Changes in density and composition of algal assemblages over time in two water purification plants

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ABSTRACT

In recent years, due to a change in the water situation in South-Africa and the effect of eutrophication in our water systems, there has been a significant increase in algal abundance and changes in species composition. The aim of this study was to investigate algal assemblages at two water purification plants with the main focus on dominant species that may pose a problem in the water purification process. Both water purification plants, especially the one at Virginia, experience problems with blue-green bacteria that are toxic and detrimental to water purification. There was also a need to determine the time of year that blooms of problematic algae occur in the system, in order to develop sufficient measures to remediate the situation. Chemical data helped with the explanation of algal tendencies.

To achieve the principal aims of the study, algal species were identified and the concentrations were determined. It was possible to relate algal assemblages, dominance and succession to the prevailing environmental variables.

Sixty three phytoplankton species, belonging to seven major algal groups, were identified. Aside from these, thirty four species were only identified up to genus level. The blue-green bacteria, diatoms and green algae were the main phytoplankton groups and constantly succeeded each other. Blooms of blue-green bacteria occurred in the raw water due to high temperatures and dissolved inorganic nitrogen concentrations in the late summer periods. These organisms did not penetrate far into the purification process, indicating that the purification procedures were sufficient for effective removal of blue-green bacteria.

Keywords: eutrophication, algae, species composition, water purification, blue-green bacteria, blooms, dissolved inorganic nitrogen.

OPSOMMING

Weens die veranderende water situasie in Suid-Afrika en die effek van eutrofikasie op ons waterstelsels, was daar onlangs ‘n merkbare toename in die hoeveelheid alge, asook veranderinge in spesie-samestelling. Die doel van die studie was om die algsamestelling en -konsentrasie by twee watersuiweringsaanlegte te ondersoek, met die fokus op dominante spesies wat probleme mag inhou in terme van die watersuiweringsproses. Beide watersuiweringsaanlegte, veral die een by Virginia, ondervind probleme met blou-groen bakterieë wat toksies en nadelig is vir watersuiwering. Daar was ook ‘n behoefte om te bepaal watter tyd van die jaar opbloeie van probleemalge mag voorkom, met die oog op die ontwikkeling van voldoende maatreëls om dit te hanteer. Chemiese data het gehelp met die waarneming van algtendense.

Om die hoofdoelwitte van die studie te bereik, was algspesies geïdentifiseer en die konsentrasies bepaal. Dit was moontlik om algsamestelling, dominansie en suksessie te vergelyk met heersende omgewingstoestande.

Drie en sestig fitoplanktonspesies, behorende tot sewe hoof alggroepe, is geïdentifiseer. ‘n Bykomende vier en dertig spesies is slegs tot op genus vlak geïdentifiseer. Die blou-groen bakterieë, diatome en groen-alge was die belangrikste fitoplanktongroepe en het mekaar gedurig opgevolg. Opbloeie van blou-groen bakterieë in die rouwater was die gevolg van hoë temperature en opgeloste anorganiese stikstof in die laat somermaande. Hierdie organismes het nie vêr gepenetreer in die watersuiweringsproses nie, wat aantoon dat die watersuiweringsprosedures voldoende is om blou-groen bakterieë effektief te verwyder.

Sleutelwoorde: eutrofikasie, alge, spesie-samestelling, watersuiwering, blou-groen bakterieë, opbloeie, opgeloste anorganiese stikstof.

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CHAPTER 1: INTRODUCTION

Water is the most precious and scarce commodity that exists in South Africa, since the country is located in the southern, dry, subtropical region of the African continent. This situation is further aggravated by the fact that our western coast is located along the cold Benguela sea current, which leads to even more arid conditions. There is also the growing threat of climate change, brought about by global warming, which is making our country even drier, especially in the south-western districts. According to DWAF (1986), South Africa has a mean annual rainfall of about 497 mm which is very low when compared to the world average of 860 mm. Rainfall is erratic, decreases from east to west and approximately 95% of the country records less than 500 mm annually. Only about 7% of South Africa has a mean annual precipitation exceeding 800 mm. Statistics indicate that KwaZulu-Natal is the wettest province, while the Western Cape has the highest variability of mean annual precipitation of all nine provinces (www.environment.gov.za). It becomes quite clear that we have to protect the quality and purity of the water resources in South Africa to prevent us from facing a dire water crisis in coming years (Ahuja, 2009).

Rivers act as drains for the land surface and are major sources of surface water, which make them an extremely important resource in a dry country such as South Africa. Since water is the factor that may limit the economic prosperity of South Africa, it is easy to understand that that our rivers will be continuously exploited to the furthest possible extent and we must understand what effects this will have on the river ecosystems (Davies & Day, 1986).

Rivers that drain the Free State, Gauteng and North West provinces produce only 4300 million cubic meters of water per annum (DWAF, 1986). It is the most important water supply system in South Africa and has to supply water to our economic heartland, the Gauteng area (Grobler *et al*., 1987). The Vaal River originates on the western slopes of the Drakensberg escarpment and flows about

900 km west-south-west across the interior plateau to join the Orange River near Douglas (Janse van Vuuren, 1996). The climatic conditions in the Upper Vaal Water Management Area (WMA) vary with the mean annual precipitation extending from 800 mm in the headwaters to 500 mm in the Middle Vaal WMA (DWAF, 2002).

Land use in the Upper Vaal WMA is dominated by sprawling urban and industrial areas in the northern and western parts of the WMA. There are also extensive gold and coal mining activities located in the Upper Vaal WMA that generate very large return flow volumes in the form of treated effluent from urban areas and mine dewatering which are all discharged into the Vaal River system. Discharges such as these are having an extensive impact on water quality in the Vaal River, through all three of the Vaal Water Management Areas (DWAF, 2004). Apart from direct uses, the entire length of the Vaal River itself is used for recreational purposes (Bruwer *et al*., 1985). Due to intense use of the Vaal River, along with excessive and increasing demands in the catchment area, the quality of the water supply becomes even more important (Basson & Van Rooyen, 1989).

Since water quality affects our lives in many ways it has to be of acceptable quality for its aesthetic value to be appreciated (Palmer, 1980). Concern for water quality increases as the use of the water system is intensified. The Vaal River is known to be enriched and polluted (Janse van Vuuren, 1996), in many cases through tributaries or point-sources, which makes it harder to find the normal patterns of river ecology found in undisturbed rivers as well as the normal river continuum process. The main water quality problems in the Vaal River system are increased salinity and eutrophication as stated by Braune & Rogers (1987).

“Eutrophication” is the ecological term that is used to describe the process where a water body becomes enriched with plant nutrients. The body of water accumulates organic matter which can be living or decaying and will progressively change character from being deep, to a wetland and eventually a terrestrial system. Eutrophication therefore describes the natural ageing process

of lakes. When the eutrophication process occurs naturally it takes place over tens of thousands of years, is dependant on the geology and natural features of the catchment and is also irreversible while continuing at a slow rate. This however has changed over the last hundred years as a result of human influences, which have remarkably sped up the rate of enrichment and which now shortens the lifespan of water bodies. The human-induced process which relates to anthropogenic activities is known as “cultural eutrophication”. It is associated with both social and economic activities but is reversible (Walmsley, 2000). Cultural eutrophication was first recognised as a problematic phenomenon when scientists perceived the link between nuisance conditions in water bodies and increased nutrient enrichment from human related activities (Steward & Rohlich, 1967; Vollenweider, 1968). The eutrophication process also became associated with a wide range of water resource problems (Dunst, 1974). Nauman (1919) and Rast & Thornton (1996) used a classification system with the following terms to describe the state of enrichment in aquatic ecosystems:

- **Oligotrophic** refers to an aquatic system with low nutrient levels and thus no water quality problems.
- **Mesotrophic** conditions describe intermediate nutrient levels and small signs of emerging water quality problems.
- **Eutrophic** systems have high levels of nutrients and an increased frequency of water quality problems.
- **Hypertrophic** is a term used when excessive nutrient levels are present and plant production is governed by physical factors. Problems with water quality are mostly unyielding.

The connection between aquatic plant production, nutrients and anthropogenic activities was first noted at the beginning of the century (Nauman, 1919). Nutrients can be defined as chemical compounds or elements that can be used directly by plant cells, for example algae and aquatic macrophytes, for growth. In terms of eutrophication, nutrients are inorganic elements that are assimilated by plant cells which are, by means of photosynthesis, used to produce and

accumulate organic material in aquatic ecosystems. For this to take place the photosynthetic cells require approximately twenty different elements while the rate and extent of growth depends on the concentration and ratios of nutrients present in the system. Growth is often limited by the concentration of the nutrient present in the least amount relative to the growth needs of the organism. This is classified as the “limiting nutrient concept” which forms a basis for eutrophication management policy. Because of nutrient supply and demand in ecosystems, it is noticed that phosphorus and nitrogen are the most frequent limiting nutrients present in freshwater systems. When either of these elements increase, the risk of experiencing eutrophication problems also increases. Therefore, when considering the nutrient limiting concept, management of phosphorus and nitrogen inputs into aquatic systems provides the solution to the eutrophication problem (Walmsley, 2000).

Eutrophication leads to the development of algal blooms which can be defined as the growth of planktonic algae which can be dense enough to give the water a distinct colour (Palmer, 1980). Algal blooms can be expected at phosphorus concentrations above 0.015 mg l^{-1} and nitrogen concentrations above 0.3 mg l^{-1} according to Sawyer (1947). Genera that fix atmospheric nitrogen usually bloom in lakes after nutrients have been depleted by blooms of other algae (Sawyer, 1947). The end of diatom blooms is usually due to silica-limitation (Müller, 1984). Gerloff & Skoog (1954) noticed that many algal species accumulate large amounts of various nutrients under favourable, nutrient-rich conditions and are therefore independent from an external medium for an extended period after nutrients subsided to sub-optimum levels. Under optimum conditions the rapid division rate of algal cells will result in dense algal blooms. Environmental variables such as flow rate, temperature and turbulence also play a part in algal bloom occurrences (Scagel *et al.,* 1972). Warm water temperatures and calm weather are especially important in initiating blue-green bacterial blooms (Palmer, 1980). Many researchers (e.g. Lefévre, 1932; Novak, 1961; Lin, 1972) discovered that algae from other phyla are present in very low densities when

blooms of blue-green bacteria occur. It has also been found that blue-green bacterial blooms usually consist of only a few species (Fitzgerald, 1964).

During the last ten years a number of events in South Africa have led to increased awareness of algae, in particular cyanobacteria or blue-green bacteria (Harding & Paxton, 2001; Downing & Van Ginkel, 2002). The Hartbeespoort Dam is well known as a cyanobacterial bloom hazard, both nationally and internationally. Many impoundments, like the Hartbeespoort, Bon Accord, Bospoort, Bronkhorstspruit, Klipvoor, Rietvlei, Roodeplaat and Voëlvlei Dams, are classified with a trophic status extending from eutrophic to hypertrophic which inevitably leads to algal and cyanobacterial blooms on a large scale (Van Ginkel *et al.,* 2001a). Noxious cyanobacterial blooms have spread to freshwater systems that have never before encountered this problem. The Orange River experiences cyanobacterial blooms on an annual basis from the year 2000. High flow conditions flushed a new invader species, *Cylindrospermopsis raciborskii*, down the lower Orange River (Van Ginkel & Conradie, 2001). This species is toxic and was responsible for various problems during the bloom in the lower Orange River (Janse van Vuuren & Kriel, 2008). There are also reports of increased *Ceratium hirundinella* blooms in the freshwater resources of South Africa (Van Ginkel *et al.,* 2001b). This dinoflagellate can be seen as a problematic organism due to the taste and odour problems it imparts to potable water and it also clogs sand filters within water purification plants (Hart & Wragg, 2009).

The species responsible for the blooms in the Hartbeespoort Dam is *Microcystis aeruginosa*, a colonial blue-green bacterium (Pieterse, 1986). *M. aeruginosa* is often forming blooms and may even secrete chemicals that inhibit other algae. They possess gas vacuoles, which enable them to remain buoyant and produce surface scums, therefore leading to great disturbances in lakes and reservoirs. Dense growths directly or indirectly cause the death of fish through suffocation (due to oxygen depletion during decomposition) or by poisoning. *Microcystis* spp

produce the polypeptide called microcystin (named after the genus *Microcystis*), which is toxic to animals that ingest the contaminated water. Human illnesses, due to this substance, include necrosis of the liver when ingested and severe dermatitis when in contact with skin surface (WHO, 1999).

Bloom-forming genera in the Vaal River system include blue-green bacteria such as *Microcystis, Oscillatoria* and *Anabaena,* green algae such as *Chlamydomonas*, as well as certain genera of centric and pennate diatoms (Pieterse, 1986). *Anabaena* produces toxins leading to problems such as dermatitis and taste and odour problems. Some species of *Oscillatoria* produce neuro- and hepatotoxins. Neurotoxins block transmission of signals between neurons and from neurons to muscles, while hepatotoxins cause liver bleeding. The toxins threaten livestock more often than humans. *Oscillatoria* causes severe dermatitis and irritates the mucous membranes of people swimming in water with high concentrations of this genus (WHO, 1999). Correlations between high concentrations of blue-green bacteria and outbreaks of gastroenteritis in humans have been reported (DWAF, 1991). Blooms of *Chlamydomonas* may be aesthetically unacceptable (Janse van Vuuren *et al.,* 2006). Blooms of the above mentioned algal groups can frequently occur in low flow sections or above weirs (Pieterse, 1986). *Actinastrum, Ankistrodesmus, Pediastrum* and *Scenedesmus* (green algae) as well as *Euglena* (euglenophyte) can also cause taste and odour problems (Tate & Arnold, 1990). Algal assemblages usually found in sewage ponds are fairly common and sometimes even dominant in the Vaal River system and is a major sign that the Vaal River is heavily polluted (Pieterse, 1994). Swanepoel *et al.* (2008) also stated that phytoplankton assemblages in water bodies can provide an indication of the prevailing water quality.

Water bodies can rapidly absorb both natural and man-made substances which will generally make the water unsuitable for drinking without some sort of treatment (Gary, 2008). To produce drinking water from eutrophic sources, phyto- and zooplankton, along with the high concentrations of algal-derived organic matter, must be removed (Visser, 1996). Low concentrations of algae

can be removed by slow sand filtration, but with higher concentrations effective flocculation is required because unflocculated algae penetrate sand filters and even the distribution network (DWAF, 1993).

Since this study focuses on sites in two water purification plants it is important to realise the importance of algae because, among other problems, they produce huge quantities of organic matter in water. Algae may clog sand filters and distribution pipes, shorten filter runs, impart unpleasant tastes and odours, resist sedimentation and interfere with industrial uses (Pieterse, 1989). Possible carcinogenic trihalomethanes may form when water from eutrophic sources is chlorinated during purification and algal growth may also appear on canal linings which can result in a loss of hydraulic capacity (DWAF, 1986). Algal blooms may cause aesthetically unacceptable conditions and can easily increase the purification costs of water for potable purposes due to the clogging of filters and the formation of scums in purification plants (Bruwer *et al*. 1985). This will increase the amount of chemicals needed, as well as the various treatment processes to remove odours, tastes and other side effects as stated by Wnorowski (1992). Algae are also seen as important factors in the supply of water because they can modify pH and alkalinity and affect the colour and turbidity of the water (Palmer, 1980).

The turbid nature of the Vaal River system partially counters the effect of eutrophication, but as salinity increases and turbidity decreases primary productivity can be enhanced (Braune & Rogers, 1987). Under eutrophic conditions, clear water will result in blooms.

For the water to be fit for human consumption it must be free from organisms capable of causing disease and free from minerals and organic substances (like algal toxins) that can cause adverse physiological effects (Tate & Arnold, 1990).
Phytoplankton (photosynthetic, free-floating organisms which are mostly microscopic - including eukaryotic algae and prokaryotic cyanobacteria) removal

is mostly inhibited by a variety of factors. This includes the specific species that are present, the total biomass of the phytoplankton in the source water, effectiveness of coagulation and flocculation unit processes and the effectiveness of the sand filtration process. That is why it is necessary to monitor phytoplankton in both raw and potable water (Swanepoel & Du Preez, 2007).

Specific algal species in the aquatic environment and in the water treatment plants may be responsible for unique problems (Janse van Vuuren, 1996), which makes it very important to identify the specific algal genera that may prove to be problematic in those areas. Algal species that is known to have caused problems elsewhere can be compared with species found in the study area to help indicate potential problems.

Water supplied by Sedibeng Water is extremely important since it is used by various municipalities (see chapter 2), mostly located in the Free State, but also in the North-West and Northern Cape provinces for various household and industrial uses. They also need to supply vast quantities of water to the mining industry. From the total purified water output of Sedibeng Water, 59% is supplied to municipalities, 36% to the mining industry, 3% to agriculture and 2% to various other recipients (www.sedibengwater.co.za).

The greatest challenge of Sedibeng Water at the Balkfontein water purification plant (near Bothaville) is finding a way to purify the water of the Vaal River as optimally as possible even though there is an extensive daily variation in water quality. The organic component (mostly phytoplankton assemblages) poses the most problems and is always symptomatic of enrichment of the river system. The organic component is characterised by high phytoplankton (mainly cyanobacteria) concentrations during certain times of the year, especially during periods of high water temperatures (M. Ludick, personal communication). Previous observations at the Balkfontein water purification plant indicated that if

algal biomass is high in the river water, it is also high in the final water (Pieterse, 1989).

The water quality at the Virginia water purification plant, also operated by Sedibeng water, is much more consistent except for the very high concentrations of phytoplankton that complicates water purification at the plant (M. Ludick, personal communication). The raw water for the Virginia water purification plant is supplied by the Allemanskraal Dam which is classified as a hypertrophic system by Van Ginkel *et al.* (2000). The high concentration of phytoplankton in the source water accounts for the problems experienced in the water purification plant.

At both water purification plants there is a great need to manage components caused by enrichment of the river systems, thus the presence of phytoplankton assemblages and their abundance, to enable them to continue supplying water of acceptable quality. Enrichment of the source water worsens the problem and increases the organic component of the raw water. Due to the degeneration of raw water quality, the amount of chemicals needed in the water purification process is increased, thus escalating the cost of water purification (www.sedibengwater.co.za).

Data generated from this study will be helpful to answer the following questions:

- Is there an observable enrichment of the source water over the fourteen month study period?
- What is the contribution of enrichment in the source water, with regard to an increase in phytoplankton abundance?
- To what extent does the phytoplankton abundance and species composition vary over time?
- What is the contribution of algal assemblages to complications in water purification and the associated cost implications?

- How does the contribution of algal assemblages compare to the problems caused by other determinants in the system?

The main aims of the study were, therefore:

- To determine changes in composition and density of algal assemblages over a fourteen month study period at two water purification plants (Virginia and Balkfontein) of Sedibeng Water.
- To compare the differences between the composition and density of algal assemblages in the source water and after sedimentation at the two water purification plants.
- To determine the differences between the composition and density of algal assemblages at the different sampling sites within each purification plant, thereby giving an idea of how the water quality changes in a given water purification plant.
- To identify the dominant genera or species that were present.
- To identify phases in water treatment where problems occur regarding certain species and the possible cause.
- To identify the correlation between algae and environmental variables (both physical and chemical) by means of multiple regression analyses.

The results of this study will play an important role in determining when and where it would not be advisable to purify the water when viewed in terms of cost effectiveness.

CHAPTER 2: STUDY AREA, MATERIALS AND METHODS

2.1 STUDY AREA

The study area is situated in the Free State province of South Africa where Sedibeng Water subtracts water from two river systems, namely the Sand and the Vaal, both originating on the western slopes of the Drakensberg escarpment. The Vaal River flows about 900 km west-south-west across the interior plateau and joins the Orange River near Douglas. Major tributaries of the Vaal River system drain the province of Gauteng in the north, the Drakensberg in the east and the Maluti Mountains in the south. The region with the most precipitation in the Drakensberg (800 to 1000 mm per annum) is the major source of water for the Vaal River system (Bruwer *et al.,* 1985). Rainfall gradually decreases to 300 mm per annum as the river flows westwards and evaporation increases. The lower reaches of the river, as a result, mostly depends on eastern catchments for water supply. The catchment of the Vaal River system has the surface area of 192 000 km² (Braune & Rogers, 1987). Van Vliet (1986) divided the Vaal River catchment into three sections, namely the upper Vaal, stretching from its source to the Barrage below the Vaal Dam, the middle Vaal, from the Barrage to Bloemhof Dam and the lower Vaal, from Bloemhof Dam to where the Vaal joins the Orange River. The study area falls within the middle Vaal River Region and the catchment is situated on the Highveld of the inland plateau. The water from the Sand and Vaal Rivers is fed to the water purification plants at Virginia and Balkfontein (Bothaville) for purification and supply to the specific region they service (Fig. 2.1.1).

Sedibeng Water supplies approximately 78 million kilolitres of water annually to municipalities, farms, mines and other industries. The North-West region receives 9 million kilolitres, the Northern Cape region 12 million kilolitres and the Free State region 57 million kilolitres of this amount (www.freestatebusiness.co.za).

The area of distribution of the two water purification plants, Balkfontein and Virginia, in the Free State region includes the Nala, Matjabeng and Maqhassi Hills municipalities. Both plants supply purified water to the same water distribution network. Balkfontein water purification plant subtracts water from the Vaal River at the Klipplaatdrif weir, and Virginia from the Allemanskraal Dam situated in the Sand River (www.sedibengwater.co.za).

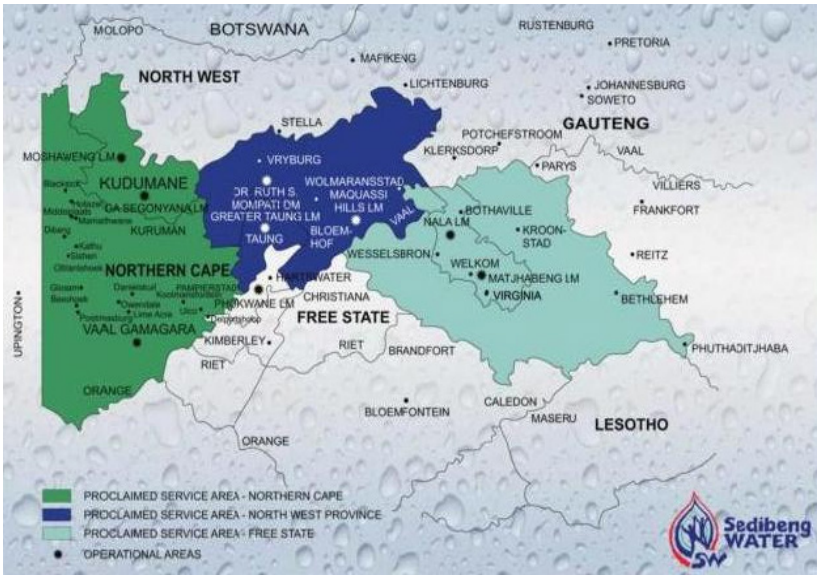
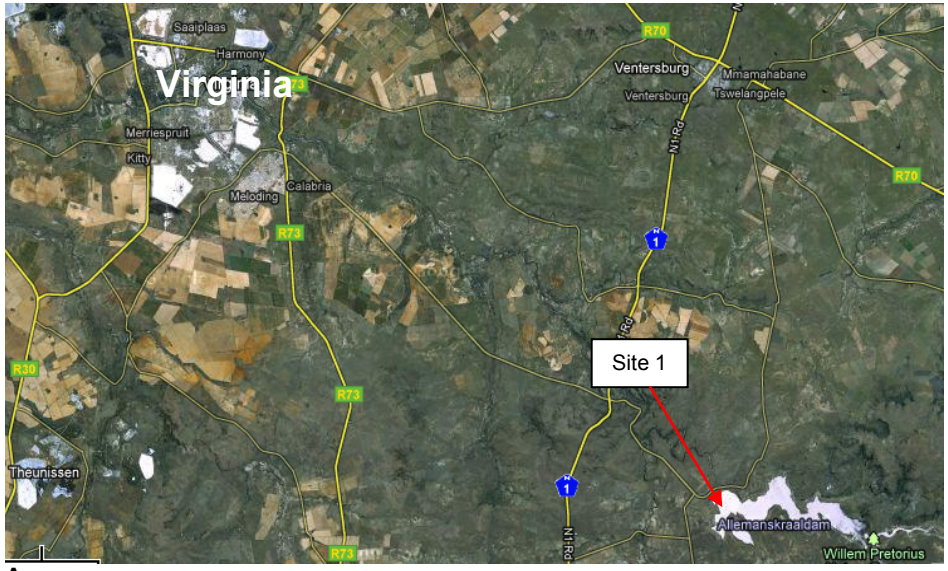


Figure 2.1.1: The main service areas of Sedibeng Water with the region serviced by the Balkfontein and Virginia water purification plants demarcated on the far right (map obtained from Sedibeng Water, www.sedibengwater.co.za).

For the water purification plant at Virginia, raw water is taken from the Allemanskraal Dam that impounds water from the Sand River system. Water from the dam is mainly used for irrigation purposes in the surrounding area. The position of Virginia relating to the location of the Allemanskraal Dam (site 1) is shown in Fig. 2.1.2A. The water from the Allemanskraal Dam is transported by means of a transfer canal (site 2; Fig. 2.1.2B) and flows into a reservoir outside Virginia (site 3; Figs. 2.1.2B and C). From the reservoir the water is pumped to the purification plant outside Virginia (sites 4 & 5; Figs. 2.1.2C and D). Fig. 2.1.2D indicates the layout of the Virginia water purification plant. Five samples were taken along the water supply route and in the main complex of the Virginia water purification plant.



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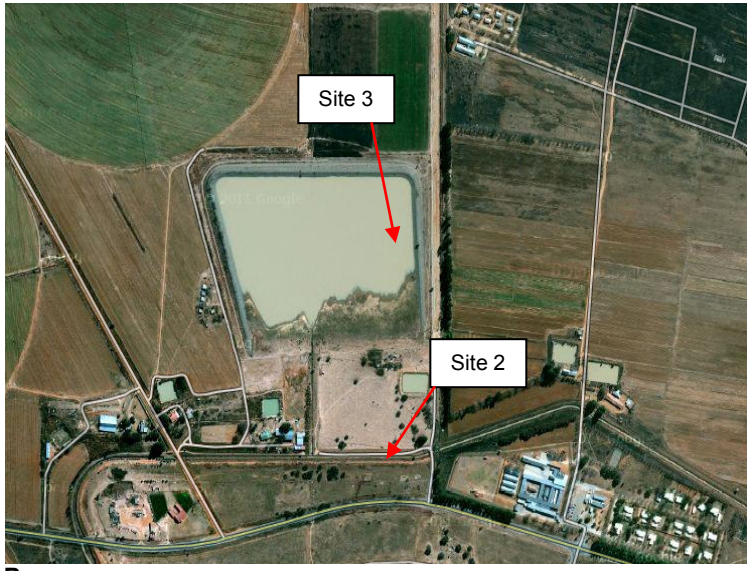


B

C

D

Figure 2.1.2: Aerial photographs indicating the water supply route to the Virginia water purification plant. **A:** Location of the Allemanskraal Dam (site 1) and Virginia; **B:** Water Transfer Canal (site 2) and Reservoir (site 3); **C:** Reservoir and Virginia water purification plant (sites 4 & 5); **D:** Primary Settlement Tanks (site 4) and Recycling Dams (site 5) (http://maps.google.co.za).

The sampling sites and direction of water flow is explained in a simplified schematic drawing in Fig. 2.1.3 and can be summarised as follows:

- Allemanskraal Dam (Aldam) – close to the dam wall at the southern shore (site 1)
- Water Transfer Canal – en route to the purification plant between Allemanskraal Dam and the reservoir (site 2)
- Reservoir – close to the purification plant outside Virginia (site 3)
- Primary Settlement Tank – at the purification plant (site 4)
- Recycling Dam – at the purification plant (site 5)

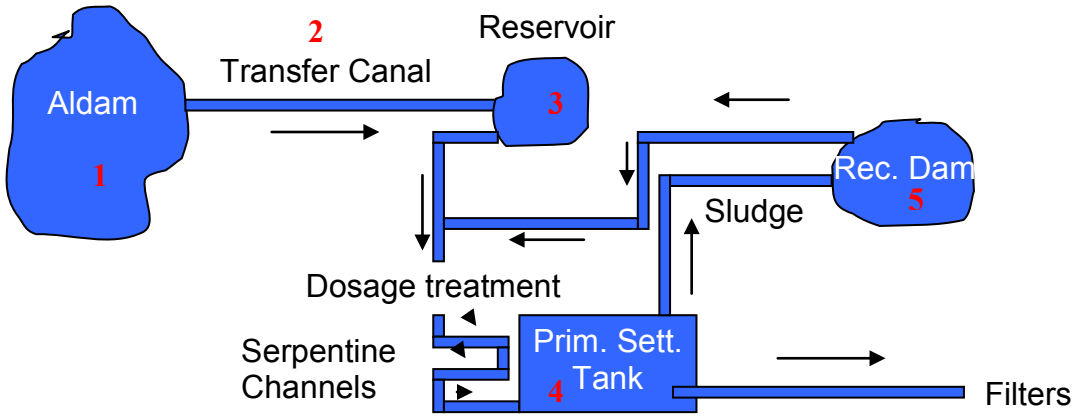


Figure 2.1.3: Schematic presentation of the Virginia water purification plant and the associated water supply route indicating the direction of water flow and position of the sampling sites.

For the water purification plant at Balkfontein raw water is taken by means of a pumping station from the Vaal River about 20 km from Bothaville, (location indicated in Fig 2.1.4A). Five samples were taken at the Balkfontein water purification plant of Sedibeng Water outside Bothaville. The position of these sites is shown in Fig. 2.1.4B. The position of the pumping station is indicated by site 6. The width of the Vaal River at the site is about 77 m, with a maximum depth of 5 m and an average depth of about 4 m (Pieterse, 1986). Site 7 is the primary settlement tank, site 8 is the secondary settlement tank while sites 9 and 10 represent the recycling dam and recycling dam outlet respectively.

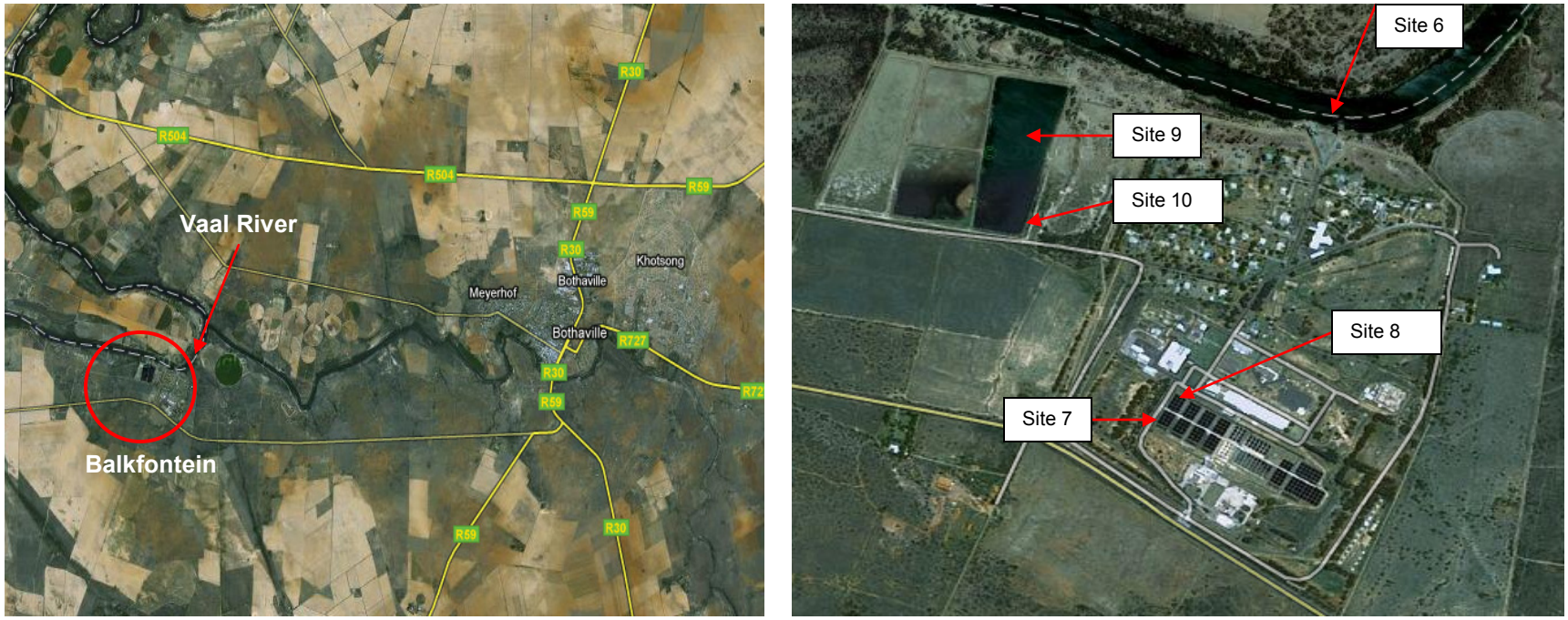


Figure 2.1.4: Aerial photographs indicating the water supply route to the Balkfontein water purification plant. **A:** Location of the Vaal River, Bothaville and the Balkfontein water purification plant; **B:** Balkfontein water purification plant including the Vaal River and Recycling Dams (http://maps.google.co.za).

Five samples were taken at the Balkfontein water purification plant. The sampling sites and direction of water flow is explained in a simplified schematic drawing in Fig. 2.1.5 and can be summarised as follows:

- Vaal River – close to the pumping station (site 6)
- Primary Settlement Tank (site 7)
- Secondary Settlement Tank (site 8)
- Recycling Dam (site 9)
- Recycling Dam Outlet (site 10)

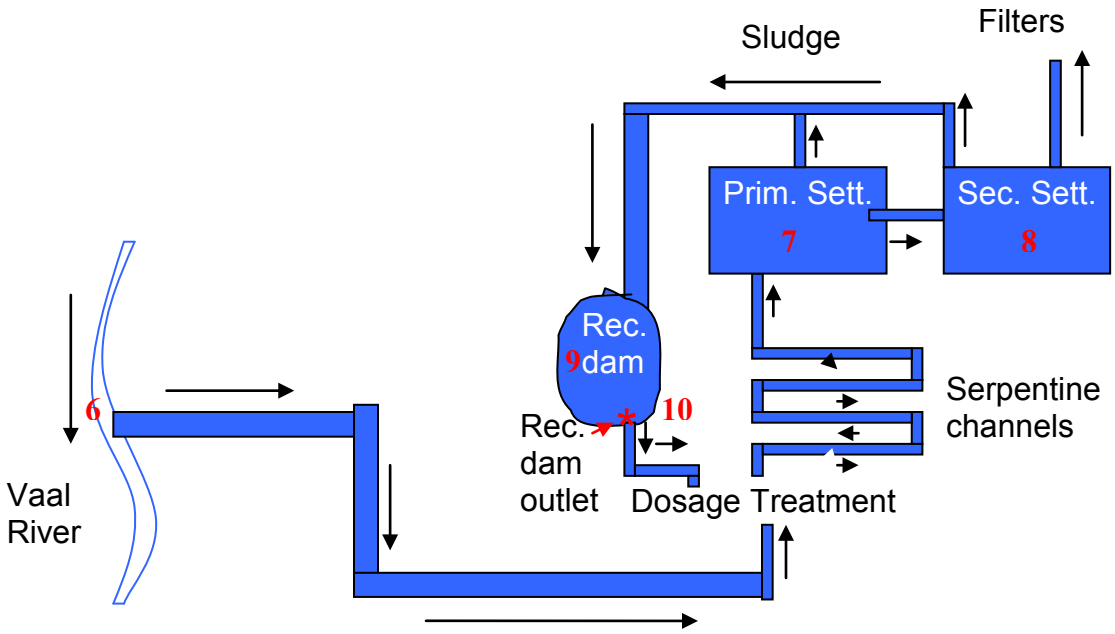


Figure 2.1.5: Schematic presentation of the Balkfontein water purification plant indicating direction of water flow and position of the sampling sites.

2.2 MATERIALS AND METHODS

2.2.1 SAMPLING

Sampling was done weekly in order to observe variation in the algal communities at the various sites over the study period. At each sampling site, grab samples were taken and preserved with 2% formaldehyde (final concentration). This report will reflect on the changes observed over a period of fourteen months, from February 2010 to March 2011.

2.2.2 PHYTOPLANKTON ANALYSES

Each sample was shaken to ensure a uniform distribution of algal cells. Gas vacuoles of cyanobacteria were pressure-deflated in a metal container with the use of a specially designed mechanical hammer that exerted a pressure of 49.5 kPa on the sample. According to Walsby (1971) this is the approximate pressure that is needed to collapse the gas vacuoles of cyanobacteria. Depending on the concentration of the algae or turbidity in each sample, 0.002 to 5 ml of each water sample was pipetted into sedimentation chambers. The remaining volume of the chambers was filled with distilled water and covered with circular glass cover slips. Algal cells were left to settle for a period of at least 48h (24h settling time per cm length of the sedimentation tube) in a desiccator with water in the base to prevent evaporation of water from the sample. The above mentioned procedures were repeated for each sample. This method is described by Utermöhl (1931, 1958) and modified by Lund *et al*. (1958).

After the settling period, algal cells were identified to genus or (where possible) species level with the use of texts such as Prescott (1978), John *et al.* (2002), Janse van Vuuren *et al.* (2006) and Taylor *et al.* (2007) and counted by means of an inverted Zeiss light microscope. The technique used for counting algal cells,

using Utermöhl sedimentation chambers, was described by Utermöhl (1931, 1958) and modified by Lund *et al*. (1958). One eyepiece of the microscope contained a Whipple grid which was used to deline the counting area. The glass bottoms of the sedimentation tubes were examined in strips and all algal cells which fell inside the grid were counted. In the case of densely packed colonies, like *Microcystis*, the cell number was estimated within each small square of the Whipple grid and then multiplied by the number of squares in the grid that the colony occupied. According to Lund *et al*. (1958) algal cells that settled randomly in the sedimentation chamber ensures that a single count is sufficient to provide an estimate of algal abundance.

Algal counts were used to determine species number, biomass (cells per millilitre), percentage composition of different algae at a given time, and successional patterns of the dominant algal species. The sub-sample volume transferred to the sedimentation tubes, cell counts and the number of strips that were counted were used to calculate the concentration of the individual algal genera or species and their percentage composition with the aid of a Microsoft Excel spreadsheet.

2.2.3 ENVIRONMENTAL VARIABLES

Physical and chemical variables were measured by Sedibeng Water on a weekly basis from March 2010 to March 2011 at the different sampling sites. The variables included pH, temperature in °C, turbidity in NTUs, conductivity measured in mS m^{-1} as well as nitrite (NO_2), nitrate (NO_3) ammonia (NH_3), and total organic carbon (TOC) in mg l^{-1}. Dissolved inorganic nitrogen (DIN) was calculated as the sum of NO_2, NO_3 and NH_3 values in mg l^{-1}. An induction coupled plasma (ICP) meter was used to measure NH_3, an ion chromatograph (IC) was used to measure NO_2 and NO_3, while a TOC analiser was used for TOC measurements.

2.2.4 STATISTICAL ANALYSIS OF DATA

Principal Component Analysis (PCA) is a statistical technique which is applied to a single set of variables when interested in discovering which variables in the set form coherent subsets that are relatively independent of one another (Tabachnick & Fidell, 2001).

A PCA plot consists of eigenvectors and eigenvalues. Eigenvectors are a set of scores, each of which represents the weighting of each of the original species or variables on each component. The eigenvector scores are scaled like correlation coefficients and range from +1.0 throughout 0.0 to -1.0. For each component every species or variable has a corresponding set of eigenvector scores and the nearer the score is to +1.0 or -1.0, which is the furthest from zero, the more important that species is in terms of weighting that component. Eigenvalues represent the relative contribution of each component to the explanation of the total variation in the data. There is one eigenvalue for each component, and the size of the eigenvalue for a component is a direct indication of the importance of that component in explaining the total variation within the data set (Kent & Coker, 1992).

A PCA is used to summarise patterns of correlations among observed variables, to reduce a large number of observed variables, to provide an operational definition for an underlying process by using observed variables, or to test a theory about the nature of the underlying processes (Tabachnick & Fidell, 2001).

Correspondence Analysis (CA) is related to the method of weighted averaging which is applied to a data matrix. Canonical Correspondence Analysis (CCA) examines the relationships between species distributions and the distribution of associated environmental factors and gradients (Kent & Coker, 1992).

PCA and CCA plots were done on the available environmental and species data with the use of the program CANOCO (canonical correspondence analyses: Ter Braak, 1988). Graphs regarding species density and composition over time as well as physical and chemical variables were done by means of MS Excel.

CHAPTER 3: RESULTS

3.1 INTRODUCTION

Rivers are confined, uni-directional systems that act as “drains” for the surrounding landscape, while lakes and wetlands are mostly “sinks” that accumulate materials brought by the wind, water and humans from their surroundings (Dallas & Day, 2004). The water chemistry of rivers reflects conditions occurring upstream and the extent to which these conditions influence those further downstream will depend largely on discharge, which varies from season to season and from year to year (Day *et al*., 1994). Activities anywhere in the upstream areas of the catchment area are reflected in a river and its associated ecosystems and alterations or perturbations, even in the upper reaches, may have an effect down the entire length of the river system (Dallas & Day, 2004).

Because water demands in the Vaal River catchment outstrips the supply, water in the Vaal River is utilised intensively, which then results in the salinisation and eutrophication of the river system (Pieterse & Kruger, 2002). Nutrient enrichment of surface waters from anthropogenic sources (cultural eutrophication) has long been recognised as a global water resource problem (Vollenweider, 1968; European Environmental Agency (EEA), 1998; United States Environmental Protection Agency (EPA), 1999). It is mostly found in highly populated and developed areas where certain agricultural practices and water-borne sewage systems contribute to increased loads of nutrients into the receiving natural water systems (Walmsley, 2000). The nutrients promote the development of both living and decaying biological material in river systems, which can cause a wide range of water quality and user problems (Dunst *et al.*, 1974). The Balkfontein water purification plant of Sedibeng Water treats water from the middle Vaal River. The

water in this section of the Vaal River contains a large fraction of recycled water which leads to a decrease in water quality (Traut, 2002).

The National Water Act (Act 36 of 1998) gave the (then called) Department of Water Affairs and Forestry (DWAF) the responsibility to develop National Monitoring Programmes such as the National Eutrophication Monitoring Programme (NEMP). During the development and implementation of this programme in 2000, it was identified that there was a need for an increase in algal identification capacity in South Africa and to report on all problems associated with eutrophication (Janse van Vuuren *et al*., 2006). Qualitative and quantitative knowledge of organisms that grow in an ecosystem are of great importance when studying the functioning of those ecosystems (Vollenweider *et al*., 1974). By determining the algal composition within water bodies, it is possible to deduce important information used to understand the quality of freshwater resources in South Africa. The use of diatom indices can now be regarded as a feasible option when determining the eutrophication status of rivers. Identification of algae, as a skill, is valued by, amongst others, the academic world, water purification institutions and governmental organisations whose operators need to be alerted to the presence of possible taste-, odour-, filter-clogging or toxin-producing algae in their source water. The presence of algae also contributes to the high cost of water purification while excessive cyanobacterial blooms produce toxins that can pose a serious risk to human health when not treated with the necessary caution and knowledge (WHO, 1999). During the last ten years a number of events in South Africa have led to an increased awareness of algae, most notably cyanobacteria (Harding & Paxton, 2001; Downing & Van Ginkel, 2002).

When determining physical and chemical variables and the associated phytoplankton composition in response to those variables, the reader needs to keep in mind that the conditions at the time of sampling may not necessarily be those conditions that caused the particular phytoplankton composition that was

collected (Janse van Vuuren, 1996). It takes time for water to move through the system and that is why conditions at a site located later in the sequence are the reflection of conditions that had occurred a few days before that specific date at a site earlier in the sequence. Sommer (1989) stated that “the most significant advances in understanding the ecology of phytoplankton will come from knowledge of how the rates of growth and attrition of individual species are affected by environmental variability”.

3.2 PHYTOPLANKTON CONCENTRATION AND COMPOSITION

Cyanobacteria and eukaryotic algae are classified into major taxa in Table 1 that shows a list of the different genera and species identified over the study period, along with their respective authors and whether the phytoplankton were found in the form of cells, colonies or filaments. Seven major phytoplankton taxa were found at the ten different sampling sites. The Cyanophyceae (cyanobacteria), Bacillariophyceae (diatoms) and the Chlorophyceae (green algae) were the most abundant in terms of concentration (total cells per milliliter) and diversity (amount of genera/species present) and they succeeded one another continually as the dominant groups. Other less numerous, but no less important groups, were the Cryptophyceae (cryptophytes), Chrysophyceae (golden algae), Dinophyceae (dinoflagellates) and the Euglenophyceae (euglenophytes).

Where possible, the cyanobacteria and algae were identified to species level before counting. More detailed light microscope and scanning electron microscope studies will be necessary in some cases to accurately identify some of the algal genera to species level. Certain algae, especially some filamentous forms, such as *Oedogonium* and *Mougeotia*, need to be observed in their sexual reproductive stages in order to identify them to species level.

TABLE 1: List of genera and species identified, along with their authors as well as the cellular arrangement of the phytoplankton found during the study period (2010 to 2011).

CYANOPHYCEAE	
Anabaena Bory de Saint-Vincent *ex* Bornet *et* Flahault	filament
Arthrospira Sitzenberger *ex* Gomont	filament
Cylindrospermopsis raciborskii (Wolosz.) Seenayya *et* Subba Raju	filament
Lyngbya C. Agardh *ex* Gomont	filament
Merismopedia minima (Beck) Meyen	colony
Microcystis aeruginosa (Kützing) Kützing	colony
Microcystis flos-aquae (Wittrock) Kirchner	colony
Microcystis wesenbergii (Komárek) Komárek	colony
Oscillatoria Vaucher *ex* Gomont	filament
Pseudanabaena Lauterborn	filament
BACILLARIOPHYCEAE	
Achnanthes minutissima Kützing	single cell
Amphipleura pellucida (Kützing) Kützing	single cell
Amphora libyca Ehrenberg	single cell
Asterionella formosa Hassall	colony
Aulacoseira granulata (Ehrenberg) Simonsen	filament
Cocconeis pediculus Ehrenberg	single cell
Cyclotella Kützing *ex* Brébisson	single cell
Cyclotella meneghiniana Kützing	single cell
Cymatopleura W. Smith	single cell
Cymbella C. Agardh	single cell
Diadesmis confervacea Kützing	filament
Diatoma vulgaris Bory de Saint-Vincent	single cell
Gomphonema Ehrenberg	single cell
Gyrosigma Hassall	single cell
Melosira varians C.Agardh	filament
Navicula Bory de Saint-Vincent	single cell
Nitzschia constricta (Kützing) Ralfs	single cell
Nitzschia Hassall	single cell
Nitzschia palea (Kützing) W. Smith	single cell
Pinnularia Ehrenberg	single cell
Pleurosigma elongatum W. Smith	single cell
Rhopalodia gibba (Ehrenberg) Müller	single cell
Surirella Turpin	single cell

Ulnaria ulna (Nitzsch) Compete	single cell

CHLOROPHYCEAE

Actinastrum hantzschii Lagerheim	colony
Ankistrodesmus Corda	colony
Carteria Diesing	single cell
Chlamydomonas Ehrenberg	single cell
Chlorella Beijerinck	single cell
Chlorococcum infusionum (Schrank) Meneghini	single cell
Closterium cornu Ehrenberg *ex* Ralfs	single cell
Coelastrum Nägeli	colony
Coelastrum pseudomicroporum Korshikov	colony
Cosmarium Corda *ex* Ralfs	single cell
Crucigenia tetrapedia (Kirchner) Kuntze	colony
Crucigeniella rectangularis (Nägeli) Komárek	colony
Eudorina elegans Ehrenberg	colony
Gonatozygon de Bary	single cell
Gonium O.F. Müller	colony
Kirchneriella Schmidle	single cell
Micractinium pusillum Fresenius	colony
Monoraphidium Komárková-Legnerová	single cell
Monoraphidium circinalis (Nygaard) Nygaard	single cell
Monoraphidium minutum (Nägeli) Komárková-Legnerová	single cell
Mougeotia C. Agardh	filament
Oedogonium Link *ex* Hirn	filament
Oocystis Nägeli *ex* A. Braun	single cell
Oocystis lacustris Chodat	single cell
Oocystis marsonii Lemmermann	single cell
Pandorina morum (O.F. Müller) Bory de Saint-Vincent	colony
Pediastrum boryanum (Turpin) Meneghini	colony
Pediastrum duplex Meyen	colony
Pediastrum simplex Meyen	colony
Pediastrum tetras (Ehrenberg) Ralfs	colony
Pteromonas aculeata Lemmermann	single cell
Pteromonas angulosa Lemmermann	single cell
Scenedesmus acuminatus (Lagerheim) Chodat	colony
Scenedesmus disciformis (Chodat) Fott *ex* Komárek	colony
Scenedesmus lefevrii Deflandre	colony
Scenedesmus lunatus (W. & G.S. West) Chodat	colony
Scenedesmus quadricauda Chodat	colony
Schroederia indica Philipose	single cell
Staurastrum Meyen *ex* Ralfs	single cell
Stigeoclonium Kützing	filament
Tetraedron caudatum (Corda) Hansgirg	single cell
Tetraedron mediocris Hindák	single cell

Tetraedron minimum A. Braun	single cell
Tetraedron planctonicum G.M. Smith	single cell
Tetrastrum Chodat	colony

CRYPTOPHYCEAE

Cryptomonas major Butcher	single cell

CHRYSOPHYCEAE

Dinobryon sertularia Ehrenberg	colony

DINOPHYCEAE

Ceratium hirundinella (O.F. Müller) Dujardin	single cell
Peridinium Ehrenberg	single cell
Sphaerodinium Woloszynska	single cell

EUGLENOPHYCEAE

Euglena Ehrenberg	single cell
Euglena oblonga F. Schmitz	single cell
Euglena pusilla Playfair	single cell
Phacus Dujardin	single cell
Phacus acuminatus Stokes	single cell
Phacus meson Pochmann	single cell
Strombomonas fluviatilis (Lemmermann) Deflandre	single cell
Strombomonas ovalis (Playfair) Deflandre	single cell
Strombomonas verrucosa (E. Daday) Deflandre	single cell
Trachelomonas intermedia P.A. Dangeard	single cell
Trachelomonas scabra Playfair	single cell
Trachelomonas volvocina (Ehrenberg) Ehrenberg	single cell

Of the different taxa the Chlorophyceae showed the highest diversity with 29 species and 16 genera which has not yet been identified to species level. This was followed by the Bacillariophyceae with 16 species and 9 genera, the Euglenophyceae with 10 species and 2 genera, the Cyanophyceae with 5 species and 5 genera, the Dinophyceae with 1 species and 2 genera and the Cryptophyceae and Chrysophyceae, each with one representative species.

During the study period 63 species were identified, along with 34 species that were only identified up to genus level.

In Table 2 the dominant algal genera/species, sampling sites where they were dominant as well as the period during which they dominated, are given. *Microcystis aeruginosa* (Cyanophyceae) was one of the most dominant species at 8 of the 10 sampling sites. It was dominant for an extensive period in the Allemanskraal Dam. It tended to dominate in the summer periods and its dominance extended well into the month of May. *Anabaena* sp. succeeded *M. aeruginosa* as the dominant algal species in the Allemanskraal Dam for the rest of the study period. *Anabaena* also gained dominance for brief periods at 4 other sites, succeeding *M. aeruginosa*. The primary settlement tank in Virginia was mostly dominated by Bacillariophyceae, specifically *Aulacoseira granulata* (April – May 2010) and *Nitzschia palea* (March 2010, July 2010 – March 2011). The unicellular centric diatom, *Cyclotella meneghiniana* of the group Bacillariophyceae, was quite prolific in the Vaal River at Balkfontein and gained dominance during May 2010 and the summer of 2011. The Chlorophyceae species, *Actinastrum* sp. was dominant in the winter of 2010 in the Vaal River and both the Balkfontein settlement tanks. *Chlamydomonas* sp. was dominant in late winter 2010 and summer 2011 in the water transfer canal and reservoir. It also dominated in the late summer and autumn period of 2010 in the Vaal River and both of the Balkfontein settlement tanks. *Pandorina morum* became dominant in the reservoir from June to July 2010 and October 2010 as well as in the primary settlement tank in Virginia during June 2010. *Pediastrum duplex* dominated in the recycling dam sites of the Balkfontein water purification plant during late winter and early spring 2010 as well as in December 2010. *Scenedesmus quadricauda* was periodically dominant in all the sites of the Balkfontein water purification plant, especially from August to December 2010 in the Vaal River. A discussion of the results presented in Tables 1 and 2 can be found in Chapter 4.

TABLE 2: List of dominant genera and species at each sample site and the months they dominated during the study period (2010 – 2011).

DOMINANTS	SAMPLE SITE	PERIOD
CYANOPHYCEAE		
Anabaena sp.	Allemanskraal Dam	Jul – Nov 2010, Mar 2011
	Water Transfer Canal	Nov 2010
	Reservoir	Feb, Sept, Dec 2010
	Recycling Dam, Balkfontein	Feb 2010
	Recycling Dam Outlet, Balkfontein	Mar 2010
Lyngbya sp.	Water Transfer Canal	Oct 2010
Microcystis aeruginosa	Allemanskraal Dam	Feb – Jun 2010, Dec 2010 – Feb 2011
	Water Transfer Canal	Feb – Jul 2010
	Reservoir	Mar – May 2010
	Recycling Dam, Virginia	Mar – May 2010
	Primary Settlement Tank, Balkfontein	Jan – Feb 2010
	Secondary Settlement Tank, Balkfontein	Dec 2010 – Jan 2011
	Recycling Dam, Balkfontein	Mar – May 2010, Jan – Mar 2011
	Recycling Dam Outlet, Balkfontein	Feb, Apr – May 2010, Jan – Feb 2011
BACILLARIOPHYCEAE		
Aulacoseira granulata	Primary Settlement Tank, Virginia	Apr – May 2010
Cocconeis pediculus	Water Transfer Canal	Dec 2010 – Jan 2011
Cyclotella sp.	Primary Settlement Tank, Balkfontein	Mar 2011
	Secondary Settlement Tank, Balkfontein	Mar 2011
Cyclotella meneghiniana	Vaal River	May 2010, Jan – Feb 2011
Nitzschia palea	Water Transfer Canal	Sept 2010
	Primary Settlement Tank, Virginia	Mar 2010, Jul 2010 – Mar 2011

CHLOROPHYCEAE		
Actinastrum sp.	Vaal River	Jun – Jul 2010
	Primary Settlement Tank, Balkfontein	Jun – Aug 2010
	Secondary Settlement Tank, Balkfontein	Jun – Aug 2010
Chlamydomonas sp.	Water Transfer Canal	Aug 2010, Feb – Mar 2011
	Reservoir	Aug, Nov 2010, Jan – Mar 2011
	Recycling Dam, Virginia	Feb 2010
	Primary Settlement Tank, Virginia	Feb 2010
	Vaal River	Feb – Apr 2010
	Primary Settlement Tank, Balkfontein	Feb – May 2010
	Secondary Settlement Tank, Balkfontein	Feb – May 2010, Feb 2011
Coelastrum sp.	Recycling Dam Outlet, Balkfontein	Nov 2010
Coelastrum pseudomicroporum	Primary Settlement Tank, Balkfontein	Oct 2010
	Secondary Settlement Tank, Balkfontein	Oct 2010
Micractinium sp.	Primary Settlement Tank, Balkfontein	Sept 2010
Pandorina morum	Reservoir	Jun – Jul, Oct 2010
	Primary Settlement Tank, Virginia	Jun 2010
Pediastrum boryanum	Recycling Dam, Balkfontein	Nov 2010
	Primary Settlement Tank, Balkfontein	Dec 2010
Pediastrum duplex	Recycling Dam, Balkfontein	Jul – Sept, Dec 2010
	Recycling Dam Outlet, Balkfontein	Jul, Sept – Oct, Dec 2010
Scenedesmus quadricauda	Vaal River	Aug – Dec 2010, Mar 2011
	Recycling Dam, Balkfontein	Jun, Oct 2010
	Recycling Dam Outlet, Balkfontein	Jun, Aug 2010
	Primary Settlement Tank, Balkfontein	Nov 2010
	Secondary Settlement Tank, Balkfontein	Sept, Nov 2010

Figure 1 shows extremely high algal concentrations in the Allemanskraal Dam for the entire study period (note the log-scale on the Y-axis). During February – June 2010 the concentration increased even more with the period of highest abundance being the month of May (maximum of 100 000 000 cells/ml). After that the algal abundance decreased (although the average concentration was still high) and remained more constant with brief escalations in September 2010 and February 2011. The group responsible for these extremely high concentrations was the Cyanophyceae (mainly *Microcystis* and *Anabaena*).

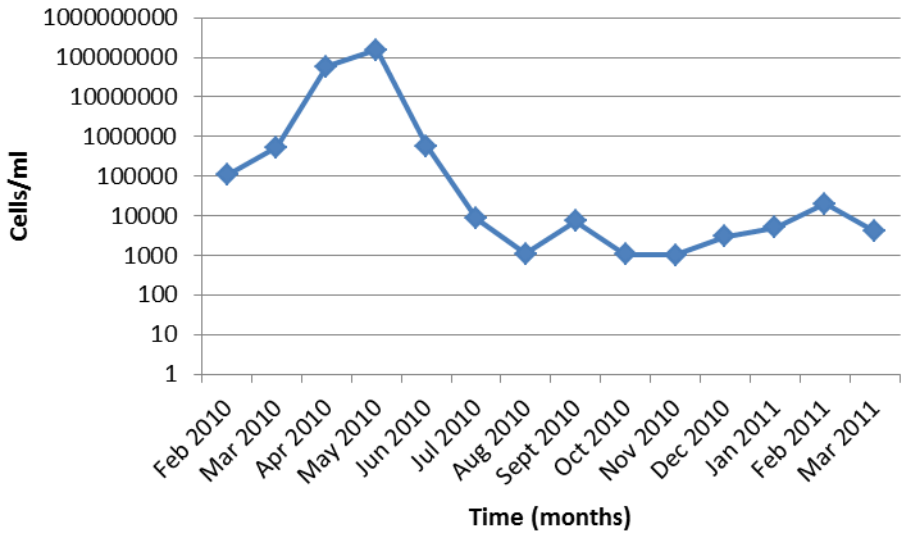


FIGURE 1: Total phytoplankton concentration (cells per milliter) over the fourteen month study period in the Allemanskraal Dam.

Figure 2 shows the algal concentration in the Vaal River at Balkfontein over the same study period. It is clear that the algal concentration in the Vaal River was, in general, much lower compared to the concentration in the Allemanskraal Dam. From February – May 2010 algal concentration was below 10 000 cells/ml, where after it escalated to reach a peak of 42 515 cells/ml in September 2010 after a brief decline in August 2010. Algal abundance showed a general decrease after that, except for an increase in December 2010 after which it decreased again reaching lower concentrations from January – March 2011. The algal genera

responsible for the peak in September were *Micractinium* and *Scenedesmus* (Chlorophyceae).

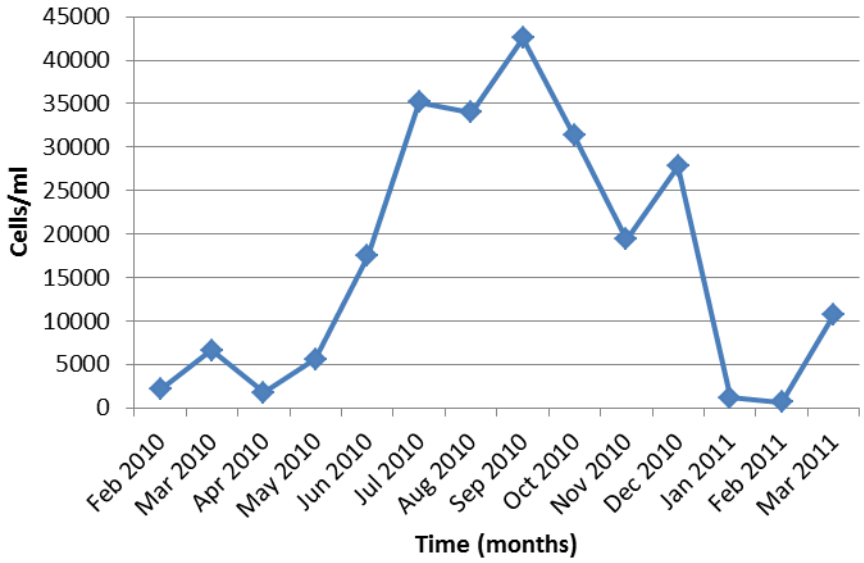


FIGURE 2: Total phytoplankton concentration (cells per milliter) over the fourteen month study period in the Vaal River at Balkfontein.

The phytoplankton composition of the source water for each purification plant is shown in Figures 3 and 4. It is clear from these figures that the Allemanskraal Dam was completely dominated by cyanobacteria (mostly *Microcystis aeruginosa*) throughout the study period (Fig. 3). A peak where the Chlorophyceae (mostly *Stigeoclonium* sp.) dominated was observed during October 2010. Abundance of the Bacillariophyceae, Chrysophyceae, Dinophyceae, Euglenophyceae and Cryptophyceae was relatively low when compared to the Cyanophyceae and, to a lesser extent, the Chlorophyceae. At Balkfontein (Fig. 4) the percentage composition of the different algal groups shows a clearly different picture. Prominent algal classes included the Chlorophyceae, Bacillariophyceae and Cyanophyceae. Of these, the Chlorophyceae was the most important and they dominated for most of the study period. Towards the end of the study period (beginning of summer) their concentration decreased in relation to that of the diatoms and at the beginning of 2011 a mixed assemblage of mostly Bacillariophyceae (diatoms), but also

Chlorophyceae and Cyanophyceae, was observed. Again, the Chrysophyceae, Dinophyceae, Euglenophyceae and Cryptophyceae were relatively scarce when compared to the 3 most common taxa (Cyanophyceae, Bacillariophyceae and Chlorophyceae).

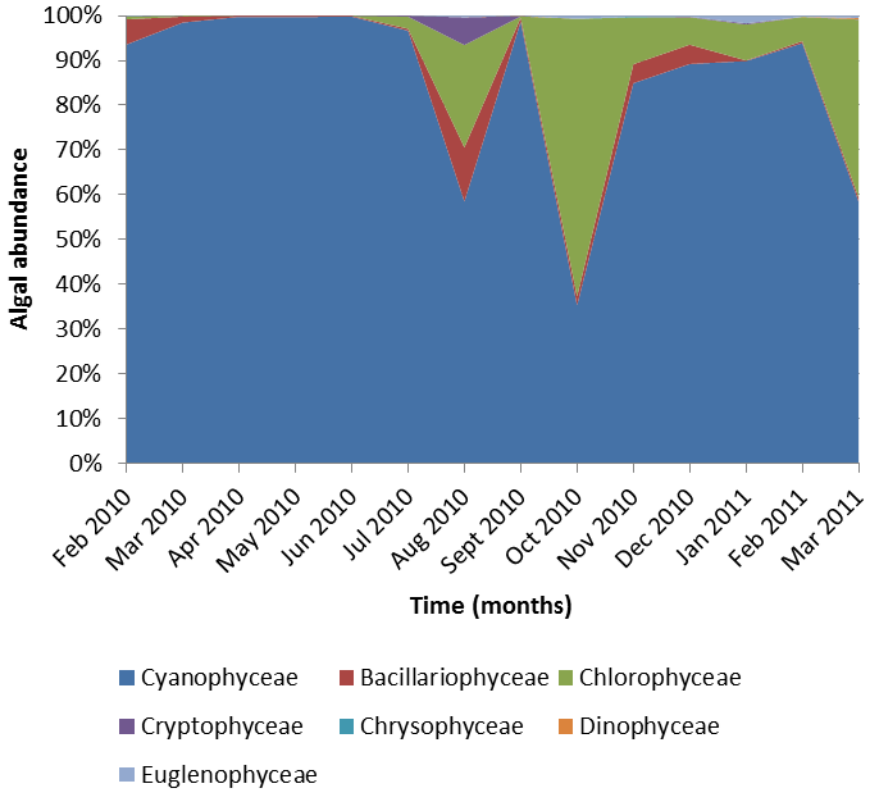


FIGURE 3: Variation in phytoplankton composition over the fourteen month study period in the Allemanskraal Dam.

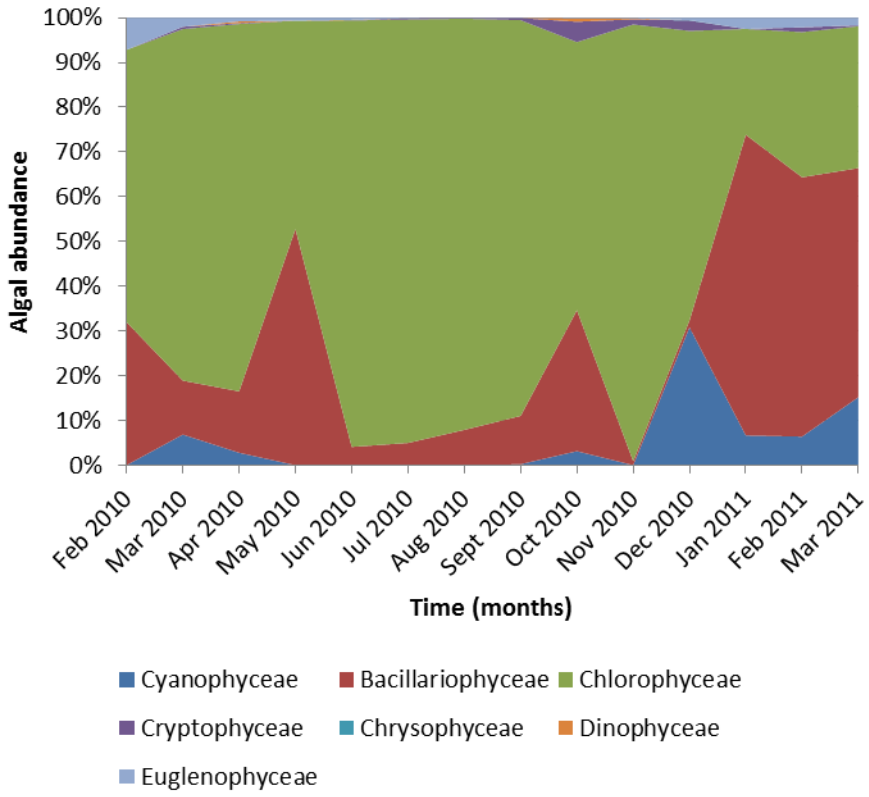


FIGURE 4: Variation in phytoplankton composition over the fourteen month study period in the Vaal River at Balkfontein.

Figures 3 and 5A show that the Cyanophyceae completely dominated in the Allemanskraal Dam. In the Water Transfer Canal, between the dam and the reservoir, the Bacillariophyceae and Chlorophyceae increased in concentration relative to the (still dominant) Cyanophyceae (Fig. 5B). When the water reached the reservoir (Fig. 5C), it was still dominated by Cyanophyceae, but the Chlorophyceae became relatively more important. During primary settlement (Fig. 5D), a change can be seen in the percentage composition of the phytoplankton. The percentage composition of the Cyanophyceae was reduced to only 15% (showing the efficiency of the sedimentation process in removing algal material). The phytoplankton was dominated mainly by Bacillariophyceae, which comprised 65% of the total phytoplankton. The Chlorophyceae was still important (19%). The remaining 1.7% consisted of Cryptophyceae, Chrysophyceae, Dinophyceae and Euglenophyceae. The tendencies seen in these figures and the efficiency of the purification plant to remove algal material will be discussed in Chapter 4.

Figure 6 shows that the Chlorophyceae dominated in the Vaal River and comprised 80% of the total phytoplankton with the Bacillariophyceae comprising (13%) and Cyanophyceae comprising (5%) respectively. The abundance of the Chlorophyceae was relatively higher in the two Settlement Tanks than in the Vaal River. The relative abundance of the Bacillariophyceae and Cyanophyceae were lower than in the Vaal River but remained constant between the two Settlement Tanks. The relative abundance of the Cryptophyceae changed little from the Vaal River to the Primary Settlement Tank but decreased towards the Secondary Settlement Tank. The tendencies seen in these figures and the efficiency of each step in the purification plant to remove algal material will be discussed in Chapter 4.

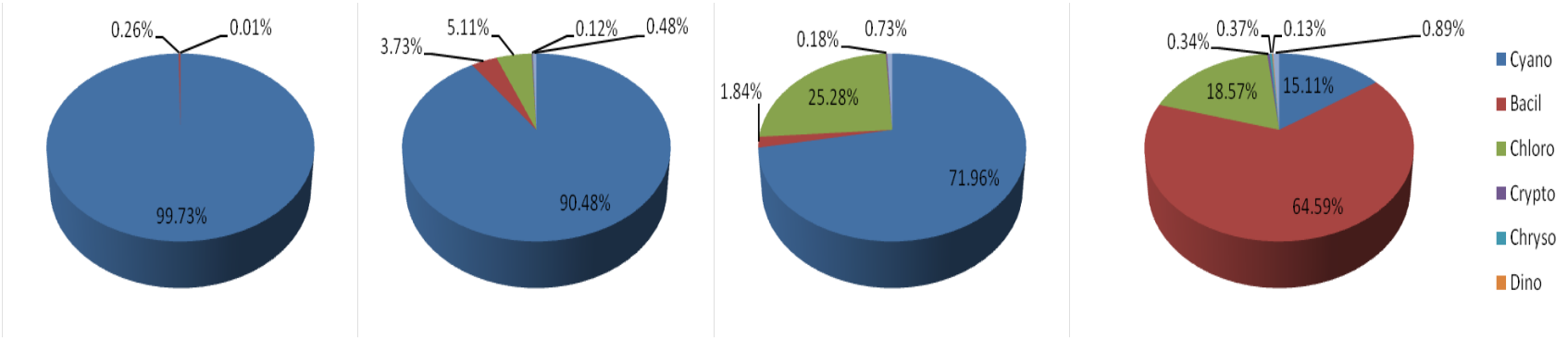


FIGURE 5: Relative abundance of the major algal groups for the study period at the different sampling sites of the Virginia water purification plant. The locations are: Allemanskraaldam (A), Transfer Canal (B), Reservoir (C) and Primary Settlement Tank (D). Cyano = Cyanophyceae, Bacil = Bacillariophyceae, Chloro = Chlorophyceae, Crypto = Cryptophyceae, Chryso = Chrysophyceae, Dino = Dinophyceae, Eugleno = Euglenophyceae.

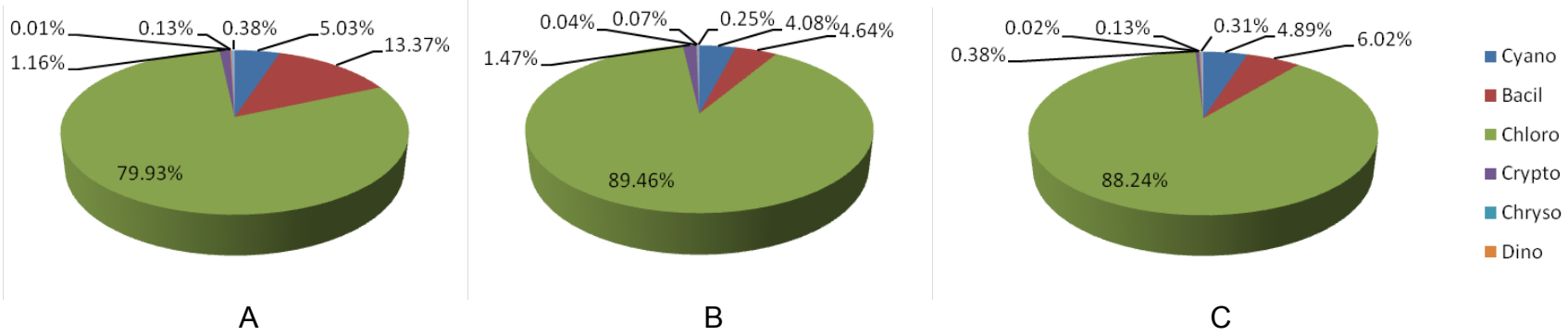
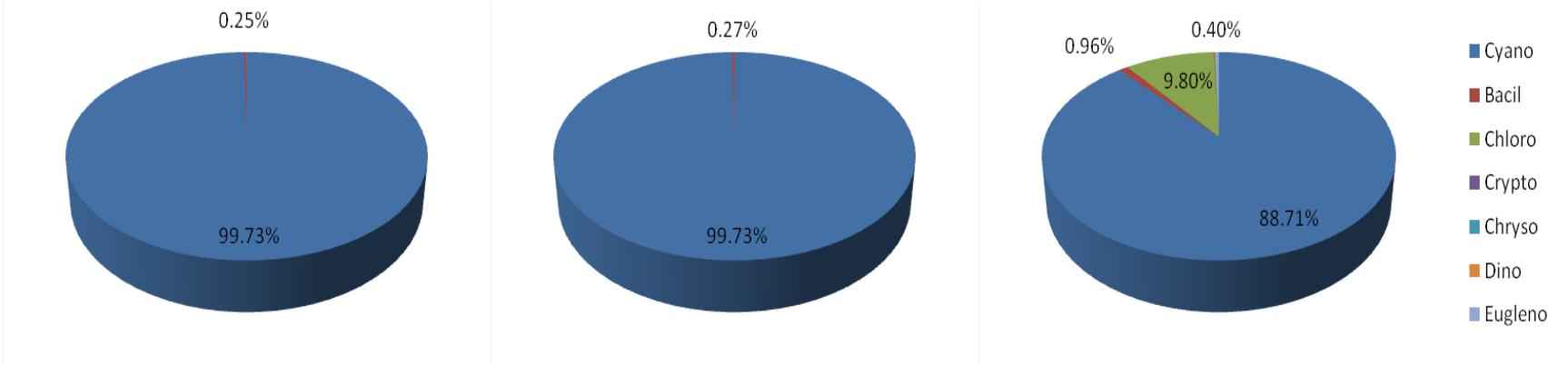


FIGURE 6: Relative abundance of the major algal groups for the study period at the different sampling sites of the Balkfontein water purification plant. The locations are: Vaal River (A), Primary Settlement Tank (B) and Secondary Settlement Tank (C). Cyano = Cyanophyceae, Bacil = Bacillariophyceae, Chloro = Chlorophyceae, Crypto = Cryptophyceae, Chryso = Chrysophyceae, Dino = Dinophyceae, Eugleno = Euglenophyceae.

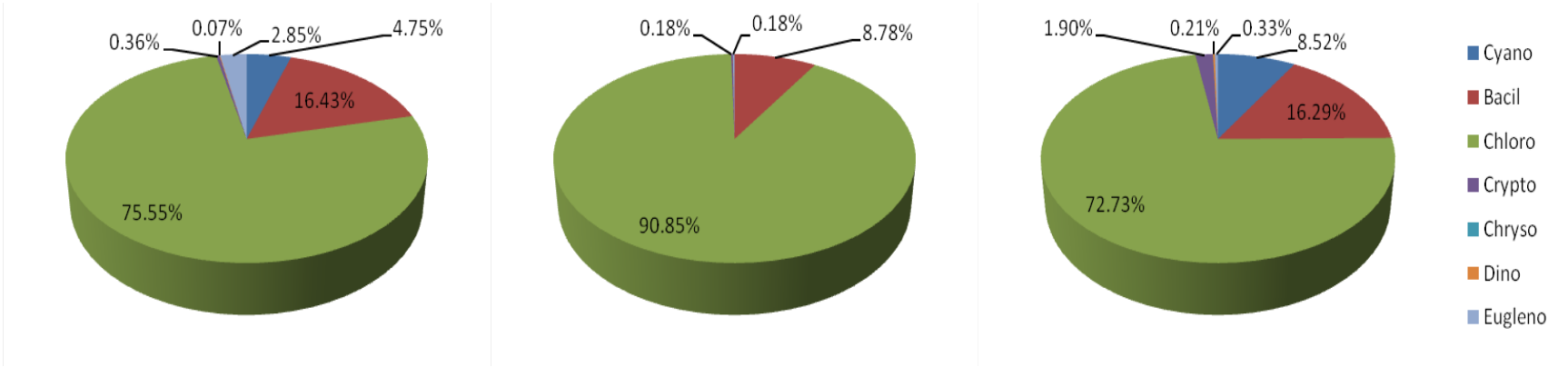
Figures 7A and B show that the Cyanophyceae completely dominated in the Allemanskraal Dam during the first two main periods of the study and thus no clear difference was observed between late summer and winter. During the following spring – summer period (Figure 7C), the Allemanskraal Dam was still dominated by Cyanophyceae, but the Chlorophyceae became more important. The percentage composition of the Cyanophyceae was reduced from 99.7% in the previous period to 89%, with the Chlorophyceae increasing to 10%.

Figure 8 shows that the Chlorophyceae dominated through all three main periods in the Vaal River. During the late summer – autumn period (Fig. 8A) the Chlorophyceae was dominant (76%), followed by Bacillariophyceae (16%) and the Cyanophyceae (5%). During the winter period (Fig. 8B) the percentage composition of the Chlorophyceae increased to 91% as a result of the simultaneous decrease in the percentage composition of the Bacillariophyceae (9%), the Cyanophyceae and the Euglenophyceae (0.2% each). During the following spring – summer period (Fig. 8C) the Bacillariophyceae became relatively more important (to the same extent as depicted in Fig. 8A) and the Cyanophyceae was more prominent in relation to other algal groups during the summer periods when compared to the winter.



A B C

FIGURE 7: Relative abundance of the major algal groups in the Allemanskraal Dam over the three main periods of the study. A = February – April 2010, B = May – August 2010, C = September 2010 – March 2011. Cyano = Cyanophyceae, Bacil = Bacillariophyceae, Chloro = Chlorophyceae, Crypto = Cryptophyceae, Chryso = Chrysophyceae, Dino = Dinophyceae, Eugleno = Euglenophyceae.



A B C

FIGURE 8: Relative abundance of the major algal groups in the Vaal River over the three main periods of the study. A = February – April 2010, B = May – August 2010, C = September 2010 – March 2011. Cyano = Cyanophyceae, Bacil = Bacillariophyceae, Chloro = Chlorophyceae, Crypto = Cryptophyceae, Chryso = Chrysophyceae, Dino = Dinophyceae, Eugleno = Euglenophyceae.

Figure 9 indicates the relative algal abundance for February – May 2010 in the recycling dam at Balkfontein (Fig. 9A), the recycling dam outlet at Balkfontein (Fig. 9B) and the recycling dam at Virginia (Fig. 9C). The Cyanophyceae clearly had the highest abundance at the Balkfontein recycling dam and recycling dam outlet, with the Dinophyceae (mostly *Ceratium hirundinella*) having a higher abundance than at any of the other sampling sites. In the recycling dam outlet it is the second most abundant group after the Cyanophyceae for this period. In the recycling dam at Virginia (Fig. 9C) the Cyanophyceae is also dominant, but with a much lower relative abundance than at Balkfontein. The Chlorophyceae and Bacillariophyceae are more abundant than at Balkfontein at 27% and 18% respectively. Dinophyceae were not prevalent in large concentrations at Virginia.

Figure 10 indicates the relative algal abundance of the recycling dam and recycling dam outlet of Balkfontein water purification plant, but – in contrast to Figure 9 - these graphs represent the entire study period. No further sampling was done in the recycling dam at the Virginia water purification plant due to the amount of sludge present. The Cyanophyceae remained the group with the highest relative abundance although it was lower in the recycling dam outlet. The Chlorophyceae was the second most abundant group with a higher relative abundance in the recycling dam outlet. If counting results were expressed in terms of biovolume in stead of cells/ml the Dinophyceae biomass (mainly large *Ceratium hirundinella* cells) would have seemed far more important than they currently do.

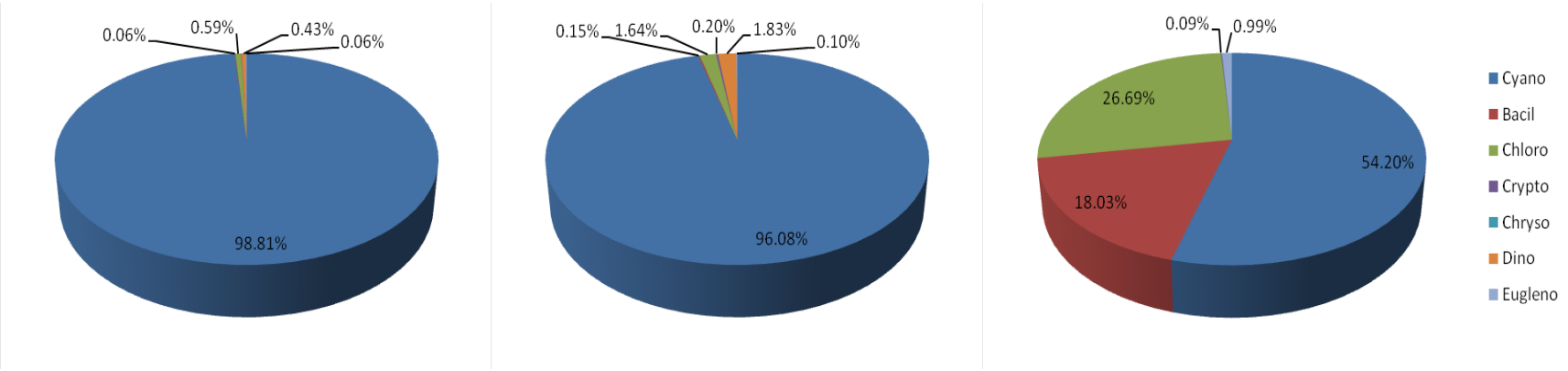


FIGURE 9: Relative abundance of the major algal groups from February – May 2010 at the Recycling Dam (A) and Recycling Dam outlet (B) of the Balkfontein water purification plant and the Recycling Dam (C) of the Virginia water purification plant. Cyano = Cyanophyceae, Bacil = Bacillariophyceae, Chloro = Chlorophyceae, Crypto = Cryptophyceae, Chryso = Chrysophyceae, Dino = Dinophyceae, Eugleno = Euglenophyceae.

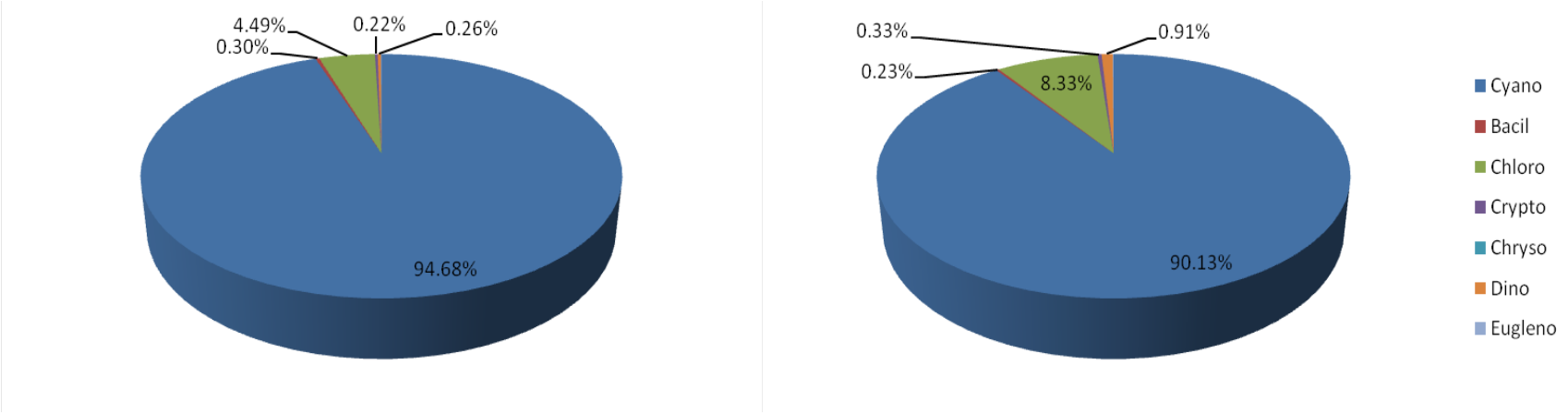


FIGURE 10: Relative abundance of the major algal groups for the study period at the Recycling Dam (A) and Recycling Dam outlet (B) of the Balkfontein water purification plant. Cyano = Cyanophyceae, Bacil = Bacillariophyceae, Chloro = Chlorophyceae, Crypto = Cryptophyceae, Chryso = Chrysophyceae, Dino = Dinophyceae, Eugleno = Euglenophyceae.

The following graphs indicate the variation in concentration of the dominant algal species during the study period at the different sites of the Virginia water purification plant.

Figure 11 shows a peak in concentration of the Cyanophyceae species *Anabaena* sp. and *Microcystis aeruginosa* in the Allemanskraal Dam between March and June 2010, reaching a peak in May. There was also a marked increase in *Chlamydomonas* sp. (Chlorophyceae) during the same period where it reached a peak in April and decreased thereafter.

Figure 12 indicates high concentrations of *Anabaena* sp. and *M. aeruginosa* between February and May 2010 in the water transfer canal, reaching a peak in April. There is a higher occurence of *Chlamydomonas* sp. in March and *Anabaena* sp. reached another peak in January 2011.

In Figure 13 clear successional patterns can be observed where *M. aeruginosa* succeeded *Anabaena* and reached a peak during April 2010 in the reservoir. *Pandorina morum* completely dominated during July 2010 and *Chlamydomonas* during November 2010. *Chlamydomonas* numbers also increased after February 2011 when it dominated again. Overall algal abundance was high in the late summer of 2010.

In Figure 14 it is illustrated that *M. aeruginosa* dominated during March 2010 and February 2011 in the primary settlement tank. *Nitzschia palea* increased during the colder months, reaching a concentration of about 200 cells/ml during October 2010. *Aulacoseira granulata* dominated in April 2010 and shows a peak in concentration during August and December 2010.

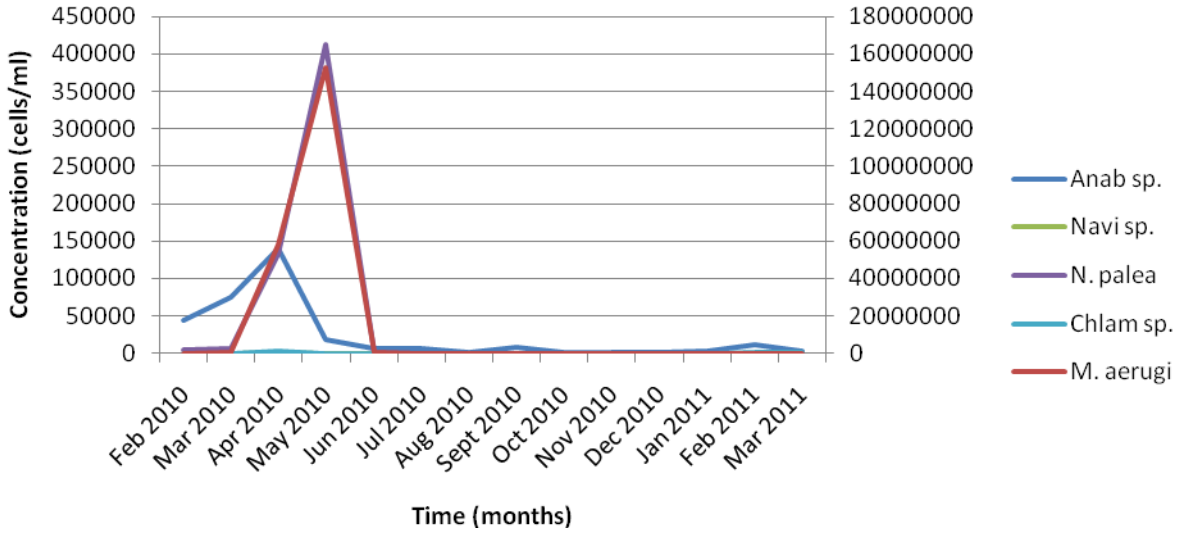


FIGURE 11: Variation in the concentration of the dominant algal species during the study period in the Allemanskraal Dam. Anab = *Anabaena*, Navi = *Navicula*, N. palea = *Nitzschia palea*, Chlam = *Chlamydomonas*, M. aerugi = *Microcystis aeruginosa*. *M. aeruginosa* is plotted on the secondary Y-axis.

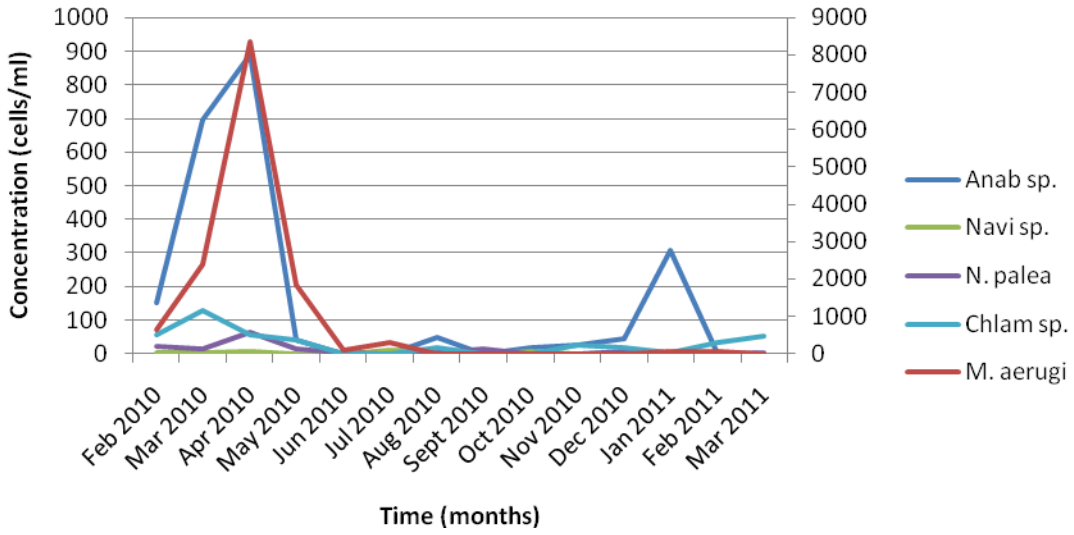


FIGURE 12: Variation in the concentration of the dominant algal species during the study period in the water transfer canal leading from the Allemanskraal Dam. Anab = *Anabaena*, Navi = *Navicula*, N. palea = *Nitzschia palea*, Chlam = *Chlamydomonas*, M. aerugi = *Microcystis aeruginosa*. *M. aeruginosa* is plotted on the secondary Y-axis.

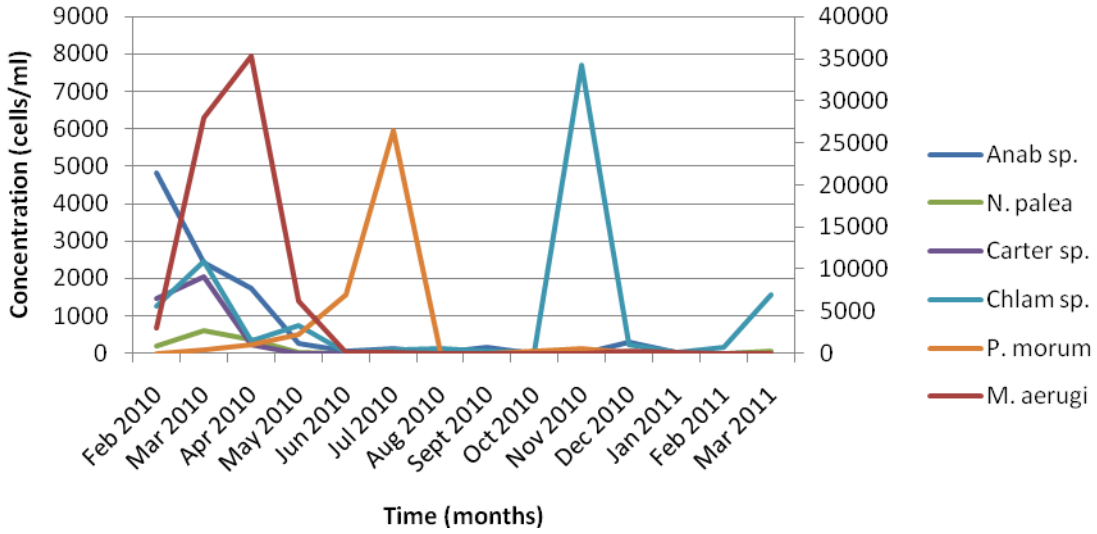


FIGURE 13: Variation in the concentration of the dominant algal species during the study period in the reservoir. Anab = *Anabaena*, N. palea = *Nitzschia palea*, Carter = *Carteria*, Chlam = *Chlamydomonas*, P. morum = *Pandorina morum*, M. aerugi = *Microcystis aeruginosa*. *M. aeruginosa* is plotted on the secondary Y-axis.

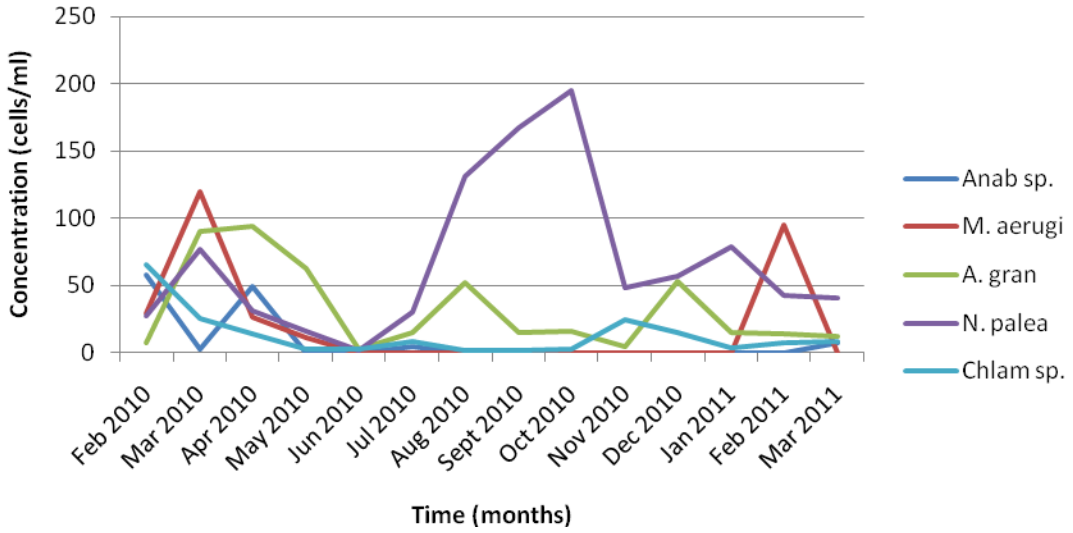


FIGURE 14: Variation in the concentration of the dominant algal species during the study period in the primary settlement tank of the Virginia water purification plant. Anab = *Anabaena*, M. aerugi = *Microcystis aeruginosa*, A. gran = *Aulacoseira granulata*, N. palea = *Nitzschia palea*, Chlam = *Chlamydomonas*.

Figs 15 to 19 illustrate the succession of the dominant species in the Balkfontein water purification plant of Sedibeng Water.

Figure 15 shows that *Actinastrum hantzschii* dominated in the winter months peaking during July 2010 in the Vaal River. It was succeeded as the dominant by *Scenedesmus quadricauda* which peaked during September 2010. A second, smaller peak in the concentration of *Actinastrum hantzschii* was observed during the same period. After that *S. quadricauda* gradually decreased towards January 2011. *Cyclotella meneghiniana* and *M. aeruginosa* became the dominants in October 2010 and December 2010 respectively. During the spring period a mixed algal assemblage consisting of Cyanophyceae, Bacillariophyceae and Chlorophyceae occurred.

In Figure 16 it is illustrated that algal abundance remained fairly low during both summer periods and increased during the winter period of 2010 in the primary settlement tank. *Actinastrum hantzschii*, transferred from the raw water, was clearly dominant, peaking in July after which it decreased to be succeeded by *Micractinium pusillum* in September. During the same time a peak in the concentration of *S. quadricauda* was also experienced.

In Figure 17 it is shown that algal abundance increased during the winter period with *A. hantzschii* being dominant from May to September 2010 (peaking in July) in the secondary settlement tank. Algal abundance of the other major species showed an increase between July and October with *M. pusillum* peaking in August and *S. quadricauda* peaking in September, succeeding *A. hantzschii* as the dominant.

In Figure 18 a clear successional pattern can be observed from cyanobacterial dominance at the beginning of the study period to green algal dominance at the end in the recycling dam. *Anabaena* was dominant at the start of the study period and then succeeded by *M. aeruginosa* in autumn 2010. A peak in *Chlamydomonas* concentration was observed in October after which it was

succeeded again by *M. aeruginosa* in January and *Pediastrum duplex* in March 2011. An increase in *Ceratium hirundinella* concentration was noticed in May, where the species briefly succeeded *M. aeruginosa*.

Figure 19 shows peaks in concentration of *Anabaena* and *M. aeruginosa* during late summer to autumn, as well as a marked increase of *C. hirundinella* that briefly dominated during May 2010, succeeding *M. aeruginosa* as the dominant in the recycling dam outlet. Algal abundance during the winter remained low with a gradual increase in the numbers of *Pediastrum duplex* that reached a peak in October when *Chlamydomonas* was dominant. A sharp decrease in algal abundance was experienced in November after which the amount of *P. duplex* increased again, peaking in January 2011 when *M. aeruginosa* was again dominant.

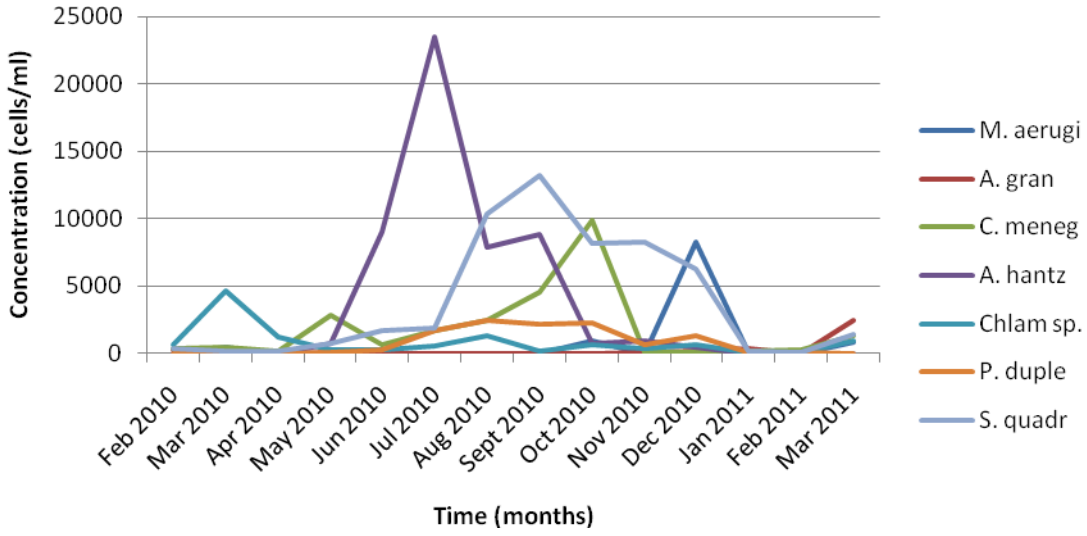


FIGURE 15: Variation in the concentration of the dominant algal species during the study period in the Vaal River at Balkfontein. M. aerugi = *Microcystis aeruginosa*, A. gran = *Aulacoseira granulata*, C. meneg = *Cyclotella meneghiniana*, A. hantz = *Actinastrum hantzschii*, Chlam = *Chlamydomonas*, P. duple = *Pediastrum duplex*, S. quadr = *Scenedesmus quadricauda*.

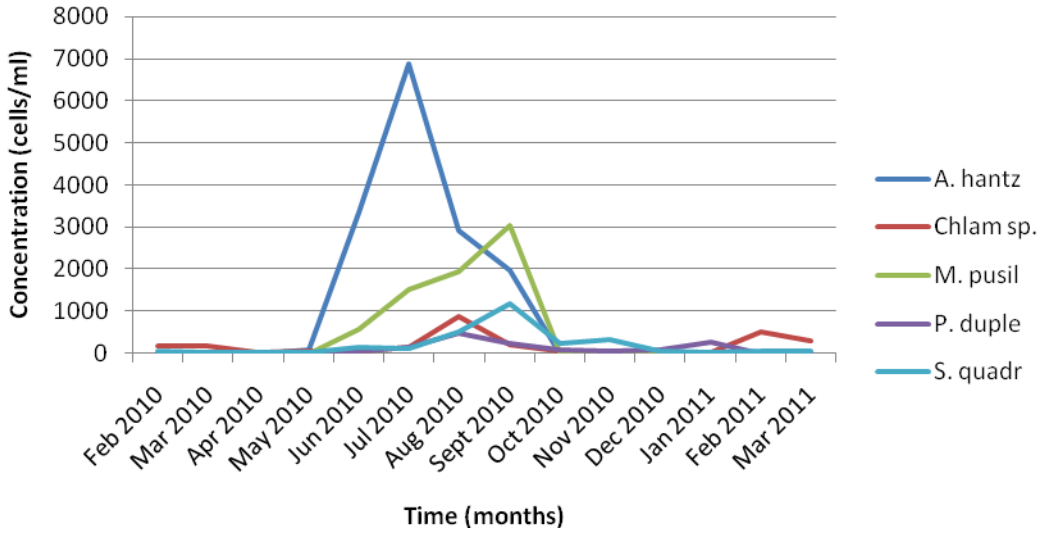


FIGURE 16: Variation in the concentration of the dominant algal species during the study period in the primary settlement tank of the Balkfontein water purification plant. A. hantz = *Actinastrum hantzschii*, Chlam = *Chlamydomonas*, M. pusil = *Micractinium pusillum*, P. duple = *Pediastrum duplex*, S. quadr = *Scenedesmus quadricauda*.

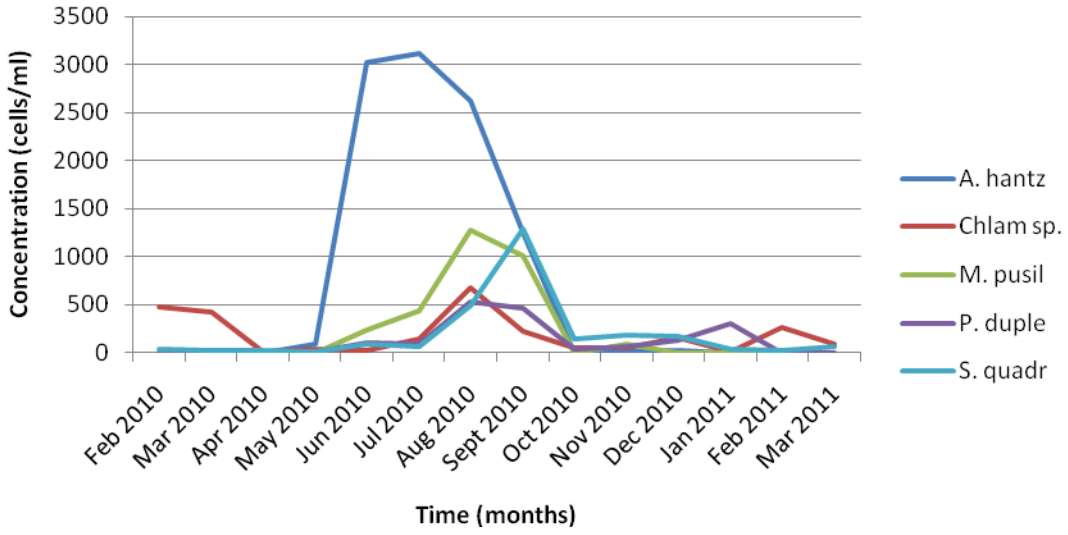


FIGURE 17: Variation in the concentration of the dominant algal species during the study period in the secondary settlement tank of the Balkfontein water purification plant. A. hantz = *Actinastrum hantzschii*, Chlam = *Chlamydomonas*, M. pusil = *Micractinium pusillum*, P. duple = *Pediastrum duplex*, S. quadr = *Scenedesmus quadricauda*.

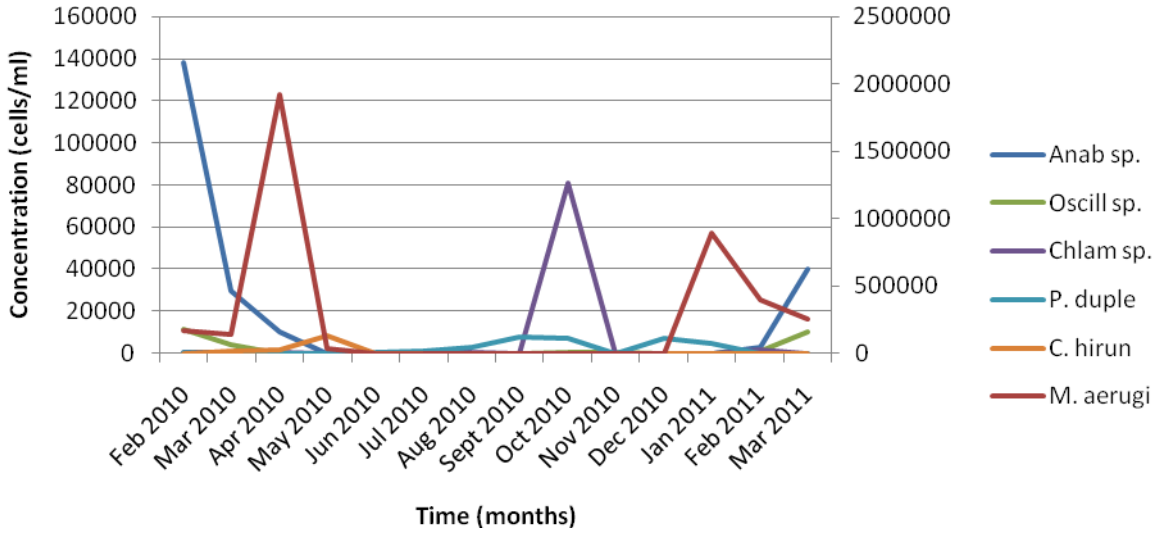


FIGURE 18: Variation in the concentration of the dominant algal species during the study period in the recycling dam of the Balkfontein water purification plant. Anab = *Anabaena*, Oscill = *Oscillatoria*, Chlam = *Chlamydomonas*, P. duple = *Pediastrum duplex*, C. hirun = *Ceratium hirundinella*, M. aerugi = *Microcystis aeruginosa*. *M. aeruginosa* is plotted on the secondary Y-axis.

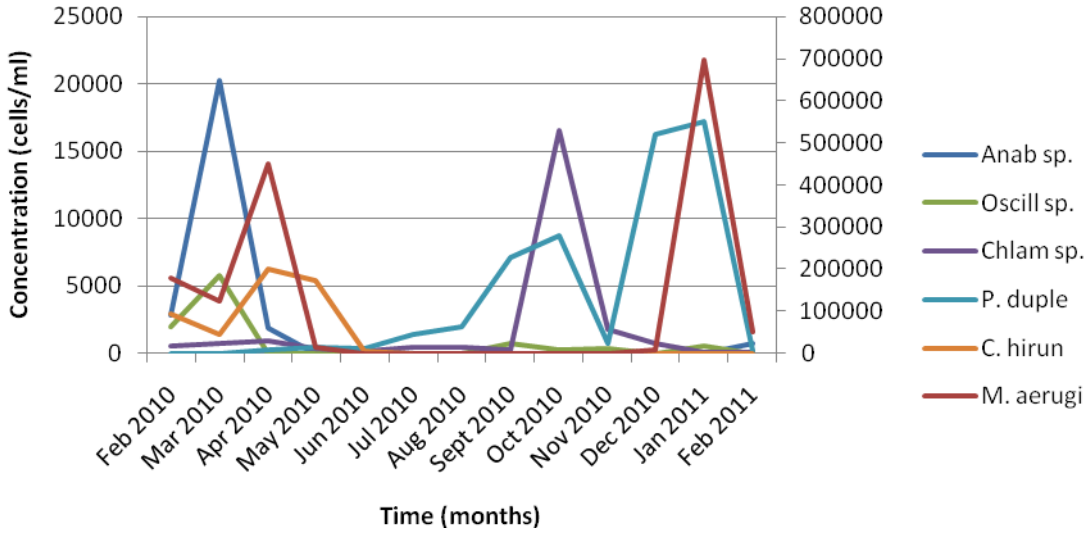


FIGURE 19: Variation in the concentration of the dominant algal species during the study period in the recycling dam outlet of the Balkfontein water purification plant. Anab = *Anabaena*, Oscill = *Oscillatoria*, Chlam = *Chlamydomonas*, P. duple = *Pediastrum duplex*, C. hirun = *Ceratium hirundinella*, M. aerugi = *Microcystis aeruginosa*. *M. aeruginosa* is plotted on the secondary Y-axis.

3.3 ENVIRONMENTAL VARIABLES

Environmental variables are divided into physical and chemical variables. Physical variables include temperature and turbidity, while chemical variables include pH, conductivity, dissolved inorganic nitrogen (DIN) and total organic carbon (TOC). Unfortunately no environmental variables are available for March 2011 in the recycling dam outlet of the Balkfontein water purification plant due to equipment that was out of order.

Figure 20 and 21 indicates that water temperature increased during the summer and decreased during the winter, as expected. Individual peaks in water temperature above the average occurred during May, June and September 2010 as well as January 2011 at the sampling sites of Virginia water purification plant. Peaks in temperature at the Balkfontein water purification plant occurred during May, September and December 2010 as well as February 2011. None of the sites were consistently warmer throughout the study period and both plants had roughly the same seasonal temperatures.

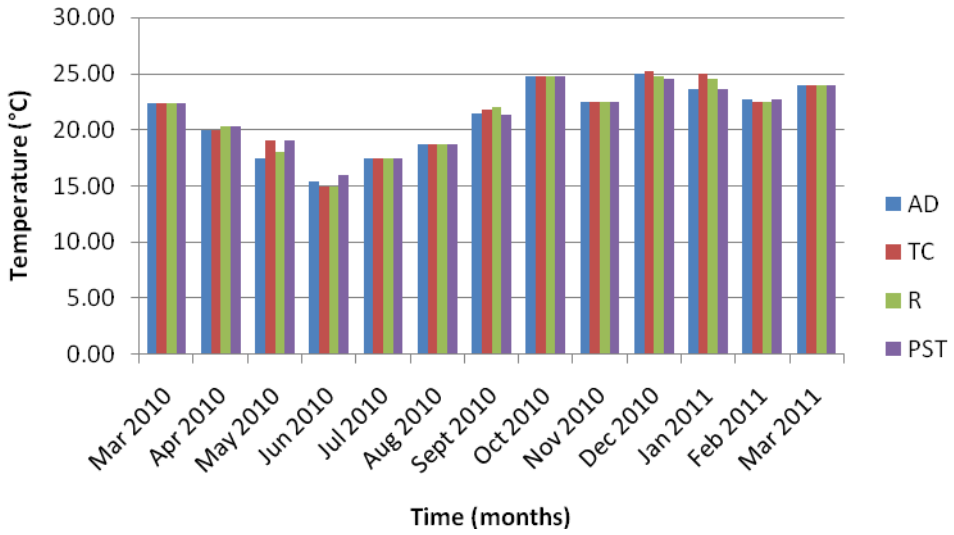


FIGURE 20: Water temperature measurements at the different sampling sites of the Virginia water purification plant. AD = Allemanskraal Dam, TC = water transfer canal, R = reservoir, PST = primary settlement tank.

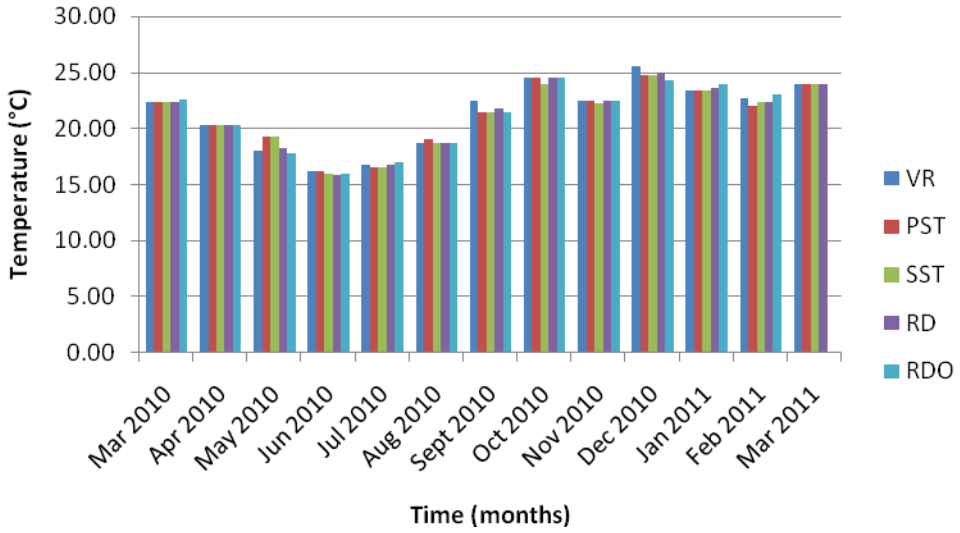


FIGURE 21: Water temperature measurements at the different sampling sites of the Balkfontein water purification plant. VR = Vaal River, PST = primary settlement tank, SST = secondary settlement tank, RD = recycling dam, RDO = recycling dam outlet.

Figure 22 shows increases in turbidity during the summer months in the Allemanskraal Dam and increased turbidity in the water transfer canal between December 2010 and January 2011. Throughout the study the turbidity in the primary settlement tank was very low except during June 2010 and February 2011. Turbidity levels in the Allemanskraal Dam and the transfer canal were usually higher or equal to that in the reservoir and the primary settlement tank with only a few exceptions.

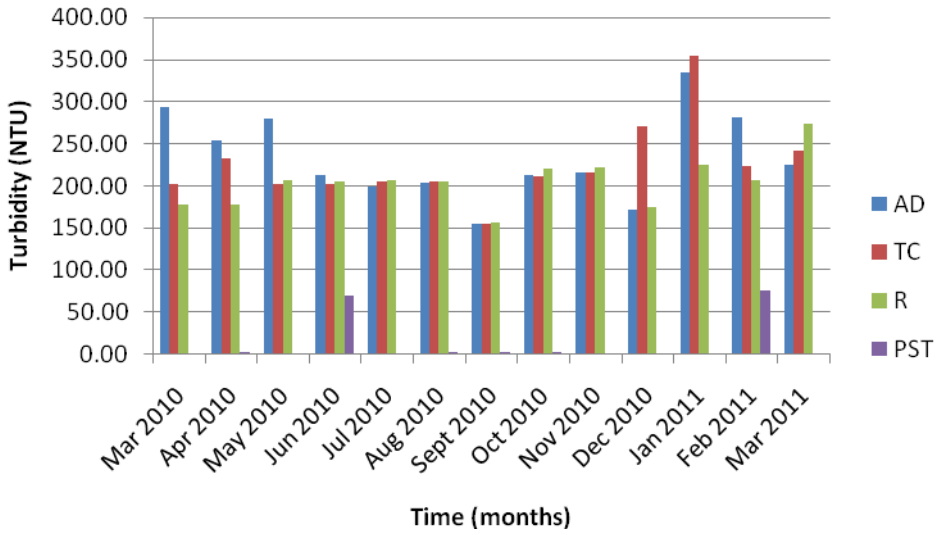


FIGURE 22: Turbidity measurements at the different sampling sites of the Virginia water purification plant. AD = Allemanskraal Dam, TC = water transfer canal, R = reservoir, PST = primary settlement tank.

In Figure 23 its easy to notice increases in turbidity during the summer and spring months in the Vaal River. During the winter turbidity remained low. The recycling dam and recycling dam outlet followed similar trends, although turbidity at those sites were lower. Turbidity in the settlement tanks were very low with the exception of the secondary settlement tank that showed a sharp increase during April 2010 and the primary settlement tank during January 2011.

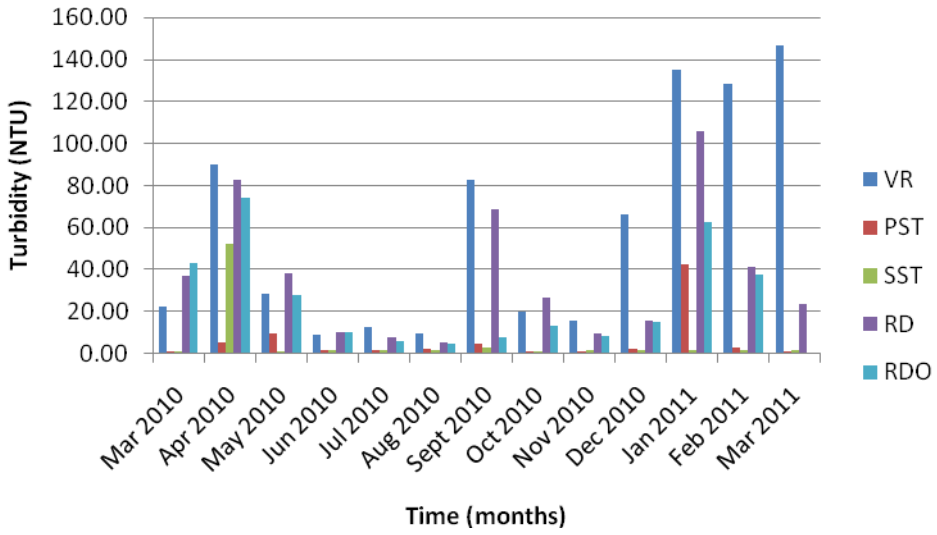


FIGURE 23: Turbidity measurements at the different sampling sites of the Balkfontein water purification plant. VR = Vaal River, PST = primary settlement tank, SST = secondary settlement tank, RD = recycling dam, RDO = recycling dam outlet.

Figure 24 indicates that pH decreased in the late summer and autumn and then returned to average (pH 8) for the rest of the period. During autumn the pH in the Allemanskraal Dam was slightly below average, reaching a low point in May while it increased above average in July and early spring.

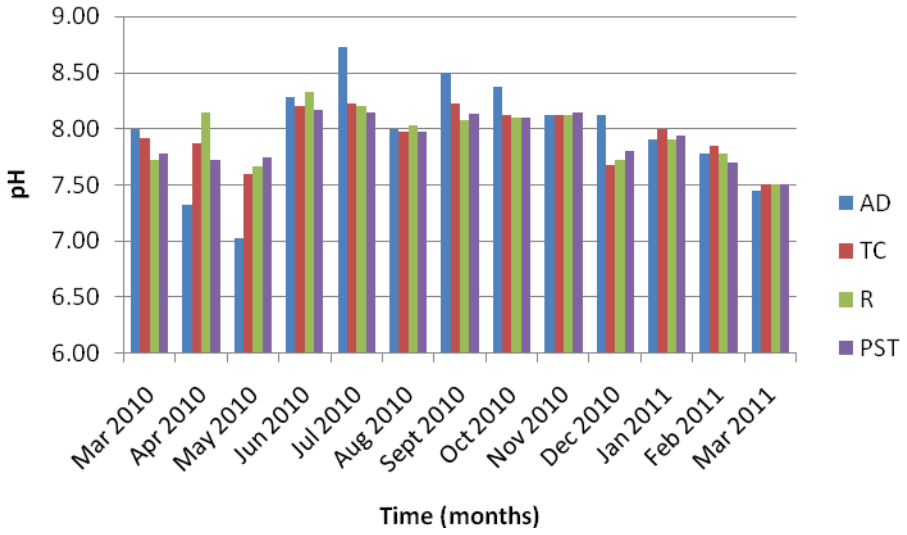


FIGURE 24: pH measurements at sampling sites of the Virginia water purification plant. AD = Allemanskraal Dam, TC = water transfer canal, R = reservoir, PST = primary settlement tank.

Figure 25 shows more variation in pH as it increased in the Vaal River during the colder months. On average the pH in the primary settlement tank was higher than in the secondary settlement tank. Prominent peaks in the pH of the recycling dam sites occurred during March 2010 and from January to March 2011.

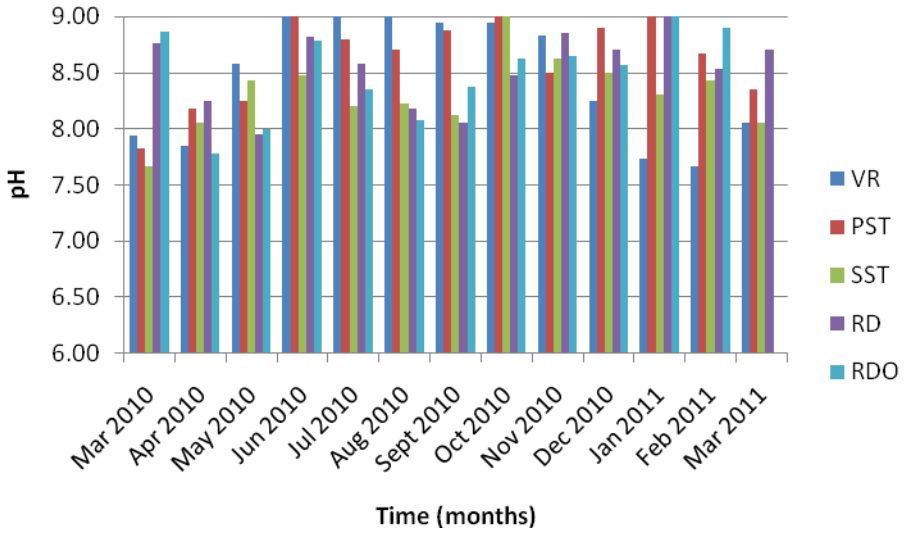


FIGURE 25: pH measurements at sampling sites of the Balkfontein water purification plant. VR = Vaal River, PST = primary settlement tank, SST = secondary settlement tank, RD = recycling dam, RDO = recycling dam outlet.

In Figure 26 it is illustrated that the average conductivity of all 4 sampling sites increased above average (15 mS/m) during April and September 2010. Peaks in conductivity in the water transfer canal can be observed during August 2010 and especially during January 2011. An increase is also visible in the reservoir during November 2010.

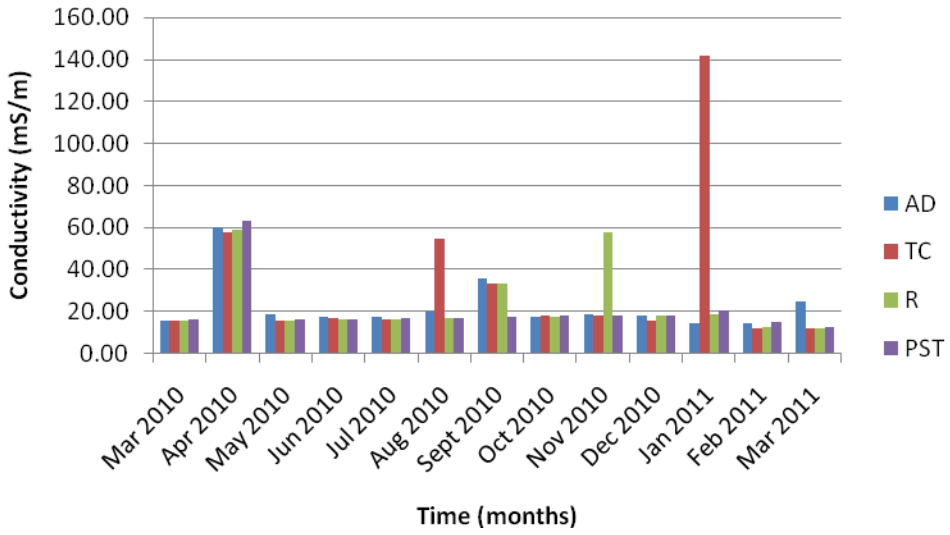


FIGURE 26: Conductivity measurements of the study at sampling sites of the Virginia water purification plant. AD = Allemanskraal Dam, TC = water transfer canal, R = reservoir, PST = primary settlement tank.

Figure 27 illustrates that the average conductivity was higher from June – December 2010 and lower during March – May 2010 and January – March 2011. The two recycling dam sites showed much higher conductivity levels between October 2010 and February 2011 although it also decreased from December to January. A decrease in conductivity in the Vaal River is observed during September 2010. Average annual conductivity levels were also much higher at the Balkfontein water purification plant (average 60 mS/m) than at the Virginia water purification plant (average 40 mS/m).

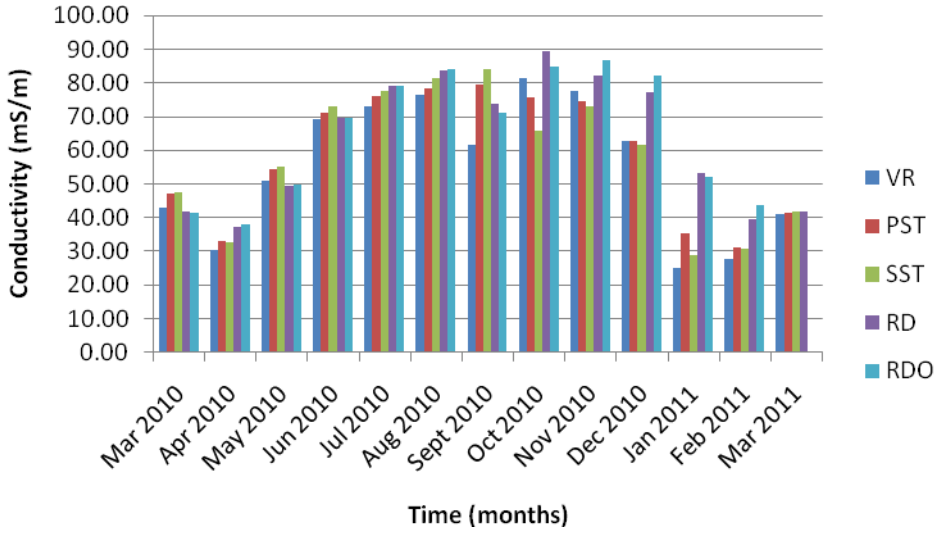


FIGURE 27: Conductivity measurements of the study at sampling sites of the Balkfontein water purification plant. VR = Vaal River, PST = primary settlement tank, SST = secondary settlement tank, RD = recycling dam, RDO = recycling dam outlet.

The Dissolved Inorganic Nitrogen (DIN) for the Allemanskraal Dam increased drastically during May 2010 after which it gradually decreased towards previous levels reaching a low in September before increasing again in October 2010 (Figure 28). High levels (above 4 mg/l) were experienced once more during January and February 2011. DIN concentration ranged between 1 and 6 mg/l with an average of 3 mg/l, higher than the Vaal River with an average of 2 mg/l. Levels were also higher in the water transfer canal and reservoir between June and October 2010 with September being the exception. Peaks can also be seen during January and February 2011 with higher levels in the water transfer canal than in the reservoir. DIN was present in low concentrations and remained constant in the primary settlement tank. Even further decreases were observed during March 2011.

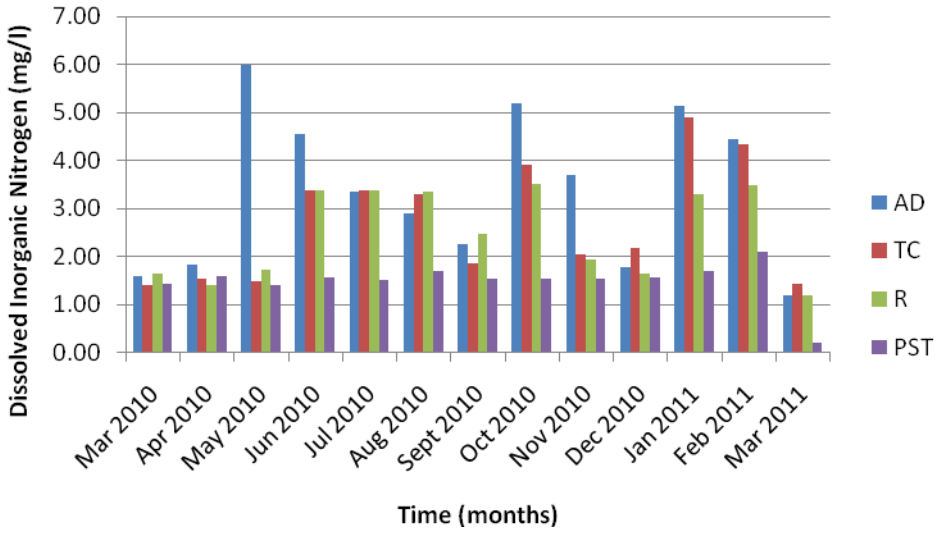


FIGURE 28: Concentration of Dissolved Inorganic Nitrogen (DIN) during the study at sampling sites of the Virginia water purification plant. AD = Allemanskraal Dam, TC = water transfer canal, R = reservoir, PST = primary settlement tank.

In Figure 29 it is shown that the DIN concentration remained fairly constant over the study period (about 1.5 mg/l). Higher levels were observed in the Vaal River and recycling dam sites. DIN concentrations >3mg/l were measured in the Vaal River during August 2010 and in the recycling dam during November 2010. Decreases in the DIN concentration of the Vaal River and primary settlement tank were seen during March 2011.

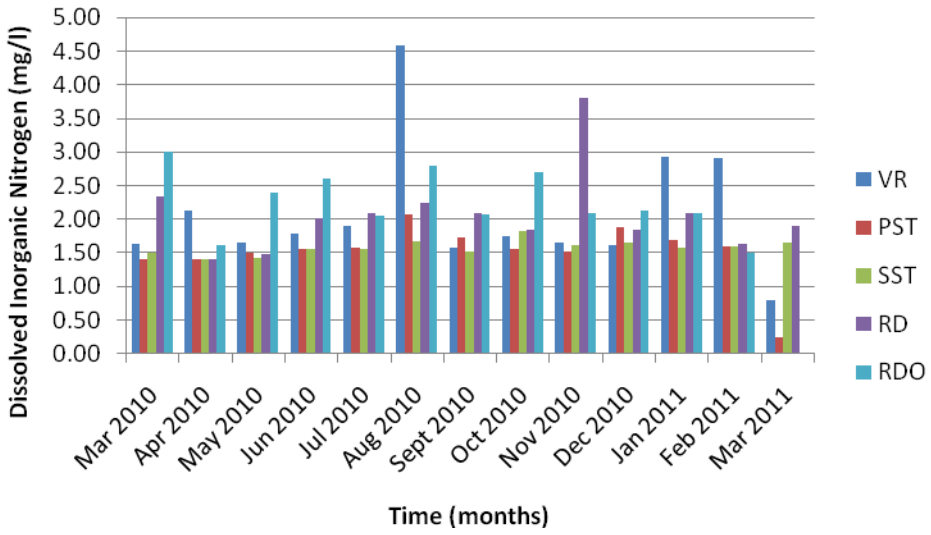


FIGURE 29: Concentration of Dissolved Inorganic Nitrogen (DIN) during the study at sampling sites of the Balkfontein water purification plant. VR = Vaal River, PST = primary settlement tank, SST = secondary settlement tank, RD = recycling dam, RDO = recycling dam outlet.

Figure 30 illustrates that Total Organic Carbon (TOC) remained constant, except for a decrease between June and September 2010. Peaks were observed in the Allemanskraal Dam during May 2010 (13.5 mg/l) and reservoir during October 2010 (12 mg/l). The TOC in the primary settlement tank remained lower than at the other sites but with a slight increase between June and October 2010.

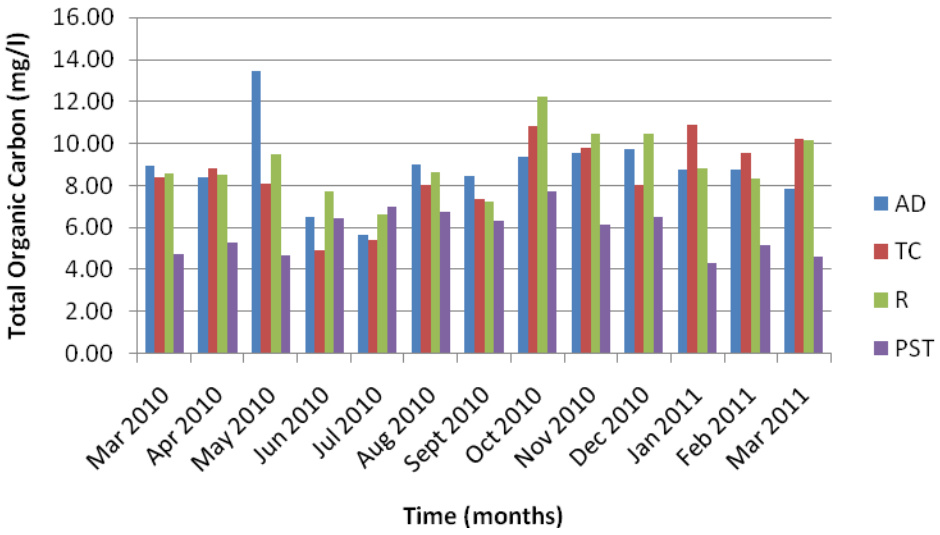


FIGURE 30: Concentration of the Total Organic Carbon (TOC) during the study at sampling sites of the Virginia water purification plant. AD = Allemanskraal Dam, TC = water transfer canal, R = reservoir, PST = primary settlement tank.

On average a decrease in TOC could be seen between June and August 2010 in Figure 31. The TOC of the recycling dam sites were much higher than the average TOC in the Vaal River. The TOC was higher during the warmer months and peaks in the TOC of the primary settlement tank were seen during January and March 2011.

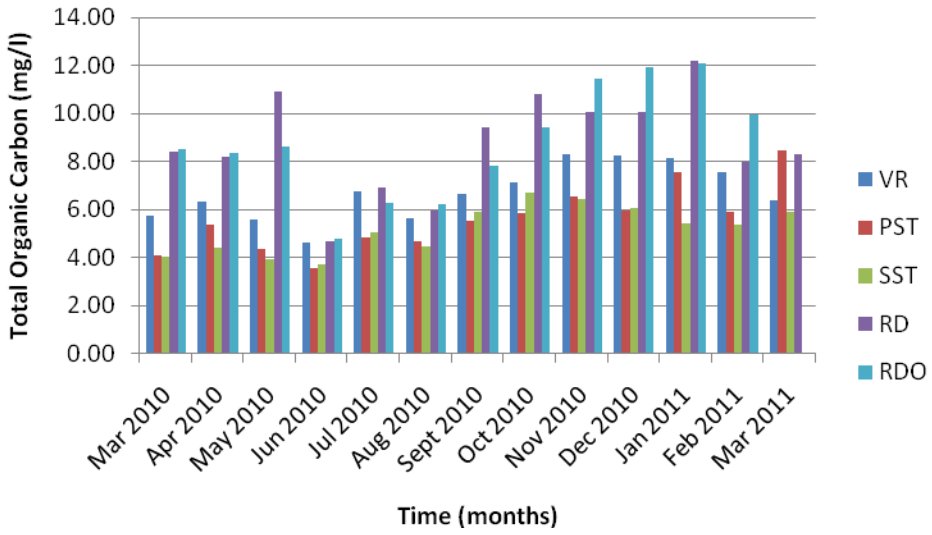


FIGURE 31: Concentration of the Total Organic Carbon (TOC) during the study at sampling sites of the Balkfontein water purification plant. VR = Vaal River, PST = primary settlement tank, SST = secondary settlement tank, RD = recycling dam, RDO = recycling dam outlet.

CHAPTER 4: DISCUSSION

4.1 PHYTOPLANKTON CONCENTRATION AND COMPOSITION

In the following paragraphs the findings of this study will be discussed after a brief review of the algal groups that have been encountered during this study. This will give the reader some background information on the characteristics and common problems of each group. Species known to be associated with problems in water purification will be mentioned, along with how we can use some species as indicators of water quality. Dominant species and associated trends in algal abundance will also be discussed after which comments will be made on phytoplankton concentration over the period at both water purification plants. Furthermore differences in percentage composition of the water purification plants, sampling sites and seasonal periods will be presented and discussed.

Organisms from the Cyanophyceae (cyano- or blue-green bacteria) have photosynthetic prokaryotic cells and are not true algae but rather ancient bacteria. Dominance of this group within the phytoplankton assemblage reflects seasonal physical, chemical and biological changes in the water environment (Van Ginkel, 2012). It may occur in the form of single cells, colonies or filaments that may be branched or unbranched. Organisms are mostly blue-green, olive-green or brown in colour and seldom bright green. The blue-green colour is due to the presence of photosynthetic pigments such as chlorophyll-*a* (green pigment) and phycocyanin (blue pigment). Some may have a red pigment, called phycoerythrin, which, when the other two pigments are present, makes the cells appear purplish. There are no flagellated stages, but some filamentous forms are motile by means of gliding. A characteristic common to Cyanophyceae is the presence of gas vacuoles in the cells that give buoyancy to the organisms. Cells are covered with a thick layered cell wall and are mostly surrounded by mucous (that may be present in varying amounts). Sexual reproduction is absent. Due to their preference for high temperatures, Cyanophyceae is more prolific during the summer months and they often dominate during this period. Other factors, such

as eutrophication and stagnant water bodies in the dry season, contribute to Cyanophyceae blooms (Janse van Vuuren *et al.,* 2006). These organisms are usually associated with eutrophication and reflect the conditions of water quality in the system which matches their optimum requirements, thus making them the ideal water quality indicators (APHA, 1980). During this study high densities of cyanobacteria were seen at different sampling sites and intervals – this is indicative of eutrophication and may pose problems with regard to water purification and health of the aquatic ecosystem.

Bacillariophyceae or diatoms are mostly single-celled organisms but adjacent cells may be attached to each other, thus forming chains. The colour of the chloroplasts varies from yellow to yellow-brown. They contain the photosynthetic pigments chlorophyll *a* and *c* and fucoxanthin with the latter often overshadowing the green of the chlorophyll to cause the yellow-green colour. The storage products are chrysolaminarin and oil droplets. Oil, being less dense than the surrounding water, aids in providing buoyancy to the cells that are very heavy due to their hard and resistant silica cell walls, called frustules. The cell walls are usually ordained with pores and striations. Diatoms are mainly identified by comparing these distinctive wall patterns. The shape of the frustule and the amount, general appearance and shape of the various marks and structures found on and within it, are used to identify and classify the numerous species. Freshwater diatoms consist of two major morphological groups, the centric and pennate diatoms. Pennate diatoms are bilateral symmetrical along the longitudinal axis and centric diatoms radial symmetrical. All centric diatoms are non-motile while most pennate diatoms have a slit-like structure called a raphe on one or both valves. In some species the raphe is situated along the side of the valve. The secretion of polysaccharides through the raphe, when in contact with a substrate, enables these diatoms to perform gliding movement. Flagella are only present on some male gametes and reproduction is mostly asexual by means of cell division. Diatoms are more prolific during the winter months which may allow them to become dominant during this period as they can compete much better than other groups during the colder water temperatures (Janse van

Vuuren *et al.,* 2006). Taylor *et al*. (2007) produced the first method manual in South Africa for the collection, preparation and analysis of diatoms. Research by de la Rey *et al*. (2004) and Taylor *et al*. (2007) led to the development of a diatom index method, based on methods tested internationally. Diatoms are very useful in this regard and can give a good indication of the conditions in the water body, as was also seen during this study.

Chlorophyceae or green algae had the highest species diversity of all the groups found during this study with some species that became quite prolific at different stages of the study period (see Table 1). Representatives of this group may be unicellular, colonial or filamentous (branched or unbranched) but can also be much more complex in structure. The chloroplasts are the most conspicuous features of the cell and are bright green in colour due to the presence of chlorophylls *a* and *b*. Some species may also appear yellow-green or blackish-green when carotenoid pigments or high concentrations of chlorophyll are present. The structure of the chloroplast varies in many cases and can be very useful for taxonomic purposes. The chloroplast normally contains one or more pyrenoids that store starch used as a food reserve. A stigma or red eye spot may be present in flagellated forms. The protoplasm is surrounded by a firm cellulose or glycoprotein cell wall that is situated just to the outside of the cell membrane. Cells are non-motile, swim by means of two to four equal anterior flagella or perform gliding movements. This is a very diverse group that often dominates during the warmer summer conditions (Janse van Vuuren *et al.,* 2006). Some green algal species indicative of eutrophication in water bodies include *Pediastrum* spp., *Oocystis* spp., *Scenedesmus* spp. and *Coelastrum* spp. (Janse van Vuuren, 2001) which have all been abundant during the course of the study in both raw water sources sampled. This is indicative of eutrophication in both river systems.

The Cryptophyceae or cryptomonads are always unicellular and in this study only one species was found (see Table 1). Cells may vary in colour from red, blue-green, olive-green to olive-brown due to the presence of chlorophyll *a* and *c*,

alpha-carotene and phycobilin pigments. Unlike Cyanophyceae the phycobilin pigments are not located within phycobilisomes but are located on the inside of the lumen of the thylacoids of chloroplasts. There are mostly two chloroplasts per cell. The cells are asymmetrical, dorsiventrally flattened and mostly heart- or leaf-shaped. Cells are motile by means of two, slightly unequal, flagella. At the anterior end of the cell there is an opening, called the gullet, which is lined with ejectisomes. Underneath the cell membrane a proteinaceous pellicle, in the form of plates, is present. Genera in this group are easy to recognise though identifying the different species may prove to be difficult (Janse van Vuuren *et al.,* 2006).

The Chrysophyceae or golden algae are mostly single cells or colonies (filaments may also occur) that are yellow or golden-brown in colour due to the presence of fucoxanthin. Cells are motile by means of two unequal flagella (Janse van Vuuren *et al.,* 2006). In this study only one chrysophyte species was found (see Table 1) in very low concentrations compared to the other groups. The species, although a colonial form, was seen as individual cells in most cases. The presence and relative abundance of chrysophytes are indicative of clean, oligotrophic water (Palmer, 1980).

The Dinophyceae or dinoflagellates are unicellular organisms that vary greatly in morphology and size and are usually brown to yellow-brown in colour due to the presence of pigments such as peridinin. Dinoflagellates are important members of the phytoplankton in marine and freshwater ecosystems though most species are confined to marine environments (Janse van Vuuren *et al.,* 2006). The name “dinoflagellate” refers to the forward whirling and spiral swimming motion of species in this group. There are two flagella implanted in grooves on the cell surface. One flagellum is pointed backwards and the other stretches around the center of the cell. Cells are often covered with armour-like cellulose plates located on the inside of the cell membrane. The presence of these thecal plates separates the dinoflagellates from other algal groups and the arrangement of the plates is used to distinguish between different genera and species within the

group. The cell covering of unarmoured species is comprised of a membrane complex. The most conspicuous species observed during this study was *Ceratium hirundinella*, described by Whittington *et al*. (2000) as a ubiquitous, relatively large (thus having an easily observable impact on biomass) and slow growing species that can usually be found during late summer (in this study late summer through autumn).

The Euglenophyceae or euglenophytes/euglenids are unicellular and mostly bright green in colour, sometimes with bright red eyespots. Chloroplasts contain chlorophyll *a* and *b* and carotenoids (concentrated when forming a red eyespot). The storage product is in the form of paramylon stored in paramylon granules. One or more contractile vacuoles are present in freshwater species. Proteinaceous pellicle strips are located on the inside of the cell membrane and enable some members in the group to perform metabolic or euglenoid movement. Motile cells usually have two flagella inserted in a pith or groove at the anterior end of the cell but only one flagellum is visible as the other is short and non-emergent. Most species live in freshwater ecosystems where they are found in hard or soft water habitats of varied pH and light levels. Populations will thrive under high nutrient conditions and can be used as bio-indicators of organic pollution for this reason (Janse van Vuuren *et al.,* 2006). During this study species with and without loricas as cell coverings were found. Some loricas were stained dark brown which is indicative of iron and/or manganese in the water system (Nudelman *et al*., 1998).

The presence of certain species in the water can be a good indication of the quality of a water body. The presence and relative abundance of cryptophytes and chrysophytes are indicative of clean, unpolluted water (Palmer, 1980). This can be seen with the high abundance of *Dinobryon sertularia* found in oligotrophic water bodies such as the Klerkskraal Dam (Van Ginkel *et al.,* 2001a) and low abundance of the same species during this study. Representatives of both these groups were found, but in low concentrations which is more indicative of pollution. Kristiansen (1986) opposed this view by pointing out that

chrysophytes (particularly scaled species) are not good oligotrophic indicators as they may reach high numbers in eutrophic ecosystems. Palmer (1980) stated that the absence of cyanobacteria was indicative of clean, unpolluted water. During this study Cyanophyceae was abundant, especially during certain periods, which supports the fact that the raw water is nutrient enriched and eutrophied.

An algal bloom can be defined as a massive or conspicuous growth of algae which visibly discolours the water. It is typically planktonic and often forms surface scums while a large percentage of the total cells are one of a few species (Janse van Vuuren *et al*., 2006). Allen (1972) stated that a bloom is defined by approximately 10^6 cells/ml. Pieterse (1974) referred to blooms as algal concentrations that were higher than the average annual algal concentration which is responsible for discolouration of the water. According to Palmer (1980) there are various algal species capable of producing blooms and genera frequently involved in bloom formation are *Microcystis, Anabaena* and *Oscillatoria* (Cyanophyceae), *Chlorella, Ankistrodesmus* and *Chlamydomonas* (Chlorophyceae), *Cyclotella* (Bacillariophyceae) and *Euglena* (Euglenophyceae). All of the above mentioned genera were present during the study period (Table 1), some which formed blooms under environmental conditions favouring their growth. Blooms cause numerous problems that range from aesthetically unacceptable conditions to the poisoning of animals by cyanobacteria, such as *Microcystis aeruginosa*. Algal species present during this study responsible for taste and odour problems include *M. aeruginosa, Anabaena* sp., *Oscillatoria* sp., *Pandorina morum* and *Ceratium hirundinella* (Table 1). Algal species present that may be responsible for clogging sand filters in water purification plants include *Aulacoseira granulata, Cyclotella meneghiniana, Ulnaria ulna, Navicula* sp., *Chlamydomonas* spp. and *C. hirundinella*. In addition *C. hirundinella* and other flagellated species are also responsible for the destabilisation of flocs during the flocculation process in water purification (Van der Walt, 2011).

Diatoms (Bacillariophyceae) and coccoid green algae (Chlorophyceae) tend to be the most common algae in rivers, and of the diatoms small centric species of the genus *Cyclotella* are undoubtedly the most common (Round, 1985). Dominance of diatoms was also found in the Vaal River and diatoms and green algae were the best represented algal groups for the period 1985 to 1986 (Pieterse & Roos, 1987) and 1991 to 1993 (Janse van Vuuren, 1996). Similar trends were also observed during this study in the Vaal River.

Janse van Vuuren (1996) and Ewerts (2010) observed that Cyanophyceae usually reached high concentrations during the summer and late summer months due to high water temperatures that favour their growth. *Microcystis aeruginosa* (Cyanophyceae) was repeatedly dominant at 8 of the 10 sampling sites (Table 2). It was dominant for an extensive period in the Allemanskraal Dam from the summer periods to well into the month of May. Temperature, however, is not the only variable influencing succession of algal species. As mentioned earlier, eutrophication and stagnant water bodies in the dry season contribute to Cyanophyceae blooms. This strongly suggests observable enrichment of the source water during this study (especially during the period when the bloom occurred) and gives a good indication of the effect this had on the system as a whole and how it might influence water purification processes. Bloom conditions may pose threats and if current purification methods prove to be inefficient, a different source of water may be required for purification to be more cost effective. The occurrence of Cyanophyceae is generally associated with high loadings of nitrogen and phosphorus that favour algae that do not require silica (Goldman & Horne, 1983). The same might be said for *Anabaena* that also gained dominance for brief periods in 4 other sites where it succeeded *Microcystis aeruginosa* as the dominant. It has been found that *Anabaena* sometimes occurs under nitrogen deficient conditions as the genus can fix their own atmospheric nitrogen as it contains heterocysts (Janse van Vuuren *et al*., 2006).

The primary settlement tank in the Virginia water purification plant was mostly dominated by Bacillariophyceae, especially *Aulacoseira granulata* (April to May 2010) and *Nitzschia palea* (March 2010, July 2010 to March 2011). Visser (1996) noticed that sedimentation was more efficient in removing centric diatoms and thus also found pennate and filamentous diatoms to be more prolific than centric diatoms after sedimentation. This was also observed during the current study where a relatively higher abundance of prominent diatom species after settlement were found. According to Lund (1962) and Rosén (1981) *Aulacoseira granulata* prefers eutrophic conditions and is rare or absent in oligotrophic conditions. The occurrence of *A. granulata* is comparable to that of the blue-green bacteria which is (as previously mentioned) a very good indicator of eutrophication (Akbay *et al.*, 1999). In this study *Nitzschia palea* was the second most abundant species in the Allemanskraal Dam after the dominant Cyanophyceae and was present in very high amounts during the *Microcystis aeruginosa* bloom in late summer and autumn. This implies that this species is also indicative of eutrophication as it flourished under similar conditions while successfully competing with *M. aeruginosa*. Diatom species that are normally indicative of eutrophication and poor water quality dominated in the primary settlement tank of the Virginia water purification plant. Their presence may imply conditions of poor water quality (Taylor *et al.,* 2007) after settlement that needs to be addressed, although this still needs to be verified with similar data on chemical and environmental variables. The fact that the algal concentration remained low after primary settlement (Figure 14), regardless of high algal concentrations in the source water, is indicative of successful purification that effectively remove problematic algae.

The unicellular centric diatom, *Cyclotella meneghiniana* is widely distributed in fresh and brackish waters, although evidence suggests that it may be strongly halophilic, sometimes needing elevated total dissolved solid levels to successfully complete its life cycle. High concentrations of this diatom coincide with high TDS (total dissolved solids) levels of the Vaal River system (compare with high turbidity measurements for the Vaal River in Figure 23).

C. meneghiniana was abundant in the Vaal River at Balkfontein and gained dominance during May 2010 and the summer of 2011. Visser (1996) also found that diatoms were dominant in the Vaal River after which it was gradually removed by the different purification processes. This trend was also observed during settlement in this study which supports the view of a somewhat lesser impact that diatoms might have during purification, aside from blocking sand filters. Dominant diatom species found during the period 1994 to 1996 by Visser (1996) were *Melosira granulata* (since reclassified as *Aulacoseira granulata*), which is indicative of eutrophied water and unidentified pennate and centric diatom species. It is suspected that the unidentified centric diatom might have been a *Cyclotella* species when compared to this study.

Representatives of the Chlorophyceae were abundant during the entire period with species like *Actinastrum* sp. and *Pandorina morum* dominating in winter and early spring, while *Pediastrum duplex* and *Scenedesmus quadricauda* dominated from late winter to mid-summer. *Chlamydomonas* sp. was periodically dominant during the study period. Janse van Vuuren (1996, 2001) also observed that the Chlorophyceae dominated throughout the year, as they can tolerate wider temperature ranges. This was also reflected in the study done by Visser (1996). As mentioned before, species of *Pediastrum* (including *P. boryanum, P. duplex, P. simplex* and *P. tetras* seen during this study) and species of *Scenedesmus* (including *S. acuminatus, S. disciformis, S. lefevrii* and *S. quadricauda*) are indicative of eutrophication (Janse van Vuuren, 2001). When present in large numbers, *Pandorina morum* may cause a fishy odour (Janse van Vuuren, 2001), which is not aesthetically acceptable. The results of this study emphasise that both river systems can be classified as eutrophic based on their algal composition and thus the purification and management of the water purification plants need to be adjusted accordingly with regards to the quality of the final water and cost effectiveness in doing so. Compounds released from algal cells during water purification that may affect the taste, odour and other aspects of the final water need to be considered and ways to effectively remove these

compounds need to be developed or implemented if the need arises. Removing the algal cells before they release these compounds remains more preferable.

The volume of water used for the purpose of algal counts during the quantification of phytoplankton assemblages will only represent a small part of the total volume of water in the river. Because of this, it is possible that certain algal cells were not detected in the water samples even though they may be present in the river system (Janse van Vuuren, 1996).

Extremely high algal concentrations were seen in the Allemanskraal Dam (Figure 1) for the entire study period due to blooms of cyanobacteria, especially *Microcystis aeruginosa* and *Anabaena* sp.. The concentration remained high from February to June 2010 (Figure 3) and approximately 100 million cells/ml were recorded during May 2010. This could not be attributed to high temperatures because of a change in seasons to winter. After June the algal abundance decreased but the average concentration remained high. The fact that cyanobacteria remained high during May 2010, can be attributed to the large inoculum that was carried over from the previous summer. After winter (when concentrations decrease dramatically) it takes longer to reach bloom-forming proportions in spring (even if the temperature is exactly the same as in autumn) because of a lower inoculum after winter. These high algal concentrations are comparable to that of Missisquoi Bay of Lake Champlain in southern Quebec (Canada) which is prone to cyanobacterial blooms because of its high nutrient loadings (Simomeau, 2007). This emphasises the effect that nutrients may have on a water system and is also indicative of high nutrient loadings in the Sand River system (compare with peaks in nutrient levels experienced in Figure 28).

The algal concentration in the Vaal River at Balkfontein was much lower (Figure 2) than in the Allemanskraal Dam. Maximum cell numbers were recorded during September 2010 (approximately 42 500 cells/ml). The high abundance in early spring was the result of increases in the concentration of *Micractinium pusillum* and *Scenedesmus quadricauda*. A similar phytoplankton

composition was seen in the Darling River (Australia) which primarily consisted of Chlorophyceae and Bacillariophyceae with irregular developments of Cyanophyceae (Hötzel & Croome, 1994). Descy (1987) observed that this type of assemblage frequently occurs in temperate, nutrient-rich rivers where hydrological conditions allow full development of true riverine plankton. Maximal densities of cells in the water column are high in these types of rivers, often exceeding 10 000 cells/ml, and can reach 100 000 cells/ml during blooms (Descy, 1987). The description of the algal assemblage of the Vaal River system is reflected in Figure 4 where the algal assemblage consists mostly of Chlorophyceae in 2010 giving way to Bacillariophyceae in 2011 although the Chlorophyceae remained important. These findings correspond well with the algal assemblage found in the Darling River as the Vaal River is situated within a similar climatic region.

The Cyanophyceae dominated in the Allemanskraal Dam (Figures 3 and 5A) while the water in the water transfer canal, between the dam and the reservoir, had an increased concentration of Bacillariophyceae and Chlorophyceae relative to the (still dominant) Cyanophyceae (Fig. 5B). This might be attributed to the fact that the withdrawal point for the transfer canal is below the surface of the water which ensures that the amount of Cyanophyceae that enters the transfer canal are less. This is due to the fact that most Cyanophyceae contain gas vacuoles that enable them to float and form surface scums. If withdrawal is done from a depth below the surface many of the cyanobacteria that float in the surface scum would be eliminated. Increased mixing in the transfer canal might also have discouraged the growth of Cyanophyceae as a surface scum could not be formed. In the reservoir (Fig. 5C), the Chlorophyceae became relatively more important relative to the dominant Cyanophyceae. This was the result of a decrease in Cyanophyceae concentration rather than an increase in Chlorophyceae concentration. During primary settlement (Fig. 5D), a dramatic change was seen in the percentage composition of the phytoplankton where the Cyanophyceae was reduced to only 15% (showing the efficiency of the settling process). The assemblage was mostly dominated by Bacillariophyceae, which

comprised 65% of the total phytoplankton, although the Chlorophyceae was still important at 19%. Not only did the percentage composition of the Cyanophyceae drastically decrease with regard to the rest of the algal assemblage, the total algal concentrations also decreased to very low levels (an average of 100 cells/ml) when compared to the source water samples (an average of 100 000 cells/ml). This indicates that the settlement processes employed at Virginia water purification plant are effective in removing phytoplankton, especially Cyanophyceae which caused the most concern due to the abundance and blooms seen in the source water. Visser (1996) observed that colonial algae (for example *Microcystis*) were removed more easily during sedimentation than unicellular algae and are present in very small amounts. This corresponds well with findings during primary settlement in Virginia. During bloom conditions in the Allemanskraal Dam the levels of Cyanophyceae in the primary settlement tank was very low which proves that there is no need to alter purification processes or to periodically cease water transport from the Allemanskraal Dam to the Virginia water purification plant.

The Chlorophyceae dominated in the Vaal River source to Balkfontein water purification plant and comprised 80% of the total phytoplankton with the Bacillariophyceae at 13% and Cyanophyceae at 5% as seen in Figure 6. Although the total Chlorophyceae concentration was lower after settlement, the percentage composition relative to the Bacillariophyceae and Cyanophyceae was higher than in the Vaal River. It then remained constant from primary through to secondary settlement with minor alterations. Relative abundance of the Cryptophyceae changed little from the Vaal River to primary settlement, but decreased after secondary settlement. Visser (1996) commented that algal cells with flagella (for example *Chlamydomonas*) are motile which makes it much more difficult to remove during sedimentation due to the movement of the flagella that prevents floc formation or break up already formed flocs. This is reflected in the relatively higher abundance of Chlorophyceae (such as *Chlamydomonas*), in relation to non-flagellated groups, after settlement. The same also applies to the flagellated Cryptophyceae and Dinophyceae cells. Chlorophyceae cells are more

buoyant than Bacillariophyceae cells with heavy frustules, making it more difficult to remove during settlement (Visser, 1996), which is why small, unicellular green algae often dominate in the final water. These results and results obtained from Visser (1996) indicate that Chlorophyceae are in general less effectively removed by the unit processes employed at Balkfontein water purification plant. The Chlorophyceae are less important with regard to the problems they cause in the final water although high densities can be aesthetically unacceptable. To improve floc formation for effectively removing Chlorophyceae, pre-ozonation might be suggested before adding flocculants.

The Cyanophyceae completely dominated in the Allemanskraal Dam during the first two seasonal periods and thus no clear difference was observed between late summer and winter (Figures 7A and B). During the following spring to summer period (Figure 7C), the Allemanskraal Dam was still dominated by Cyanophyceae although the Chlorophyceae gained more importance relative to the Cyanophyceae. The extremely high algal abundance during the first two seasonal periods was due to bloom conditions probably caused by elevated nutrient conditions during that period (Figure 28). The amount of Cyanophyceae present provides a good indication of eutrophication.

The Chlorophyceae dominated through all three seasonal periods in the Vaal River. During the late summer to autumn period (Fig. 8A) the Chlorophyceae composed 76% of the algal assemblage, after which their percentage composition increased to 91% during the winter period (Fig. 8B) as a result of the simultaneous decrease in the percentage composition of the Bacillariophyceae to 9%. During the following spring to summer period (Fig. 8C) the Bacillariophyceae became relatively more important (to the same level as the previous warm water period) and the Cyanophyceae became relatively more important when compared to both previous periods. The traditional seasonal paradigm of periodicity (e.g. Fogg, 1975; Reynolds, 1984) states that classically the onset of spring is accompanied by increased development of diatoms (Bacillariophyceae). Diatoms are best suited for survival during the cold winter months which imparts

selective advantage in terms of cell numbers present at the onset of spring (Janse van Vuuren, 1996). This was not the case during this study where diatoms were more prevalent during the warmer periods. Diatom development is usually terminated by depletion of silicon (Müller, 1984).

The highest abundance of Cyanophyceae in the Balkfontein water purification plant was found in the recycling dam and recycling dam outlet (Figure 9). The Dinophyceae (mostly *Ceratium hirundinella*) was also the most proliferate at these two sites. In the recycling dam outlet Dinophyceae was the second most abundant group from February to May 2010 after the Cyanophyceae. Concentrations of *C. hirundinella* seemed low because it were expressed in terms of cells/ml, but if the cell biomass (expressed as cell volume) would have been measured much higher abundances would have been recorded. Concentrations expressed in terms of cells per ml have serious limitations especially when considering cell size and volume. Biomass determinations will give more accurate results. It can thus be reasoned that it is inaccurate to compare one small cell of *Microcystis aeruginosa* with one very large cell of *Ceratium hirundinella* during a cell count, which equalises their value although it is not the case when the difference in biomass is taken into account. *C. hirundinella* seems to be a new invader species in the Vaal River system as neither Pieterse and Janse van Vuuren (1997) or Janse van Vuuren (2001) listed it in their species list of algae present in the Vaal River system from 1991 to 1997. Visser (1996) also did not find it in the Vaal River between 1994 and 1996. Kruskopf (2002) indicated that *C. hirundinella* was detected in the Vaal River during 1999 and 2000, specifically at the Loch Vaal. Although *C. hirundinella* has been found periodically dominant in South African impoundments of different trophic status, like the Klipvoor and Boskop Dams (Van Ginkel, 1999), it has not been previously associated with extreme bloom conditions (Van Ginkel, 2001b). A lake-wide bloom of *C. hirundinella* in Albert Falls Dam, KwaZulu-Natal, during October 2006 exposed a significant ecological change indicative of reduced water quality in a reservoir that was historically viewed as mesotrophic (Hart & Wragg, 2009). A comparison between Kruskopf (2002), Carrim (2006) and

Morrison (2009) indicates that *C. hirundinella* was found in the Vaal River from 1999 onwards, while Morrison (2009) detected high concentrations of *C. hirundinella* during 2007 and 2008. Although there was very little *C. hirundinella* seen in the Vaal River during this study, the sludge water from the years when they were abundant in the Vaal River moved through the recycling dams, probably introducing the species. It was found that conditions at certain times of the year were beneficial for the increase of *C. hirundinella* in the recycling dam sites, probably as a result of the germination of cysts introduced during previous years. This may have a negative impact since water from the recycling dams is recycled and purified along with the raw water from the Vaal River and the presence of *C. hirundinella* may lead to taste and odour problems in the final water. The very high abundance of Cyanophyceae in the recycling dam and recycling dam outlet may be the result of high nutrient conditions provided by the sludge particles to which nutrients often adhere as well as possible higher temperatures due to a corresponding increase in turbidity (see section 4.2). The higher temperature and shallowness of these two sites, especially the recycling dam outlet, may lead to an increased rate of evaporation leaving nutrients behind to accumulate. Recycling dams are also typical nutrient traps as water containing less sludge leaves the dams by means of an overflow.

In the recycling dam at Virginia (Fig. 9C) the Cyanophyceae was also dominant, but with a much lower relative abundance than at Balkfontein. The Chlorophyceae and Bacillariophyceae were relatively more abundant than at Balkfontein at 27% and 18% respectively. Low concentrations of the Dinophyceae were noticed at Virginia. High abundance of Cyanophyceae at the recycling dam sites is important since the water is recycled and purified along with the raw water. Algal concentrations in the recycling dam escalated to as high as 3 383 cells/ml in March 2010 but unfortunately no data was available after May 2010 as a result of sludge preventing viable algal samples from being attained.

Relative algal abundance in the recycling dam and recycling dam outlet of Balkfontein water purification plant for the complete period (Figure 10) indicates that the Cyanophyceae remained the group with the highest relative abundance (although slightly lower in the recycling dam outlet). The Chlorophyceae was the second most abundant group with a slightly higher relative abundance in the recycling dam outlet. Algal concentrations in the recycling dam reached a peak during April 2010 with 1 944 157 cells/ml and in the recycling dam outlet during January 2011 with 720 100 cells/ml. Algal concentrations were much higher in the recycling dam sites at Balkfontein than in the recycling dam site at Virginia but further data comparisons could not be made due to the lack of data from the recycling dam at Virginia. Many of the samples were saturated with sludge which made algal identification and enumeration difficult.

4.2 PHYTOPLANKTON AND ENVIRONMENTAL VARIABLES

The concentration of *Anabaena* sp. and *Microcystis aeruginosa* increased in the Allemanskraal Dam between March and June 2010, reaching a peak during May 2010 and there was also a marked increase in *Chlamydomonas* sp. (Chlorophyceae) during the same period reaching a peak during April (Figure 11). This correlates with high water temperatures for the period (Figure 20) and was expected since Cyanophyceae abundance increases with an increase in temperature. It is known that the growth of *Microcystis* spp. can become inhibited at temperatures below 15°C, causing cell losses through death and sedimentation (Zohary & Robarts, 1989). Turbidity (Figure 22) was also above average for the same period which might to explain higher than average temperatures when compared to the other sites. The Dissolved Inorganic Nitrogen (DIN) concentration was very high during May 2010 (Figure 28) and since nitrogen is an important nutrient, it might help to explain the peak in algal abundance during that month. Total Organic Carbon (TOC) also increased during May. The pH of April and May was lower than usual. More data is needed to determine why such a bloom commenced at the start of the study and reached such high levels in autumn when temperatures were low. Data on total

phosphorus for the period would have been very helpful in formulating an explanation.

High concentrations of *Anabaena* sp. and *Microcystis aeruginosa* were present between February and May 2010 in the water transfer canal (Figure 12) and it reached a maximum during April. The peak in the canal was observed a month before a similar peak during May in the Allemanskraal Dam. The same tendency can be seen with an increase in *Anabaena* sp. in January 2011, a month before a similar increase in the Allemanskraal Dam during February 2011 (Figure 11 and 12). The reason for this might be that dormant stages or cysts of the dominant species entered the water transfer canal where conditions for a bloom might have been better than in the Allemanskraal Dam. There was a higher occurence of *Chlamydomonas* sp. in March and *Anabaena* sp. reached another peak in January 2011. The high concentrations of Cyanophyceae did not correlate with higher concentrations of DIN during the same period so it might be ascribed to cells entering the transfer canal from the Allemanskraal Dam.

Microcystis aeruginosa succeeded *Anabaena* sp. and the concentration also reached a peak during April 2010 in the reservoir (Figure 13) which compares well with what was seen in the Allemanskraal Dam and especially the water transfer canal that had a similar peak in April. Concentrations of *Microcystis aeruginosa* decreased rapidly from approximately 150 million cells/ml in the Allemanskraal Dam to approximately 8 300 cells/ml in the water transfer canal during autumn 2010. This might be attributed to a high mixing rate. The concentration of *M. aeruginosa* in the reservoir during the same period was 35 000 cells/ml, probably as a result of the reservoir being a stagnant water body. The high concentration of Cyanophyceae in the reservoir was not a result of changes in key environmental variables like temperature and plant nutrients, but probably the result of the bloom in the Allemanskraal during the same period and dormant stages that was transported to the reservoir by means of the water transfer canal. It can be noted that the peak of the bloom (Figure 13) was also a month ahead of the peak of *M. aeruginosa* that occurred in the Allemanskraal Dam (Figure 11). *Pandorina morum* completely dominated during July 2010

which was the result of an increase in the DIN of the reservoir during the same period. *Chlamydomonas* dominated during November 2010 after an increase in DIN during October 2010. *Chlamydomonas* numbers also increased after February 2011 when it dominated again as a result of an increase in DIN during the same month. Increases in algal abundance occurred after increases in the necessary nutrients. Overall algal abundance was high in the late summer of 2010 which can be attributed to the high algal abundance in the Allemanskraal Dam for that period although other species were outcompeted by *Microcystis aeruginosa*.

In the primary settlement tank *Microcystis aeruginosa* dominated during March 2010 and February 2011 (Figure 14), probably as a result of the bloom that occurred in the Allemanskraal Dam. The concentrations were extremely low (approximately 100 cells/ml) when compared to the concentrations in the source water (approximately 150 milion cells/ml during May 2010) which is a very good indication of how effective the Virginia water purfication plant is in the removal of this problematic group. *Nitzschia palea* increased during the colder months, reaching a concentration of about 200 cells/ml during October 2010. *Aulacoseira granulata* dominated in April 2010 and showed a peak during August and December 2010. Both of these diatom species are indicative of an eutrophic system but their concentration in the primary settlement tank was low, again underlining effective algal removal procedures.

Actinastrum hantzschii dominated during the winter months peaking during July 2010 in the Vaal River (Figure 15). According to Palmer (1980), this species is particularly common in eutrophic freshwater ponds, lakes and slow-flowing rivers and in this study it seemed to have a preference for colder climatic conditions. It was succeeded as the dominant by *Scenedesmus quadricauda* which peaked during September 2010 during a corresponding peak in the turbidity in the Vaal River (approximately 80 NTU's). A second, smaller peak in the concentration of *Actinastrum hantzschii* was observed during the same period. After that *Scenedesmus quadricauda* gradually decreased towards January 2011 even though turbidity increased again after December 2010. Increases in

S. quadricauda and increases in turbidity were, therefore, not related. *S. quadricauda* occurs in all climates but prefers eutrophic to hypertrophic waters with slight acidity and low salinity (John *et al*., 2002). This was also not the case in this study as the Vaal River is alkaline. No salinity comparisons could be made due to a lack of salinity data during this study. *Cyclotella meneghiniana* was dominant in May and October 2010 and through the rest of the year it maintained a constant presence due to the fact that it is such a cosmopolitan species also present in other similar river systems. *Microcystis aeruginosa* was dominant in December 2010 as a result of the higher summer temperatures and sustainable nutrient concentrations that were experienced.

Actinastrum hantzschii was dominant in the primary settlement tank at Balkfontein, reaching a peak of approximately 7 000 cells/ml in July (Figure 16). This corresponds with the peak during the same period in the Vaal River of approximately 23 000 cells/ml showing that it was introduced via the source water. Settlement in the primary settlement tank was successful in removing two thirds of its concentration. There are no known water purification and human health problems associated with this species. It was succeeded by *Micractinium pusillum* in September 2010 (3 000 cells/ml) during which time a peak in the concentration of *Scenedesmus quadricauda* was also experienced.

In the secondary settlement tank algal abundance again increased during the winter period with *Actinastrum hantzschii* being dominant from May to September 2010 with a peak of approximately 3 100 cells/ml in July (Figure 17). The concentration was similarly high for June and August although concentrations decreased after secondary settlement for the period. When compared to concentrations of algal cells after primary settlement at Virginia this is relatively high and thus a revision of flocculation and sedimentation methods, especially with regard to green algae, should be considered at Balkfontein. In the study done by Visser (1996), it was found that different algal species or morphological groups required different treatment conditions to be successfully removed during water purification and the same can be said for the results of this study. Algal

abundance of the other major species showed an increase between July and October with *Micractinium pusillum* peaking in August and *Scenedesmus quadricauda* peaking in September, succeeding *Actinastrum hantzschii* as the dominant.

In the recycling dam at Balkfontein, cyanobacterial dominance at the beginning of the study period gave way to green algal dominance at the end of the period (Figure 18). High DIN concentrations throughout the study correlate with the corresponding high cyanobacterial abundance in the recycling dam as it is a vital nutrient that often results in blooms when present in high concentrations. TOC concentrations were also very high throughout the period. *Anabaena* sp. was succeeded by *Microcystis aeruginosa* during autumn 2010. A peak in *Chlamydomonas* sp. concentration was observed during October during a peak in DIN concentration (approximately 3.75 mg/l). It was succeeded again by *Microcystis aeruginosa* in January which was probably due to the high water temperatures during the summer of December 2010 (Figure 21) and *Pediastrum duplex* in March 2011 which can easily form blooms under these nutrient rich conditions. An increase in *Ceratium hirundinella* concentration was noticed in May, where the species briefly succeeded *Microcystis aeruginosa*. Additional biomass determinations would have provided a clearer perspective on the impact of the *Ceratium hirundinella* increase during autumn 2010 and this should be amended with future studies that should include biomass measurements.

The species composition in the recycling dam outlet was similar to that of the recycling dam but algal concentrations were lower (compare peaks in *Microcystis aeruginosa* concentrations in Figures 18 and 19). This is preferable as fewer cyanobacteria and *Ceratium hirundinella* cells are returned to the purification cycle. An increase in the amount of *Pediastrum duplex* cells were detected after November 2010 which might be the result of a high DIN concentration during that month in the recycling dam causing corresponding increases of *P. duplex*.

CHAPTER 5: STATISTICAL ANALYSES OF DATA – PHYTOPLANKTON AND ENVIRONMENTAL VARIABLES

5.1 MULTIVARIATE ANALYSES

Multivariate analyses allow simultaneous analyses of environmental data and biological (species/abundance/sites) data (Harper *et al*., 2000). Canonical ordination is a combination of ordination and multiple regression and has the advantage of combining the simplicity of regression models with the power of ordination techniques (Ter Braak, 1988; Varis, 1989). The use of canonical ordination greatly improves the power to detect the specific effects or factors one is interested in. Ordination techniques, such as Principal Components Analyses (PCA), are commonly used to reduce the variation in community composition to the scatter of samples and species in an ordination diagram. Canonical correlation analyses finds the maximum between-set correlation between linear combinations of variables, extracting the linear combination which is most dependent on the values of the other data-set (Varis *et al*., 1989). Canonical correlation analyses appear to be a useful approach in phytoplankton community analyses when compared with several other ordination techniques (Varis *et al*., 1989).

Data analyses by canonical ordination can be exploratory or confirmatory. When used in an exploratory way, it leads to an ordination diagram of samples, species and environmental variables, which optimally displays how community composition varies with environmental conditions. When used in a confirmatory capacity, it leads to statistical tests of the effects of particular environmental variables on community composition while taking into account the effect of all other variables in the data-set (Ter Braak & Prentice, 1988).

Two data-sets, one on phytoplankton and the other one on environmental variables, were used in this study. Data were analysed using Principal Components Analyses (PCA) and Canonical Correspondence Analysis (CCA).

The PCA was used to summarise patterns of correlations among observed variables and to reduce the large number of observed variables. The CCA examined the relationships between species distributions and the distribution of associated environmental variables and gradients. Before commencing the multivariate analyses, excessively rare algal species (low abundance) were removed from the original data-set because including these species would have weakened correlations and confounded the total analyses (Austin & Greig-Smith, 1968; Allen & Koonce, 1973). The most important variables were used for further analyses based on their dominance, abundance and ecological importance.

In Figure 32 a positive correlation was found between *Microcystis aeruginosa* and environmental variables such as dissolved inorganic nitrogen (DIN) and turbidity (NTU). The high density of *M. aeruginosa* can, therefore, be attributed to plant nutrients such as DIN and this leads to increased (organic) turbidity in the system. *M. aeruginosa* was very prolific in the Allemanskraal Dam and this is also indicated by means of the correlation between *M. aeruginosa* and the Allemanskraal Dam samples. *Nitzschia palea* and *Aulacoseira granulata* showed a positive correlation with temperature. The green algae, on the other hand, showed a negative correlation with temperature, supporting previous findings that stated a preference to colder water conditions for species like *Actinastrum hantzschii* and *Pandorina morum.* In Figure 33 the trends are similar and *Microcystis aeruginosa* is more closely grouped together with the raw water samples, especially the Allemanskraal Dam, when compared to the samples taken in the settlement tanks. This is indicative of effective removal of this problematic species and proves that the current sedimentation processes are effective.

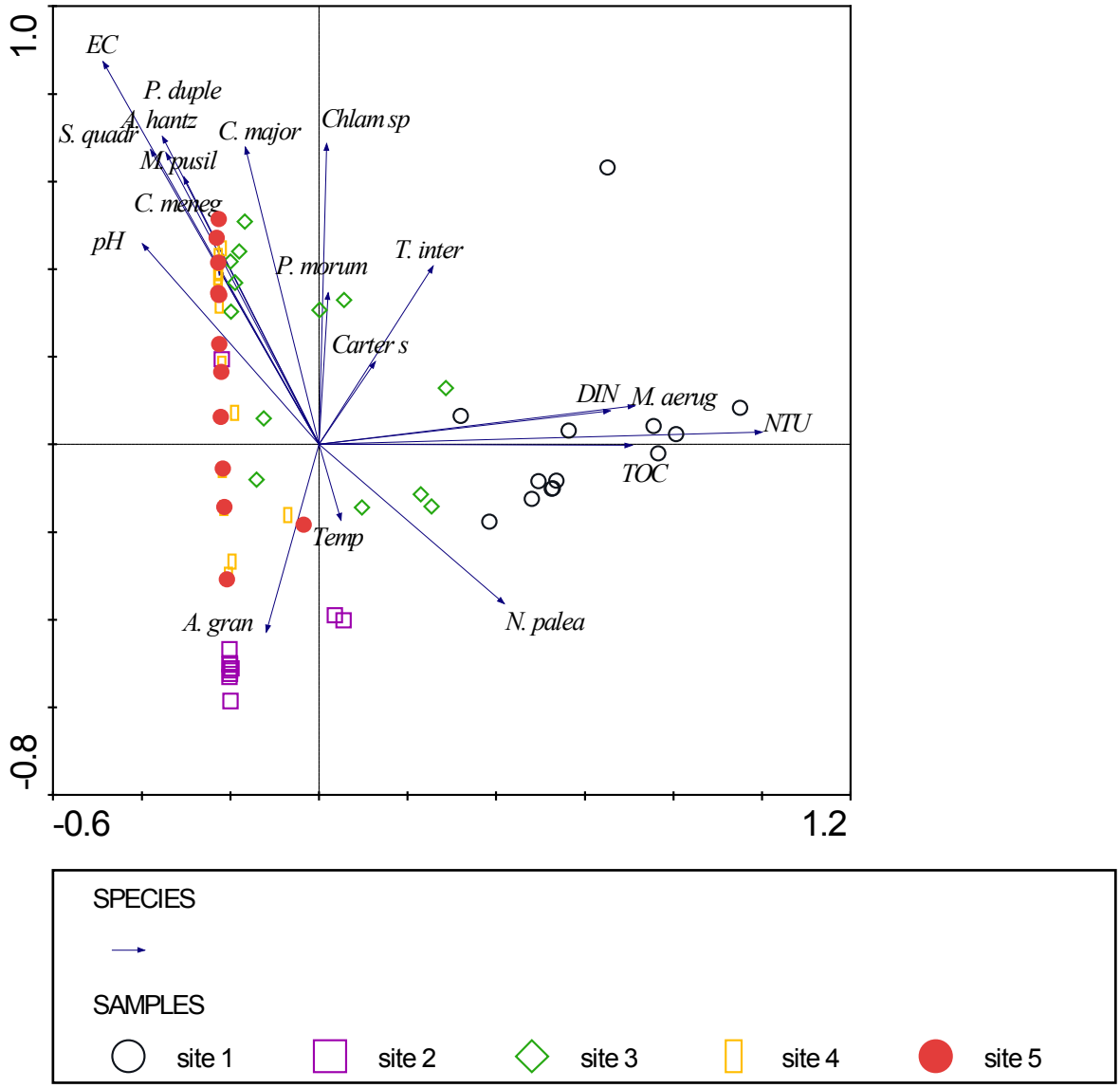


FIGURE 32: Bi-plot PCA ordination diagram showing the correlation of major algal species, environmental variables and 5 sampling sites over the study period. Site 1 = Allemanskraal Dam, site 2 = primary settlement tank Virginia, site 3 = Vaal River, site 4 = primary settlement tank Balkfontein, site 5 = secondary settlement tank Balkfontein.

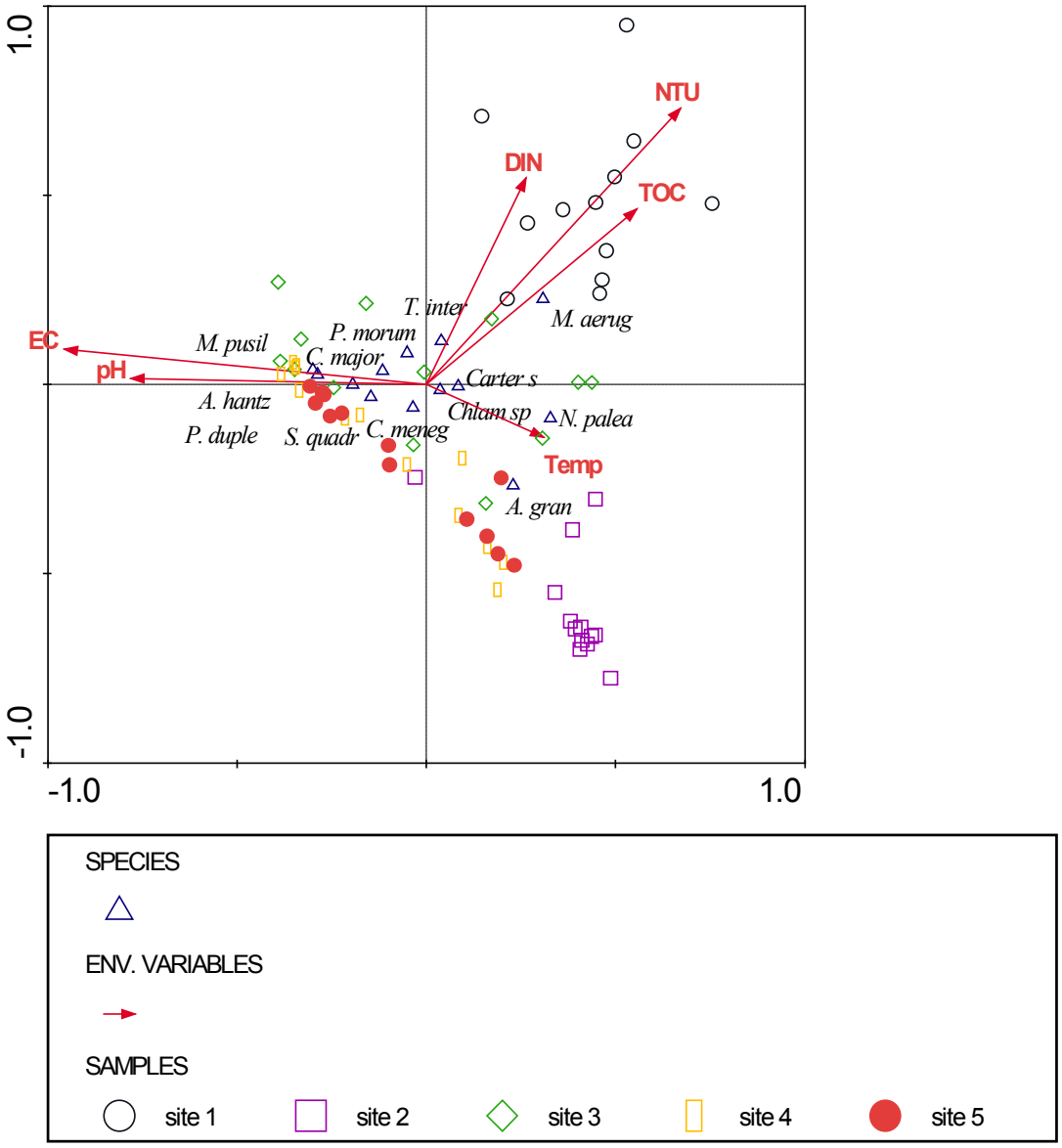


FIGURE 33: CCA ordination diagram showing the correlation between major algal species, environmental variables and 5 sampling sites. Site 1 = Allemanskraal Dam, site 2 = primary settlement tank Virginia, site 3 = Vaal River, site 4 = primary settlement tank Balkfontein, site 5 = secondary settlement tank Balkfontein.

5.2 STATISTICAL ANALYSES

Statistical analyses were done on the principal algal components found in the source water and water after settlement at both water purification plants to test some of the results in the previous chapters. This was done by means of some exploratory data analyses.

The mean was found by summing all the values of each variable and dividing it by the total number of observations. This gave a good indication of the algal abundance at each sampling site and these values were particularly affected by some of the extreme values that occurred in the source water. The standard deviation was taken as a measure of dispersion, indicating to which degree the values in the data set deviated from the mean. At Virginia the T-test and Wilcoxon Matched Pairs test were used to indicate the difference between the means of the dependent samples.

TABLE 3: Results of dependent T-test and Wilcoxon Matched Pairs test (WMP) for the difference between source water and water after sedimentation at Virginia water purification plant. Values indicated in red means they are statistically significant ($p<0.05$).

Species	Source water		Primary Settlement		T-test	WMP
	Mean	Standard Deviation	Mean	Standard Deviation	p-value	p-value
Anabaena sp.	22133	39957	9	19	0.058663	0.000982
Microcystis aeruginosa	15132368	42467111	20	39	0.205337	0.000982
Navicula sp.	143	484	6	9	0.307955	0.008775
Nitzschia palea	40061	112926	68	58	0.208019	0.396727
Chlamydomonas sp.	513	930	13	17	0.065521	0.001225

At Balkfontein the repeated measures and Friedman ANOVA were used. The reason for this was that it took less time for algae to move through the system at Balkfontein than at Virginia and these tests were deemed to be more suitable.

TABLE 4: Results of dependent repeated measures (RM) ANOVA and Friedman ANOVA for the difference between source water and water after sedimentation at Balkfontein water purification plant. Values indicated in red means they are statistically significant ($p<0.05$).

Species	Means			Mean Square Error	RM ANOVA	Friedman ANOVA
	Source water	Primary settlement	Secondary settlement		p-value	p-value
Cyclotella meneghiniana	1794	20	34	0.006392	0.022509	0.00001
Actinastrum hantzhii	3727	1091	727	0.027598	0.054693	0.00050
M. pusilllum	1456	513	220	0.012155	0.045789	0.20340
P. duplex	792	103	126	0.001499	0.007035	0.02461
Scenedesmus quadricauda	3749	191	183	0.000713	0.010035	0.00010

When data is indicated in red the P-value is smaller than 0.05, indicating that the specific data is statistically significant in showing an improvement in water quality after settlement. When data is indicated in black, the data might have proved to be more statistically significant had more data been available. Values were expressed as dependant data for every month at each site due to the time it takes for water to move through the system. The mean values also indicate a clear decrease in algal concentration from raw water to water after settlement at both the water purification plants. This is in accordance with the results found in Chapter 3.

CHAPTER 5: CONCLUSIONS

Seven major phytoplankton taxa were found at the ten different sampling sites namely the Cyanophyceae, Bacillariophyceae, Chlorophyceae, Cryptophyceae, Chrysophyceae, Dinophyceae and Euglenophyceae. The Cyanophyceae, Bacillariophyceae and the Chlorophyceae were the most abundant in terms of concentration and diversity and they succeeded one another continually as the dominant groups. The other groups were less numerous, though not less important during the study period.

The Chlorophyceae showed the highest diversity with 29 species and 16 genera not identified to species level. This was followed by the Bacillariophyceae with 16 species and 9 genera, the Euglenophyceae with 10 species and 2 genera, the Cyanophyceae with 5 species and 5 genera, the Dinophyceae with 1 species and 2 genera and the Cryptophyceae and Chrysophyceae, with one representative species each. During the study period a total of 63 species were identified, along with 34 species that were only identified up to genus level.

Algal species capable of producing blooms and genera frequently involved in bloom formation are *Microcystis, Anabaena* and *Oscillatoria* (Cyanophyceae), *Chlorella, Ankistrodesmus* and *Chlamydomonas* (Chlorophyceae), *Cyclotella* (Bacillariophyceae) and *Euglena* (Euglenophyceae). All of the above mentioned genera were present during the study period, some forming blooms under environmental conditions favouring their growth. Blooms cause numerous problems that range from aesthetically unacceptable conditions to the poisoning of animals by cyanobacteria, such as *Microcystis aeruginosa*. Algal species present during this study responsible for taste and odour problems include *M. aeruginosa, Anabaena* sp., *Oscillatoria* sp., *Pandorina morum* and *Ceratium hirundinella*. Algal species present that may be responsible for clogging sand filters in water purification plants include *Aulacoseira granulata, Cyclotella meneghiniana, Ulnaria ulna, Navicula* sp., *Chlamydomonas* spp. and *Ceratium*

hirundinella. In addition *C. hirundinella* and other flagellated species are also responsible for the destabilisation of flocs during the flocculation process in water purification.

Microcystis aeruginosa dominated at 8 of the 10 sampling sites. It was dominant for an extensive period in the Allemanskraal Dam from the summer periods to well into the month of May. Bloom conditions may pose threats and if current purification methods prove to be inefficient, a different source of water may be required for purification to be more cost effective. *Anabaena* also gained dominance for brief periods in 4 sites where it succeeded *Microcystis aeruginosa* as the dominant. *Nitzschia palea* was the second most abundant species in the Allemanskraal Dam after the dominant Cyanophyceae and was present in very high concentrations during the *Microcystis aeruginosa* bloom. This implies that this species is also indicative of eutrophication as it flourished under similar conditions, while successfully competing with *M. aeruginosa*.

The primary settlement tank at the Virginia water purification plant was mostly dominated by Bacillariophyceae, especially *Aulacoseira granulata.* Sedimentation is more efficient in removing unicellular centric diatoms and as a result, pennate and filamentous diatoms are more prolific. The occurrence of *A. granulata* and *Nitzschia palea* is comparable to that of the Cyanophyceae which is a very good indicator of eutrophication. The fact that the algal concentration remained low, regardless of high algal concentration in the source water, is indicative of successful settlement processes that effectively remove problematic algae.

The Bacillariophyceae, especially small centric *Cyclotella* species and the Chlorophyceae tend to be the most common algal groups in the Vaal River. The Bacillariophyceae was gradually removed by the different purification processes at the Balkfontein purification plant and this is indicative of the smaller impact that diatoms have during purification, aside from blocking of sand filters.

The Chlorophyceae was abundant during the entire period with species like *Actinastrum* sp. and *Pandorina morum* dominating in winter and early spring, while *Pediastrum duplex* and *Scenedesmus quadricauda* dominated from late winter to mid-summer. *Chlamydomonas* sp. was periodically dominant during the study period. The Chlorophyceae dominated throughout the year, with different representatives that can tolerate different temperature ranges. The species of *Pediastrum* and *Scenedesmus* that were abundant during the study are indicative of eutrophication in the Vaal River system.

The results of this study emphasise that both river systems are eutrophic, based on their algal composition and thus the purification and management of the water purification plants need to be adjusted accordingly to produce a final water of good quality in a cost effective way. Compounds released from algal cells during water purification that may affect the taste, odour and other aspects of the final water need to be considered and ways to effectively remove these compounds need to be developed or implemented if the need arises. Removing the algal cells before they release these compounds remains more preferable.

Extremely high algal concentrations were seen in the Allemanskraal Dam for the entire study period due to blooms of cyanobacteria, especially *Microcystis aeruginosa* and *Anabaena* sp. between February and June 2010 with a peak during May. Peak concentrations could not be attributed to high temperatures because of a change in seasons to winter. It emphasises the effect that nutrients have on a water system and is also indicative of high nutrient loadings in the Sand River system. The algal concentration in the Vaal River at Balkfontein was much lower than in the Allemanskraal Dam.

There was an increased concentration of Bacillariophyceae and Chlorophyceae relative to the (still dominant) Cyanophyceae in the water transfer canal. This might be attributed to the fact that the withdrawal point for the transfer canal is below the surface of the water which decreases the amount of Cyanophyceae that may enter the next part of the system. Increased mixing in the transfer canal

might also have discouraged the growth of Cyanophyceae as a surface scum was not formed. In the reservoir the Chlorophyceae became relatively more important due to a decrease in Cyanophyceae concentration rather than an increase in Chlorophyceae concentration. During primary settlement a dramatic change was seen in the percentage composition of the phytoplankton where the Cyanophyceae was reduced to only 15%. This indicates that the Virginia water purification plant is effective in removing problematic phytoplankton groups and there is no need to alter purification processes or to periodically cease water transport from the Allemanskraal Dam to the Virginia water purification plant provided that the abstraction point stays well below the water surface.

In the Vaal River at Balkfontein the total Chlorophyceae concentration was lower after settlement, although the percentage composition relative to the Bacillariophyceae and Cyanophyceae was higher than in the Vaal River. This remained constant from primary through to secondary settlement with minor alterations. The Chlorophyceae are less important with regard to the problems they cause in the final water although high densities can be aesthetically unacceptable. To improve floc formation for effectively removing Chlorophyceae, pre-oxidation in the form of pre-ozonation is suggested before adding flocculants.

The highest abundance of Cyanophyceae in the Balkfontein water purification plant was found in the recycling dam and recycling dam outlet. *Ceratium hirundinella* was also more proliferate in these two sites. In the recycling dam outlet Dinophyceae was the second most abundant group (after the Cyanophyceae) from February to May 2010. Conditions at certain times of the year were beneficial for the increase of *C. hirundinella*, probably as a result of the germination of cysts introduced during previous years. The high numbers of *C. hirundinella* cells may have a negative impact since water from the recycling dams is recycled and purified along with the raw water from the Vaal River and the presence of *C. hirundinella* may lead to the disturbance of flocs, clogging of sand filters and taste and odour problems in the final water. Little effect of the Dinophyceae was noticed at Virginia.

In the Allemanskraal Dam the Dissolved Inorganic Nitrogen (DIN) concentration was very high during May 2010 and it helps to explain the peak in algal abundance during that month. Total Organic Carbon (TOC) also increased during May. The pH during April and May was lower than usual. More data is needed to determine why the bloom of *Microcystis aeruginosa* commenced at the start of the study and reached such high levels in autumn when temperatures were low. Data on total phosphorus for the period would have been very helpful in formulating an explanation.

Peaks in the concentration of *Anabaena* sp. and *Microcystis aeruginosa* in the water transfer canal were observed a month before similar peaks in the Allemanskraal Dam. The same tendency can be seen with an increase in *Anabaena* during January 2011, a month before a similar increase in the Allemanskraal Dam during February 2011. The reason for this may be that dormant stages (or cysts) of the dominant species entered the water transfer canal where conditions for a bloom might have been better than in the Allemanskraal Dam. The high concentrations of Cyanophyceae did not correlate with high concentrations of DIN during the same period so it might be ascribed to cells entering the transfer canal from the Allemanskraal Dam. Concentrations of *Microcystis aeruginosa* decreased rapidly from approximately 150 million cells/ml in the Allemanskraal Dam to approximately 8 300 cells/ml in the water transfer canal during autumn 2010. This might be the result of a high mixing rate.

The high concentration of Cyanophyceae in the reservoir was not a result of changes in key environmental variables like temperature and plant nutrients, but probably the result of the bloom in the Allemanskraal Dam during the same period and dormant stages that were transported to the reservoir by means of the water transfer canal. *Pandorina morum* dominated during July 2010, the result of an increase in the DIN concentration of the reservoir during the same period. *Chlamydomonas* dominated during November 2010 after an increase in DIN concentration during October 2010. *Chlamydomonas* numbers also increased

after February 2011 when it dominated again as a result of an increase in DIN concentration during the same month. Increases in algal abundance were observed after increases in nutrient concentrations.

Actinastrum hantzschii, normally common in eutrophic freshwater systems, dominated during the winter months, peaking during July 2010 in the Vaal River. In this study it seemed to have a preference for colder climatic conditions.

Concentrations of *Scenedesmus quadricauda* peaked during September 2010 during a simultaneous peak in the turbidity in the Vaal River. Increases in *S. quadricauda* and increases in turbidity were later found to not be related after further comparisons were made. *S. quadricauda* occurs in all climates but prefers eutrophic to hypertrophic waters, again indicative of the Vaal River's eutrophic nature. *Cyclotella meneghiniana* was dominant in May and October 2010 and through the rest of the year it maintained a constant presence due to the fact that it is such a cosmopolitan species. *Microcystis aeruginosa* was dominant in December 2010 as a result of the higher summer temperatures and sustainable nutrient concentrations that were experienced.

Actinastrum hantzschii was dominant in the primary settlement tank at Balkfontein. This corresponds with the peak during the same period in the Vaal River, showing that it was introduced via the source water. Settlement in the primary settlement tank was successful in removing two thirds of the cells.

In the secondary settlement tank algal abundance again increased during the winter period with *Actinastrum hantzschii* being dominant from May to September 2010. The concentration was also high during June and August although concentrations decreased after secondary settlement for the period. When compared with concentrations of algal cells after primary settlement at Virginia, this is quite high and a revision of flocculation and sedimentation methods, especially with regard to green algae, should be considered at Balkfontein.

In the recycling dam at Balkfontein, cyanobacterial dominance at the beginning of the study period gave way to green algal dominance at the end of the period. High DIN concentrations throughout the study correlate with the corresponding high cyanobacterial abundance in the recycling dam as it is a vital nutrient that often results in blooms when present in high concentrations. TOC concentrations were also very high throughout the period possibly due to high algal biomass.

An increase in *Ceratium hirundinella* concentration was noticed in May in the recycling dam at Balkfontein, where the species briefly succeeded *Microcystis aeruginosa* as the dominant. Additional biomass determinations would have provided a clearer perspective on the impact of the *Ceratium hirundinella* increase during autumn 2010 and this should be amended with future studies that include biomass measurements.

The species composition in the recycling dam outlet was similar although the species concentration was lower than in the recycling dam. This is preferable as fewer cyanobacteria and *Ceratium hirundinella* cells will be returned to the head of works for purification.

Multivariate analyses showed positive correlations between *Microcystis aeruginosa* and environmental variables such as dissolved inorganic nitrogen (DIN) and turbidity (NTU). The high density of *M. aeruginosa* can, therefore, be attributed to plant nutrients such as DIN and this leads to increased (organic) turbidity in the system. *Nitzschia palea* and *Aulacoseira granulata* showed a positive correlation with temperature. The green algae, on the other hand, showed a negative correlation with temperature, supporting previous findings that stated a preference to colder water conditions. *Microcystis aeruginosa* is more closely grouped together with the raw water samples, especially the Allemanskraal Dam, than the samples taken in the settlement tanks. This is indicative of effective removal of this problematic species and proves that the current sedimentation processes are effective.

The statistical analyses gave a good indication of the algal abundance at each sampling site and these values were particularly affected by some of the extreme values that occurred in the source water. Values were expressed as dependant data for every month at each site due to the time it takes for water to move through the system. The mean values indicated a clear decrease in algal concentration from raw water to water after settlement at both of the water purification plants.

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