHYCEAE	*Chaetophora* Schrank
Anabaena Bory *ex* Bornet *et* Flahault	*Characium* Braun
Aphanocapsa Nägeli	*Chlamydomonas* Ehrenberg
Arthrospira Sitzenberger *ex* Gomont	*Chlorella* Beijerinck
Calothrix Agardh *ex* Bornet *et* Flahault	*Chlorococcum* Meneghini
Gloeocapsa Kützing	*Chlorolobion* Korshikov
Merismopedia Meyen	*Closteriopsis* Lemmermann
Microcystis Kützing *ex* Lemmermann	*Closterium* Nitzsch *ex* Ralfs
Nostoc Vaucher *ex* Bornet *et* Flahault	*Coelastrum* Nägeli
Oscillatoria Vaucher *ex* Gomont	*Cosmarium* Corda *ex* Ralfs
Phormidium Kützing *ex* Gomont	*Crucigenia* Morren
BACILLARIOPHYCEAE	*Dictyosphaerium* Nägeli
Achnanthes Kützing	*Didymogenes* Schmidle
Achnanthidium Kützing	*Elakatothrix* Wille
Amphiprora Ehrenberg	*Golenkinia* Chodat
Aulacoseira Thwaites	*Goniochloris* Geitler
Cocconeis Ehrenberg	*Kirchneriella* Scmidle
Cyclotella Kützing *ex* Brébisson	*Lagerheimia* Chodat
Cymbella Agardh	*Micractinium* Fresenius
Diadesmus Kützing	*Monoraphidium* Komárková-Legnerová
Fragilaria Lyngbye	*Oocystis* Braun
Gomphonema Ehrenberg	*Pandorina* Bory de Saint-Vincent
Gyrosigma Hassall	*Pediastrum* Meyen
Melosira Agardh	*Pteromonas* Seligo
Navicula Bory	*Scenedesmus* Meyen
Nitzschia Hassall	*Spirogyra* Link
Surirella Turpin	*Staurastrum Meyen* ex Ralfs
CHLOROPHYCEAE	*Tetraedron* Kützing
Actinastrum Lagerheim	*Tetrastrum* Chodat
Ankistrodesmus Corda	*Ulothrix* Kützing
Asterococcus Scherffel	**CHRYSOPHYCEAE**
Carteria Diesing	*Dinobryon* Ehrenberg

APPENDIX A.3: CONTINUE.

GENUS
Mallomonas Perty
DINOPHYCEAE
Ceratium Schrank
Peridinium Ehrenberg
CRYPTOPHYCEAE
Cryptomonas Ehrenberg
EUGLENOPHYCEAE
Euglena Ehrenberg
Phacus Dujardin
Strombomonas Deflandre
Trachelomonas Ehrenberg

APPENDIX A.4: Eigenvalues and related percentages on all 28 contributing principal components.

Principal components	Eigenvalue	% Total variance	Cumulative Eigenvalue	Cumulative %
1	5.759234	20.56869	5.75923	20.5687
2	4.060394	14.50141	9.81963	35.0701
3	3.249376	11.60491	13.06900	46.6750
4	1.805225	6.44723	14.87423	53.1222
5	1.662344	5.93694	16.53657	59.0592
6	1.558836	5.56727	18.09541	64.6265
7	1.191844	4.25658	19.28725	68.8830
8	1.074705	3.83823	20.36196	72.7213
9	0.956795	3.41712	21.31875	76.1384
10	0.851673	3.04169	22.17043	79.1801
11	0.788497	2.81606	22.95892	81.9962
12	0.768034	2.74298	23.72696	84.7391
13	0.693136	2.47548	24.42009	87.2146
14	0.590030	2.10725	25.01012	89.3219
15	0.535697	1.91320	25.54582	91.2351
16	0.481181	1.71850	26.02700	92.9536
17	0.406869	1.45310	26.43387	94.4067
18	0.334038	1.19299	26.76791	95.5997
19	0.273305	0.97609	27.04121	96.5758
20	0.257385	0.91923	27.29860	97.4950
21	0.210920	0.75329	27.50952	98.2483
22	0.196301	0.70107	27.70582	98.9493
23	0.145926	0.52116	27.85174	99.4705
24	0.074428	0.26582	27.92617	99.7363
25	0.049667	0.17738	27.97584	99.9137
26	0.013561	0.04843	27.98940	99.9621
27	0.008814	0.03148	27.99821	99.9936
28	0.001787	0.00638	28.00000	100.0000